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Mammary-specific Deletion of PTHrP Reduces Bone Turnover and Preserves Bone Mass During Lactation. J. VanHouten,*[1] P. Dann,*[1] A. Stewart,*[2] C. Watson,*[3] A. Karaplis,*[4] J. Wysolmerski.*[1] [1]Yale Medical School, New Haven, CT, USA, [2]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [3]University of Cambridge, Cambridge, United Kingdom, [4]McGill University, Montreal, Canada.

Lactating mammals secrete large amounts of calcium into milk. Much of this calcium appears to come from the maternal skeleton, for lactation is associated with reversible increases in bone resorption and reductions in bone mass. Neither PTH nor 1,25 dihydroxyvitamin D are necessary or sufficient to account for the mobilization of calcium during lactation, and the regulation of calcium transfer from mother to milk remains poorly understood. The PTHrP gene is highly expressed in mammary epithelial cells (MEC) during lactation, and milk is the most abundant physiological source of PTHrP. It has been suggested that PTHrP might contribute to the regulation of bone turnover during lactation, but because mammary development fails in the absence of PTHrP, traditional PTHrP knockout mice have not addressed this question. In order to examine this issue, we utilized Cre-lox technology to delete the PTHrP gene in MEC, but only at the onset of lactation after development was complete. We created mice that were hemizygous for an ovine β-lactoglobulin (BLG) promoter-driven Cre transgene and that were compound heterozygotes at the PTHrP locus, with one allele containing a disrupted exon 4 and the other containing an exon 4 flanked by loxP sites. In these Cre-lox mice, upon induction of the BLG milk gene promoter during lactation, the one functioning PTHrP allele is removed by Cre-mediated recombination specifically in MEC. Littermate controls had identical PTHrP loci, but no BLG-Cre. Nearly all MEC of lactating Cre-lox, but not virgin Cre-lox or control mice, expressed Cre. PTHrP RNA was almost completely absent in mammary glands from lactating Cre-lox mice. Milk PTHrP was reduced by 75% in these mice, but significant amounts (4.7ng/ml) remained. Circulating levels of PTHrP were reduced, as was urinary cAMP excretion. In addition, compared to controls, lactating Cre-lox mice had lower rates of bone resorption as measured by histomorphometry and urinary excretion of type I collagen fragments, and higher bone mass. In summary, we have successfully deleted the PTHrP gene within the lactating mammary gland. This lowers PTHrP levels in milk and in the maternal circulation. It also lowers maternal bone resorption rates and preserves maternal bone mass. We conclude that MEC secrete PTHrP into both milk and the maternal circulation during lactation. This mammary-derived PTHrP increases bone resorption and leads to a decrease in bone mass, thus contributing to the efflux of calcium from the maternal skeleton during lactation.

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Essential Role of HIF-1alpha in Growth Plate Development. E. Schipani,[1] H. E. Ryan,*[2] T. Kobayashi,[1] M. Knight,*[1] R. S. Johnson.*[2] [1]Endocrine Unit, MGH-Harvard Medical School, Boston, MA, USA, [2]Division of Biology, UCSD, San Diego, CA, USA.

The growth plate is an avascular tissue. However, no data are available that directly address the issue of the degree of oxygenation of the growth plate during embryogenesis, and the role of hypoxic response in that tissue.To ascertain the presence of hypoxia in mammalian fetal cartilage, we injected a marker for bio-reductive activity into pregnant female mice E15.5. This marker, the nitroimidazole EF5, allowed us to study distribution of the molecule in the fetal growth plate via a rhodamine-coupled anti-EF5 antibody .We found that the fetal growth plate is hypoxic in its interior but not at its periphery, indicating that there is a gradient of oxygenation evolved during the growth of this tissue.In vitro the transcripion factor HIF-1alpha is a major modulator of the trancriptional response to hypoxia, by regulating expression of genes involved in metabolic processes and angiogenesis.We thus postulated that HIF-1alpha could be critical for growth plate development. Mice nullizygous for HIF-1alpha die at approximately E9. Therefore, in order to determine the role of HIF-1alpha in chondrocyte activity and, in general, to investigate its role in survival of hypoxic cells in vivo, we conditionally inactivated HIF-1 alpha in growth plate chondrocytes. Newborn mutant mice lacking HIF-1 alpha in the growth plate were smaller than control littermates with characteristic shortening of the limbs and died within a few hours of birth. Their growth plates were misshapen and disorganized. In particular, the center of both the proliferative and the hypertrophic zones was either remarkably hypocellular or occupied by abnormal cellular elements with irregular nuclei.The presence of cells expressing typical chondrocyte markers such as collagen type II and/or collagen type X at the periphery of the mutant growth plate indicated that the lack of HIF-1alpha had not significantly altered the process of chondrocyte proliferation and differentiation per se. However, the absence of collagen type II mRNA expression and the presence of numerous TUNEL positive cells in the central core of the growth plate provided evidence that chondrocytes in the core of the mutant cartilaginous element underwent massive cell death and that, therefore, HIF-1alpha was required for survival of hypoxic chondrocytes. This severe and unique phenotype was associated with downregulation of mRNAs encoding both VEGF and enzymes of the glycolytic pathway.This is the first in vivo model that demonstrates the physiological role of HIF-1alpha in cellular adaptation to hypoxia during fetal development; as such, it points out the essential role micro-physiological response plays in mammalian ontogeny.

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Heterozygous PPARγ-Deficient Mice Exhibit High Bone Mass with Increased Osteoblastic Differentiation from Bone Marrow Progenitors. T. Akune,[1] N. Kubota,*[2] Y. Harada,[3] Y. Azuma,[3] T. Kadowaki,*[2] K. Nakamura,[1] H. Kawaguchi.[1] [1]Orthopaedic Surgery, University of Tokyo, Tokyo, Japan, [2]Metabolism, University of Tokyo, Tokyo, Japan, [3]Teijin Co. Ltd., Tokyo, Japan.

Possible regulation of osteoblastic differentiation by peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor that plays a pivotal role in adipocyte differentiation, has been reported in several mesenchymal cell cultures. To investigate the role of endogenous PPARγ in vivo, we created mice lacking the PPARγ gene by homologous recombination in mouse embryonic stem cells. Although homozygous PPARγ-deficient embryos died at 10-11 dpc due to placental dysfunction, heterozygous PPARγ-deficient (+/-) mice developed normally except for lower fat mass than wild-type (+/+) mice on a high-fat diet. However, bone densitometry and 3D-μCT analyses revealed significant increases of both trabecular and cortex bone volumes in +/- mice compared to +/+ littermates. Histomorphometric analysis at 8 weeks showed that bone volume (BV/TV) was 38% higher and parameters for osteoblast number (Ob.S/BS, OS/BS, & MS/BS) were 92-114% higher in +/- mice. On the other hand, bone formation parameters normalized to the number of osteoblasts (MAR & BFR/Ob.S) and bone resorption parameters (Oc.N/B.Pm & ES/BS) were slightly (5-12%) but not significantly higher in +/- mice. In bone marrow cell culture derived from +/- mice, osteoblastogenesis determined by ALP-positive CFU-F and Alizarin red / von Kossa-positive CFU-OB was markedly increased compared to that in +/+ marrow cell culture. Troglitazone, a ligand of PPARγ, notably stimulated adipogenesis (oil red O-positive CFU-AD) and inhibited osteoblastogenesis in +/+ marrow cell culture, while both effects were much milder in +/- marrow cell culture. +/- marrow cells showed lower PPARγ but higher Cbfa1 mRNA levels than +/+ marrow cells. Osteoblastic proliferation and matrix synthesis in the primary calvarial osteoblast culture did not differ between +/- and +/+ cultures. Osteoclastogenesis in the M-CSF-dependent bone marrow macrophage culture in the presence of soluble RANKL, osteoclastogenesis / resorbed pit formation in the co-culture of osteoblasts and marrow cells, and isolated osteoclast survival were not different between +/- and +/+ cultures. We conclude that deficiency of PPARγ in +/- mice increases bone mass by stimulating osteoblastogenesis from bone marrow progenitors without affecting mature osteoblasts or osteoclast-lineage cells. Because human aging is associated with a reciprocal increase in adipocytes and a decrease in osteoblasts in bone marrow, PPARγ may be clinically involved in the pathophysiology of bone loss with aging as a suppressor of osteoblastogenesis.

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Dysfunctional Osteoblasts, Impaired Osteoblastogenesis, Reduced Bone Formation and Severe Osteoporosis in a Calcineurin Aα Null Mouse. Molecular Implications for Understanding Immunosuppressant Bone Loss. L. Sun,[1] O. A. Adebanjo,[1] X. H. Liu,*[1] B. S. Moonga,[1] A. Inzerillo,*[1] A. Koval,*[1] X. Y. Wu,*[1] X. B. Wu,*[1] P. J. R. Bevis,[1] H. C. Blair,[2] D. Conner,*[3] J. Seidman,*[3] B. R. Troen,[1] S. Epstein,[1] E. Abe,[1] M. Zaidi.[1] [1]The Mount Sinai Bone Program and Bronx VA GRECC, NY, USA, [2]University of Pittsburgh, PA, USA, [3]Harvard Medical School, MA, USA.

The widely used immunosuppresants, cyclosporine A (CsA) and tacrolimus (FK506) cause profound bone loss. Both compounds potently inhibit calcineurin A, a calmodulin-activated cellular phosphatase. We report here that: (a) calcineurin Aα null mice are severely osteoporotic with reduced bone formation; (b) calcineurin Aα directly stimulates bone formation; and (c) the genes for calcineurin A and its signaling molecules, NFAT1c and ryanodine receptors are co-regulated. Calcineurin Aα null mice exhibited runted long bones (~20%) and reductions in tibial metaphyseal diameter (~15%) and diaphyseal cortical thickness (~40%). Femoral bone mineral density was significantly decreased. Dual tetracycline labeling indicated a ~40% reduction in bone formation *in vivo* consistent with diminished osteoblast maturation (CFU-F formation) *in vitro.* Together, the findings provided compelling evidence for a role for calcineurin A in osteoblast formation and thus prompted further studies. We therefore cloned both calcineurin Aα and Aβ genes from a cDNA library. Immunostaining, confocal imaging, Western immunoblotting, RT-PCR and single cell *in situ* RT-PCR indicated that calcineurin Aα was localized to both osteoblasts and osteoclasts. We next synthesized and purified a TAT-calcineurin Aα fusion protein. Note that the fusion of any protein with TAT, a 12 amino acid long Arg-rich sequence, results in its receptor-less movement across cell membranes. Likewise, MC3T3.E1 osteoblastic cells were transduced with high, ~90%, efficiency with the TAT-calcineurin Aα. Noteworthy is that the transduced fusion protein dramatically enhanced osteoblastic maturation (alkaline phosphatase positivity). This, together with the bone formation defect seen in the calcinueirn Aα null mouse, suggested clearly that calcineurin Aα was directly anabolic to bone. Finally, we found that the calcineurin A signaling molecules, ryanodine receptor and NFAT1c, were co-regulated upon calcineurin Aα transfection of MC3T3.E1 cells as well as in the calcineurin Aα null mouse. Taken together, the evidence strongly favors a new role for the calcineurin pathway in osteoblastic bone formation. The inhibition of this pathway may underlie the low turnover osteoporosis seen with CsA and FK506.

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Stimulated Osteoclastogenesis is Impaired in NF-κB Inducing Kinase Knockout Mice Despite an Intact NF-κB Signaling Pathway. D. V. Novack, L. Yin,* R. D. Schreiber,* F. P. Ross, S. L. Teitelbaum. Pathology, Washington University, St. Louis, MO, USA.

RANKL activates NF-κB in osteoclast precursors. Mice lacking RANKL, RANK, or both the p50 and p52 subunits of NF-κB fail to generate osteoclasts (OCs). NF-κB inducing kinase (NIK) is a non-receptor serine/threonine kinase thought to link a number of cell surface receptors with the NF-κB pathway. Given the central role of the RANKL/RANK/NF-κB pathway in osteoclastogenesis, we asked if NIK is essential for OC formation. Concentrations of RANKL as high as 10 fold the optimal dose for wild-type (WT) cells fail to induce OC differentiation of marrow macrophages derived from $NIK^{-/-}$ mice ($p<0.001$ compared to WT). Those $NIK^{-/-}$ OCs that do form do not spread normally nor resorb bone-like substrates. Because TNFα and TGFβ synergize with suboptimal doses of RANKL in normal cells, we asked if these cytokines would enhance osteoclastogenesis in NIK-deficient cultures. TNFα, and especially TGFβ, substantially rescues the capacity of $NIK^{-/-}$ macrophages to form functional OCs, but only when combined with doses of RANKL 5-10 times higher than those needed in WT cultures. OC number is completely normalized by the combination of TNFα and TGFβ in the presence of high dose RANKL. Having established that absence of NIK impairs osteoclastogenesis in vitro, we turned to the role of the kinase in vivo. OCs are present in normal numbers in the bones of unmanipulated, NIK-deficient mice, perhaps due to the presence of TNFα and/or TGFβ in vivo. However, these animals fail to respond to the potent osteoclastogenic agent parathyroid hormone (adminis-

tered over the calvarium every 6 hours for 3 days), with either stimulated osteoclastogenesis or enhanced resorption, as determined by calvarial OC number and porosity. Studies performed in other cell types, and involving other membrane receptors, have linked NIK with the NF-κB pathway at various levels. Absence of NIK, however, does not diminish NF-κB activation in OC precursors in response to RANKL. RANKL induces normal IκBα degradation in $NIK^{-/-}$ precursors, with subsequent nuclear translocation of NF-κB and up-regulation of the NF-κB responsive genes IκBα, ICAM, and TLR-2. Moreover, NIK has also been implicated as the molecule required for processing of p100 to p52, but we find normal p52 levels in RANKL-stimulated OC precursors. These events establish that, despite their inability to undergo osteoclastogenesis, $NIK^{-/-}$ macrophages show full activation of NF-κB by RANKL. Thus NIK, while not required for basal osteoclastogenesis, is essential for stimulated osteoclastogenesis and bone resorption, and has critical functions in OC recruitment beyond the activation of NF-κB.

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Osteoclast Inhibitory Lectin – A Family of New Osteoclast Inhibitors. H. Zhou, V. Kartsogiannis, J. M. W. Quinn, J. Elliott,* C. Ly,* K. W. Ng, M. T. Gillespie. St. Vincent's Institute of Medical Research, Melbourne, Australia.

Osteoclast Inhibitory Lectin (OCIL) is a predicted type II membrane-bound molecule of 207aa containing a C-lectin domain in its extracellular domain. Recombinant protein to the extracellular domain of mOCIL inhibits osteoclast formation in murine osteoblast with spleen cell cocultures, murine spleen and adherent spleen cell cultures treated with RANKL and M-CSF, and in human monocyte cultures treated with M-CSF and RANKL. These results suggest that OCIL acts upon hemopoietic cells to inhibit osteoclast formation.We have identified two novel C-lectins that share substantial identity with murine OCIL. These new molecules have been designated OCILrp1 and OCILrp2. The novel OCIL-related proteins were identified by nucleic acid sequence analyses of RT-PCR products derived from RNA extracted from murine osteoblasts treated with IL-11 for 24 hr. Full length OCILrp1 and OCILrp2 were cloned and predicted to encode type II membrane-bound C-lectins of 218 and 217aa, respectively. The predicted intracellular domains of OCILrp1 and OCILrp2 shared 83% identity, but showed no identity with the intracellular domain of OCIL. The extracellular domains of OCILrp1 and OCILrp2 share 83% and 75% identity, respectively, with the extracellular domain of OCIL. The extracellular domains of OCILrp1 and OCILrp2 were expressed in E. coli, and each protein inhibited osteoclast formation in murine spleen cultures treated with M-CSF and RANKL with similar potencies to OCIL (IC50 0.2 ng/ml).Immunohistochemistry using antipeptide antibodies to the intracellular domain of OCILrp1/2 and to OCIL demonstrated that OCIL and OCILrp1/2 were concordantly expressed in osteoblasts, chondrocytes and in extraskeletal tissues. Further, their cellular distribution was identical to that of RANKL. Distinct genes encoded the three OCIL family members. OCIL and OCILrp1 genes were of 6 and 9kb, containing 5 and 6 exons, respectively, with 4 intron/exon boundaries conserved suggesting gene duplication. A TATA promoter controlled OCIL expression and a GC-rich promoter controlled OCILrp1. Consistent with these promoters, OCIL mRNA expression in primary murine osteoblasts was upregulated by calciotropic agents (PTH, 1,25(OH)2 vitamin D3 and IL-11), while OCILrp1 and OCILrp2 were constitutively expressed. Notably, osteoblast OCIL was upregulated by calciotropic agents that also upregulate RANKL; in contrast these factors typically down regulate OPG production by osteoblasts.The identification of three distinct genes and OCIL proteins implies redundancy for OCIL, and their concordant expression with that of RANKL suggests that the RANKL : OPG axis may be further influenced by OCIL family members.

Disclosures: Pfizer Ltd.,2.

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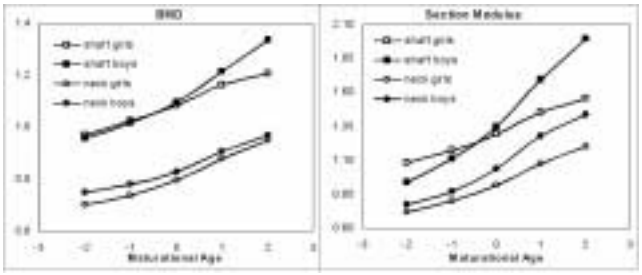
Gender Differences In Bone Geometry During The Adolescent Growth Spurt. D. A. Bailey,[1] T. J. Beck,[2] M. R. Forwood,[3] R. L. Mirwald,*[1] W. A. Wallace,*[1] R. A. Faulkner.*[1] [1]College of Kinesiology, University of Saskatchewan, Saskatoon, SK, Canada, [2]Radiology, The Johns Hopkins University, Baltimore, MD, USA, [3]Anatomical Sciences, The University of Queensland, Brisbane, Australia.

To investigate whether there are gender differences in the bone geometry of the proximal femur during the adolescent years we used an interactive computer program ?Hip Strength Analysis? developed by Beck and associates (Beck et al., Invest Radiol. 1990,25:6-18.) to derive femoral neck geometry parameters from DXA bone scans (Hologic 2000, array mode). We analyzed a longitudinal data-set collected on 70 boys and 68 girls over a seven year period. Distance and velocity curves for height were fitted for each child utilizing a cubic spline procedure and the age of peak height velocity (PHV) was determined. To control for maturational differences between children of the same chronological age and between boys and girls, section modulus (Z) an index of bending strength, cross sectional area of bone (CSA), sub-periosteal width (SPW), and BMD values at the neck and shaft of the proximal femur were determined for points on each individual?s curve at the age of PHV and one and two years on either side of peak. To control for size differences, height and weight were introduced as co-variates in the two-way analyses of variance looking at gender over time measured at the maturational age points (-2, -1, age of PHV, +1, +2). The following figure presents the results of the analyses on two variables, BMD and Z at neck and shaft regions:After the age of peak linear growth (PHV), independent of body size, there was a gender difference in BMD at the shaft but not at the neck. Section modulus at both sites indicated that male bones became significantly stronger after PHV. Underlying these maturational changes, male bones became wider (SPW) after PHV in both the neck and shaft and enclosed more material (CSA) at all maturational age points at both regions. These results call into question the emphasis on using BMD as a measure of skeletal integrity in growing children.



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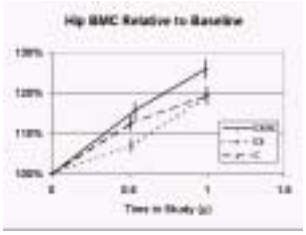
Exercise without Sufficient Calcium Does Not Increase Rate of Bone Mass Accrual in Pubertal Females. J. M. Lappe, J. A. Stubby,* K. M. Davies, R. R. Recker. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

The link between optimal development of the skeleton in childhood and prevention of fractures late in life is widely acknowledged. Although it is recognized that exercise is necessary for optimal skeletal development, our understanding of the effects of exercise on bone strength of growing females is incomplete. Further, we do not know whether increased exercise enhances skeletal development in the absence of sufficient calcium. These questions are particularly salient in light of the current low levels of physical activity and calcium intake in American youth. The purpose of this study is to determine if weight-bearing exercise increases bone mass accrual in pubertal females and whether the combination of weight-bearing exercise and a high calcium diet has a greater effect than exercise alone. The convenience sample includes 101 healthy premenarchal females who were 9 to 10 years old and in Tanner Stage 1 upon entry into study. Participants were randomly assigned to 1 of 3 groups: 1. (EX) participate in a weight-bearing exercise program that meets 3 times/week and consume usual diets; 2. (EXHC) participate in the weight-bearing exercise program and consume at least 1500 mg/day of calcium from foods; or 3. (C) consume usual diet and maintain customary activity level. Bone mineral content (BMC) is measured with dual energy absorptiometry (DXA) at the hip, spine, radius and total body. Calcium intake is determined by 3-day diet diaries. This is an interim report made 1 yr. into a 3-yr study.There were no differences in anthropometric or bone mass values at baseline. Mean baseline hip bmc by group was: EX 15.6(3.4) g; EXHC 14.4(3.0) g; and C 15.2(3.3) g. Mean daily intake of calcium by group during the study was: EX-898 mg; EXHC-1644 mg; and C-847 mg. One way ANOVA indicated that after 1 year of treatment the EXHC group demonstrated a significantly greater rate of increase in hip BMC than both the EX and the C groups ($p<0.05$). (See figure). Rate of gain of BMC at the other sites did not differ between groups. We conclude that exercise-related increases in bone mass accrual in pubertal girls are dependent on sufficient calcium intake.



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The Effect of Dietary Sodium on Calcium Retention in Black and White Female Adolescents. K. Wigertz,[1] C. Palacios,[1] A. Kempa-Steczko,*[1] B. Martin,[1] G. McCabe,*[1] M. Peacock,[2] J. H. Pratt,*[2] C. M. Weaver.[1] [1]Purdue University, West Lafayette, IN, USA, [2]IU School of Medicine, Indianapolis, IN, USA.

Dietary sodium is believed to be a major determinant of calcium excretion in urine. A metabolic study was undertaken to test the hypothesis that high sodium intake may lead to decreased calcium retention in adolescent girls. A total of 36 girls (age 12 ± 1 years in blacks and 13 ± 1 years in whites) participated in two sessions of summer camp, simulating a free-living environment. Each session of the camp was three weeks long separated by a two-week wash-out period. The controlled diet consisted of 815 mg calcium and one of two levels of sodium (1.3 g and 4.0 g). The diet was also constant in magnesium, potassium, phosphorus, protein, fat, and fiber. The two levels of sodium were tested using a randomized crossover design. The study indicated that black girls had significantly ($P < 0.05$) higher calcium retention than white girls regardless of dietary sodium level. The daily urinary calcium output in white girls was significantly ($P < 0.05$) higher with the high sodium diet than with the low sodium diet. Black girls excreted significantly ($P < 0.01$) less calcium in the urine than white girls on the high sodium diet. This indicates a possible genetic predisposition to the effect of salt on the kidney in the white population. In summary, increased dietary sodium led to increased urinary calcium in the white adolescent girls only. No effect of salt intake on urinary calcium was seen in the blacks.

Table 1. Daily urinary and fecal calcium excretion, calcium retention, and apparent calcium absorption in black and white adolescent girls

	Race	Low Sodium diet	High sodium diet

Urinary calcium excretion (mg/day)	Blacks	44 ± 39	48 ± 36**
	Whites	62 ± 35a	101 ± 41 a**
Fecal calcium excretion (mg/day)	Blacks	292 ± 136*	325 ± 153
	Whites	480 ± 260*	508 ± 202
Calcium retention (mg/day)	Blacks	516 ± 158**	469 ± 182*
	Whites	265 ± 246**	213 ± 219*
Apparent calcium absorption (%)	Blacks	23 ± 13	20 ± 13
	Whites	12 ± 21	17 ± 20

Mean ± SD
[a] Group means were statistically significant at P < 0.05 between diets
* Group means were statistically significant at P < 0.05 between races
* * Group means were statistically significant at P < 0.01 between races

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Underdiagnosis of Vertebral Fractures Is a Worldwide Problem: The IMPACT Study. P. D. Delmas,[1] N. Watts,[2] R. Eastell,[3] G. Von Ingersleben,*[4] L. van de Langerijt,*[5] D. L. Cahall.[6] [1]University Claude Bernard, Lyon, France, [2]Emory University, Atlanta, GA, USA, [3]University of Sheffield, Sheffield, United Kingdom, [4]Synarc, San Francisco, CA, USA, [5]Aventis Pharma, Hoevelaken, The Netherlands, [6]Aventis Pharma, Bridgewater, NJ, USA.

Two thirds of fragility vertebral fractures do not come to clinical attention. In addition, it has been reported in retrospective studies that vertebral fractures are underdiagnosed in radiology reports. We have looked at the accuracy of the radiology report of vertebral fractures in a large prospective trial, the IMPACT study. The primary aim of the study is to assess the impact of using bone turnover marker monitoring of risedronate treatment on patient compliance and persistence. Over 7166 women aged 65-80 yr were screened for osteoporosis by DXA in 172 centers in Europe, North and Latin America. 2386 women that had osteoporosis, i.e. a T score ≤ -2.5 at the spine and/or hip or a T score between -2.5 and -1 with a peripheral fragility fracture, and who were willing to be enrolled into the trial, had lateral radiographs of the thoracic and lumbar spine that were subsequently reviewed centrally by an expert radiologist and classified according to the semiquantitative method of Genant. The presence of a vertebral fracture was not a criteria for study entry. Out of 1860 evaluable radiographs analyzed centrally so far, 592 women (32%) had one or more vertebral fracture(s). In 252 of them the diagnosis of vertebral fracture was not made by the local radiologist, i.e. a false negative rate (FNR) of 43%. The underdiagnosis of vertebral fractures was highly prevalent worldwide, with a FNR of 57% in centers in North America, 35% in centers in Europe and 62% in Latin America. Conversely, 117 women without vertebral fractures according to the central reading were diagnosed locally as having vertebral fracture(s), leading to a false positive rate of 9.2% globally, 9.4% and 1% in North and Latin America respectively, and 10.2% in Europe. We conclude that vertebral fractures are markedly underdiagnosed in the radiology report, a pattern that probably contributes to the undertreatment of postmenopausal osteoporosis.

Disclosures: Aventis Pharma,3.

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Long-Term Prediction of Incident Hip Fracture Risk in Older White Women. B. C. Taylor,*[1] P. J. Schreiner,[1] K. L. Stone,[2] S. R. Cummings,[2] M. C. Nevitt,[2] K. E. Ensrud.[3] [1]University of Minnesota, Minneapolis, MN, USA, [2]University of California, San Francisco, CA, USA, [3]VA Medical Center & University of Minnesota, Minneapolis, MN, USA.

Previous studies have shown bone mineral density (BMD), age and other factors to be strong predictors of incident hip fracture over relatively short follow-up periods. Using risk factor data from the Study of Osteoporotic Fractures (SOF), we examined the relationship of BMD and other factors to hip fracture risk during long-term follow-up.At the second SOF exam (1988-90), we measured total hip BMD by DXA in 8,070 women age 67 years and older. We then followed these women for an average of 8.5 (+/-2.4) years; during this time 444 incident hip fractures occurred. At the first and second exams, risk factors for fracture, including anthropometry, muscle strength, a variety of physical performance measures, self-reported personal and family fracture history, reproductive history, health status, personal habits, cognitive function, medication use, functional status and other measures were collected. Measurements from the first exam included parity, maternal history of hip fracture, height at age 25 and type 2 diabetes status; the remainder were from visit 2.Using Cox regression, best subset and stepwise techniques were used to identify independent risk factors. Variables that remained significantly related to hip fracture (p<0.05) in a multivariate model are shown in the table below with and without adjustment for total hip BMD.

Variable (unit change)	HR Without BMD (95% CI)	HR With BMD (95% CI)
Mom hip fracture after 50 (yes/no)	1.64 (1.24, 2.16)	1.51 (1.14, 2.00)
Age at viswit 2 (5 years)	1.54 (1.39, 1.71)	1.42 (1.28, 1.57)
Fractured any bone after age 50 (yes/no)	1.50 (1.23, 1.84)	1.27 (1.04, 1.56)
He8ght at age 25 (6cm)	1.18 (1.07, 1.31)	1.21 (1.10, 1.34)
Digit SWymbol Test (12 completed)	0.83 (0.74, 0.93)	0.84 (0.75, 0.94)
Walking speed (0.22 m/s)	0.77 (0.69, 0.86)	0.80 (0.71, 0.89)
Percent weight change 25 to visit 2 (20%)	0.75, (0.67, 0.84)	0.95 (0.84, 1.07)
Parity: 1-3 children vsw no children and 4 or more children vs no children	0.76 (0.60, 0.96) 0.49 (0.34, 0.69)	0.75 (0.59, 0.94) 0.48 (0.34, 0.69)
Total hip BMD (013g/cms2)		0.53 (0.47, 0.61)

Type 2 diabetes, current smoking and thyroid medication use were also significantly associated with increased rates of hip fracture. When total hip BMD was added to the multivariate model, all variables remained significant except for current smoking and percent weight change from age 25.We conclude that while BMD is strongly related to hip fracture risk in elderly white women, other risk factors are also independent predictors of long-term risk and may provide additional insight into prevention of fracture in high risk women

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Bone Mineral Density and Verbal Memory Impairment: The Third National Health and Nutrition Examination Survey (NHANES III). Y. Q. Zhang,[1] S. Seshadri,*[2] R. C. Ellison,*[3] T. Heeren,*[4] D. T. Felson.[3] [1]Arthritis Center, Boston University, Boston, MA, USA, [2]The Framingham Heart Study, Framingham, MA, USA, [3]Boston University School of Medicine, Boston, MA, USA, [4]Boston University School of Public Health, Boston, MA, USA.

While numerous studies have examined the relation of either a single measurement of endogenous estrogen levels or estrogen replacement therapy to the risk of poor cognitive function among women, the results have been inconclusive. No study has examined this relation in men. Bone mineral density (BMD) has been proposed as a marker of cumulative estrogen exposure, with several studies showing that high BMD is associated with an increased risk of some estrogen-related diseases, such as breast cancer. We studied the association between BMD and the prevalence of verbal memory impairment in both men and women. 2,115 men and 2,189 women in NHANES III (age 60-91 years) had BMD measurement and verbal memory assessment between 1988 and 1994. BMD was measured in five regions of the proximal femur with dual-energy x-ray absorptiometry. Verbal memory was assessed using the 3-delayed item recall and the 6-delayed story recall, with a combined score ranging from 0 to 9. The memory impairment was defined as a combined score < 4 (Pekins, et al, Am J Epidemiol 1999;150:37-44). Since sex, age and ethnicity are important determinants of memory and are strongly associated with BMD, we created age, sex, and ethnicity-specific quintiles of BMD. We used SUDDAN statistical software to incorporate sample weights and to account for the complex survey design in the variance estimates. 305 men and 231 women had verbal memory score less than 4. Prevalence decreased from 8.35% in the lowest quintile of femoral neck BMD to 5.74, 5.22, 5.00 and 3.38% among increasing quintiles of BMD in women, and 11.54, 7.27, 8.47, 6.29, and 5.89% among men, respectively. After adjusting for age, sex, ethnicity, education, income, smoking, alcohol consumption, history of stroke and hypertension, the prevalence ratios of verbal memory impairment for men and women combined from the lowest to the highest quintiles of BMD at femoral neck were 1.0, 0.64, 0.65, 0.55, and 0.44, respectively (p for trend < 0.001). A similar association was also found when BMD assessed at other proximal femoral sites were used. The relationship was consistent across almost all strata of other potential risk factors assessed above, and was robust when different cut-points, i.e., combined score < 3 or combined score < 5, were used to define the verbal memory impairment. The results from NHANES III showed that BMD in elderly men and women is strongly associated with performance on tests of verbal memory, suggesting that cumulative exposure to estrogen may play a role.

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C-Type Natriuretic Peptide (CNP) as Novel Positive Regulator of Endochondral Ossification - The Analysis of CNP Knock Out Mice. H. Chusho,* Y. Komatsu, N. Tamura,* Y. Ogawa,* A. Yasoda,* M. Suda,* T. Miyazawa,* M. Miura,* K. Tanaka, K. Nakao.* Medicine and Clinical Science, Kyoto University, Kyoto, Japan.

We have reported that the transgenic mice with elevated plasma level of B-type natriuretic peptide, and with targeted overexpression of C-type natriuretic peptide (CNP) in the growth plate both exhibit marked skeletal overgrowth accompanied by increased endochondral ossification. We also showed the *in vivo* CNPmRNA expression in the growth plate chondrocytes, suggesting that CNP can affect the endochondral ossification. However, the physiological role of CNP in the bone is undefined. To investigate the physiological significance of CNP *in vivo*, we generated mice with a disrupted CNP allele (*Nppc*$^{-/-}$ mice) by gene targeting in 129/Sv mice-derived embryonic stem cells. Neither *Nppc*mRNA, nor CNP protein was detected in the cerebellum or cartilage from *Nppc*$^{-/-}$ mice. In *Nppc*$^{-/-}$ mice, dwarfism with short tails and extremities became prominent as they grew. The naso-anal lengths of *Nppc*$^{-/-}$ mice were 60-70% of those of *Nppc*$^{+/+}$ mice at the age of 4-10 weeks. Soft x-ray analysis revealed that the longitudinal growth of vertebrae and tail and limb bones is affected in *Nppc*$^{-/-}$ mice. Histological analysis showed that *Nppc*$^{-/-}$ mice display striking narrowing of the growth plate of vertebrae and long bones at the age of 7 days. *In situ* hybridization analysis revealed no appreciable difference in the intensity of type II and type X collagen mRNA expression between genotypes. However the width of cell layers expressing Indian hedgehog was narrowed in *Nppc*$^{-/-}$ mice. The ratio of the height of the hypertrophic zone to that of the proliferative zone was decreased by 50% in *Nppc*$^{-/-}$ mice. There was a significant reduction of BrdUrd-labeled cells in *Nppc*$^{-/-}$ mice relative to *Nppc*$^{+/+}$ mice, demonstrating that CNP promotes chodrocyte proliferation *in vivo*. Expression of mRNA for the CNP receptor, GC-B was detected predominantly in the proliferative and prehypertrophic zone in both *Nppc*$^{-/-}$ and *Nppc*$^{+/+}$ mice. The organ culture study using the tibiae from 16.5-d fetus of *Nppc*$^{-/-}$ mice with 10^{-7} CNP achieved the 35%

increase of the total length, comparable to the result of *Nppc*$^{+/+}$ mice, suggesting that local application of CNP can rescue the skeletal defect in *Nppc*$^{-/-}$ mice. This study demonstrates that CNP is the novel endogenous positive regulator of endochondral ossification *in vivo.*

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C-type Natriuretic Peptide is Essential for Post-Natal Skeletal Growth. M. E. Steinhelper,*[1] L. M. Libby,*[2] A. D. Garcia,*[1] R. A. Rees,*[2] J. Rosser,*[3] B. Story,*[3] M. C. Naski,[4] L. F. Bonewald,[3] S. W. M. John.*[2] [1]Physiology, UTHSCSA, San Antonio, TX, USA, [2]HHMI, The Jackson Laboratory, Bar Harbor, ME, USA, [3]Medicine, UTHSCSA, San Antonio, TX, USA, [4]Pathology, UTHSCSA, San Antonio, TX, USA.

Extracellular signaling molecules that increase intracellular cGMP are likely to regulate postnatal skeletal development. The particulate or membrane guanylate cyclases include ligand-activated receptors for the natriuretic peptides (NPs). To investigate the potential role of the most evolutionarily conserved member of the NP gene family, C-type natriuretic peptide (CNP), we generated by homologous recombination mice with a deletion of Nppc exon 2 that encodes CNP. At birth, the expected Mendelian ratio was observed, and null mice had similar body mass (1.18 +/- 0.05) as compared with their wild-type (1.32 +/- 0.05) and heterozygous siblings (1.34 +/- 0.07). Alcian blue-alizarin red stained skeletons showed no obvious structural or developmental patterning defects in cartilage and skeleton during the embryonic period or at birth. Postnatal growth of heterozygotes was not different from wild-type controls, however, nulls showed markedly reduced postnatal growth. Appendicular bones of nulls showed significant 20-30% reductions in length at day 5, decreased longitudinal growth (30% reduction in crown-rump length), a characteristic "dome-shaped" cranium and shortened mandible which likely contributed to malocclusion, although tooth eruption appeared normal. Survival of the homozygous mice was significantly reduced (median survival of 21 days) as compared to their wild-type and heterozygous littermates (>150 days). Radiography showed a significant 37% reduction in femoral and tibial cortical thickness in the nulls on postnatal day 10. At 20 days, femoral cortical thickness was reduced significantly by 52%, as was tibial cortical thickness, 45%. These findings indicate reduced cortical bone growth during the postnatal period. Histomorphometric analysis showed a marked decrease in the width of the hypertrophic zone of growth plates at 10 and 20 days of age. New trabecular bone was severely reduced at the distal femoral and proximal tibial growth plates consistent with the reduction in hypertrophic chondrocytes. Similar changes were observed in vertebral growth plates. In conclusion, the present study demonstrates that CNP is essential for normal postnatal skeletal development in mice. Further studies will be necessary to determine the specific cellular targets of CNP action within the developing bone as well as at extraskeletal sites that contribute to the development of chondrodysplasia, dwarfism, and lethality in these mice.

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Interleukin-4 Reversibly Inhibits Osteoclastogenesis via Inhibition of NF-κB and MAP Kinase Signaling. S. Wei, M. W. H. Wang, S. L. Teitelbaum, F. P. Ross. Pathology, Washington University, St. Louis, MO, USA.

Interleukin-4 (IL-4), a Th2 cytokine, inhibits osteoclastogenesis, in vitro and in vivo, and this study was designed to investigate the mechanism(s) mediating this event. We find that IL-4 inhibits formation of osteoclasts (OCs) when primary bone marrow macrophages (BMMs) are treated with M-CSF plus RANKL. The T cell-derived cytokine is effective only when added during the first two days of culture, suggesting it targets early OC precursors. Withdrawal of IL-4, up to five days after its addition to OC-generating cultures, restores OC differentiation, revealing that inhibition of OC formation is reversible. To define the molecular mechanism(s) by which IL-4 inhibits osteoclastogenesis, we examined RANKL-induced signaling pathways. Immunoblot analysis reveals that IL-4 does not alter levels of RANK, TRAF6 or IKKβ, all components of NFκB and/or MAP kinase activation. In contrast, pretreatment of BMMs with IL-4 decreases RANKL-induced IκBα phosphorylation, thus blocking degradation of this protein. As a consequence, IL-4 inhibits NFκB activation by 70%, as assessed by EMSA of nuclear extracts. Consistent with this finding, IL-4 down-regulates RANKL-induced expression of the NFκB-dependent genes IκBα, ICAM-1 and TLR-2. Turning to the MAP kinase pathway, pretreatment of BMMs with IL-4 inhibits RANKL-dependent activation of JNK, p38 and ERK signals. The abnormalities in NF-κB and MAP kinase activation, which mirror the effect of IL-4 on osteoclastogenesis, can be reversed by withdrawal of the cytokine for 24 hours. Consistent with these observations, and in contrast to previous reports, we find that the MEK inhibitors PD98059 and U0126 dose-dependently block both OC differentiation and BMM proliferation. Withdrawal of the MEK inhibitors partially rescue OC formation, demonstrating ERK activation is required for osteoclastogenesis. To further dissect the signaling pathway mediating the inhibitory effect of IL-4 on osteoclast formation, we used mice lacking either the transcription factor STAT6 or the phospholipid 5' phosphatase SHIP, both of which transduce signals from the IL-4 receptor. IL-4 fails to inhibit RANKL/M-CSF-induced osteoclastogenesis by BMMs derived from STAT6, but not SHIP, knockout mice, despite a ten fold increase in OC number in the latter. In keeping with this observation, the inhibitory effects of IL-4 on RANKL-induced NFκB and MAP kinase activation are STAT6-, but not SHIP-, dependent. We conclude that IL-4 reversibly arrests osteoclastogenesis in a STAT6-dependent manner by 1) preventing IκBα phosphorylation and thus NFκB activation, and 2) blockade of the JNK, p38 and ERK MAP kinase pathways.

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Stimulation of Bone Resorption and Repression of Bone Formation are Key Mechanisms by which Interleukin-7 Plays a Central Role in the Pathogenesis of Ovariectomy Induced Bone Loss. M. N. Weitzmann, C. Roggia, L. Weitzmann, R. Pacifici. Division of Bone & Mineral Diseases, Washington University & Barnes-Jewish Hospital, St. Louis, MO, USA.

Estrogen (E2) deficiency leads to bone loss as bone formation, although enhanced, is unable to keep pace with increased rates of bone resorption. The mechanism driving this uncoupling of bone formation from bone resorption remains poorly understood. The cytokine Interleukin-7 (IL-7) is a powerful inducer of osteoclast formation in vitro and bone resorption in vivo. The mechanism by which IL-7 induces bone resorption involves production of the key osteoclastogenic cytokine RANKL by T cells. We now show that ovariectomy (ovx) increases bone marrow IL-7 mRNA by 2 fold, as measured by semi-quantitative RT-PCR, resulting in a >30% increase in IL-7 protein levels in the bone marrow, as determined by a murine IL-7 ELISA. This data suggests that IL-7 may play an important role in the bone loss associated with E2 deficiency. In support of this notion, neutralization of IL-7 in vivo, by injection of anti-IL-7 antibody into mice, completely prevented ovx induced bone loss, as measured by pQCT and DEXA. IL-7 neutralization prevented bone loss by blunting bone resorption and enhancing bone formation. These data suggest that in vivo, IL-7 limits the ovx induced increase in bone formation thus preventing bone formation from reaching the magnitude necessary to compensate for enhanced bone resorption. We further characterized this novel inhibitory effect of IL-7 on bone formation in vitro and found that IL-7 completely suppressed the formation of new bone growth in neonatal calvarial organ cultures. In addition, IL-7 inhibited both osteocalcin mRNA, determined by semi-quantitative RT-PCR, in the osteoblastic cell line ROS 17/2.8, and alkaline phosphatase activity in purified calvarial osteoblasts in vitro, demonstrating that IL-7 is a powerful inhibitor of bone formation. Consistent with this data, IL-7 was found to inhibit promoter activity of the key osteoblast specific factor, CBFA-1 by 50%, when transiently transfected into ROS 17/2.8 cells. In addition IL-7 blocked by 50% the transactivation of a minimal osteocalcin promoter driven by CBFA-1 responsive elements. In conclusion, our data suggests that IL-7 disrupts bone homeostasis by upregulating osteoclastic bone resorption, while simultaneously limiting the magnitude of the compensatory rise in osteoblastic bone formation characteristic of E2 deficiency. The net effect of IL-7 action is thus the uncoupling of bone formation from bone resorption, leading to the reduced bone mass characteristic of postmenopausal osteoporosis.

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Neuropeptide Y2 Receptor Has a Role in Bone Homeostasis Independent of Body Weight. P. A. Baldock,*[1] G. P. Thomas,*[1] A. Sainsbury,*[2] M. Couzens,*[3] R. F. Enriquez,*[1] H. Herzog,*[3] E. M. Gardiner.[1] [1]Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, Australia, [2]Metabolism Program, Garvan Institute of Medical Research, Sydney, Australia, [3]Neurobiology Program, Garvan Institute of Medical Research, Sydney, Australia.

Absence of leptin signalling has been shown to increase bone mass in mice by a central regulatory mechanism, possibly through neuropeptide Y (NPY) signalling. Leptin-responsive neurons in the arcuate nucleus, a hypothalamic structure, co-express both the leptin and the neuropeptide Y2 receptors. The role of NPY in bone was therefore assessed in mutant mice lacking the Y2 receptor (Y2 KO), either as a germline null mutation or as a conditional knockout induced by bilateral injection of CRE recombinase-expressing adenovirus into the arcuate nucleus of adult mice carrying Lox P-modified Y2 receptor genes. Distal femurs of 4 month-old male Y2 KO and control mice were histomorphometrically assessed by light and UV microscopy.The germline Y2 KO animals had elevated trabecular bone volume (12.4% ± 1.7 vs 5.3% ± 0.2; P<0.05). This difference was associated with increased thickness of trabeculae (29.9μm ± 2.0 vs 18.9μm ± 0.7; P<0.005) and a non-significant increase in trabecular number (4.0/mm ± 0.3 vs 2.8/mm ± 0.002; P<0.06). These bone changes were similar to those described for the leptin knockout (ob/ob) mouse but occurred in the absence of obesity, as the germline Y2 KO mice tended to low body weight.To assess the central nature of this regulation, bones from arcuate-specific Y2 receptor knockouts were examined. Five weeks after injection of CRE-adenovirus, results were similar to the previous observations in germline Y2 KO mice, with greater trabecular bone volume (9.9% ± 1.0 vs 5.5% ± 0.1; P<0.01) and trabecular thickness (30.9μm ± 0.3 vs 19.6μm ± 0.3; P<0.0005) than in CRE-adenovirus injected wildtype controls. Bone formation in the arcuate-specific knockout mice was elevated due to an increase in mineral apposition rate (1.4μm/d ± 0.1 compared to controls, 0.96μm/d ± 0.03; P<0.02). Bone resorption was not altered in either germline or arcuate-specific Y2 knockout mice.NPY signalling through the Y2 receptor is therefore strongly implicated in the control of bone formation. The bone effect was independent of body weight, consistent with the previous report that the high bone mass phenotype in ob/ob mice preceded the onset of obesity. The results of the arcuate-specific knockout are consistent with a central action of NPY through the Y2 receptor that can indirectly modulate bone formation. The NPY pathway thus appears to mediate a central anabolic effect on bone.

Disclosures: AZA Research,2.

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Statins Stimulate Bone Formation by Enhancing eNOS Expression. I. R. Garrett, G. Gutierrez, D. Chen, A. Escobedo,* D. Horn,* J. Esparza,* G. R. Mundy. OsteoScreen, San Antonio, TX, USA.

Statins, drugs used to lower cholesterol, have recently been shown to enhance bone formation. The mechanism by which they elicit this effect is not clear. Statins reduce cholesterol production by inhibiting the rate-limiting enzyme in the mevalonate pathway, hepatic HMG-CoA reductase. Statins stimulate bone formation in murine neonatal calvaria and in vivo by increasing BMP2 protein, since noggin, the endogenous inhibitor of BMPs, blocks effects in vitro, and lovastatin (10mg/kg/day) was ineffective in stimulating bone formation in vivo in transgenic mice with truncated mutant unresponsive BMP receptors targeted to the osteoblast lineage. To determine the mechanism by which the statins increase BMP2 expression, we examined the effects of immediate downstream metabolites of the HMG-CoA reductase enzyme, mevalonate (100uM) and GGPP (10uM), and found they inhibited bone formation and BMP-2 transcription stimulated by the statins. Previous studies have indicated that statins mediate beneficial effects on ischemic stroke through enhanced expression of eNOS in endothelial cells and do so by effects on prenylated proteins such as Rho. We therefore examined a specific inhibitor of Rho, clostridium botulinium C3 transferase (50ug/ml), which stimulated bone formation in organ cultures, suggesting the involvement of Rho GTPase and subsequent prenylated proteins. We next examined the

effects of NOS inhibitors on statin-mediated bone formation. We found that a preferential inhibitor of eNOS (L-NAME - 2uM) inhibited bone formation by 86% in calvarial cultures treated with statins whereas a less potent inhibitor of eNOS (1400) did not. We further found statins increased NO production and enhanced the expression of eNOS mRNA and protein in osteoblastic 2T3 cells. To confirm the role of eNOS in mediating statin effects on bone, we examined calvarial cultures from eNOS -/- mice, which have recently been shown by others to have a bone phenotype characterized by decreased bone formation, and compared the effects of simvastatin to those in bone cultures from control eNOS+/+ mice. We found that calvaria from null mutant mice showed reduced bone formation responses. We conclude that statins mediate effects on bone formation by inhibition of the enzyme HMG-CoA reductase in osteoblasts. Inhibition of this pathway leads to a reduction in Rho prenylation, which enhances expression and activity of eNOS in osteoblasts. In turn, increased generation of NO from eNOS enhances BMP2 expression, which causes osteoblast differentiation and bone formation. These results suggest additional molecular targets for drug discovery for anabolic agents and the central role of eNOS in statin-stimulated bone formation.

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Null Mutation in Collagenase-3 (Matrix Metalloproteinase[MMP]-13) Affects Maturation of Growth Plates. Y. Wang, M. H. Byrne,* M. Inada, M. U. Rahman,* S. M. Krane. Medicine, Massachusetts General Hospital, Boston, MA, USA.

Remodeling of the extracellular matrix (ECM) of cartilage and bone during embryonic development is an important event. To assess the role of collagenases in ECM resorption, we introduced, by homologous recombination in ES cells, a null mutation into the gene encoding MMP-13 and obtained germline transmission. The mutation results in splicing out Exon 5 (Exon 5 encodes the critical, catalytic, Zn-binding domain) which would lead to an inactive enzyme. Heterozygous MMP-13 -/+ mice were bred and viable MMP-13 -/-, -/+ and +/+ offspring were obtained. By in situ hybridization using a riboprobe specific for Exon 5, MMP-13 expression was observed in distal growth plate and primary centers of ossification in the MMP-13 -/+ and +/+ mouse embryos (E) 15.5 through early adulthood but not in the MMP-13 -/- mice; using ribroprobes for portions of MMP-13 mRNA other than for Exon-5, expression of MMP-13 was observed (although at a markedly decreased level) in the MMP-13 -/- mice as well, indicating reduced transcription or increased degradation of mRNA. Using riboprobes for MMP-8, -9 and -14, we observed increased skeletal expression in the MMP-13 -/- mice; e.g., a signal for MMP-8 was seen in MMP-13 -/- embryos at E 15.5 and 17.5 but not in +/+ embryos, but appeared at a detectable although lower level in newborn MMP-13 +/+ mice. Expression of MMP-14 was also increased in MMP-13 -/- vs. MMP-13 +/+ mice. The width of the total growth plate was increased by ~ 50% and the width of the hypertrophic zone by ~ 100% in MMP-13 -/- vs. +/+ E-15.5, 17.5 and newborn mice; this was associated with increased uptake of BrdU in the proliferating zone in the MMP-13 -/- mice. Increased width of the growth plate persisted through ~4-6 weeks of age. In newborn MMP-13 +/+ mice, there was staining with Mab 9A4 for the collagenase-cleavage epitope in type II collagen and with a polyclonal antibody to mouse MMP-13 in the septae of the distal growth plates; no staining with Mab 9A4 or MMP-13 antibody was seen in the MMP-13 -/- mice. That the increased width of the growth plates in the MMP-13 -/- mice was associated with decreased maturation is supported by ~ 8-9 % decrease in femoral length in 1 and 3 month-old MMP-13 -/- vs. +/+ mice. MMP-13 is normally expressed predominantly in the distal growth plates and centers of ossification in developing embryos. Thus, a targeted mutation in the MMP-13 gene, with decreased transcription and translation of a protein that is no longer a proteinase, results in increased width of the hypertrophic zone of growth plates and decreased maturation. MMP-13-mediated degradation of skeletal ECM must be important in normal endochondral ossification.

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Induction of RANKL and MMPs in Bone with Metastasis of Breast Cancer. C. Miyaura, T. Ohshiba,* M. Inada, A. Ito.* Biochemistry, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Bone metastasis of breast cancer induces severe osteolysis with increased bone resorption, and is one of the most serious problems in breast cancer patients. Osteoclast differentiation regulated by receptor activator of NF-kB ligand (RANKL) in osteoblasts and matrix degradation induced by matrix metalloproteinase (MMPs) are thought to be involved in the process of bone resorption, but the role of RANKL and MMPs in bone metastasis is not known. In this study, we investigated the expression of RANKL and MMPs in bone with metastasis. In vivo study, nude mice were injected with human breast cancer cells (MDA-MB-231), and metastases in the femur and tibia were identified by soft-X ray and morphological analysis. When the MDA tumor was close to the bone surface, severe osteolysis occurred with increased bone resorption. The number of TRAP-positive osteoclasts and resorption pits were markedly elevated, and the degradation of bone matrix markedly progressed. Expression of RANKL, MMP-13 (collagenase 3) and MT1-MMP mRNA was markedly elevated in tibia and femur with metastasis compared with control bone. In vitro, MDA cells were embedded in type-I collagen gel and cultured on calvaria collected from 5 days-old mice. Some MDA cells in the gel were in contact with the surface of the calvaria, and co-culture with MDA markedly induced calcium release from calvaria and osteoclast formation. Numerous osteoclasts were detected on the surface of calvaria attached to MDA-gel. After co-culture, MDA cells in collagen gel were removed from calvaria, and the expression of RANKL and MMPs in calvaria was examined. We detected the marked induction of RANKL, MMP-2, MMP-9 and MMP-13 in calvaria after co-culture with MDA. Adding OPG, a decoy receptor for RANK, and BB94, an inhibitor of MMPs, significantly suppressed bone resorption induced by co-culture with MDA. The separation of MDA-gel from calvaria showed decreased bone resorption in the co-cultures, suggesting that cell-to-cell and/or cell-to-matrix interaction is essential for cancer-induced resorption in bone. These results suggest that RANKL-induced osteoclast formation and MMP-dependent matrix degradation are associated with osteolysis due to bone metastasis.

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Bone Sialoprotein Binds to and Modulates Pro-Matrix Metalloproteinase-2 Activity. N. S. Fedarko,*[1] A. Jain,*[1] A. Karadag,*[2] L. Fisher.[2] [1]Department of Medicine, Johns Hopkins University, Baltimore, MD, USA, [2]Craniofacial and Skeletal Diseases Branch, NIDCR, NIH, Bethesda, MD, USA.

Bone sialoprotein expression is induced in osteoblasts, osteoclasts and in many cancers. Also, BSP has been shown to be angiogenic in vivo. We have previously reported a mechanism for BSP-mediated protection of cells from humoral surveillance involving an initial binding of BSP to the vitronectin receptor (alphaVbeta3), subsequent binding of the complement-dampening protein Factor H, which then results in quenching of complement activity. BSP could also compete with pro-matrix metalloproteinase-2 (pro-MMP-2) that has been reported to be bound to the vitronectin receptor. If this were the case then BSP expression would not only confer enhanced survival via protection from complement surveillance, but also contribute to MMP release from the osteoblast, osteoclast or tumor cell surface into the extracellular environment. To test this possibility, normal and cancer cell lines were treated with recombinant BSP and the release of MMPs into the medium was followed as a function of time. Treatment of human marrow stromal fibroblasts with normal BSP caused a large increase in pro-MMP-2 in the medium in as little as 2 minutes. This suggests that the release is not a downstream event requiring synthesis and secretion, but rather a direct consequence of BSP binding. Addition of BSP in which the integrin-binding RGD was changed to the inactive KAE did not cause the release of the pro-MMP-2. KG1A cells (a CD4 positive human hemopoetic precursor cell line) express proMMP-2, but treatment with rBSP did not cause MMP-2 release. KG1a cells express alpha4beta1 and alpha5beta1 but not the alphaVbeta3 integrin so this is consistent with the BSP-mediated release of MMP-2 involving the alphaVbeta3 integrin. Kinetic studies using a fluorescein-labeled gelatin substrate and pure pro-MMP-2 revealed a BSP-dependent activation of gelatinase activity that could be blocked by the addition of 1,10 phenanthroline. The binding of BSP to pro-MMP-2 was characterized by titration studies utilizing the intrinsic tryptophan fluorescence of pro-MMP-2 and revealed 1:1 stoichiometry and a binding constant in the nM range. BSP did not bind to pro-MMP3 or pro-MMP-9. Our results indicate that BSP selectively interacts with pro-MMP-2 and modulates its activity. Thus, BSP expression may lead to local release and activation of pro-MMP-2 in both normal bone and in many tumors

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Targeted Expression of Constitutively Active BMP Receptor 1A in Chondrocytes Causes Lethal Skeletal Dysplasia with Shortening of Growth Plate. T. Kobayashi,[1] A. P. McMahon,*[2] H. M. Kronenberg.[1] [1]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [2]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA.

Bone morphogenetic proteins (BMPs) play crucial roles in skeletal development. In addition to their roles in early limb bud outgrowth, several lines of evidence suggest that BMP signaling stimulates formation of the mesenchymal condensation as well as differentiation of mesenchymal cells into the chondrocytic lineage. However, little is known about BMP action on differentiated chondrocytes in vivo. Overexpression of a constitutively active mutant BMP receptor 1A (caBMPR1A) delayed hypertrophic differentiation in chick developing limbs after recombinant retroviral infection (Zou H et al, 1997 Genes Dev. (11) 2191). To extend these observations to the setting of mammalian development, we expressed caBMPR1A in mouse growth plate chondrocytes. Since initial experiments suggested that transgenic expression of this gene was lethal, a bigenic system was used to allow the reproducible generation of the transgenic mice. Gal4 transgenic mice express yeast Gal4, a transcription activator, under the control of the rat type II collagen promoter. UAS-caBMPR1A transgenic mice carry caBMPR1A cDNA downstream of UAS sequences (Gal4 binding sites), followed by a Wnt –1 minimal promoter. In the double transgenic mice, GAL4 transactivates UAS-caBMPR1 gene transcription exclusively in chondrocytes. Double trangenics die perinatally, showing skeletal abnormalities including short limbs and domed skulls. Histological examination at E 17.5 showed shortening of the growth plates, especially the columnar proliferating layer. After BrdU labeling, chondrocyte proliferation was unchanged, and a pulse-chase analysis revealed that hypertrophic cells were produced at a reduced rate in the mutant. At day E 12.5, mutant bones were already smaller than controls. In conclusion, missexpression of caBMPR1A in growth plate chondrocytes leads to short bones through reduction of the initial mass of the cartilage premordia and reduced rate of hypertrophic cells produced.

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Osteopontin-Deficiency Induces PTH-Activation of Cortical Bone Formation and Enhances PTH-Activation of Cancellous Bone Formation in Mice. K. Kitahara,*[1] M. Ishijima,[1] S. R. Rittling,[2] K. Tsuji,[1] H. Kurosawa,*[3] A. Nifuji,[1] D. T. Denhardt,*[2] M. Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Rutgers University, Piscataway, NJ, USA, [3]Juntendo University, Tokyo, Japan.

Treatment of osteoporosis patients, especially those who already have lost significant amount of bone, requires therapeutic measures to increase bone mass by enhancing formation of bone. Intermittent administration of parathyroid hormone (PTH) has been known to be one of the promising treatments for osteoporosis to enhance bone formation. Recent observations on the transgenic mice expressing constitutively active PTH/PTHrP receptor indicates that PTH signaling increases cancellous bones while it reduces cortical bones. However, the downstream events which are involved in the differential PTH activation of bone formation is still unclear. Osteopontin (OPN) is a non-collagenous bone matrix protein and cytokine, and PTH suppresses OPN gene expression via cyclic AMP and protein kinase A in vitro. We hypothesized that OPN could be one of the target molecules that may be involved in PTH actions to increase bone in vivo. Therefore, we examined the effect of PTH on the bone metabolism in OPN-deficient mice in comparison to its effect on bone in wild type mice. PTH treatment was conducted by injecting 80mg/kg PTH (1-34) subcuta-

neously, daily for 5 days a week for 4 weeks. In wild type mice, as reported previously, treatment with PTH for 4 weeks increased cancellous bone volume. In contrast, OPN-deficiency further enhanced the PTH-dependent increase in trabecular bone volume more than the increase observed by PTH treatment in wild type mice. PTH did not alter cortical bone volume, cortical bone thickness and bone marrow area in wild type mice. In sharp contrast to such inability of cortical bones to respond to PTH in wild type mice, OPN-deficient mice showed increase in cortical bone volume and cortical bone thickness, and decrease in bone marrow area in response to PTH treatment for 4 weeks. DEXA analyses indicated that the total BMD (bone mineral density) in the whole femur was not increased by the treatment with PTH in wild type. In the absence of OPN, however, BMD of the whole femur was increased significantly by PTH treatment. TRAP-positive multinucleated cell number per area but not per bone surface in the trabecular bone was increased by the treatment with PTH in osteopontin-deficient mice. Our data indicated that OPN -deficiency potentiated PTH activation of bone formation in the cortical bone. Therefore, the long-standing question about how cortical bone differs from cancellous bones with regard to their responses to PTH could be explained at least in part by the presence of OPN.

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Long Bone Development in Transgenic Mice Overexpressing Human Osteoblast Stimulating Factor-1. G. Li,*[1] S. McQuaid,*[1] P. Masterson,*[1] M. Mushipe.*[2] [1]Department of Trauma and Orthopaedic Surgery, The Queen's University of Belfast, Belfast, United Kingdom, [2]Department of Mechanical Engineering, The Queen's University of Belfast, Belfast, United Kingdom.

Osteoblast stimulating factor-1 (osf-1), a heparin binding Lys-rich 18 Kda protein, was first identified as a neurogenic growth factor, and later found to have potent effects on regulation of osteoblast recruitment, proliferation and differentiation. Osf-1 is highly expressed in the mesenchymal tissues prior to bone formation, and overexpression of osf-1 results in an increase in total bone mass. The aim of this study is to elucidate the effects of osf-1 overexpression on long bone development in a transgenic mouse model.Trangenic mice expressing the human osf-1 gene under the control of the human osteoclacin promoter were made (Masuda, et al., Biochem Biophy Res Comm, 1997). Male osf-1 mice and the background BDF1mice were used and 4 mice from each group were killed at age of 1, 2, 4 and 6 months. Femora and tibiae were collected, digital radiographs were taken and images were analyzed using NIH software Scion Image. The length of the tibia and femur, diameters of both femoral and tibial metaphysis, diaphysis and cortical thickness were measured. The bone samples were also examined using peripheral quantitative computed tomography (XCT960). Total bone mineral density, trabecular and cortical density, trabecular percentages were recorded. Finally, the bone samples were subject to 3-point bending test, and the maximum load of failure was recorded. The osf-1 mice were found to have longer, thicker, denser and stronger femora in comparison with the BDF1 mice. The femora of the osf-1 mice have narrower bone marrow spaces, and the osf-1 overexpression appeared to enhance cortical bone maturation in femora resulting in a significant reduction of the femoral trabeculae percentages. However, in the tibiae, the observation was opposite to what was found in the femora. At 1, 2 and 4 months the tibiae of the osf-1 mice appeared to be longer but thinner, lighter and weaker than that of the BDF1 mice, and the bone marrow spaces were wider but all of the difference diminished at 6 months between the two groups. The development of the femora and tibiae in the osf-1 overexpression mice over the initial 6 months exhibited a transiently different pattern. The results suggest that the effects of osf-1 overexpression on long bones may be linked with other factors, such as abundance of bone precursor cells in surrounding tissues, sufficient blood supply and the mechanical environment. The current study highlights the important role of osf-1 in regulating long bone development and its possible interactions with other local factors.

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Leptin Prevents Disuse-Induced Bone Loss in Tail-Suspended Female Rats. T. Thomas, R. De Vittoris,* V. N. David,* L. Vico, M. Lafage-Proust, C. Alexandre. LBBTO, INSERM E9901, St-Etienne, France.

Beside leptin effects on central nervous system in modulating bone metabolism, there is growing evidence that leptin also exerts direct and positive effects on bone cells. Thus, we tested the hypothesis that leptin could at least in part prevent trabecular bone loss and microarchitecture disruption induced by local disuse in the tail-suspended rat model as early as one week after suspension begins. The uncoupling pattern of bone remodeling in this specific model of bone loss is of great interest since it allows to separately studying the effects on either bone resorption or formation. Fifty female 12-week old Wistar rats (weight 250 ± 10 g) were randomly assigned to 1 of the following groups: tail-suspended rats treated with leptin (A) or vehicule (B), non-suspended rats treated with leptin (C) or vehicule (D), and baseline group (E). After one week of acclimatation, rats were tail-suspended and human recombinant leptin (0.35 mg/kg/d) was continuously administered using intraperitoneal Alzet osmotic pumps. Bone parameters were measured by double energy X-ray absorptiometry using PIXImus (Lunar Corp.). Animals were measured at baseline and day 7, and bone samples were collected for histomorphometry analysis after sacrifice at day 7. After 7 days of tail suspension, group B presented a decrease in bone mineral density (BMD) at the tibial proximal metaphysis, a trabecular bone site (0.162 ± 0.003 vs 0.173 ± 0.004; P<0.01), whereas no change was observed at the femoral diaphysis, a cortical bone site (0.195 ± 0.005 vs 0.190 ± 0.004; NS) as compared to baseline. Leptin treatment prevented disuse-induced BMD loss in the group A at the tibial metaphysis (0.181 ± 0.005 vs 0.179 ± 0.003; NS), and induced an increase in BMD at the femoral diaphysis (0.188 ± 0.003 vs 0.183 ± 0.003; P<0.05). Interestingly, leptin treatment significantly increased BMD in the non-suspended group C (0.182 ± 0.003 vs 0.178 ± 0.003 and 0.194 ± 0.003 vs 0.183 ± 0.002 at tibial metaphysis and femoral diaphysis bone sites, respectively; P<0.01) while no change was observed in the group D. These data suggest that peripheral administration of leptin could either prevent the increase in trabecular bone remodeling induced by immobilization and/or stimulate cortical bone modeling. In addition, leptin may have positive indirect effects by inhibiting the transitory and initial phase of stress previously described in these experimental conditions, as suggested by the prevention of weight loss observed at day 3 in group B (-5% as compared to baseline, P<0.05). Further histomorphometric studies are needed to clarify the putative dual leptin effects on bone depending on the route of administration, the stage of bone growth and the targeted bone tissues.

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Transforming Growth Factor-ß Prevents and Reverses Bone Marrow Adipogenesis Induced by Skeletal Unloading in Rats. S. Ahdjoudj,*[1] F. Lasmoles,[1] X. Holy,*[2] E. Zérath,[2] P. J. Marie.[1] [1]Hopital Lariboisiere, INSERM U349, Paris Cedex 10, France, [2]IMASSA, Bretigny, France.

Skeletal unloading in rats induces osteopenia resulting from decreased osteoblastogenesis. We determined the effects of skeletal unloading on bone marow stromal cell differentiation into adipocytes and the responsiveness and mechanisms of action of transforming growth factor-ß2 (TGF-ß2) on adipogenesis. Skeletal unloading was induced by hindlimb suspension for 7 days in 4-weeks old rats. Suspended rats were treated continuously with rhTGF-ß2 (2 µg/kg bw /day, Novartis, Basel) by osmotic minipump, given either as a preventive treatment (0-7 days of suspension) or as a curative treatment (4-7 days of suspension). At 0, 4 and 7 days, long bones from suspended and control rats were processed for bone histomorphometry, and mRNA in metaphyseal bone and marrow stroma were prepared separately. Skeletal unloading for 4-7 days markedly decreased bone formation and bone volume in long bone metaphysis compared to control rats. This was associated with a marked reduction in mRNA levels for Cbfa1/Runx2, osteocalcin, type I collagen and TGF-ß signaling receptor II in metaphyseal osteoblasts, as shown by RT-PCR analysis. Conversely, unloading for 4-7 days induced a 10-fold increase in mRNA levels for PPARγ, a specific adipocyte transcription factor, in the marrow stroma. This was associated with a 5-fold increase in lipoprotein lipase (LPL) and aP2 mRNA levels, and a 2-3-fold increase in adipocyte number and volume in the marrow stroma at 7 days of unloading. Both preventive and curative treatments with TGF-ß2 corrected the abnormal expression of Cbfa1/Runx2, osteocalcin and type I collagen mRNAs, and normalized the reduced bone formation in unloaded metaphyseal bone. Furthermore, treatment of unloaded rats with TGF-ß2 decreased PPARγ and C/EBPα mRNA levels at 4 and 7 days, corrected aP2 and LPL overexpression and normalized adipocyte number and volume in the bone marrow stroma at 7 days. These results show that skeletal unloading in rats enhances adipocyte differentiation of marrow stromal cells concomitantly with reduction of osteoblastogenesis. Moreover, exogenous TGF-ß prevents and reverses the increased bone marrow adipogenesis by inhibiting adipocyte gene expression in marrow stromal cells, and concomitantly corrects the defective osteoblastogenesis by stimulating osteoblast marker genes in metaphyseal osteoblasts. We suggest that the reduced endogenous TGF-ß expression and signaling induced by unloading leads to promote adipocyte differentiation and to turn down the osteoblast differentiation program, which provides a mechanism for the altered osteoblastogenesis and adipogenesis induced by skeletal unloading.

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Defects in In Vitro Mineralization and Osteoclast-Spreading Are Associated with Resistance to Unloading-Induced Bone Loss in Osteopontin-Deficient Mice. M. Ishijima,[1] S. R. Rittling,[2] T. Yamashita,[1] K. Tsuji,[1] H. Kurosawa,*[3] A. Nifuji,[1] D. T. Denhardt,*[2] M. Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Rutgers University, Piscataway, NJ, USA, [3]Juntendo University, Tokyo, Japan.

We recently reported that the presence of osteopontin (OPN) was required for the effects of mechanical stress on bone as OPN-null (OPN-/-) mice showed neither enhancement of bone resorption nor suppression of bone formation when they were subjected to unloading by tail-suspension (J. Exp. Med. 2001. 193. 399-404). However, no further mechanisms underlying the phenomenon have been known. To obtain further insight into the role of OPN in mediating mechanical stress effect on bone, we examined the in vitro mineralization and osteoclast-like cell formation in bone marrow cells and the in vivo vascular systems in hindlimb bones of OPN-/- mice after tail-suspension. OPN-/- and wild type mice were subjected to tail-suspension for 2 weeks. After tail-suspension, bone marrow cells obtained from the femora were cultured in the presence of ascorbic acid and beta-glycerophosphate for mineralized nodule formation assay or for osteoclast-like cell formation assay in the presence of M-CSF and RANKL. We also counted the number of vessels in the secondary spongiosa in the tibiae. The numbers of mineralized nodules formed in cultures tended to be less in the marrow cells from tail-suspended mice compared to the cells from loaded mice in wild type. In OPN-/- mice, the numbers of mineralized nodules were less than those in wild type regardless of the tail-suspension or loading (about 70% reduction, p<0.05). The maximal diameters of the osteoclast-like cells developed in the marrow cells from tail-suspended mice were significantly greater (about 50%, p<0.05) than those from loaded mice in wild type. Spreading of these cells was significantly reduced in the bone marrow cells obtained from loaded OPN-deficient mice compared to loaded wild type mice (about 50% reduction, p<0.05). Unloading-induced enhancement of spreading of these cells was still observed compared to the cells from loaded OPN-/- mice (about 50% enhancement, p<0.05). The numbers of vessels within the secondary spongiosa in the tibiae were similar between loaded and suspended mice in both genotypes. These data revealed that the defects in in vitro mineralization by osteoblasts and in osteoclast spreading were associated with the resistance to unloading-induced bone loss in OPN-/- mice.

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Modulating Osteotomy Gap Healing with Motion Exercise. K. M. O'Connor,[1] S. H. Park,*[2] M. C. Salud.*[1] [1]Biokinesiology and PT, University of Southern California, Los Angeles, CA, USA, [2]J. Vernon Luck Orthopaedic Research Center, Orthopaedic Hospital, Los Angeles, CA, USA.

The purpose of this pilot study was to ascertain whether the application of recoverable axial interfragmentary motion (RAIM) exercise would promote significant ingrowth of new bone into diaphyseal osteotomy gaps in the tibia of skeletally mature rabbits. Osteoto-

mies with 10 mm interfragmentary gaps were surgically created in the left tibia of 6 rabbits and stabilized with four-pin double-bar external fixators to heal for 6 weeks. Three rabbits received daily RAIM exercise beginning 4 days after surgery. RAIM exercise consisted of unlocking a telescoping mechanism in the fixator to permit 4 mm of axial sliding at the interfragmentary gap of the osteotomy (40% strain). Motion was generated as the investigator gently moved the hind limb through 20 passive range of motion cycles, each beginning at knee and ankle extension and moving into knee and ankle flexion. Control animals received exercise while the fixator remained locked. Axial excursion during the exercise sessions was monitored by a strain transducer attached to the fixator. Ingrowth of mineralized tissue into the osteotomy gap was quantified with peripheral quantitative computer tomography (pQCT). The gap region was divided into 5 cross-sectional slices of 2 mm thickness (voxel size: .2x.2x2 mm). The threshold was set at 200 mg/cm^3, which excluded fibrous tissues but included low density mineralized tissues. No areas within the gap region measured at or above 900 mg/cm^3 indicating the absence of cortical bone. Mean axial excursion during RAIM exercise declined from 3.76 mm at post-op day 4, to 3.05 mm at 3 weeks and 1.02 mm at 6 weeks ($p = 0.0001$). Axial excursion of the control group (noise level) remained constant at a mean of 0.14 mm throughout the healing period ($p = 0.66$). The space volume created by the 10 mm gap was 500 mm^3. The volume of mineralized tissue within the gap that was detected at a threshold of 200 was 87 mm^3 (17% filled) for the control group compared with 413 mm^3 (83% filled) for the RAIM group ($p = 0.002$). Mineral content within the gap was 33 mg and 196 mg for the control and RAIM groups respectively ($p = 0.004$). The volumetric density of the mineralized tissue that formed in the gap was higher in the RAIM group (471 mg/cm^3 vs. 385 mg/cm^3) but the associated p-value was marginal at 0.07. Mineral content in all specimens declined toward the center of the gap ($p = 0.025$) indicating that bone ingrowth occurred from both the proximal and distal cortical surfaces. These observations suggest that RAIM exercise, administered at an initial strain near 40%, is a potent stimulus for osteogenic ingrowth in diaphyseal osteotomies with gap size of approximately 1 cm.

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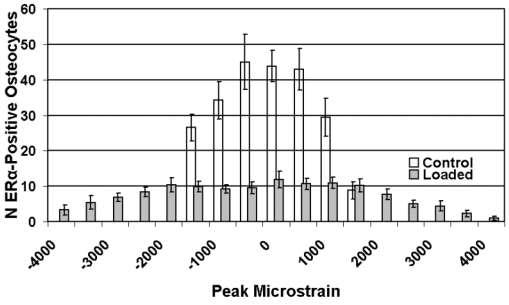
Mechanical Strain Decreases the *in vivo* Expression of Estrogen Receptor-α in Osteocytes. P. J. Ehrlich,*[1] B. S. Noble,[2] H. L. Jessop,*[1] H. Y. Stevens,[3] J. R. Mosley,*[2] L. E. Lanyon.[1] [1]Royal Veterinary College, London, United Kingdom, [2]Univ. of Edinburgh, Edinburgh, United Kingdom, [3]Univ. of California, San Diego, CA, USA.

We have previously reported that estrogen and mechanical strain: 1) independently stimulate proliferative responses that can be blocked by estrogen receptor (ER) modulators[a], 2) stimulate ERK-mediated phosphorylation of ERα[b] and, 3) increase activity of estrogen response elements[c]. We hypothesize that ERα provides a common pathway through which both estrogen and strain regulate bone remodelling activity. If true, reduction in ERα number could explain bones' limited ability for adequate mechanically adaptive remodelling after menopause.As osteocytes are ideally located to respond to strain, we examined whether their *in vivo* strain environment influenced the expression of ERα. We examined the immunofluorescent labeling of rat cortical bone osteocytes in sections from normal neonatal and adult, male and female rat ulnae (n=3 of each gender and age), and adult male rat ulnae which, in addition to normal locomotor loading, had been axially loaded daily as previously described[d] for 10 minutes at 2 Hz to generate peak strains of either -3000 (n=3) or -4000 (n=5) microstrain. In normal neonatal and adult bones 14 +/- 1.3% (mean +/- SEM) of all osteocytes were positive for ERα with no influence of either gender or age. In loaded bones, there were significantly fewer osteocytes expressing ERα (7.5 +/- 0.9%) than in contralateral controls (14 +/-1.7%) ($p=0.01$, median diff=6.4, 95% confidence interval=2.6, 10.3). Within both loaded and control bones, the number of osteocytes labeled for ERα was inversely related to the peak strains engendered at that location (Fig). These data support the hypothesis that ERα is involved in bone cells' responses to strain, with high strains decreasing the number of osteocytes expressing ERα. Reductions in ERα accompanying the high strains assumed to occur in osteoporosis may compound the reduction of ERα expression associated with estrogen deficiency[e] thus further impairing the capacity for appropriate adaptive remodelling following the menopause. [a]Damien, JBMR 15:2169-77; 2000, [b]Jessop, JBMR 16(6): *in press*; 2001, [c]Zaman, Bone 27:233-9; 2000, [d]Mosley, Bone 20:191-8; 1997, [e]Hoyland, J Path 188:294-303; 1999



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Mechanical Strain Rapidly Activates Osteoblastic Protein Kinase B (PKB): An Upstream Modulator of eNOS Activity. T. S. Grewal,*[1] R. J. Tolley,*[1] S. C. F. Rawlinson,*[2] L. E. Lanyon,[2] T. M. Skerry.[1] [1]Biology, University of York, York, United Kingdom, [2]Royal Veterinary College, London, United Kingdom.

Protein Kinase B (PKB/Akt) has been shown to mediate the activation of eNOS-dependent release of NO from endothelial cells in response to fluid flow. We therefore hypothesised that a similar mechanism may also be responsible for the obligatory NO release from osteoblasts in response to mechanical strain. The PKB intracellular signalling pathway is initiated by activation of phosphatidylinositol-3-kinase (PI-3-K). The resultant phosphorylation of PKB at Ser473 and Thr308 leads to its activation. The major downstream targets of activated PKB include eNOS, the pro-apoptotic proteins Bad/Bax, and the forkhead family of transcription factors. To investigated the role of PKB in osteoblasts, we subjected cells to mechanical strain *in vitro* using either a FX-3000 Flexercell or a four-point bending system. Cells (SaOS-2, TE-85 and calvarial-derived primary rat osteoblasts (ROBs)) were mechanically stimulated with cyclical strain (four-point bending: 1,100-3,800με; FX-3000: 4,500-7,000 με) of 1 Hz for 10 minutes and total protein extracted at various times post-stimulation. In the case of the primary ROBs, cells were either maintained in medium alone or supplemented with osteoinductive media to induce osteoblast differentiation for 7 days prior to mechanical stimulation. Protein lysates were analysed by immunoblotting using specific phospho-PKB (Ser473) antibody. Immunoblots were subsequently stripped and analysed with an antibody to PKBα to control for protein loading. In clonal cells (SaOS-2 and TE-85), a significant 2.5-fold increase in phosphorylation of PKB at Ser473 was evident within 2 minutes of the end of the straining regimen and peaked at 20-40 minutes. In all cases this response to strain was completely blocked by pre-treatment of cells for 30 minutes with specific PI-3-K inhibitors (wortmannin, 500 nM or LY294002, 10 μM) but unaffected by GF109203X (a PKC inhibitor). In primary ROBs, PKB phosphorylation was significantly increased (3-fold) in differentiated osteoblasts subjected to mechanical strain, but not undifferentiated ROBs subject to the same stimulation. These findings demonstrate that in osteoblasts, PKB is rapidly phosphorylated in response to physiological levels of mechanical strain. Additionally, differentiating primary osteoblasts show a much greater mechanical induction of PKB activity than less differentiated cells. These finding suggest 1) a mechanism for regulation of the eNOS activity known to be an obligate step in the adaptive mechanism, and 2) that even early commitment towards the osteoblastic lineage sensitises cells to the effects of strain.

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Smoothened Is Required for Osteogenesis in Endochondral Bone Development. U. Chung,[1] F. Long,*[2] J. McMahon,*[2] A. McMahon,*[2] H. Kronenberg.[1] [1]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [2]Harvard University, Cambridge, MA, USA.

It has been recently shown that Indian hedgehog (Ihh), synthesized by prehypertrophic and hypertrophic chondrocytes, is locally required for maturation of osteoblasts in the bone collar, the precursor of cortical bone. It is, however, not clear yet whether the same Ihh is required for osteoblast development in the primary spongiosa, the precursor of trabecular bone, as well. To address this question, chimeric mice containing wild-type cells and cells homozygous for a null-mutation in the *Smoothened* gene (which encodes a membrane bound protein mediating Hedgehog signaling) were generated by blastocyst injection. The mutant cells were marked with a β-galactosidase transgene engineered to be expressed ubiquitously, and their contribution to osteoblasts in the primary spongiosa was analyzed by standard histology and in situ hybridization. *Smo*-deficient cells can contribute to mesenchymal tissues such as the muscle, tendon and cartilage. However, even though mutnat cells contribute to the perichondrium, they fail to contribute to bone-forming osteoblasts, while wild-type cells become osteoblasts and form bone collar in the perichondrium adgacent to prehypertrophic and hypertrophic chondrocytes. This finding further supports the crucial role of Ihh in bone collar formation. Furthermore, *Smo*-deficient cells fail to contribute to osteoblastic cells in the primary spongiosa of the chimeric mice so far generated (n=4), even though the mutant cells can contribute to vascular endothelial cells, hematopoietic cells as well as osteoclasts. These data strongly suggest that the Hh signal is required for osteoblast development in the primary spongiosa as well. Ihh from prehypertrophic and hypertrophic chondrocytes is the likely source of this Hh signal. This Ihh provides a crucial signal necessary for osteogenesis in both the primary spongiosa and the bone collar. Ihh, which also regulates chondrogenesis, thereby links chondrogenesis and osteogenesis during endochondral bone development.

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Phosphatidylinositol 3 Kinase (PI 3-K) and Akt Serine Threonine Kinase Regulate BMP-2-Induced Osteoblast Differentiation and Smad-Dependent Transcription of BMP-2 Gene. N. Ghosh-Choudhury,*[1] S. L. Abboud,[2] G. Ghosh Choudhury.*[3] [1]Pathology, Univ. Tx. Hlth. Sc. Cntr., San Antonio, TX, USA, [2]UTHSCSA, San Antonio, USA, [3]GRECC, STVA Hospital, San Antonio, USA.

Interplay of different signal transduction pathways converge into the nucleus to induce transcription of specific genes necessary for lineage specific cellular differentiation. Bone morphogenetic protein-2 (BMP-2) stimulates osteoblast differentiation of progenitor cells. The precise mechanism by which different signaling pathways regulate BMP-2-induced osteogenesis has not been explored. We investigated the involvement of PI 3-K in BMP-2-induced osteoblast differentiation of 2T3 mouse mesenchymal precursor cells. BMP-2 stimulated PI 3-K activity in PI 3-K and anti-phosphotyrosine immunoprecipitates in a time-dependent manner, indicating BMP-2-induced association of PI 3-K with tyrosine phosphorylated proteins. Incubation of 2T3 cells with a pharmacologic inhibitor of this enzyme, Ly 294002 (Ly), prevented BMP-2-induced PI 3-K activity. Ly blocked BMP-2-induced mature bone nodule formation in 2T3 cells. Osteoblastic differentiation induced by BMP-2 precedes expression of specific marker protein, such as alkaline phosphatase. Ly as well as expression of dominant negative PI 3-K inhibited BMP-2-induced alkaline phosphatase expression, indicating that PI 3-K is necessary for expression of osteoblast-specific protein marker. One of the downstream target of PI 3-K is the serine threonine kinase Akt which regulates many biological activities of PI 3-K. BMP-2 stimulated Akt in a PI 3-K-dependent manner. Inhibition of Akt activity by expression of a dominant negative mutant abolished BMP-2-induced alkaline phosphatase expression. In contrast, a constitutively active mutant of Akt increased BMP-2-induced alkaline phosphatase. We previously demonstrated that BMP-2 stimulates BMP-2 mRNA expression during osteoblast differentiation. Expression of dominant negative PI 3-K inhibited BMP-2-induced BMP-2 gene transcription. In fact the transcriptional effect of BMP-2 is mediated by BMP

specific Smads. Expression of either Smad 1 or Smad 5 stimulated BMP-2 gene transcription independent of addition of the ligand. To investigate the cross talk between PI 3-K and Smad signaling, we used dominant negative PI 3-K in transcriptional assay. Dominant negative PI 3 kinase inhibited Smad 1 as well as Smad 5-dependent transcription of BMP-2 gene. Together these data provide the first evidence that activation of BMP receptor serine threonine kinase activates PI 3 kinase/Akt pathway and define a role for this signaling cascade for BMP-2-induced osteoblast differentiation and Smad-dependent gene transcription.

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Regulation of Osteoblast Differentiation by Proteasome Control of Smad1. D. Chen,[1] M. Zhao,[1] M. Qiao,*[1] R. Garrett,[1] Z. Mi,*[1] C. Crews,*[2] G. Mundy.[1] [1]Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [2]Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA.

Recently we have found that proteasome inhibitors (PIs) stimulate bone formation in vitro and in vivo. Since the effects of PIs mimic those of BMP-2 and proteasome inhibitor 1 (PS-1) enhanced BMP2-induced bone formation in vitro and in vivo, in the present studies, we examined the effects of PIs on Smad1, an essential molecule in the BMP signal pathway. We found that overexpression of Smad1 in C2C12 myoblast/osteoblast precursor cells induced osteoblast differentiation and enhanced BMP-2 responsiveness as assessed by increased alkaline phosphatase (ALP) activity and osteocalcin production. Smad1 degradation was induced by Smurf1, an ubiquitin E3 ligase, in a dose-dependent manner. In contrast, Smad2 degradation was not affected by Smurf1. When Smad1 was co-transfected with mutant Smurf1 (C710A) in C2C12 cells, the steady-state levels of Smad1 were not significantly changed. In the mutant Smurf1, the cysteine residue in the hect domain that is responsible to form a thiolester bond with ubiquitin was replaced by alanine. These results suggest that ubiquitin is required for Smad1 degradation. Overexpression of mutant Smurf1 acted in a dominant-negative fashion and increased ALP activity, osteocalcin production and BMP-2 responsiveness in C2C12 cells. Structurally unrelated PIs, MG-132 (2.5 μM), lactacystin (10 μM), epoxomicin (20 nM) and PS-1 (50 nM), completely prevented Smurf1-mediated Smad1 degradation. Addition of these PIs at same concentrations also enhanced endogenous Smad1 protein levels up to 6-fold in C2C12 cells. To determine which specific activity of the proteasome is responsible for Smad1 degradation, two different substrate specific PIs, Ac-hFLFL-epoxide (chymotryptic specific) and YU-102 (post-glutamyl peptide hydrolyzing specific) were utilized. Ac-hFLFL-epoxide significantly reversed Smurf1-mediated Smad1 degradation in a dose-dependent manner. In contrast, YU-102 had no significant effect on Smad1 degradation in C2C12 cells, suggesting that chymotryptic activity of the proteasome is responsible for Smad1 degradation. These results are consistent with the results that inhibition of chymotrypsin-like activity caused new bone formation in bone organ culture assay. Our findings indicate that PIs may enhance effects of BMP-2 by preventing Smad1 degradation and increasing intracellular steady-state levels of Smad1. The increase of Smad1 leads to enhancement of BMP-2 signaling and subsequent osteoblast differentiation and bone formation.

Disclosures: OsteoScreen Inc.,1,3.

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Identification of a Novel BMP-2 Induced Gene, which Dramatically Accelerates Osteoblast Differentiation. F. Gori, M. B. Demay. Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

Endochondral bone formation is a complex process controlled by a wide variety of factors including transforming growth factor (TGF)-βετα superfamily members and more specifically the bone morphogenetic proteins (BMPs). Using differential display (dd) PCR, we have isolated and cloned a novel gene, named BIG-3 (BMP-2 Induced Gene 3kb), that is induced as a murine prechondroblastic cell line, MLB13MYC clone 17, acquires osteoblastic features in response to BMP-2 treatment. The 3 kb mRNA encodes a 33 KDa protein belonging to the family of WD-40 repeat proteins, which have a wide variety of cellular functions including signal transduction, mRNA processing and gene regulation. Northern analyses using RNA isolated from MLB13MYC clone 17 cells revealed that BIG-3 was induced 3.5-fold after 24 h of BMP-2 treatment. BIG-3 mRNA was also expressed in conditionally immortalized murine bone marrow stromal cells, osteoblasts, osteocytes and growth plate chondrocytes as well as in primary calvarial osteoblasts. By Northern analyses BIG-3 mRNA expression was detected in mouse brain, heart, kidney, muscle, skin, spleen, thymus and testis. In addition, Western analyses using cell lysate from MLB13MYC clone 17 cells treated with BMP-2 (0-200 ng/ml), showed that BIG-3 protein was also induced after 24 h of treatment. To characterize the functional role of BIG-3 in osteoblast differentiation, MC3T3E1 cells were stably transfected with the full length coding region of BIG-3 (MC3T3E1-BIG3) cloned into an expression vector (pcDNA3.1) under a CMV promoter. Three pools of 10 clones for both MC3T3E1-BIG3 and cells transfected with the empty vector (MC3T3E1-EV) were initially characterized in these studies. Pooled clones transfected with BIG-3 expressed alkaline phosphatase activity earlier and, at 7 d achieved a peak level of activity 28-fold higher than MC3T3E1-EV cells. In addition, cAMP production in response to PTH was increased 10 and 14-fold at 7 d and 14 d, respectively in MC3T3E1-BIG3 cells, relative to MC3T3E1-EV cells. Overexpression of BIG-3 also accelerated formation of mineralized nodules (21 d) compared to MC3T3E1-EV cells (35 d) as assessed by Alizarin Red S and increased calcium accumulation in matrix by 12 and 44-fold at 21 d and 25 d, respectively, as assessed by methylthymol blue. In conclusion, we have identified a novel WD-40 protein induced in response to BMP-2 treatment, which dramatically accelerates the program of osteoblastic differentiation in stably transfected MC3T3E1 cells.

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Δ2ΔFosB Expression Positively Regulates BMP Signalling Pathways in Osteoblast Cell Lines *In Vitro*. G. Sabatakos,[1] M. Kveiborg,[1] M. Wu,*[1] S. Roman-Roman,[2] W. C. H. Horne,[1] R. Baron.[1] [1]Yale School of Medicine, New Haven, CT, USA, [2]Aventis Pharma, Paris, France.

The AP-1 family of transcription factors consists of Fos- and Jun-related proteins that have been shown to be important regulators of bone cell differentiation both *in vivo* and *in vitro*. We have reported previously that overexpression of δFosB, which arises from alternative splicing of the *fosB* transcript, in transgenic mice leads to increased bone formation and progressive osteosclerosis. *Ex vivo* osteoblast cultures and *in vitro* overexpression studies have shown that δFosB isoforms stimulate osteoblast marker gene expression and bone formation in a cell-autonomous manner. The aim of the present study was to further elucidate the molecular mechanism by which δFosB isoforms regulate osteoblast differentiation and function *in vitro*. For this purpose we have generated stable transfectants of δFosB, as well as of mutants expressing only the full length δFosB or the N-terminally truncated δ2δFosB isoform in the bipotential cell lines C2C12 and ST2. Analysis of the effect of these mutants on osteoblast differentiation in response to BMP-2 demonstrated an increase in the expression of osteoblast markers of only in cells expressing the truncated δ2δFosB isoform. Moreover, studies on the downstream Smad signalling pathway demonstrated that the levels of BMP-2 as well as of its receptors (BMPR Ia and Ib) or their phosphorylation were not affected by δFosB isoforms. In contrast, the expression of the receptor activated Smads 1, 5 and 8 and of Smad4 were differentially regulated by δFosB isoforms. In particular the mRNA levels of Smad1 were increased only in cells expressing δ2δFosB and this increase correlated with earlier and higher translocation of phospho-Smad1 to the nucleus. This response was not due to new protein synthesis in response to BMP-2 but rather to a direct effect on Smad1 transcription. In addition the ability of δFosB isoforms to regulate inhibitors of Smad signalling such as Smad6 and Tob was studied. The levels of both Smad6 and Tob were dramatically down-regulated in response to BMP-2 in cells expressing the truncated δ2δFosB isoform, suggesting an additional role for δ2δFosB as a suppressor of inhibitors of Smad pathway. Since osteocalcin expression is regulated by BMP-2, we then analyzed the osteocalcin promoter. EMSA binding on specific AP-1 elements and transient transactivation studies revealed higher binding activity and transactivation potential only in cells expressing the δ2δFosB isoform. It is therefore concluded that the δ2δFosB truncated isoform of δFosB is a potent regulator of osteoblast differentiation *in vitro* which differentially regulates both activators and inhibitors of the BMP-2 signalling pathway.

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COX-2 is Critical for Mesenchymal Cell Differentiation During Skeletal Repair. X. Zhang,* L. Xing, B. F. Boyce, J. E. Puzas, R. N. Rosier, E. M. Schwarz, R. J. O'Keefe. Center for Musculoskeletal Research, University of Rochester, Rochester, NY, USA.

NSAIDs are among the most commonly used drugs in our society. While agents specific for the inhibition of cyclooxygenase-2 (Cox-2) have recently been introduced and have an improved safety profile, it has become apparent that cyclooxygenase activity is important in skeletal reparative processes. However, the relative role of Cox-1 and Cox-2 isoforms, and the target cells and tissues involved in this process have not been clearly defined. In this study we used both in vivo and in vitro models in wild type and Cox-2 -/- mice to demonstrate that COX-2 plays a critical role in mesenchymal cell differentiation during skeletal reparative processes. Osteoblast and chondrocyte differentiation was examined in vitro in isolated bone marrow stromal cell cultures obtained from wild type and Cox-2 -/- mice. The expression of the bone cell and chondrocyte differentiation markers, osteocalcin and col X were much greater in cultures of wild type cells under both basal conditions and following BMP-2 induction. In vivo skeletal reparative responses were examined in wild type and Cox-2 -/- animals using 2 models. In the first model, closed intramedullary-pin-stabilized tibia fractures were created in 2-3 month old mice and fracture healing evaluated by radiographs and histomorphometry. At day 7, both wild type and Cox-2 -/- animals had abundant mesenchymal cell proliferation and little or no bone formation. However, while cartilage was forming in wild type animals, less cartilage was found in Cox-2 -/- animals. By 14 days, 70% of the callus in wild type animals was bone, compared to only 30% in Cox-2 -/- animals (n=3, $p<0.001$), while mesenchymal cells predominated in the Cox-2 -/- callus (60% in Cox-2 -/- and 13% in wild type; $p< 0.001$). By 21 days, cartilage was completely replaced by bone in wild type mice. In contrast, Cox-2 -/- mice now had significantly more cartilage, as well as three times more mesenchymal tissue at the fracture site (n=8, $p<0.001$). There was less bone, and less radiographic evidence of healing. Osteoclast numbers were also reduced by 57% (n=5, $p<0.001$), indicating a deficiency in osteoclastogenesis in COX-2 -/- mice. In another model, recombinant human fibroblast growth factor-1 (FGF-1) was injected subcutaneously (1ug/day x 3 days) onto the calvaria to examine growth factor induced intra-membranous bone formation. The Cox-2 -/- mice had 60% less (n=5, $p<0.0001$) new bone formation, as well as markedly reduced calvarial osteocalcin expression compared to wild type animals. Altogether, these studies provide direct evidence for a critical role of COX-2 in the differentiation of mesenchymal cells, with subsequent effects on bone formation and bone healing.

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Estrogen and Testosterone have Opposite Effects on Circulating OPG Levels Following Induction of Hypogonadism and Aromatase Inhibition in Normal Elderly Men: Potential Mechanism for Differential Effects of Estrogen Versus Testosterone on Bone Resorption. S. Khosla,[1] E. J. Atkinson,*[2] C. R. Dunstan,*[3] W. M. O'Fallon,*[2] B. L. Riggs.[1] [1]Endocrinology, Mayo Clinic, Rochester, MN, USA, [2]Biostatistics, Mayo Clinic, Rochester, MN, USA, [3]Amgen, Thousand Oaks, CA, USA.

Previously, we demonstrated that in normal elderly men, estrogen (E) was the dominant sex steroid regulating bone resorption (JCI 106:1553, 2000). Since both E and testosterone (T) directly inhibit osteoclastic activity and IL-6 production by osteoblastic lineage cells, it

is unclear why T is less potent than E in inhibiting bone resorption in vivo. Osteoprotegerin (OPG) is a soluble neutralizing receptor that binds to and inactivates RANK ligand (RANK-L), the final mediator of osteoclastogenesis. We have previously reported that E increases and T decreases OPG production by osteoblastic cells, but whether this occurs in humans in vivo is unclear. In the present study, we analyzed serum for OPG levels from our previous study in which 59 elderly men were made acutely hypogonadal using a GnRH agonist and were also placed on an aromatase inhibitor, letrozole, to block conversion of androgens to estrogens. Concomitant with the induction of hypogonadism, they were fully replaced for 3 weeks with T and E. Following baseline studies, they were then randomized to 4 groups and restudied 3 weeks later: Group A (-T, -E) had withdrawal of both the T and E patches; Group B (-T, +E) stopped the T patch; Group C (+T, -E) stopped the E patch; and Group D (+T, +E) continued both patches. The table below shows the baseline values and percent changes in serum OPG levels over the 3 week intervention period in the 4 groups (mean ± SEM).

Group	Baseline serum OPG, pg/ml	Serum OPG, % change from baseline	P-value
A (-T, -E)	115 ± 14	+10.0 ± 6.5	0.145
B (-T, +E)	104 ± 11	+18.6 ± 7.9	0.033
C (+T, -E)	115 ± 10	-10.0 ± 7.9	0.256
D (+T, +E)	97 ± 8	-3.2 ± 6.1	0.606

As evident, the group treated with E alone (group B) had a significant increase in serum OPG levels. By contrast, OPG decreased by an average of 6.4 ± 5.0% in the +T groups (C and D) whereas it increased by 14.5 ± 5.1% in the the -T groups (A and B), and using a 2-factor ANOVA model, there was a highly significant T effect (P = 0.006) on decreasing serum OPG levels. In this analysis, the T effect on decreasing OPG levels dominated, since the effect of E in the ANOVA model was not significant. Taken together, however, these data suggest that, in vivo, T decreases OPG levels, whereas E tends to have the opposite effect. These differential effects of E versus T on OPG production may, in part, explain why T has weaker effects than E on inhibiting bone resorption in vivo in humans

Disclosures: Amgen Corporation,3.

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Vitamin D Dose Response Relationships. R. P. Heaney,[1] M. J. Barger-Lux,[1] K. M. Davies,[1] T. C. Chen,[2] M. F. Holick.[2] [1]Creighton University, Omaha, NE, USA, [2]Boston University, Boston, MA, USA.

Vitamin D inputs required to achieve or maintain any given serum 25(OH)D level are not known. Our objective was to establish the quantitative relationship between controlled steady state vitamin D input and resulting serum 25(OH)D concentration, and to estimate the proportion of the daily requirement during winter that is met by vitamin D reserves in body fat stores. We studied four groups of 16-18 men each, living at 41.5° N. latitude (age 38.7 ± 11.2), with habitual milk intakes of one serving per day or less. We gave oral doses of 0, 25, 125, and 250 µg vitamin D per day for 20 weeks across the winter months (when concurrent cutaneous production is close to zero at this latitude). We measured the time course of serum 25(OH)D concentration at intervals over the course of dosing. From a mean baseline value of 93.2 nmol/L, serum concentration of 25(OH)D fell on the zero dose, as is commonly observed. At the other doses, 25(OH)D rose to respective plateau values by 20 weeks, and the resulting equilibrium values were in direct proportion to dose (slope = 0.970 nmol/L for each 1 µg vitamin D input). The calculated oral vitamin D input required to sustain the autumn serum 25(OH)D level was 11.5 µg (460 IU)/d, while the total vitamin D input for a constant 25(OH)D level from all sources (supplement, food, fat stores) in these men, was ~96 µg (~3800 IU)/d. Since dietary intake was under 5 µg/d, by difference therefore, fat stores were providing ~80-84 µg vitamin D/d. These findings indicate that in healthy young adults probably better than 80% of their winter vitamin D utilization comes from cutaneous solar synthesis that had occurred during the preceding summer months. It follows that currently recommended vitamin D intakes are inadequate to maintain serum 25(OH)D concentration in the absence of substantial cutaneous production of vitamin D.

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Homocysteine Serum Levels Predict the Risk of Osteoporotic Fractures in Postmenopausal Women. J. B. J. van Meurs,*[1] M. van der Klift,*[2] R. de Jonge,*[3] J. Lindemans,*[3] J. Witteman,*[2] A. Hofman,*[2] J. P. T. van Leeuwen,[1] A. G. Uitterlinden,[1] H. A. P. Pols.[1] [1]Internal Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands, [2]Epidemiology and Biostatistics, Erasmus University Rotterdam, Rotterdam, The Netherlands, [3]Clinical Chemistry, Academic Hospital Dijkzigt, Rotterdam, The Netherlands.

Very high levels of homocysteine (homocystinuria) are associated with a high prevalence of vascular disease, mental retardation and osteoporosis. Mild increases in serum homocysteine (Hcy) give rise to increased risk for artherosclerotic and thromboembolic disease. Homocysteine has also been suggested to interfere with the crosslinking of collagen type I, the major matrix component of bone.This raises the question whether mildly elevated Hcy levels increase the risk for osteoporosis. We therefore examined total serum homocysteine at baseline and determined its relationship to baseline bone mineral density (BMD) and risk of incident fractures. Hcy levels were determined in a population based sample of 429 women (aged 55-85 years), randomly drawn from a cohort of 4250 women participating in a large prospective follow-up study. BMD was measured at the lumbar spine and femoral neck by DEXA. Incident non-vertebral fractures were recorded over a mean follow-up period of 6 years. For 264 women lateral radiographs of the spine from the T4 to L5 were available and analyzed for the presence of vertebral fractures. We divided the women in three categories according to serum Hcy. Women in the highest tertile were 5 years older (p<0.001), had a higher body mass index (BMI) (p=0.03) and had lower mean dietary calcium intake (p=0.001). Mean femoral neck and lumbar spine BMD (crude or adjusted for age, BMI and dietary calcium intake) was not significantly different in the three groups. However, we observed an overrepresentation of fractures in women in the upper tertiles of the homocysteine level. Logistic regression showed that in comparison with the lowest tertile, women in the middle tertile had a 1.5 times increased risk (95% CI 0.6-4.0) for non-vertebral fractures and a 4 times increased risk for vertebral fractures (95%CI: 1.3-12.8). This increased to 2.7 (95%CI: 1.1-6.9) and 6.1 (95%CI: 1.9-19.5) in the highest tertile. These relative risks did not change essentially after adjustment for potential confounders like age, BMI, BMD, or dietary calcium intake. This study suggests that elevated serum homocysteine level is associated with increased risk for osteoporotic fractures in postmenopausal women. This risk is largely independent of differences in age or bone mineral density.

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Bone Response to Estrogen Replacement in OVX Double Estrogen Receptor-(α and β) Knockout Mice. M. A. Gentile, H. Zhang,* S. Harada, G. A. Rodan, D. B. Kimmel. Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA, USA.

α and β isoforms of the estrogen receptor (ER) are known, but their role in bone has not been fully established. The object here was to evaluate the skeletal effects of 17-β-estradiol (E2) in ovariectomized (OVX) double ER knockout ERα(-/-)β(-/-) (DRKO) mice obtained by F2 crosses of ERα(+/-)β(-/-) founders, derived from original ERα and ERβ null lines.DRKO and age-matched wild type (WT) mice were OVX or Sham-OVX at age 29 weeks. OVX groups were given 0, 0.04, or 0.2mg/kg 3X/wk E2 in sesame oil for eight weeks. Uterine weight (UWt, mg), femur length and distal 20% bone mineral density (DF-BMD, mg/cm2; pDXA, Norland) were measured after necropsy. The four groups within each genotype (all N=~11) were compared by Kruskal-Wallis ANOVA with Student Neuman-Keuls post-hoc test. All DRKO mice were compared to all WT mice for femur length (t-test of means).

Geno/Surg	E2	UWt	DF-BMD	F-Lgt
WT/Sham	0	143±50#	64±8#	16.2±0.3
WT/OVX	0	23±5	57±6	16.5±0.4
WT/OVX	0.04	127±29#	63±6#	16.1±0.3
WT/OVX	0.2	165±30#	67±9#	16.4±0.4
DRKO/Sham	0	47±25#*	64±7#	15.6±0.7
DRKO/OVX	0	18±7	57±5	16.1±0.4
DRKO/OVX	0.04	21±4	58±5	15.6±0.5
DRKO/OVX	0.2	63±38#	64±6#	15.8±0.4

Mean±SD; #diff from OVX/same genotype (P<.04) *Less than WT/Sham (P<.001)

KO/Sh mice had lower UWt and shorter femurs (P<.005), but similar DF-BMD, compared to WT. As expected, OVX reduced UWt in WT, but also in DRKO (P<.001) and lowered DF-BMD (P<.04) similarly in both genotypes. While 0.04mg/kg E2 protected against loss of UWt and DF-BMD in WT, 0.2mg/kg E2 was required in DRKO mice, that have high E2 levels prior to OVX. We conclude that: 1) OVX causes bone loss in DRKO; and 2) OVX-induced bone loss is prevented by high E2 (0.2mg/kg) in the absence of ERα and β. The higher E2 dose required in DRKO than WT suggests that E2 may act via conversion to testosterone or other non-ER mediated mechanism.

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Gender-Independent Suppression of CFU-OB Replication by Either Estrogens or Non-Aromatizable Androgens in Bone Marrow Cultures from Males and Females. G. B. Di Gregorio, R. L. Jilka, S. Kousteni, S. C. Manolagas. Division of Endocrinology & Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, and the Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Estrogens can restore bone mass in males with androgen deficiency, while non-aromatizable androgens can protect the female skeleton against the adverse effects of estrogen deficiency. However, the mechanisms of these phenomena are unknown. Based on evidence that a key mechanism of the anti-remodeling effects of estrogens in females may be the suppression of osteoblast progenitor (CFU-OB) replication, we examined whether androgens have similar effects to estrogens on replication of CFU-OB from females and whether estrogens have similar effects to androgens on the replication of CFU-OB from males. In these experiments CFU-OBs were identified in bone marrow cultures maintained for 25 days in the presence of ascorbate-2-phosphate, by their ability to form von Kossa stained colonies of mineralized matrix. CFU-OB replication was determined by measuring the difference in the number of colonies in these cultures, versus cultures from the same marrow maintained for 6 days in collagen gels, and then replated for an additional 25 days as above. The number of CFU-OB increased by 5-8 fold in cultures from adult female or male Swiss Webster mice maintained without sex steroids. However, in cultures maintained in the presence of either 17β-estradiol (E_2) or dihydrotestosterone (DHT), at doses ranging from 0.01 to 100 nM, CFU-OB replication was suppressed dose-dependently by E_2 or DHT irrespective of the sex of the bone marrow donor. In the case of either class of sex steroids the maximal inhibition of replication was 40-60%. Semi-quantitative RT-PCR analysis of ERα, ERβ and AR transcripts in 5-day cultures of bone marrow cells revealed similar levels for each one of the three receptors in cultures from females or males. These results suggest for the first time a mechanism by which the increased remodeling caused by estrogen deficiency can be reversed by androgens and vice versa. Moreover, in view of previous evidence that the suppressive effect of E_2 on CFU-OBs was abrogated in mice lacking ERα, our findings suggest that gender-independent suppression of remodeling by

either estrogens or non-aromatizable androgens results from sex steroid actions through their respective receptors. Whether suppression of remodeling alone can account for the anti-osteoporotic efficacy of androgens in females and estrogens in males, or additional gender-independent effects on the life span of osteoblasts and osteoclasts are involved will require future studies.

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Tumor Necrosis Factor-a (TNF) Plays a Prominent Role in Protein Undernutrition-induced Bone Resorption. P. Ammann,[1] I. Garcia,[2] J. Bonjour,[1] R. Rizzoli.[1] [1]University Hospital, Div of Bone Diseases, Geneva, Switzerland, [2]Dpt of Pathology, Geneva, Switzerland.

Protein undernutrition is associated with an accelerated bone loss, resulting from an unbalance in bone turnover, with increased bone resorption and decreased bone formation. Whereas the latter could be ascribed to a marked decrease in serum IGF-I, the mechanisms underlying the increased bone resorption are still not understood. In analogy with sex hormone deficiency-induced bone loss, where TNF plays a crucial role, we investigated whether TNF would be involved in protein undernutrition-stimulated bone resorption, by evaluating the effects of isocaloric low (2.5%, LP) or normal (15%, NP) protein diets in 6-month old female transgenic mice expressing high levels of soluble TNF receptor-1 fusion protein, which blocks the actions of TNF (TNFtrans). Negative littermates (NegLit) were used as controls. We measured tibia areal BMD in vivo by DXA, and midshaft tibia bone strength after 16 weeks of isocaloric low or normal protein diets, together with serum IGF-I and osteocalcin. The BMD decrease (% of baseline) was significantly less pronounced in TNFtrans (-6.4±3.1*° vs +7.9±5.4, LP vs NP) than in NegLit (-19.6±2.6* vs +7.0±3.6 %) (*, p<0.05 vs NP;°, vs LP NegLit, by ANOVA, x±SEM). Bone strength was significantly decreased in NegLit (8.5±0.6* vs 11.2±0.8 N), but not in TNFtrans (9.2±1.1 vs 10.9±0.3, NS). Tibia midshaft diameter was similarly lowered in both NegLit (1.12±0.02* vs 1.25±0.01 mm) and TNFtrans (1.18±0.01* vs 1.25±0.02) mice fed the low protein diet. This suggests a reduced periostal apposition. Similar decrease in IGF-I (88±3* vs 161±7 in NP, and 93±4* vs 176±17), and in osteocalcin (80±9* vs 126±13, and 81±16* vs 152±13) occurred in NegLit and TNFtrans fed the isocaloric low protein diet. Thus, the increased bone loss and decreased bone strength induced by protein undernutrition were markedly attenuated by blocking TNF. In contrast TNF did not seem to be involved in the inhibition of bone formation. This is the first evidence of a causal relationship between a bone resorption stimulating cytokine and bone loss induced by protein undernutrition.

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Pamidronate to Prevent Bone Loss in Men Receiving Gonadotropin-Releasing Hormone Agonist Therapy for Prostate Cancer. M. R. Smith,*[1] F. J. McGovern,*[1] A. L. Zietman,*[1] M. A. Fallon,*[1] D. L. Hayden,*[1] D. A. Schoenfeld,*[1] P. W. Kantoff,*[2] J. S. Finkelstein.[1] [1]Massachusetts General Hospital, Boston, MA, USA, [2]Dana Farber Cancer Institute, Boston, MA, USA.

Gonadotropin-releasing hormone agonist treatment decreases bone mineral density and increases the risk of osteoporotic fracture in men with prostate cancer. There have been no controlled studies for the prevention or treatment of osteoporosis associated with gonadotropin-releasing hormone agonist administration in men.In a 48 week open label study, we randomly assigned 47 men with locally advanced, node-positive, or recurrent prostate cancer to receive either leuprolide 3-month depot (22.5 milligrams intramuscularly every 12 weeks) alone or leuprolide 3-month depot and pamidronate (60 milligrams intravenously over 2 hours every 12 weeks). Men with bone metastases or secondary causes of osteoporosis were excluded. Bone mineral densities of the posterior-anterior lumbar spine, total hip, and total body were measured by dual energy x-ray absorptiometry. Trabecular bone mineral density of the lumbar spine was measured by quantitative computed tomography.Mean changes in bone mineral density at the posterior-anterior lumbar spine, trochanter, and total hip and trabecular bone mineral density of the lumbar spine differed significantly between the two groups (P<0.05 for each comparison). In men treated with leuprolide alone, mean (± SE) bone mineral density decreased by 3.3 ± 0.7 percent in the posterior-anterior lumbar spine, 2.1 ± 0.6 percent in the trochanter, and 1.8 ± 0.4 percent in the total hip (P= 0.001 for each comparison) and mean trabecular bone mineral density of the lumbar spine decreased by 8.5 ± 1.8 percent (P<0.001) over the 48 week study period. In contrast, mean bone mineral density did not change significantly at any skeletal site in the men treated with both leuprolide and pamidronate (P>0.05 for each comparison).Pamidronate prevents bone loss in the hip and lumbar spine of men receiving gonadotropin-releasing hormone agonist treatment for prostate cancer.

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A Pilot Investigation Evaluating Progression of Coronary Artery Calcification in Patients Taking Alendronate for Osteoporosis. J. A. Hill,*[1] J. G. Goldin,*[1] H. Yoon,*[2] L. D. Greaser,*[1] D. Gjertson,*[3] A. M. Emerick,*[1] B. Hu,*[4] D. R. Aberle,*[1] J. S. Adams.[5] [1]Radiological Sciences, UCLA School of Medicine, Los Angeles, CA, USA, [2]Radiology, University of Utah School of Medicine, Salt Lake City, UT, USA, [3]Bioststatistics, UCLA School of Medicine, Los Angeles, CA, USA, [4]Pathology, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA, [5]Burns and Allen Research Institute, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA.

Subendocardial atherosclerotic plaque calcifies as it matures. Resorption of hydroxyapatite in plaque is controlled by macrophage-like 'foam' cells. In vitro 'foam' cells undergo apoptosis when exposed to physiological concentrations of aminobisphosphonates (Adams et al, unpublished). We hypothesized that bisphosphonate-induced apoptosis of plaque 'foam' cells would inhibit resorption of calcium from plaque, thereby increasing plaque mass. To evaluate this hypothesis in vivo we under took a prospective pilot study tracking coronary artery calcification (CAC) progression by elecron beam computed tomography (EBCT) and bone mineral density (BMD) by dual energy x-ray absorptiometry (DEXA) over a 5-year period in 59 patients taking alendronate 70 mg per week for treatment of osteoporosis. Rates of progression of in patients taking alendronate were compared to those from a larger, sex-matched "control" cohort (n=225) as well as to 59 CAC-score-age-, sex-, and cardiac risk factor-matched control subjects not taking alendronate. As anticipated, the alendronate-treated group experienced a significant increase in femoral neck and lumbar spine DEXA scores (paired t-test p<0.004 and p<0.001, respectively). There was significant progression of CAC scores in the alendronate-treated (p<0.05) and both control groups (p<0.02), most notably in those patients whose baseline CAC score was above 100. No statistical difference was detected in the absolute change or rate of change among the alendronate-treated and control cohorts. The results of this pilot suggest that there is no increase in the progression of CAC in patients treated with alendronate for osteoporosis; similar observations have recently been made in alendronate-treated, hypercholesterolemic pigs with baloon injury-induced arterial plaque formation (Hill et al, unpublished). We theorize that the discrepancy between our clinical findings and those in vitro with isolated 'foam' cells is failure of the bisphosphonate to penetrate the vascular endothelial barrier which separates circulating drug from the exposed hydroxyapatite matrix of plaque.

Disclosures: Merck,5; Procter and Gamble,5.

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Novel, Non-steroidal Selective Androgen Receptor Modulators (SARMs) Increase Bone Mass and Reduce Androgenic Virilizing Effects in Adult Osteoporotic Rats. K. Furuya,* N. Yamamoto,* H. Nejishima,* K. Ichikawa,* S. Amano,* M. Tsunashima,* Y. Sumita,* K. Inoguchi,* M. Miyakawa,* T. Nakamura,* K. Hanada.* Drug Discovery Research Department, Kaken Pharmaceutical, Co., Ltd., Kyoto, Japan.

Cumulative evidences clearly indicate that androgens stimulate bone formation and increase bone mass in osteoporotic animal models and patients. However, androgen therapy has been hampered by its undesirable, dose-limiting side effects such as prostate stimulation and other virilizing effects. We have successfully generated a novel, non-steroidal small molecule library containing several hundreds of compounds that can bind androgen receptor (AR) as strongly as non-steroidal antiandrogens, casodex. To find out bone-selective AR agonists, these compounds were evaluated for tissue selectivity in comparison with testosterone propionate (TP) and DHT in orchiectomized (ORX) rats. The compounds were administrated subcutaneously (sc) or orally into ORX rats once daily for 4 weeks. Bone mineral density (BMD) of femurs was measured by dual energy X-ray absorptiometry (DEXA) and ventral prostates were weighed for representative index of virilizing effects. Among our non-steroidal library, Compound K1 and K2 (30mg/kg) dose-dependently increased in the BMD as markedly as TP and DHT, but did not elevate prostate weight over the normal at any doses tested. In contrast, TP and DHT showed 1.5-2 fold elevation in prostate weight as compared with the normal rats at efficacious dose of BMD increase in ORX rats (1~10mg/kg). To further analyze the virilizing effects, Compounds were given to the normal rats for 4 weeks and evaluated for prostate stimulation. The Compounds did not affect the prostate size, whereas the prostates of DHT-treated rats were about 1.5-fold larger than those of the normal. When the Compounds were administrated into ORX rats with TP, they competitively prevented prostate hypertrophy induced by TP and restored prostate to normal size. These data strongly suggested that our Compounds had the similar bone formation activity as natural androgen steroids, but the lessor virilizing effects than them. To confirm bone anabolic effects, the Compound K1 was given sc to ovariectomized (OVX) rat for 2 months and BMD of femurs was measured. The Compound significantly increased in BMD of femoral midshaft (cortical bones) as well as DHT and TP, whereas estrogen, anti-bone resorptive hormone, did not. Collectively, our novel Compounds were candidates of SARMs which had unique tissue selectivity with high potency for bone formation and lower impact upon prostate. The candidates are promising drugs for osteoporosis and androgen-deficient disease (androgen replacement therapy).

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Osteoprotegerin (OPG) Prevents Bone Loss in a Rat Model of Glucocorticoid-Induced Osteopenia. S. Morony,[1] J. Lu,*[1] C. Capparelli,[1] C. R. Dunstan,[2] D. L. Lacey,[1] P. J. Kostenuik.[1] [1]Pharmacology/Pathology, Amgen, Inc., Thousand Oaks, CA, USA, [2]Development, Amgen, Inc., Thousand Oaks, CA, USA.

The prolonged use of glucocorticoids such as prednisolone (Pred) often results in osteoporosis by mechanisms which include osteoclast stimulation and osteoblast suppression. The osteoclast response to glucorticoid therapy may be mediated by increased osteoblast expression of OPGL/RANKL (a physiological activator of osteoclasts) and decreased expression of OPG (a physiological inhibitor of osteoclasts) (Hofbauer et al, Endocrinology 140: 4382, 1999). Recent reports indicate that antiresorptives suppress bone resorption and increase bone mass in the glucocorticoid-treated skeleton. We therefore tested the effects of recombinant OPG in male SD rats (8-10 weeks old, n=6-7/group) which were implanted SC with pellets containing placebo or Pred (1 or 2 mg/kg/day). Animals were treated (SC) for 45 days with vehicle (PBS) or recombinant OPG (1 mg/kg, 3X/week) starting immediately after pellet implantation. On days 0, 15, 30 and 45, BMD was measured by DEXA and blood was drawn for biochemical markers of bone turnover. Both doses of Pred caused significant decreases in BMD in the femur/tibia compared to placebo-challenged rats. The Pred-related decrease in BMD was not accompanied by a histological decrease in bone volume, suggesting that the bone formed during Pred therapy may be hypomineralized. OPG treatment prevented the Pred-induced decrease in BMD, and restored BMD to levels significantly greater than in normal untreated rats. Pred challenge was associated with a significant increase in osteoclast surfaces, and OPG treatment reduced osteoclast surfaces to levels lower than in normal untreated rats. OPG also caused significant reductions in serum TRAP (a marker of bone resorption) in both Pred- and placebo-challenged rats. These anti-osteoclast effects were associated with significant increases in bone volume. Osteoblast suppression may also have contributed to bone loss

in this model, as evidenced by a significant reduction in serum osteocalcin in Pred-challenged rats. OPG did not reverse the Pred-related decrease in osteocalcin. These data suggest that Pred-induced bone loss in rats is mediated by a combination of increased bone resorption and suppressed bone formation. OPG treatment reversed the Pred-related increases in bone resorption, and reduced osteoclast numbers to subphysiological levels. These effects translated into increased BMD and bone volume in the tibia/femur to levels greater than in untreated normal rats. Recombinant OPG therapy may therefore represent a novel therapeutic approach for glucocorticoid-induced bone loss.

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Subcutaneous Administration of Insulin-Like Growth Factor (IGF)-II/ IGF Binding Protein-2 Complex Stimulates Bone Formation and Prevents Loss of Bone Mineral Density in a Rat Model of Disuse Osteoporosis . C. A. Conover,*[1] R. T. Turner,[1] E. W. Johnstone,*[1] F. J. Ballard,*[2] P. M. Doran,[1] S. Khosla.[1] [1]Mayo Clinic, Rochester, MN, USA, [2]GroPep, Adelaide, Australia.

We have previously reported that elevation in serum levels of IGFBP-2 and a precursor form of IGF-II is associated with marked increases in bone formation in patients with hepatitis C-associated osteoscelerosis (J Clin Invest 101:2165, 1998). Furthermore, in vitro studies indicated that IGF-II in complex with IGFBP-2 has high affinity for bone matrix and is able to stimulate osteoblast proliferation. The purpose of this study was to determine the ability of IGF-II/IGFBP-2 complex to stimulate bone formation in vivo. Osteopenia of the femur was induced by unilateral sciatic neurectomy in rats. At the time of surgery, 14-day mini-osmotic pumps containing vehicle (NaCl/1% BSA, n=6) or 2 µg IGF-II/100 g body weight/day + 9 µg IGFBP-2 (n=5) were implanted under the neck skin. Bone mineral density (BMD) measures were taken immediately before surgery and 14 days later using a PIXImus small animal densitometer. Fluorochromes were administered on day 8 and day 13 for bone histomorphometry. Results for the BMD (mean ± SE) are presented in the Table.

	R Femur (sciatic neurectomy)		**L Femur (control)**	
	Day 0	Day 14	Day 0	Day 14
Vehicle	0.217 ± 0.005	0.197 ± 0.007*	0.215 ± 0.003	0.216 ± 0.004
II/BP-2	0.214 ± 0.004	0.220 ± 0.003	0.214 ± 0.006	0.233 ± 0.003*

*P<0.05 vs Day 0

Neurectomy (R femur) resulted in a 10% decrease in BMD that was prevented by the IGF-II/BP-2 complex. Of note, even in the control (L femur), the IGF-II/BP-2 complex resulted in a significant (9%) increase in BMD. Our preliminary bone histomorphometric analyses indicate an increase in endocortical bone formation rates with IGF-II/IGFBP-2 treatment. These results demonstrate that short-term administration of IGF-II/IGFBP-2 complex can prevent loss of BMD associated with disuse oteoporosis and stimulate new bone formation in adult rats. Success in further preclinical trials may indicate a novel approach to stimulating new bone formation and to increasing bone mass in humans with osteoporosis

Disclosures: GroPep,2.

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Novel Bone-Targeted Src Tyrosine Kinase Inhibitors Prevent Bone Loss and Stimulate Osteoblast Activity. B. Boyce,[1] L. Xing,*[1] S. Bain,*[2] V. Shen,*[2] C. Liu,*[2] H. Chen,*[2] Y. Wang,*[3] W. Shakespeare,*[3] C. Metcalf,*[3] R. Sundaramoorthi,*[3] T. Keenan,*[3] R. Bohacek,*[3] M. R. van Schravendijk,*[3] D. Dalgarno,[3] J. Iuliucci,[3] M. Weigele,*[3] T. Sawyer.[3] [1]Univ. Rochester Med Ctr, Rochester, NY, USA, [2]SkeleTech, Bothell, WA, USA, [3]ARIAD Pharmaceuticals, Cambridge, MA, USA.

Src is a non-receptor tyrosine kinase required for osteoclast (ocl) activation and bone resorption. Thus, it is a well-validated therapeutic target for prevention of postmenopausal and other forms of bone loss. To this end, we have used rational drug design to develop novel, potent (IC50s in nM range) non-peptide Src tyrosine kinase inhibitors (MW < 500) selective against several other protein kinases. We targeted them to bone by specific chemical moieties with high affinity for hydroxyapatite and tested their effects on bone resorption. Selected lead compounds (AP23286; AP23317; AP23381; AP23451): 1) dose-dependently inhibited ocl resorption *in vitro* (pit area and number reduced by 55-97% at 1 µM and by 98-100% at 10 mM) after pre-incubation on bone slices and 6d treatment, and without pre-incubation, at 10 µM (AP 23317 and AP 23381), effects comparable to alendronate at 1 and 10 mM; 2) prevented PTH-induced hypercalcemia (50-100% inhibition) and bone resorption (osteoclast number/mm calvarial bone surface (4.5+/-1.3 AP23381 + PTH vs 11.1+/-1.5 PTH alone; AP23451: 1.7+/-1.7 vs 14.4+/-3.3) when given twice daily s.c(10 mg/kg) to mice for 5d; 3) prevented Ovx-induced vertebral (DEXA: .063+/-.0001 vs .056+/-.002 g/cm^2) and femoral metaphyseal (pQCT: 571+/-8 vs 455+/-14 g/cm^3) bone loss, with associated decreased ocl activity (Oc.S/BS: 4+/-0.3 vs 17+/-1.5%) and increased vertebral breaking strength (12.9 +/- 1.3 vs. 6.5 +/- 0.9 N/mm^2) and elastic modulus (300 +/- 27 vs. 139 +/- 16 MPa) (AP 23317, 10mg/kg s.c. once daily for 35d). In addition to its required role in osteoclasts, Src also negatively regulates bone formation. In preliminary studies to examine this latter role for Src, 6d treatment of fetal rat calvarial cells with Src inhibitors increased alkaline phosphatase activity 2-fold. Furthermore, AP23317 given once daily to intact mice for 35d increased bone mass in vertebrae (DEXA: .066+/-.002 vs. .059+/-.002 g/cm^2) and in femoral metaphyses (pQCT: 639+/-9 vs 447+/-22 mg/cm^3; cancellous bone volume: 15.7+/-1.3% vs 7.8+/-1.0%). Our findings provide the first evidence that this specifically-designed class of bone-targeted Src tyrosine kinase inhibitors prevent bone loss and maintain bone strength by inhibiting osteoclastic resorption, and suggest that they also stimulate bone formation. Thus, they may have a dual action on bone, suggesting efficacy not only in the prevention of osteoporosis, but also in its treatment.

Disclosures: ARIAD Pharmaceuticals,5.

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Activation of Unliganded AF2 Defective Vitamin D Receptor Mutant by Phosphorylation. F. Barletta,*[1] L. P. Freedman,[2] S. Christakos.[1] [1]Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School, Newark, NJ, USA, [2]Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

The vitamin D receptor (VDR) normally acts in the presence of 1,25dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] as a ligand inducible transcription factor by binding as a heterodimer with the retinoid X receptor to hormone response elements in target genes. The ligand dependent transcriptional activation function (AF2) is located in the ligand binding domain (LBD). The C terminal helix 12, the core AF2 domain, contains residues that function as an interaction surface for coactivators. In this study COS-7 cells were cotransfected with 500 ng wild type (WT) VDR or the AF2 defective mutant L417S (the leucine residue 417 at the C terminus of the predicted helix 12 in VDR was mutated to serine) and 3µg of a chloramphenicol acetyltransferase (CAT) reporter plasmid containing multiple copies of the rat 24(OH)ase vitamin D response element (VDRE) (-151/-137) or the mouse OPN VDRE (-757/-743). Cells transfected with WT VDR exhibited a 9.4±0.2 fold and a 5.6±0.9 fold induction in CAT activity respectively after treatment with 1,25$(OH)_2D_3$ (10^{-8}M for 24h). In cells transfected with L417S mutant VDR, vitamin D dependent transactivation was abolished. In contrast, in the presence of the phosphatase inhibitor okadaic acid (OA; 50nM, 24h), the AF2 defective mutant was able to activate the VDRE tk CAT constructs in a ligand independent manner (3 -4 fold induction compared to basal, $p < 0.05$). These results show that activation by phosphorylation can be independent of the conserved leucine in the AF2 domain. In addition, phosphorylation rescued the recruitment of DRIP205, a subunit of the DRIP coactivator complex, to the AF2 defective mutant. COS cells transfected with L417S mutant VDR were treated with vehicle, 1,25$(OH)_2D_3$ or OA (50 nM) for 24h. Nuclear extracts containing equal amounts of L417S mutant VDR were incubated with immobilized GST-DRIP205 and bound L417S was subsequently visualized by Western analysis. The defective receptor was unable to bind to DRIP205 in the presence or absence of 1,25$(OH)_2D_3$. However, treatment with OA was able to cause a ligand independent recruitment of DRIP205 to the AF2 defective VDR. Binding of L417S mutant VDR to DRIP205 was further increased in the presence of both OA and 1,25$(OH)_2D_3$. These findings suggest that phosphorylation results in a conformational change in the AF2 defective mutant that creates an active interaction surface with coactivators. These results demonstrate a novel mechanism by which an inactive VDR can be converted to an effective transcriptional activator.

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Osteopenia in Male Androgen Receptor Deficient Mice. H. Kawano,[1] T. Sato,*[1] H. Kawaguchi,[2] S. Kato.[1] [1]Institute of Molecular and Cellular Biosciences, Univ. of Tokyo/ CREST, Tokyo, Japan, [2]Orthopaedic Surgery, Univ. of Tokyo, Tokyo, Japan.

Androgen is physiologically important for male skeletal formation and reproductive organs; however, its action on bone at molecular levels remains largely unknown, mainly due to the lack of androgen receptor-knock out (ARKO) mouse line. As ARKO males are expected to exert testicular feminization mutant (Tfm) abnormalities with infertility, it is impossible to generate ARKO mouse lines by either conventional KO methods or natural mutations. To avoid this problem, we applied a Cre/loxP system, and succeeded in generating an ARKO mouse line. We further performed in vivo and in vitro analyses of the bone of ARKO males. We first generated the floxed AR mice, in which the AR gene locus was flanked by loxP sites. The floxed mice were fertile and expressed AR protein normally. By mating them with CMV-Cre transgenic mice expressing Cre recombinase ubiquitously, the AR gene was disrupted during embryogenesis. ARKO males grew normally with typical features of Tfm abnormalities, and genital organs were atrophic with a marked decrease in the serum testosterone level. Bone densitometry and 3D-µCT analyses revealed significant decreases of both trabecular and cortex bone volumes in ARKO males compared to wild-type (WT) littermate males at 6-16 weeks of age. Histomorphometric analysis at 8 weeks showed that bone volume (BV/TV) was 29% lower. Parameters for both bone formation (Ob.S/BS, MAR) and resorption (Oc.N/B.Pm , ES/BS) were higher in ARKO males than WT littermates, and the increase in bone resorption (40-50%) exceeded that in formation (15-20%), indicating a state of high turnover osteopenia. To investigate the cellular mechanism of this osteopenia, we compared functions of bone cells derived from ARKO and WT males. Neither osteoclastogenesis in M-CSF-dependent bone marrow macrophage culture in the presence of soluble RANKL nor isolated osteoclast survival rate was different between ARKO and WT cultures, indicating that AR signaling in cells of osteoclastic lineage is not important. However, osteoclastogenesis and resorbed pit formation in the co-culture system of osteoblasts and marrow cells were up-regulated only when osteoblasts were derived from ARKO mice independently of the origin of marrow cells, and ARKO osteoblasts showed higher RANKL mRNA expression. These results demonstrate that AR deficiency in osteoblasts stimulates the supporting ability of osteoclastogenesis causing high turnover osteopenia. Since the serum estrogen levels were similar between ARKO and WT males, androgen signaling was shown to play a critical role in maintaining male bone turnover and preventing bone loss in an independent mechanism of estrogen signaling.

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Osteoblast-Targeted Expression of Stretch Activated Cation Channels Enhances the Anabolic Effect of Mechanical Stimulation on Bone. N. L. Kizer,[1] U. Alvarez,*[2] J. L. Chaudhary,[1] K. Tustison,*[2] T. Clemens,[3] J. W. Pike,[3] K. Hruska.[1] [1]Internal Medicine/Renal, Washington University, Saint Louis, MO, USA, [2]Internal Medicine/Renal, Barnes-Jewish Hospital, Saint Louis, MO, USA, [3]Molecular/Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA.

Osteoblasts respond to chronic mechanical stimulation by increasing matrix protein synthesis and increasing the activity of plasma membrane stretch-activated cation channels

(SA-cat). We hypothesize that SA-cat channels function as a mechanosensor mediating exercise-induced bone growth. To investigate the role of the SA-cat channel in vivo we have generated transgenic (SA-cat Tg) mice that over-express the channel in osteoblasts and osteocytes. Northern analyses demonstrated that osteoblasts from SA-cat Tg mice expressed nearly 5 fold more SA-cat mRNA. SA-cat Tg specific RT-PCR indicated that the additional channel transcripts were due to transgenic mRNA. SA-cat Tg +/- mice express age dependent increases in bone strength, toughness and flexibility. To test the hypothesis that expression of the transgene increases the sensitivity of bone to mechanical stimulation, we subjected the SA-cat Tg +/- and wild type mice to in vivo tibial flexing. The left tibia was flexed in the medial direction using four-point bending with a bending moment of 12 Nmm applied at 2 Hz for 36 repetitions. The animals were stimulated each day for two weeks. Visual inspection of the left (flexed) tibia indicated periosteal hypertrophy in many of the bones. Tibia from transgenic mice averaged 100% more hypertrophy than their wild type counterparts as measured by maximum thickness of the tibia in the area subjected to 4 point bending ($P<0.05$). Application of 4-point pressure without bending did not result in hypertrophy of the tibia. Analysis of flexed and non-flexed bones from wild type and transgenic mice using pQCT revealed an 8.3% reduction ($p < 0.01$) of cortical thickness in the transgenic mice while cortical area and density remained unchanged. The change in cortical geometry was further reflected by a 13.9% ($p < 0.05$) increase in cross-sectional moment of inertia and an increase of 8.7% ($p < 0.02$) in polar moment of inertia. Furthermore, calcein labeling indicated that bone turnover was accelerated in the transgenic mice. These data taken together with our previous findings, lead us to conclude that the SA-cat channel functions as a mechanosensor in vivo, participating in the regulation of bone mass and strength. Overexpression of SA-cat lowers the set point for mechanotransduction resulting in a fundamental alteration of cortical bone material and geometry leading to a more flexible and stronger bone that retains bone properties of much younger animals.

Disclosures: Isto Technologies,5; Kizer Instrumentation,4.

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Mice with Null Mutation in Collagenase-3 (Matrix Metalloproteinase[MMP]-13) Exhibit Altered Bone Remodeling and Increased Bone Mass. M. Inada,[1] Y. Wang,[1] M. H. Byrne,*[1] C. Miyaura,[2] S. M. Krane.[1] [1]Medicine, Massachusetts General Hospital, Boston, MA, USA, [2]Biochemistry, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

We have previously described abnormal bone remodeling, including decreased bone-resorptive responses to parathyroid hormone (PTH) in mice with a targeted mutation in Col1a1 that encodes resistance to collagenase cleavage at the helical locus of type I collagen. MMP-13 can degrade collagen at neutral pH and is expressed in growth plates and bone during embryonic development and postnatally. To determine the specific role of MMP-13 in bone remodeling, we targeted a null mutation in the MMP-13 gene that results in splicing out Exon 5 which encodes the critical catalytic, Zn-binding domain leading to an inactive enzyme. Homozygous mutant mice (MMP-13 -/-) demonstrated increased width and delayed maturation of the growth plate, using histological analysis of tibias and femurs. In mice > 1 month of age there was an apparent increase in mass of metaphyseal bone. Furthermore, soft X-ray analysis of femurs from 1 and 3 month- old mice revealed a decrease in length, increase in width and increase in metaphyseal trabecular bone in MMP-13 -/- vs. MMP-13 +/+ mice. Changes in bone mineral density (BMD) of femurs were quantified using DEXA. In 1 month-old mice, mean distal BMD was 33.0 mg/cm2 ± 1.70 (SEM) in MMP-13 +/+ vs. 37.5 ± 0.72 in MMP-13 -/- ; in 3 month-old mice, distal BMD was 39.2 mg/cm2 ± 1.60 in MMP-13 +/+ vs. 48.2 ± 1.18 in MMP-13 -/-. Organ cultures of calvariae from newborn mice incubated for 4 days were used to assess bone resorption responses to PTH and interleukin-1a (IL-1a). In 3 separate experiments, the responses to PTH and IL-1a measured by changes in medium [Ca]were significantly suppressed ($p<0.01$) in MMP-13 -/- vs. MMP-13 +/+ mice. Western blot analysis showed a marked increase in medium MMP-13 protein induced by PTH or IL-1a in cultures of MMP-13 +/+ calvariae; in contrast, no detectable MMP-13 protein was induced by PTH or IL-1a in cultures of MMP-13 -/- calvariae. Nevertheless, expression of other MMPs (e.g. MMP-2 and -9), measured using casein and gelatin zymography, was induced by PTH or IL-1a in cultured calvariae from MMP-13 -/- as well as +/+ mice.In conclusion, disruption of the function of MMP-13 in mice results in abnormalities of bone remodeling with increases in metaphyseal BMD. Although MMP-13 is expressed predominantly in osteoblasts and related cells, osteoclastic bone resorption appears to be decreased in the MMP-13 -/- mice. Compensatory increases in other collagenolytic MMPs may partially ameliorate the effects of loss of function of MMP-13.

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RANK Ligand Stimulates Anabolic Bone Formation. J. Lam,[1] F. P. Ross,[1] S. L. Teitelbaum.[1] [1]Washington University School of Medicine, Saint Louis, MO, USA.

We have made the surprising observation that murine RANK ligand (RANKL), the key osteoclastogenic cytokine, when administered as an amino-terminal glutathione-S-transferase (GST) fusion protein, profoundly stimulates anabolic bone formation in vitro, ex vivo, and in vivo. Diurnal subcutaneous injection of GST-RANKL (158-316) (5 μg/kg to 4.5 mg/kg) for 7 days in mice results in a dose-dependent net increase in the number of activated osteoblasts in long bones, maximizing at 25-fold relative to control (624 ± 43 ob/mm^2 experimental vs. 28 ± 4 ob/mm^2 control, $p < 0.001$). In contrast, GST-RANKL fails to affect osteoclast number (45 ± 10 oc/mm^2 experimental vs. 39 ± 5 oc/mm^2 control). Systemic administration of GST-RANKL for 7 days induces as much as a 3-fold increase in cortical bone thickness, structural augmentation of the microarchitecture of the primary spongiosa, and a 10% increase ($p < 0.01$) in bone mineral density by DEXA. Dual fluorochrome labeling in animals receiving 1.5 mg/kg/day GST-RANKL for 7 days demonstrates a mineral apposition rate (MAR) of 3.59 ± 0.18 μm/day in the parietal bones of GST-RANKL-treated animals, compared with 0.18 ± 0.03 μm/day in the parietal bones of control animals, ($p < 0.001$). Marrow derived from mice administered 1.5 mg/kg/day GST-RANKL for 14 days exhibits a 100-fold increase in mineralizing bone nodules, when cultured under osteoblastogenic conditions ex vivo. Furthermore, administration of GST-RANKL for 12 hours, given on days 1 and 4 of ex vivo whole organ culture, induces a dose-dependent increase in the thickness of calvaria, maximizing at 2-fold (46.1 ± 3.97 μm experimental vs. 20.7 ± 1.5 μm control, $p < 0.001$). Consistent with the osteogenic properties of GST-RANKL, we find that its receptor RANK is expressed by primary mesenchymal osteoprogenitor cells, and that these cells respond to GST-RANKL with activation of the NFκB and MAPK pathways. Attesting to the function of RANKL-RANK signaling in osteoprogenitor cells, expression of the osteoblast transcription factor Cbfa1 is enhanced within 1 hour of in vivo treatment with GST-RANKL. Thus, a chimeric derivative of the pro-resorptive cytokine RANKL can induce bone formation by a mechanism involving RANK signaling in early osteoblast precursors to enhance Cbfa1 expression and induce commitment to the osteogenic phenotype. RANKL, or derivatives thereof, therefore presents itself as a potential anabolic agent for bone.

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Cells of the Osteoblastic Lineage from Transgenic Mice Over-Expressing Cbfa1 Induce Increased Bone Resorption in Vitro. V. Geoffroy,*[1] M. Kneissel,[2] B. Fournier,*[2] P. Matthias.*[1] [1]Friedrich Miescher-Institute, Basel, Switzerland, [2]Bone Pharma, Novartis, Basel, Switzerland.

The transcription factor Cbfa1 is required for bone formation. It acts as a differentiation factor during mesenchymal condensation and is also important for proper osteoblastic function, but its role in adult bone remodeling is not fully understood. To address this question we generated transgenic mice overexpressing Cbfa1 under the control of the rat collagen type I promoter. These mice present a severe osteopenic phenotype associated with high bone turn-over, cortical bone loss, and multiple fractures.To understand the origin of the increased bone resorption, we developed bone marrow stromal cell cultures and reciprocal co-culture of primary osteoblasts and spleen cells from wild type and transgenic mice followed by TRAP staining. The culture of bone marrow stromal cells under osteoclastogenic conditions showed that stromal cells of transgenic genotype induced an increased number of TRAP positive multinucleated cells compared to wild-type cells. To confirm these results we performed co-culture experiments from osteoblasts derived from calvaria and spleen cells. As expected primary osteoblasts derived from transgenic mice trigger the generation of more TRAP positive osteoclastic cells suggesting that primary osteoblasts as well as bone marrow stromal cells from transgenic mice have stronger osteoclastogenic properties compared to cells derived from wild-type animals. We then investigated the candidate genes that could trigger this increase of TRAP positive osteoclasts and analyzed the expression of bone markers in calvaria-derived cells and stromal cells isolated from wild type and transgenic animals. In these cells, we showed by semi-quantitative RT-PCR experiments, that the transgene was expressed in both cell populations and that the expression of RANK-ligand and collagenase 3, two factors involved in formation-resorption coupling, were markedly increased in transgenic cells. These results are consistent with an increase of these two factors measured in RNA prepared from long bone of transgenic animals. Our data rather suggest that the overexpression of Cbfa1 in cells of the osteoblastic lineage, including bone marrow stromal cells, have a positive effect on osteoclast differentiation and consequently on bone resorption. These findings also indicate that the increase of bone turn over observed in the mice overexpressing Cbfa1 under the control of the collagen type I promoter is partly due to the high expression level of RANK-ligand and collagenase 3 in bone marrow stromal cells.

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Longitudinal Transmenopausal Changes in Three-Dimensional Trabecular Microarchitecture and Connectivity of Human Iliac Crest Bone Biopsies. Y. Jiang,[1] J. Zhao,[1] R. R. Recker,[2] M. W. Draper,[3] H. K. Genant.[1] [1]Osteoporosis and Arthritis Research Group, University of California, San Francisco, CA, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [3]Lilly Research Laboratories, Indianapolis, IN, USA.

This study was designed to capture true longitudinal transmenopausal changes in three-dimensional (3D) trabecular architecture, which may improve our ability to understand the pathophysiology of osteoporosis and other bone disorders, and to estimate bone biomechanical properties in terms of fracture resistance as the mechanical competence of trabecular bone is a function of its apparent density and 3D distribution. During aging and diseases such as osteoporosis, trabecular plates are perforated and connecting rods are dissolved, with a continuous shift from one structural type to the other. Such changes can not be evaluated by 2D histological sections. In histomorphometry, there is debate about whether trabecular thinning occurs, or rather trabecular disappearance occurs with aging and/or menopause based on 2D sections using the parallel plate model. We examined paired bone biopsies from the iliac crest, not a primary weight bearing anatomical site, from 20 Caucasian women. The first biopsy was from normal, premenopausal women, age 46.3 – 53.5 years (mean ± SD, 49.1 ± 2.7 years), and the second biopsy from the same group of women, but 12 months postmenopausal, occurring 2.3 – 8.4 years (5.0 ± 1.7 years) after the first biopsy. The specimens were scanned using a micro computed tomography scanner (μCT 20, Scanco) with isotropic resolution of 20 μm. 3D trabecular structural parameters were directly measured without stereological model assumption. Values of 0 and 3 for the structure model index represent an ideal plate structure and rod structure, respectively, while values ranging from 0 to 3 indicate a structure with both plates and rods of equal thickness, depending on the volume ratio of rods and plates. After menopause, there was a significant change in 3D trabecular bone volume fraction (-5.4%/yr), trabecular number (-1.2%/yr), trabecular thickness (-3.3%/yr), trabecular separation (+2.0%/yr), structure model index (+11.3%/yr), degree of anisotropy (-0.7%/yr), and connectivity density (-2.0%/yr). The percentage change over the mean 5-year period was greater in 3D trabecular thickness (-16.4%) than in trabecular number (-6.1%) and trabecular separation (+9.9%). Thus, there is a rapid deterioration of 3D trabecular structure and connectivity in the iliac crest in the initial postmenopausal year. Trabecular thinning does occur and trabeculae dramatically shift from a plate-like structural type to a rod-like pattern, and become more isotropic.

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A Novel Therapeutic Vaccine That Prevents Pathological Bone Destruction in Models of Osteoporosis and RA. T. Juji,[1] K. Aoki,[2] D. Horie,*[2] K. Ohya,[2] M. Herz,*[3] A. Gautam,*[3] S. Mouritsen,*[3] H. Oda,*[1] K. Nakamura,[1] S. Tanaka.[1] [1]Department of Orthopaedic Sugery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan, [2]Section of Pharmacology, Department of Hard Tissue Engineering, Graduate school, Tokyo Medical and Dental University, Tokyo, Japan, [3]M & E Biotech A/S, Hørsholm, Denmark.

The receptor activator of NF-kappaB ligand (RANKL) is a novel member of tumor necrosis factor family cytokines, which is critically involved in osteoclast differentiation and activation, and therefore important for normal bone development. There is accumulating evidence that RANKL also plays important roles in the pathological bone destruction such as osteoporosis and rheumatoid arthritis. The natural inhibitor of RANKL, osteoprotegerin (OPG), has potent therapeutic effects on such conditions. However, repeated administration of OPG in larger dosescould potentially be immunogenic and may elicit antibody responses, limiting its long-term effectiveness. Here we describe a simple and effective method of active immunization against self RANKL as a possible treatment of bone diseases. RANKL protein vaccines were generated by inserting a promiscuous foreign T helper (Th) peptide into the RANKL cDNA. Immunization with these vaccines resulted in a rapid and sustainable polyclonal anti-RANKL antibodies in mice. No apparent macroscopic abnormality was observed in any organs of the immunizedanimals. To determine the therapeutic effects of theses vaccines, we utilized ovariectomy and arthritis models. Female BALB/c mice were immunized with either control antigen or the Th peptide-modified RANKL vaccine four times at two week intervals before subjecting them to ovariectomy. Mice immunized with RANKL vaccines were resistant to bone loss in response to ovariectomy. Importantly, both osteoclast numbers as well as bone resorption surface were significantlyreduced in RANKL vaccinated mice. We next examined the effect of the vaccine on SKG mice, a natural mutant of BALB/c background, that develops spontaneous rheumatoid arthritis-like inflammatory joint disorders and bone destruction. Immunization with RANKL vaccines almostcompletely prevented the bone destruction in these mice. Osteoclast numbers in various regions of bones were also dramatically reduced following this vaccination. These results demonstrate that a therapeutic vaccine approach targeting RANKL can be used to inhibitbone destruction in a variety of pathological bone loss conditions such as osteoporosis and rheumatoid arthritis.

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Targeted Deletion of the Histidine Decarboxylase Gene in Mice Increases Bone Formation and Protects Against Ovariectomy-Induced Bone Loss. C. Horvath,[1] A. Falus,*[2] E. Buzas,*[2] A. Mester,*[3] A. Nagy,*[4] L. Fitzpatrick,[5] J. Barsony.[6] [1]1st Dept Internal Medicine, Semmelweis University, Budapest, Hungary, [2]Dept Biology, Semmelweis University, Budapest, Hungary, [3]Dept Radiology, Semmelweis University, Budapest, Hungary, [4]Samuel Lunenfeld Institute, Mount Sinai Hospital, Toronto, Canada, [5]Dept Medicine, Mayo Clinic, Rochester, MN, USA, [6]LCBB, NIH/NIDDK, Bethesda, MD, USA.

Few studies have suggested a role for increased histamine production in osteoporosis and a bone-protective effect of antihistamines. To understand the role of histamine, we developed a knockout mouse model by targeted disruption of the histidine decarboxylase (HDC) gene, the only enzyme responsible for histamine production. Dramaticly decreased tissue histamine levels, decreased mast cell number and degranulation, impaired passive cutaneous anaphylaxis, decreased gastric acid secretion and nocturnal locomotor activity indicated the validity of the histamine deficient model (KO). The bone phenotype was investigated by measurements of bone mineral content by DEXA (BMC), computer tomography (CT), double calcein labeling, histomorphometric analysis, and laboratory parameters of calcium homeostasis. Groups of 5-7 wild-type (WT) and KO animals were also evaluated 45 and 90 days after sham-operation or ovariectomy. BMC was higher in KO than in WT animals and was also higher in KO after ovariectomy ($p<0.002$). Radiographs of femora showed increased cortical bone thickness in KO mice, as early as 45 days after ovariectomy. Quantitative CT also showed an increased cortical thickness in sham operated and ovariectomized KO mice compared to WT. Histology revealed a marked increase in cortical bone in the femoral diaphysis of KO mice by endochondral ossification, while the epiphysis was unchanged from the WT. Morphometric measurements showed increases in mineralizing surface ($p<0.004$) and in surface or volume-based parameters of bone formation rates ($p<0.006$; $p<0.007$). These differences were augmented after ovariectomy. Serum calcium, phosphorus, and 25-hydroxyvitamin D levels were the same in WT and KO animals and were within normal ranges. Serum alkaline phosphatase and 1,25-dihydroxyvitamin D levels were significantly elevated, and PTH levels were lower ($p<0.001$) in KO, consistent with increased bone formation. These results show that histamine deficiency causes an increase in cortical bone mass, bone formation rate, and reduces bone loss after ovariectomy. Our findings indicate that histamine negatively regulates bone formation and support the potential use of antihistamines for the treatment of postmenopausal osteoporosis.

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Demonstration that FGF-23, a Factor Produced by Tumors Associated with Phosphate Wasting, Inhibits Phosphate Transport *In Vitro*. S. M. Jan de Beur,*[1] A. E. Bowe,*[2] J. Y. Cho,*[1] R. Finnegan,*[2] R. Kumar,[3] M. A. Levine,[1] S. C. Schiavi.*[2] [1]Medicine and Pediatrics, The Johns Hopkins University, Baltimore, MD, USA, [2]Genzyme, Framingham, MA, USA, [3]Medicine, Mayo Clinic, Rochester, MN, USA.

Oncogenic osteomalacia (OOM) is an acquired hypophosphatemic syndrome that resembles genetic forms of hypophosphatemic rickets but which is associated with primitive mesenchymal tumors. The clinical and biochemical similarity of the two disorders has led to the hypothesis that the secreted protein produced by mesenchymal tumors in OOM is also responsible for the phosphaturia present in patients with the inherited forms of hypophosphatemic rickets, Autosomal Dominant Hypophosphatemic Rickets (ADHR) and X-linked Hypophosphatemic Rickets (XLH). The recent identification of activating mutations of the FGF-23 gene in ADHR has led to speculation that this secreted protein might also play a role in XLH and OOM. Using serial analysis of gene expression (SAGE), we previously demonstrated that FGF-23 was over-expressed in hemangiopericytomas but not sarcomas removed from patients with OOM. Using RT-PCR to analyze total tumor RNA, we now show that FGF-23 is expressed in 7/7 OOM tumors and 0/7 control hemangiopericytomas. Sequence analysis of the FGF-23 cDNA from three OOM tumors did not reveal any mutations in the coding region. To test the ability of FGF-23 to inhibit sodium-dependent phosphate transport, we seeded opossum kidney cells (OK) into 24-well dishes at densities ranging from 2,500/well to 80,000/well. OK cell cultures were incubated for 3 hours with conditioned media from COS-7 cells that had been transfected with empty vector or recombinant vectors expressing V5-tagged wild type FGF-23, FGF-23 R179Q (ADHR mutation), or PTH(1-34). Compared to conditioned medium from COS-7 cells transfected with vector only, medium containing wild type or R179Q FGF-23 inhibited radiolabeled phosphate uptake by 34-78%, which was comparable to that observed with medium containing PTH(1-34). Inhibition of phosphate uptake was inversely proportional to the density of OK cells. The addition of 10 ug/ml heparin sulfate, which binds FGF, abolished the inhibition of phosphate transport. Because increasing pH inhibits phosphate transport by ubiquitous sodium-dependent phosphate transporters but not the proximal tubular type II sodium-dependent phosphate transporter (NaPi-II), we performed the radiolabelled phosphate uptake at pH 9.5. The FGF-23 mediated inhibition was maintained suggesting that FGF-23 inhibits NaPi-II. These findings indicate that FGF-23 can inhibit renal phosphate uptake, and supports a functional role of FGF-23 in acquired and inherited forms of renal phosphate wasting.

Disclosures: Proctor and Gamble,8.

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Transgenic Mice Expressing Fibroblast Growth Factor 23 (FGF23) Demonstrate Hypophosphatemia With Low Serum 1,25-dihydroxyvitamin D [1,25(OH)2D] and Rickets/Osteomalacia. T. Shimada,*[1] T. Yoneya,*[1] R. Hino,*[1] Y. Takeuchi,[2] S. Fukumoto,[3] T. Yamashita.[1] [1]Pharmaceutical Research Laboratories, KIRIN Brewery CO.,LTD., Takasaki, Japan, [2]Division of Endocrinology, Department of Medicine, University of Tokyo School of Medicine, Tokyo, Japan, [3]Department of Laboratory Medicine, University of Tokyo Branch Hospital, Tokyo, Japan.

We have recently cloned FGF23 as a causative factor of tumor-induced rickets/osteomalacia (TIO). Administration of recombinant human FGF23 reduced serum phosphate and 1,25(OH)2D in mice. Furthermore, nude mice implanted with CHO cells stably expressing human FGF23 showed bone abnormalities resembling rickets/osteomalacia (T. Shimada et al, PNAS in press). In the present study, biochemical and histological features of transgenic mice expressing human FGF23 were analyzed to clarify the developmental and biological effects of FGF23 and the mechanism of hypophosphatemia by FGF23. Human FGF23 cDNA driven by CAG promoter was injected into 233 fertilized eggs and they were impregnated into 9 BDF1 mice. Thirteen genetically positive mice were obtained in 61 born mice. Before weaning, 3 transgenic mice died from unknown reasons. At 8 weeks old, most transgenic mice demonstrated retarded growth with curving backbones (body weight 17.9+/-1.4 vs 24.4+/-1.2 g, $p<0.005$). Serum phosphate and 1,25(OH)2D levels of transgenic mice were significantly reduced compared to those of FGF23-negative littermates (phosphate 3.41+/-0.45 vs 7.49+/-0.26 mg/dL, $p<0.0001$, 1,25(OH)2D 29.8 vs 103.3 pg/mL). Immunohistochemistry for renal Npt2, which is believed to be a responsible transporter for physiological phosphate reabsorption, indicated that expression levels were decreased in transgenic mice. However, serum PTH levels of transgenic mice were not different from those of control mice. Histological analysis of sternum and femur showed marked increase of osteoid and widening of growth plate. Thus, overexpression of FGF23 during fetal life does not result in lethality. It is shown that FGF23 induces hypophosphatemia by reducing expression of Npt2 without changing PTH levels. In addition, it is confirmed that FGF23 induces pathophysiological changes similar to those of TIO. Therefore, FGF23 seems to play a key role in maintaining phosphate homeostasis in mammals.

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Trabecular Bone from Two Strains of Mice is Differentially Mechanosensitive at the Tissue and Molecular Level. S. Judex,[1] M. Hadjiargyrou,[1] L. Donahue,[2] C. Rubin.[1] [1]Biomedical Engineering, SUNY Stony Brook, Stony Brook, NY, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA.

The identification of molecular mechanisms underlying mechanically stimulated bone formation and disuse related bone loss will be critical for the discovery of novel drug targets and effective physical interventions. Here, we subjected two distinct strains of mice to hindlimb suspension and extremely low level but high frequency mechanical stimulation (<10 microstrain) and cross-compared changes in trabecular bone formation rates (BFR) as well as altered transcription levels of two candidate genes. Female 16wk old Balb/cByJ and C57BL/6J mice were assigned to control, mechanically stimulated, and disuse groups (n=13 each). Mice in the mechanically stimulated group were placed on a vibrating plate (45 Hz, 0.25g) for 10 min/d. Disuse animals were subjected to tail suspension. Four animals per group were culled 4d into the protocol for determining gene expression levels (semi-quantitative RT-PCR) while the remaining animals were sacrificed after 21d for the assessment of bone formation rates (BFR). In Balb/c mice, hindlimb suspension caused a 52% decrease in trabecular BFR of the proximal tibia while low level mechanical stimulation increased BFR by 30%. In contrast, trabecular bone of C57BL/6 mice was unresponsive to both mechanical stimulation and disuse. After 4d of disuse, tibial type I collagen mRNA expression was significantly reduced by 35% in Balb/c mice but not in C57BL/6 mice. Inducible nitric oxide synthase (iNOS) transcription levels were significantly lower (25%) in mechanically stimulated mice of both strains than in controls. These data demonstrate that trabecular bone from Balb/c and C57BL/6 mice is differentially mechanosensi-

tive. While both the histomorphometric and molecular data are intriguing, together they reveal a great limitation when correlating gene expression levels with mechanically related bone formation without an appropriate negative control model. For example, iNOS was significantly down-regulated by mechanical vibration in both strains, yet only one mouse strain responded to the mechanical stimulus with new bone formation, suggesting a limited role of iNOS in mechanically mediated bone formation at the time point considered. This opportunity of eliminating candidate genes by cross-comparing a mechanically sensitive to a mechanically unresponsive (at the tissue level) strain will be particularly critical in future studies in which large numbers of candidate genes are considered (i.e., microarrays). Furthermore, the differential tissue level response can be exploited for searching for the genomic basis of bone's mechanosensitivity (e.g., quantitative trait loci).

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Low Bone Mass, Low Body Weight and Abnormal Eye Vascularization in Mice Deficient in Lrp5, the Gene Mutated in Human Osteoporosis Pseudoglioma Syndrome (OPS). R. Levasseur,*[1] M. Kato,[2] M. S. Patel,[1] L. Chan,*[2] G. Karsenty.[1] [1]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA, [2]Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA.

Bone formation by osteoblasts balanced by bone resorption by osteoclasts maintains normal bone mass. Little is known about extra to intracellular signal transduction pathway(s) favoring bone formation. Here we show that low density lipoprotein receptor-related protein-5 (Lrp5), a homolog of Drosophila Arrow and a putative Wnt coreceptor, regulates bone formation. Lrp5-deficient mice develop an osteoporosis resulting from decreased osteoblast proliferation and function. Skeletal preparation analyses and histomorphometric studies revealed that Lrp5-deficient mice have a delay of osteogenesis already at birth and a decrease in bone formation that worsen over time. In addition Lrp5-deficient mice have a persistence of embryonic vascularisation of the eye, a feature observed in osteoporosis-pseudoglioma patients (OPS). *LRP5* is the gene mutated in OPS patients. Statins completely correct the bone loss of mutant mice indicating that they could be used to treat human patients. Additionally, Lrp5-deficient mice are abnormally lean, confirming the existence of a coregulation of bone mass and body weight. We suggest that unidentified Wnt protein(s), acting postnatally, regulate(s) bone formation, body weight and eye vascularization.

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X-linked Recessive Hypoparathyroidism Is Caused by a Molecular Deletional-insertion Involving Chromosomes Xq27 and 2p25. M. R. Bowl,[1] M. A. Nesbit,*[1] B. Harding,*[1] E. Levy,*[2] D. Schlessinger,*[3] M. P. Whyte,[4] R. V. Thakker.[1] [1]Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom, [2]Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom, [3]National Institutes of Health and Ageing, Baltimore, MD, USA, [4]Center for Metabolic Research, Shriners Hospitals for Children, St Louis, MO, USA.

X-linked recessive hypoparathyroidism (XLHPT), which is due to isolated congenital absence of the parathyroids, has been reported in two families from Missouri, USA, and mitochondrial DNA studies have established that these kindreds are related. Affected individuals, who are males, suffer from potentially lethal epilepsy due to hypocalcaemia during infancy, whilst the females are healthy and normocalcaemic. Genetic mapping studies have located XLHPT to chromosome Xq27 and defined a 1.5 million base pair (Mbp) region flanked centromerically by Factor IX and telomerically by DXS984. DNA sequence analysis of 4 candidate genes (proto-dbl, AS6, U7snRNA and SOX3) did not reveal abnormalities, and the occurrence of deletions of this entire critical region in some haemophilia B patients who do not have XLHPT, indicated that other mechanisms may cause XLHPT. We therefore embarked on characterising this region further, in 6 affected males, by combined analysis of single nucleotide polymorphisms (SNPs) and sequence tagged sites (STSs). This approach identified a centromeric recombinant that reduced the interval containing XLHPT to 1.3 Mbp, and revealed a telomeric molecular deletion of <30 Kbp. However, this deletion did not contain any genes, and further characterisation using DNA fibre-fluorescence in situ hybridisation (FISH) and pulsed field gel electrophoresis revealed that the abnormality also contained an insertion that was >75kb. Analysis of 48 members (6 affected males, 18 carrier females, and 24 unaffected members) established co-segregation of this deletional-insertion with XLHPT, with a peak LOD score >+5 at 0% recombination. The combined use of a flow-sorted X chromosome specific library generated from an affected male, a modified Vectorette PCR protocol, and DNA sequence analysis enabled the centromeric and telomeric breakpoints of this deletional-insertion to be characterised. The additional use of a mono-chromosomal somatic cell hybrid DNA panel, and FISH using metaphase spreads of chromosomes revealed that the insertion originates from chromosome 2p25. Thus, XLHPT is caused by a molecular deletional-insertion involving chromosomes Xq27 and 2p25. These results, which identify a novel genetic abnormality causing hypoparathyroidism, will help to advance our understanding of the molecular basis of parathyroid development.

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A Large-Scale Whole-Genome Linkage Scan Identifies Several Genomic Regions with QTLs Underlying Bone Size Variation. H. Shen,*[1] F. H. Xu,*[1] H. T. Zhang,*[1] H. Y. Deng,*[2] T. Conway,[2] Q. H. Xia,*[1] J. Chen,*[1] Y. Z. Liu,*[1] Y. J. Liu,*[1] Q. Y. Huang,*[1] K. M. Davies,[1] R. R. Recker,[1] H. W. Deng.[1] [1]Osteoporosis Research Center and Biomedical Sciences, Creighton University, Omaha, NE, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Bone size is an important determinant of osteoporotic fractures. Using a whole-genome linkage scan, we have set out to identify genomic regions that may contain QTLs underlying variation in bone area (DXA). For a sample of pedigrees that includes 1,249 sib pairs, 1,098 grandparent-grandchildren pairs and 1,172 first cousin pairs, we performed a whole-genome scan using 400 microsatellite markers (with an average heterozygosity of ~0.75 and spaced ~10cM apart throughout the whole genome) from the ABI PRISMTM Linkage Mapping Set Version 2 (Applied Biosystems, Foster City, CA). For chromosomes 9-22 and chromosome X, the genotyping has been completed with a rate of missing and error data (determined by sample replication and pedigree consistency check) of ~0.3%. For chromosomes 1-8, the genotyping finished with one round of repeat experiments yielding a rate of ~3% for missing and error genotype data. Further rounds of repeat experiments are being performed to reduce the missing and error data rate from the current 3.0% to ~0.3% for chromosomes 1-8. Adjusting for age, sex, weight and other significant co-varieties, we conducted two- and multi-point linkage analyses to identify genomic regions that may contain QTLs underlying bone size variation. Several genomic regions were identified. For example, the genomic region near the marker D17S787 on chromosome 17 may contain a QTL for wrist (ultra distal) bone size variation (with a two-point analysis LOD score of 3.98 and a multi-point analysis LOD score of 3.00). The genomic region near the marker D11S4046 on chromosome 11 may contain a QTL for spine bone size variation (with two-point analysis LOD score of 2.78). The genomic region identified on chromosome 17 for wrist bone size seems to be consistent with that identified for the region identified for femur head width (Koller et al. 2000, JBMR 15 (supplement), 1094). Our results are compared with one earlier study and discussed.

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The Naturally Occurring Autosomal Dominant Hypophosphatemic Rickets (ADHR) Mutations Stabilize Full-Length FGF-23. K. E. White,[1] G. Carn,*[1] B. Lorenz-Depiereux,*[2] T. Meitinger,*[2] T. M. Strom,*[2] M. J. Econs.[1] [1]Indiana University School of Medicine, Indianapolis, IN, USA, [2]Institut fuer Humangenetik, Neuherberg, Germany.

We identified the gene for the phosphate wasting disorder ADHR as FGF23, a secreted protein related to the fibroblast growth factors (FGFs). In previous studies, missense mutations R176Q, R179W, and R179Q were detected in FGF23 from ADHR kindreds, however the relationships between the mutations and the etiology of ADHR are unknown. The amino acid substitutions replace critical R residues within the possible FGF-23 subtilisin-like proprotein convertase (SPC) cleavage site 176RHTR179 (RXXR motif), therefore our goal was to determine if the mutations lead to FGF-23 protease resistance. The mutations were introduced separately into the human FGF-23 cDNA and were transiently transfected into HEK293 cells. Western blots of conditioned media using an antibody directed downstream of the SPC site revealed that native FGF-23 protein resolved as two species, a full-length 32 kD band and a 12 kD band. In contrast, the mutants appeared only as the intact, full-length 32 kD species. To test for the presence of an upstream cleavage fragment, an N-terminal FLAG-FGF-23 and FLAG-R176Q were transfected into HEK293 cells and the media analyzed with an anti-FLAG antibody. The native FLAG-FGF-23 was again detected as two species, the full-length 36 kD (32 kD plus the FLAG tag) protein and a pronounced 26 kD cleavage fragment. FLAG-R176Q resolved essentially as the full-length species, with only a faint band at 26 kD, indicating that the mutants are resistant to proteolysis. To determine the cellular localization of cleavage, native FGF-23 conditioned media was applied to HEK293 cells. There was no difference in the relative levels of the 32 and 12 kD bands after 24 h, supporting the idea that FGF-23 can be processed before or during secretion by HEK cells. FGF-23 and mutant conditioned media were also incubated with heparin sepharose to compare their relative heparin binding abilities. The full-length native and mutant proteins bound heparin equally, indicating that gross loss of FGF-23 integrity due to the mutations does not occur. In summary, we demonstrated that R176Q-, R179W-, and R179Q-FGF-23 could be produced by mammalian cells at levels similar to wild type FGF-23. These mutations, however, create analogs far less sensitive to proteases than native FGF-23. Thus, the naturally occurring ADHR missense mutations protect FGF-23 from proteolysis, thereby potentially elevating circulating concentrations of FGF-23 in ADHR patients, which leads to phosphate wasting.

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Paget's Disease of Bone: Mapping of Two Loci at 5q35-tel and 5q31 and Genetic Heterogeneity. N. Laurin,*[1] J. P. Brown,[2] A. Lemainque,*[3] A. Duchesne,*[1] D. Huot,*[4] Y. Lacourcière,*[5] G. Drapeau,*[6] J. Verreault,*[7] V. Raymond,*[1] J. Morissette.*[1] [1]Centre de recherche en endocrinologie moléculaire et oncologique, CHUL Research Center, Ste-Foy, PQ, Canada, [2]Rheumatology - Immunology, CHUL Research Center, Ste-Foy, PQ, Canada, [3]Centre national de génotypage, (CNG), Evry, France, [4]Centre hospitalier de la région de l'Amiante, Thetford Mines, PQ, Canada, [5]CHUL Research Center, Ste-Foy, PQ, Canada, [6]Hôtel-Dieu d'Arthabaska, Victoriaville, PQ, Canada, [7]Centre universitaire de santé de l'Estrie, Sherbrooke, PQ, Canada.

Paget's disease of bone is characterized by focal increases of the bone remodeling process. It is the second most common metabolic bone disease after osteoporosis. Genetic factors play a major role in the aetiology of Paget's disease of bone and two loci have been mapped for the disorder: PDB1 at 6p and PDB2 at 18q21-q22. The gene(s) causing the typical form of the disorder still remain to be characterized. To decipher the molecular basis of Paget's disease of bone, we performed genetic linkage analysis in 24 large French-Canadian families (479 individuals) in which the disorder was segregating as an autosomal dominant trait. After excluding PDB2, a genome-wide scan with 379 microsatellite markers was performed on the three most informative family nuclei. Regions with LOD score values between 1.0 and 3.0 were assessed by higher-density mapping and haplotype analysis in the extended families. Genetic heterogeneity was investigated using the HOMOG program. We report strong evidence for linkage at chromosome 5q35-tel in eight of the kindreds. Significant evidence for heterogeneity was observed at D5S408 (c2=3.86; p<0.05). Under heterogeneity, the maximum LOD score value of 8.71 was obtained at D5S2073. The same characteristic haplotype was shown by all pagetic patients in these eight families, suggesting a founder effect. Recombination event in a key family confined the disease region to a 6 cM interval between D5S469 and the telomere. The 16 other fam-

ilies, with very low conditional probability of linkage at 5q35-tel, were further used to map a second locus at 5q31. Under heterogeneity, a maximum LOD score value of 3.70 was detected at D5S500 with q=0.00 (c2=10.13; p<0.0002). Key recombination events in the two families refined the 5q31 disease region to a 11.2 cM interval between D5S642 and D5S1972. Finally, two other kindred were excluded from both the 5q35-tel and 5q31 loci. These observations demonstrate the mapping of two novel loci for Paget's disease of bone and provide further evidence for the genetic heterogeneity of this disorder.

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Assessing the Genetic Determinants of Vertebral Trabecular Bone Density and Microarchitecture in Mice. M. L. Bouxsein,[1] R. Mueller,[2] T. Uchiyama,*[2] J. Mytar,*[3] C. H. Turner,[4] L. R. Donahue,[3] C. J. Rosen,[3] W. G. Beamer.[3] [1]Orthopedic Biomechanics, Beth Israel Deaconess Med Center, Boston, MA, USA, [2]Beth Israel Deaconess Med Center, Boston, MA, USA, [3]Jackson Laboratory, Bar Harbor, ME, USA, [4]Indiana University Medical School, Indianapolis, IN, USA.

Genetic factors have been shown to play an important role in the regulation of bone mineral density (BMD). Yet, in addition to BMD, other traits that affect bone strength, such as trabecular architecture, may be genetically regulated. To identify heritable determinants of vertebral trabecular architecture, we evaluated adult female mice from the F2 intercross of C57BL/6J (B6) and C3H/HeJ (C3H) progenitor strains. Micro-computed tomography (μCT, 17 μm resolution) was used to assess trabecular bone volume fraction (BV/TV), thickness (Tb.Th), separation (Tb.Sp), and number (Tb.N) in the 5th lumbar vertebral body of the progenitors (n = 8/strain) and B6C3H-F2 progeny (n=914). B6 mice had greater vertebral trabecular BV/TV (+114%), Tb.N (+68%), and lower Tb.Sp (-42%) compared to C3H (p<0.001 for all). F2 progeny were genotyped for all autosomes using PCR and regression analysis used to find quantitative trait loci (QTL) contributing to the vertebral trabecular bone traits. Whole genome scans revealed that 6 to 10 QTL's associated with each trait. LOD scores ranged from 3.2 to 14.4. QTL's on Chr 4 and 8 were common for all the vertebral trabecular traits. BV/TV, Tb.N, and Tb.Sp also shared common QTL's on Chr 1, 9, 12, and 13. For BV/TV, the LOD scores, variance explained in the F2 population by the major QTL's, and allele effects are shown in the Table. Note that C3H alleles, which have previously been associated with high cortical bone mass, had both positive (Chr 1, 4) and negative (Chr 6, 8, 9, 12, and 13) effects on trabecular BV/TV. Several of the QTL's identified here were previously identified as major regulators of vertebral BMD (Chr 1, 4, 6, 9, and 13). However, two of them (Chr 8 and 12) appear to be unique to vertebral trabecular architecture, and two (Chr 8 and 9) appear to influence vertebral architecture independently of femoral BMD. In summary, we identified several genetic determinants of vertebral trabecular bone density and architecture in mice, and showed that C3H alleles contribute both positively and negatively to trabecular bone density. LOD score,% variance, and allele effects (mean ± SD) for the major QTL's for BV/TV (%) .

	Chr 1	Chr 4	Chr 8	Chr 9	Chr 12
LOD	5.2	10.7	10	9.8	12.5
Variance (%)	3.5	5.5	4.5	6.3	6
B6/B6	21.0 ± 0.4	20.4 ± 0.4	23.7 ± 0.4	24.1 ± 0.5	24.6 ± 0.4
B6/C3H	22.2 ± 0.3[b]	22.5 ± 0.3[b]	22.4 ± 0.3 [b]	22.2 ± 0.4 [b]	22.2 ± 0.3 [b]
C3H/C3H	24.1 ± 0.4 [a, c]	24.8 ± 0.5 [a,c]	21.3 ± 0.4 [a,c]	20.3 ± 0.6 [a,c]	20.7 ± 0.4 [a,c]

a: C3H/C3H vs B6/B6; b: B6/C3H vs B6/B6; c: C3H/C3H vs B6/C3H (p<0.05)

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Secreted Frizzled-related Protein (sFRP-1) Binds to RANKL to Inhibit Osteoclast Formation. K. D. Hausler,*[1] N. J. Horwood,[1] A. Uren,*[2] J. Ellis,*[2] C. Lengel,*[2] T. J. Martin,[1] J. S. Rubin,*[2] M. T. Gillespie.[1] [1]St. Vincent's Institute of Medical Research, Melbourne, Australia, [2]National Cancer Institute, NIH, Bethesda, MD, USA.

sFRP-1 is a secreted member of the Frizzled (Fz) family of proteins that binds to wnt proteins to modulate their activity. Wnt signaling is crucial in the processes of organogenesis and limb development, and the development of colorectal cancer.After isolating cDNA for sFRP-1 during mRNA analysis of osteoblastic stromal cells, we noted that sFRP-1 was differentially expressed in osteoblastic cell lines and was elevated in those capable of supporting osteoclast formation. Furthermore, in situ hybridization studies showed that sFRP-1 mRNA was expressed in osteoblasts and chondrocytes in murine bone. We therefore investigated the possibility that sFRP-1 might influence osteoclast formation.Neutralizing antibodies against sFRP-1 enhanced TRAP positive mononuclear and multinuclear osteoclast formation (3 and 2-fold, respectively) in cocultures of murine osteoblasts with spleen cells treated with PGE2 (10-7M) and 1,25 (OH)2 vitamin D3 (10-8M). This effect was more pronounced (10 and 7-fold) under conditions of submaximal osteoclast formation. Recombinant sFRP-1 was able to dose dependently inhibit osteoclast formation in either osteoblast / spleen cocultures, RANKL+M-CSF-treated splenic cultures or in RANKL-treated RAW264.7 cell cultures, indicating a direct action of sFRP-1 upon hematopoietic cells. Although RAW264.7 cells expressed sFRP-1, its production was lower than that of T cells and this, along with other data, suggests that sFRP-1 derived from T cells or tonically expressed by stromal osteoblasts exerts inhibitory control upon osteoclast formation. Consistent with the likely lack of a dynamic controlling function from the osteoblastic source is the fact that sFRP-1 mRNA expression was only marginally enhanced by dexamethasone or IL-11 (2-fold by 2 and 8hr, respectively), whilst many other osteotropic agents (eg., IL-1, IL-6, calcitrol, PTH) were without any effect.sFRP-1 is thought to act as a decoy receptor binding to wnts and thus regulating the interaction of wnts with their transmembrane Fz signaling receptors. However, another mechanism appears to account for the ability of sFRP-1 to inhibit osteoclast formation. Screening of a peptide phage display library revealed a sFRP-1 binding motif that corresponds to a sequence present in RANKL. Subsequent experiments demonstrated that recombinant RANKL bound specifically to sFRP-1 in an ELISA format. This finding raises the possibility that sFRP-1 limits osteoclast formation by binding directly to RANKL, and suggests that sFRP-1 may act as a decoy receptor mimicking OPG activity.

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Deletion of the Genes Encoding c-Cbl or Cbl-b Alter the Ability of Osteoclasts to Migrate During Development, Delaying Resorption and Ossification of Cartilage in Long Bones. R. Chiusaroli,[1] J. Juel,*[1] L. Neff,[1] A. Sanjay,[1] M. Naramura,[2] H. Gu,[2] W. C. Horne,[1] R. Baron.[1] [1]Yale University, New Haven, CT, USA, [2]NIH, Bethesda, MD, USA.

Osteoclast (OC) recruitment and migration through the perichondrium and the bone collar is required for the vascular invasion of the cartilaginous anlage and the formation of the primary and secondary centers of ossification. We have previously shown that c-Cbl lies downstream of the vitronectin receptor and forms a complex with c-Src and Pyk2 in a signalling pathway that regulates cell adhesion and motility. Furthermore, OCs that lack either c-Src or c-Cbl display decreased migration in vitro. Since migration is essential in order for OC precursors to reach the bone collar and for newly formed OCs to penetrate the hypertrophic cartilage during long bone development, we analyzed these events in c-Cbl and Cbl-b knockout mice to determine whether the decreased motility we observed in vitro translated into decreased cell migration in vivo. Analysis of metatarsals at E18 showed that the initiation of vascularization and replacement of cartilage by bone are delayed in c-Cbl -/- mice. This delay is apparently due to the decreased ability of osteoclasts to invade the hypertrophic cartilage through the bone collar. Metatarsals from c-Cbl +/- and c-Cbl -/- E18 mouse embryos were analyzed to determine the numbers of large TRAP+ cells localized both outside the bone collar and inside, in contact with and resorbing the hypertrophic cartilage. In c-Cbl +/- metatarsals, the numbers of TRAP+ cells/total area (mm^2) were 11.4 ± 0.7 and 22.4 ± 10.1 outside and inside the bone collar, respectively, whereas in c-Cbl -/- metatarsals the numbers of TRAP+ cells/total area were 24.5 ± 7.7 outside and only 6.2 ± 5.8 inside the bone collar (p<0.05 vs. c-Cbl +/- for both outer and inner TRAP+ cells). The total number of large TRAP+ cells was not changed (33.8 ± 10.5 vs. 30.7 ± 5.1, p = n.s.). Furthermore, at day P10 both c-Cbl -/- and Cbl-b -/- mice display a delay in the formation of the secondary center of ossification in the tibia proximal epiphysis, which is also dependent on osteoclast invasion of the mineralized cartilage. Detailed histomorphometric analysis of adult c-Cbl -/- mice failed to demonstrate any significant changes in bone volume or in bone resorption parameters, except that the hypertrophic zone of the growth plate was thicker in c-Cbl -/- 18 day old mice (119.8 ± 17.7 vs. 90.8 ± 14.8 μm, p<0.05), also suggesting a slowing down of osteoclast resorption of the calcified cartilage. Thus, the observed decrease in the in vitro motility of the c-Cbl-/- osteoclasts may result in a decreased ability of osteoclasts to invade and resorb the mineralized cartilage in the bone primordium and the growth plate.

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***c-myc* Is Required for Osteoclastogenesis.** R. Battaglino,*[1] D. Kim,*[2] P. Stashenko.*[1] [1]Cytokine Biology, The Forsyth Institute, Boston, MA, USA, [2]Harvard School of Dental Medicine, Boston, MA, USA.

RANKL (receptor activator of NF-kB ligand), a TNFα-related cytokine, is essential for osteoclast formation. RANKL is secreted by osteoblasts in response to specific signals and binds to its receptor, RANK, expressed in osteoclast precursors. Even though the role of RANKL in osteoclastogenesis has been established, the activated downstream signaling pathways remain largely unknown. In order to identify genes that play a role in osteoclastogenesis we developed and characterized a model that reproduces *in vitro* the differentiation of multinucleated osteoclasts from mononucleated precursors. RAW 264.7 mouse monocytes were induced to differentiate into osteoclast-like cells (OCL), after culturing them for four days in medium supplemented with RANKL. Morphologically, OCL were TRAP positive multinucleated giant cells. Northern Blot analysis showed that OCL differentially expressed a number of markers for osteoclast differentiation, among them: *trap* , *gelatinase B* , *cathepsin K* , *atp6i* , the 116KDa subunit of an osteoclastic proton pump and the protooncogene *c-src*. OCL were also able to form resorption pits on sub-micron calcium phosphate films. Conversely, only undifferentiated RAW 264.7 cells expressed *CD-14* , a macrophage surface marker. Using gene arrays we found that the protooncogene *c-myc* was strongly up regulated in RANKL-induced OCL, but was absent in undifferentiated cells. Northern blot analysis showed *c-myc* mRNA expression as early as one hour after RANKL stimulation. *c-myc* expression declined after four days of stimulation. NF-kB activation, a step required for osteoclast differentiation, follows RANKL stimulation. TPCK, an inhibitor of RANKL-induced NF-kB activation, was able to block *c-myc* expression, suggesting that RANKL-induced *c-myc* upregulation required NF-kB activation. Expression of c-Myc partners, Max and Mad, was constant during OCL differentiation, suggesting that the c-Myc-Max-Mad system is primarily regulated at the level of c-myc transcription. The expression of *c-myc* in response to RANKL stimulation, suggested a role for *c-myc* in OCL formation. To test that hypothesis, we generated RAW 264.7 cell lines stably transfected with an expression vector carrying a dominant negative version of c-Myc. Our results showed that a cell line that expressed high levels of the dominant negative message was unable to fully differentiate into OCL upon RANKL stimulation. Furthermore, Northern Blot analysis revealed a delay and a significant reduction in the level of mRNA for TRAP and Cathepsin K. We conclude that RANKL-induced *c-myc* expression requires NF-kB activation and is required for osteoclastogenesis.

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Upregulation of α2β1 Integrin in β3 Null Type Glanzmann Thrombasthenia Enables Bone Resorption. M. A. Horton,[1] U. Seligsohn,*[2] H. M. Massey,*[3] A. M. Flanagan.[3] [1]Medicine, University College London, London, United Kingdom, [2]Chaim Sheba Medical Center, Tel-Hashomer, Israel, [3]Histopathology, University College London, London, United Kingdom.

A large body of evidence supports a central role for αvβ3 in the development, activation and function of osteoclasts (OCs); inhibition of αvβ3 results in reduced bone resorption in vitro and in various animal models of osteoporosis. The development of integrin antagonist drugs for bone disease has stressed the need to elucidate the impact of chronic inhibition/lack of αvβ3 integrin (vitronectin receptor) upon OC function. Most patients with Glanzmann Thrombasthenia (GT) of Iraqi-Jewish origin have complete absence of the integrin β3 chain due to an 11bp deletion in exon 12 of the β3 (CD61/GPIIIa) gene. This results in defective platelet function and a haemorrhagic diathesis, the clinical hallmark of GT, due to lack of the platelet fibrinogen receptor, αIIbβ3 integrin. We examined the impact of the absence of β3, and hence of αvβ3, in OCs generated by in vitro culture of peripheral blood monocytes (PBM) from patients with GT. These were cultured on bone slices in the presence of 30ng/ml RANKL and 25ng/ml M-CSF for up to 14d and examined by confocal microscopy for expression of OC integrins (αv, αvβ3, α2, β1) and macrophage proteins and other integrins (β5, α3, α5; CD11b, 14, 44, 68). Bone resorption was assessed by reflection and scanning electron (SEM) microscopy. 5 patients with β3 null GT were compared with 2 cases of αIIb deficient GT and 3 normal controls. Phenotypically and functionally normal OCs were generated from normal and αIIb deficient GT; both expressed similar levels of αvβ3. In contrast, OCs derived from β3 null GT PBMs failed to express αvβ3 (<1% control levels); the normally low level of αvβ5 in OCs was not increased; quantification of α2 and β1 levels showed significant 2.4 - 4.3-fold increases ($p<0.01$) in expression. β3 null osteoclasts resorbed less bone (44% and 59% decreases in pit number and depth, respectively, $p<0.001$). Examination of resorption lacunae by SEM showed shallower pits with less defined edges when compared with normal controls; the balance between removal of collagen and mineral, though, appeared grossly similar. The OC phenotype caused by the β3 null mutation in humans is thus similar to that of the β3 knockout mouse. We conclude that chronic deficiency of αvβ3 in human OCs is accompanied by a compensatory rise in α2β1 expression. This is sufficient to enable bone resorption to proceed, albeit to a sub-maximal extent, explaining why affected individuals do not present with osteopetrosis.

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Granzyme A Stimulates Osteoclast Formation by Inducing TNF-α Production. C. Menaa,[1] J. Lieberman,[2] S. M. Sprague.[3] [1]Evanston Hospital, Evanston, IL, USA, [2]Harvard Medical School, Boston, MA, USA, [3]Northwestern University Medical School, Evanston, IL, USA.

T cells play a major role in rheumatoid arthritis (RA) and postmenopausal osteoporosis (PO). T cell-secreted products have been shown to affect bone remodeling through their ability to regulate osteoclast (OC) activity. Granzyme A (GrA), a serine proteinase which induces apoptosis, is secreted from activated T cytotoxic and natural killer cells. Evidence suggesting that GrA plays a role in the development of RA includes the following: 1) GrA expression and concentration are elevated in serum and synovial fluid of patients with RA; 2) GrA can activate thrombin receptor, a pathway reported to regulate OC formation and/or activation; and 3) GrA induces matrix dissolution and inflammatory cytokine production (i.e. IL-6, TNF-α and IL-8). TNF-α and IL-6 are responsible, in part, for bone loss in PO and RA. The role of GrA in bone remodeling has not been elucidated. Therefore, the effect of GrA on OC formation was determined. Treatment of mouse bone marrow cells with recombinant GrA induced multinucleated cells which satisfied the major criteria of OC, including, TRAP activity, expression of β-integrin and calcitonin receptor, and the ability to form resorption pits on dentine slices. This effect was dose dependent (0.01 to 7 μg/ml) and independent of RANK/RANKL signaling pathway, as osteoprotegerin, at a dose sufficient to block the effect of 10 ng/ml of RANKL, was unable to abrogate OC formation. To determine whether this effect required accessory cells, highly purified OC precursors (CFU-GM or RAW 264.7) were incubated with GrA. GrA stimulated OC formation without osteoblast/stromal cells demonstrating a direct effect. The formation of OC appeared to be mediated by TNF-α, as the stimulatory effect of GrA on OC formation was inhibited by neutralizing antibody to TNF-α. Furthermore, GrA induced TNF-α expression by osteoclast precursors suggesting an autocrine/paracrine effect. To further examine the role of GrA activity, including whether the effect is thrombin receptor mediated, Raw cells were stimulated with human thrombin and with inactive GrA (mutation of the active site serine to alanine). Thrombin (10 U/ml) was unable to induce OC formation and the osteoclastogenesis effect of GrA was not related to the enzyme activity. In conclusion, these data demonstrate that GrA is a potent osteoclastogenesis factor. Its effect is not related to the enzyme activity, is RANK/RANKL independent and requires the induction of TNF-α production by osteoclast precursors, which induces these precursors to differentiate into mature osteoclasts via a paracrine/autocrine fashion. These data suggest that GrA plays a crucial role in the development of bone destruction in RA.

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The HIV Protease Inhibitor Ritonavir Inhibits Osteoclast Differentiation and Function, in Vitro and in Vivo, Via the NF-κB and AKT Pathways. M. W. H. Wang, R. Faccio,* S. L. Teitelbaum, F. P. Ross. Pathology, Washington University, St. Louis, MO, USA.

We find that HIV infected patients treated with protease inhibitors (PIs) have diminished bone mineral density t ($p=0.02$) and Z ($p=0.04$) scores relative to similarly-infected individuals not receiving these drugs. Because accelerated resorptive activity may contribute to systemic bone loss, we studied the effects of two commonly used PIs, Ritonavir and Indinavir, on osteoclast (OC) differentiation and function, in vitro and in vivo. We find that neither PI enhances OC number in cultures consisting of OC precursors, in the form of bone marrow macrophages, cultured with RANKL and M-CSF. Surprisingly, however, Ritonavir, but not Indinavir which blocks bone formation (this meeting), virtually eliminates osteoclastogenesis in these cultures. The 50% inhibitory dose of Ritonavir occurs at a pharmacologically relevant concentration of 10μg/mL. The effect of the drug is not due to toxicity, as its removal from culture completely normalizes osteoclast formation. To determine if Ritonavir impacts OC function, as well as differentiation, the drug was added to cultures of mature OCs maintained on dentin slices. In this circumstance, OC actin ring formation is disrupted and dentin resorption arrested, even though OC number is unchanged. Confirming that Ritonavir is bone sparing, the drug, systemically administered, completely blunts PTH-induced osteoclastogenesis in mice. We next asked if Ritonavir blunts signaling induced by the key osteoclastogenic cytokine RANKL, which stimulates the MAPK, NF-κB, and Akt pathways. Ritonavir has no effect on RANKL-induced activation of the p38, SAPK/JNK, or ERK1/2 components of the MAPK pathway. While serine phosphorylation of IκBα remains intact, the protein is not degraded in the presence of the drug, suggesting the inhibitory effect is at the level of a ubiquitin ligase or the proteosome. Consistent with a lack of cytoplasmic IκBα degradation in cells treated with Ritonavir, the drug decreases nuclear translocation of NFκB, as measured by EMSA. Ritonavir also suppresses the third osteoclastogenic signaling pathway, Akt activation, as measured by its phosphorylation at threonine 308, with a resultant failure to phosphorylate an Akt substrate, Forkhead. This event is RANKL specific, as Ritonavir does not block M-CSF induced Akt activation. Thus, Ritonavir arrests osteoclastogenesis by blocking RANKL induction of the NF-κB and Akt pathways. Because it is specifically bone sparing, Ritonavir presents itself as a promising choice to prevent PI-induced osteopenia in HIV infected patients.

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Relative Contributions of Bone Density, Bone Turnover and Clinical Risk Factors to Long-Term Fracture Prediction. L. J. Melton III,[1] C. S. Crowson,*[1] W. M. O'Fallon,*[1] M. K. O'Connor,*[2] B. L. Riggs.[3] [1]Health Sciences Research, Mayo Clinic, Rochester, MN, USA, [2]Radiology, Mayo Clinic, Rochester, MN, USA, [3]Endocrinology, Mayo Clinic, Rochester, MN, USA.

The long-term predictability of osteoporotic fractures using bone mineral density (BMD) remains controversial, as does the additional contribution from assessing bone turnover or clinical risk factors. Although theoretical analyses suggest that fracture predictability degrades rapidly with time, no population-based prospective data on hip or spine BMD extend beyond a decade. We assessed bone density at various sites (including proximal femur and lumbar spine) on an age-stratified sample of 304 Rochester, MN women in 1980. We also made baseline measurements with biochemical markers of bone turnover and assessed over 150 clinical variables exclusive of laboratory data. Multiple fractures per subject were accounted for using the Anderson-Gill model, an adaptation of the Cox proportional hazards model. The 225 women who were postmenopausal at baseline were followed subsequently for 2982 person-years (median, 16 years per subject; range 54 days to 19 years) during which time they experienced 289 new fractures; 56% involved the proximal femur, thoracic or lumbar vertebrae or distal forearm, and 80% resulted from only minimal to moderate trauma. Moderate trauma fractures in aggregate were best predicted by baseline femoral neck BMD (age-adjusted relative hazard [RH] per 1 SD decrease, 1.41; 95% CI, 1.17-1.69), while forearm fractures due to moderate trauma (n = 18) were best predicted by distal radius BMD, hip fractures (n = 27) and vertebral fractures (n = 95) by femoral neck BMD and other fractures (n = 92) by midradius BMD. Compared to the first decade of follow-up (RH, 1.29; 95% CI, 0.99-1.67), femoral neck BMD performed better in predicting overall fracture risk more than 10 years after baseline (RH, 1.58; 95% CI, 1.21-2.05). The biochemical markers available at the time (serum osteocalcin and alkaline phosphatase, urinary hydroxyproline) were not independently predictive of long-term fracture risk, but modern assays were not evaluated. Other than bone density, consistent risk factors for the different fractures were not identified but statistical power was limited. A weighted clinical risk factor score (median, 6; range, 0 to 38.5) predicted fractures in general (RH per unit increase, 1.02; 95% CI, 1.003-1.04) and vertebral fractures specifically in multivariate modeling. The clinical approach to fracture prevention requires knowledge of the patients at greatest risk, but identifying them many years in advance is a challenge. It is reassuring that femoral neck BMD can predict the overall risk of moderate trauma fractures even a decade later.

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Natural History of Kyphosis: A Prospective Study of Change in Thoracic Curvature in Men and Women in The Framingham Study. E. J. Samelson,[1] H. N. Rosen,[1] I. D. Iloputaife,*[1] M. T. Hannan,[1] D. T. Felson,[2] D. P. Kiel.[1] [1]Research & Training Inst, Hebrew Rehab Ctr, Boston, MA, USA, [2]Bos Univ Sch Med, Boston, MA, USA.

Kyphosis has been related to aging, vertebral fracture (VF), and height loss, as well as to adverse outcomes, including reduced lung and physical function, pain, and deformity. Little is known about natural history of kyphosis in women and men. The purpose of this study was to describe natural history of kyphosis in a population-based group of older women and men followed for 17 years.Thoracic spine x-rays, obtained in 1975 and repeated in 1992, were available for 275 cohort members of the Framingham Study (193 women, 82 men; mean baseline age, 63 years; range, 50-79). Thoracic vertebrae were classified as normal or fractured using a semiquantitative method. An adjustable triangle was used to measure kyphosis angle (KA) determined by lines drawn from the superior border of T4 and inferior border of T12. Change in kyphosis was calculated by subtracting baseline KA from follow-up KA, such that increase in KA corresponds to increase in kyphosis, and decrease in KA corresponds to decrease in kyphosis. Mean KA at baseline was 39.6º in women and 34.0º in men. Curvature increased an average of 10.9º in women and 6.7º in men. Improvement of >5º in KA occurred in a small number of women (2%) and men (6%). Baseline age did not influence change in KA. Mean change in KA was similar for women with incident VF (12.5º), prevalent VF (10.8º), and without VF (10.6º). Similarly, mean change in KA did not differ between men free of VF (5.6º) and with prevalent VF (7.8º), but KA change was twice as high in men with incident VF (12.0º) than without VF, although the difference did not reach significance ($p=.08$). About ½ the women and ¼ the

men, regardless of VF status, increased KA >10º. When women and men were combined, prevalent VF did not increase risk of increasing KA >20º (OR=1.7; 95% CI=0.6-5.2), although incident VF was associated with >20º increase in KA (OR=3.6; 95% CI=1.4-9.5). The combined influence of both prevalent and incident VF accounted for < 3% of the variance in KA change. Baseline KA did not affect KA change in women (trend, p=.97), but men with low baseline KA had greater increase in KA change (10º vs. 4º for lowest vs. highest quartile of baseline KA; trend, p=.009).This study provides a unique description of change in thoracic curvature for a population-based cohort of elderly women and men followed for nearly two decades. Results indicate increase of >10º in curvature occurs in ½ of older women and ¼ of older men whether or not they have prevalent or incident VF. Moreover, kyphosis is largely determined by factors other than VF. These findings need to be extended to determine clinical implications related to longitudinal change in kyphosis.

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Excess Bone Loss Attributable to Hip Fracture. J. Magaziner,[1] W. Hawkes,*[1] K. Stone,*[2] M. Hochberg,*[1] L. E. Wehren,[1] J. R. Hebel,*[1] L. Fredman,*[3] D. Orwig,*[1] S. I. Zimmerman.*[4] [1]University of Maryland Baltimore, Baltimore, MD, USA, [2]University of California, San Francisco, CA, USA, [3]Boston University, Boston, MA, USA, [4]University of North Carolina, Chapel Hill, NC, USA.

Although hip fracture is associated with substantial bone loss in the next year, most women who sustain hip fractures do not receive interventions to improve bone mineral density (BMD). The extent of bone loss that is attributable to the hip fracture itself, beyond losses due to normal aging in the frail elderly, is unknown. To evaluate this, 84 white female hip fracture patients participating in the Baltimore Hip Studies who had BMD measurements of the contralateral hip at time of fracture and 12 months later were matched with 168 control subjects from the Study of Osteoporotic Fractures (SOF). Matching factors included age and total hip BMD. Mean age of the combined sample was 79.0 years; mean BMD was 0.602 gm/cm2. Expected one-year bone loss at the total hip and femoral neck was estimated in the SOF subjects and compared to observed losses in the fracture subjects, controlling for baseline weight and smoking. BMD loss in SOF subjects was 0.6% at the total hip and 0.4% at the femoral neck. In contrast, the observed BMD loss in hip fracture subjects was 3.5% at the total hip and 4.7% at the femoral neck, with adjusted excess loss of 2.9% (95% CI 2.2%, 3.5%) at the total hip and 4.3% (95% CI 3.1%, 5.5%) at the femoral neck. BMD loss in the year after hip fracture was, therefore, 5.4 times the expected amount at the total hip and 12.5 times that expected at the femoral neck. Viewed another way, the 1-year impact of hip fracture on BMD is equivalent to aging 5.4 to 12.5 years. This finding may partially explain the high rate of subsequent hip fracture seen in those who have hip fractures and strongly suggests that interventions should be targeted to increasing BMD.

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Weight Loss in Elderly Women Increases the Risk of Hip Fracture Irrespective of Current Weight and Weight Loss Intention. K. E. Ensrud,[1] S. K. Ewing,*[2] K. L. Stone,[2] P. J. Bowman,*[1] T. M. Knudson,*[1] J. A. Cauley,[3] S. R. Cummings.[2] [1]VA Med Center & Univ of MN, Minneapolis, MN, USA, [2]Univ of CA, San Francisco, CA, USA, [3]Univ of Pittsburgh, Pittsburgh, PA, USA.

Weight loss is a strong risk factor for hip fracture (fx) in older women, but the effects of current weight and weight loss intention on this relationship are uncertain. To test the hypothesis that women with recent weight loss are at increased risk of hip fx irrespective of current weight or weight loss intention, we measured weight at baseline and 4th exams (average 5.7 years between exams) in a cohort of 6785 women age ≥65 years at baseline participating in the Study of Osteoporotic Fractures. During this time, 16% of participants gained ≥5% of their baseline weight (weight gain), 55% had <5% change from their baseline weight (stable weight), and 29% lost ≥5% of their baseline weight (weight loss). After an average of 5.6 years of 98% complete follow-up, 259 (4%) women suffered a first hip fx. Proportional hazards models were used to analyze the association between weight change and hip fx; all analyses were adjusted for age, health status, smoking status, medical conditions, prior fx, estrogen use, physical activity, falling, height, and hip BMD. Recent weight loss was a strong risk factor for hip fx. Among both heavier and thinner women, women with weight loss compared with women with stable weight had increased risks of subsequent hip fx (p>0.24 for interaction between weight loss and current weight). Similarly, weight loss increased the risk of hip fx irrespective of weight loss intention (p>0.93 for interaction). Among heavier women trying to lose weight, women with weight loss compared with those with stable weight had a 3-fold increased risk of hip fx (RH 3.17, 95% CI 1.39-7.20).

	Relative Hazards (RH) of Hip Fx (95% Cl)		
	Weight Loss	**Stable Weight**	**Weight Gain**
Overall Cohort	1.8 (1.4-2.4)	1.0 (Referent)	0.9 (0.6-1.5)
Current Weight 65+ kg*	2.6 (1.6-4.3)	1.0 (Referent)	0.6 (0.3-1.3
Current Weight <65 kg*	1.5 (1.1-2.2)	1.0 (Referent)	1.6 (0.9-2.8)
Trying to Lose Weight	2.1 (1.1-4.1)	1.0 (Referent)	0.6 (0.3-1.6)
Not Trying to Lose Weight	1.7 (1.3-2.4)	1.0 (Referent)	1.1 (0.7-2.0)

*Median weight=65 kg

Weight loss in later years is associated with increased hip fx risk irrespective of current weight or weight loss intention. We conclude that even voluntary weight loss in overweight women increases the risk of hip fracture.

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A Large Proportion of Fractures in Postmenopausal Women Occur with Baseline Bone Mineral Density T-score >-2.5. S. A. Wainwright,*[1] K. R. Phipps,[1] J. V. Stone,*[1] J. A. Cauley,[2] M. T. Vogt,[2] D. M. Black,[3] E. S. Orwoll.[1] [1]Oregon Health Sciences University, Portland, OR, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA, [3]University of California at San Francisco, San Francisco, CA, USA.

While low bone mineral density (BMD) measurements are related to increased fracture risk, not all elderly women who fracture have low BMD. To determine the proportion of elderly women who fracture despite having baseline BMD measurements higher than generally considered to place these women at risk, we analyzed data from the Study of Osteoporotic Fractures (SOF). Measurements of hip and lumbar spine BMD were obtained using DXA. Incident non-traumatic hip and non-vertebral fractures occurred in 469 and 2055 subjects, respectively, during the 10 years after BMD measurement.
In the 5 years following baseline BMD measurement, the probability of incident hip fracture was 8.5% for those with total hip BMD (TBMD) T-score ≤-2.5 and 2.1% for those with T-score >-2.5. However, the majority of those with incident hip fracture (54%) or any incident non-vertebral fracture (74%) had baseline TBMD T-score >-2.5 (Table). Additionally, this phenomenon was noted even when a combination of BMD sites was assessed. Forty two percent of those with hip fracture and 53% of those with any non-vertebral fracture had both total hip and lumbar spine BMD T-scores >-2.5. Many fractures occurred at an even higher baseline BMD. Thirty two percent of those with hip fracture and 54% of those with any non-vertebral fracture had baseline TBMD T-score >-2.0. Results for those with hip and any non-vertebral fracture were similar if observation was continued for 10 years after DXA measurement.

Table: Proportion of Fractured Subjects With Baseline BMD T-score >-2.5

BMD Site	**Hip Fracture**	**Any Non-vertebral Fracture**
Total Hip	54%	74%
Lumbar Spine	54%	60%
Total Hip and Lumbar Spine	42%	53%

In summary, a large proportion of elderly women who suffer hip or any non-vertebral fracture have BMD measures higher than conventional osteoporosis diagnostic thresholds. As a result, a significant number of these fractures are not adequately predicted by BMD measures. New methods to identify women with higher BMD at risk for fracture are essential to direct effective preventive strategies

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Bone Loss Predicts Cognitive Decline in Older Women. L. L. Lui,[1] K. L. Stone,[1] J. A. Cauley,[2] T. A. Hillier,[3] K. Yaffe.*[1] [1]University of California, San Francisco, San Francisco, CA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA, [3]Kaiser Center for Health Research, Portland, OR, USA.

Previous studies have suggested that low bone mineral density (BMD) is associated with poor cognitive function and a greater risk of cognitive deterioration in elderly women and men. However, it is not known whether bone loss predicts subsequent cognitive decline independently of current bone mass. Prior studies have been limited by using a single BMD measurement or assessing bone loss and cognitive decline in the same period of time. No studies have examined whether rate of recent change in BMD is associated with subsequent cognitive decline among elderly women.We studied 4462 women age 70 and older (mean age = 75.8 years old), who were part of the Study of Osteoporotic Fractures (SOF). Total hip BMD was measured at 2 and 6 years after enrollment (mean follow-up 3.5 years) and we expressed rate of annualized % change in BMD in quartiles. We administered the modified Mini-Mental Status Exam (mMMSE) at 6 and 10 years after enrollment (mean follow-up 4.5 years). Cognitive decline was defined as a >= 3-point decline on repeat mMMSE score. Women with more rapid hip BMD loss were more likely to experience subsequent cognitive decline. Cognitive decline occurred in 12%, 14%, 16% and 20% of women in the lowest, second, third and highest hip BMD change quartiles, respectively (p for trend <0.001). The trend remained significant after adjustment for age, education, history of stroke, functional status, body mass index and smoking. Compared to women in the lowest quartile, those in the highest quartile of BMD change were almost 50% more likely to develop cognitive decline (table).

Annualized % change in hip BMD	**Adjusted OR (95% CI) for cognitive decline**
Lowest Quartile (mean increase 1.11%)	1.0 (reference)
2nd Quartile (mean decrease 0.04%)	1.20 (0.93 – 1.55)
3rd Quartile (mean decrease 0.78%)	1.36 (1.06 – 1.75)
Highest Quartile (mean decrease 2.12%)	1.46 (1.14 – 1.86)

This relationship was similar after further adjustment for estrogen use and final BMD (measured at the same visit as the initial cognitive score). Results remained the same if we substituted absolute change instead of % change in BMD.Women with more rapid hip bone loss were more likely to experience cognitive decline than those with less hip BMD loss or gain. This association was not explained by differences in functional status and comorbidities. Further investigation is needed to determine the underlying mechanisms that explain this relationship, and to determine whether therapies to retard bone loss may also prevent cognitive decline

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Familial Isolated Hyperparathyroidism: Clinical and Genetic Characteristics of Thirty-Six Kindreds. W. F. Simonds,[1] L. A. James-Newton,*[1] S. K. Agarwal,*[1] B. Yang,*[2] M. C. Skarulis,*[3] G. N. Hendy,[2] S. J. Marx.[1] [1]Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA, [2]Departments of Medicine, Physiology and Human Genetics, McGill University, Montreal, Canada, [3]Division of Intramural Research, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA.

The identification of the gene for multiple endocrine neoplasia type 1 (MEN1) has allowed genetic screening to define a population of patients with familial hyperparathyroidism due mainly to other etiologies. Thirty-six kindreds with apparent familial isolated hyperparathyroidism (FIH) were studied at the National Institutes of Health. Each index case and other relatives had detailed biochemical and bone imaging tests, as well as mutation testing of the MEN1 and calcium-sensing receptor (CaSR) genes. None had evidence of MEN1 by clinical, biochemical and mutational criteria. During this time approximately 100 kindreds with MEN1 were diagnosed, but none had FIH. Approximately 60 kindreds with familial hypocalciuric hypocalcemia (FHH) were diagnosed by biochemical criteria, and excluded. Unexpected mutations in the coding region of the CaSR gene, inactivation of which accounts for most cases of FHH, were found in the probands of five of the FIH kindreds included in our study. Based on the presence of parathyroid carcinoma, bilateral renal cysts and/or fibro-osseous jaw tumors, three other kindreds had the hyperparathyroidism-jaw tumor syndrome (HPT-JT). The remaining twenty-eight kindreds with FIH exhibited a spectrum of manifestations, some with associated non-parathyroid tumors. No kindred was large or distinctive enough to define a new clinical category. One kindred showed cosegregation of parathyroid tumors and breast cancer in three generations. Two kindreds each had renal angiomyolipomas or lipomas in association with FIH. In conclusion, most occurrences of FIH appear to result from mutation of unknown genes. Further study of such families may expand the range of phenotypes associated with HPT-JT, and help to identify other novel tumor suppressors or oncogenes.

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Vitamin D Levels and Prevalence of Secondary Hyperparathyroidism in a Spanish Population-Based Sample Aged 54 to 89. C. Gómez-Alonso, M. Naves-Díaz,* J. L. Fernández-Martín,* J. B. Díaz-López,* M. T. Fernández-Coto,* J. B. Cannata. Servicio de Metabolismo Oseo y Mineral, Hospital Central de Asturias, Oviedo, Spain.

Vitamin D deficiency is common in elderly people and it may contribute to increase the prevalence of metabolic bone disorders. Serum 25-hydroxyvitamin D (25OHD) is a good indicator of the vitamin D status. However, due to several reasons there is no clear threshold to separate "normal" and "abnormal" values. It is accepted that levels of 25OHD <5 ng/ml leads to osteomalacia, <10 ng/ml leads to secondary hyperparathyroidism, and >18ng/ml could be considered "normal". The purpose of this study was to assess the vitamin D status and the prevalence of secondary hyperparathyroidism in a random sample of 312 people (159 women and 153 men, 68±9 years old; ranged 54 to 89) who participated in the European Vertebral Osteoporosis Study (EVOS) in who we performed a study including among others, BMD, 25OHD, calcitriol, iPTH, alkaline phosphatase. From this EVOS sample we included in this analysis only those people who never received any kind of treatment for osteoposis (n=268, 86% of the sample). There were no differences in age, sex or season of blood withdrawal between the 44 treated (any kind of therapy), and the 268 non-treated people. Nevertheless, serum 25OHD was higher in the treated people (24.5±11.7 vs 15.9±8.7 ng/ml, p<0.005).In the 268 people analysed in this abstract, serum 25OHD showed a significant seasonal relationship, observing lower values in winter (12.9±5.9 ng/ml) and spring (14.4±7.4 ng/ml) respect to summer (20.9±8.8 ng/ml) and autumn (18.7±7.8 ng/ml) without differences between sexes. We observed a weak relationship between age and serum 25OHD (r=-0.19, p<0.02) that disappeared when season was included in the analysis.Serum 25OHD showed a significant relationship with iPTH (r=-0.3, p<0.01) and calcitriol (r=0.3, p<0.01) and 25OHD was the strongest independent factor in the multivariate analysis. Serum 25OHD levels were "deficient" (<10 ng/ml) in 27% of people, "borderline" (10-18 ng/ml) in 40% and "normal" (>18 ng/ml) in 33% of people. The prevalence of secondary hyperparathyroidism (iPTH ≥65 pg/ml) according to 25OHD levels was 33% (<10ng/ml), 16% (10-18 ng/ml) and 12% (>18 ng/ml). There was no a single case of secondary hyperparathyroidism with 25OHD levels ≥40ng/ml.In conclusion, the 25OHD levels currently used in clinical practice to define "normal" or "abnormal" status needs to be reviewed and redifined in order to avoid a non negligible amount of secondary hyperparathyroidism likely due to "inadequately low" levels of 25OHD.

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PTH(1-84) and N-truncated PTH Fragment in the Serum and Parathyroid Glands of Patients with Primary Hyperparathyroidism. A. Cohen,*[1] P. LoGerfo,*[1] P. Gao,[2] I. B. Brown,*[1] S. Trokan,*[1] T. Cantor,[2] J. P. Bilezikian,[1] S. J. Silverberg.[1] [1]College of Physicians & Surgeons, Columbia University, New York, NY, USA, [2]Scantibodies Lab Inc., Santee, CA, USA.

The new immunoradiometric assay (wPTH), using antigenic determinants at the extreme N-terminal end (1-4) of the PTH molecule, detects only whole PTH(1-84). This assay is more sensitive for the diagnosis of primary hyperparathyroidism (PHPT) than the "intact" PTH IRMA (iPTH), which measures PTH(1-84) as well as the large N-truncated PTH fragment (i.e. PTH[7-84]) which is present in serum from normal subjects, and in patients with renal failure. As this fragment does not have classic PTH-like activity, the ratio wPTH/iPTH provides an index of the percent of biologically active PTH. In this study, we report on the presence of whole PTH(1-84) and N-truncated PTH fragment in adenomatous parathyroid glands, and in the circulation of patients with PHPT. 21 consecutive patients undergoing surgery for PHPT (ages 43-89; 76% female) were enrolled, and levels of wPTH (nl: 7-36 pg/ml) and iPTH (nl: 10-65 pg/ml) were measured in serum and in the parathyroid gland. Pathology revealed adenomas in 18 patients and hyperplasia in 3 patients (latter excluded from analysis). Baseline serum calcium was 11.5 ± 0.1 mg/dl (nl: 8.7-10.8). In the parathyroid adenomas, iPTH was 1.8-fold higher than wPTH (697,090 ± 267,061 vs. 390,605 ± 116,385 pg/ml), although these values were highly correlated (R=+0.963, p <0.0001). In the serum, PTH levels were also higher (1.3-fold) by the iPTH assay (132 ± 14 pg/ml; wPTH: 96 ± 10 pg/ml; p<0.0001). Glandular PTH levels correlated with serum PTH in the wPTH (R=+0.578, p<0.05) but not the iPTH assay. Serum calcium levels correlated with glandular PTH in both assays (wPTH: R=+0.569, p<0.05; and iPTH: R=+0.544, p<0.05), but not with either PTH measurement in the serum. The mean percent of bioactive PTH(1-84) in the adenomas was 66 ± 3%, not different from bioactive hormone levels in the serum of these patients (73 ± 2%; paired T-test P=NS), and similar to previously reported amounts in normal serum (65%). While the serum of normal and uremic patients shows wide variability (20-90%) in percent of bioactive PTH(1-84), all patients with PHPT had >60% bioactive hormone. The data suggest that 1) the proportion of PTH(1-84) and N-truncated PTH fragment in the adenomatous gland is similar to the proportion found in the serum; 2) the peripheral metabolism of hormone and fragment, in the liver or kidney, does not alter the proportion of bioactive PTH in the circulation. Finally, this study provides the first evidence that both whole PTH(1-84) and large N-truncated PTH fragments are produced in the adenomatous glands of patients with PHPT.

Disclosures: Scantibodies Lab Inc.,3,4.

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Osteoprotegerin Inhibits Artery Calcification Induced by Warfarin and by Vitamin D. P. A. Price, J. R. Buckley,* H. H. June,* M. K. Williamson. Division of Biology, University of California, San Diego, La Jolla, CA, USA.

Previous studies have shown that doses of the amino bisphosphonates alendronate and ibandronate that inhibit bone resorption will potently inhibit the calcification of arteries induced by treatment with warfarin (Arterioscler. Thromb. Vasc. Biol.(2001) 21(5) in press). These observations support the hypothesis that artery calcification is linked to bone resorption. In the present investigations we have carried out an additional test of this hypothesis by determining whether the selective inhibition of bone resorption with osteoprotegerin will also inhibit artery calcification.

In the first test, artery calcification was induced by treating 22-day-old male rats with warfarin, a procedure that inhibits the γ-carboxylation of matrix Gla protein and thereby inactivates the calcification inhibitory activity of the protein and causes progressive calcification of the artery media. Compared with rats treated for 1 week with warfarin alone, rats treated with warfarin plus osteoprotegerin at a dose of 1 mg/kg/day had dramatically reduced Alizarin red staining for calcification in the aorta and in the carotid, hepatic, mesenteric, renal, and femoral arteries, and 90% lower levels of calcium and phosphate in the abdominal aorta (p< 0.001) and in tracheal ring cartilage (p< 0.01).

More rapid artery calcification was induced by treating 49-day-old male rats with high doses of vitamin D. Rats treated for 96h with vitamin D alone had widespread Alizarin red staining for calcification in the aorta and the femoral, mesenteric, hepatic, renal, and carotid arteries, while rats treated with vitamin D and osteoprotegerin had no evidence for Alizarin red staining for calcification in any of these arteries. Chemical analysis further showed that rats treated with vitamin D alone and examined at 96h had 6-fold higher levels of calcium and 20-fold higher levels of phosphate in their abdominal aorta than found in control rats (p< 0.001), while the levels of calcium and phosphate in the abdominal aorta of rats treated with vitamin D plus osteoprotegerin were at control values. The ability of osteoprotegerin to inhibit artery calcification in vitamin D-treated rats cannot be explained by an effect on serum calcium, since serum calcium values in rats treated with vitamin D alone (14.6 ± 0.5mg/dl) and with vitamin D plus osteoprotegerin (14.9 ± 0.8mg/dl) were the same.

We conclude that doses of osteoprotegerin that inhibit bone resorption are able to potently inhibit the calcification of arteries that is induced by warfarin-treatment and by vitamin D treatment. These results support the hypothesis that artery calcification is linked to bone resorption.

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Paresis of a BMP4 Antagonist Response In Fibrodysplasia Ossificans Progressiva. J. Ahn,* L. Serrano de la Pena,* E. M. Shore, F. S. Kaplan. Orthopaedic Surgery (JA, LS, EMS, FSK), Genetics (EMS) and Medicine (FSK), University of Pennsylvania, Philadelphia, PA, USA.

Formation of the human skeleton requires inductive signals that are meticulously balanced with antagonist signals in a highly regulated negative feedback system. Patients with fibrodysplasia ossificans progressiva (FOP), a clinically catastrophic genetic disorder, form an ectopic skeletal system post-natally as muscle and deep connective tissues are replaced with heterotopic bone. Recombinant bone morphogenetic protein-4 (BMP4), a potent osteogenic morphogen, can induce endochondral osteogenesis at an ectopic site in a manner identical to that seen in FOP. Moreover, BMP4 mRNA and protein are uniquely overexpressed in lymphocytes and lesional cells from patients who have FOP. However, the BMP4 gene is not mutated in FOP, and the BMP4 locus has recently been excluded from linkage to the condition.The cellular effects of bone morphogenetic proteins (BMPs) are specified in part in a dose-dependent fashion by tightly-regulated morphogenetic gradients of BMPs and secreted antagonists such as noggin, chordin, and gremlin. Although the various BMP antagonists are unique proteins, they share the functional property of binding specifically to extracellular BMPs preventing them from interacting with their transmembrane receptors. Several recent studies have indicated that BMP4 upregulates expression of noggin and gremlin, thereby establishing an autoregulatory negative feedback loop.We hypothesized that a defect in the feedback pathway between BMP4 and one or more of its extracellular antagonists could plausibly contribute to elevated BMP4 activity in fibrodysplasia ossificans progressiva. Therefore, we investigated basal and BMP4-induced expression of noggin, chordin, and gremlin mRNA in control and FOP cell lines (LCLs).We found that cells from patients with FOP fail to upregulate the expression of noggin and gremlin in response to a BMP stimulus; control cells exhibit marked increases in noggin and gremlin mRNA expression (as measured by RT-PCR) whereas patient-derived cells

exhibit a dramatically attenuated response. Our data suggest that a loss of negative feedback due to an insufficient BMP antagonist response may account in part for increased BMP4 activity in FOP. Such a defect suggests the loss of a critical negative feedback mechanism by which cells normally regulate the magnitude and boundaries of ambient morphogenetic signals. These findings from a disabling human disease support the importance of a critical balance between an inductive morphogen and its secreted antagonists in the formation of an ectopic organ system and suggest the potential of BMP antagonist-based strategies in the therapy of FOP.

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Platelet-Derived Growth Factor A Chain (PDGF-A) Antagonism with Triazolopyrimidine (Trapidil) Inhibits Marrow Fibrosis in a Rat Model for Osteitis Fibrosa Cystica. S. Lotinun, G. L. Evans,* M. Zhang,* R. T. Turner. Department of Orthopedics, Mayo Clinic, Rochester, MN, USA.

The skeletal disorder osteitis fibrosa cystica is associated with hyperparathyroidism. Similar disorders (e.g. renal osteodystrophy) are associated with chronic secondary hyperparathyroidism. The histological presentation of these diseases suggests that excess parathyroid hormone (PTH) results in local release of one or more growth factors by cells of the osteoblast lineage which in turn stimulate fibroblast over growth. The purpose of this study was to identify the putative causative factor. First, we established a rat model for osteitis fibrosa cystica. Adult rats were infused continuously for one week with human PTH (1-34) (40 mg/kg/d) delivered using sc implanted ALZET osmotic pumps. This treatment resulted in moderate hypercalcemia and bone histological changes similar to patients with primary hyperparathyroidism. The changes included increased bone formation, focal bone resorption and severe peritrabecular marrow fibrosis. Next, we compared gene expression between rats given continuous PTH and those given the hormone once daily sc. The latter treatment induces bone formation without detrimental side effects. Using a cDNA microarray having over 5000 genes, we found a set of genes differentially regulated by continuous PTH. One of these genes was PDGF-A, a known mitogenic and chemotactic factor for fibroblasts. Verification of the regulation of PDGF-A expression was accomplished using an RNase protection assay which showed that continuous PTH increased PDGF-A mRNA levels 3-fold ($p < 0.001$), whereas pulsatile PTH had no effect. Finally, we investigated the effect of trapidil on our rat model for osteitis fibrosa cystica. Four groups of animals were studied: (i) vehicle, (ii) trapidil, (iii) PTH pump, (iv) PTH pump and trapidil. Trapidil, itself did not have any effect on bone histomorphometry. The combination of PTH and trapidil showed a dramatic 74% ($p < 0.001$) reduction in marrow fibrosis compared to continuous PTH, whereas the stimulatory effects of PTH on bone formation were maintained. These results suggest that excess PDGF-A is responsible for PTH-induced marrow fibrosis and that drugs which target PDGF-A can reduce or prevent development of skeletal pathology occurring in hyperparathyroid patients.

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Distinct and Overlapping Actions of PTH and PTHrP in Bone and Cartilage Development. D. Miao, B. He,* A. C. Karaplis, D. Goltzman. Dept. of Medicine, McGill University, Montreal, PQ, Canada.

To assess the role of PTH and PTHrP in bone and cartilage development we analyzed the skeletons of newborn mice homozygous for targeted ablation of the genes encoding PTH (PTH-/-), PTHrP (PTHrP-/-) or PTH and PTHrP (PTH-/-;PTHrP-/-) and compared these to each other and to wild type mice. Although PTH-/- mice are viable, PTH-/-;PTHrP-/- mice died at birth with skeletal malformations including short-limbed dwarfism, which were more severe than in PTHrP-/- animals. Analysis of the tibial growth plate revealed a marked decrease in the proliferation zone in both PTHrP-/- and PTH-/-;PTHrP-/- mice, which was not seen in PTH-/- animals. This was associated with diminished proliferating cell nuclear antigen positive chondrocytes and increased apoptosis in the PTHrP deficient animals. In contrast, the hypertrophic zone was increased in all three models but in the two PTHrP deficient animals it contained increased type X collagen, and was disorganized. Mineralization of the cartilage matrix, as assessed by van Kossa staining, was reduced in the two PTH-deficient mice but enhanced in PTHrP-/- animals. In long bone, cortical thickness was increased in all three models compared to wild type mice. In sharp contrast, trabecular bone volume was diminished in the PTH-/- mice and the PTH-/-;PTHrP-/- mice, but was slightly increased in the PTHrP-/- mice at birth. TRAP positive osteoclast number and size were reduced in all three models but osteoblast number was diminished in the two PTH deficient models and increased in the PTHrP-/- mice. Overall, the cartilage phenotype of the double "knockout" mice approximated more closely the PTHrP-/- mice, whereas the bone phenotype resembled more closely the PTH-/-animals. These results suggest that PTH and PTHrP have discrete and overlapping effects in skeletal development, which require coordinated action to achieve normal cartilage and bone growth. The unique effects of each molecule may reflect temporal or spatial differences in exposure of skeletal target cells to the two molecules or differences in molecular action of the two peptides despite their capacity to interact with a common receptor.

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Mice Lacking Both PTH and PTHrP Have Novel Defects in Fetal Growth and Bone Mineralization. C. S. Kovacs,[1] L. L. Chafe,*[1] N. Fudge,*[1] N. R. Manley.*[2] [1]Faculty of Medicine, Memorial University of Newfoundland, St. John's, NF, Canada, [2]Institute of Molecular Medicine and Genetics, Medical College of George, Augusta, GA, USA.

In fetal life, both PTH and PTHrP are present in the circulation, and both appear to contribute to the regulation of fetal calcium metabolism. We have previously shown that lack of PTH (in *Hoxa3* null mice) causes hypocalcemia and hyperphosphatemia, but no effect on placental calcium transfer. Lack of PTHrP (in *Pthrp* null mice) also causes hypocalcemia and hyperphosphatemia, and placental calcium transfer is reduced. In the current study, we have examined the relative contributions of PTH and PTHrP to fetal calcium and bone metabolism by creating double-knockouts for *Hoxa3* and *Pthrp*. Compared to wt siblings, single mutant *Hoxa3* null fetuses had a normal weight, crown-rump length and limb length; single mutant *Pthrp* null fetuses also had a normal weight and crown-rump length but displayed the previously described chondrodysplasia with shortened and misshaped limbs. Double-knockout fetuses were significantly smaller with lower body weight, crown-rump length and markedly foreshortened limbs. Whole mount skeleton preparations showed the double-knockout to have abnormal mineralization of costal cartilages, similar to the *Pthrp* null. In addition, growth plates of the double-knockout showed more severe shortening and disorganization as compared to the *Pthrp* null. Alizarin red and von Kossa studies demonstrated that the *Hoxa3* null and the double-knockout both had accreted less skeletal mineral, while the *Pthrp* null had normal skeletal mineral content. Wt fetuses had a blood calcium of 1.70 ± 0.04 mmol/l; single mutant *Pthrp* null fetuses had a modestly reduced blood calcium (equal to maternal) of 1.40 ± 0.03 mmol/l; single mutant *Hoxa3* null fetuses had a markedly reduced blood calcium (below maternal) of 1.20 ± 0.04 mmol/l; and double-mutant fetuses had the lowest blood calcium at 1.02 ± 0.02 mmol/l (ANOVA: $p<0.01$ among all 4 genotypes). Our results show that lack of both PTHrP and PTH resulted in more severely reduced blood calcium compared to the single mutants, as well as new defects in skeletal mineral accretion and overall fetal growth. These findings suggest: 1) that PTH may play a more dominant role than PTHrP in regulating the fetal blood calcium; 2) that the blood calcium level is an important determinant of skeletal mineral accretion independent of placental calcium transfer rate; and 3) that lack of both PTH and PTHrP (but not either alone) will cause fetal growth restriction.

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Altered Bone Mass and Microarchitecture in Beta-Arrestin2 KO Mice. S. L. Ferrari,[1] F. Lin,*[2] R. J. Lefkowitz,*[2] M. L. Bouxsein.[3] [1]Div. of Bone Diseases, University Hospital, Geneva, Switzerland, [2]Howard Hughes Medical Institute Laboratories, Duke University Medical Center, Durham, NC, USA, [3]Orthopedic Biomechanics Laboratory, Beth Israel Deaconess Medical Center, Boston, MA, USA.

Beta-arrestins are cytoplasmic molecules involved in intracellular trafficking and signaling of G protein-coupled receptors (GPCRs). Beta-arrestins are expressed in osteoblasts and we have previously shown that beta-arrestin2 (b-arr2) mediates endocytosis of agonists--parathyroid hormone (PTH)/PTH-related protein (PTHrP) receptor and regulates PTH-stimulated cAMP signaling. Mice null for b-arr2 develop normally and show no gross phenotypic abnormalities. However, due to the central role of PTHrP and PTH on bone development and remodeling, respectively, we hypothesized that bone mass and microarchitecture might be altered in these mice. Adult b-arr2 KO mice and their wild-type (wt) littermates were evaluated for whole body and femoral bone mineral density (BMD) by pDXA (PIXImus®). High resolution micro-computed tomography was used to evaluate trabecular and cortical bone mass and architecture at the proximal tibia and femoral midshaft, respectively.No differences in skeletal morphology were observed between six adult b-arr2 KO and wt mice on conventional radiographs. In contrast, compared to wt, b-arr2 KO mice had lower whole body and femoral BMD (-9%), trabecular number (TbN, -7%) and connectivity (-119%), increased trabecular separation (TbSp, +7%), and decreased cortical bone volume (BV, -13%) (all $p<0.05$ by nonparametric Mann-Whitney U-test). There also was a trend for lower trabecular bone volume fraction (BV/TV, -17%) and thickness (TbTh, -8%), as well as cortical thickness (Th, -6%) in b-arr2 KO mice. Body composition also markedly differed in b-arr2 KO and wt mice, as fat mass was 33% lower in the former ($p<0.05$). Since beta-arrestin1 (b-arr1) is also expressed in bone and could partly compensate for the loss of b-arr2 function, we further evaluated twelve mice generated by crossing b-arr2 KO with b-arr1 KO mice. Double homozygous b-arr1/b-arr2 KO mice were embryonnically lethal. However, in b-arr1 (+/-)/b-arr2 KO mice, BMD was decreased by up to 20%, TbTh by 17% (both $p<0.05$), and cortical BV and Th by up to 18% ($p=0.12$) compared to b-arr1(+/-)/b-arr2(+/-) mice.In summary, adult mice null for b-arr2 exhibit reduced BMD, cortical bone mass, and altered trabecular microarchitecture. These features appeared to be even more prominent with the additional loss of one b-arr1 allele. These in vivo findings confirm the important role of beta-arrestins in regulating GPCRs activity in bone and suggest that the skeletal phenotype observed in b-arr2 KO mice may result from a sustained activity of PTH.

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ADAMTS-1: A Cellular Disintegrin and Metalloprotease with Thrombospondin Motifs Is Essential for Normal Bone Growth and PTH Regulated Bone Metabolism. J. E. Onyia, Y. L. Ma, E. Galbreath,* Q. Zhang,* Q. Zeng, R. Cain,* J. Hoover,* R. R. Miles,* D. L. Halladay,* L. V. Hale,* R. F. Santerre, A. K. Harvey, S. Chandrasekhar, N. Fox,* D. D. Yang.* Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN, USA.

Recently, we discovered ADAMTS-1 as potential PTH target gene in genomic analysis of bone response to PTH. ADAMTS-1, a new secreted member of the ADAM (A disintegrin and metalloprotease) gene family, is selectively up-regulated in rat and mouse bones by parathyroid hormone. In vivo, acute exposure to anabolic PTH results in a rapid and transient increase in ADAMTS-1 expression while catabolic continuous PTH infusion leads to a rapid and sustained induction of ADAMTS-1. To validate the function of ADAMTS-1 and understand the biology in an animal model, we generated ADAMTS-1 knockout mice by gene targeting. ADAMTS-1(-/-) mice are viable, but suffer from growth retardation, impaired female fertilization with delayed sexual development, and renal abnormalities that involve enlargement of renal calices and reduction of corticomedullary tissue. Dual energy X-ray (DEXA) analyses showed a significant reduction in whole body bone mineral content in ADAMTS-1(-/-) mice. Histomorphometric analyses of the proximal tibia of 6 month old mice showed decreases in trabecular bone volume (TBV), thickness (Tb.Th), and number (Tb.N) in ADAMTS-1(-/-) mice. No significant difference was observed in mineralizing surface (MS/BS), but there was an increase in matrix apposition rate (MAR) in ADAMTS-1(-/-) mice. Osteoclast number normalized to bone surfaces (N.OC/BS) or on trabecular area (N.OC/T.Ar) was significantly increased in ADAMTS-1(-/-) mice. Histomorphometric differences between mutant and wildtype mice were diminished in tibia from 9 month old female mice due to normal age related bone loss in wild type animals. Intermittent PTH challenge increased trabecular bone mass and markedly

upregulated serum osteocalcin (a marker of osteoblast differentiation) in mutant mice. Additionally, ADAMTS-1(-/-) mice were resistant to bone resorption induced by continuous PTH infusion. These data underscore an essential role for ADAMTS-1 in bone metabolism and are consistent with the hypothesis that ADAMTS-1 is a critical mediator of PTH actions in bone. We speculate that under normal physiological conditions ADAMTS-1 is an inhibitor of PTH-induced bone formation and is required for PTH-induced resorption.

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The Protooncogene c-*fos* Is Critical for Anabolic Effects of Parathyroid Hormone in Bone. B. Demiralp,*[1] H. Chen,[1] A. J. Koh,*[1] C. Chen,*[1] J. Dai,*[2] E. T. Keller,[2] L. K. McCauley.[1] [1]Perio/Prev/Geriatrics, Univ Michigan, Ann Arbor, MI, USA, [2]Unit for Lab Animal Medicine, Univ Michigan, Ann Arbor, MI, USA.

Parathyroid hormone (PTH) has both anabolic and catabolic actions in bone that are not well understood. AP-1 family members such as c-*fos* are critical transcriptional mediators, and are highly regulated by PTH. The purpose of this study was to examine the anabolic mechanisms of PTH, and specifically the role of *c-fos* during endochondral bone growth. Mice, c-*fos* (-/-), wildtype (+/+), and heterozygous (+/-) littermates, were genotyped by PCR. An anabolic regime of PTH (0.05 µg/g) was administered s.c. from postnatal d4-23, or a single PTH (20 µg) was administered. FAXITRON radiography, dual x-ray absorptiometry, histology, serum and bone ash biochemistry and whole organ gene expression by northern blot were performed. Effects of PTH *in vivo* were dramatically different in wild-type and heterozygous vs. c-*fos* deficient mice. The +/+ mice had increased bone mineral density (BMD), and bone mineral content (BMC) with daily PTH; whereas PTH caused a reduction in BMD and BMC in -/- mice ($p<0.05$). Daily PTH treatment also decreased the calcium/ash weight of -/- femurs but not +/+ or +/- ($p<0.01$). Serum calcium levels of +/+ mice were higher than -/- mice ($p<0.05$), and with anabolic PTH administration, calcium levels decreased in +/+ but increased in -/- mice. Histologically, PTH resulted in an increase in proliferating chondrocytes in +/+ mice, but a decrease in proliferating chondrocytes and an increase in hypertrophic chondrocytes in -/- mice. This resulted in an exacerbation of the already widened growth plate in -/- mice. Daily PTH also increased the thickness of calvaria in +/- mice but not -/- mice. The c-*fos* -/- mice had lower bone sialoprotein (BSP) and osteocalcin (OCN) ($p<0.05$) mRNA expression in calvaria than c-*fos* +/+ mice but there was no difference in PTH-1R mRNA expression in calvaria and kidneys of c-*fos* +/+ or -/- mice suggesting the PTH-1 receptor was not altered by the c-*fos* deficiency. Short term PTH administration (8h) resulted in an increase in BSP ($p<0.01$), a decrease in OCN ($p<0.01$), and no change in PTH-1R mRNA expression in calvaria of +/- and +/+ mice whereas there was no alteration in gene expression for BSP, OCN or PTH-1R in -/- mice. These studies suggest that c-*fos* is critical for gene expression of BSP and OCN in bone. *In vitro* studies with an inhibitor of c-*fos* and c-*jun* (U0126) and pre-osteoblastic cells similarly indicated that AP-1 signaling is required for basal OCN expression and for PTHrP downregulation of OCN. These data suggest that c-*fos* is critical for anabolic effects of PTH and that this role of c-*fos* may be associated with genes that regulate extracellular matrix mineralization.

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Direct Binding of the PTH/PTHrP Receptor (PTH1R) to the Na-H Exchange Regulatory Factor 2 (NHERF2) Selectively Transfers Signaling from Adenylate Cyclase to Phospholipase C Pathways. M. J. Mahon,*[1] M. Donowitz,*[2] G. V. Segre.[1] [1]Endocrine Unit, MGH, Harvard University, Boston, MA, USA, [2]Department of Gastroenterology, Johns Hopkins University, Baltimore, MD, USA.

The intracellular carboxyl-terminal "tail"(C-tail) of the PTH1R, when used as "bait" in the yeast two-hybrid system, binds to NHERF2 through an interaction with an atypical PDZ consensus motif (ETVM) and the more C-terminal of two PDZ domains in NHERF2. NHERFs are known to function as scaffolding proteins capable of binding several molecules. A GST-fusion protein containing the PTH1R C-tail strongly interacted with NHERF2 in overlay assays. In contrast, PTH1R C-tail containing a C-terminal 20 amino-acid truncation or single alanine point-mutations at M591 T589, or E588, located on the C-terminus disrupted binding to NHERF2. However, V590A replacement had no effect on this interaction, which is consistent with known properties of PDZ binding. Furthermore, the PTH1R C-tail exclusively interacted with NHERF2's C-terminal PDZ domain, not with the N-terminal PDZ domain. The specificity of these interactions was confirmed and extended in mammalian cells with full length wild-type (WT) and mutant PTH1Rs and NHERF2 by co-immunoprecipitation. WT PS 120 cells are fibroblasts devoid of Na-H Exchangers (including NHE3), NHERFs and PTH1Rs. Stable cells lines expressing NHE3 were first generated, and then modified to stably express either the WT PTH1R or PTH1R lacking a functional PDZ interaction motif (PTH1R-Cdelta20 and M591A), and in the absence or presence of NHERF2. Stable cells co-expressing the WT PTH1R and NHERF2 generated only 2-fold increases in cAMP when treated with 100 nM PTH (1-34). In contrast, cells co-expressing either PTH1R-Cdelta20 or M591A and NHERF2, or cells co-expressing WT PTH1R in the absence of NHERF2 displayed PTH-mediated increases in cAMP of 10-15 fold. Importantly, cell lines co-expressing WT PTH1R and NHERF2 preferentially signaled via the IP pathway, displaying a PTH-mediated increase of 25-30 fold. However, cell lines co-expressing PTH1R-Cdelta20 or M591A and NHERF2, or stables lines expressing WT PTH1R in the absence of NHERF2 displayed only a 2-4 fold increase in IPs in response to PTH (1-34). In summary, PTH1R's C-terminal recognition domain directly interacts with NHERF2 via its C-terminal PDZ domain. This interaction results in a NHERF2-dependent shift in PTH-mediated signaling from the adenylate cyclase pathway to the phospholipase C pathway. Thus, NHERF2 functions as "switch" to select the signaling pathway activated by the PTH1R. Studies are in progress to examine the physiological significance of this interaction in cells not over expressing these molecules.

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Modulation of T Cell TNF Production via Antigen Presenting Cells: A Novel Immune Mechanism by which Estrogen Prevents Bone Loss. S. Cenci, C. Roggia, G. Toraldo, Y. Gao, M. N. Weitzmann, J. L. Kindle,* F. Starkey,* R. Pacifici. Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA.

T cells are essential players in the mechanism by which ovariectomy (ovx) induces bone loss, as demonstrated by the failure of ovx to induce bone loss in T cell deficient (nude) mice. Since ovx increases T cell TNF production in vitro, we investigated if ovx induces bone loss in vivo through upregulated T cell TNF production. Nude mice were reconstituted with T cells from wild-type (WT) or TNF deficient (-/-) donor mice and bone density measured by pQCT on excised tibiae. In nude mice reconstituted with T cells from WT mice, ovx caused a 25 % bone loss in 4 weeks. In contrast, ovx caused no bone loss in nude mice reconstituted with TNF -/- T cells. Thus, increased T cell TNF production is crucial for ovx induced bone loss in vivo. Ovx upregulated TNF production by increasing T cell activation, a phenomenon leading to an increased number of bone marrow TNF producing T cells. In vitro estrogen (E2) treatment did not repress T cell proliferation and TNF production, suggesting that E2 regulates T cells indirectly, either targeting accessory cells or T cell precursors, which become E2-insensitive when reach maturity. Injection of mature T cells from ovx mice into sham nude recipients caused a 2-week period of rapid bone loss, due to T cell TNF production. Between week 2 and 4 there was no additional bone loss due to T cell deactivation. Injection of sham T cells into ovx nude mice induced no bone loss in the first 2 weeks, T cell proliferation at 2 weeks, and rapid bone loss thereafter. Thus, endogenous E2 regulates mature T cells in vivo. Since antigen (Ag) presenting cells (APC) are central modulators of T cell activation, we investigated if APC mediate the effects of ovx in vivo. Thus, bone density was measured in ovx DO11.10 mice, a strain with impaired cross-talk between APC and T cells. DO11.10 mice were completely protected against ovx induced bone loss, a finding which establishes the central role of APC in ovx induced bone loss. Finally, we found that ovx increases APC activity by twofold, as assayed in vitro by measurement of proliferation of target T cells in presence of the whole unprocessed Ag. In contrast, no difference between sham and ovx APC activity was observed when the immunogenic fragments of the Ag were used, indicating that E2 deficiency increases Ag presentation by enhancing Ag processing. Together, the data demonstrate that ovx induced bone loss is a consequence of upregulated T cell TNF production resulting from increased APC activity. The observed link between the immunosuppressive and the bone-sparing effects of E2 provides a novel paradigm as it identifies immune cells as an essential target of E2 in bone.

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Rapid Activation of MAP Kinases by Estrogens or Androgens Leads to Potent Downstream Regulation of the Transcription of the Serum Response Element and AP-1: A Link Between Nongenotropic and Genotropic Functions of their Classical Receptors. S. Kousteni, L. Han, M. E. McIntire,* T. Bellido, R. L. Jilka, S. C. Manolagas. Division of Endocrinology & Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Estrogens or androgens activate the Src/Shc/ERK signaling pathway via an extranuclear function of the classical estrogen (ER) or androgen (AR) receptors. Based on this and evidence that ERK or JNK kinases activate Elk-1 or c-fos/c-jun, respectively, we investigated whether nongenotropic activation of MAP kinases by ER or AR leads to activation of these transcription factors. HeLa cells were transiently transfected with either ERα or AR, or a construct consisting of the ligand binding domain (E) of the ERα fused either to a membrane (E-Mem) or a nuclear localization (E-Nuc) sequence together with one of two reporter plasmids carrying promoters interacting with either Elk-1 or c-fos/c-jun. Binding of Elk-1 to the cis serum response element (SRE), or c-jun/c-fos to an AP-1 site in these constructs drives the expression of secreted human alkaline phosphatase (SEAP). 17β-estradiol (E_2) or dihydrotestosterone (DHT) at 10^{-8} M upregulated (~8-fold) the transcriptional activity of Elk-1. On the other hand, either steroid downregulated (~7-fold) the AP-1-dependent transcription from the AP-1-SEAP. These effects could be demonstrated in cells transfected with the wild type ERα, or with the AR, or with the E-Mem mutant; but, were completely eliminated in cells containing the E-Nuc. Moreover, these effects resulted from downstream activation of kinases, as co-transfection with dominant negative mutants for Src, Shc or ERK activation completely abrogated them. In agreement with the evidence that Elk-1 and c-fos/c-jun transcriptional regulation is a consequence of a nongenotropic kinase activation, an estren which has no transcriptional activity via the ER but is a potent inducer of ERK activation, also stimulated SRE-SEAP activity. On the other hand, a pyrazole with potent transcriptional activity, but no effects on ERK activation was unable to induce SRE-SEAP activity via either receptor. These results demonstrate that rapid signals originating from membrane-associated receptors influence transcription. Nonetheless, in contrast to the present evidence that rapid activation of MAP kinases by membrane-associated ERα suppresses AP-1 activity, E_2-activated ERα stimulates AP-1 activity directly. Therefore, the response of a target cell to sex steroids may be determined by the balance between membrane- and nucleus-associated receptor actions.

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Gender-Independent Induction of Murine Osteoclast Apoptosis In Vitro by Either Estrogens or Non-Aromatizable Androgens. J. R. Chen, S. Kousteni, T. Bellido, L. Han, G. B. Di Gregorio, R. L. Jilka, S. C. Manolagas. Division of Endocrinology & Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

In rodents and humans sex steroids influence the survival of osteoclasts and osteoblasts in opposite directions: they promote osteoclast, but prevent osteoblastic/osteocytic cell

apoptosis. The latter effect evidently results from a nongenotropic, sex-nonspecific action of the classical estrogen (ER) or androgen (AR) receptor and it can be transmitted by either the ER or the AR with similar efficiency irrespective of whether the ligand is an estrogen or an androgen. To determine the mechanism, and in particular the sex steroid- and gender-specificity of the pro-apoptotic effect of estrogens and androgens on osteoclasts we examined the effect of 17β-estradiol (E_2) or the non-aromatizable dihydrotestosterone (DHT) on the survival of osteoclasts derived from bone marrow cell cultures from 4-8 week old C57Bl/6 male or female mice. In one set of experiments non-adherent cells were harvested 2 days following the establishment of the culture and placed in medium containing 30 ng/ml M-CSF and soluble RANK ligand; in a second set both adherent and non adherent cells were cultured together in the presence of 10^{-8} M 1α,25-$(OH)_2D_3$. Cultures were grown until a maximum number of multinucleated osteoclasts were observed (7-8 days from female-derived cells and 5-8 days for male-derived cells) and were then treated with vehicle or 10^{-10} to 10^{-7} M E_2 or DHT alone or in the presence of equimolar concentrations of the estrogen receptor antagonist ICI 182,780 or the androgen receptor antagonist flutamide. 24 h later apoptosis was assessed both by direct visualization of apoptotic features in TRAP-positive cells and by measuring caspase 3 activity in cell lysates. Both E_2 or DHT stimulated osteoclast apoptosis in a dose-dependent manner, by as much as 3 -fold over vehicle, in either assay. E_2 or DHT were equally effective irrespective of whether the donor mouse was a male or a female, or whether stromal/osteoblastic support cells were present or absent from the cultures. More important and as shown before in the case of the anti-apoptotic effects of sex steroids on osteoblasts, the pro-apoptotic effect of E_2 was abrogated by ICI as well as flutamide and the effect of DHT by flutamide as well as ICI. These results strongly suggest that the opposite effects of sex steroids on osteoclast and osteoblast apoptosis are mediated via a similar mechanism of action and that in both cases are sex-nonspecific and gender-independent.

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A Pure Anti-estrogen ICI 182780 Inhibits Osteosclerotic Bone Metastases in an Animal Model of Breast Cancer. B. Yi, P. J. Williams,* M. Niewolna,* T. Yoneda. Medicine/Endocrinology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Breast cancer has a predilection for spreading to bone. Several clinical studies have reported that estrogen receptor (ER)-positive breast cancers more preferentially spread to bone than ER-negative breast cancer. Although ER-positive breast cancers respond well to anti-estrogen therapy, the effects of anti-estrogens on bone metastases have not been specifically explored to date. Here, we tested ICI 182780, a new compound with pure anti-estrogen activity, for its potential to suppress bone metastases caused by ER-positive breast cancer. To approach this, we established an animal model in which the ER-positive MCF-7 human breast cancer cells overexpressing the oncogene Neu (MCF-7/Neu) developed osteosclerotic bone metastases following left cardiac ventricle inoculation in female nude mice with no requirement for estrogen supplementation. The ICI 182780 was found to profoundly inhibit transcriptional activity of ER using an ERE-CAT reporter construct in MCF-7/Neu cells. Histomorphometric examination showed that ICI 182780 (5mg/mouse, sc, once a week) significantly decreased metastatic tumor burden in bone and tumor-induced osteosclerotic lesions compared with control mice. ICI 182780 given according to this protocol was shown to have little effects on bone in control non-tumor-bearing mice radiologically and histologically. Since it has been shown that MCF-7/Neu cells produce PDGFBB in culture and that PDGFBB plays a critical role in the development of osteosclerotic bone metastases in mice, the effects of ICI 182780 on PDGFBB production in MCF-7/Neu cells were next examined. We found that ICI 182780 dose-dependently decreased the production of PDGFBB. Furthermore, histological examination demonstrated that the conditioned media harvested from ICI 182780-treated MCF-7/Neu cells had reduced bone-forming activity as assessed in organ cultures of mouse neonatal calvariae. Finally, ICI 182780 inhibited anchorage-independent growth of MCF-7/Neu cells. In summary, our results suggest that a pure anti-estrogen ICI 182780 decreased osteosclerotic bone metastases caused by MCF-7/Neu human breast cancer with inhibition of cell growth and production of osteosclerotic PDGFBB through suppressing transcriptional activity of ER. The new anti-estrogen ICI 182780 may be an effective agent in endocrine therapy for bone metastases in ER-positive breast cancers.

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The Role of Estrogen Receptor-beta (ER-b) in the Early Age-Related Bone Gain and Later Age-Related Bone Loss. H. Z. Ke, K. L. Chidsey-Frink, H. Qi, D. T. Crawford, H. A. Simmons, T. A. Brown, D. N. Petersen, M. R. Allen, J. D. McNeish, D. D. Thompson. Pfizer Global Research and Development, Groton Labs, Groton, CT, USA.

The mechanism of action of estrogens in skeletal tissue remains unclear. The purpose of our study was to understand the role of ER-b on cortical and cancellous bone during growth and the aging process by comparing the bone phenotype of 6- and 13-month-old female mice with or without ER-b. Groups of 11-14 wild-type controls (WT) and ER-b knockout female mice (BERKO) were necropsied at 6 and 13 months of age. At both ages, BERKO did not differ significantly from WT mice in uterine weight, indicating that ER-beta does not regulate the growth of uterine tissue. At 6 months of age, pQCT analysis of the distal femoral metaphysis (DFM) showed that BERKO mice had significantly higher total content (+24%), total bone area, cortical content (+19%), cortical area (+16%), and periosteal circumference (+7%) as compared with WT controls. Femoral length increased significantly by 5.5% in BERKO compared with WT. These results demonstrated that ER-b plays a role in periosteal bone formation, and longitudinal and radial bone growth during the growth period. There was no difference between BERKO and WT in trabecular density and marrow cavity area by pQCT, or trabecular bone volume (TBV), formation and resorption indices by histomorphometry of DFM at 6 months of age. In WT mice, TBV (-41%), trabecular density (-27%) and cortical thickness (-18%) decreased while marrow cavity area (+18%) and endocortical circumference (+19%) increased significantly at 13 months of age as compared with those at 6 months of age. These results indicate an age-related decrease in cancellous and endocortical bone in WT mice. These age-related changes did not occur in BERKO mice between 6 and 13 months of age. At 13 months of age, BERKO mice had significantly higher TBV (+157%), total content (+30%), total density (+24%), cortical content (+32%), cortical area (+36%), cortical thickness (+54%) and trabecular density (+48%) in DFM as compared with WT controls. Furthermore, BERKO mice had significantly lower osteoclast surface (-51%), osteoclast number (-50%) and bone turnover rate (-85%) in DFM compared with WT at 13 months of age. These results indicate that ER-b knockout protected against age-related bone loss in the cancellous and endocortical bone by decreased bone resorption and bone turnover in the aged female mice. These data demonstrate that estrogen receptor-beta plays an inhibitory role in periosteal bone formation, longitudinal and radial growth during the growth period, while it plays a role in stimulation of bone resorption, bone turnover and bone loss on cancellous and endocortical bone surfaces during the aging process.

Disclosures: Pfizer Global Research and Development,3.

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Estrogen Receptor-β Is the Key Receptor Supporting Protection of Bone Mass by Estrogen. L. Cao, R. M. McKeon, R. Bu, H. C. Blair. Pathology and Cell Biology & Physiology, University of Pittsburgh, Pittsburgh, PA, USA.

How estrogens or estrogen-like molecules protect skeletal mass remains controversial. We studied the dependence of osteoblast function and osteoclast differentiation on estrogen receptors [ERs]-α and -β by modifying the human osteosarcoma cell line MG63 to make stable cell lines expressing only ERα, ERβ, or both receptors. Cells where ER-α or ER-β were not expressed at detectable levels by Western analysis were made using plasmids with neomycin resistance and strong viral promoters driving antisense ERs. We examined estrogen-dependent protein expression, and support of osteoclast differentiation from human monocytes, by these cells. ER-α negative cells supported reduced osteoclast formation in co-cultures with normal human monocytic precursors when 10 nM estradiol was added, but there was no detectable difference between osteoclasts produced by co-cultures of these cells and control cells. However, when ER-β was not expressed, estrogen (estradiol, 10 nM) no longer affected osteoclast differentiation measurably. Surprisingly, RANKL and CSF-1 expression, with or without estrogen, did not change significantly in ER-α or -β-negative cells, suggesting that accessory growth factors are important in estrogen effects. ERs also affect expression of several key proteins in osteoblastic differentiation and induction of bone turnover. These were studied by Western analysis or semi-quantitative PCR. No significant differences between cell types, with or without estrogen, were seen when analyzing osteoprotegerin or BMPs 2 and 4. Collagen and alkaline phosphatase responded significantly to estrogen in control cells and ERα negative cells, but not in ERβ negative cells. It has also been questioned whether estrogen affects osteoclast formation from the standpoint of the monocytic precursors. While we have not tested effects of estrogen on promonocyte proliferation, no effect of estrogen on osteoclastic differentiation was seen when differentiation of human monocytic precursors was supported with CSF-1 and RANKL (without osteoblasts). Monocytes were derived from peripheral blood by apheresis. This suggests that estrogen effects, reported using marrow-derived monocytic cells, may result from mixed cell populations or may reflect effects on earlier stages of monocyte growth and differentiation. We conclude that, in a human osteoblast-like osteosarcoma-derived cell line that retains key features of osteoblastic differentiation, the major estrogen response in bone synthesis and protection from bone degradation is dependent on ER-β, and that the major effects on osteoclastic differentiation are mediated by the osteoblast, not by ERs in pre-osteoclastic monocytic cells.

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Conditional Mutagenesis of the IGF1 Receptor Gene in Osteoblasts Reduces Cancellous Bone Volume and Increases Turnover. M. Zhang,*[1] S. Xuan,*[2] M. L. Bouxsein,[3] D. von Stechow,*[3] M. Faugere,[4] H. Malluche,[4] G. Zhao,*[1] C. J. Rosen,[5] A. Efstratiadis,*[2] T. L. Clemens.[1] [1]Department of Medicine, University of Cincinnati, Cincinnati, OH, USA, [2]Department of Genetics and Development, Columbia University, New York, NY, USA, [3]Department of Orthopedics, Beth Israel Deaconess Medical Center, Boston, MA, USA, [4]Department of Medicine, University of Kentucky, Lexington, KY, USA, [5]Department of Medicine, St. Joseph's Hospital, Bangor, ME, USA.

Insulin like growth factor 1 (IGF1) exerts anabolic effects on bone and is thought to amplify other osteogenic signals. However, the cellular and molecular mechanisms that mediate the anabolic actions of IGF1 have been difficult to address experimentally primarily due to the complexity of the IGF system. To study the paracrine actions of IGF1 in skeletal tissue in a physiological context, we have used Cre-mediated recombination to disrupt selectively in mouse osteoblasts the gene encoding the type 1 IGF receptor (IGF1R). Mice expressing the site-specific recombinase gene (*cre*) under the control of the human osteocalcin (OC) promoter were crossed with Z/AP reporter mice which express β-galactosidase in the absence of Cre, but human placental alkaline phosphatase in its presence. Offspring from these matings demonstrated high levels (>90%) of recombination in bone osteoblasts and osteocytes. The OC-*cre* mice were then crossed with mice in which the 3rd exon of both *Igf1r* alleles was flanked by *loxP* sites ("floxed"; *Igf1r* $^{flox/flox}$). Progeny with an OC-*cre/Igf1r* $^{flox/+}$ genotype were then intercrossed. PCR analysis using DNA templates from tissues of OC-*cre/Igf1r* $^{flox/flox}$ offspring demonstrated that Cre-mediated recombination had occurred exclusively in bone. Six week old mice (*Igf1rδflox/δflox* in bones) were of normal size and weight. Micro CT analysis on femurs in groups of female mice (N=6) revealed a striking decrease in cancellous bone volume (78% of control), connectivity (53%), and trabecular number (86%), and an increase in spacing (119%). No significant changes were observed in cortical bone. The reduction in cancellous bone volume was confirmed by histomorphometric analysis. Surprisingly, however, the conditional *Igf1r* mutants had significantly greater osteoblast surface (146% of control), osteoid volume (170%), and increased osteoclast number (144%) and osteoclast erosion surface (185%). Thus, disruption of IGF1 signaling in mouse osteoblasts appears to cause a state of accelerated cancellous bone turnover possibly due to loss of inhibitory effects of IGF1 on bone resorption. These results establish a valuable model to investigate further the role of IGF1 signaling in bone development and turnover.

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Mice Lacking Insulin Receptor Substrate-2 Exhibit Osteopenia with Decreased Bone Formation and Increased Osteoblast-Mediated Osteoclastogenesis. T. Akune,[1] N. Ogata,[1] K. Hoshi,[1] N. Kubota,*[1] T. Kadowaki,*[1] H. Takagi,[2] Y. Azuma,[2] K. Nakamura,[1] H. Kawaguchi.[1] [1]Univ. of Tokyo, Tokyo, Japan, [2]Teijin Co. Ltd., Tokyo, Japan.

Insulin receptor substrates (IRS-1 and -2) are essential for intracellular signaling by IGF-I and insulin, anabolic regulators of bone metabolism. We previously reported that IRS-1 is important for maintaining bone turnover as a signaling molecule of IGF-I / insulin using IRS-1 deficient mice. We this time created mice lacking the IRS-2 gene (-/-) by homologous recombination in mouse embryonic stem cells, and investigated the role of IRS-2 in bone. Although -/- mice developed normally, significant decreases in both trabecular and cortex bone volumes were observed by bone densitometry and 3D-μCT analyses compared to wild-type (+/+) littermates at 4-16 weeks of age. Histomorphometric analysis at 8 weeks showed that bone resorption parameters (Oc.N/B.Pm & ES/BS) were approximately 40% increased in -/- mice compared to +/+ mice. Although osteoblast number was proportionally increased in -/- mice, bone formation parameters (MAR & BFR/BS) were 30-40% decreased. To investigate the cellular mechanism, we compared functions of primary calvarial osteoblasts (OBs), M-CSF-dependent bone marrow macrophages (BMMϕ), and osteoclasts isolated from co-culture of OBs and marrow cells (OCLs) derived from -/- and +/+ littermates. Although IRS-2 was expressed in all +/+ cells but none of the -/- cells, IRS-1 was expressed only in OBs and showed no compensatory increase in -/- OBs. Cultured -/- OBs showed similar proliferation ([^{3}H]-TdR) but markedly reduced differentiation and matrix synthesis as determined by ALP, Alizarin red, and von Kossa stainings, compared with +/+ OBs. IGF-I or insulin stimulated differentiation, matrix synthesis, and tyrosine phosphorylation of intracellular proteins in +/+ OBs, but not in -/- OBs. Neither osteoclastogenesis from soluble RANKL-stimulated BMMϕ nor OCL survival rate was different between -/- and +/+ cultures, indicating that IRS-2 signaling in cells of osteoclastic lineage is not important. Osteoclastogenesis and resorbed pit formation by $1,25(OH)_2D_3$, PGE_2 or IL-11 in co-culture of OBs and marrow cells were up-regulated only when OBs were derived from -/- mice independently of the origin of marrow cells, and -/- OBs showed greater induction of RANKL mRNA by these resorptive factors. It is concluded that IRS-2 deficiency causes osteopenia through impairment of bone formation and enhancement of osteoblast-mediated osteoclastogenesis. We propose that osteoblastic IRS-2 has anabolic actions on bone not only as a signaling molecule for IGF-I / insulin but also as an inhibitor of RANKL independently of IGF-I / insulin or IRS-1 signaling pathway.

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p57^{Kip2}, and p107 & p130, Regulate Chondrocyte Proliferation *in Vivo*, and May Be Targets of PTHrP Action. H. E. MacLean,[1] D. Cobrinik,*[2] P. Zhang,*[3] H. M. Kronenberg.[1] [1]Endocrine Unit, Mass. General Hospital & Harvard Medical School, Boston, MA, USA, [2]Medicine, Columbia University College of Physicians & Surgeons, New York, NY, USA, [3]Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA.

Targeted ablation of the negative cell cycle regulators, p57^{Kip2}, and p107 and p130, have shown that they are required for normal chondrocyte function. We have examined the chondrocyte phenotype of p57 knockout mice, and of p107/p130 double knockout mice, and explored possible relationships between PTHrP signaling and expression of p57, p107 and p130. Absence of either p57, or both p107 and p130, causes similar abnormalities in chondrocytes. In both cases, knockout chondrocytes show increased cell density, with prolonged proliferation at E14.5, delayed appearance of hypertrophic chondrocytes, and delayed mineralization of bone collar. These data are consistent with the hypothesis that p57, p107 and p130 are required to coordinate the cessation of chondrocyte proliferation, and transition to differentiation. We have previously shown that PTHrP maintains chondrocytes in the proliferative pool, with absence of PTHrP causing accelerated transition of chondrocytes from proliferation to differentiation, and shortened zones of round proliferative and columnar chondrocytes. We have also shown that overexpression of a constitutively active PTH receptor in transgenic mice results in decreased p57 expression. Therefore, we studied PTHrP/p57 double knockout mice, and PTHrP/p107/p130 triple knockouts, to determine if absence of these cell cycle factors could compensate for loss of PTHrP. Absence of either p57, or p107 and p130, causes partial reversal of the accelerated chondrocyte differentiation that occurs in PTHrP null mice. In the tibia, ulna, vertebrae and ribs of PTHrP/p57 double knockouts, or PTHrP/p107/p130 triple knockouts, the region of chondrocyte proliferation is expanded compared to PTHrP null littermates. There is a concomitant reversal of the accelerated differentiation that occurs in the PTHrP knockouts. The onset and extent of expression of hypertrophic chondrocyte markers including collagen X, are relatively normal compared to the early, expanded expression seen in PTHrP knockouts. The ulna, vertebrae and ribs of double and triple knockouts also show partial restoration of the zones of round and columnar proliferating chondrocytes, that are reduced in the PTHrP null. These results indicate that loss of either p57, or p107 and p130, can partially compensate for the absence of PTHrP *in vivo*. This suggests a potential model in which PTHrP is upstream of p57, p107 and p130, with PTHrP acting in part through down-regulation of these negative cell cycle factors.

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Targeted Disruption of the PTH Gene Leads to Abnormalities in Skeletal Development and Calcium Homeostasis. D. Miao,[1] B. He,*[1] B. Lanske,[2] D. Goltzman,[1] A. C. Karaplis.[1] [1]Dept. of Medicine, McGill University, Montreal, PQ, Canada, [2]Molecular Endocrinology, Max-Planck-Institut für Biochemie, Munich, Germany.

Parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP) bind to and activate the same PTH/PTHrP receptor. Targeted disruption of either the PTHrP gene or the PTH/PTHrP receptor gene leads to acceleration of differentiation of growth plate chondrocytes. To explore further the functional relationships of PTHrP, PTH, and the PTH/PTHrP receptor, we deleted the PTH gene using embryonic stem cell technology and investigated the consequences associated with PTH deficiency. Heterozygous mice were phenotypically normal. Crosses between these animals produced mice homozygous for the null PTH allele at the predicted Mendelian frequency. Although severe hypocalcemia and lethality were anticipated based on studies of Gcm2-null animals, over 95 percent of PTH-deficient mice were viable and had mild to moderate hypocalcemia and hyperphosphatemia associated with complete absence of circulating PTH, as determined by ELISA. Moreover, they developed normally and were fertile, although females had difficulty maintaining pregnancies and nursing their pups. Histological analysis of the parathyroids showed massive, diffuse enlargement of the glands consistent with their continuous stimulation by hypocalcemia. Since PTH has major effects on bone remodeling, we then analyzed the skeletons of PTH-negative animals at E18.5 and postnatally at 2, 4, 6 and 9 months of age. Cartilage development prenatally was characterized by a modest increase in the length of the growth plate compared to wild-type littermates, which was attributed primarily to an increase in the zone of hypertrophy. These changes, however, were not evident at any of the post partum periods examined and there were no other cartilage abnormalities. In mutant fetuses, trabecular bone volume was decreased whereas cortical thickness was increased. Postpartum, however, both trabecular and cortical bone consistently increased, and were associated with a low turnover state.These findings indicate that: first, in view of the normal viability of the mutant mice, the PTH gene is unlikely to encode the only thymus-derived calcium regulating factor; second, the effect of PTH on cartilage plate development prenatally is distinct from that of PTHrP and diminishes postpartum; and third, PTH exerts influences on bone remodeling which differ in the fetus and in growing animals. In the fetus, PTH appears to be necessary for promoting trabecular bone formation while limiting the development of cortical bone, whereas in the postpartum period it is required for establishing normal bone turnover and sustaining calcium homeostasis.

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The Transcription Factor C/EBP beta Promotes the Differentiation of Pluripotent Mesenchymal Stem Cells toward Osteoblasts. K. Hata, F. Ikeda,* T. Hiraga, K. Yamashita,* T. Nokubi,* R. Nishimura, T. Yoneda. Biochemistry, Osaka University Graduate School of Dentistry, Osaka, Japan.

C/EBP beta (C/EBPb) is a transcription factor which plays a contributive role in controlling the differentiation of mesenchymal stem cells into adipocytes in cooperation with PPAR gamma and C/EBP delta. Accumulating evidence that the mesenchymal stem cells are pluripotent and capable to differentiate into osteoblasts as well as adipocytes raises the possibility that C/EBPb also plays a role in osteoblastic differentiation. We studied this yet-unexplored hypothesis using the C3H10T1/2 pluripotent mesenchymal stem cells which differentiate into osteoblasts and adipocytes in the presence of appropriate stimuli. Osteoblastic and adipocytic differentiation were assessed by determining alkaline phosphatase activity and oil-red O staining, respectively. Upon treatment with BMP2, C3H10T1/2 cells showed osteoblastic and adipocytic differentiation with up-regulated C/EBPb expression. Overexpression of C/EBPb using an adenovirus vector induced not only adipocytic but also osteoblastic differentiation in the absence of BMP2. Promotion of osteoblastic differentiation by C/EBPb was enhanced in the presence of BMP2 or by co-infection of Smad1 and Smad4, whereas Smad6 blocked it. Moreover, co-infection of Cbfa1 with C/EBPb enhanced the osteoblastic differentiation, while dominant-negative Cbfa1 blocked it, suggesting an interaction between C/EBPb and Cbfa1. Consistent with this notion, we found that there was a physical association between C/EBPb and Cbfa1 as determined by co-immunoprecipitation experiments and that C/EBPb together with Cbfa1 activated osteocalcin gene transcription using a luciferase reporter construct. To further define the role of C/EBPb in the osteoblastic differentiation, we next examined the effects of a naturally-occurring isoform of C/EBPb ,LIP, which inhibits intact C/EBPb in a dominant-negative fashion. Surprisingly, introduction of LIP in C3H10T1/2 cells promoted osteoblastic differentiation in the absence of BMP2 and this effect was enhanced in the presence of BMP2 or by Cbfa1 overexpression. LIP also formed a complex with Cbfa1. Of note, LIP inhibited adipocytic differentiation induced by BMP2 or overexpression of C/EBPb and C/EBPdelta. In conclusion, our results suggest that C/EBPb, which has been thought to be an adipogenesis-regulating transcription factor, also promotes osteoblastic differentiation of mesenchymal stem cells with or without collaboration with Smad and Cbfa1. The results also suggest that the isoform of C/EBPb, LIP, may play a key role in directing the differentiation of the mesenchymal stem cells toward osteoblasts and adipocytes.

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Col1a1 Promoter-Targeted Expression of p20C/EBPbeta, a Truncated C/EBPbeta Isoform, Causes Osteopenia in Transgenic Mice. J. R. Harrison,*[1] P. L. Kelly,*[1] Y. Huang,*[2] D. J. Adams,*[3] M. Nahounou,*[3] G. Gronowicz,[3] S. H. Clark.*[4] [1]Department of Orthodontics, University of Connecticut Health Center, Farmington, CT, USA, [2]Department of Oral Diagnosis, University of Connecticut Health Center, Farmington, CT, USA, [3]Department of Orthopedics, University of Connecticut Health Center, Farmington, CT, USA, [4]Department of Genetics, University of Connecticut Health Center, Farmington, CT, USA.

Osteoblasts (OB) and adipocytes (AD) share a common progenitor in bone marrow, and C/EBP transcription factors are known to promote AD differentiation. We therefore hypothesized that overexpression of dominant negative p20C/EBPbeta in pluripotent progenitors might enhance OB differentiation by blocking adipogenesis. A construct was prepared in which the 3.6 kb Col1a1 promoter and first intron drive expression of FLAG-tagged p20C/EBPbeta. Previous studies have shown that pOBCol3.6 is expressed early in the OB lineage, prior to expression of differentiated OB markers. Four lines of pOBCol3.6-FLp20C/EBPbeta transgenic (TG) mice were established. Analysis of FLp20C/EBPbeta mRNA expression indicated that the transgene is targeted to bone, with low levels of expression in lung, skin and adipose tissue. Line 00-63 TG mice were obtained in Mendelian ratios, whereas survival was around 72% in lines 00-50-9 and 00-50-10, and 23% in line 00-65. TG mice from lines 00-50-10 and 00-65 were reduced in size compared to wild-type (WT) littermates, while lines 00-50-9 and 00-63 were of normal size. The abdominal fat pads in line 00-50-10 and 00-65 TG mice were noticeably reduced, indicating that adi-

pogenesis was inhibited. These lines also showed a dental phenotype manifested by malocclusion, overgrowth and breakage of incisors. Surprisingly, all four lines of TG mice showed evidence of osteopenia ranging from moderate to severe. By microCT, femoral trabecular bone volume (TBV) at 6 weeks of age was 19% in WT mice, compared to 10%, 5% and 1.5% in TG mice from lines 00-63, 00-50-10 and 00-65, respectively. TBV in line 00-50-9 TG mice was not different from WT at 6 weeks, but histomorphometry on 5 month-old TG mice revealed a significant decrease in trabecular bone area. No increase in osteoclast number per unit bone surface was observed in the lines examined (00-50-9 and 00-50-10), suggesting that osteopenia is not due to increased bone resorption. Moreover, long bones and calvariae of TG mice showed reduced OB marker mRNA levels, including COL1A1 and osteocalcin, consistent with an inhibition of bone formation in this model. These data suggest that C/EBP transcription factors, which are known regulators of AD differentiation, might also function as important determinants of OB differentiation and bone mass.

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The Role of Infection in Gender Differences in Mortality after Hip Fracture. L. E. Wehren,[1] W. Hawkes,*[1] D. L. Orwig,*[1] J. R. Hebel,*[1] S. I. Zimmerman,*[2] J. Magaziner.[1] [1]University of Maryland Baltimore, Baltimore, MD, USA, [2]University of North Carolina, Chapel Hill, NC, USA.

At present, men sustain 25-30% of all hip fractures and are approximately twice as likely to die during the 1 to 2 years following hip fracture as women. In a cohort of 804 community-dwelling men and women in the Baltimore Hip Studies, this difference was not explained by differences in age, pre-fracture functional ability or comorbid illness, type of fracture or surgery, or postoperative complications. The objective of this report is to compare cause-specific mortality during the 2 years following hip fracture for men and women. Overall mortality was relatively greater for men than women when compared to age- and gender-specific mortality in the general, with the most marked difference seen during year 1. Among men, the largest increases in cause-specific mortality relative to the general population of males aged 65+ were seen in infections: pneumonia, influenza, and septicemia. To determine whether gender differences in cause-specific mortality exist, death certificates for women from this cohort who died within 2 years of hip fracture were reviewed and death rates compared to cause-specific rates for the general population of females aged 65+. As had been seen for men, rate ratios for women were highest for septicemia and pneumonia/influenza during year 1, with some decline in year 2. If deaths due to these infections are omitted, mortality for men and women after fracture is much more similar and excess mortality is limited to the first year after fracture. Deaths attributable to infection appear to explain the gender difference in mortality in these elderly patients. These results suggest that immune competence may be differentially compromised in men and women after fracture, and that increased surveillance for infection may be warranted for both genders. Rate ratios (95% CI) for death compared to general population aged 65+

		Year 1	Year 2
All cause	Men	5.19 (2.77, 9.74)	1.31 (0.69, 2.47)
	Women	3.24 (2.18, 4.82)	1.55 (1.01, 2.38)
Pneumonia, influenza	Men	23.81 (12.81, 44.25)	10.38 (3.35, 32.19)
	Women	7.46 (6.51, 8.55)	4.00 (3.46, 4.62)
Septicemia	Men	87.91 (16.49, 175.80)	31.95 (7.99, 127.76)
	Women	36.72 (28.16, 47.87)	13.33 (10.16, 17.48)
Other causes	Men	3.46 (1.79, 6.67)	0.96 (0.48, 1.91)
	Women	2.47 (1.63, 3.72)	1.26 (0.80, 1.98)

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Recombinant Human Parathyroid Hormone (1-34) Therapy Reduces the Incidence of Moderate/Severe Vertebral Fractures in Men with Low Bone Density. E. Orwoll,[1] W. H. Scheele,*[2] A. D. Clancy,*[2] S. Adami,*[3] U. Syversen,*[4] A. Diez-Perez,*[5] S. L. Myers,*[2] B. H. Mitlak.[2] [1]Oregon Health Sciences University, Portland, OR, USA, [2]Eli Lilly and Company, Indianapolis, IN, USA, [3]University of Verona, Valeggio sul Mincio (VR), Italy, [4]University Hospital, Trondheim, Norway, [5]Hospital del Mar, Barcelona, Spain.

Recombinant human parathyroid hormone (1-34) [rhPTH(1-34)], given once daily, increased vertebral bone mineral density (BMD) and reduced vertebral fractures by 65-69% in postmenopausal women with osteoporosis (19-month median PTH exposure). Osteoporosis is common in men but there have been few large trials of therapies to prevent fractures in men. Thus, we performed a randomized, double-blind, placebo-controlled clinical trial of the effects of rhPTH(1-34) on bone mineral density in 437 men (mean age 59 yr) with spine or hip BMD >2 SD below male young adult mean. Men were randomly assigned to receive either placebo (n=147), PTH 20µg/day (n=151) or PTH 40 µg/day (n=139) by once-daily subcutaneous injections. The median study drug exposure was 11 months. After stopping therapy, 81% (355) of the men volunteered for an 18-month observation study of subsequent effects on BMD (data not shown) and fractures. During treatment and observation periods, all subjects received daily calcium and vitamin D supplements. Other osteoporosis treatment use was reported by 22% (79/355) of the men (placebo, 29%; PTH20, 16%; PTH40 22%) during the observation period. Vertebral fracture incidence was assessed from lateral spinal radiographs using a semi-quantitative grading score in 269 men with radiographs at both the original study baseline and 18 months after discontinuation of rhPTH(1-34) (placebo, 101; PTH20, 87; PTH40, 81). At baseline, 41% of the men had ≥1 prevalent vertebral fractures. During the entire study period (median 30 months), 22 men had at least one new vertebral fracture [placebo =12 (12%) and combined PTH = 10 (6%)]. For the combined PTH treatment groups, the relative risk of new vertebral fractures was 0.50 (P=0.086; 95% CI, 0.23-1.12), comparable to that found in women during a similar observation period (RR=0.53; P<0.001; 95% CI, 0.42-0.68). There were fewer men with moderate or severe vertebral fractures in the PTH treatment groups than in placebo [placebo=7 (7%); combined PTH =2 (1%)]; relative risk 0.17 (P=0.029; 95% CI, 0.04-0.81). Therefore, rhPTH(1-34) treatment over 11 months significantly reduced the risk of moderate or severe vertebral fracture during a 30 month period including 18 months following discontinuation of parathyroid hormone treatment. rhPTH(1-34) is an effective therapy for osteoporosis in men.

Disclosures: Eli Lilly and Company,2,5; Merck,2,5; Proctor & Gamble,2,5; Roche,5.

1105

Incident Vertebral Fractures During an 18-Month Observation Period Following Discontinuation of LY333334 [Recombinant Human Parathyroid Hormone (1-34), rhPTH(1-34)] Use in Postmenopausal Women with Osteoporosis. R. Lindsay,[1] W. H. Scheele,*[2] A. D. Clancy,*[2] B. H. Mitlak.[2] [1]Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Eli Lilly and Company, Indianapolis, IN, USA.

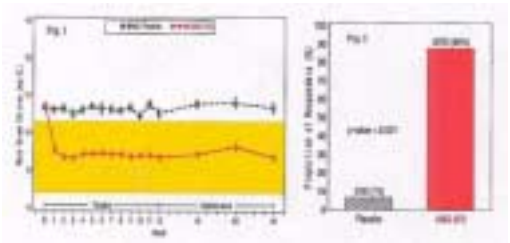
In a controlled clinical trial, 20 (PTH20) or 40 (PTH40) µg of parathyroid hormone (1-34) [rhPTH(1-34)] was shown to significantly reduce vertebral fractures by 65-69% in 1637 postmenopausal women with osteoporosis (19-month median PTH exposure). Lumbar spine bone mineral density (BMD) increased from baseline by 1.1% in the placebo group, compared with significant increases of 9.7% and 13.7% with PTH use (P<0.001). Of the original study population, 77% (1262/1637) of the women volunteered for an 18-month observation study to determine the effects of discontinuation of PTH on vertebral bone density and fractures. At 6 months after discontinuation of therapy, 29% of the women reported use of other forms of osteoporosis therapy; by 18 months after discontinuation, 54% of the women (57%, 53%, 52% for placebo, PTH20, PTH40, respectively, P=0.35) reported use of other therapies. Use of other treatments was permitted based on the treating physician's judgment. The primary analysis for incident vertebral fractures was performed without adjusting for other prescriptions. Vertebral fracture incidence was assessed from lateral spinal radiographs using a semi-quantitative grading score (Genant et al. JBMR 1993) in 1043 women with radiographs from original study endpoint and 18-month observation period. During the treatment period (baseline to 21 months), participants had an absolute risk reduction for ≥1 incident vertebral fracture of 9% and 10% for PTH20 and PTH40, respectively, and after an additional 18 months of observation (baseline to 39 months), the absolute risk was 13% for each PTH group. For new moderate and severe fractures, the absolute risk reduction was 8% and 6% for PTH20 and PTH40, respectively, after the 21-month treatment period. For moderate or severe incident vertebral fractures during the 39-month period (21 months treatment plus 18 months observation), the absolute risk reduction was 11% for both PTH study groups. Therefore, there is evidence of durable effects of treatment of rhPTH(1-34) for vertebral fracture efficacy even after treatment is stopped.

Disclosures: Eli Lilly and Company,5,8; Proctor & Gamble,2,5,8; Aventis,8; Wyeth-Ayerst,2.

1106

The Calcimimetic AMG 073 Reduces Serum Calcium (Ca) in Patients with Primary Hyperparathyroidism (PHPT). M. Peacock,[1] D. M. Shoback,[2] W. E. Greth,[3] T. A. Binder,*[4] T. Graves,*[4] R. M. Brenner,*[4] S. A. Turner,*[4] R. Marcus.[5] [1]Indiana Univ Sch Med, Indianapolis, IN, USA, [2]SF Veterans Affairs Med Ctr, UCSF, San Francisco, CA, USA, [3]Clinical Research Ctr of Reading, LLP, Reading, PA, USA, [4]Amgen Inc., Thousand Oaks, CA, USA, [5]Veterans Affairs Med Ctr, Palo Alto, CA, USA.

Calcimimetics reduce PTH secretion, and subsequently serum Ca, by increasing the sensitivity of the parathyroid calcium-sensing receptor to extracellular Ca. In a prospective, double-blind, 24 week trial, 78 patients with PHPT and Ca levels > 10.3 mg/dL and ≤ 12.5 mg/dL were randomized to receive twice daily oral doses of 30 mg AMG 073 or placebo. Twenty-three percent of patients had recurrent PHPT after surgical parathyroidectomy. Patients were dose titrated to a maximum dose of 50 mg bid AMG 073/placebo to achieve a serum Ca within the normal range. They were then treated with a fixed dose for 12 weeks in a maintenance phase (MP). The effects of AMG 073 and placebo on predose serum Ca are shown (Figure 1). The normal range is shaded. In the AMG 073 group, 88% of patients ("responders") experienced a reduction in predose serum Ca of at least 0.5 mg/dL and a mean predose serum Ca over the MP of ≤ 10.3 mg/dL as compared with 5% of placebo-treated patients (Figure 2). Maximum reductions in mean PTH were observed 2 to 4 hours after AMG 073 dosing (approx. 50%). During the MP, there was a 7.6 (± 22.9)% reduction in mean (SD) PTH at 12 hours post-dose in the AMG 073 group as compared with an increase of 7.7 (± 26.6)% in the placebo group (p=0.009). No differences between the two groups were observed in the change from baseline in 24-hour urine calcium/creatinine ratio.In this 24-week study of patients with PHPT, AMG 073 was well-tolerated; adverse events were similar in the two treatment groups. In this study, AMG 073 effectively reduced serum Ca to the normal range with concurrent reductions in PTH in patients with PHPT.



Disclosures: Amgen Inc.,3,5.

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Antifracture Efficacy of Risedronate: Prediction by Change in Bone Resorption Markers. R. Eastell,[1] I. Barton,[2] R. A. Hannon,[1] P. Garnero,[3] A. Chines,[2] S. Pack,[2] P. D. Delmas.[3] [1]Bone Metabolism Group, Division of Clinical Sciences (North), University of Sheffield, Sheffield, United Kingdom, [2]Procter and Gamble Pharmaceuticals, Cincinnati, OH, USA, [3]INSERM, Lyon, France.

The early decrease in vertebral fracture risk with antiresorptive therapy could be explained by the early decrease in bone resorption markers. We have tested this directly by measuring the bone resorption marker, N-telopeptide of type I collagen (NTX) in the VERT study. We studied 2442 women with at least one vertebral deformity (mean age, 69 years, SD 7) who were treated with calcium supplement (and vitamin D if required) and placebo or risedronate 5 mg/day for 3 years. Samples were collected at baseline, 3 and 6 months on a subset of 28% of the total. We measured NTX in second morning void urine samples using an automated immunoassay analyser (Ortho Clinical ECi, CV<4%) and expressed the result as a ratio to creatinine. Vertebral fracture was assessed by morphometric and semiquantitative approaches (with adjudication in discrepant cases)-the overall reduction over the 3 years was 43%. We examined the relationship between NTX/Cr and fracture risk using non-parametric (Classification and Regression Tree, CART) and parametric (logistic regression) analyses. The median NTX/Cr at baseline was 70 nmol BCE/mmol and the median decrease at 6 months with calcium and placebo was 22% and with calcium and risedronate 55%. A baseline NTX/Cr of more than 49 nmol BCE/mmol in the calcium and placebo group (or more than 64 in the calcium and risedronate group) predicted increased risk of fracture (CART analysis P<0.05). There was a significant association with decrease in NTX/Cr at 3 months in the calcium and risedronate group (logistic regression, P<0.05). This predicted that for a 30% reduction in NTX/Cr (placebo vs. risedronate difference) there would be a 17% decrease in vertebral fracture risk. Thus, 40% of the effect of risedronate on vertebral fracture risk could be explained by the change in NTX/Cr at 3 months (17%/43%=40%). The relationship between the change in NTX/Cr and fracture risk was best fit by a cubic logistic regression model, especially at the 6-month time point. There was little further improvement in fracture benefit below a decrease of 60% in NTX/Cr. Thus high bone resorption predictes increased vertebral fracture risk in the placebo group; decrease in bone resorption markers predict up to 40% of the effect of risedronate on vertebral fracture risk; there may be no further benefit in decreasing bone resorption to very low levels.

Disclosures: Procter and Gamble Pharmaceuticals,2.

1108

Fall Prevention by Vitamin D and Calcium Supplementation: A Randomized Controlled Trial. H. A. Bischoff,[1] H. B. Staehelin,*[2] W. Dick,*[3] R. Akos,*[3] R. Theiler,[4] M. Pfeiffer,[5] B. Begerow,[5] R. A. Lew,*[1] M. Conzelmann.*[6] [1]Rheumatology, Immunology and Allergy; the Robert B. Brigham Multipurpose Arthritis Center, Brigham and Women's Hospital, Boston, MA, USA, [2]Geriatrics, University Basel, Basel, Switzerland, [3]Orthopeadics, University Basel, Basel, Switzerland, [4]Rheumaltology, Kantonsspital Aarau, Aarau, Switzerland, [5]Institute for Clinical Osteology, Bad Pyrmont, Bad Pyrmont, Germany, [6]Geriatrics, Felix Platter Spital, Basel, Switzerland.

We prospectively studied the combined effect of vitamin D and calcium supplementation upon the risk of falling and muscle strength in the elderly. In a double-blind randomized controlled trial we studied 122 elderly women in long-stay geriatric health care institutions (mean age in years: 85.3; range: 63-99). Falls were recorded for a 6 week pretreatment and for a 12 week treatment period. Subjects received either 1200 mg calcium plus 800 IU cholecalciferol (Cal+D-group; n = 62) or 1200 mg calcium (Cal-group; n = 60) per day. Poisson-adjusted number of falls (having 0, 1, 2-5, 6-7, >7 falls) were compared between groups. Adjustments were performed for observation time during treatment, number of falls during the pretreatment period, number of fallers during the pretreatment period, age, body mass index, days in the institution before study entry, use of a walking aid, Folstein Mini Mental Status, Charlson Comorbidity Index, number of medications, baseline 25-hydroxyvitamin D, baseline 1,25-dihydroxyvitamin D and baseline musculoskeletal function, expressed as a summed score of knee flexor and extensor strength, grip strength and the timed up&go test. Before treatment, 22 falls occurred in the Cal+D-group and 20 in the Cal-group. After treatment, there were 25 falls in the Cal+D-group and 55 in the Cal-group. The Cal+D treatment accounted for a 49% reduction of falls (95% Confidence Interval [14%; 71%]; p < 0.01). Also, musculoskeletal function improved significantly in the Cal+D-group (p = .0094). Among subjects in the Cal+D-group, there were significant increases in median serum 25-hydroxyvitamin D (+ 71%) and 1,25-dihydroxyvitamin D (+ 8%), respectively significant decreases in serum iPTH (-29%), alkaline phosphatase (-7%), urinary dpd (- 30%) and urinary N-telopeptides (-26%). A single intervention with vitamin D plus calcium over a 3 month period reduced the risk of falling by 49%. This effect could be explained by an improvement in musculoskeletal function.

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BMP-antagonist Sclerostin is Expressed in Mineralized Bone and Blocks BMP-induced Bone Formation In Vitro. R. L. van Bezooijen,[1] M. Karperien,[1] A. Visser,*[1] H. Hamersma,*[2] D. Winkler,*[3] T. Hayes,*[3] J. Skonier,*[3] K. Staehling-Hampton,*[3] J. A. Latham,[3] S. E. Papapoulos,[1] C. W. G. Lowik.[1] [1]Dept. of Endocrinology, Leiden Univ. Med. Center, Leiden, The Netherlands, [2]Florida Park, Roodepoort, South Africa, [3]Celltech, Bothell, WA, USA.

Sclerosteosis is a skeletal dysplasia characterized by progressive skeletal overgrowth. Bone formation is largely increased while resorption is not affected or slightly inhibited. Sclerosteosis results from loss of the SOST gene product sclerostin, a protein that shares similarity with cystine knot-containing factors belonging to the BMP-antagonist family. In the present study, we studied SOST expression in relation to bone formation and the effect of sclerostin on BMP-activity.Mouse bone marrow cells and osteoblastic KS483 cells were cultured under bone formation inducing conditions (ascorbic acid and beta-glycerophosphate). In these cultures, SOST mRNA expression was found after induction of osteocalcin mRNA expression and onset of mineralization, i.e. from day 14 and 17 onwards in KS483 and mouse bone marrow cultures, respectively. In human bone marrow cultures, which form a mineralized matrix without nodules in the presence of ascorbic acid, beta-glycerophosphate, and dexamethasone, SOST mRNA was at low levels and less clearly expressed, but tended to be higher after onset of mineralization. Stimulation with BMP-4 (50 ng/ml) or BMP-6 (100 ng/ml) induced SOST mRNA expression after 24 and 72 hours in undifferentiated human bone marrow cells.Alkaline phosphatase (ALP) activity induced by BMPs was blocked by recombinant sclerostin produced by SF-9 cells. Sclerostin blocked BMP-6 (100 ng/ml)-induced ALP activity dose-dependently with an IC50 of 100 ng/ml. Sclerostin, up to 5 µg/ml, decreased BMP-4 (50 ng/ml)-induced ALP activity by only 56%.Expression of SOST mRNA was further studied by in situ hybridization (ISH) in fetal and neonatal bones and by RT-PCR in adult bone. In 17.5-day-old mouse embryos, SOST expression was found in all mineralized bones of both intramembraneous and endochondral origin. The other tissue with significant SOST mRNA expression were arteries adjacent to the heart. In 5-day-old neonatal radii and ulnae, expression was again found within mineralized bone. Finally, SOST expression was found in RNA isolated from tibia of adult mice and a human femur head.In summary, in vitro and in vivo SOST mRNA expression data suggest that expression of sclerostin is restricted to embryonic and adult mineralized bone, possibly by mature osteoblasts and/or osteocytes. Blocking of BMP-induced ALP activity by sclerostin in osteoblastic KS483 cells suggests that increased bone formation in sclerosteosis is due to unopposed anabolic BMP activity.

1110

JAB1 Promotes Smad4 Degradation through Proteasome-Ubiquitin Pathway. M. Wan, X. S. Cao,* Y. Wu,* S. Bai, L. Wu,* X. Shi, X. Cao.* Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Smad4 is the common mediator in the TGF-β, activin and BMP signaling pathways. Degradation of Smad proteins is an important means of regulating functional activity in the TGF-β superfamily. Smurfs have been shown to mediate the degradation of both regulatory Smads (R-Smads) and inhibitory Smads (I-Smads), but not common partner Smad4. The regulation of Smad4 protein steady-state level remains unknown. Here we report the mechanism of Smad4 degradation. JAB1 (a protein initially identified as a co-activator of c-Jun) directly interacts with Smad4 and induces its ubiquitination for degradation. To examine the mechanism of JAB-1 that opposes TGF-β activity, we tested potential interaction between JAB-1 and Smads in yeast two-hybrid assays. Results of β-gal activity indicated that JAB-1 interacts with Smad4, but neither Smad1 nor Smad3. We then examined the effects of the interaction on endogenous Smad4 steady-state level. HA-JAB1 expression plasmid was transfected in Mv1Lu cells treated with or without TGF-β. We found JAB1 dose-dependently down-regulates Smad4. Lactacystin and LLM, inhibitors of the 26S proteasome, were found to inhibit degradation of Smad4, indicating that Smad4 is targeted for degredation by JAB1 through the ubiquitin-proteasome pathway. Moreover, pulse-chase assay was also performed to detect the effect of JAB1 on Smad4 stability. JAB1 largely enhanced the protein degradation rate of newly synthesized proteins. Again lactacystin treatment of COS-1 cells transfected with JAB1 yielded a decrease in the rate of protein degradation of newly synthesized proteins in comparison with untreated cells. Proteins destined for degradation by the 26S proteasome are marked by covalent attachment of ubiquitin chains, which can be recognized by the 26S proteasome. Therefore, we examined whether JAB-1 induces ubquitination of Smad4. Conjugated ubiquitin of Smad4 is detected when cells are transfected with ubiquitin expression plasmid together with HA-JAB1, confirming that the degradation of Smad4 by JAB1 is through proteasome-ubiquitin pathway. Finally, we determined the functional relevance of JAB1-mediated Smad4 down-regulation by luciferase assay. As we expected, JAB1 dose-dependently inhibited TGF-β-induced gene transactivation in Mv1Lu cells. Our data suggest that JAB-1 antagonizes TGF-β function by inducing degradation of Smad4 through Proteasome-Ubiquitin Pathway. These findings suggest that JAB1 may play a critical role in regulating cell proliferation and cell differentiation in response to TGF-β and BMP through a distinct degradation pathway.

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Functional Analysis of GDF-6/BMP-13 During Skeletogenesis. L. W. Gamer,*[1] K. A. Cox,[1] G. Hattersley,[2] V. Rosen.[3] [1]Genetics Institute, Cambridge, MA, USA, [2]Millenium Pharmaceuticals, Cambridge, MA, USA, [3]Forsyth Institute, Boston, MA, USA.

Bone Morphogenetic Proteins (BMP) and Growth and Differentiation Factors (GDF) play important roles in formation of the embryonic skeleton. During limb development, Gdf-5 is expressed in the interzone of all joints while the highly related Gdf-6/Bmp-13 localizes only to the forming wrists and ankles. Gdf-5 has been shown to be essential for appendicular skeletal development, acting during the initial stages of chondrogenesis to promote cell adhesion and later during joint formation to control the growth of the epiphyseal cartilages. While mutations in the Gdf-5 gene in mice in humans have identified a direct role for Gdf-5 in skeletal patterning, the role of Gdf-6/Bmp-13 in the skeleton remains unclear. To understand the function of GDF-6/BMP-13 in the appendicular skeleton, we overexpressed the protein in chick limb by bead implantation. BMP-13 increased the size of the cartilage elements but did not cause the induction of ectopic of joint tissue. Mesenchymal cells around the BMP-13 bead appeared disorganized and immature and in situ hybridization studies revealed that BMP-13 caused a delay or disruption of chondro-

cyte maturation as measured by reduced levels of ihh and collagen type X. Unlike GDF-5, BMP-13 did not induce more cartilage by increasing cell number. Instead, BMP-13 treatment appeared to divert mesenchymal cells into the chondrocyte lineage, as we saw an inhibition of the muscle marker, MyoD in BMP-13 treated limb after 24 hrs. We next looked at the pathway of BMP-13 induced chondrogenesis by testing the effects of BMP-13 on MLB13MYC, a skeletal progenitor cell line. When MLB13MYC cells were treated with BMP-13 protein, they expressed cartilage markers collagen type II and aggrecan, but failed to express markers of more mature cells like ihh, PTH/PTHrP receptor, or collagen type X. Even at high doses and long culture periods, BMP-13 treatment did not result in a bone phenotype or the expression of joint markers such as chordin or Gdf-5. Our data indicate that BMP-13 induces more cartilage in the limb in two ways: early in limb formation it recruits stem cells into chondrocytes; and, later in limb development it delays chondrocyte maturation, resulting in radial thickening of the skeletal elements. In comparison, GDF-5 causes a similar enlargement of the skeletal elements through a different pathway, increasing proliferation and adhesiveness in the affected cartilage cells. Taken together, our data suggest that individual GDFs play multiple roles in the later stages of joint formation, functioning as secondary secreted signals from the interzone region that act on the adjacent cartilages to control their growth and differentiation.

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An Osteoblast Phage Display Library Identifies TRIP to Have High Affinity for TRAP. J. E. Puzas, T. J. Sheu,* E. M. Schwarz, R. J. O'Keefe, R. N. Rosier. Orthopaedics, University of Rochester, Rochester, NY, USA.

In normal bone remodeling, bone resorption is coupled to bone formation at all sites in the adult skeleton. This coupling occurs in both a temporally and spatially coordinated fashion. In order to investigate the site specificity of this process, we have used a T7 phage-display library expressing proteins produced by osteoblasts to probe components of an osteoclast resorption surface. Our initial studies have documented that TRIP (TGF beta receptor interaction protein) has a very high affinity for the osteoclast lysosomal enzyme, TRAP (type V tartrate resistant acid phosphatase). The phage-display cDNA library utilizes T7 phage to express proteins from a cDNA osteoblast library. The phage are then used to detect specific protein-protein interactions with a biopanning technique. The target material is purified TRAP immobilized on plastic culture dishes. After 3 rounds of selection/amplification, individual clones are characterized by DNA sequencing. In order to test if TRAP could activate TGF beta signaling pathways we exposed osteoblasts to soluble TRAP and measured parameters of cell differentiation. We also transfected osteoblasts with a TGF beta reporter (P3TP-Lux) and a dominant negative type II TGF beta receptor construct to characterize the effect of TRAP on signaling. A mammalian two-hybrid system was used to verify the association between TRIP and TRAP. We sequenced the clones that demonstrated high affinity binding. The DNA sequence that demonstrated one of the highest affinities for TRAP was TRIP. TRIP is known to bind to the type II TGF beta receptor and modulate its activity. Our results show that both TGF beta and TRAP strongly stimulate alkaline phosphatase activity, osteoprotegerin expression and cbfa1 levels in osteoblasts. TRAP also strongly up regulates TGF beta reporter activity and this effect can be negated by the co-transfection of a dominant negative TGF beta type II receptor construct. Co-transfection of a gal-4 fusion protein containing TRIP with a VP16 fusion protein containing TRAP into MG-63 cells in a mammalian two-hybrid system demonstrated a positive protein/protein interaction between TRIP and TRAP. TRIP was selected from an osteoblast phage display library as having high affinity for TRAP (tartrate resistant acid phosphatase). This affinity was verified in a mammalian two hybrid system. Functionally, TRAP appears to activate the TGF beta signaling pathway and can mimic the effects of TGF beta on osteoblast differentiation. From these data we conclude that TRAP can have a growth factor-like effect in osteoblasts. Given its localization within resorption lacunae TRAP may act as a site-directing molecule for bone formation.

1113

c-fms and the αvβ3 Integrin Collaborate During Osteoclast Differentiation. R. Faccio,*[1] S. Takeshita,[1] D. V. Novack,[1] X. Feng,[1] A. Zallone,[2] F. P. Ross,[1] S. L. Teitelbaum.[1] [1]Pathology, Washington University, St. Louis, MO, USA, [2]Human Anatomy, University of Bari, Bari, Italy

While the αvβ3 integrin is important for osteoclast (OC) function we also find that the number of OCs generated, in vitro, from αvβ3-/- marrow macrophages is substantially diminished (10 fold, $p<0.001$). Thus, the αvβ3 integrin also modulates OC differentiation. Reflecting this differentiation arrest, RT-PCR demonstrates a decrease in calcitonin receptor (12 fold), TRAP (2 fold) and cathepsin K (3 fold) expression in αvβ3 deficient OCs precursors. Moreover, the mRNA level of microphthalmia, a transcription factor involved in formation of mature OCs, is also dampened in β3-/- cells (5 fold) after 3 days in culture. Interestingly, we find that, while high doses of RANKL have no effect, increasing the concentration of M-CSF from 10 ng/ml - to 100 ng/ml completely rescues the differentiation defect in β3-/- OC precursors, normalizing the number of TRAP positive cells, and restoring expression of the above OC markers. Thus, signals transduced by the M-CSF receptor, c-fms, and the αvβ3 integrin collaborate in osteoclast formation. To identify the residues within the β3 integrin responsible for its ability to compensate for c-fms, various β3 mutants were retrovirally transduced into the β3-/- OC precursors. While expression of wild type β3 integrin restores OC differentiation, deletion of the entire β3 integrin cytoplasmic tail abrogates this effect. Point mutational analysis demonstrates OC differentiation is mediated by S752 in the β3 cytoplasmic domain. We next turned to the component of c-fms critical for osteoclastogenesis in the absence of the β3 integrin. To this end, we retrovirally transduced OCs precursors with a chimeric receptor consisting of the external domain of the erythropoietin (Epo) receptor and the transmembrane and cytoplasmic domains of c-fms (EpoR/c-fms). Cells bearing this chimeric receptor differentiate into OCs in the presence of Epo and RANKL, without the necessity for M-CSF. In this circumstance, Epo activates c-fms mediated signals via the chimeric, but not endogenous, M-CSF receptor. We then mutated 6 tyrosines in the c-fms cytoplasmic tail of this chimeric receptor, a strategy known in other circumstances, to dampen M-CSF signaling. Only one EpoR/c-fms mutant, Y697F, differentially affects the osteoclastogenic capacity of β3+/+ and β3-/- cells. Specifically, while not impacting β3+/+ cells, this mutation diminishes the capacity of β3-/- macrophages to differentiate into OCs by 3 fold ($p<0.001$), even at high doses of Epo. Thus, c-fms, via Y697, and the β3 integrin, via S752, compensate for each other in the osteoclastogenic process.

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The Crystal Structure of RANKL Reveals Determinants of Receptor-Ligand Specificity. J. Lam, C. A. Nelson,* F. P. Ross, S. L. Teitelbaum, D. H. Fremont.* Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis, MO, USA.

To determine the precise structural elements of RANK ligand (RANKL) that mediate its osteoclastogenic properties, we have crystallized the ectodomain of murine RANKL and solved its structure. RANKL (158-316) crystallized by vapor diffusion as two forms: rhombohedral space group R3 with 7 molecules per asymmetric unit (ASU), arranged as 2 trimers with a seventh monomer situated on a 3-fold axis of rotational symmetry, creating an additional trimer; and orthorhombic space group $P2_12_12_1$ with 1 trimer per ASU. The structure of RANKL was solved by molecular replacement and refined to a resolution of 2.6 Å (crystallographic R factor 23.5% and R-free 28.0%). Each RANKL monomer comprises a β-sandwich conforming to a jellyroll topology, with 2 antiparallel β-pleated sheets formed by strands A, H, C, and F in one sheet, and strands B, G, D, and E in the other. The inner AHCF β-sheet forms the trimeric interface, while the BGDE β-sheet forms the outer surface. RANKL monomers self-associate tightly along a 3-fold axis of rotational symmetry to form a bell-shaped homotrimer, assembling such that one edge of the β-sandwich (strands E and F) in each monomer packs against the inner hydrophobic face of the AHCF β-sheet of the neighboring monomer. Importantly, within the TNF family, the RANKL structure displays unique conformations of the AA' (170-193), CD (224-233), DE (245-251), and EF (261-269) loops. These solvent-accessible surface loops and flanking regions of adjacent β-strands form unique structural patterns of electrostatic charge in two regions that may act as interfacial sites for the receptor RANK. To assess the biological relevance of this structural prediction, and to identify regions of RANKL that are necessary for biological activity, a structure-based mutagenesis was performed to delete a portion of the AA' loop (δ177-183), swap the AA' loop of RANKL with that of TNF (δLS), or induce a single amino acid substitution in the DE loop (I248D). The δ177-183 and δLS mutants demonstrate a 90-100% decrease in osteoclastogenic capacity relative to native RANKL, while the I248D mutant exhibits an 8-fold decrease in potency (100 ng/ml ED_{50} for I248D RANKL vs. 12 ng/ml ED_{50} for native RANKL). Furthermore, the morphology of osteoclasts generated with I248D RANKL does not recapitulate that of resorptive polykaryons generated with native RANKL. These observations confirm a critical role for the AA' and DE loops in conferring the osteoclastogenic capacity of RANKL. Such structural determinants of receptor-ligand specificity may be of relevance in the design of therapeutic compounds for osteopenic disorders.

1115

Integration of Age, Prevalent Fracture Status and DEXA Bone Density to Provide Precise 5 Year Risks of Fracture. R. L. Prince,[1] D. Doherty,*[1] S. S. Dhaliwal.*[2] [1]Dept. of Medicine, Univ. of Western Australia, Perth, Australia, [2]Dept. of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, Australia.

Appropriate clinical care for osteoporosis is directed at management to prevent fractures occurring in the future. As with any disease the intervention must be appropriate to the risk. We have recently published precise 5-year risks of appendicular and axial fracture by age dichotomised by the presence or absence of previous fracture (Doherty et al Osteoporo. Int. 2001;12:16-23). We now include DEXA bone density measurement in the risk evaluation. Using data from Marshall et al (BMJ. 1996;312:1254-59) we have selected the total hip site as representative of the approach. At this site the change in risk per SD change in BMD is 2.6. We apportioned the population risk of fracture by bone density SD (Z score) taking into account the proportion of the population in each SD category. We then calculated the proportion of fracture patients in each Z score category. Finally we have illustrated the effects in a population of 75 to 79 year old women without prevalent spine fracture who have a 5-year risk of first clinical spine fracture of 3.3%.

SD group (Z score)	Relative risk of fracture (2.6/SD)	Proportion of total population	5 year Z score group risk (%) for a total population risk of 1%	5 year Z score group risk (%) for a total population risk of 3.3%
3.5 - 2.5	0.057	0.006	0.03	0.1
2.5 - 1.5	0.148	0.061	0.09	0.3
1.5 - 0.5	0.385	0.242	0.24	0.8
0.5 - -0.5	1.000	0.383	0.61	2.0
-0.5 - -1.5	2.600	0.242	1.59	5.3
-1.5 - -2.5	6.760	0.061	4.14	13.7
-2.5 - -3.5	17.576	0.006	10.77	35.5

Calculation of age specific fracture rates which include DEXA bone density Z scores and previous history of fracture provide a simple clinical tool to make treatment decisions in consultation with the patient. Large controlled trials have illustrated the treatment benefit of therapy in patients with a T score of –2.5 which corresponds to a Z score of –1 in 75 to 79 year old women. In these patients spine fracture rates are reduced by up to 50 % of baseline risk. Thus the absolute fracture risk reduction should be a saving of approximately 2.8 to 17.6 fractures per 100 women per 5 years depending on BMD category. Similar simple risk calculations can be made for other fracture sites at other ages. These calculations should aid clinical decision-making.

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Standardised Fracture Risk Criteria: Alternative to T-scores for Identifying Subjects at Risk. C. C. Glüer,[1] D. M. Reid,[2] R. Eastell,[3] D. Felsenberg,[4] C. Roux.[5] [1]University Hospital Kiel, Kiel, Germany, [2]University of Aberdeen, Aberdeen, United Kingdom, [3]University of Sheffield, Sheffield, United Kingdom, [4]Free University Berlin, Berlin, Germany, [5]René Descartes University, Paris, France.

Different diagnostic approaches identify different subjects as "osteoporotic" when using T-scores for diagnosis of osteoporosis. We developed standardised fracture risk criteria based on the probability of having a vertebral fracture as predicted by dual x-ray absorptiometry (DXA) or quantitative ultrasound (QUS). Probability of fracture *p(fx)* was modelled using age-adjusted logistic regression. We defined a standardised risk classification criterion p_{cc}, as the cut-off level of p(fx) at which the number of cases considered "at risk" ($p(fx)>p_{cc}$) approximates the true number of cases with prevalent deformities for the most predictive techniques. Using the same p_{cc} cut-off for all techniques we calculated sensitivity, specificity, and Kappa scores among techniques. To assess a technique's power for classification beyond age-related risk estimates, we derived a multiplicative model for the odds of having a fracture: ODDS (total) = ODDS (age) * ODDS (method). The equivalent Z-score derived from this model reflects the difference in BMD or QUS results (expressed in units of standard deviations of age-adjusted population variance) associated with a doubling of the risk of having a vertebral deformity. The model was tested using data from a population-based sample of 1236 women, (aged 55 to 80 years), 195 of whom had a prevalent vertebral fracture, who participated in the Osteoporosis & Ultrasound (OPUS) study. All subjects had DXA of the lumbar spine and the total hip and QUS of the heel on 4 devices (Lunar Achilles+, Osteometer DTU-one, Quidel/Metra QUS-2, and DMS UBIS 5000) and of the finger on one device (IGEA DBM Sonic BP). For a cut-off level of p_{cc} = 0.25, corresponding to a T=-2.5 at age 65 for BMDhiptot NHANES III data, the number of women classified as "at risk" of vertebral deformity was close to the true number with some techniques (Table). Applying our new technique resulted in Kappa scores ranging from 0.43 to 0.63 compared to 0.07 to 0.35 when using T-scores.

Technique	no."at risk"*	Sensitivity*	Specificity*	Equivalent Z-score
DXAspine	187	32%	88%	1.53
DXAhiptot	198	34%	87%	1.71
DXAhipneck	171	29%	89%	1.86
Achilles SOS	194	29%	87%	1.63
DTU-one SOS	187	28%	87%	1.88
UBIS 5000 SOS	185	29%	88%	1.83
QUS-2 BUA	167	28%	89%	2.12
DBM AD-SoS	165	22%	88%	2.92

*: "at risk" criterion: p(fx)>0.25 The new approach provides a coherent framework for fracture risk assessment across different techniques and reduces discrepancies in classification.

Disclosures: Igea,2; Quidel,2; Osteometer,2; DMS,2.

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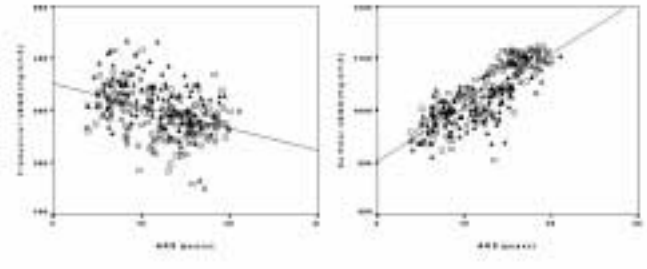
Distal and Mid-Shaft Tibia Bone Mass Assessment Using Peripheral Quantitative Computed Tomography (pQCT) in Healthy Children and Adolescents. L. J. Moyer-Mileur,* R. Roberts,* S. D. Ball.* Pediatrics, University of Utah, Salt Lake City, UT, USA.

This cross-sectional study used pQCT to evaluate the influences of age, gender, body size, and puberty on bone acquisition in healthy children and adolescents. The pQCT technique provides analyses of volumetric bone mineral density (vBMD, gm/cm^3) for total bone and bone compartments and bone strengthexpressed as polar strength strain index (PSSI, mm^2). Bone mass of the non-dominant tibia by pQCT (XCT 2000, Norland Medical Systems, Inc) was measured in 287 healthy Caucasian children and adolescents (166 girls; 121 boys; 4 to 19 y). Measurements were obtained at 4% and 66% from the distal end plate. The distal site (4%) assessed trabecular bone, and the mid-shaft site (66%) assessed total and cortical bone and muscle cross-sectional area (MCSA). Age, gender, weight, height, body mass index (kg/m2), and pubertal stage were recorded. Mean age, weight and height were 12.2 ± 4.2 y; 46.7 ± 21.7 kg, and 1.46 ± 0.2 m, respectively and pubertal stage distribution (Tanner 1-5) was 47%, 10%, 15%, 11%, and 17%, respectively. Age, gender, weight and height correlated with total, cortical, and trabecular bone mineral content (BMC, mg), bone area (BA, cm^2), vBMD, PSSI, and MCSA. Only cortical vBMD was influenced by puberty. Multiple regression found 69% of the variance in cortical vBMD explained by pubertal stage and age, 23.5% of the variance in trabecular vBMD explained by age and gender, and >75% of the variance in PSSI and MCSA explained by body weight ($p<0.001$). Boys had greater trabecular vBMD values than girls (317.9 vs 284.8 mg/cm^3; $p=0.001$) while total and cortical vBMD values were similar. Both genders had a 10% decrease in trabecular vBMD and 14% increase in total and cortical vBMD with age (Graph). We conclude that bone measurements by the pQCT technique provides information on bone acquisition, architecture, and strength that may prove useful in the assessment of normal bone growth and development as well as pediatric bone disease.



1118

A Prospective Study of Dry Calcaneal Quantitative Ultrasound and Fracture Risk in Older Women: The Study of Osteoporotic Fracture. D. C. Bauer,[1] L. Palermo,[2] D. M. Black,[2] T. A. Hillier,[3] J. A. Cauley.[4] [1]Department of Medicine, UCSF, San Francisco, CA, USA, [2]Department of Epidemiology and Biostatistics, UCSF, San Francisco, CA, USA, [3]Kaiser Center for Health Research, Portland, OR, USA, [4]Department of Epidemiology, University of Pittsburgh, Pittsburgy, PA, USA.

Prospective studies have found that low calcaneal quantitative ultrasound (QUS) is associated with an increased risk of hip and non-spine fractures. Newer dry devices have not been evaluated prospectively. We studied the ability of a dry QUS device and BMD to predict fractures in older women as part of the Study of Osteoporotic Fractures (SOF).4,153 women (mean age 80.0 ± 4.6 yr.) were examined at four clinical centers. Duplicate calcaneal QUS measurements (Hologic Sahara) and femoral neck BMD (Hologic QDR 1000) were obtained. Calcaneal BMD (Dove Medical) was measured at 3 clinics. Subsequent hip and other non-spine fractures were documented by central review of x-rays or x-ray reports. The relationships between QUS, BMD, and fracture were examined with proportional hazard models adjusted for age and clinic; results are presented as relative risk (RR) with 95% confidence intervals (CI).Over a mean follow-up of 2.3 years, a total of 216 non-spine fractures were confirmed, including 41 wrist fractures and 39 hip fractures. Both low QUS and low BMD were associated with an increased risk of any non-spine fracture (Table), while only QUS was associated with wrist fracture risk, and only BMDfn was associated with hip fracture risk.In this study of very elderly women, calcaneal QUS measured with a dry device, and BMD measured at either the calcaneous or hip, were similarly associated with the short-term risk of any non-spine fracture. These preliminary findings suggest that dry QUS measurements do not predict hip fracture risk in this population, but these results need to be confirmed in other studies and with longer follow-up.

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Experimental Determination of Mechanical Failure Loads at the Femur, Spine and Radius, and their Correlation with DXA, QCT, pQCT, and Quantitative Ultrasound. F. Eckstein,[1] R. Mueller,[2] E. M. Lochmüller.*[3] [1]Musculoskeletal Research Group, Institute of Anatomy, München, Germany, [2]OBL, BIDMC and Harvard Medical School, Boston, MA, USA, [3]1. UFK, LMU, München, Germany.

The purpose of this study was to test the hypotheses that mechanical failure loads in elderly individuals display substantial heterogeneity among clinically relevant sites, that site-specific measurements permit to predict mechanical failure strength significantly better than non-site-specific analysis - even in situ with spatially variable soft tissue errors, and that ultrasound adds significant independent information to site-specific analysis. In 120 cadavers (age 80 ± 10.6 years, 78 female, 42 male), DXA measurements were obtained including intact soft tissues in the femur, lumbar spine, and distal radius. pQCT was performed at the distal radius and tibia, QCT at the lumbar spine, and quantitative ultrasound (QUS) at the calcaneus. The bones were then excised and mechanically tested to failure, using a material testing machine (Zwick 1445). The femora were tested in a side-impact configuration, two vertebrae (one thoracic, one lumbar) in axial compression (3-segment method), and the distal radius also in axial compression. The failure loads displayed only very moderate correlations among the femur, spine, and radius (r2 =30% to 37%). Site-specific in situ DXA predicted 53% (r2) of the variability in failure loads at the femur, 64% in the spine, and 71% in the distal radius. Spinal QCT (65%) and radial pQCT (69%) were equivalent to site-specific DXA, but pQCT at the distal tibia (16%) was significantly inferior ($p < 0.01$; Fischer z-transformation) to femoral DXA. Non-site-specific measurements displayed significantly ($p < 0.01$) lower correlations with failure loads at all sites. The correlation between QUS and failure load was 27% for the femur, 26% for the spine, and 35% for the radius. These coefficients were significantly ($p < 0.01$) lower than those for site-specific DXA, and they were either equivalent or inferior to non-site-specific DXA. In multiple regression models, QUS contributed significant information to site-specific data for failure loads of the distal radius, but not for the femur or spine. We conclude that loss of mechanical competence in osteoporosis is governed by strong regional variation and does not appear to represent a strictly systemic process. Site-specific measurements, therefore, predict mechanical strength significantly better that non-site specific measurements, despite spatially variable soft tissue errors. Quantitative ultrasound is able to add significant independent information to site-specific analysis in the radius, but not in the spine or femur.

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Vitamin D Status, Sex Hormone Binding Globulin, IGF-1 and Markers of Bone Turnover as Determinants of Bone Mass and Fractures in the Longitudinal Aging Study Amsterdam. P. T. A. Lips,[1] S. M. F. Pluijm,*[2] C. Popp-Snijders,[1] J. H. Smit.*[3] [1]Department of Endocrinology, Vrije Universiteit Medical Center, Amsterdam, The Netherlands, [2]EMGO-Institute, Amsterdam, The Netherlands, [3]Department of Sociology and Social Gerontology, Vrije Universiteit, Amsterdam, The Netherlands.

Endocrine diseases cause osteoporosis and fractures by increasing bone resorption and decreasing bone formation. The role of vitamin D status, parathyroid hormone (PTH), sex hormone binding globulin (SHBG), insulin-like growth factor 1 (IGF-1), and markers of bone turnover as determinants of bone mass and fractures was assessed in a cohort of 1350 community-dwelling women and men (≥ 65 years) from the Longitudinal Aging Study Amsterdam. Fasting blood and urine samples were obtained in 1995-96 and serum 25(OH)D, PTH, SHBG, IGF-1, osteocalcin and urinary deoxypyridinolin (DPD)/creatinine were measured by (radio)immunochemical methods. Broadband ultrasound attenuation (BUA) and velocity of sound (VOS) were measured in the heel using the CUBA Clinical instrument. Serum 25(OH)D was 53.2±24.1 nmol/l, serum PTH was 3.7±2.5 pmol/l, serum SHBG was 48.4±22.4 nmol/l, serum IGF-1 was 14±5 nmol/l, serum osteocalcin was 2.2±1.1 nmol/l and urine DPD/creat was 5.7±2.7 nmol/mmol. BUA of the heel was significantly predicted by serum 25(OH)D, IGF-1, SHBG, osteocalcin and urine DPD/creat ($P<0.001$). VOS through the heel was predicted by the same variables and serum PTH ($P<0.001$). Most associations remained significant after adjustment for age and sex. During 3 years of follow-up 88 subjects reported 108 fractures, i.e. 23 hip, 26 wrist and 59 other fractures. The following variables were associated with an increased fracture risk: serum 25(OH)D<30 nmol/l (vs >30 nmol/l) RR 1.65 (CI: 1.02-2.68); a high serum osteocalcin in men, RR 1.32 per SD increase (CI: 1.04-1.63); A high urinary DPD/creat, RR 1.30 per SD increase (CI:1.04-1.63). A high serum osteocalcin was protective, RR 0.78 per SD increase (CI: 0.62-0.98). In conclusion, the assessed biochemical variables are significant determinants of bone mass in elderly living in the community. A low serum 25(OH)D and high concentration of bone turnover parameters can predict fractures, whereas a high serum IGF-1 appears to be protective.

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Genome Screen for Quantitative Trait Loci Contributing to Bone Mineral Density: Framingham Osteoporosis Study. D. Karasik,[1] R. H. Myers,*[2] M. T. Hannan,[1] D. R. Gagnon,*[3] L. A. Cupples,*[3] A. Herbert,*[2] D. P. Kiel.[1] [1]Hebrew Rehab Ctr for Aged & Harvard Med Sch, Boston, MA, USA, [2]Neurogenetics, BU Sch of Med, Boston, MA, USA, [3]Epidem/Biostat, BU Sch of Public Health, Boston, MA, USA.

To identify chromosomal regions that might be linked to normal bone mineral density (BMD), considered as a quantitative trait, we performed a genome-wide scan in a randomly ascertained set of 330 Caucasian families (1326 persons with BMD measures) from the population-based Framingham Osteoporosis Study. A set of 401 microsatellite markers (set 8A) spaced at a 10 cM average density throughout the genome were typed by the Mammalian Genotyping Service (Marshfield, WI). BMDs at lumbar spine, trochanter, femoral neck, and Ward's area, were adjusted for age, age2, body mass index, height, alcohol and caffeine consumption, calcium and vitamin D intake, smoking status, physical activity, and estrogen use in females, by each sex and generation, separately, and these adjusted measures were used for linkage analyses. A strong familial resemblance (heritability values between 0.526 and 0.633) was found for all sites. Two-point and multipoint quantitative linkage analyses were performed for each BMD site using the maximum likelihood variance components method as implemented in the computer package SOLAR. By two-point screening, loci of suggestive linkage were identified on chromosomes 6, 12, and 21. The maximum two-point LOD score was 2.41 for trochanter with D21S1446 (62 cM). Chromosome 6pter showed evidence of linkage with both femoral neck and lumbar spine BMDs, at D6S2427 (LOD score 2.20 and 1.88, respectively, 54 cM). Lumbar spine BMD was also linked to chromosome 12q23 with LOD score 2.08 (137 cM). Ward's area was weakly linked to D8S373 (LOD = 1.63, 164 cM).Multipoint linkage analysis revealed suggestive linkage of trochanteric BMD at a broad (~20 cM) interval on chromosome 21q, with the peak linkage located close to D21S1446 (LOD = 3.10). Another region of suggestive linkage was at chromosome 14q with lumbar spine BMD (LOD > 1.90, 50-52 cM). No significant multipoint linkage was shown for femoral neck BMD. For Ward's area, again low LOD was obtained at 8q24 in multipoint analysis (LOD = 1.76).Possible candidates in the chromosomal regions are bone morphogenetic protein 6 (BMP-6) at 6p24, osteoprotegerin at 8q24, and BMP-4 at 14q22. The region of large size on chromosome 21q (~20 cM) is suggestive of linkage but it is still premature to address candidate genes at the current time. To our knowledge, this is the largest genome screen to date for genes underlying normal variation in BMD in population sample and represents an important step toward identifying genes contributing to osteoporosis in the general population.

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A Large-Scale Whole-Genome Scan Suggests Several Genomic Regions Containing QTLs for BMD Variation. H. W. Deng,[1] F. H. Xu,*[1] H. Shen,*[2] H. T. Zhang,*[1] H. Y. Deng,*[2] T. Conway,[2] Q. Y. Huang,*[1] J. Chen,*[1] Q. H. Xia,*[1] Y. Z. Liu,*[1] Y. J. Liu,*[1] K. M. Davies,[2] R. R. Recker.[2] [1]Osteoporosis Research Center and Biomedical Sciences, Creighton University, Omaha, NE, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Osteoporosis is an important health problem, particularly in the elderly women. Bone mineral density (BMD) is a major determinant of osteoporosis. We have set out to identify genomic regions that may contain QTLs underlying BMD variation using a whole-genome scan. For a sample of pedigrees that contains 1,249 sib pairs, 1,098 grandparent-grandchildren pairs and 1,172 first cousin pairs, we performed a whole-genome linkage scan using 400 microsatellite markers (with an average heterozygosity of ~0.75 and spaced ~10cM apart throughout the whole genome) from the ABI PRISMTM Linkage Mapping Set Version 2 (Applied Biosystems, Foster City, CA). Each pedigree was ascertained through a proband with BMD Z-scores < -1.28 or > 1.28 at the hip or spine, i.e., the proband belongs to the bottom or top 10% of the population BMD variation. For chromosomes 9-22 and chromosome X, the genotyping has been completed showing a rate of missing and error genotype data (determined by sample replication and pedigree consistency checks) of ~0.3%. For chromosomes 1-8, the genotyping has been finished with some repeat experiments to be done to reduce the missing and error data rate from the current ~3.0% to ~0.3%. Using the raw BMD values adjusted for age, sex, weight and other significant covarieties, we conducted two- and multi-point linkage analyses to identify genomic regions that may contain QTLs underlying BMD variation. Several genomic regions were identified. For example, the genomic region near the marker D3S1580 on chromosome 3 may contain a QTL for hip BMD variation (with two-point analysis LOD score of 2.14 and multi-point analysis LOD score of 1.99). The genomic region near the marker D12S1723 on chromosome 12 and marker D13S285 on chromosome 13 may contain QTLs for spine BMD variation (with two-point analysis LOD score of 2.17 and 1.77 and multi-point analysis LOD score of 2.96 and 2.38, respectively). The genomic regions identified will be subject to extension studies with more samples and denser markers for confirmation. Once confirmed, subsequent fine mapping studies will be pursued using methods we developed to pinpoint the QTLs to genomic regions smaller than 1cM for eventual identification of the major functional genes involved. The inconsistency of the genomic regions identified in this and earlier few reports was explored and explained, from statistical genetics point of view, in terms of sample size and statistical power in whole-genome linkage studies.

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A Quantitative Trait Locus on Chromosome 4p Influences Variation in Bone Mineral Density at the Wrist and Hip. B. D. Mitchell,*[1] C. M. Kammerer,[2] J. L. Schneider,*[2] S. A. Cole,*[2] J. E. Hixson,*[3] R. Perez,*[4] R. L. Bauer.[4] [1]Medicine, University of Maryland School of Medicine, Baltimore, MD, USA, [2]Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA, [3]University of Texas Health Science Center at Houston, Houston, TX, USA, [4]University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

The San Antonio Family Osteoporosis Study (SAFOS) was designed to identify the genetic determinants of bone mineral density in large Mexican American families. Between 1996 and 2000, a total of 898 individuals from 34 large families were recruited into the SAFOS. As part of this study, we conducted a genome-wide linkage analysis using 389 highly polymorphic microsatellite markers spaced approximately 9.9 cM apart to locate and identify quantitative trait loci (QTLs) that affect bone mineral density (BMD). These analyses are based on 657 subjects aged 20 years and over (399 women, 258 men) from the 23 largest families included in a genome scan. The mean age of study subjects was 42.7 yrs. We measured BMD using dual x-ray absorptiometry, at 11 skeletal sites: radius (3 sites), ulna (3 sites), femur (4 sites) and spine. Heritabilities of all traits ranged between 0.40±0.07 and 0.75±0.08. To locate quantitative trait loci (QTLs), we performed a multipoint genome scan using maximum-likelihood based, variance components linkage analysis methods. We obtained strong evidence that a QTL affects BMD of the radius midpoint and is located on chromosome 4p (multipoint lod= 4.05; empirically estimated p-value=0.00001). Peak evidence for linkage occurred at marker D4S2639 at position 40 cM from pter. Additionally, peak evidence for linkage to BMD at the hip (intertrochanter) was observed in this region (multipoint lod = 2.2). We found little evidence for QTLs affecting BMD at any site linked to chromosomes 1, 5, or 11, as has been reported in other studies. Interestingly, in men, but not women, there was moderate evidence for linkage (lod = 3.1, empirically estimated p-value=0.00008) to BMD at the hip (intertrochanter) to a region on chromosome 13 near marker D13S800, approximately 67 cM from pter, which has been linked to variation in forearm BMD in a previous study of Chinese families. We conclude that QTLs influencing variation in BMD may be located on chromosome 4p, and possibly on chromosome 13q.

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Mapping of Quantitative Ultrasound of the Calcaneus to Chromosomes 1 and 5 by Genome-Wide Linkage Analysis. D. Karasik,[1] R. H. Myers,*[2] M. T. Hannan,[1] D. R. Gagnon,*[3] R. R. McLean,[1] L. A. Cupples,*[3] D. P. Kiel.[1] [1]Hebrew Rehab Center for Aged & Harvard Med Sch, Boston, MA, USA, [2]Neurogenetics Dept., BU Sch of Medicine, Boston, MA, USA, [3]Epidemiology/Biostat Dept, BU Sch of Public Health, Boston, MA, USA.

Quantitative ultrasound (QUS) may predict the risk of fracture independent of bone density. Evidence suggests that QUS phenotypes are largely determined by genetic factors, yet little is known of chromosomal localization of these gene(-s). The aim of this study was to identify, using quantitative trait linkage analysis, chromosomal regions that might contain genes influencing variation in calcaneal QUS in a set of families from the general population. To accomplish this aim, we conducted a genome-wide autosomal scan in 330 Caucasian families (1187 measured individuals) from the Framingham Osteoporosis Study, using a set of 401 Marshfield microsatellite markers with a 10 cM average density map. QUS measurements included broadband ultrasound attenuation (BUA), speed of sound (SOS), and quantitative ultrasound index (QUI). These phenotypes were regressed on age, age2, body mass index, height, alcohol and caffeine consumption, smoking status, physical activity, as well as estrogen use in females, by each sex and generation, separately. Standardized residuals were then used in the quantitative genetic analyses by the maximum likelihood variance components method (SOLAR package). Estimating familial resemblance of QUS phenotypes demonstrated a strong heritability ranging from 0.45 for SOS to 0.52 for BUA. By two-point genome screening, principal loci of possible linkage were identified on chromosomal regions 1p36.3 and 5p15.2. The maximum LOD score attained was 2.74 for BUA with D1S468 (4 cM) and 2.69 for SOS with D5S817 (23 cM). Both identified loci were phenotype-specific. QUI, a linear combination of the SOS and BUA, showed linkage with both markers: maxima were obtained for D1S468 (LOD = 2.1) and D5S817 (LOD = 2.2). Multipoint linkage analysis of BUA also revealed a peak linkage located close to D1S468 (LOD = 2.4). However, no indication of suggestive multipoint

linkage (LOD < 1.9 by Lander-Kruglyak criteria) was obtained with any chromosomal location for either SOS or QUI. The loci for QUS do not overlap with those seen in our genome scan for BMD in the same pedigrees. To our knowledge, this study is the only genome screen for QUS phenotypes in the general population, and our results suggest that there may be genetic determinants for QUS phenotypes separate from BMD. These results should encourage further investigations of the genetic source of QUS variability.

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Linkage Disequilibrium Mapping at Chromosome 1p36.2 for Bone Mineral Density. L. D. Spotila,[1] H. Rodriguez,*[2] M. Koch,*[2] S. Stoltzfus,*[2] W. Epley,*[2] H. S. Tenenhouse,[3] A. Tenenhouse.[4] [1]Bioscience and Biotechnology, Drexel University, Philadelphia, PA, USA, [2]Drexel University, Philadelphia, PA, USA, [3]McGill University, Montreal, PQ, Canada, [4]Montreal General Hospital, Montreal, PQ, Canada.

Two candidate genes located on chromosome 1p36.2 have been investigated for linkage and association to low bone density. Non-parametric linkage analysis has delimited a 30 cM interval defined by markers D1S468 (distal) and D1S507 (proximal) that is potentially linked to low bone density. The physical distance of this interval is 12 Mb. There are at least two genes with potential significance for bone function and metabolism located within 1 Mb of one another: TNFRSF1b (tumor necrosis factor receptor 2) and PLOD (lysyl hydroxylase). There is significant association of the polymorphism within the 3' untranslated region (UTR) of the TNFRSF1b gene to low bone density (Spotila et al., JBMR, 2000; Albagha et al., CTI, 2000). We have scanned the exons and flanking intronic regions of the genes TNFRSF1b and PLOD for single nucleotide polymorphisms (SNPs) in a population of 160 unrelated individuals selected for low bone density. In addition to known sequence variants in the 3' UTR and within intron 4 of TNFRSF1b, we have examined the frequency of a third variant within exon 6 (T677G) that alters a methionine codon to arginine (M196R). There were no additional sequence variants within TNFRSF1b that occurred with a frequency greater than 1% in the test population. The variant M196R however, is associated with low bone density of the spine ($p = 0.001$). This site is in significant linkage disequilibrium with the intron 4 microsatellite and the SNP at position 188 of the 3' UTR in the test population ($p<0.0001$ and $p = 0.001$, respectively). In contrast to TNFSF1b, the PLOD gene has 12 SNPs within the 19 coding exons and flanking intronic regions as well as a complex repeated sequence within intron 16. Of these variants, 3 are the most likely to be of functional significance: a G/A variant in exon 3 that coverts an alanine codon to threonine (A99T), a T/G SNP at position -17 from the 3' splice junction of intron 6, and a C/T SNP in exon 14 that alters an arginine codon (R512C). Genotypes at these three sites within the PLOD gene were not individually associated with bone density after correction for multiple testing. However there is significant linkage disequilibrium between the exon 3 and intron 6 SNP's although they are separated by 4.9 kb ($p<0.0001$).In summary, we have utilized linkage, association and linkage disequilibrium analysis to examine the potentially important contribution of chromosome 1p36.2 to variation in bone density. Additionally, that variation may reflect contribution from two individual genes.

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Major Genetic Influence on Femoral Stiffness and Strength Identified on Mouse Chromosome 4. C. H. Turner,[1] Q. Sun,*[1] M. L. Bouxsein,[2] C. J. Rosen,[3] L. R. Donahue,[3] K. L. Shultz,[3] W. G. Beamer.[3] [1]Orthopaedic Surgery, Indiana University, Indianapolis, IN, USA, [2]Beth Israel/Deaconess Medical Center, Boston, MA, USA, [3]The Jackson Laboratory, Bar Harbor, ME, USA.

The C3H/HeJ (C3H) inbred mouse strain has superior femoral bone biomechanical properties, compared to C57BL/6J (B6) mice. Genetic analyses of femoral biomechanical properties from 906 B6C3H-F2 adult female mice identified 11 quantitative trait loci (QTLs) linked to strength, stiffness or work to failure on chromosomes 1, 4, 6, 8, 10, 11, 12, 13, 14, 17 and 18. Of these, the QTL on Chr 4 had the strongest influence on femoral biomechanics. A congenic strain of mice was developed by transferring the Chr 4 QTL region from C3H onto the B6 background by 6 generations of selective backcrossing. The new congenic strain (denoted B6.C3H-4t) contains the C3H Chr 4 QTL on a 98% B6 background. Excised femora from this congenic strain of mice were analyzed biomechanically in 3-point bending to test the effect of the donated C3H QTL on bending force to fracture (F), stiffness (S) and work to failure (U) of the mid-diaphyseal region. The C3H alleles in the Chr 4 QTL significantly influenced the mechanical properties of the femoral midshaft, particularly stiffness (Table 1). Average biomechanical properties for the B6.C3H-4t congenic strain were compared to B6, C3H, and B6C3H-F1 hybrid mice (N=10-16). In Table 1, significant differences ($p<0.05$) from B6, C3H, and B6C3H-F1 are denoted "b", "c", and "f", respectively.Compared to B6 mice, C3H had 76% greater femoral strength (F), 35% greater stiffness (S), and 105% greater work to failure (U). The B6.C3H-4t congenic mice also had improved femoral biomechanical properties: F was greater than B6 by 21%, S by 35%, and U by 29%. Consequently, the Chr 4 QTL donated from C3H accounted for between 28% and 100% of the difference in biomechanical properties between B6 and C3H mice. The observation that femoral stiffness for B6C3H-F1 hybrid mice was equal to C3H suggests that there is a dominant influence of the C3H allele on femoral stiffness. Interestingly, this dominant effect appeared to be captured completely within the Chr 4 QTL. The Chr 4 QTL spans over 30 cM and contains hundreds of genes, so it is unclear at this point which gene is responsible for the effect on femoral stiffness and strength. Nevertheless, there is a strong genetic influence on femoral bone biomechanics within the defined QTL region on mouse Chr 4.

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Telomerase Expression Extends Life Span and Prevents Senescence-Associated Impairment of Human Osteoblast Functions *in Vitro* and *in Vivo*. J. L. Simonsen,*[1] C. Rosada,*[1] J. Justesen,*[1] K. Stenderup,*[1] C. Bischoff,*[2] F. Dagnaes-Hansen,*[3] E. F. Eriksen,[1] S. I. S. Rattan,*[4] T. G. Jensen,*[2] M. Kassem.[1] [1]University Department of Endocrinology and Metabolism, University Hospital of Aarhus, Aarhus, Denmark, [2]Institute of Human Genetics, University of Aarhus, Aarhus, Denmark, [3]Department of Medical Microbiology and Immunology, University of Aarhus, Aarhus, Denmark, [4]Department of Molecular and Structural Biology, University of Aarhus, Aarhus, Denmark.

Cellular senescence of human osteoblasts is associated with limited proliferative potential, substantial alterations in gene expression, and impaired osteoblast functions. These changes are associated with telomere shortening suggesting that cellular senescence may be related to telomere effects. Thus, we studied the effects of maintaining telomere length on cellular characteristics of the human bone marrow stromal cells (hMSCs). hMSCs cultured from bone marrow aspirates, were stably transduced by a retroviral vector containing the catalytic subunit of the human telomerase gene (TERT) driven by a Moloney murine leukemia virus. Efficiency of transduction was close to 100%, as assessed by green fluorescence protein expression. The presence of an active telomerase enzyme in hMSC-TERT$^+$ cells was demonstrated by the telomerase repeat amplification protocol (TRAP) and mean telomere length, determined by terminal restriction fragment analysis, was maintained during continuous *in vitro* culture. The hMSC-TERT$^+$ cells exhibited extended life span *in vitro* and have, until now, undergone more than 130 population doublings (PDs) while control cells exhibited senescence-associated proliferation arrest at 25 PDs. Morphologically, the hMSC-TERT$^+$ cells are similar to early passage young cells and they do not stain for senescence-associated β-galactosidase. Analysis of gene expression of hMSC-TERT$^+$ cells demonstrates the maintenance of gene expression of osteoblastic markers such as Cbfa1, collagen type I, alkaline phosphatase, and osteocalcin during continuous subculturing. The hMSC-TERT$^+$ cells combined with a hydroxyapatite-tricalciumphosphate vehicle and implanted subcutaneously in immunodeficient mice, were able to form normal lamellar bone quantitatively similar to what was formed by young cells and there was no evidence for tumor formation. Furthermore, chromosome analysis using the comparative genomic hybridization technique showed minimal evidence of chromosomal abnormalities. Our findings demonstrate that maintenance of the telomere pathway can prevent senescence-associated impairment of osteoblast functions and provide important targets for pharmacological intervention to increase bone formation.

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Cell-Autonomous and Endocrine Deficiencies Contribute to the Hypoplastic Skeletal Phenotype of Ku70 – Deficient Mice. D. M. Willis, A. Loewy,* J. Shao,* M. Bidder, D. M. Ornitz,* D. A. Towler. Washington University Medical Center, St. Louis, USA.

Basic fibroblast growth factor (FGF2) stimulates the proliferation and recruitment of undifferentiated calvarial osteoprogenitors to the mature osteoblast lineage, reflected in the upregulation of osteocalcin (OC) gene expression. Previously, we mapped a transcriptional response to fibroblast growth factor receptor activation to a GCAGTCA motif (OC FGF response element; OCFRE) at nucleotides -144 to -138 relative to the rat OC promoter transcription initiation site. We purified the OCFRE DNA binding activity (OCFREB) from MG63 osteosarcoma cells, and identified Ku70, Ku80, and a novel acetyltransferase as core constituents of the OCFRE DNA binding complex using techniques of reverse phase liquid chromatography-triple quadrupole mass spectrometry fingerprinting and sequencing. In phenotypically immature MC3T3E1 cells and primary murine calvarial osteoblasts, FGF2 regulates both the accumulation (increased) and serine phosphorylation (decreased) of Ku70, but does not regulate Ku80. To further define the role of Ku70 in skeletal physiology, we have initiated the characterization of mice homozygous for disruption of the Ku70 gene. Compared to Ku70 /+ littermates, Ku70 -/- mice are small, discernable ca. 7 days post partum and persistent throughout life in both genders. Tibiae and femurs from Ku70 -/- mice are more gracile than those of Ku70/+ littermates. Primary calvarial osteoblasts derived from E18.5 Ku -/- mice exhibit significant osteogenic deficiency in vitro. As compared to Ku70 +/+ mice, the formation of mineralized nodules by Ku70 -/- calvarial osteoblasts is decreased by 50% ($p = 0.07$), quantified by von Kossa staining and digital image analysis. Moreover, both mineralized nodule area ($p = 0.006$) and intensity ($p = 0.02$) are decreased by 80% in Ku70 -/- calvarial osteoblasts cultured under mineralizing conditions. Consistent with these in vitro observations, serum alkaline phosphatase levels -- a reflection of bone formation and osteoblast numbers -- are decreased in 1 month old Ku -/- mice, but to a greater extent in female mice (31%, $p = 0.01$) than in male mice (15%; $p = 0.06$). Notably, levels of serum IGF1 -- a major skeletal growth factor -- are also reduced by 30% in Ku -/- mice (269 +/- 104 ng/ml) compared to Ku +/+ mice (386 +/- 49 ng/ml; $p = 0.03$), consistent with smaller size and global deficiencies in growth. In toto, these data confirm the role of Ku70 in osteoblast gene expression, mineralization, and skeletogenesis. Deficiencies in both cell-autonomous control and endocrine regulation of bone growth and net osteoblast synthetic functions contribute to the hypoplastic skeletal phenotype of Ku70 -/- mice.

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Secreted Frizzled-Related Protein (SFRP)-1: A Novel Regulator of Osteoblast and Osteocyte Apoptosis. P. V. N. Bodine,[1] R. A. Moran,*[1] H. E. Ponce-de-Leon,*[1] S. A. McLarney,*[1] J. Green,*[2] G. S. Stein,[2] J. B. Lian,[2] B. S. Komm.[1] [1]Women's Health Research Institute, Wyeth-Ayerst Research, Radnor, PA, USA, [2]Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA.

We have used differential display-polymerase chain reaction analysis to characterize osteogenic pathways in a collection of conditionally immortalized adult human osteoblast (HOB) cell lines. This collection contains representatives of 4 stages of differentiation: pre-osteoblasts (pre-OBs), mature osteoblasts (OBs), pre-osteocytes (pre-OCYs) and mature osteocytes (OCYs). These cell lines were treated for 24 hr with various osteogenic agents including prostaglandin E2 (PGE2) and transforming growth factor-beta1 (TGF-beta1). Eighty-two differentially expressed genes were identified from these experiments. Of these genes, only the secreted Wnt antagonist, SFRP-1, was regulated by numerous osteogenic agents and in multiple stages of differentiation. Basal SFRP-1 mRNA levels increased up to 24-fold during HOB differentiation from pre-OBs to pre-OCYs. Expres-

sion of SFRP-1 mRNA was increased up to 38-fold following PGE2 treatment of pre-OBs and OBs, but not after treatment of pre-OCYs. PGE2 treatment of primary rat and normal human OBs also increased SFRP-1 mRNA levels over 10-fold, supporting the developmental regulation of this gene. In contrast, SFRP-1 expression was down-regulated by as much as 80% following TGF-beta1 treatment of pre-OCYs. Additional growth factors, cytokines, and hormones also regulated SFRP-1 expression in osteoblasts. Message levels for this gene were increased up to 5-fold in various HOB cell lines after treatment with interleukin-1beta and retinoids, but were decreased by as much as 75% following treatment with bone morphogenetic protein-2, insulin-like growth factor-1, vitamin D3, dexamethasone and fetal bovine serum. Over-expression of SFRP-1 in HOB cells accelerated the rate of cell death 3-fold, and this was blocked by treatment with an SFRP-1 antibody. Consistent with this finding, treatment of pre-OBs and OBs with PGE2 increased apoptosis up to 3-fold, while treatment of pre-OCYs with TGF-beta1 decreased cell death by 50%. Finally, transfection of HOB cells with SFRP-1 suppressed Wnt signaling by as much as 70%. These results demonstrate for the first time that SFRP-1 is a key regulator of osteoblast and osteocyte survival, and that Wnt proteins are important modulators of bone forming cells.

Disclosures: Wyeth-Ayerst,1,2,3,5.

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Connexin43/Src Interaction and Src Activity Link Connexin43 Hemichannels with the ERK Pathway: Mechanism of Anti-Apoptosis by Bisphosphonates in Osteocytes. L. I. Plotkin,[1] J. Davis,*[1] R. Civitelli,[2] S. C. Manolagas,[1] T. Bellido.[1] [1]Endo/Metab. Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Bone & Min. Diseases, Washington Univ. School of Medicine, St. Louis, MO, USA.

Preservation of the mechanosensory function of osteocytes by inhibiting their apoptosis might contribute to the anti-fracture efficacy of bisphosphonates (BPs). These agents prevent apoptosis of MLO-Y4 osteocytic cells by opening connexin43 (Cx43) hemichannels and subsequently activating the extracellular signal regulated kinases (ERKs). However, the mechanism by which Cx43 hemichannel opening leads to ERK activation is unknown. We report that embryonic fibroblasts and osteoblastic cells derived from Cx43 deficient mice or osteoblastic cell lines with very low levels of Cx43 were not protected from apoptosis by BPs. This effect was specific for Cx43, as transfection of Cx43 - but not Cxs 26, 31, 32, 37, 40, or 45 - into HeLa cells rendered them responsive to the anti-apoptotic effect of BPs. The cytoplasmic C-terminus is unique to each Cx, and Cx43 contains Src SH2 and SH3 binding sites in this region. Therefore, we examined whether opening of Cx43 hemichannels by BPs may activate ERKs and prevent apoptosis by activating Src. We found that a Cx43 mutant, which forms channels with normal permeability but lacks the C-terminus, did not confer BP-induced anti-apoptosis. On the other hand, co-expression of this mutant together with the C-terminus of Cx43, but not the C-terminus alone, did confer BP-induced anti-apoptosis. Hence, both the pore forming and the C-terminus regions of Cx43 are required for anti-apoptosis. BPs also prevented apoptosis in HeLa cells transfected with Cx43 and wild type Src, but not Src mutants lacking either the SH2 or SH3 domains or kinase activity. Consistent with this, anti-apoptosis and ERK activation by BPs in osteocytes were abolished by the inhibitor of Src kinases PP1 and were absent in embryonic fibroblasts derived from Src deficient mice. These results suggest strongly that cell communication with the extracellular space is not sufficient for anti-apoptosis by BPs and that interaction between Cx43 and Src and the resulting activation of Src are required. Lastly, we determined that ERK activation lies downstream of Cx43, by showing that a dominant negative MEK that prevents ERK activation abolished Cx43-mediated survival and a Cx43 mutant that cannot be phosphorylated by ERKs conferred anti-apoptosis as effectively as wild type Cx43. These findings reveal a novel and gap junction-independent function of Cx43 in the regulation of survival signaling pathways.

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ERKs as well as PI3K/Akt, but not p38, Mediate the Anti-apoptotic Effect of Sex Steroids in Osteoblasts and Osteocytes, in Part by Phosphorylating Bad. S. Kousteni, T. Bellido, L. Han, R. L. Jilka, S. C. Manolagas. Division of Endocrinology & Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

MAP or PI3 kinases transduce chemical and physical signals from the cell surface to the nucleus thereby controlling cell proliferation, differentiation and survival. A particular cellular fate may depend on the dynamic balance between different MAP kinases rather than on the activation of a particular family. ERKs, JNK and p38 MAPKs as well as PI3K can all be regulated by estrogen through a nongenotropic mechanism; and estrogens acting via a nongenotropic ERK-mediated mechanism protect osteoblasts and osteocytes from apoptosis via physical association of their receptor with kinases within pre-assembled scaffolds. Based on these lines of evidence, we have investigated whether other kinases besides ERKs play a role in mediating the anti-apoptotic effect of estrogens. The ability of 17β-estradiol (E_2) to prevent etoposide-induced apoptosis was assessed in calvaria-derived murine osteoblastic cells in the presence or absence of the specific inhibitor of PI3K, wortmannin, or the p38 inhibitor, SB203580. Wortmannin dose-dependently attenuated the anti-apoptotic effect of E_2 with maximal efficacy at a concentration of 30nM. On the other hand, SB203580 at concentrations as high as 30μM did not interfere with the anti-apoptotic effect of E_2. Activated ERKs and PI3K/Akt phosphorylate the pro-apoptotic protein Bad, a member of the Bcl-2 family of proteins that control release of caspase activating factors from the mitochondria. Bad phosphorylation prevents Bad from activating the death program, thereby rescuing cells from apoptotic stimuli. In support of the contention that the anti-apoptotic effects of estrogen are mediated by kinases which in turn phosphorylate Bad, we found that a dominant negative Bad lacking the ability to undergo phosphorylation at serines 112, 136 and 155 (because of substitution by alanine) abrogated the anti-apoptotic effect of estrogen in ERα-transiently transfected cells. These results suggest that activation of both the ERK and PI3K pathways by estrogens is indispensable for their anti-apoptotic effects; while the p38 cascade is not involved in these effects. Moreover, Bad phosphorylation by at least one of these pathways is required for the anti-apoptotic action of estrogens.

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Osteoprotegerin (OPG) binds TNF Family Proteins Including TNFα and Protects Osteoblasts from TNFα-Induced Apoptosis: A Complementary Function in Addition to Effects of OPG on Osteoclast Differentiation via RANKL. R. Bu, R. M. McKeon, L. Cao, H. C. Blair. Pathol and Cell Biol & Physiol, U Pitt, Pittsburgh, PA, USA.

Bone turnover is balanced by osteoclast/ osteoblast activity via cytokine interactions that control differentiation and activity of both cell types. Degradation requires osteoclasts, but recent work points to involvement of osteoblast/ osteocyte apoptosis. Osteoclasts may promote apoptosis, in part, by TNF secretion. TNFα in particular promotes apoptosis in osteoblasts. The potential importance of osteoclast TNF secretion was confirmed by Western analysis of supernatants of human osteoclast cultures, which showed TNF α at concentrations exceeding 10 ng/ml. Osteoblastic cells have been noted to secrete both RANKL and OPG, a paradoxical situation, since RANKL is the key cytokine for terminal fusion and osteoclast differentiation, while OPG is the most efficient factor protecting bone from degradation, including RANKL binding that blocks the activation of RANK. Our studies confirmed that both RANKL and OPG are expressed by osteoblasts. However, interaction of monocyte precursors with RANKL-expressing cells mediated osteoclast differentiation on mineralized substrate efficiently despite significant OPG. In light of recent findings of an interaction of OPG with TRAIL (another TNF family protein), these results suggested that OPG also interacts with additional TNFs in bone. ELISAs with OPG as the target, or immunoprecipitation using TNFα, RANKL, or TRAIL as the targets, showed OPG binding to TNFα and RANKL at similar affinities, and weaker binding of OPG to TRAIL. RANKL competitively inhibited TNFα binding to OPG at ~equimolar concentrations, confirming these results and suggesting that binding of TNFα by OPG involves a common molecular site. However, whether this interaction is physiologically relevant was uncertain. We examined the affect of OPG on TNFα-induced osteoblast apoptosis, using annexin assays or TUNEL/cell survival to determine early and late apoptosis. TNFα at 3-10 ng/ml induced apoptosis in cultures of MG63 cells in 2-4 h, but this effect was dramatically diminished by OPG at 10-30 ng/ml. MG63 cells in contiguous clusters were affected, involving 20-50% of cells; ~2% background apoptosis of individual cells occurred with or without TNFα and was not affected by OPG. Our studies suggest that, in addition to effects on osteoclast differentiation, OPG protects osteoblasts from TNFα-induced apoptosis. These results reconcile the co-expression of OPG and RANKL, and indicate that OPG is of more general importance in balancing bone formation and catabolism than has been indicated by models focusing solely on its interaction with RANKL.

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Incidence and Risk Factors for Second Hip Fracture. The Study of Osteoporotic Fractures. R. D. Chapurlat, D. C. Bauer, M. C. Nevitt, K. L. Stone, T. Blackwell, S. R. Cummings. Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA, USA.

Women with hip fracture have an increased risk of second hip fracture. But few of them currently receive adequate therapy, so that many remain at high risk of second hip fracture. The epidemiology of second hip fracture, however, is poorly understood as few studies, mostly retrospective, have addressed this issue.To determine the incidence and risk factors for second hip fracture, we conducted a prospective cohort study in postmenopausal women aged 65 years and older. Among 9,704 community-dwelling participants recruited at baseline, 632 women suffered a first hip fracture after enrollment. These 632 hip fracture cases were then followed for occurrence of a second hip fracture for an average of 3.7 years (0.1-12.7). Clinical risk factors and bone mineral density (BMD) were assessed at the baseline for the study. We estimated the incidence rate of second hip fractures among women who had a first incident hip fracture, and we used a stratified proportional hazards analysis with robust estimate of variance to identify independent risk factors of the second hip fracture.Fifty three second hip fractures occurred during an average 2.3 years following the first fracture. Women with first hip fracture had a 2.3% per year risk of second hip fracture. In multivariate analysis, women with a first hip fracture who walked for exercise at baseline were less likely to sustain a second hip fracture (RR=0.49 [0.27-0.90]), as were those who had normal depth perception (RR=0.51 [0.29-0.92]). Women who either did not gain or lost weight after age 25 had an increased risk of second incident hip fracture compared to the others (RR = 2.69 [1.56-4.62]). Risk of second hip fracture was also increased in those who had the lowest calcaneal BMD (RR=1.47 [1.11-1.96] per SD decrease in BMD). Current use of estrogen replacement therapy at baseline was protective (RR=0.49 [0.26-0.93]) up to 2 years of follow-up after baseline. Age was not a significant predictor of the risk of second hip fracture.In conclusion, women with a first hip fracture have a high risk of second hip fracture, especially if they are inactive, have impaired depth perception, have low BMD or if they have lost weight. These risk factors may be affected by simple interventions.

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Risk Factors for Falls in Older Women in Residential Care in Australia. L. Flicker,[1] K. Mead,*[2] C. Nowson,*[3] S. Scherer,*[4] M. Stein,*[2] J. Thomas,*[1] J. L. Hopper,*[5] J. D. Wark.[2] [1]Medicine, University of Western Australia, Perth, Australia, [2]Medicine, University of Melbourne, Melbourne, Australia, [3]Deakin University, Melbourne, Australia, [4]Royal Freemasons Homes of Victoria, Melbourne, Australia, [5]Public Health, University of Melbourne, Melbourne, Australia.

Approximately one third of all hip fractures occur in women in residential care, and most of these fractures are preceded by a fall. Residential care in Australia is divided into high and low levels based on disability. Common risk factors for falls and bone fragility in these women may produce a deleterious synergy, magnifying the risk for osteoporotic frac-

ture.This prospective study spanned three states of Australia and 2454 female high level and 1797 low level residents were approached. Informed consent was obtained from the facility, the subjects, and where appropriate, their caregivers, with 952 high level and 667 low level residents consenting to the study, yielding a response rate of 38%. Subjects had their age, weight, cognitive function (using the Abbreviated Mental Test Score (AMTS)), walking ability, psychotropic drug use and previous fractures recorded. Vitamin D nutrition was measured by serum 25-hydroxyvitamin D level (25D) using the Incstar assay (lower limit of reference range, 25nmol/l). Residential care supervisors recorded falls in monthly falls diaries for a mean period of 159 days and there was on average 1.5 falls per year, which occurred in 26% of the residents after a median period of 44 days.Mean age of residents was 83.7 years. Median vitamin D level were 34.6 nmol/l for the low level and 26.1 nmol/l for the high level residents with 22% and 45% having frank vitamin D deficiency respectively. Using a Cox proportional hazards multivariate model with time to fall as the outcome measure, the following variables were associated with falls, after excluding those individuals who were bedbound:

	Hazard Ratio	95% CI	p
Natural Log 25D	0.64	0.47-0.89	0.004
Natural Log Weight	0.42	0.21-0.83	0.013
Past Colles Fracture	2.43	1.43-4.16	0.001
Neuroleptic Use	1.95	1.37-2.76	< 0.001
Walking with a frame	1.43	1.04-2.00	0.030
Mild impairment AMTS	1.68	1.20-2.33	0.002
Severe impairment AMTS	1.52	1.04-2.22	0.029

Vitamin D level was independently and inversely associated with falls in women in residential care even allowing for fall risk factors and may interact with known deleterious effects on bone to produce a markedly increased risk of fracture.

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Risk Factors for Hip Fractures in Predominantly African-American Veteran Male Population. E. Barengolts,[1] D. Karanouh,[2] L. Kolodny,[3] S. Kukreja.[4] [1]University of Illinois and VA CHCS, Chicago, IL, USA, [2]University of Illionois, Chicago, IL, USA, [3]University of Illinois, Chicago, IL, USA, [4]VA CHCS, Chicago, IL, USA.

We performed a case-control study to determine potential risk factors for hip fractures in VA male patients. Charts of 218 patients admitted to VA Chicago Health Care System over 1988-2000 period for hip fractures and 218 race- and age-matched controls were reviewed. For this analysis we chose several risks prevalent in our population and known as predictors of hip fractures from previous studies. These included age, height, weight, body mass index (BMI), smoking, alcohol use; medical conditions: stroke, dementia, Parkinson's disease, and seizures; medications: hydrochlorthiazide (HCTZ), phenytoin, statins, and levothyroxine; and laboratory values reflecting nutritional status and disease burden: albumin, creatinine, blood urea nitrogen, cholesterol, and hemoglobin. Racial distribution reflected distribution in our VA population, 137 men were African-American (AAM, 63%) and 81 men were Caucasian (CM, 37%). For cases and controls mean values ± SD were similar for age (69.9 ± 11.2 vs 69.6 ± 10.5 years, due to matching). Body weight (68.8 ± 14.5 vs 82.3 ± 18.7 kg) and BMI (22.6 ± 4.7 vs 27.2 ± 6.1 kg/m2) were significantly lower in cases than in controls ($p < 0.01$ for both). In multivariate logistic regression analysis significant independent predictors of hip fracture were identified (Table). Higher BMI and albumin, and use of HCTZ and statins were protective (Table).

Risks	OR, Odds ratio	95% CI, confidence interval	P value
Alcohol	3.38	1.56-7.33	0.0008
Cigarette smoking	2.59	1.14-5.89	0.0321
Dementia	12.77	3.03-53.77	0.0005
Phenytoin	28.44	4.05-199.84	0.0008
HCTZ	0.34	0.12-0.98	0.0467
Statins	0.21	0.05-0.86	0.0295
BMI	0.89	0.83-0.97	0.0075
Albumin	0.41	0.21-0.82	0.0115

Comparison of CM and AAM groups showed some difference in hip fracture predictors. For CM significant independent predictors included alcohol: OR 7.3, CI 1.6-34.8, cigarette smoking: OR 5.8, CI 1.4-25.2, and dementia: OR 25.5, CI 3-218.7, while protective factors included higher albumin: OR 0.2, CI 0.04-0.8 and use of HCTZ: OR 0.1, CI 0.004-0.8. For AAM these predictors included alcohol: OR 3.4, CI 1.4-8.3, dementia: OR 14, CI 1.3-147, and phenytoin OR 24, CI 2.2-261, while protective factors were higher BMI: OR 0.84, CI 0.46-0.93, albumin: OR 0.26, CI 0.11-0.61, and use of statins: OR 0.6, CI 0.01-0.42. This is the first study of hip fracture risks in predominantly AAM population. It shows differences in risks and protective factors between CM and AAM. Further investigation is necessary to explain roles of various factors predicting risks and providing protection for hip fractures in diverse populations

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Prevalent Vertebral Fractures in African-American Women. J. A. Cauley,[1] K. Stone,[2] S. Ewing,[2] M. C. Nevitt,[2] T. Hillier,[3] D. M. Black,[2] M. Vogt.*[1] [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of California, San Francisco, CA, USA, [3]Kaiser Center for Health Research, Portland, OR, USA.

Vertebral fractures (VFx) are strong predictors of future fractures. Little is known about the prevalence and correlates of VFx among African American women. As part of the Study of Osteoporotic Fractures (SOF), we enrolled 662 African American (AA) women, age 61 to 94, into a longitudinal study of bone loss. Of these, lateral spine x-rays were available on 481 (73%) women. Prevalent VFx were ascertained by morphometry using identical methods as in our study of SOF women and defined as a vertebra race-specific height ratio >3 SD below the mean for normals. Total Hip BMD (DXA) and calcaneal ultrasound (Sahara) were measured. A total of 55 (11.4%) of AA women had a VFx. This compares to a VFx prevalence of 20% in white women in SOF ages 65-99. Among AA women, prevalent VFx increased with age: age <70 year, 3.6%; 70-74, 9.1%; 75-59, 11.6%; 80+, 20%. AA women with a VFx in comparison to AA women without a VFx, respectively, were older (77y vs. 75y, p=0.02); weighed less (70 kg vs. 76 kg, p=0.002), and reported higher caffeine intake (141 mg/d vs. 111 mg/d, p=0.11). There was no difference in physical activity, alcohol intake or cigarette smoking between the 2 groups. AA women with a VFx in comparison to AA women without a VFx, respectively, were more likely to report poor/fair health status (32% vs 24%, p=0.006), falling in the past 12 months (42% vs 27%, p=0.02), a fracture since age 50 (33% vs 21%, p=0.04) and performed more poorly on several performance measures of function (grip strength, 17 kg vs 20 kg p=0.0002). Total hip bone mineral density (BMD) was lower among women with a VFx compared to women without a VFx: 0.75 g/cm2 and 0.83 g/cm2 p=0.0001, respectively. A one standard deviation (SD) decrease in BMD or ultrasound parameter was associated with increased odds of having a VFx (table). Table: Age Adjusted Odds Ratio (OR)(95% Confidence Intervals) per one SD Decrease

Skeletal Parameter	OR	95%CI
Total Hip BMD	1.99	(1.4-2.8)
Broadband Ultrasound Attenuation	1.80	(1.1-3.0)
Speed of Sound	1.75	(1.1-2.9)

In conclusion, the prevalence of VF x in AA women is about 50% lower than white women. Correlates of VFx in AA women are similar to those observed for white women.

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Vertebral Deformities in Urban Chinese Men: The Beijing Osteoarthritis Study. M. Nevitt,[1] S. Bent,*[1] L. Lui,[1] Y. Zhang,[2] W. Yu,*[3] S. Cummings,[1] D. T. Felson,[2] L. Xu.*[3] [1]Univ. of California, San Francisco, San Francisco, CA, USA, [2]Boston University, Boston, MA, USA, [3]Peking Union Medical College Hospital, Beijing, China

The prevalence and correlates of vertebral deformity among men in mainland China have not been studied. Prior studies indicate similarities in the prevalence of vertebral deformity between Chinese and Caucasian women, and between Caucasian women and men. It is uncertain if prevalence rates are similar in Chinese men and women. We conducted a population-based study of older men living in Beijing to determine the prevalence of vertebral deformity and correlation with potential risk factors and outcomes of the disease. Beijing residents 60 years and older were recruited door-to-door in randomly selected neighborhoods. 588 men (82% of those contacted) completed an interview, a clinic exam, and lateral spine x-rays. Radiographic methods, assessment and definition of deformity, and risk factor and outcome measurements were identical to those used in our previous studies of older women in Beijing (Xu, et al. JBMR 2000;15: 2019)*. We defined a vertebral deformity as a reduction of > 3 SDs (severe deformity > 4SDs) from level-specific normal means of vertebral height ratios derived from the Beijing men. The prevalence of vertebral deformity in men increased with age and was nearly identical to the prevalence in women in Beijing, with an age standardized prevalence ratio for men vs. women = 1.0, 95% CI 0.8 to 1.2.

Prevalence of Vertebral Deformity in Beijing by Age and Gender

Age Group (n of men)	Men (95% CI)	Women* (95% CI)
60-69 (363)	9.9% (6.8, 13.0)	10.5% (4.6, 16.3)
70-79 (184)	16.3% (11.0, 21.6)	15.0% (8.0, 22.0)
80+ (41)	24.4% (11.2, 37.5)	31.2% (21.8, 40.6)

Verrtebral deformity in men was associated with low total hip BMD (age-adjusted OR per -1 SD: 1.8; 95% CI 1.4 to 2.4) and with values below the median for quadriceps strength (OR 2.2; 1.3, 3.8) and BMI (OR 1.7; 1.0, 2.8), but not with low spine BMD, smoking, alcohol use, or heavy manual labor. Deformity was associated with an increased time to complete five chair stands (P=0.01), and there was a non-significant association of severe deformity with back pain (1.7; 0.9, 3.3). Relationships were similar for severe deformity and after exclusion of possible nonosteoporotic deformities (12% of total).We conclude that the prevalence of vertebral deformity in older Beijing men is the same as in Beijing women, and similar to that reported in studies of Chinese men living in Hong Kong and Taiwan and Caucasian men in Europe. The association of deformity with low hip BMD suggests that osteoporosis is an important contributing factor. Vertebral deformity is common, associated with poorer physical function, and is likely to become an increasingly important health problem in China as the population ages

Disclosures: Eli Lilly and Company,2.

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Carotid Atherosclerosis Is Associated with Increased Bone Mineral Density (BMD) in Mexican American Men, but Decreased BMD in Women. C. M. Kammerer,[1] D. L. Rainwater,*[1] J. L. Schneider,*[1] D. H. O'Leary,*[2] R. L. Bauer,[3] B. D. Mitchell,*[4] [1]Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA, [2]Tufts-New England Medical Center, Boston, MA, USA, [3]University of Texas Health Science Center, San Antonio, TX, USA, [4]Medicine, University of Maryland School of Medicine, Baltimore, MD, USA.

Several previous studies, the majority conducted in women, have reported an association between osteoporosis and/or bone mineral density (BMD) and markers of sub-clinical atherosclerosis. We investigated this relationship in men and women studied as part of the San Antonio Family Osteoporosis study, a population-based genetic epidemiology study of BMD and its correlates in large Mexican American families. We measured BMD using dual x-ray absorptiometry at 11 skeletal sites: radius (3 sites), ulna (3 sites), femur (4 sites) and spine. Sub-clinical atherosclerosis was assessed by ultrasound measurement of the intimal-medial thickness (IMT) of the far wall of the carotid artery and measurements were Z-transformed. Measurements of both BMD and IMT were available on 460 women and 290 men (average age 42.7 years) from 34 families. We used maximum likelihood methods, conditioned on the pedigree structure to account for familial relationships, to estimate correlations between BMD and IMT, while accounting for potential confounders, including age, body mass index, diabetes, physical activity, and, in women, menopause and breast feeding. Men and women were analyzed separately. In women > 40 years of age (n=246), IMT was negatively correlated with BMD at two ulna sites and the spine ($P < 0.05$ at each site), a finding that is consistent with other reports. In contrast, we observed positive relationships in men (n=344; both older and younger) between IMT and BMD in 5 of 10 arm and femur sites ($P < 0.05$ at each site). We further examined the relationship between BMD and low-density lipoprotein particle size (LDL size), a strong risk factor for coronary heart disease, and observed strong correlations between atherogenic small LDL size and high BMD ($p < 0.05$ in 9 of 11 sites), again supporting a link between atherosclerosis and high BMD in men. LDL size was not correlated with BMD in women. Thus, compared to women, the relationship between BMD and CHD risk factors in men is stronger, but in the opposite direction. These results imply that if common etiologic factors affect BMD and CHD, the effects are mediated differently in men and women.

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Role of T Cells and T to B Signaling Through CD40 in the Pathogenesis of Estrogen Deficient Osteoporosis. K. Watanabe,[1] Y. Uchiyama,[2] A. Sugita,*[2] S. Takeda,*[2] A. Hishiya,*[1] K. Ikeda.[1] [1]Dept of Geriatric Res, Natl Inst for Longevity Sci, Obu, Japan, [2]Chugai Pharmaceutical, Gotemba, Japan

We have previously demonstrated that B220-positive cells, which are increased in bone marrow in estrogen deficiency, contribute to accelerated osteoclastic bone resorption by presenting RANKL on their cell surface (JBMR 2000) and differentiating into osteoclasts themselves (JBMR 2001). In order to clarify the role of T cells in the pathogenesis of osteoporosis, we studied bone metabolism in nude mice (defective in T cells), SCID mice (defective in both T and B cells) and Balb/c mice as their control. Following ovariectomy (OVX), the number of B cells in bone marrow increased substantially in Balb/c mice, whereas this increase was not seen in nude or SCID mice. Accordingly, both nude and SCID mice were resistant to bone loss following OVX, suggesting that T cells play a role in bone loss in estrogen deficiency presumably by signaling to B cells. Next, the involvement of CD40, a receptor molecule on B cells which is liganded by CD40L on T cells and important for the growth and activation of B cells, was therefore investigated by using stimulating as well as blocking antibody, and CD40 knockout mice. When anti-CD40 activating antibody was administered into intact mice, an increase in B cells in bone marrow was observed concomitantly with a decrease in BMD at L2-4 (with intact ovary). Unexpectedly, however, when OVX mice were treated with anti-CD40L antibody that inhibits the activation of CD40, bone loss was even more severe than in untreated OVX mice, raising the possibility that CD40 may act to protect against bone loss in estrogen deficiency. Interestingly, it was confirmed that bone loss after OVX was also exaggerated in CD40 knockout mice. The mechanism by which CD40 knockout mice are more sensitive to estrogen deficiency is being investigated by detailed histomorphometry. In conclusion, our results suggest that in addition to B cells, T cells in bone marrow regulate BMD, and that, in estrogen-replete animals, CD40 activation causes bone loss by increasing the number of B cells in bone marrow, while CD40 may protect bone loss in estrogen deficiency.

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Both Estradiol and Testosterone Play Fundamental Roles in the Maintenance of Normal Bone Turnover in Men. B. Z. Leder,*[1] D. A. Schoenfeld,*[2] K. LeBlanc,*[1] J. S. Finkelstein.[1] [1]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [2]Dept. of Biostatistics, Massachusetts General Hospital, Boston, MA, USA.

Hypogonadism is an important cause of osteoporosis in men, but the mechanisms underlying hypogonadism-induced bone loss are incompletely understood. A fundamental unresolved question is whether androgens, estrogens, or both are involved in the physiological maintenance of normal bone turnover in men. To address this question, we randomized 55 men between the ages of 20 and 44 (mean +/- SE, 32 +/- 1) to receive one of three treatment regimens:

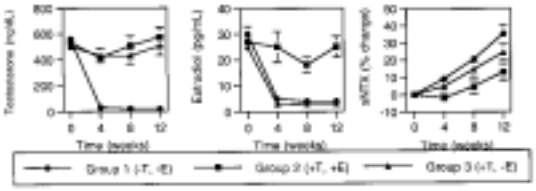
Group 1 (n=20): a GnRH analog (goserelin acetate 3.6 mg SC q 4 wks) alone for 12 weeks (to suppress endogenous gonadal steroids to pre-pubertal levels).

Group 2 (n=17): goserelin plus transdermal testosterone (Androderm 5 mg topically QD) (to normalize circulating testosterone (T) and estradiol (E) levels).

Group 3 (n=18): goserelin plus testosterone plus an aromatase inhibitor (anastrozole 1 mg po QD) (to induce selective E deficiency).

To assess the effects of these regimens on bone resorption, serum N-telopeptide (sNTX) levels were measured every 4 weeks for 12 weeks.

Baseline serum T and E levels and sNTX levels were similar in all groups. Over the 12-week treatment period, mean +/- SE sNTX increased by 35 +/- 5%, 13 +/- 5%, and 24 +/- 5% in the hypogonadal (Group 1), eugonadal (Group 2) and selectively estrogen deficient subjects (Group 3), respectively. Changes in T, and E, and sNTX are shown in the figure.



Between group differences were assessed by a random slopes models and the corresponding P values (1-sided) are shown in the table.

	T	E	sNTX
Group 1 vs. 2	<0.001	<0.001	<0.001
Group 1 vs. 3	<0.001	0.490	0.033
Group 2 vs. 3	0.364	<0.001	0.043

These data demonstrate that the selective E deficiency increases bone resorption in men. Bone resorption increases even further, however, in men with both T and E deficiency. Thus, these data strongly suggest that both estradiol and testosterone play fundamental and independent roles in the maintenance of normal bone turnover in men.

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Estrogen Withdrawal Augments PTH-Induced IL-6 and IL-6 Soluble Receptor Production by the Liver. U. S. Masiukiewicz,[1] M. Mitnick,[2] A. Grey,[2] L. Rios-Velez,[2] K. Augustyn,[2] K. Insogna.[2] [1]Endocrinology, Yale University, New Haven, CT, USA, [2]Yale University, New Haven, CT, USA.

Increased skeletal sensitivity to the resorbing actions of PTH may be one mechanism for estrogen-deficiency bone loss. Interleukin-6 (IL-6) and its soluble receptor (IL-6sR) appear to be key mediators of PTH-induced bone resorption in vivo. Continuous PTH administration increases circulating levels of IL-6 and IL-6sR, and these increases correlate with accelerated bone turnover in rodents, and in human. The magnitude of this increase is considerably augmented following estrogen withdrawal. The tissue source(s) of circulating IL-6 and IL-6sR, released in response to PTH are uncertain but it has recently been reported that PTH induces production of large amounts of immuno- and bioactive IL-6 and IL-6sR by the liver.The current study was designed to determine whether estrogen withdrawal affects PTH-induced hepatic IL-6 and IL-6sR production. Ex-vivo perfusion with 100 pM (1-84)hPTH for 130 minutes induced substantially greater release of IL-6 by livers isolated from ovx as compared to sham/ovx rats. In ovx animals IL-6 rose by 51.8 pg/ml (3.9Æ55.7) while it only increased by 26.1 pg/ml in the sham/ovx animals (3.9Æ30) ($p<0.001$, by 2-way ANOVA). PTH perfusion led to a more rapid increase in IL-6sR production by livers from ovx animals such that by 10 min the mean value in hepatic effluent was 65.3 pg/ml as compared to a value of 7.0 pg/ml in livers from vehicle-perfused ovx animals and 7.0 pg/ml in PTH-perfused livers from sham/ovx animals. Peak IL-6sR values were also highest in the PTH-perfused livers from ovx animals, 186.3 pg/ml vs 59.3 pg/ml and 141.6 pg/ml in vehicle-perfused ovx and PTH-perfused sham/ovx groups respectively ($p<0.001$ for effect of ovx and PTH by 2-way ANOVA). Since hepatocytes are the predominant cell type in the liver, the effect of estrogen withdrawal was investigated in these cells. Cultured primary hepatocytes isolated from ovx rats demonstrated a significantly greater release of IL-6 following PTH treatment than did cells isolated from sham/ovx animals, 3.8-fold vs. 1.7-fold ($p<0.001$).These data indicate that estrogen withdrawal sensitizes the liver to PTH and may account for the greater increase in circulating levels of IL-6 and its receptor seen following PTH exposure in the estrogen-deficient state. We conclude that increased skeletal sensitivity to PTH, associated with estrogen withdrawal, may be mediated, in part, by the liver.

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The Effect of Gonadectomy and Growth Hormone Deficiency on Periosteal and Endocortical Growth, Cortical Thickness, Ash Density and the Breaking Strength of the Femoral Midshaft in Male and Female Rats. B. T. Kim,*[1] L. Mosekilde,[2] X. Z. Zhang,*[1] L. Tornvig,*[2] J. S. Thomsen,*[2] E. Seeman.[1] [1]Department of Endocrinology, Austin & Repatriation Medical Centre, University of Melbourne, Melbourne, Australia, [2]Department of Cell Biology, Institute of Anatomy, University of Aarhus, Aarhus, Denmark.

To study the sexual dimorphism in the growth of the femoral midshaft and its regulation, the effects of gonadectomy (Gx) at 4 weeks of age were studied in 200 male and female growth hormone replete (GH+) and deficient (GH-) rats. During 8 months, periosteal expansion was ~5 times greater in males than females producing a wider bone; periosteal expansion accounted for 95% of the increase in cortical thickness placing bone further and further from its neutral axis. Medullary diameter changed little in males but contracted in females so that endocortical contraction accounted for 70% of the increase in cortical thickness in females and placed most of the bone nearer the neutral axis. Gx diminished some of these sexually dimorphic features; Gx GH+ males reduced periosteal expansion by ~50%, endocortical diameter was unaffected; the smaller bone had a thinner cortex and reduced strength relative to nonGx GH+ males. Gx GH+ females increased periosteal expansion and abolished endocortical contraction; cortical thickness was reduced but greater periosteal expansion maintained bone strength similar to nonGx GH+ females. GH-

had less deleterious effects in males than females; periosteal expansion was not abolished in males; endocortical diameter remained unchanged; the smaller bone had reduced cortical thickness and strength relative to GH+ males. In GH- females, periosteal expansion and medullary contraction were abolished; the smaller bone had reduced cortical thickness and strength relative to GH+ females. Bihormonal deficiency in Gx GH- males and females abolished periosteal expansion and increased endocortical contraction in both sexes. In summary, sexual dimorphism in the size, mass and strength of the femur is the result of greater periosteal expansion in males, and greater endocortical contraction in females. In males, GH and androgens independently promote periosteal expansion; abolition of growth occurs only when both regulators are absent (in Gx GH- males). Estrogen inhibits periosteal expansion and promotes GH dependent endocortical contraction; periosteal expansion in estrogen deficiency is also GH dependent. Sex hormone deficiency has a less severe effect in females than males because of the release from inhibition of periosteal expansion while GH deficiency has more deleterious effects in females than males because of the dual independent control of the periosteum in males.

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Tob-deficient Mice Exhibit Preservation of Bone Mass Even After Ovariectomy-Induced Bone Loss. M. Usui,[1] Y. Yoshida,*[2] K. Tsuji,[1] A. NIfuji,[1] T. Yamamoto,*[2] M. Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]University of Tokyo, Tokyo, Japan.

Tob(transducer of ErbB2) is a member of novel antiproliferative family consisting of Tob, Tob2, ANA/BTG3, BTG2/PC3/TIS21, and BTG1. Tob-deficient mice reveal high levels of bone mass in adult, due to increase in bone formation without major alteration in bone resorption. Further analyses revealed that Tob acts as an antagonist against BMP2-induced Smad signaling to activate transcription via association with Smads in vitro and that in vivo BMP function to enhance orthotopic bone formation is also enhanced by the absence of Tob (Yoshida Y. et al., Cell, 2000). Although Tob-deficiency revealed its phenotype in adult mice, the role of Tob in osteoporosis such as those after ovariectomy (OVX) – induced alteration in bone metabolism has not yet been understood. Thus, this paper examined the effect of Tob-deficiency on OVX-induced bone loss. Tob-deficient and wild-type female mice (9 weeks old) were ovariectomised (OVXed) and control mice were subjected to sham operation. OVX resulted in reduction in uterine weight by about 80% in both Tob-deficient and wild-type mice compared to sham operated mice. Quantification of the two-dimentional trabecular bone volume in the distal end of the femur was conducted by using automated image analyzer. Trabecular bone volume in the wild type mice was significantly reduced 4 weeks after OVX compared to that in the sham-operated wild-type mice (3% vs 8% respectively). In contrast, although bone volume in Tob-deficient mice was reduced after OVX (7%) compared to sham-operated Tob-deficient mice (12%), the fianal levels were still comparable to the sham-operated wild-type mice (7% vs 8%). We further examined bone mineral density (BMD) of the femur. The BMD of femur of wild-type mice after OVX was significantly less than that in sham-operated wild-type mice, and such reduction was also abserved after OVX in Tob-deficient mice. However, even after OVX, BMD levels in Tob-deficient bone were comparable to those in sham operated wild-type bones, and was higher than that in OVXed wild type bones (wild-type mice, OVX;48.5mg/cm2, Tob-deficient mice, OVX;50.8mg/cm2). Futhermore, cortical bone volume in OVXed wild-type mice was less than that of sham operated mice and that of OVXed tob-deficient mice, and no significant difference was observed between the cortical bone volume levels in sham-operated wild-type mice and OVXed Tob-deficient mice. These data indicate that Tob-deficiency preserves bone mass even after the bone loss due to OVX, suggesting that modulation of the Tob fnction could give a clue to develop novel therapeutic measures for the treatment of osteoporosis.

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Parallel Development of Vascular Calcification and Osteoporosis in Hyperlipidemic Mice: Evidence for the Role of Lipid Oxidation Products. F. Parhami,[1] A. D. Watson,*[1] A. Dominguez,*[1] W. G. Beamer,*[2] S. Hama,*[1] M. Navab,*[1] T. A. Drake,*[3] X. Wang,*[1] Y. Tintut,*[1] T. J. Hahn,*[4] L. L. Demer.*[1] [1]Medicine, UCLA, Los Angeles, CA, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA, [3]Pathology, UCLA, Los Angeles, CA, USA, [4]Medicine, West LA VA Medical Center, Los Angeles, CA, USA.

Vascular calcification and osteoporosis are debilitating diseases that have been linked with atherogenesis and hyperlipidemia in humans. Common mechanisms that could explain the parallel development of these diseases are not known. We have recently reported that atherogenic oxidized lipids induce osteoblastic differentiation and mineralization of calcifying vascular cells, whereas the same lipids inhibit those in osteoblastic cells. Therefore we hypothesized that formation and accumulation of atherogenic oxidized lipids in the artery wall and in bone may be common mediators that contribute to vascular calcification and osteoporosis, respectively. In this report we present evidence that diet-induced hyperlipidemia promotes both the development of vascular calcification and osteoporosis in low density lipoprotein receptor (LDLr) null mice. Eight 1-month old male LDLr null mice were placed on high fat or control diets for 11 and 22 weeks. Vascular calcification was assessed by histologic examination and quantitative area measurement. Bone mineral density (BMD) was determined in the femurs by quantitative CT scanning. Aortic calcification was induced in the animals on the high fat diet, occurred in close proximity to atherosclerotic lesions, and was greatly increased with time (mean calcified area vs. 309±550 vs. 9944±7630 mm2/section at 11 and 22 weeks, respectively). BMD was also reduced during the experiment, and at 22 weeks animals on the high fat diet had 8% lower BMD than those on the control diet (471±45 vs. 512±55 mg/mm3; $p<0.01$ by paired analysis). These changes in vascular calcification and BMD in the high fat fed mice were associated with increased circulating lipid hydroperoxides (LOOH) compared to animals on control diet (26.6 vs. 1.9 mg/mg protein). In a separate experiment we found that LOOH and cholesterol increased 2-3 fold in bone marrow from C57BL/6 mice fed an atherogenic high fat vs. control diet for 5 weeks. Furthermore, mass spectrometric analysis of fatty acids from those mice after 8 weeks of high fat diet showed accumulation of oxidized lipids similar to those previously identified in oxidized LDL and in atherosclerotic arteries. Altogether, these observations further support the hypothesis that lipid oxidation may be a common mechanism underlying atherosclerotic vascular calcification and age-related osteoporosis, as well as providing evidence for the accumulation of oxidized lipids in bone.

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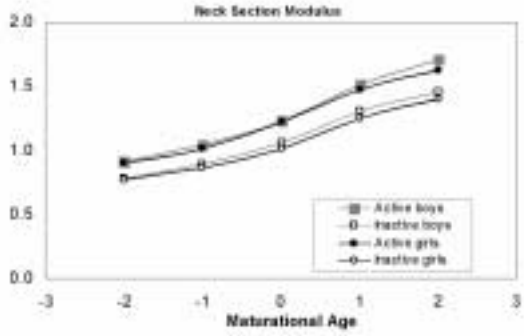
Physical Activity and Bone Strength at the Proximal Femur during the Adolescent Growth Spurt. M. R. Forwood,[1] D. A. Bailey,[2] T. J. Beck,[3] R. L. Mirwald,*[2] W. A. Wallace,*[2] T. L. Oreskovic.*[3] [1]Anatomical Sciences, University of Queensland, Brisbane, Australia, [2]Kinesiology, University of Saskatchewan, Saskatoon, SK, Canada, [3]Radiology, Johns Hopkins University, Baltimore, MD, USA.

To investigate the influence of physical activity on bone strength during the adolescent years we analyzed seven years of longitudinal data from 70 boys and 68 girls who were part of a longitudinal study monitoring bone mineral and bone geometry changes in healthy, growing children. Physical activity and dietary information along with anthropometric measurements were secured every six months and DXA bone scans of the total body, hip and lumbar spine were measured annually (Hologic QDR2000, array mode). Applying the Hip Strength Analysis software protocol developed by Beck and associates (Beck et al., Invest Radiol. 1990, 25:6-18) to our hip DXA scans allowed us to estimate important structural determinants of bone strength including the section modulus at the femoral neck. Distance and velocity curves for height were fitted for each child utilizing a cubic spline procedure and the age of peak height velocity (PHV) was determined. To control for maturational differences between children of the same chronological age, section modulus values were determined for points on each individual's curve at the age of PHV and one and two years on either side of peak. Subjects were categorized into activity groupings using all their physical activity assessments as measured by the (PAC-Q) physical activity inventory score. This was transformed into an age specific mean Z-score. On the basis of mean Z-score ranking, subjects were compared between the most and least active activity quartiles. There were no statistical differences between these activity groups in terms of attained height and weight at the age of PHV. Section modulus of the femoral neck for the active and inactive groups of boys and girls is shown in the figure below.Two way analyses of variance revealed significant ($p<.05$) activity and maturational age effects in both boys and girls. These results suggest that a modifiable lifestyle factor like physical activity may play an important role in the optimization of bone strength at the proximal femur during the adolescent years.



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Bone Mineral Change in Prepubertal Asian and Caucasian Boys: Effects of Jumping Intervention, Body Mass, and Ethnicity. K. J. MacKelvie, H. A. McKay, M. A. Petit, K. M. Khan. Human Kinetics, University of British Columbia, Vancouver, BC, Canada.

Only one study has investigated the effects of an exercise intervention on bone mass in an independent group of prepubertal boys, and none have reported an ethnic-specific response. Therefore, we asked 2 questions: 1) Does a 7-month, school based load-bearing exercise program benefit bone mineral content (BMC, g) and areal density (aBMD, g/cm^2) in prepubertal boys and 2) Do Asian and Caucasian boys respond differently to this intervention? Boys from 14 elementary schools were randomized to control (Con) and intervention (Int) groups. Intervention boys participated in a 10 minute, circuit-training jumping intervention, 3 x/week. Primarily, we investigated outcomes in boys who were below the 75th percentile (40.6 kg) of the cohort mean for baseline body mass. There were 41 Asian (19 Con, 22 Int) and 50 Caucasian (25 Con, 25 Int) prepubertal (Tanner stage 1) boys. Age and weight did not differ between groups, and boys were 10.2 (0.6) yrs old and weighed 31.7 (4.1) kg, on average. We measured BMC and aBMD by DXA (Hologic QDR 4500) for the total body (TB), lumbar spine (LS), proximal femur (PF), femoral neck (FN) and trochanter (TR) at 0 and 7 months. Lean mass (g) and fat mass (g) were DXA-derived. Calcium intake and physical activity were assessed by questionnaire. Changes in height, weight, lean mass, fat mass, and average yearly calcium intakes and physical activity did not differ between Int/Con or ethnic groups (ANOVA). We used ANCOVA (with baseline weight, final Tanner stage, change in height, and physical activity as covariates) to analyze change in bone. Intervention boys gained significantly more TB BMC (9.6 vs. 7.7%, $P<0.01$, PF aBMD (3.2 vs. 1.8 %, $P<0.05$), and TR aBMD (3.4 vs. 1.4 %, $P<0.01$) compared with controls; change in LS BMC approached significance (10.0 vs. 8.2 %, $P=0.050$). Change in bone mineral did not differ between Asian and Caucasian boys. Secondarily, we investigated the bone mineral change in those prepubertal boys who were in the highest quartile for baseline body mass. Control (N=16, 10.5 (0.6) yr and 50.3 (8.8) kg) and intervention (N=14, 10.4 (0.7) yr and 47.8 (6.8) kg) boys did not differ in changes in height, weight, lean mass, fat mass, and average yearly calcium intakes and physical activity. Using a similar ANCOVA analysis as described above, 7-month change in bone mineral did not differ between control and intervention groups at any site. These data suggest that jumping exercise augments bone mineral accrual at several regions equally in prepu-

bertal Asian and Caucasian boys who are of average or low body mass. However, the intervention offered no bone mineral advantage to prepubertal boys who were in the highest quartile (>40.6 kg) for body mass.

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Bone Structural Adaptation to Exercise in Pre- and Early- Pubertal Girls: A Randomized Intervention Trial. M. A. Petit,[1] H. A. McKay,[1] K. J. MacKelvie,[1] A. Heinonen,[1] K. M. Khan,[1] T. J. Beck.[2] [1]Human Kinetics, University of British Columbia, Vancouver, BC, Canada, [2]Radiology, Johns Hopkins University, Baltimore, MD, USA.

Growing bone may conceivably respond to loading by altering its geometric properties independent of changes in bone mass. However, very little is known about changes in bone strength in response to load-bearing in pediatric groups. Therefore, we compared total bone cross-sectional area, subperiosteal and endosteal width and section modulus (bone structural variables) at the proximal femur at baseline and after 7 months of load bearing exercise in 174 pre- and early- pubertal girls. Girls rated as Tanner breast stage 1 at baseline were considered prepubertal (PRE; n = 43 intervention (I), n = 25 control (C)); while Tanner stage 2 and 3 girls were classified as early-pubertal (EARLY; n = 43 I, n = 63 C). Mean (SD) age was 10.0 (0.6) and 10.5 (0.6) for the PRE and EARLY groups, respectively. Schools randomized to intervention group participated in a 10-minute, 3x per week high impact, progressive circuit training program. Physical activity and calcium intake were assessed by questionnaire 3 times during the year. Control schools conducted regular elementary school PE. We used proximal femur DXA scans (Hologic QDR-4500) and Beck et al's. hip structural analysis (HSA) program to estimate cross-sectional geometry and BMD in narrow regions across the femoral neck (FN), intertrochanter (IT), and femoral shaft (FS). There were no significant differences between I and C groups for baseline height, weight, calcium intake or physical activity or for change over 7-months ($p > 0.05$). We used ANCOVA to examine group differences for change in bone structural variables adjusting for baseline weight, height change, Tanner breast stage, and physical activity. There were no significant group differences for change in adjusted bone structural variables at any site in the pre-pubertal girls. However, the more mature girls (EARLY) in the intervention group showed significantly greater gains in FN (+2.6%, p=0.03) and IT (+1.7%, p=0.02) BMD. Underlying these changes were increased bone cross-sectional area and reduced endosteal expansion in the intervention group. Changes in subperiosteal width did not differ at any site. Structural changes led to a greater increase in bone bending strength (section modulus) at the FN (+4.0%, p=0.04), but not the IT region. There were no differences at the primarily cortical femoral shaft. These data highlight the complexity of the bone adaptation to mechanical loading which varies depending on the bone site and region and the characteristics of the participants (pubertal stage).

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A Randomized, Placebo Controlled, Pilot Trial of Low Magnitude, High Frequency Loading Treatment of Low Bone Mineral Density in Children with Disabling Conditions. K. A. Ward,*[1] C. W. Alsop,*[1] S. Brown,*[2] J. Caulton,*[3] J. E. Adams,[1] Z. Mughal.*[4] [1]Clinical Radiology, Imaging Science and Biomedical Engineering, University of Manchester, Manchester, United Kingdom, [2]Smith and Nephew Group Research Centre, York, United Kingdom, [3]School of Physiotherapy, Central Manchester Healthcare Trust, Manchester, United Kingdom, [4]Pediatric Medicine, Saint Marys Hospital, Manchester, United Kingdom.

Children with certain disabling conditions, such as cerebral palsy, are prone to atraumatic fractures, which are associated with low bone mineral density. The aim of this randomized, double blind, placebo controlled, pilot trial (RCT) was to determine whether low magnitude, high frequency loading would lead to an increase in tibial and spinal volumetric trabecular bone mineral density (vTBMD, mg/ml) in children with disabling conditions.A heterogeneous group of 20 pre-or post pubertal disabled but ambulant children (14 males, 6 females; mean age 9.1 ± 4.3 years, range 4-19 years) took part in this RCT. They were randomized to standing on active (n = 10; 0.3G, 90Hz,) or placebo (n = 10) devices (Exogen Optimass Device, Smith & Nephew) for 10 minutes/day, 5 days/week for 6 months. This G-force results in approximately 5 microstrain in the tibia of a standing sheep (Dr C Rubin, Personal Communication). Quantitative computer tomography (Philips SR-400), in conjunction with a spiral 3-D software (QCT-Pro, Mindways Software Inc) was used to measure vTBMD of the proximal tibia and the lumbar spine (L2). Repeat analysis precision was 0.9% for proximal tibia and spine. Median duration of treatment received by subjects in the active and placebo groups was 35.5 days (range 15 - 117 days); median standing time 481 minutes (range 88 - 1206 minutes). Mean proximal tibial vTBMD showed a significant effect of treatment (p=0.0036) after adjusting for covariates of body weight, disability category, pubertal status, calcium intake, baseline vTBMD and number of days in the study. Mean change in proximal tibial vTBMD in children who stood on active plates was +11.6 mg/ml; and in those who stood on placebo plates it was - 6.7 mg/ml. The net increase in mean proximal tibial vTBMD was 18.2 mg/ml (95% CI: 7.3 to 29.1); in spinal vTBMD was 3.8 mg/ml, (95% CI: -6.4 to 13.9; p=0.43). Based on the results of this pilot RCT we conclude that low magnitude, high frequency loading treatment administered by the Exogen Optimass Device offers a non-invasive and non-pharmacological approach to improving trabecular bone mineral density in children with disabling conditions.*Exogen Optimass Devices used in this study were on loan from Smith & Nephew, Memphis, USA.*

Disclosures: Smith & Nephew Inc, Memphis, Tennessee, USA,2.

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Bone Mineral Gain Following Calcium Supplementation in Teenage Girls is Reversed Two Years After Withdrawal of the Supplement. K. D. Ghatge,*[1] H. L. Lambert,*[2] M. E. Barker,*[1] R. Eastell.[2] [1]Centre for Human Nutrition, University of Sheffield, Sheffield, United Kingdom, [2]Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Supplementation with milk or calcium during childhood and adolescence enhances bone mineral accrual. The extent to which this beneficial effect persists beyond the period of supplementation is less clear. The relative merits of milk and elemental calcium as a supplement are also not known. We conducted an 18-month randomised, controlled calcium supplementation trial in 86, 11-12 year-old girls, with follow-up 2 years after stopping the supplement. The supplement was 800mg/day calcium in the form of citrate malate (CCM). Subjects were randomised to a calcium or control group, stratified by baseline calcium intake and pubertal stage (Tanner breast stage). Calcium intake was assessed by weighed intake (7-days at 0 and 18 months, 4-days at 42 months). Total body bone mineral content (TBBMC) and bone mineral density (BMD) of the total body (TB), lumbar spine (LS) and total hip (TH) were measured by DXA at baseline, at the end of supplementation (18 months) and 2 years after stopping the supplement (42 months). The mean baseline calcium intake was 658mg/day. After 18 months this had increased to 972mg/day in the calcium group, with no change in the control group. At 42 months the mean calcium intake was 652mg/d, and with no differences between the groups.At the end of the supplementation period the calcium group had gained significantly more TBBMD than the control group ($P<0.05$, t-test). This effect was completely reversed 24 months after stopping the supplement (table 1). The results for TBBMC, LSBMD and THBMD showed a similar trend.

Table 1. Percent change in BMD: mean (SD)

	TBBMD		LSMBD		THBMD	
Group	**0-18 months**	**18-42 months**	**0-18 months**	**18-42 months**	**0-18 months**	**18-42 months**
Calcium	8.4 (3.1)	5.9 (3.2)	15.2 (4.8)	9.5 (7.0)	12.8 (5.6)	5.4 (5.5)
Control	6.8 (2.9)	7.9 (3.2)	13.2 (6.6)	13.7 (8.1)	11.9 (7.4)	7.5 (6.8)
t	2.21	-2.31	1.49	-2.33	0.53	-1.46
p	0.03	0.02	0.14	0.02	0.60	0.15

Two-tailed, unpaired t-test

The pattern of the effect of CCM suggests that the observed gain in BMD is likely due to suppression of bone remodelling, and a contraction of the remodelling space, which is reversed when the supplement is withdrawn. This pattern is not seen with milk[1], indicating that the mechanism for the effect of this supplement differs to that of elemental calcium. We plan to confirm the effect of the contribution of the remodelling space using longitudinal change in biochemical marker levels.[1]Cadogan J., Eastell R., Jones N. and Barker M.E. (1997) Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trail. *British Medical Journal*, **315**, 1255-1260.

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The Growing Skeleton: A Comparison of MRI, DXA, and Cross-Sectional Geometric Changes at the Proximal Femur Over 7-Months. H. A. McKay,[1] A. Heinonen,[1] M. A. Petit,[1] K. M. Khan,[1] K. Forkheim,*[2] B. Forster,*[2] [1]Human Kinetics, University of British Columbia, Vancouver, BC, Canada, [2]Radiology, University of British Columbia, Vancouver, BC, Canada

Bone may alter its geometric properties independent of changes in bone mass. Further, two-dimensional measurement modalities such as DXA are confounded by size increases during growth and do not allow us to study the structural basis of skeletal change. Therefore, we assessed 7-month changes in bone structural parameters at the femoral neck by magnetic resonance imaging (MRI) and with the Hip Structural Analysis program (HSA), and total bone area, bone mineral content and BMAD by dual energy X-ray absorptiometry (DXA) in 18 prepubertal girls. Total bone cross-sectional area was also calculated from high resolution T1-weighted MRI oblique axial images of the femoral neck. We used proximal femur DXA scans (Hologic QDR-4500) and Beck et al's HSA program to estimate bone cross-sectional area (CSA), subperiosteal bone width, and section modulus at the femoral neck. We used ANCOVA to examine group differences (controlling for age and change in lean body mass) to assess percent change (95% confidence intervals. We also determined short-term precision for MRI with repositioning on 10 adult subjects. Mean percent change (95% CIs) from baseline in TBA, adjusted for covariates was 8.5% (4.9 to 12.1%) as measured by MRI and 5.1% (0.8-9.5%) by DXA. A significant increase was also noted for BMC (9.6% (6.0-13.2%)) and cross-sectional geometry (subperiosteal width +2.4% (0.2-4.6%), cortical thickness +5.2% (1.9-8.6%), section modulus +11.3% (6.9-15.6%)) between trials. BMAD showed a non-significant change (2.1% (-0.9 to 5.0%)). Spearman rank order correlation was significant between absolute values for total bone area measured by MRI and total bone area by DXA ($r=0.45$, $p<0.10$), section modulus ($r = 0.40$, $p<0.10$), and subperiosteal width ($r=0.45$, $p<0.10$) by HSA. Precision (CV%) for total bone area, cortical and trabecular bone CSA by MRI were 1.5%, 6% and 1.8%, respectively. This is the first prospective study in a pediatric group to utilize MRI to assess change in bone parameters at the proximal femur in growing girls. Findings highlight the independent change in bone mass and size at the femoral neck by MRI with no concomitant change in bone mineral apparent density (by DXA). MRI is a reproduceable method to assess total bone and trabecular bone change at the proximal femur.

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Effect of Age, Size and Muscularity on Peripheral Quantitative Computed Tomography (pQCT) Measures in Children. B. Zemel,[1] M. C. Nelson,*[1] A. V. Owen,*[1] V. A. Stallings,[1] M. B. Leonard.[2] [1]Division of GI and Nutrition, The Children's Hospital of Philadelphia, Philadelphia, PA, USA, [2]Division of Nephrology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA.

Assessment of bone health in children is complicated by the effects of growth and changing body composition on bone dimensions. These influences are important in light of the altered growth and body composition that commonly occur in children with chronic diseases for whom bone health is a significant concern. Size-related effects in DXA bone density measurements are recognized, but are less well understood for pQCT. The goal of this preliminary study was to examine the effects of age, body size and muscularity on pQCT measures in healthy prepubertal children. Healthy children, ages 7.0 to 10.9 years were recruited. Exclusion criteria included obesity, chronic illnesses or medication use affecting growth, bone density or dietary intake. pQCT measurements of the left distal tibia were obtained using a Norland-Stratec XCT2000. At the 4% site, trabecular bone mineral content (BMC), area and density (BMD) was measured, as were cortical BMC, area, and BMD, and the polar moment of inertia (PMI) at the 20% site. Height and tibia length (TL) were assessed. Upper arm muscle area (UAMA) was derived from arm circumference and triceps skinfold measures. UAMA z-score was based on NCHS reference data. Grip strength was measured using a hand-held dynamometer. Data were analyzed in Stata 6.0 (College Station, TX) using t-tests and regression analysis. A dummy variable for pQCT analysis mode at the 4% site was included in regression models as appropriate. 51 children (30F) were evaluated. Age was significantly associated with most pQCT measures except for trabecular and cortical BMD, and TL was a better predictor than age (see Table). Gender differences were not evident. Grip strength and UAMA-Z were also significantly associated with BMC, area and PMI but not BMD. Regression models using TL and grip strength were significant, with R^2 = 80% for cortical BMC and 72% for PMI. TL and UAMA-Z were significant predictors of cortical area (R^2 = 60%). Table: Percent of variance explained (R^2) by age and size for pQCT measures

	4% distal Tibia*			20% distal Tibia			
	BMC	**Area**	**BMD**	**BMC**	**Area**	**BMD**	**Polar MI**
Age	0.51	0.56	ns	0.37	0.29	ns	0.34
TL	0.64	0.68	ns	0.69	0.57	0.10	0.69

Note: *adjusted for pQCT analysis mode at 4% site; $p<0.05$, ns = not significant Consistent with other reports, in prepubertal children, BMD is not associated with age. Size and muscularity are significant predictors of BMC, area and PMI (an indicator of bone strength). These findings underscore the importance of body size and composition in assessing bone health even within a narrow age range of prepubertal children

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The Use of Inhaled Corticosteroids Is Not Associated with an Increased Risk of Fracture in Children. T. P. van Staa,[1] C. Cooper,[2] H. G. M. Leufkens,*[3] N. Bishop.[4] [1]Procter & Gamble Pharmaceuticals, Staines, United Kingdom, [2]University of Southampton, Southampton, United Kingdom, [3]Utrecht Institute for Pharmaceutical Sciences, Utrecht, Netherlands Antilles, [4]Sheffield University, Sheffield, United Kingdom

Oral corticosteroids are known to increase the risk of fractures. It is unknown whether children using inhaled corticosteroids have an increased risk of fracture due to systemic absorption. We undertook a population-based nested case-control study using medical records of general practitioners in the UK (from the General Practice Research Database). Children aged 4 to 17 years taking inhaled corticosteroid were compared to children taking only noninhaled/nonsystemic corticosteroid prescriptions (topical, aural, ophthalmic, or nasal). Incidence rates of fracture during follow-up were estimated for each cohort. Each fracture case was then matched by age, sex, practice, and calendar time to one child without a fracture.It was found that the incidence of fracture for both the reference and inhaled corticosteroid cohorts rose before puberty, reached a peak around puberty, and fell thereafter. The risk of fractures for boys was generally higher than that for girls (rate of 2.1 fractures per 100 person-years in boys versus 1.3 in girls). There were 97 387 children taking inhaled corticosteroids, of whom 3754 suffered a fracture during treatment. With an average daily dose of 200 µg/day or less (standardized to beclomethasone dipropionate), the crude odds ratio for fracture relative to children taking only noninhaled/nonsystemic corticosteroids was 1.09 (95% CI 0.98-1.21), with 201 to 400 µg 1.05 (0.95-1.15) and over 400 µg 1.22 (1.06-1.41). The excess risk in the high daily dose group disappeared after adjustment for indicators of asthma severity (adjusted odds ratio of 1.02 [95% CI 0.87-1.20]). There were also higher risks of fracture in children with more previous cumulative exposure to inhaled corticosteroids, but this also disappeared after adjustment.Asthma itself, rather than the use of inhaled corticosteroids, is associated with an increased risk of fracture in childhood. The mechanism underlying this increase in risk remains to be determined.

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CIZ, a Transcription Factor Shuttling between Nuclei and Cell Adhesion Plaque, Enhances Cbfa1 (Runx 2) Gene Expression but Counteracts against BMP Signaling to Resume Osteopontin and to Suppress Collagen Gene Expression in Osteoblast Like Cells. Z. Shen,[1] K. Tsuji,[1] T. Nakamoto,[2] H. Hirai,[2] A. Nifuji,[1] M. Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]University of Tokyo, Tokyo, Japan.

CIZ (Cas interacting zinc finger protein) (NP/NMP4) is a nucleocytoplasmic shuttling protein, which binds to p130CAS at focal adhesion plaques and also binds to DNA to regulate expression of genes encoding MMPs and type-I collagen. Thus, CIZ could act as a novel type of transcription factor expressed in osteoblast. Although osteoblastic functions are regulated by matrix-signaling through their adhesion to the ECM proteins (out-side-in signaling) and the matrix formation and reorganization are tightly controlled by osteoblasts (in-side-out signaling) under the control of cytokine signals including BMP, the molecular mechanisms involved in this relationship between matrix-induced signals and gene regulation in osteoblasts are not well understood. Here, we examined cross talk between the actions of CIZ and BMP signaling in MC3T3E1 osteoblastic cells. CIZ was overexpressed stably in MC3T3E1 cells and clonal cell lines were established using geneticin selection. Overexpression of CIZ enhanced Cbfa1 (Runx 2) gene expression in the clonal cell lines. Promoter sequence analysis of Cbfa1 gene also identified CIZ-binding sites of G/CAAAAA. BMP treatment enhanced Cbfa1, alkaline phosphatase (ALP), and osteocalcin (OC) expression in control MC3T3E1 cell lines transfected with an empty vector. In contrast, CIZ overexpression suppressed the BMP-induced enhancement of Cbfa1, ALP, and OC gene expression. BMP2 treatment also enhanced type-I collagen mRNA expression over 7.8-fold in the control MC3T3E1 within 3-days and this enhancement was still observed at 7 and 14 days. In contrast, CIZ overexpression completely blocked the BMP2 effects on type-I collagen mRNA expression at all the time points. These data reveal the presence of CIZ inhibition of BMP signaling. Either CIZ overexpression or BMP2 treatment alone suppressed osteopontin (OPN) expression while the presence of both signaling systems cancelled out the suppressive effects of each other ending up with the recovery of basal level of OPN expression, indicating another line of the interference between the two signaling systems. Thus, our data indicate the dual role of CIZ, i.e activaion of Cbfa1 gene expression in the absence of BMP2 and inactivation of BMP signaling-induced events. As Cbfa1 and BMP signaling are required for osteoblastic differentiation and function, CIZ would act as a critical modulator to balance the two major signaling systems in osteoblasts under the control of matrix.

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Runx2-C/EBP Molecular Interactions and Functional Synergism Support Maximal Expression of Osteocalcin in Osteoblasts. A. Javed,* S. E. Gutierrez,* D. K. Tennant,* M. van Rees,* M. Montecino,* A. J. van Wijnen, G. S. Stein, J. B. Lian, J. L. Stein.* Cell Biology, UMASS Medical School, Worcester, MA, USA.

CCAAT/enhancer binding proteins (C/EBP) members of the leucine zipper family of transcription factors are critical for normal cellular differentiation, although their functional role(s) in osteoblast development have not been determined. Here we show that C/EBP β and C/EBP δ mRNAs are expressed from growth to maturation stages with an increase (3-5 fold) at the onset of osteocalcin transcription. Peak expression of both mRNAs occurs during the mineralization stage. Furthermore, vitamin D_3, a physiologic regulator of osteoblastic gene expression, mediates 3-6 fold upregulation of C/EBP β and δ, suggesting that C/EBP(s) contribute to osteogenic differentiation. We characterized a C/EBP regulatory element in the proximal promoter of the rat osteocalcin gene. Mutational analyses and forced expression studies demonstrate that this element mediates a 4-8 fold transcriptional enhancement in both osseous and non-osseous cells. This enhancer activity is equivalent to that mediated by the Runx2 transcription factor. Functional studies show that C/EBP and the bone-related Runx2/Cbfa1 transcription factor interact synergistically to enhance OC transcription (35-40 fold). We established by point mutations that this synergism is mediated through the C/EBP responsive element in the OC promoter. Furthermore mutant Runx2 protein lacking the C/EBP interaction domain fails to exhibit synergism. Taken together, our findings indicate that C/EBP and Runx2, are both principal transactivator of the OC gene that directly interact and cooperate to regulate physiologic induction of the bone-tissue specific osteocalcin gene during osteoblast differentiation.

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FHL-2, A LIM Only Transcription Factor, Is a Binding Partner for IGF Binding Protein (BP)-5 in Normal Human Osteoblasts (OB). Y. Amaar, G. Thompson,* T. A. Linkhart, S. T. Chen,* D. J. Baylink, S. Mohan. MDC, Pettis VAMC, Loma Linda, CA, USA.

BP-5 stimulates bone formation parameters and correlates with BMD, thus suggesting BP-5 is an important bone formation regulator. Recent studies using IGF-I knockout mice demonstrate that BP-5 itself is a growth factor with cellular effects not dependent on IGFs. Based on these findings and the findings that BP-5 contains nuclear localization sequence that mediates transport of BP-5 into the nucleus, we propose that BP-5 interacts with transcription factors to stimulate transcription of genes that lead to increased OB proliferation and/or differentiated functions. As a means of testing this hypothesis we undertook studies to identify proteins that bind to BP-5 using BP-5 as bait in the yeast two hybrid screen of U2 human osteosarcoma cDNA library. Five clones were identified that interacted strongly with the bait under high stringency conditions that corresponded to FHL2 gene (also known as SLIM-3), which contains four and a half LIM domains. One clone (1.4 kb) was full length encoding 297 amino acids while the other four were partial clones encoding the C-terminal region of FHL2. The smallest clone (0.8 kb) encoded LIM domains 3 and 4, thus suggesting that the C-terminal region of FHL2 containing the last two LIM domains is sufficient for FHL2 interaction with BP-5. In order to determine the specificity of FHL2 interaction with BP-5, we expressed FHL2 in E.coli and performed interaction studies between purified FHL2 and various IGFBPs *in vitro* using FHL2 antibody. We found that FHL2 interacted with BP-5 but not with BP-4 or BP-6. We next determined if FHL2 is expressed in normal human OBs and localized in the nucleus. Northern blot analysis showed that FHL2 is strongly expressed in normal human OBs derived from calvaria and rib besides U2 and SaOS-2 human osteosarcoma cells. Western immunoblot analysis using cytoplasmic and nuclear extracts revealed that the FHL2 protein is present in both of these extracts. Furthermore, nuclear localization of BP-5 in OBs was identified by immunofluorescence and by western immunoblot analysis using BP-5 specific antibodies, thus raising the possibility that the interaction between BP-5 and FHL2 could occur in the nucleus. Conclusions: 1) FHL2, a LIM only protein, has been identified as a binding partner for BP-5 in OBs. 2) Interaction between FHL2 and BP-5 is specific and involves C-terminal

region of FHL2. 3) Based on the findings that BP-5 stimulates bone formation and that LMP-1, another LIM-domain protein, is a positive regulator of bone formation, we predict FHL2 to be an important bone cell regulatory protein that functions to mediate the effects of BP-5 and other osteoregulatory agents in bone cells.

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Normalizing the Decreased Circulating Levels of Leptin in ΔFosB Transgenic Mice Fails to Rescue the Osteosclerotic Phenotype. M. Kveiborg, R. Chiusaroli, J. Juel,* G. Sabatakos, W. C. Horne, R. Baron. Yale University School of Medicine, New Haven, CT, USA.

Transgenic mice overexpressing δFosB isoforms exhibit an osteosclerotic phenotype (Sabatakos et al. Nature Med 6: 985-990, 2000). This effect was shown to be cell-autonomous, i.e. reproducible in vitro with osteoblasts isolated from transgenic animals or by transfecting δFosB isoforms in osteoblastic cell lines. We also observed that abdominal fat as well as numbers of marrow adipocytes were decreased in δFosB mice, as were serum leptin levels. Increased bone mass has been linked to deficient leptin and leptin receptor signaling in Ob/Ob and Db/Db mice (Ducy et al. Cell 100: 197-207, 2000), raising the possibility that, in addition to the direct effect of δFosB isoforms in cells of the osteoblast lineage, the reduction in leptin contributes to the increased bone mass. We therefore tested the hypothesis that, in addition to affecting directly osteoblastogenesis and bone formation, δFosB isoforms might increase bone mass indirectly via a decrease in leptin. For this purpose, we restored normal circulating levels of leptin in δFosB mice by infusing leptin via subcutaneous osmotic pumps for periods of 4 weeks. Infusion of 200 ng/hr restored circulating levels of leptin to the normal range in δFosB mice (saline: 0.94 ± 0.29 ng/ml vs leptin: 2.62 ± 0.70 ng/ml), but did not affect circulating leptin levels in control mice (saline: 2.00 ± 0.76 ng/ml vs leptin: 2.73 ± 0.76 ng/ml), due to decreased fat: Leptin infusion caused a significant decrease in abdominal fat in control mice (saline: 0.465 ± 0.040 g vs leptin: 0.256 ± 0.090 g), but did not further decrease fat weight in transgenic mice (saline: 0.117 ± 0.037 g vs 0.103 ± 0.053 g). Complete histomorphometric analysis demonstrated that trabecular bone volume was unchanged in the control mice (saline: 9.47 ± 1.29 BV/TV (%) vs leptin: 10.08 ± 3.70 BV/TV (%)) as well as in transgenic mice (saline: 28.60 ± 7.04 BV/TV (%) vs leptin: 22.70 ± 5.78 BV/TV (%)), independent of gender. Furthermore, bone formation parameters were unaffected by leptin treatment (MAR control mice, saline: 1.47 ± 0.55 μm/day vs leptin: 1.53 ± 0.46 μm/day; MAR δFosB mice, saline: 1.23 ± 0.22 μm/day vs leptin: 1.17 ± 0.27 μm/day) (BFR control mice, saline: 213.63 ± 99.06 $\mu m^3/\mu m^2$/year vs leptin: 195.60 ± 43.13 $\mu m^3/\mu m^2$/year; BFR δFosB mice, saline: 176.90 ± 89.90 $\mu m^3/\mu m^2$/year vs leptin: 170.10 ± 108.87 $\mu m^3/\mu m^2$/year). These results demonstrate that restoring circulating levels of leptin in δFosB transgenic mice failed to rescue the bone phenotype, further indicating that the marked increase in bone formation is autonomous to the osteoblast lineage.

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AP-1/Interleukin (IL)-11 Signaling Cascades May be Involved in PTH-induced Bone Formation: ERK-dependent Induction of Delta-fosB and IL-11 by hPTH(1-34). S. Kido, D. Inoue, T. Matsumoto. First Department of Internal Medicine, University of Tokushima School of Medicine, Tokushima, Japan.

Intermittent PTH administration increases bone mass by stimulating bone formation in vivo. However, PTH-induced osteogenic signals remain to be determined. We have shown that delta-fosB, a fosB splicing variant, and IL-11, both of which have been shown to stimulate bone formation by in vivo over-expression in transgenic mice, are rapidly induced by mechanical stress both in vitro and in vivo osteoblasts. We have also demonstrated that IL-11 transcription is totally dependent on AP-1 and that impaired bone formation in the aged is associated with reduction in AP-1 activities and IL-11 expression in marrow stromal cells. These findings suggest that AP-1 and its target IL-11 may play universal roles in divergent osteogenic signaling pathways. We therefore tested a hypothesis that PTH induces bone formation via AP-1/IL-11 induction. Consistent with our hypothesis, we found that treatment of mouse primary osteoblasts (POB) with 100 ng/ml hPTH (1-34) induced delta-fosB mRNA expression in 30 min and IL-11 at 3 hrs. Intermittent PTH treatment (6 hrs/48 hrs x 3 cycles) of osteoblasts resulted in accumulation of Delta-FosB and Delta2-Delta-FosB, which is produced from the same delta-fosB mRNA by alternative ATG codon usage, in nuclear extracts. Furthermore, IL-11 neutralizing antibodies partially blocked PTH induction of ALP in POB. We also demonstrated that PTH activated ERK in POB and that PTH induction of delta-fosB/IL-11 was completely blocked by an ERK1/2 inhibitor U0126, but not by a p38 kinase inhibitor SB203580, indicating that PTH induction of delta-fosB is dependent on ERK. Interestingly, in vivo treatment of mice with PTH by peritoneal injection induced IL-11 mRNA expression in limb bones within several hours, and this induction was partially inhibited by pre-injection of dexamethasone (Dex). In vitro, pre-treatment of POB with 100 nM Dex completely blocked the PTH induction of IL-11, but not that of delta-fosB, consistent with the ligand-dependent inhibition of AP-1 activities by glucocorticoid receptors. IL-11 expression was also induced by a Ca-ionophore A23137 and DBcAMP, which was again completely inhibited by Dex. Taken together, these results suggest that the ERK/AP-1 (delta-fosB)/ IL-11 cascade participates in PTH-induced signaling pathways that leads to increased bone formation. Our findings also imply that suppression of IL-11 expression may contribute to the pathogenesis of glucocorticoid-induced osteoporosis. Thus, the AP-1/IL-11 cascade appears to be a critical component of various osteogenic signaling pathways under both physiological and pathological conditions.

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Transcriptional Repression of Osteocalcin Gene Expression by Delta EF1. K. Sooy,* M. B. Demay. Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

The first intron of the rat osteocalcin gene contains a complex suppressor region from +106 to +142. Previous studies have identified unique silencer elements in this region that have a suppressive effect on rat osteocalcin-reporter fusion gene transcription. A search of the transcription factor database, "Transfac", using the sequence of the first intron of the rat osteocalcin gene, suggested several candidate transcription factors that may interact with the sequences in intron 1. The consensus sequence for one of these factors, delta EF1 (dEF1), showed extremely high homology with two pyrimidine rich repeats (CCTCCT). DNAse I protection analysis also demonstrated that these CCTCCT motifs were important contact sites for nuclear protein-DNA interactions. dEF1 was initially identified as a repressor of the chicken delta 1 crystallin gene enhancer. dEF1, a member of the zinc finger-homeodomain protein family and a vertebrate homolog of zfh-1 in Drosophila, is inversely expressed with type II collagen in chick limb bud mesenchymal cells and is able to repress Col2a1 promoter activity. Additionally, dEF1 knockout mice exhibit a severe skeletal phenotype, suggesting that dEF1 has a critical role in regulating skeletal gene expression. In order to further investigate a potential role of dEF1 in regulating osteocalcin gene expression, the sequence of intron 1 of the rat osteocalcin gene was placed upstream of the native promoter (-306 to +29) fused to a luciferase reporter gene, to determine whether overexpression of dEF1 is able to increase transcriptional repression by these sequences. Cotransfection of this fusion gene (wtOC-luc) with a dEF1 expression vector led to a 30% decrease in luciferase activity as compared to wtOC-luc cotransfected with empty vector. This repressive effect was not observed when the dEF1 vector was cotransfected with an osteocalcin luciferase fusion gene with mutations in both motifs (CCTCCT to atgCaT; mOC-luc), indicating that intact CCTCCT motifs are necessary for dEF1-mediated repression by these DNA sequences. In gel retardation assays, a wild type dEF1 consensus sequence was able to compete with the wild type osteocalcin oligonucleotide probe containing both CCTCCT motifs, for the generation of the DNA-protein complex. Mutations in the CCTCCT motif which abolished suppressor activity, also abolished competition for the DNA-protein complex, as did analogous mutations in the dEF1 consensus oligonucleotide. These data suggest that dEF1 interacts specifically with the CCTCCT motif and contributes to transcriptional repression of the rat osteocalcin gene in ROS 17/ 2.8 cells.

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Localized Expression of Hypoxia Inducible Factor Family and Differential Expression of VEGF Splice Isoforms in Neovascularization and Bone Formation During Regenerating Bone and Bone Marrow After Drill-Hole Injury. S. Uchida,[1] H. Kudo,*[2] A. Sakai,[1] K. Narusawa,*[1] M. Tanaka,*[1] M. Watanuki,*[1] T. Nakamura.*[1] [1]Orthopedic Surgery, University of Occupational and Environmental Health, Kitakyushu, Japan, [2]Department of Anatomy, University of Occupational and Environmental Health, Kitakyushu, Japan.

The purpose of this study is to clarify the cellular events and the expression of Vascular Endothelial Growth Factor (VEGF) and Hypoxia Inducible Factor (HIF) on bone cells and vascular cells in the regenerating bone and bone marrow following drill-hole injury by RT-PCR, immunocytochemistry and in situ hybridization. Wistar rats aged 12-13 weeks were used in the present experiments. A hole of approximately 2.5 mm in diameter penetrating the bone marrow was drilled in the diaphysis of bilateral femur using a surgical drill. At days 1, 3, 5, 7, 11, 14, and 21 after surgery, the rats were sacrificed and bone wound regions were isolated and provided for the experiments Abundant ALP positive osteoprogenitor cells existed in the endosteum surrounding wound region at day 3. At the same period, growing capillaries invaded the wound region from the adjacent endosteum and bone marrow. Some ALP positive cells in the endosteum showed immunoreactivity for Flk-1 as a marker of angioblasts. At day 5, osteoblasts that had begun to express osteocalcin mRNA actively participated in bone formation. Between days 7 and 11, sinusoidal capillaries extended to interstices between trabecular bone networks of medullary callus. The expression of VEGF120 and VEGF165 mRNA were detected from day 3, and further VEGF189 mRNA began to exhibit from day 5. These expressions gradually declined after day 11. VEGF immunoreactivity and gene expression were strongly detected on angioblasts, osteoprogenitor cells and osteoblasts from days 3 and 7. Then the expression gradually declined thereafter. Immunoreactivity for Flk-1 was detected on vascular cells and immunoreactivity for Flt-1 was also detected on both bone cells and vascular cells between days 3 and 7. On the other hand, the gene expression of HIF-1 alpha was detected constitutively between day 1 and day 21 by RT-PCR. Immunoreactivity for HIF-1alpha and HIF-1beta was observed on vascular cells and growing capillaries from day 1 to day 7. However, these expressions were not detected on osteoprogenitor cells and osteoblasts. These findings suggest that alternative splicing of VEGF mRNA may play important role in the transformation of osteoprogenitor cells to osteoblast during bone formation, and HIF family may induce the expression of VEGF mRNA in neovascularization during regenerating bone and bone marrow following Drill-Hole injury.

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Expression of Serotonin Receptors in Bone. J. Klein-Nulend,[1] I. Westbroek,*[2] A. van der Plas,*[2] C. M. Semeins,*[1] E. H. Burger,[1] P. J. Nijweide.[2] [1]Oral Cell Biology, ACTA-Vrije Universiteit, Amsterdam, The Netherlands, [2]Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands.

The 5-hydroxytryptamine (5-HT) receptors 5-HT2A, 5-HT2B, and 5-HT2C belong to a subfamily of serotonin receptors. Their amino acid and mRNA sequences have been published for several species, including man. They have been described to act on nervous, muscle, and endothelial tissues. Here we report the presence of 5-HT2B receptors in chicken and mouse bone cells and on the possible functions of serotonin in bone. Using suppression subtractive hybridization we identified a large number of genes preferentially expressed in chicken osteocytes as compared to osteoblasts. One of the genes was the 5-HT2B receptor. RT-PCR and Northern blot analysis of chicken osteocytes, osteoblasts, and osteoblast precursor cells confirmed the preferential but not exclusive expression of 5-HT2B mRNA in osteocytes. To study the possible functions of serotonin in bone, we tested whether serotonin stimulated osteoblast precursor cell proliferation, as has been reported for vascular smooth muscle cell proliferation. We also examined whether serotonin modulated bone cell mechanosensitivity, which could offer a role for the relatively high 5-HT2B

receptor expression in osteocytes, the putative mechanosensory cells of bone. α-Methyl-5-HT, a 5-HT analogue which preferentially binds to 5-HT2 receptors, dose-dependently stimulated chicken osteoblast precursor cell proliferation. Similar effects were found with the 5-HT2B receptor-selective agonist BW723C86. An effective inhibitor of proliferation appeared to be SB215505, a 5-HT2B receptor-selective antagonist. However, ketanserin, a 5-HT2A receptor-selective antagonist, also inhibited cell proliferation to some extent. These pharmacological studies clearly indicate that the 5-HT2B receptor is involved in serotonin-regulated osteoblast precursor cell proliferation, but a possible role of 5-HT2A receptor is not excluded. mRNA expression of both 5-HT2A and 5-HT2B receptor variants was found in primary cultures of adult trabecular bone-derived mouse bone cells. These cells respond to pulsating fluid shear stress *in vitro* by rapid release of nitric oxide (NO) as we have reported earlier. Addition of α-methyl-5-HT to the flow medium reduced both basal and PFF-stimulated NO release by approximately 50%.These results suggest that serotonin has a functional role in bone metabolism by regulating bone precursor cell proliferation. The high expression of serotonin receptor in osteocytes, combined with the modulating effect of serotonin analogue on bone cell mechanosensitivity, suggest a role for serotonin in modulating bone adaptation to stress.

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TRAF6 Expression Promoted by RANKL/RANK Signaling Plays an Important Role in Osteoclast Differentiation. Y. Kadono,* T. Akiyama,* A. Yamamoto, T. Ogata,* I. Nakamura, H. Oda, K. Nakamura, S. Tanaka. Department of Orthopaedic Surgery, The University of Tokyo, Tokyo, Japan.

Recent studies showed that RANKL (receptor activator of nuclear factor-kappa B ligand) and its receptor RANK play central roles in osteoclast development. Members of the family of TRAF (tumor necrosis factor receptor-associated factor) proteins are cytoplasmic adaptor proteins that have been shown to interact with the intracellular domains of cell surface receptors, and we and other groups demonstrated that TRAF6 is involved in RANKL/RANK signaling and plays an essential role in osteoclast differentiation. Although TRAF6 is also recruited to interleukin (IL)-1/IL-1 receptor signaling, RANKL cannot be substituted by IL-1 for osteoclast differentiation. When bone marrow cells cultured in the presence of macrophage colony-stimulating factor (M-BMMϕ) were stimulated with RANKL, the expression level of TRAF6, as detected by Western blotting, was elevated, while its expression did not change in response to IL-1 stimulation. To investigate the role of TRAF6 induction in osteoclast differentiation, we constructed a retrovirus vector carrying TRAF6 cDNA, and overexpressed TRAF6 in M-BMMϕ to the comparable level to that observed in RANKL-stimulated M-BMMf. Surprisingly, mere overexpression of TRAF6 in M-BMMϕ was sufficient to induce osteoclast-like multinucleated cell (OCL) formation. The OCLs formed in response to TRAF6 overexpression was positive for tartrate-resistant acid phosphatase activity, showed actin-ring, and expressed mRNA specific for osteoclasts such as MMP-9, cathepsin K and calcitonin receptors. When cultured on dentine slices, they formed resorption pits, satisfying major criteria of osteoclasts. Osteoprotegerin treatment did not suppress the OCL formation induced by TRAF6 overexpression, indicating that this OCL formation was not due to the intrinsic RANKL production by M-BMMϕ. In contrast, overexpression of either TRAF2 or TRAF5 did not induce OCL differentiation, nor did the overexpression of TRAF6 deleted either RING finger domain or TRAF-C domain. These results suggest that RANKL-induced TRAF6 upregulation is sufficient and essential for differentiation of osteoclasts. Further investigation is required to clarify the molecular mechanism of TRAF6-mediated osteoclast differentiation.

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A TNF-α-Related Peptide Inhibits Bone Resorption in Vivo and Osteoclastogenesis in Vitro by Interfering with RANK/RANKL Interaction. K. Aoki,[1] Y. Suzuki,*[1] T. Shibata,*[1] R. Murali,*[2] W. C. Horne,[3] M. I. Greene,*[2] K. Ohya,[1] R. Baron.[3] [1]Tokyo Medical and Dental University, Tokyo, Japan, [2]University of Pennsylvania, Philadelphia, PA, USA, [3]Yale University, New Haven, CT, USA.

Therapeutic peptidomimetics that interfere with the TNF/TNF receptor(I) binding have been developed based on the crystal structures of TNF-α and the TNF-β/TNF receptor(I) complex (Nature Biotech. 15: 1266, 1997). The cyclized peptidomimetic WP9QY (YCWSQYLCY), which was designed to mimic the most critical TNF-α recognition site on the TNF receptor(I), efficiently antagonized the effects of TNF-α binding to the TNF receptor(I). We have previously reported that this peptide antagonist blocks vitamin D_3/PGE_2- and sRANKL-induced osteoclastogenesis in vitro (J. Bone Miner. Res. 14: S178, 1999). However, reports that TNF-α enhances RANKL-dependent OC formation raised the possibility that the effect of the peptide was due to an inhibitory effect on TNF-α/TNFR interactions and not to the inhibition of RANKL/RANK binding. We therefore examined the effect of WP9QY on sRANKL-induced osteoclast differentiation of bone marrow cells derived from wild type animals and TNF receptor (I)-deficient or TNF receptor (II)-deficient mice. In both cases, WP9QY inhibited osteoclastogenesis to the same extent in the KO cultures as in wild-type bone marrow, while a control peptide had no effect. These data further show that in this in vitro system the inhibitory effect of the peptide is due to the inhibition of RANKL/RANK interaction and not to an inhibition of TNF signaling. We further examined the effect of WP9QY on bone resorption in vivo. Male CD-1 mice (7 week-old) were fed with either a diet containing 0.5% Ca (normal Ca group) or a diet with 0.05% Ca (low Ca group) for 48 hours, a time sufficient to observe increases in bone resorption and bone loss. Half of the low Ca group received subcutaneous injections of WP9QY (9 or 24 mg/kg) every 3 hours. No apparent change of body weight was observed in the peptide-treated group. Trabecular bone density of tibial metaphyses, measured by pQCT, was significantly reduced from 190 mg/cm^3 in the normal Ca group to 156 mg/cm^3 in the untreated low Ca group. Most interestingly, WP9QY inhibited the trabecular bone loss in a significant and dose-dependent manner (-17.6% in untreated low Ca mice vs -1.1% and +9.3% with treatment). Histomorphometric analysis revealed that treatment with WP9QY, in addition to protecting bone volume, prevented both the increased osteoclast number and the increased osteoclast surface that were induced by the low Ca diet. These results indicate that the WP9QY peptide antagonizes osteoclastogenesis by blocking RANK/RANKL interaction, and is able to exert this effect in vivo as well as in vitro.

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Inhibition of JNK1/c-Jun Signaling Pathway Decreases RANKL-induced Osteoclastogenesis. F. Ikeda,*[1] K. Hata,*[1] K. Yamashita,*[1] T. Watanabe,*[2] T. Kukita,[2] K. Yoshioka,*[3] R. Nishimura,[1] T. Yoneda.[1] [1]Dept Biochem, Osaka Univ Grad Sch Dent, Osaka, Japan, [2]Second Dept of Oral Anat, Kyushu Univ Grad Sch of Dent, Fukuoka, Japan, [3]Mol Pathol, Cancer Res Inst, Kanazawa Univ, Kanazawa, Japan

Recent studies suggest RANKL stimulates osteoclastogenesis through concomitant activation of the transcription factor NF-kB and a family member of MAP kinase JNK1. This notion is supported by the fact that mice deficient in NF-kB (p50/p52) show osteopetrosis due to impaired osteoclastogenesis. On the other hand, the role of JNK1 in osteoclastogenesis has not been established yet, since JNK1 knockout mice failed to show skeletal phenotype presumably due to compensation by other JNK family members and JNK1/JNK2 double knockout mice are embryonic lethal. Here, we studied the role of JNK1/c-Jun signaling pathway in osteoclastogenesis using the preosteoclastic cell line RAW-D that we recently cloned from the murine monocytic cell line RAW264. Osteoclastic differentiation was quantified by counting the number of tartrate-resistant acid phosphates-positive multinucleated cells (TRAP(+) MNC). Treatment with soluble RANKL (sRANKL) induced the differentiation of RAW-D cells into TRAP(+) MNC accompanied with an activation of JNK1, implying JNK1 in osteoclastic differentiation. To examine whether activation of JNK1 is critical to sRANKL-induced TRAP(+) MNC formation, we generated kinase-dead JNK1 (KD-JNK1) by introducing a point mutation in the ATP-binding 55 lysine. The KD-JNK1 showed no kinase activity and interfered with endogenous JNK1 activation in a dominant-negative manner. Expression of KD-JNK1 into RAW-D cells using an adenovirus vector markedly inhibited sRANKL-induced TRAP(+) MNC formation. In contrast, the dominant-negative or constitutively-active mutant of MKK3, which is a kinase specifically regulating another MAP kinase family member p38, had little effects on TRAP(+) MNC formation, suggesting a specific role of JNK1 for osteoclastic differentiation. Since JNK1 activates c-Jun and RAW-D cells expressing KD-JNK1 exhibited decreased TRAP(+)MNC formation and impaired c-Jun activation, we next examined the effects of c-Jun activation on TRAP(+) MNC formation. RAW-D cells were introduced with a dominant-negative c-Jun (DN-c-Jun) lacking the transactivation domain. We found these RAW-D cells showed a dramatic reduction in TRAP(+) MNC formation in response to sRANKL. In conclusion, our results suggest that activation of JNK1 and c-Jun by RANKL is critical to osteoclastogenesis and that identification of a target gene for JNK/c-Jun as well as NF-kB is important to further understand the molecular mechanism of osteoclastogenesis.

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Activation of c-Src, c-Cbl and RhoA, but not PYK2, in Osteoclasts, Requires the αvβ3 Integrin. R. Faccio,*[1] D. V. Novack,[1] X. Feng,[1] T. Kunicki,*[2] A. Zallone,[3] F. P. Ross,[1] S. L. Teitelbaum.[1] [1]Pathology, Washington University, St. Louis, MO, USA, [2]The Scripps Research Institute, La Jolla, San Diego, USA, [3]University of Bari, Bari, Italy

The FAK-like kinase, Pyk2, believed central to the mechanisms by which osteoclasts (OCs) resorb bone, is activated when OCs are plated on ligands recognized by the αvβ3 integrin. This observation has prompted the hypothesis that the defective bone resorptive capacity of αvβ3 deficient OCs reflects their failure to activate Pyk2. Surprisingly, we find that Pyk2 activation, as manifest by its phosphorylation, occurs equally in both β3-/- and β3 WT pre-osteoclasts plated on vitronectin for 30 and 60 min, but not in suspended cells. Thus, Pyk2 activation requires cell-matrix recognition, but does not depend upon αvβ3 occupancy. In contrast to tyrosine phosphorylation of Pyk2, activation of other components of the focal adhesion complex essential for bone resorption, namely c-src and c-cbl, is dependent on specific αvβ3 binding, as it does not occur in adherent β3-/- pre-OCs. In order to identify the structural components of the β3 subunit mediating these resorptive signals, we generated mutants known, in other systems, to affect integrin function and retrovirally expressed them in β3-/- OCs. Establishing the requirement for the β3 cytoplasmic domain, a construct consisting only of the extracellular and transmembrane domains (hβ3δc) does not rescue c-src and c-cbl activation. The point mutants S752P and Y747F/Y759F both impair platelet function but only S752P blocks adhesion-induced c-src and c-cbl activation, while Pyk2 phosphorylation remains intact. Consistent with this observation, hβ3δc and β3 S752 P, unlike WT and Y747F/Y759F β3, fail to mediate growth factor-induced (HGF or M-CSF) conformational change of αvβ3 from the basal to the activated state, as determined by the Ligand Induced Binding Site Ab, AP5. The functional consequences of the failure of αvβ3 activation, by β3 integrin mutation, include loss of growth factor induced adhesion and migration onto osteopontin in pre-OCs, and failure of αvβ3 integrin to localize to membrane ruffles in mature OCs. Reflecting these cytoskeletal inhibitory events, the same inactivating mutations specifically prevent HGF or M-CSF induced RhoA activation, assayed by affinity precipitation with the GTP-bound Rho and the Rho binding domain of Rhotekin coupled to GST. In conclusion, c-cbl , c-src, and RhoA, but not PYK-2, activation, in OCs, requires αvβ3 -transduced signals, including conformational change of the integrin, itself. These events are specifically mediated by β3 S752.

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Tyrosine 371 and the Phosphorylation of c-Cbl by c-Src are Required for the Ubiquitination of Both Proteins and for Src Kinase Down Regulation. A. Sanjay,[1] M. Yokouchi,*[1] T. Kondo,*[1] A. Bruzzaniti,[1] H. Zhang,*[1] W. C. Horne,[1] R. Baron.[2] [1]Yale University, New Haven, CT, USA, [2]Yale University, New Haven, USA.

Cbl is a Src substrate in osteoclasts that is required for in vitro bone resorption and for osteoclast motility. Cbl negatively regulates kinase activity of both receptor and non-recep-

tor tyrosine kinases either by binding to the regulatory tyrosine (as in the case of Src) via the PTB domain or by inducing their ubiquitination via a RING finger (RF)-dependent ubiquitin ligase activity. Ubiquitination of proteins involves sequential actions of the Ub–activating (E1), Ub-conjugating (E2) and Ub-protein ligase (E3) enzymes. E3 enzymes determine the specificity for the substrate and are ultimately responsible of the transfer of ubiquitin from E2 to the substrate. Using recombinant Cbl and Src proteins in an in vitro ubiquitination assay, we have shown that Cbl ubiquitinates both itself and Src. The process required the RF domain of Cbl as well as the kinase activity of Src. Recent crystal structure analysis of the Cbl PTB and RF domain bound to the E2 UbCH7 revealed that the linker region between the Cbl PTB and RF domains provides multiple sites of contact with both the PTB domain and UbCH7. We hypothesized that a mutation in the linker region of Cbl, (Y371F) that abolishes EGF receptor ubiquitination might disrupt the interaction between Cbl and UbCH7. As with EGF receptor, the Y371F mutation prevented the ubiquitination of both Src and Cbl. We next investigated whether the failure of Cbl Y371F to ubiquitinate is due to its inability to recruit UbCH7. Using the yeast two-hybrid assay we have previously shown that Cbl constitutively interacts with UbCH7 in a RF-dependent manner. In vitro binding assays using purified proteins show that in the absence of Src both Cbl and Cbl Y371F form stable complexes with UbCH7, indicating that the substitution at Y371 does not abrogate the ability of Cbl to recruit UbcH7 to the complex. Surprisingly, the presence of Src and ATP destabilizes Cbl-UbCH7 complex whereas addition of PP1 (2 uM) to the assay restores the binding of Cbl to UbCH7. Thus, these results demonstrate that the interaction of Src and Cbl, which occur after activation of the vitronectin receptor for instance, not only lead to the PTB-dependent inhibition of Src kinase activity but also to the ubiquitination of active Src and Cbl, probably leading to the degradation of the molecular complex. Given that Src and Cbl deletions result in reduced motility of osteoclasts, a process dependent on the rapid turnover of the podosomes, we propose that Cbl regulates cell adhesion not only by decreasing the Src kinase activity, but also by ensuring its ubiquitination and rapid degradation during the turnover of adhesion sites.

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The Protein-Tyrosine Phosphatase (PTP) Activity of PTP-oc Is Required for the Dephosphorylation of PY-527 and Activation of c-Src in Osteoclasts. K. H. W. Lau, S. M. Suhr, L. W. Wu,* D. J. Baylink. Pettis Mem. VAMC, Loma Linda, CA, USA.

We have cloned an osteoclastic PTP, termed PTP-oc, which is expressed predominantly in osteoclasts and precursors. Because the c-Src signaling pathway plays a pivotal role in osteoclastic resorption and the c-Src activity is regulated negatively by the protein-tyrosine phosphorylation (PY) of Y-527 of c-Src, this study tested the hypothesis that PTP-oc is a broad regulator of osteoclastic resorption (responsive to both resorption activators and inhibitors) and that its PTP activity is required for the dephosphorylation of PY-527 of c-Src in osteoclasts. Three sets of experiments were done to test this hypothesis. In the first set of experiments, we found that resorption activators [PTH, PGE2 and 1,25(OH)2D3 (1,25D)], which stimulated osteoclastic resorption (by 2-fold, P<0.001 for each), each increased PTP-oc expression (up to 75%, P<0.01 for each) and reduced (to as low as 20% of the vehicle control, P<0.01 for each) the PY level of c-Src in rabbit osteoclasts. Conversely, resorption inhibitors [calcitonin and alendondrate], which reduced osteoclastic resorption (by up to 50%, P<0.05), each decreased PTP-oc expression (by up to 20%, P<0.05) and increased (3- to 5-fold, P<0.01) the PY527 level of c-Src. That both resorption activators and inhibitors altered the PY-527 level of c-Src, apparently through changing PTP-oc expression suggests that PTP-oc is a broad regulator of osteoclastic resorption. We next tested if reduction in PTP-oc expression by PTP-oc antisense would increase the PY527 level of c-Src. We found that treatment with a PTP-oc antisense oligo reduced PTP-oc expression (by 70%, P<0.001) and increased PY527 level of c-Src (by 4-fold, P<0.001) without affecting the c-Src protein level in rabbit osteoclasts, suggesting that PTP-oc is a key regulator of c-Src in osteoclasts. To evaluate if the PTP activity of PTP-oc is required for c-Src activation, we tested if overexpression of the wild-type (wt) and dominant-negative (dn) PTP-oc in human U-937 cells would alter the PY527 level of c-Src. The dn PTP-oc overexpressing cells had only one-third of c-src protein as that in vector-transfected cells, but the PY-527 phosphorylated c-Src in these cells were 130% (P<0.05) of that in the control cells. In contrast, while the c-src protein in the wt PTP-oc overexpressing cells was similar to that in the control cells, the PY-527 level was only 24.5% (P<0.001) of that in control cells. Thus, the PTP activity of PTP-oc is clearly required for the dephosphorylation of PY-527 and the activation of c-Src in osteoclasts. In conclusion, we presented strong circumstantial evidence that PTP-oc is a broad regulator of the c-Src signaling pathway in osteoclasts.

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Identification of Putative c-Fos Kinases in Osteoclast Progenitors. J. David, D. Mehic,* E. F. Wagner.* IMP, Research Institute for Molecular Pathology, VIENNA, Austria.

c-Fos knock-out mice develop osteopetrosis due to a block in osteoclast differentiation. Since Fos activity can be modulated by phosphorylation of a conserved motif located in the C-terminal domain (serine 362 and serine 374), the activation of putative Fos kinases in response to the osteoclastogenic signals M-CSF and RANKL was investigated in M-CSF-dependent bone marrow derived monocytes (MBMMs). The MAP kinases, ERK1/2, and the Phospho-inositol-3 kinase (PI-3 kinase) are strongly co-activated upon treatment of MBMMs with M-CSF alone, or with M-CSF and RANKL. Consequently, the MAP kinase activated protein, RSK2, a downstream target of ERK1/2 and PI-3-kinase, is activated. Both ERK1/2 and RSK2 have been put forward as putative c-Fos kinases in cultured fibroblasts. Using in-gel kinase assays, three M-CSF-inducible proteins of molecular weight of 90, 70 and 65 kDa which are able to phosphorylate a c-Fos peptide containing serine 362 were identified. The 90 kDa protein is not detected when serine 362 is mutated to alanine or after RSK2 immunodepletion confirming that RSK2 is, indeed, the main kinase phosphorylating c-Fos on serine 362. Using immunoprecipitation kinase assays, the 70 kDa protein was shown to be the P70S6 ribosomal kinase. We also demonstrate by immunodepletion that ERK1/2 is the main kinase specifically phosphorylating c-Fos on serine 374 in response to M-CSF.The M-CSF-dependent activation of ERK1/2 and RSK2 is blocked by pre-treatment of MBMMs with PD98059, a specific inhibitor of the MAP-kinase-kinase, MEK1. PD98059 does not affect c-fos induction by M-CSF at the mRNA level, but inhibits the induction of c-Fos protein. Thus, c-Fos phosphorylation by ERK1/2 and RSK2 might modulate post-transcriptionally the level of c-Fos induction by M-CSF. Functionally, PD98059 blocks the formation of osteoclasts generated either in co-culture of osteoclast bone marrow progenitors with supportive osteoblasts, or by direct treatment of hematopoietic osteoclast precursors with M-CSF and RANKL.These data provide the first evidence that c-Fos phosphorylation by ERK1/2 and its downstream kinase RSK2 may be a mechanism in which M-CSF regulates monocyte/osteoclast differentiation.

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Dysfunctional Osteoclasts and Osteopetrosis in an ADP-Ribosyl Cyclase (CD38) Null Mouse. X. Y. Wu,*[1] O. A. Adebanjo,[1] B. S. Moonga,[1] A. Zhou,*[1] X. B. Wu,*[1] P. J. R. Bevis,[1] K. Jepsen,[2] M. Schaffler,[2] D. Kimmel,[3] A. Inzerillo,*[1] S. Epstein,[1] B. R. Troen,[1] E. Abe,[1] H. C. Blair,[4] M. Zaidi,[1] L. Sun.[1] [1]The Mount Sinai Bone Program and the Bronx VA GRECC, NY, USA, [2]Department of Orthopedics, Mount Sinai Medical Center, NY, USA, [3]Merck Research Laboratories, PA, USA, [4]University of Pittsburgh, PA, USA.

Previous physiological studies have predicted that the cyclic ADP-ribose (cADPr) generating enzyme, CD38, regulates osteoclastic bone resorption (J. Cell Biol. 142: 1349). Here we provide the first evidence for impaired osteoclastic bone resorption in the absence of CD38. CD38 null mice showed significant, ~15%, increases in bone mass and bone stiffness compared with wild type littermates. The most dramatic feature, however, was a >50% decrease in their resorptive areas (6.1% *versus* 13.9% in wild type controls). Equally impressive was a ~40% reduction in bone resorption by marrow-derived, CD38 null osteoclasts in the pit assay. This compelling evidence for a role of CD38 in bone resorption prompted us, next, to: (a) understand the structure-function relationship of the CD38 protein through deletion mutagenesis and (b) study gene regulation through promoter cloning and reporter-promoter assays. Deletion of 49 amino acids constituting the intracellular and transmembrane domains of CD38 expectedly prevented its plasma membrane localization. However, its ability to release Ca^{2+} from intracellular stores through cADPr remained intact. Attachment of a farnesylation or myristoylation signal to the N- or C-terminus respectively resulted in the membrane localization of CD38, *albeit* in an opposite topology. However, again its Ca^{2+} releasing activity remained conserved. Thus, contrary to previously held views, the membrane localization of CD38 was shown *not to be necessary* for its cADPr-generating and Ca^{2+} releasing function. We next cloned the 1.6 kb CD38 promoter from a rabbit genomic DNA library and established its transcriptional activity in a luciferase reporter assay. Truncation deletion of the promoter revealed a possible negative regulatory region (-1149 to -931 bp) that was rich in AP-1 sites likely responsive to NFκB, an osteoclast activator. Positive regulation was restored between -588 and -180 bp. The osteopetrotic phenotype of the CD38 null mouse together with the deletion mutagenesis and promoter analysis experiments strongly implicate CD38 as a critical regulator of osteoclastic bone resorption. By acting as a NAD^+ sensor/converter, CD38 likely couples cellular metabolism to Ca^{2+} signaling and bone resorption.

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An Inverse Relationship between Prevalence of Osteoblast Apoptosis and Rate of Bone Formation with Intermittent, but not Sustained, Elevation of PTH in Mice. A. A. Ali, C. A. O'Brien, R. S. Weinstein, P. Roberson,* S. C. Manolagas, R. L. Jilka. Div. Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. of Arkansas for Med. Sci., Little Rock, AR, USA.

The ability of PTH to inhibit osteoblast apoptosis provides a potential explanation for its anabolic effect on the skeleton when given intermittently. To firm the relationship between suppression of osteoblast apoptosis and stimulation of bone formation, we conducted detailed dose-response and time kinetic studies. Swiss-Webster mice received daily s.c. injections of vehicle, or 3, 10, 30, 100 or 300 ng hPTH(1-34)/g body weight for 28 days. BMD was measured at the beginning and end of the experiment, and histomorphometric studies were performed on cancellous bone in the distal femur. We found that the minimal effective dose for both apoptosis suppression and the anabolic effect was 10 ng/g/d, and the maximal was 100 ng/g/d. More important, there was a significant inverse relationship between the prevalence of osteoblast apoptosis and osteoblast number (P<0.01), and bone formation rate (P<0.02). Kinetic studies showed that 100 ng hPTH(1-34)/g/d inhibited osteoblast apoptosis and stimulated bone formation as early as 7 days after initiation of treatment. We next examined whether similar relationships are seen when PTH is continuously elevated. Mice placed on a low Ca diet for 7 days exhibited a 3-fold increase in serum PTH as compared to mice on a normal diet. This sustained PTH elevation caused bone loss, with increased RANKL mRNA expression, osteoclast number and bone turnover; but serum Ca remained normal. However, unlike 7 days of intermittent PTH, sustained PTH elevation did not reduce the prevalence of osteoblast apoptosis or increase the number of osteoblasts. It is possible that the 3-fold elevation in PTH, though sufficient to stimulate the synthesis of RANKL, was insufficient to inhibit osteoblast apoptosis; but both are stimulated by cAMP, making this explanation unlikely. Studies presented elsewhere in this meeting show that addition of PTH to cultured osteoblastic cells induced transient cAMP-dependent survival signaling lasting ~ 12 hours, most likely due to desensitization of the PTH receptor. However, prolonged desensitization would not occur with intermittent PTH because the hormone is cleared rapidly from the circulation. We propose that the different effects of intermittent versus sustained PTH are due to differences in the frequency of the activation of anti-apoptotic signaling: repeated bursts in the former versus a single episode in the latter resulting in little, if any, effect on osteoblast life span.

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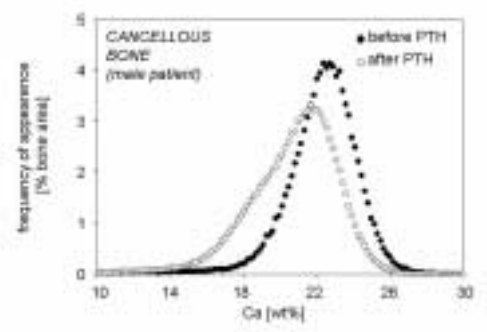
Influence of Intermittent PTH Treatment on Mineral Distribution in the Human Ilium: A Paired Biopsy Study Before and After Treatment. P. Roschger,*[1] B. M. Grabner,*[1] P. Messmer,*[1] D. W. Dempster,[2] F. Cosman,[2] J. Nieves,[2] R. Lindsay,[2] J. P. Bilezikian,[3] E. S. Kurland,[3] E. Shane,[3] P. Fratzl,*[4] K. Klaushofer.[1] [1]Ludwig Boltzmann Institute of Osteology & 4th Med. Dept., Hanusch Hospital & UKH Meidling, Vienna, Austria, [2]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [3]College of Physicians and Surgeons, Columbia Univ., New York, NY, USA, [4]Erich Schmid Inst. of Mat. Sci., Austrian Acad. Sci. & Univ. of Leoben, Leoben, Austria.

Parathyroid hormone (PTH) plays an essential role in Ca homeostasis by promoting both bone resorption and formation. Intermittent administration of low dose PTH has been reported to increase bone density and to improve trabecular architecture and cortical width [1]. The aim of the present work was to study the influence of intermittent PTH on the bone mineralization density distribution (BMDD). Pairs of transiliac biopsies (7 men with osteoporosis, mean age = 49 and 6 estrogen-treated women with osteoporosis, mean age = 54) obtained before and after treatment (25 micrograms PTH(1-34)/d for 18 months (men) and 36 months (women) were studied by quantitative backscattered electron imaging (qBEI) [2]. Two parameters were obtained from the BMDD: CaMaxFreq (position of the peak = the typical Ca concentration) and FWHM (peak width of the distribution). qBEI revealed significant alterations in the BMDD due to PTH treatment (see Fig.). In cortical bone from the male patients, CaMaxFreq was slightly, but significantly reduced (-3.32%, p=0.02) and FWHM was increased (+18.80%) after treatment. Similarly, cancellous bone showed a tendency towards lower mineralization (CaMaxFreq -2.12%) and a significant increase in FWHM (+19.65%, p=0.02). In the PTH + estrogen-treated women CaMaxFreq was not altered, but FWHM increased for cortical (+18.14%, p=0.005) and trabecular bone (+21.59%). These data are highly consistent with the observation that intermittent PTH treatment stimulates bone turnover and new bone formation in cortical and cancellous bone. The significant broadening of the BMDD indicates a higher percentage of newly formed, but still not fully mineralized bone. Recent fracture studies indicate that, although not yet completely mineralized, this new bone contributes significantly to bone strength. [1] Dempster et al., JBMR 15, Suppl 1:S194 (2000) ; [2] Roschger et al., Bone 23: 319-326 (1998)



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PTH Treatment Directly Stimulates Bone Formation in Cancellous and Cortical Bone in Humans. D. W. Dempster,[1] H. Zhou,[1] F. Cosman,[1] J. Nieves,[1] J. D. Adachi,[2] L. J. Fraher,[3] P. H. Watson,[3] R. Lindsay,[1] A. B. Hodsman.[3] [1]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Dept. of Medicine, University of Western Ontario, London, ON, Canada, [3]Dept. of Medicine, St. Joseph's Health Ctr., London, ON, Canada.

The anabolic action of PTH is well established but the cellular mechanisms are poorly understood. Bone marker studies suggest that PTH initially stimulates bone formation without prior resorption. We tested this hypothesis by morphometric analysis of bone samples obtained after only 28 days of PTH treatment. Postmenopausal women (n=27) with severe osteoporosis (OP) were given hPTH (1-34)(50 mcg/d s.c) for 28 d. Tetracycline(tet)was given on days 12-14 and 26-28 and a biopsy was obtained on day 28. Results were compared with those from untreated, age-matched patients with severe OP. Pairs of adjacent sections were obtained. One was mounted unstained to reveal tet labels; the other was stained to reveal packets and cement lines. Forming packets were identified based on the presence of a double tet label. Three types of packets were observed on the cancellous (Cn) and endocortical (Ec) surfaces: 1. Packets with a scalloped reversal line indicating prior resorption (Remodeling-Based Formation, RBF). 2. Packets in which the reversal line was smooth indicating no prior resorption (Modeling-Based Formation, MBF) and packets with clearly visible arrest lines, indicating Resumption of Formation (RF).On the periosteal(Ps) surface, none of these types of packet could be reliably identified. In the PTH-treated patients, bone formation rate (BFR) was increased on Cn, Ec and Ps surfaces. On the Cn surface, biopsies from PTH-treated patients displayed packets with MBF and RF, both of which were completely absent in controls. On the Ec surface, PTH treatment increased the number of packets with RBF. This was found to be due an increase in the formation period in packets in which formation was underway at the start of PTH treatment.In conclusion, we have demonstrated that PTH treatment directly stimulates bone formation without prior resorption on Cn and En surfaces. We have also shown for the first time in humans that PTH stimulates bone formation on both the En and Ps surfaces of the cortex. This challenges the traditional view that PTH acts primarily on cancellous bone in humans and provides a further explanation for its potent anti-fracture efficacy.

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A Randomized Controlled Clinical Trial to Compare the Efficacy of LY333334 [Recombinant Human Parathyroid Hormone (1-34)] and Alendronate Sodium in Postmenopausal Women with Osteoporosis. J. J. Body,[1] G. A. Gaich,[2] W. H. Scheele,*[2] P. D. Miller,[3] P. M. Kulkarni,*[2] A. B. Hodsman.[4] [1]Inst. J. Bordet, Univ. Libre de Bruxelles, Brussels, Belgium, [2]Eli Lilly and Company, Indianapolis, IN, USA, [3]Colorado Center for Bone Research, Lakewood, CO, USA, [4]St. Joseph's Health Care, London, ON, Canada.

Parathyroid hormone (PTH), given once daily, stimulates new bone formation and increases bone mass. A randomized, double-blind, placebo-controlled clinical trial was conducted to compare the effects of 40 µg of recombinant human parathyroid hormone (1-34) [rhPTH(1-34)] and 10 mg of alendronate sodium in postmenopausal women with osteoporosis (spine or hip T-score <-2.5). The women were randomized to either once-daily subcutaneous injection of rhPTH(1-34) plus placebo pill (PTH40, n=73) or alendronate plus placebo injection (ALN10, n=73). The median treatment period was 14 months. Treatment with rhPTH(1-34) increased bone mass significantly more than alendronate at the spine, proximal femur, and total body. At the lumbar spine, BMD increased significantly more in the PTH40 group than in the ALN10 group at 3 months and at every visit thereafter. There was no significant difference in BMD at the ultradistal radius but there was a significant decrease at the midshaft radius in the PTH40 group, compared with ALN10. Ten women (14%) in the ALN10 group sustained nonvertebral fractures compared with only 3 women (4%) in the PTH40 group (P=0.042). Back pain was reported significantly less frequently by women in the PTH40 group (6%) than in the ALN10 group (19%, P=0.012). Leg cramps were reported significantly more frequently by patients in the PTH40 group (8%) than in the ALN10 group (0%, P=0.012). There were no clinically significant increases in serum or urine calcium. Markers of bone remodeling were increased in the PTH40 group and decreased in the alendronate group. In summary, treatment with rhPTH(1-34) is well tolerated, stimulates new bone formation, increases bone remodeling, and produces larger and more rapid increases in BMD than alendronate, which reduces bone remodeling.

Skeletal site (% change from baseline to final visit)	PTH40 (mean ± SD)	ALN10 (mean ± SD)
Lumbar spine	12.2 ± 9.4*	5.6 ± 5.0
Total hip	4.0 ± 5.7*	2.5 ± 3.2
Femoral neck	4.8 ± 6.5*	1.7 ± 4.3
Ultradistal radius	0.2 ± 6.6	1.4 ± 5.1
Midshaft radius	-3.4 ± 3.8*	-0.2 ± 3.3
Total body BMC	3.5 ± 3.6*	1.9 ± 4.4

*P<0.05 PTH40 vs ALN10

Disclosures: Eli Lilly and Company,5,8; Merck,5.

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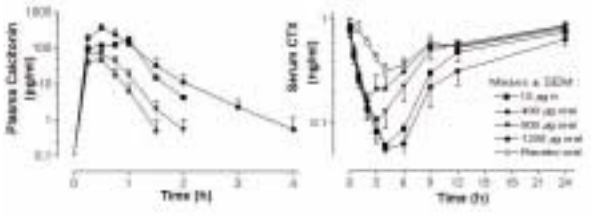
Oral Calcitonin Inhibits Bone Resorption: A Pilot Dose-Finding Study. M. Cosma,*[1] T. Buclin,*[1] P. Burckhardt,[1] M. Azria,[2] M. Attinger,*[2] J. McLeod.[2] [1]Department of Medicine, University Hospital, Lausanne, Switzerland, [2]Novartis Pharma Inc., Basle, Switzerland.

An oral formulation of calcitonin was developed using a specific carrier. To investigate its pharmacokinetics and dose-effect relationships on bone resorption markers, 3 single oral doses of calcitonin (400 µg, 800 µg and 1200 µg), one oral placebo and one intravenous dose (10 µg = 50 IU) were administered to 8 healthy male adults (means ± SD: age = 26±6) in a randomised, cross-over, double-blind (except iv) 5-period study, under standardised diet.The calcitonin plasma concentrations reached a plateau during the iv infusion, followed by a rapid decay (half-life = 0.19±0.02 h). The oral doses were absorbed rapidly (Tmax for 400, 800 and 1200 µg: 0.38±0.13, 0.44±0.12 and 0.50±0.13 h, Cmax: 60±31, 93±66 and 370±350 pg/ml respectively). The absolute oral bioavailability was 0.54% for the 400 µg, 0.43% for the 800 µg and 1.53% for the 1200 µg dose (p=0.03, Friedman's test). All oral doses produced a marked drop (ca. 90%) in serum C-terminal telopeptide (CTX); ionized calcium concentrations decreased accordingly. Both variables displayed dose-related changes, with the effects of 1200 µg exceeding those of 10 µg iv. The CTX nadir increased from 2 h post-dose after 400 µg to 4 h after 1200 µg and 10 µg iv. The effects of 1200 µg lasted for ca. 12 h. Urinary CTX excretion changed similarly. Circadian fluctuations of CTX were observed after placebo. All calcitonin doses were well tolerated, with slight gastro-intestinal events usually associated with calcitonin treatment such as nausea and diarrhoea being observed mainly after the highest oral dose.In conclusion, various doses of a novel oral formulation of calcitonin displayed a significant absorption, with a dose ratio of approximately 100:1 compared to iv calcitonin. After 1200 µg, the inhibition of bone resorption markers appeared similar to the effect of 10 µg iv calcitonin. Thus, oral administration of calcitonin is feasible and could become a promising alternative for

the treatment of chronic conditions associated with bone loss.



Disclosures: Novartis Pharma Inc.,2.

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Vitamin D Treatment Best for the Frailest to Prevent Hip Fractures?A Prospective Controlled Clinical Trial. O. Johnell,[1] M. Billsten,*[2] I. Sernbo,*[1] I. Rodine,*[2] E. Ornstein.*[2] [1]Department of Orthopaedics, Malmö University Hospital, Malmö, Sweden, [2]Department of Orthopaedics, Hässleholm-Kristianstad Hospital, Hässleholm, Sweden.

At present there are some studies with Vitamin D which show fracture reduction. The purpose of this study was to study the effect of Vitamin D on the frailestMethod. In a prospective controlled clinical trial, we investigated the effects of oral vitamin D supplements on hip fractures and other non-vertebral fractures. We included 2404 women 50 years of age and older (mean age 85.8±6.3) living in 174 nursing homes in the southern part of Sweden. The patients were followed for three years. One district was selected as treatment area and the surrounding districts as control areas.1594 women (men age 85.7 ±6.5) were assigned to receive 21000 IU vitamin-D3 once per month. The dose was distributed by a nurse as three drops of ergocalciferol (294000IU/ml) per month. In the control group we included 854 women (men age 85.9 ±5.9). The average daily dietary calcium intake was 650 mg. After 12 months 120 hip fractures had occurred with an odds ratio 0.66 (0.46-0.93) and after 36 months 220 with an odds ratio 0.85 (0.65-1.11) for the Vitamin D group, similar findings for other fracturesResults. Hip fracture: In a subgroup analysis we studied the effect of Vitamin D in those with at baseline a prevalent hip fracture N=383, after 12 months 42 had had a new hip fracture odds ration 0.28 (0.14-0.59). Interaction term P=0.0009. After 36 months 50 had had a new hip fracture odds ration 0.28 (0.14-0.59). Interaction term P=0.000. In those who had died during the 36 months follow-up N=1106 odds ratio for vitamin D treated group was 0.64 (0.43-0.96). Conclusion. The frailest women living in nursing homes have a greater chance to die without a hip fracture if they take three drops of Vitamin D per month.

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Impact of Adding Medroxyprogesterone Acetate (MPA) to Multiple Doses of Conjugated Equine Estrogens (CEE) on Bone Density in Postmenopausal Women. R. Lindsay,[1] J. C. Gallagher,[2] M. Kleerekoper,[3] J. H. Pickar.*[4] [1]Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Creighton University, Omaha, NE, USA, [3]Wayne State University, Detroit, MI, USA, [4]Wyeth-Ayerst Research, Philadelphia, PA, USA.

The impact of adding a progestin to currently prescribed and lower doses of estrogens on BMD in healthy postmenopausal women has not been thoroughly investigated. A 2-year substudy of 749 women enrolled in the double-blind, placebo-controlled, multicenter Women's Health, Osteoporosis, Progestin, Estrogen (Women's HOPE) study was conducted to evaluate the effects of commonly prescribed and lower doses of CEE, either alone or with one of two daily doses of MPA, on BMD. Postmenopausal women 40-65 years with normal BMD were randomized to receive CEE 0.625 mg/day, CEE 0.625/MPA 2.5 mg/day, CEE 0.45 mg/day, CEE 0.45/MPA 2.5 mg/day, CEE 0.45/MPA 1.5 mg/day, CEE 0.3 mg/day, CEE 0.3/MPA 1.5 mg/day, or placebo. All women also received calcium carbonate (600 mg elemental calcium daily). Women were between 1 and 4 years post menopause at enrollment.Lumbar spine (L2-L4), total hip, and total body BMD (Lunar DPX-L) were measured at baseline and every 6 months for 2 years. BMD scans were sent to a central site responsible for all analyses and quality control of BMD measurements. Data were evaluated in an intent-to-treat population with last observation carried forward. BMD changes from baseline were compared between groups using an analysis of covariance procedure with study site, years since menopause, and body weight at baseline included in the model. Statistical significance was set a priori at P<.05.By one year of treatment, all doses of CEE and CEE/MPA produced significant increases (P<.05) in BMD at all sites relative to placebo. Increases in BMD ranged from 1.1%-3.6% at the spine, 0.9%-2.8% at the hip, and 0.4%-1.1% for total body. A dose-response relationship was apparent for the spine, but not hip or total body. Placebo-treated patients lost 0.7%-2.6% of BMD at all sites over 2 years. The addition of MPA (2.5 mg/day) to CEE resulted in greater gains in spine BMD compared with CEE alone for the CEE 0.625 and CEE 0.45 groups. At 2 years, the increase in spine BMD was greater for the CEE 0.625/MPA 2.5 group (3.6%) compared with the CEE 0.625 group (2.4%, P<.05); for the 0.45/MPA 2.5 group, the increase in spine BMD (3.0%) was greater than that observed in the CEE 0.45 group (1.9%, P<.05). Although similar trends were apparent for the total hip and total body sites, statistical significance was not achieved.These data suggest that the addition of MPA may positively affect BMD in women administered CEE in the early postmenopausal years. Lower doses of CEE or CEE/MPA preserve BMD in healthy postmenopausal women.

Disclosures: Wyeth-Ayerst Research,5.

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Effect of Alendronate and Estrogen Replacement on Periosteal Bone Formation in Postmenopausal Women. R. Recker,[1] T. Coble,*[1] A. Burshell,*[2] A. Lombardi,*[3] G. Rodan,[3] D. Kimmel,[3] J. Yates.*[3] [1]Medicine, Creighton University, Omaha, USA, [2]Ochsner Clinic, Philadelphia, PA, USA, [3]Merck, Philadelphia, PA, USA.

Alendronate (ALN) and estrogen are antiresorptive agents used to prevent and treat postmenopausal osteoporosis. Both decrease bone resorption and formation at trabecular and endocortical surfaces. The purpose of this study was to evaluate the effect of ALN and estrogen on periosteal bone formation in women with postmenopausal osteoporosis. 98 postmenopausal women (age 67±3.5yrs) were treated for two years with: a)placebo (PBO), b) Premarin (0.625mg/d) (PREM), c) ALN 10mg once daily (ALN), or d) both (COMB). In vivo dual fluorochrome labeling was given to all. Transilial biopsy specimens were obtained, fixed in 70% ethanol, and processed undecalcified. All periosteal surface in two unstained 8µm sections separated by 250µm was studied for perimeter (P.Pm, mm), double label, single label, and mineral apposition rate (MAR, µm/d). Mineralizing surface (double plus half single label) (MSBS, %) was calculated. Groups were compared by Kruskal-Wallis ANOVA with Student Neuman-Keuls post-hoc test and two-factor (ALN or estrogen) ANOVA. Percentage of subjects with double label was lower in PREM and COMB than in ALN subjects. Similarly, MSBS was lower in both PREM and COMB than in ALN subjects. Mineral apposition rate was not affected significantly by either treatment. Two-factor ANOVA revealed a significant PREM reduction in periosteal formation (P<0.03) with no ALN effect.

Vbl	PBO (N=9)	PREM (N=30)	ALN (N=24)	COMB (N=35)
P.Pm	24±7	24±7	27±4	26±6
%	33	20*	62	17*
MSBS	1.1±2.7	0.5±1.3*	0.8±0.9	0.2±0.3*
MAR	0.83±0.08	0.72±0.46	0.71±0.45	0.55±0.20

Mean±SD; *less than ALN (P<.001); %= Percentage of subjects showing any double label

These data suggest that ALN permits continued periosteal apposition at a faster rate than does estrogen treatment, in agreement with observations in rats. This may play a role in the sustained rise in bone mineral density seen with up to seven years of ALN treatment

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Muscle Stem Cells Genetically Engineered With Osteogenic Factors Induce Bone Formation and Improve Bone Healing. H. Peng, V. Wright,* A. Usas, B. Gearhart, J. Cummins, J. Huard. Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA, USA.

Muscle stem cells with osteogenic potential have been isolated from mice in our laboratory. These muscle stem cells can undergo osteogenic differentiation upon stimulation with recombinant human bone morphogenetic protein-2 (BMP-2). When transduced with adenoviral vector expressing BMP-2, muscle stem cells induced de novo bone formation in mice, both at the injection sites and in diffusion chambers implanted subcutaneously in SCID mice. These transduced muscle stem cells were shown to differentiate into osteoblasts and osteocytes. Transduced muscle stem cells expressing BMP-2 also induced orthotopic bone formation and completely healed calvarial defects created in SCID mice. However, it has been problematic to extend this study to normal animals, mainly because of the immune response triggered by viral proteins. This problem has been overcome by using retroviral vector, which does not express viral products. Muscle stem cells were transduced with retroviral vector CLBMP-2/4, which contained a BMP-2/4 hybrid construct and led to high-level human BMP-4 secretion from transduced cells. The efficiency of transduction was higher than 80%. The level of BMP-4 secretion was 250 to 612 ng/106 cells/24 hr. Most of the transduced muscle stem cells became osteogenic, as evidenced by alkaline phosphatase expression.To determine the in vivo bone induction ability of muscle stem cells transduced with retroviral vector expressing BMP-4, transduced cells were implanted together with collagen type I matrices into the muscle of normal mice. Chondrogenesis was found within the implanted matrices 1 week after implantation. Endochondral bone formation occurred within 2 weeks after implantation. Transduced muscle stem cells were also found within the osteoid, and they expressed osteocalcin, suggesting their differentiation into osteocytes. Interestingly, de novo bone formation occurred only within the collagen matrices, but not in the surrounding muscle or distant organs. In summary, this study demonstrates that muscle stem cells can be efficiently transduced by retroviral vector, are capable of osteogenic differentiation, and, more importantly, induce de novo site-directed bone formation in immune-competent animals.

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Fracture Healing Impairment in Rats by an Angiogenesis Inhibitor Indicates a Requirement for Vasculature in Early Fracture Callus Development. M. R. Hausman,* M. B. Schaffler, R. J. Majeska. Leni and Peter W. May Department of Orthopaedics, Mount Sinai School of Medicine, New York, NY, USA.

Angiogenesis inhibitory drugs have shown promise in treating cancer and other diseases; however, these agents may also impair normal physiological processes like fracture healing, which require new blood vessel formation. The purpose of this study was to test the effects of the angiogenesis inhibitor TNP-470 (provided by TAP Pharmaceutical Products, Lake Forest, IL) on fracture healing in an established rat model system. Closed, internally fixed femoral fractures were created in adult female Sprague-Dawley rats. Beginning one day after fracture, animals received alternate day subcutaneous injections containing

either vehicle or a dose of TNP-470 (30 mg/kg) known to block angiogenesis and tumorigenesis in vivo. Fracture healing was assessed morphologically by histology and radiography at 7, 14 and 21 days post-fracture, and functionally by biomechanical testing at 24 days post-fracture. In addition, RNA isolated from both treated and control fractures on day 3 was reverse-transcribed and hybridized to filters containing cDNA's coding for angiogenesis-related genes (Superarray Inc., Bethesda, MD). TNP-470 completely blocked fracture healing by all criteria examined. Treated animals failed to develop either a cartilaginous callus or subperiosteal woven bone, indicating suppression of both endochondral and intramembranous pathways of bone formation. In treated animals, a fibrous tissue at the fracture site resembled that seen clinically in atrophic nonunion fractures. Biomechanical testing showed that the treated animals recovered only 15% of the strength ($p<0.01$ vs control) and 7% of the stiffness ($p<0.001$ vs control) of intact bone, compared with 56% and 50% recovery, respectively, in non-treated bones. TNP-470 had no effect on the mechanical properties of intact bones. Of 19 genes whose expression was examined, 10 were up-regulated due to TNP-470 treatment, 5 were down-regulated and 4 (including GAPDH) remained largely unchanged. Genes up-regulated at Day 3 by TNP-470 treatment included VEGF, VEGF-B, VEGF-C and VEGF-D. Down-regulated genes included FGFs-1 and 2. These data support the concept that angiogenesis is essential for proper fracture healing, and further indicate that its importance in the earliest phases of the healing process. This demonstration is also of clinical interest since patients likely to receive antiangiogenic agents in cancer chemotherapy are also prone to undergo atraumatic fractures. Finally, the identification of early changes in gene expression may lead to a greater understanding of the mechanisms by which vasculature regulates fracture healing.

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Insulin Receptor Substrate-1 (IRS-1) Is Essential for Bone Repair through Stimulation of Cell Proliferation at Its Early Stage. T. Shimoaka,[1] K. Hoshi,[1] T. Kadowaki,*[2] Y. Terauchi,*[2] K. Nakamura,[1] H. Kawaguchi.[1] [1]Orthopaedic Surgery, University of Tokyo, Tokyo, Japan, [2]Metabolic Diseases, University of Tokyo, Tokyo, Japan.

Insulin receptor substrate-1 (IRS-1) is an essential molecule for intracellular signaling by IGF-I and insulin both of which are potent anabolic regulators of bone and cartilage metabolism. We previously reported that IRS-1 is essential for maintaining bone turnover using IRS-1 deficient (-/-) mice. This study investigated the role of IRS-1 in bone repair by comparing the healing process of fracture created at the midshaft of the tibia and stabilized with an intramedullary nail between -/- and wild-type (+/+) mice at 8 weeks of age (n=15 each). This model was confirmed to show the fracture healing process with both endochondral and intramembranous ossification reproducibly in a definite temporal sequence in +/+ mice. Soft X-ray examination revealed that callus formation was detected at 1 week, and bony bridging at the fracture site appeared at 3 weeks after operation in all +/+ mice. After the callus size reached maximum around 4 weeks, it decreased due to bone remodeling throughout the observation period (up to 10 weeks). In contrast, fracture healing was extremely impaired in -/- mice: 13 out of 15 mice remained in a nonunion state even at 10 weeks, and 2 showed much weaker union with smaller callus than those of +/+ mice. Histologically, the retardation of the fracture healing was observed as early as 1 week after fracture in -/- mice. The number of both mesenchymal cells and chondrocytes associated with intramembranous and endochondral ossification, respectively, were markedly decreased, resulting in the reduced size of soft and hard callus. Few PCNA-positive cells were seen around the fracture site. In the small cartilageous tissue of -/- mice, type X collagen-positive hypertrophic chondrocytes were seen at 1 week, and some of them were TUNEL-positive, suggesting the acceleration of differentiation and apoptosis without sufficient proliferation of chondrocytes. Similar findings were observed in the growth plate of -/- mice: a smaller proliferative zone with decreased PCNA-positive cells and a wider hypertrophic zone with increased TUNEL-positive cells, as compared to that of +/+ mice. These results demonstrate that IRS-1 deficiency notably impairs the fracture healing ability by inhibiting the proliferation of mesenchymal cells and chondrocytes at the earlier stage. We propose that IRS-1 is essential for bone repair and can be clinically applied as a target molecule for bone regeneration such as fracture healing.

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An Excessive Activation of Signal Transducer and Activator of Transcription 1 (Stat1) Accelerates Differentiation of Chondrocytes in FGFR3-Related Short-limb Dwarfisms. Y. Yamanaka,[1] H. Tanaka,[1] M. Koike,*[1] R. Nishimura,[2] Y. Seino.[1] [1]Pediatrics, Okayama University, Okayama, Japan, [2]Biochemistry, Osaka University Faculty of Dentistry, Osaka, Japan

Constitutively activated mutations in FGFR3 result in disturbing the growth of long bones in achondroplasia (ACH) and related disorders. We and others demonstrated that Stat1 is constitutively activated in chondrocytes of a severe type of ACH, thanatophoric dysplasia (TD), and implicated in inhibition of proliferation caused by this mutation in FGFR3. Little is, however, known about the role of Stat1 in differentiation of chondrocytes. Therefore, we investigated the functional role of Stat1 in FGFR3 signaling pathway by using chondrogenic cell lines, ATDC5 cells which were transfected the FGFR3 mutants, including G380R (ACH) and K650E (TD type II: TDII). The TDII receptor exhibited 61-fold greater phosphorylation of Stat1 than WT receptor, determined by immunoblot with anti-phospho-Stat1 antibody. In ACH cells, Stat1 was modestly phosphorylated. Immunoprecipitation assay and GST-Stat1 pull-down assay revealed that Stat1 bound FGFR3 (TDII). Preincubation with a synthetic phsphopeptide corresponding to codon 643-654 of FGFR3 including the major autophsphorylation site inhibited the binding GST-Stat1 fusion protein to FGFR3 (TDII). TDII cells exhibited 5-fold greater expression of type X collagen mRNA than WT cells, determined by real-time RT-PCR analysis. Importantly, introduction of dominant-negative (DN)-Stat1 into TDII cells suppressed type X collagen expression, while DN-Stat5a or DN-Stat5b had no effect (Figure). Consistently, constitutively active mutant of Stat1 increased 2.5-fold greater levels of type X collagen expression than an empty-vector. Furthermore, infection of an adenovirus vector carrying FGFR3 (ACH) in ATDC5 promoted alcian blue stain-positive cartilage formation whereas induction of DN-Stat1 inhibited cartilage formation in ACH cells.In conclusion, an excessive activation of Stat1 induced by the FGFR3 mutants accelerated differentiation of chondrocytes. Thus, our data suggest that Stat1 may account for the disturbance of endochondral bone formation in achondroplasia and its related disorders.

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Postpubertal Improvement in Bone of Knock-in Mouse Model (Brittle Mouse) for Osteogenesis Imperfecta Is Due to Fundamental Alterations in Bone Material Properties. K. M. Kozloff,*[1] E. P. Frankenburg,*[1] M. F. Spurchise,*[1] C. Berwitz,*[2] A. Forlino,*[2] J. C. Marini,[2] S. A. Goldstein.[1] [1]Orthopaedic Research Labs, U Michigan, Ann Arbor, MI, USA, [2]Sect on Connective Tissue Disorders, HDB, NICHD, Bethesda, MD, USA.

The Brittle mouse (Brtl) is a knock-in murine model for non-lethal osteogenesis imperfecta type IV. It carries a point mutation in one COL1A1 allele, resulting in a classic glycine substitution, G349C, in 50% of the alpha 1(I) collagen chains. Brtl reproduces the molecular, biochemical and skeletal features of type IV OI and provides a means to examine the natural history of the mechanical properties of OI bone, as well as to compare them to the well-known clinical finding of decreased fractures after puberty. We examined a total of 78 Brtl males and normal littermates (WT) at 1, 2, 6 and 12 months of age. Geometric properties of femoral mid-diaphyses were examined using micro CT. Biomechanical properties were tested by whole bone 4 point bending to failure; material properties of ultimate strength and elastic modulus were predicted using standard mechanics of materials equations. At 1 month, analogous to childhood in humans, Brtl bone shows a trend of reduced strength from 4-point bending compared to WT. At 2 months, comparable to pubertal age, Brtl bone is weaker in 4 point bending ($p<.05$) and shows a strong trend toward reduced stiffness compared to WT. This is likely explained by geometrical properties, as bending moment of inertia (MOI) was decreased ($p<.05$), while predicted ultimate strength and elastic modulus were comparable to WT. At 6 months, comparable to a mature young adult, Brtl bone achieves parity with WT in both strength and stiffness from 4 point bending. MOI is still reduced ($p<.05$), but Brtl predicted elastic modulus ($p<.05$) and ultimate strength are greater than WT at this age. This material property alteration may be explained by BMD measures. DEXA findings show that while Brtl femoral BMD is reduced at 1 and 2 months, by 6 months it equals WT. This matrix alteration may allow material properties to overcome geometric insufficiencies, leading to observed comparable stiffness and strength between Brtl and WT. At 1 year, Brtl bone is comparable to WT in stiffness and strength, and levels suggest a weakening vs. 6 months. Displacement ratios suggest that Brtl bone fails in a brittle fashion when compared to WT. These studies demonstrate that Brtl has a post-pubertal adaptation to increase bone structural integrity, as does the type IV OI patient, and that the mechanism is likely a fundamental alteration in bone material properties rather than mid-shaft geometry. Brtl is thus an excellent model to evaluate bone structure/function relationships and therapeutic interventions in OI.

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Measles Virus Preferentially Induces Vitamin D Receptor Gene Activation in Pagetic Osteoclast Precursors. N. Kurihara, S. V. Reddy, H. Maeda, G. D. Roodman. Medicine/Hematology, UTHSCSA/VAMC, San Antonio, TX, USA.

Osteoclasts (OCLs) cultured from marrow of patients with Paget's disease (PD) differ from normal OCLs. They contain paramyxovirus-like nuclear inclusions and are hypersensitive to 1,25-(OH)2D3. Normal OCL precursors transduced with the measles virus nucleocapsid gene (MVNP) form OCLs that are very similar to OCLs formed in PD. These cells are also hypersensitive to 1,25-(OH)2D3 and form OCLs at physiologic concentrations of 1,25-(OH)2D3 that are 1-2 logs less than required for normal OCL formation. To test the hypothesis that the increased sensitivity to 1,25-(OH)2D3 may result from induction of a coactivator of vitamin D receptor (VDR) by MV, GST-VDR binding studies were performed with cell lysates from bone marrow from PD patients and empty vector (EV)- or MVNP-transduced normal OCL precursors. A 100-kDa and 17-kDa peptide that bound VDR were detected in OCLs from PD patients, MVNP-transduced normal OCL precursors, but not EV-transduced normal OCL precursors. The 17-kDa protein was identified as TAFII-17/TAFII-20 by sequence analysis, a component of the TFIID transcription complex. TAFII-17 mRNA was highly expressed in MVNP-transduced normal OCL precursors and pagetic OCL precursors treated with 1,25-(OH)2D3 compared to controls. We then determined if MV preferentially activated VDR responsive genes. A luciferase reporter vector containing DR-3, the vitamin D response elements, or DR-5, the retinoic acid response elements, was transfected into NIH3T3 cells stably transduced with the MVNP gene (NIH3T3-MVNP), TAFII-17 gene (NIH3T3-TAFII-17) or empty vector (NIH3T3-EV) and then treated with 1,25-(OH)2D3 or retinoic acid. The MVNP-transduced cells and the TAFII-17 transduced cells were hypersensitive to 1,25-(OH)2D3 and showed increased reporter activity at 1,25-(OH)2D3 concentrations that were 1-2 logs less than required for the EV-transduced cells. In contrast, transduction of DR-5 into NIH3T3-MVNP, NIH3T3-TAFII-17 or EV cells demonstrated that although basal transcription was increased in these cells, the retinoic acid dose response curve of MVNP- and TAFII-17-transduced cells did not differ from that of EV-transduced cells. These data suggested that the enhanced response of NIH3T3-MVNP, NIH3T3-TAFII-17 cells to 1,25-(OH)2D3 is not simply due to a globally enhanced transcriptional response to steroid hormones, but rather to preferential transcription of VDR responsive genes. These data suggest that enhanced sensitivity of pagetic OCL precursors to 1,25-(OH)2D3 may be due to increased expression of a specific VDR coactivator or transcription of TAFII-17 by the MVNP gene and support a potential role for MV in the pathogenesis of PD.

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Osteoclasts Stimulate Myeloma Cell Growth through Direct Cellular Interactions via Production of Osteopontin and Interleukin (IL)-6. M. Abe,[1] D. Inoue,[1] T. Hashimoto,*[1] S. Kido,[1] Y. Shibata,*[1] T. Oshima,*[1] S.

Ozaki,*[1] K. Hiura,[2] K. Moriyama,[2] T. Matsumoto.[1] [1]1st Dept. of Int. Med., University of Tokushima, Tokushima, Japan, [2]Orthodontics, University of Tokushima, Tokushima, Japan.

Multiple myeloma (MM) develops and expands in the bone marrow, causing bone destruction by inducing osteoclasts in their close vicinity. Therefore, interplays among the cellular components of the marrow microenvironment, i.e., osteoclasts, MM and stromal cells, appear to be critical to the development of myeloma bone lesions. We have found and reported that the CC chemokines macrophage inflammatory protein (MIP)-1alpha and beta play an important role in the pathogenesis of MM bone lesions. These chemokines are secreted by most MM cells, and induce MM cell adhesion to stromal cells via VCAM-1/VLA-4 interactions and stromal cell expression of RANKL, thereby enhancing osteoclast formation and function. Moreover, we demonstrated that thus induced osteoclasts (OCs) in turn supported MM cell growth and survival in a cell-cell contact dependent manner, suggesting formation of a vicious cycle. In the present study, we investigated the mechanism of MM growth enhancement by OCs. First we examined a role of IL-6, a major MM growth factor, and found that direct contact with MM cells induced production of IL-6 by OCs. However, the OC-mediated MM growth enhancement was only partially inhibited by an anti-IL-6 neutralizing antibody, suggesting a minor contribution of IL-6 at least under our experimental conditions. We then turned to osteopontin (OPN), a major extracellular matrix protein produced by OCs. We found that co-cultures with MM cells also increased OC production of OPN in a contact-dependent manner. Treatment with OPN resulted in significant enhancement of MM cell growth and/or survival in the presence of IL-6. Furthermore, MM cells strongly expressed an OPN receptor, CD44, which has been shown to be involved in survival signals in other hematopoietic cells. These results suggest that the osteoclast lineage cell also contributes to establishment of a microenvironment suitable for MM expansion at least partially through induction of OPN and IL-6 production by OCs. Consistent with critical roles of such cellular interplays, a bisphosphonate minodronate (YM529) almost completely suppressed the enhancement of MM cell growth by rabbit OCs on dentine slices at 0.1 to 1.0 microM, concentrations too low to induce apoptosis of MM cells. Taken together, these results suggest a role for the osteoclast lineage cells not only in bone destruction but also in MM cell growth and expansion, and provide a molecular basis of bisphosphonate therapy for MM.

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Osteoprotegerin Gene Transfer into Muscle by Electroporation in Vivo as Efficient Gene Therapy for Osteolytic Bone Diseases. T. Michigami, M. Yamagata,* K. Ozono. Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan.

The direct injection of plasmid DNA into muscle is a simple, inexpensive and safe technique for gene transfer in vivo. Although the application of this method has been limited by the relatively low expression of the transferred gene, it has recently been reported that in vivo electroporation markedly increases the efficiency. In the current study, we have investigated the effects of osteoprotegerin (OPG) gene transfer by direct injection of plasmid DNA into muscle followed by in vivo electroporation, using several kinds of animal models which manifest increased bone resorption. To obtain high levels of OPG expression, we utilized pCAGGS vector, which carries the CAG (cytomegalovirus immediate-early enhancer-chicken beta-actin hybrid) promoter. Plasmids were dissolved in saline, and injected into the muscles of hindlimbs of mice (50 microgram/leg) or rats (150 microgram/leg). For electroporation, stainless steel plate electrodes were utilized. The condition of electroporation was optimized by histochemical staining for beta-galactosidase activity in the muscle after transfer of pCAGGS-lacZ DNA with or without electroporation, and it has revealed the increase in the expression level of the gene by electric pulses of 50 V. Electrotransfer of pCAGGS-PTH[1-34] caused transient hypercalcemia, suggesting that muscles can function as a secretory organ when plasmid encoding secretory protein is transferred. Then, we have constructed pCAGGS-OPG-FLAG, which contains full-length rat OPG cDNA and FLAG-tag fused to the C-terminus of OPG cDNA in frame. In vitro osteoclastogenesis assay revealed that the product derived from pCAGGS-OPG-FLAG exhibited inhibitory activity against osteoclast formation. Then electro-transfer of pCAGGS-OPG-FLAG into muscles was performed in two kinds of rodent models of humoral hypercalcemia of malignancy. We found that serum Ca levels were restored in both models within a week after pCAGGS-OPG-FLAG gene transfer, but not when empty vector was transfected. Western blot analysis detected OPG-FLAG in the blood of the animals transferred pCAGGS-OPG-FLAG. Furthermore, OPG knockout mice undergone electro-transfer of pCAGGS-OPG-FLAG exhibited higher bone mineral density compared with the mice received pCAGGS empty vector, which was determined by DXA. Taken together, electrotransfer of OPG expression plasmid into skeletal muscles may provide an efficient nonviral gene thrapy for osteolytic bone diseases.

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Alopecia in VDR Null Mice Is Secondary to a Keratinocyte Defect and Can Be Prevented by Targeting Expression of the Human VDR to Keratinocytes in Vivo. C. Chen, Y. Sakai, M. B. Demay. Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

Mice with targeted ablation of the Vitamin D receptor (VDR) develop alopecia regardless of mineral ion status. Previous studies in neonatal keratinocytes isolated from these mice failed to demonstrate a defect in keratinocyte proliferation or in acquisition of markers of keratinocyte differentiation. However, the VDR null mice do not respond to anagen induction, after hair morphogenesis is completed, demonstrating that the alopecia is secondary to a hair cycle defect. Normal hair cycling depends upon reciprocal interactions between the epidermal (keratinocyte) and mesodermal (dermal papilla) components of the hair follicle. To identify which of these cell populations was responsible for the defect in anagen initiation, hair reconsitution assays were performed in nude mice, using keratinocytes and activated dermal papilla cells isolated from neonatal mice. These studies demonstrated that morphogenesis was not affected by VDR status of either cell population. However, follicles reconstituted with VDR null keratinocytes did not respond to anagen initiation, regardless of the VDR status of the dermal papilla cells.Studies were, therefore, undertaken to examine whether expression of the VDR in the keratinocytes of the VDR null mice could prevent the development of alopecia in vivo. The human VDR was inserted into a cassette containing Keratin 14 upstream regulatory elements and the human growth hormone poly A region. Seven potential founders were mated with VDR null mice and offspring were characterized for expression of the transgene in skin, using rt-PCR with human VDR-specific primers. Further matings were performed in three transgenic lines, two with transgene expression and one without, to obtain transgene positive VDR null mice.VDR null mice from the control line, in which the transgene is not expressed, develop progressive hair loss and do not respond to anagen induction. In contrast, the VDR null mice who express the transgene do not develop alopecia and have hair regrowth in response to anagen induction.These studies demonstrate that the absence of the VDR in keratinocytes is responsible for the alopecia in the VDR null mice and that the defect can be prevented by targeting expression of the human VDR to the keratinocytes of the VDR null mice.

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Expression of Human Vitamin D Receptor in the Skin Prevents the Development of Alopecia in Vitamin D Receptor Null Mice and Promotes the Re-initiation of the Hair Cycle. J. Kong,*[1] X. J. Li,[2] D. Gavin,*[2] Y. Jiang,*[1] Y. C. Li.[1] [1]The University of Chicago, Chicago, IL, USA, [2]Genetics Institute, Andover, MA, USA.

In addition to impaired mineral ion homeostasis, alopecia is a predominant feature of vitamin D receptor (VDR) null mice and hereditary hypocalcemic vitamin D-resistant rickets patients. Recent studies have suggested that the alopecia is probably due to the failure of the initiation of the postnatal hair cycle. To directly determine the role of VDR in the regulation of hair growth, we used the human keratin 14 promoter to generate transgenic mice expressing human VDR (hVDR) in the skin as well as to generate VDR null mice that carry the hVDR transgene through breeding. Parallel studies were carried out in littermates of wildtype (WT), VDR null (KO), wildtype transgenic (TG) and VDR null mice expressing the hVDR in the skin (KO/hVDR) in two transgenic lines. The TG mice were grossly normal. The KO and KO/hVDR mice were growth-retarded, and developed hypocalcemia and secondary hyperparathyroidism after weaning. X-ray radiography and histological analyses of their skeleton revealed rickets and osteomalacia. However, in contrast to the KO mice, the KO/hVDR mice did not develop alopecia. The skin histological structure of the TG and KO/hVDR mice was indistinguishable from that of the WT mice. Immunohistochemical analyses revealed that hVDR was highly expressed in the basal layer of the epidermis and the outer root sheath of the hair follicle. In the first postnatal hair cycle, no major histological differences were seen in the skin of WT, KO, TG and KO/hVDR mice. When anagen was induced by depilation at 18 days after birth, the KO mice failed to initiate the hair cycle, whereas the KO/hVDR mice displayed the same pattern of anagen follicle initiation as the WT mice. Interestingly, the TG mice initiated the hair cycle one to two days earlier than the WT and KO/hVDR mice. These data demonstrate that the expression of VDR in the hair follicles prevents the development of alopecia in VDR null mice, and VDR plays a key role in the re-initiation of the hair cycle after birth.

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A Novel Mechanism of Estrogen Receptor Regulation by Protein Turnover Mediated by Human *Seven in Absentia* (SIAH) Homologs. D. G. Monroe, S. A. Johnsen,* T. C. Spelsberg. Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA.

The actions of estrogen receptor (ER) isoforms, α and β, in osteoblasts (OBs) have important roles in the physiology and remodeling of the skeleton. Previous studies have indicated that the steady-state levels of ER-isoforms change throughout OB differentiation suggesting that the modulation of ER protein levels are important in the regulation of genes involved in OB function. Other studies have demonstrated that ERα is ubiquitinated and degraded by the proteasome-dependent pathway. Therefore, to understand the possible mechanisms involved in ER turnover during OB differentiation and OB function, we investigated the effects of overexpressing specific ubiquitin ligases on the function and steady-state levels of ERα and ERβ. SIAH-1 and -2 are the human homologs of the *Drosophila seven in absentia* ubiquitin ligase and are involved in proteasome-dependent degradation of specific proteins. Our data indicate that overexpression of SIAH-1 or -2 attenuates estrogen (E)-dependent transactivation of both ERα and ERβ by 50% on an ERE-reporter construct in transient transfection assays. Furthermore, western blot analysis demonstrates that SIAH-1 and -2 overexpression results in marked reduction of ERβ levels, whereas the reduction of ERα protein occurs only in the presence of SIAH-2. This suggests that SIAH-1 and SIAH-2 attenuate ERβ-dependent transactivation by directly degrading ERβ protein, whereas the turnover of ERα is dependent upon SIAH-2 and not by SIAH-1. These data also suggest that since SIAH-1 overexpression attenuates ERα transactivation but does not cause turnover of ERα protein, an alternate mechanism may be involved in which ERα recruits SIAH-1 to specifically degrade other components of the ERα transactivation machinery (e.g. steroid receptor coregulators or SRCs). This hypothesis is further strengthened by the observation that coimmunoprecipitation studies demonstrate a direct interaction of ERα with SIAH1. This represents a novel interaction with ER and a ubiquitin ligase. Collectively, these data are consistent with a model where SIAH-2 is the primary player in the attenuation of ERα and ERβ through receptor degradation, and that SIAH-1 may assist by degrading other components involved in the ER transcriptional machinery. Current studies testing the interaction of ERα with SIAH2, the interaction of ERβ with both SIAH homologs, and the effects of SIAH homologs on steady-state levels of the SRCs, are underway. In conclusion, these results demonstrate that SIAH-1 and -2 may be involved in the modulation of ER-dependent transcription in OBs through degradation of ERs by the ubiquitin-proteasome pathway.

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Histone Deacetylase (HDAC) 2 Interacts With the Half Site of DR-3 Type Negative Vitamin D Response Element (nVDREm) in the Chromatin Immunoprecipitation Assay—The Link Between Chromatin

Hypoacetylation and Transcriptional Repression by Vitamin D. T. Okazaki,[1] E. Ogata,[2] T. Fujita.*[1] [1]Endocrine unit, Internal Medicine, University of Tokyo School of Medicine, Tokyo, Japan, [2]Cancer Institute Hospital, Tokyo, Japan.

We have previously shown that vitamin D alters VDR conformation by modulating its phosphorylation status through the action of DNA-dependent protein kinase. The “altered” VDR, in turn, exerts negative gene regulation through its monomeric interaction with nVDREm, the "half" site of DR-3. Further, our transfection assay suggested that negative gene regulation by the liganded monomeric VDR requires the recruitment of HDAC (histone deacetylase) 2, but not HDAC1.Here, we employed chromatin immunoprecipitation assay to directly examine the mechanism of selective recruitment of HDAC2 to the liganded VDR and nVDREm. COS7 cells were transfected with VDR-expressing plasmid along with nVDREm-reporter TK plasmid. Chromatin was immunoprecipitated from these cells treated with no or 10 nM of vitamin D for 40 hours. In this assay, HDAC1, HDAC2 and acetylated histone H3 were immunoprecipitated with the respective antibody, followed by analysis for the presence of the reporter DNA fragment by semi-quantitative PCR. As expected, vitamin D treatment decreased the degree of histone H3 acetylation, while it recruited HDAC2, but not HDAC1. These results imply that specific HDAC2 recruitment led to hypoacetylation of chromatin in the presence of vitamin D. Trichostatin A, an HDAC inhibitor, reversed acetylation status of histone H3 and blocked recruitment of HDAC2 in this assay, suggesting that the recruited HDAC2 possessed HDAC activity. On the other hand, when we used DR-3 positive VDRE-TK plasmid as a reporter, vitamin D treatment neither affected histone H3 nor recruited HDAC2, further supporting our hypothesis that there is selective interaction between nVDREm and HDAC2 in the negative gene regulation by vitamin D. We next performed gel shift assay by using nVDREm as a probe. In this assay, whole cell proteins from COS7 cells into which VDR-expression plasmid was introduced yielded several bands. One of them was block-shifted by anti-VDR antibody. The intensity of this band was augmented by vitamin D added in vitro. Of interest, anti-HDAC2 antibody also blocked the formation of this band only when the proteins were treated with vitamin D in vitro. In the absence of vitamin D, anti-HDAC2 antibody did not affect the formation of this band. Our results raise the possibility that not only unliganded but also liganded VDR utilize the activity of histone deacetylase, HDAC2, to exert negative gene regulation by vitamin D.

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The VDR Heterodimer Interacts with BAF60a, a Component of the Mammalian SWI/SNF Complex. N. Koszewski,[1] K. Henry,*[2] E. Lubert,*[2] D. Noonan.*[2] [1]Division of Nephrology, Bone & Mineral Metabolism, University of Kentucky, Lexington, KY, USA, [2]Department of Biochemistry, University of Kentucky, Lexington, KY, USA.

Previous studies have identified a direct repeat vitamin D response element (VDRE) from the avian PTH (aPTH) promoter that bound the VDR/RXRα heterodimer complex in vitro and conferred vitamin D-dependent repression of gene transcription. Based on these results, a modified yeast one-hybrid screen was used to isolate proteins capable of interacting with the heterodimer complex while driving expression from the negative VDRE. A commercial HeLa cell cDNA/Gal 4 activation domain (AD) hybrid library was screened for proteins capable of interacting with the full-length human heterodimer partners expressed in yeast. Positive clones were identified based on their ability to activate expression of a β-galactosidase reporter vector linked to the aPTH VDRE. Over 150,000 transformants were screened and, following secondary screening and analysis, a total of 10 independent colonies were isolated, plasmid DNA recovered and subjected to sequence analysis. Three of the ten isolates possessed an in-frame coding sequence for the identical full-length protein, while a fourth clone corresponded to an alternatively spliced isoform of the same protein. A database search matched these clones with the BAF60a (or SMARCD1) protein, a component of the mammalian SWI/SNF complex. Deletion studies in yeast were unable to localize a unique region of BAF60a responsible for interaction with the heterodimer complex as only the full-length protein would support β-galactosidase gene expression. GST pull-down analyses revealed that BAF60a failed to interact specifically with either recombinant VDR- or RXRα-containing extracts alone, but displayed strong interactions with the intact heterodimer complex. Transient transfection analysis in HeLa cells indicated that increased expression of BAF60a resulted in decreased transcriptional activity from a VDRE, but not from an estrogen responsive element. In summary, we have identified BAF60a as a factor that specifically interacts with the VDR heterodimer complex using a modified yeast one-hybrid selection strategy. This suggests that the VDR complex may also be a target for the mammalian SWI/SNF chromatin remodeling complex through its interaction with BAF60a.

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Inhibition of 1,25-Dihydroxyvitamin D3-dependent Transcription By Synthetic Peptide Antagonists That Target the Activation Domain of Retinoid X Receptor. P. Pathrose,*[1] C. Y. Chang,*[2] D. P. McDonnell,*[2] J. W. Pike.*[1] [1]Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA, [2]Pharmacology and Cancer Biology, Duke University, Durham, NC, USA.

The vitamin D receptor (VDR) is known to mediate the biological actions of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) through its actions on hormone-sensitive genes. Accordingly, the hormone-activated receptor binds to specific DNA sequences located within the promoter region of regulatable genes, recruits additional coregulatory molecules essential to the process, and initiates quantitative changes in gene output. The transcriptionally active form of VDR is believed to be a VDR/retinoid X receptor (RXR) heterodimer, which binds to vitamin D response elements (VDREs) in vitro with high affinity and selectivity. The absolute requirement for RXR in VDR-mediated transcription in mammalian cells has not been determined, however, due to the absence of a cellular model that is devoid of endogenous RXR expression. In order to determine the role of RXR in VDR action directly, we first identified a subset of novel peptide antagonists (LXXLL-motifs previously identified through phage display screening) that were selective in a hormone-dependent manner for the AF-2 region of either VDR or RXR using a mammalian COS 7 cell two-hybrid system. Peptides were introduced into cells and targeted to the nucleus through the use of an expression vector that encoded the specific peptide sequence fused to the C-terminus of the DNA binding domain of Gal4. LXXLL peptide sequences derived from the nuclear receptor (NR) boxes of both SRC-1 and GRIP interacted strongly with VDR as well as RXR. This screen revealed four categories of peptides: those that interacted with both VDR and RXR, those which were selective for either VDR or RXR and those which did not interact with either receptor. Antagonist peptides were then examined for their ability to block 1,25(OH)2D3-dependent transactivation of an osteocalcin promoter-luciferase reporter chimeric gene following transfection into osteoblastic MC-3T3-E1 cells. Peptides that interacted with both VDR and RXR, including those derived from the NR boxes of SRC-1 and GRIP blocked 1,25(OH)2D3-dependent transcription by up to 85% at the highest peptide concentrations examined. Interestingly, while several peptides that interacted selectively with RXR were inactive, two were as effective in blocking 1,25(OH)2D3 response as those that bound directly to VDR. These studies provide the first direct evidence that RXR participates in transcriptional regulation by 1,25(OH)2D3 in intact cells and may represent a novel approach for assessing the contribution of accessory proteins in nuclear receptor response.

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Shuttling Vitamin D Receptors Transport the Hormone Into and Out of the Nucleus. J. Barsony, K. Prufer, C. Segovis.* LCBB, NIH/NIDDK, Bethesda, MD, USA.

Two alternative hypotheses have been put forth to explain the mechanism of steroid hormone uptake into the nucleus and release from the nucleus: one is based on free diffusion of the hormone, the other is based on carrier mediated import. We used advanced microscopy methods to investigate vitamin D receptor (VDR) shuttling and its possible role in transporting the hormone across the nuclear membrane. To detect the spatial and temporal relationship between calcitriol and VDR in living cells, we stably expressed green fluorescent protein chimera of the VDR (GFP-VDR) in rat osteosarcoma (ROS17/2.8) and porcine kidney (293) cells and developed biologically active red BODIPY labeled calcitriol (red calcitriol). Real-time multicolor confocal laser scanning microscopy allowed us to monitor the nuclear import of hormone and receptor simultaneously. These recordings revealed that calcitriol uptake into the cytoplasm is very rapid (half maximal at 5min). In contrast, hormone uptake into the nucleus was much slower (half maximal at 20min) and proceeded in parallel with GFP-VDR translocation into the nucleus. The intranuclear distribution patterns of VDR and red calcitriol were also similar. These results indicated a role for VDR translocation in nuclear hormone uptake. This was further investigated by mutational analysis. Mutations in the ligand-binding domain known to be important for hormone binding prevented red calcitriol accumulation in the nucleus. To evaluate the impact of receptor shuttling, we identified a region within the ligand-binding domain that confers nuclear import, and also used the import mutant that we generated earlier. GFP-VDR export from the nucleus was perturbed by a combination of point mutations or by leptomycin B treatment of the cells expressing the wild-type GFP-VDR (10nM for 2h). Preventing either nuclear import or export decreased nuclear hormone uptake by at least 70%. Final evidence for the VDR mediated calcitriol import and export came from a special type of fluorescence energy transfer experiment. This method visualized the interaction between VDR and red calcitriol by way of making the liganded receptors much brighter than the unliganded receptors in a small area of the nucleus or the cytoplasm upon the photobleaching of the red calcitriol. The movements of the liganded receptors from the cytoplasm into the nucleus and from the nucleus into the cytoplasm were then recorded. These experiments proved that the hormone is both imported and exported together with the receptor. Our studies gave new insights into the mechanisms of receptor subcellular trafficking and demonstrated that VDR shuttling controls the access of calcitriol to the nucleus.

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Characterization of 5’-Flanking Region of Human RANK Ligand Gene. K. Kajimoto,* R. Kitazawa, S. Maeda,* S. Kitazawa. Division of Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan.

RANK ligand (RANKL), or osteoclast differentiation factor, has been identified as a prerequisite to osteoclastogenesis. Most of the bone resorptive factors, including 1α,25dihydroxyvitamin D_3 ($1,25(OH)_2$ D_3), are thought to modulate bone resorption by regulating RANKL expression on osteoblasts and stromal cells. To elucidate the mechanism regulating human RANKL gene expression, we first examined the effect of $1,25(OH)_2$ D_3 on the RANKL gene by a nuclear run-on analysis, and found that it accelerated the transcription rate of the RANKL gene in the SaOS2 cell line. We then focused on the promoter structure and cis-acting elements of the human RANKL gene that conducts $1,25(OH)_2$ D_3 signaling. From the human genomic library, we cloned a 12.5kb fragment containing the 5'-flanking region of the human RANKL gene and accessed its promoter activity by transient transfection assay. The basic promoter of human RANKL gene comprises inverted TATA- and CCAAT-boxes and putative RUNX2 binding sites. Three putative vitamin D responsive elements (VDRE) are located at -1585/-1571 (VDRE1), -1419/-1405 (VDRE2), and -1384/-1370 (VDRE3) from the transcription start site. Radio-labeled double stranded oligonucleotides corresponding to the three putative VDREs were generated, mixed with nuclear extract from $1,25(OH)_2$ D_3 -treated SaOS2 cells, and subjected to gel motility shift assay. Specific binding of the VDR and RXRα heterodimers to VDRE1 was observed as supershifted bands by anti-VDR and -RXRα antibodies. The presence of the functional VDRE was assessed by transient transfection studies. Promoter-luciferase constructs with (Luc-1787) and without (Luc-1041) VDRE1 were transfected into SaOS2 cells. The transfected cells were then treated with 10 nM $1,25(OH)_2$ D_3 for 6, 12, 24, and 48h prior to the luciferase assay. The treatment increased RANKL promoter activity to 160% in Luc-1784, which was almost nullified in Luc-1041. Introduction of mutation to the VDRE1 site of the Luc-1787 construct also nullified the inductive effect of $1,25(OH)_2$ D_3 on the RANKL promoter. These data indicate that the human RANKL gene promoter shares striking structural similarities with the mouse promoter, and that $1,25(OH)_2$ D_3 transactivates human RANKL gene through cis-acting VDRE located at -1585/-1571.

F002

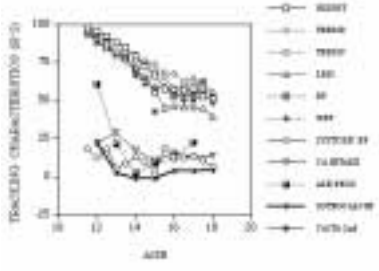
Low 25-Hydroxy-Vitamin D Concentrations Are Associated With Lower Cortical Bone Mass In 10-13 Year Old Girls (Calex-Study). C. Lamberg-Allardt,[1] H. Kröger,[2] A. Koistinen,[3] M. Kärkkäinen,*[4] A. Mahonen,[2] F. Tylavsky,[5] S. Cheng.[3] [1]Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki, Finland, [2]University of Kuopio, Kuopio, Finland, [3]University of Jyväskylä, Jyväskylä, Finland, [4]University of Helsinki, Helsinki, Finland, [5]University of Tennessee, Memphis, TN, USA.

The aim of this investigation was to study the association of serum25-OHD concentration with bone mass at different bone sites in growing girls. The subjects were 10-13 year-old girls (n=104)who presented with Tanner stage I- II and enrolled in an intervention study (CALEX). Serum(S) concentration of 25(OH)D and PTH was assessed with RIA (Diasorin) and IRMA(Nichols)respectively). Bone properties of the total body, hip, spine, radius and tibia were measured using DXA (Prodigy, Lunar) and pQCT (XCT 2000, Stratec). The mean concentration of 25-OHD was 36 nmol/l with a minimun of 19 nmol(l and maximum at 66 nmol/l(reference range 25-125 nmol/l).In a regression model we did not find any association between S-25-OHD and bone mineral content(BMC) or bone mineral density(BMD) at any site. However, when the subjects were divided into groups based on their serum 25-OHD concentration (25 nmol/l to be sufficient)we found lower cortical BMD measured with pQCT in the tibia (p=0.04) and radius(p=0.04) in those girls with S-25-OHD 25 nmol/l (n=82). When Tanner stage and age was introduced as covariates in the model the cortical bone density in the tibia was significantly lower (p=0.001) and tended to be so in the radius(p=0.07) in the group with S-25-OHD <25 nmol/l than in the group with values above this level. There was no difference in the trabecular BMD at any of these sites and at the total body, hip and spine measured with DXA. S-PTH was not associated with BMD.The results suggest that 20 % of the girls in this study may be compromising cortical bone mineralization due to marginal vitamin D status.

F004

Tracking Characteristics of Bone Mass, Body Composition, and Bone Biomarkers From Childhood to Young Adulthood. V. Matkovic,[1] N. E. Badenhop-Stevens,[1] J. D. Landoll,[1] S. L. Mobley,[1] E. J. Ha,[1] A. C. Clairmont,*[2] L. Nagode,*[2] J. L. Roehrig.*[1] [1]Bone and Mineral Metabolism Laboratory, OSU, Columbus, OH, USA, [2]OSU, Columbus, OH, USA.

We previously showed that peak bone mass is one of the main determinants of osteoporotic fracture in the population and that timing for peak bone mass occurs relatively early in life. Assuming minimal change in bone mass during skeletal maturity (age 18 to 50) it, therefore, seems plausible to consider the distribution of bone mass early in life as a good indicator of osteoporosis risk at the time of menopause, assuming that no intervention is applied to change its course. We, therefore followed a cohort (N=185) of healthy Caucasian females from age ~11 to ~18, or during the time period when more than 40% of total body bone mass is accumulated. Bone mass measurements of the total body (TBBMC & TBBMD) and of the distal forearm were measured annually for 7 years in addition to the radiogrammetry of the second metacarpal bone, basic anthropometry, dietary calcium, blood pressure, body composition, and bone biomarkers. Each set of measurements at subsequent time points was regressed on the baseline values and R^2 of each variable was plotted against age (Figure 1). The change in R^2 with time indicates a tracking characteristic of the variables from early puberty to late adolescence and young adulthood. R^2 for bone mass, stature, and body composition decreases with age and reaches a plateau by the age 15-16 with minimal change thereafter. TBBMD continues to decrease up to the age of 18 primarily due to the continuation of bone consolidation after bone modeling has been completed. The tracking characteristics of blood pressure, dietary calcium, and bone biomarkers remain to be significant after 7 years of follow-up, although at a much lower level. The above research data may have implications with regard to the primary prevention of osteoporosis, obesity, and hypertension in the population.



F005

Changes in Lumbar Spine Bone Mineral Density of Healthy Men and Women in the Third Decade of Life: A 10-year Longitudinal Study. R. K. Evans,* K. M. Sheehan,* B. C. Nindl,* C. J. Baker-Fulco,* R. P. Mello,* M. M. Murphy,* C. E. Wade,* C. R. Scoville.* Military Performance Division, USARIEM, Natick, MA, USA.

Maximizing peak bone mass during the growing years is advocated to prevent osteoporosis, yet there is no consensus on the age at which peak bone mass is reached. To effectively apply intervention strategies, a greater understanding of skeletal growth patterns is important. This longitudinal study followed healthy male (n = 48) and female (n = 59) West Point cadets from the age of 18 to 29 years to assess bone mineral changes in the lumbar spine. Dual-energy X-ray absorptiometry (LUNAR, Madison, WI) was used to assess lumbar spine (L2-4) bone mineral density (BMD), bone mineral content (BMC), and bone area biannually during college, and 6 years after graduation. Body mass and percent body fat assessed by skinfold (SF) were also examined. Time points used for analyses were: baseline (1989; age 18), senior year (1992-3, age 21), and 6 years following graduation (1999, age 29). A repeated-measures analysis of variance assessed time and gender effects. Our findings are presented in the following table.

	Men (n=48)			Women (n=59)		
Variable	**1989**	**1992-3**	**1999**	**1989**	**1992-3**	**1999**
BMD ($g \cdot cm^2$)	1.30 ± 0.14^a	1.36 ± 0.15^b	1.31 ± 0.15^a	1.26 ± 0.11^a	1.30 ± 0.12^b	1.31 ± 0.11^b
BMC (g)	63.1 ± 9.9^a	65.5 ± 10.5^b	63.9 ± 10.8^a	53.3 ± 7.0^a	54.6 ± 7.6^a	56.4 ± 7.5^b
Area (cm^2)	48.5 ± 4.8^{ab}	48.1 ± 4.7^a	48.9 ± 5.1^b	42.1 ± 3.3^a	42.0 ± 3.5^a	43.0 ± 3.7^b
Body mass (kg)	73.0 ± 8.5^a	78.0 ± 8.3^b	83.3 ± 13.9^c	60.8 ± 6.3^a	63.0 ± 6.0^a	66.3 ± 9.1^b
Body fat (%)	13.9 ± 3.7^a	15.4 ± 4.1^a	18.2 ± 5.9^b	26.1 ± 4.3^a	25.0 ± 3.9^a	26.3 ± 4.8^a
Suprailiac SF (mm)	12.3 ± 5.6^a	17.7 ± 7.9^b	20.5 ± 11.8^b	16.4 ± 6.3^a	14.7 ± 6.4^a	14.7 ± 7.7^a

Values are means ± SD. Same superscripted symbol across time points denotes lack of statistical significance (p>0.05). Female BMD increased (2.8 ± 4.5%) while at West Point, and was maintained at follow-up. Male BMD also increased while at West Point (4.7 ± 4.4%) but significantly decreased on follow-up (3.8 ± 5.1%). The increase in BMD in active men and women from the age of 18 to 21 suggests that bone mass continues to accumulate in the college-aged adult. Additionally, our sample exhibited a gender difference in skeletal growth patterns during the third decade of life. In the absence of any biological explanation for this gender difference, these findings should be interpreted cautiously. This apparent bone loss in the men could represent artifact produced by increases in male pattern intra-abdominal fat deposition. Men, but not women, accumulated significant body fat from age 21 to 29, although this was not correlated with BMD. Further studies are needed to determine the factors affecting bone mineral acquisition and loss in the young adult population.

F007

Late Normal Puberty in Men Is Associated With 1 SDS Lower Bone Mass in Spine and Hip at 21 Years of Age. A Longitudinal Study Among Young Healthy Men Between 16 and 21 Years of Age. M. G. Stenstrom,[1] E. Norjavaara,*[2] L. Hulten,*[3] L. Hallberg,[3] K. Albertsson-Wikland,*[4] D. Mellstrom.[1] [1]Geriatric Medicine, Goteborg University, Goteborg, Sweden, [2]Pediatric Medicine GP-GRC, Goteborg University, Goteborg, Sweden, [3]Clinical Nutrition, Goteborg University, Goteborg, Sweden, [4]Pediatric Medicine, Goteborg University, Goteborg, Sweden.

The effect of puberty and sex steroids on acquisition of bone mineral density is well known, but whether normal variations on the start of puberty influence the peak bone mass is not known. Both normal and decreased bone mineral densities have been reported for men with delayed puberty. The purpose of this study is to compare whether late onset of pubertal growth spurt influence the acquisition of bone mineral density. In the community of Göteborg, Sweden 250 teenagers of both sexes 16 years old were randomly selected and invited to participate in a longitudinal study with reapeated examination of bone mineral density, body composition and blood samples. 238 subjects initially responded (95%). The young men and women were examined at the age of 16, 17, 18, 19 and 21. All examinations and procedures were done at late afternoon in the same month every year. The young men were divided in quartiles regarding their height growth during five years (16-21). The quartile with the largest change in height growth was compared with the others, thus having less change in height. 54 young men were followed throughout the study. All of the subjects had started their puberty at age 16 indicated by serum testosterone, testicular volume and Tanner-score. At age 21 final height was reach for all subjects (change in height less than 1 cm). Mean growth in height among the quartile with the highest height velocity after the age of 16 was 10.8 cm versus 2.2 cm for the other quartiles. At the age of 21 the mean height were 181 cm versus 181.3 cm although a fully catch up in length the young men with late onset height growth were significantly slender, mean weight 68.0 kg versus 79.2 kg respectively. BMD, BMC and area were significantly lower at age 16 in men with late onset of the pubertal growth spurt. This differance in BMD and BMC persisted but there was no significant difference in area in spine and hip at age 21. Adjusted fore both weight and area bone mass was significantly lower at age 21 in men with late height growth. The difference in BMD in spine and hip at age 21 was >1 SDS below those men with earlier onset of pubertal growth spurt and also significantly lower than mean BMD (spine and hip) in young women at age 21. Late onset of puberty within the normal range for start of puberty is associated with 1 SDS lower bone mass in spine and hip at age 21.

F010

Estrogen Inhibits Adipogenesis of Mesenchymal Progenitor Cells: Possible Mechanism of Action. Z. C. Dang,[1] R. L. van Bezooijen,[1] M. Karperien,[1] C. Oliveira,*[2] K. Willems van Dijk,*[2] S. Papapoulos,[1] C. Lowik.[1] [1]Endocrinology, Leiden University Medical Center, Leiden, The Netherlands, [2]Anthropogenetics, Leiden University Medical Center, Leiden, The Netherlands.

Osteoblasts and adipocytes arise from a common mesenchymal progenitor cell in bone marrow. Whether estrogen (E2) regulates the differentiation of progenitor cells into osteoblasts or adipocytes remains unknown. For this, we have used the mouse clonal cell line KS483 which differentiates into osteoblasts and adipocytes when cultured in charcoal-stripped FBS. E2 from 10-13 M to 10-6 M increased alkaline phosphate activity and nodule formation and stimulated mRNA expression of Cbfa1, PTH/PTHrP-receptor and osteocalcin. In contrast, E2 concurrently decreased adipocyte numbers and decreased mRNA expression of PPARg2, aP2 and lipoprotein lipase. The above E2 action was estrogen receptor (ER) mediated as shown by specific ER antagonists. Similar results were obtained in mouse bone marrow cell cultures, showing an ER-dependent reciprocal control of osteoblast and adipocyte differentiation by E2. Immunohistochemical staining showed the pres-

ence of ERa and ERb in osteoblasts and adipocytes. We identified a mouse splice variant ERb2 and found that E2 downregulated mRNA expression of ERb1 and ERb2. Transfection studies showed that ERb functioned as a transdominant inhibitor of ERa induced gene transcription in KS483 cells. The known adipogenic mixture of isobutylmethyl-xanthine, dexamethasone, and insulin (IDI) induced adipogenesis in KS483 cells and this induction was inhibited by E2. However, the PPARg agonist indomethacin induced adipogenesis more potently and this was only slightly inhibited by E2. Interestingly, overexpression of a constitutively active ERa by adenoviral mediated gene transfer completely blocked adipogenesis induced by indomethacin. These results suggested the existence of cross-talk between ERa and PPARg. PGC-1 is a common coactivator for PPARg and ERa. PGC-1 mRNA was abundantly expressed in KS483 cells and was upregulated by E2 and IDI during the first 11 days of culture and decreased afterwards. E2 activated an ERE-luc reporter construct. Cotransfection of ERa strongly enhanced ERE reporter activity. In contrast, cotransfection of PPARg2 into KS483 exposed to indomethacin inhibited E2 stimulated activition of an ERE-luc report construct. In conclusion, our results demonstrated that E2 stimulated osteogenesis and concurrently inhibited adipogenesis of mesenchymal progenitor cells in an ER-dependent way. This inhibition may occur at the transcriptional level by competition between PPARg and ERa for common coactivators like PGC-1.

F012

Noggin is a Negative Regulator of Bone Remodeling (Formation and Resorption) in Adult Mice: Evidence from Noggin Null Mice and Noggin Overexpressing Mice. X. B. Wu,*[1] L. Sun,[1] L. J. Brunet,*[2] R. M. Harland,*[2] E. Abe.[1] [1]The Mount Sinai Bone Program and the Bronx VA GRECC, New York, NY, USA, [2]Department of Biochemistry and Molecular Biology, University of California, Berkeley, CA, USA.

To date, studies on bone morphogenetic protein (BMP) and its inhibitor, noggin, have focused mainly on their role during skeletal development and fracture healing. Here we report potent inhibitory effects of noggin on the proliferation and differentiation of osteoblasts in *adult* bone. The expression of noggin was enhanced during osteoblast maturation, but with a different time course to BMP-2/4. Noggin expression was also detected in cells of the macrophage lineage, but not in mature osteoclasts. Furthermore, BMP-2/4 and not TGF-β enhanced noggin expression. Levels were also elevated by ~2-fold in bone tissues and bone marrow cell cultures from 20 month-old mice and senescence accelerated mice (SAM-P6) compared with 'young' controls, namely 4 month-old and SAM-R1 mice, respectively. This suggested that elevated noggin levels, by reducing BMP-induced osteoblast maturation, could potentially contribute to involutional osteopenia. We thus hypothesized that the balance between BMP and noggin production might in fact regulate bone remodeling. To investigate this, we developed several transgenic mouse lines that overexpressed noggin in mature osteoblasts under the control of the osteocalcin promoter. Noggin expression in bone tissue and calvarial cell cultures was 2 to 3-fold higher in transgenic mice compared with wild type littermates. However, up to 2 months, bone mineral density remained normal. We next deleted the noggin gene, but found that the homozygotes did not survive postnatally. They had no osteoblasts and in addition displayed abnormal joint formation, likely due to a cartilage abnormality. The regulation of noggin was then examined *in vivo* in heterozygotes by studying the expression of LacZ, a transgene that was incorporated at the site of gene deletion. Consistent with the *in vitro* data, LacZ expression was enhanced by BMP-2/4 in both osteoblasts and macrophage-like cells. Finally, and importantly, bone marrow cells isolated from the noggin heterozygotes showed a significant, ~30%, reduction in their ability to form alkaline phosphatase positive fibroblastoid colonies (or CFU-Fs) as well as TRAP-positive osteoclasts. Collectively, the data confirms a *hitherto* uncharacterized action of noggin (and BMP-2/4) on adult bone remodeling, notably on both formation and resorption. This is not only of physiological significance, but is also a prelude to future therapeutic intervention.

F014

The Type I BMP Receptors ALK3 & ALK6 Function Similarly in Controlling Osteoblast and Adipocyte Differentiation from Human Mesenchymal Stem Cells. J. G. Emery,[1] A. S. Mathur,*[1] D. J. Rieman,[1] M. T. Kaing,*[1] K. A. Geubtner,*[1] C. N. Bender,*[1] M. Gowen,[1] M. Lark,[1] L. J. Suva.[2] [1]Musculoskeletal Diseases, GlaxoSmithKline, King of Prussia, PA, USA, [2]Center for Orthopaedic Research, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Osteoblasts and adipocytes are derived from common mesenchymal precursor cells. Several lines of evidence suggest the existence of a reciprocal relationship between osteoblast and adipocyte differentiation. Bone morphogenetic proteins (BMPs) stimulate both osteoblast and adipocyte differentiation. In 2T3 murine calvarial cells, activation of the type IA BMP-receptor (ALK3) stimulates adipogenesis, whereas activation of the type IB BMP-receptor (ALK6) stimulates osteoblast differentiation (Chen et al., 1988, J. Cell Biol. 142:295). To investigate the role of BMP signaling during osteoblast and adipocyte differentiation in human cells, we established in vitro models of osteoblast and adipocyte differentiation from primary human mesenchymal stem cells (MSCs) and have examined the roles of BMPs, receptors, and Smads in these pathways. To determine if certain BMPs, receptors, or Smads are specific to a particular differentiation pathway, we examined the expression of each during osteoblast and adipocyte differentiation using real-time quantitative RT-PCR. BMP4 is the predominant BMP expressed in differentiating MSCs. ALK3, ALK6, and the type II BMP receptor are expressed in all conditions, although ALK6 levels are 10-fold lower than BMPR-II and ALK3. All Smads examined, including BMP pathway specific, common, and inhibitory Smads, were expressed in differentiating MSCs, although the level of expression was variable. For all genes examined, pathway-specific expression was not detected, suggesting that the balance between osteoblast and adipocyte differentiation is not determined merely by steady-state RNA levels of BMP signaling components. We next examined the roles of ALK3 and ALK6 in human MSC differentiation. Human MSCs were infected with recombinant adenovirus expressing dominant negative (DN) or constitutively active (CA) mutants of ALK3 or ALK6 and cultured under osteoblast or adipocyte differentiation conditions. DN mutants of either receptor inhibited BMP4-stimulated adipocyte or osteoblast differentiation, whereas CA mutants stimulated adipocyte or osteoblast differentiation in the absence of BMP4. However, in adipocyte medium, high concentrations of CA mutants caused the cells to elongate and inhibited adipogenesis. These data suggest that in contrast to their roles in murine cells, ALK3 and ALK6 are functionally redundant in controlling osteoblast and adipocyte differentiation from human MSCs.

Disclosures: GlaxoSmithKline,3.

F016

HDAC-1 Mediates the Repressive Function of Smad6 in BMP Signaling. S. Bai, X. Cao. Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Smad proteins transduce BMP signaling from the cell membrane to the nucleus. Smad6 is one of the inhibitory Smads that preferably inhibits BMP signaling. Smad6 interacts with the activiated type I receptors and blocks the phosphorylation of the receptor regulated Smads. Smad6 was also identified as interacting with the phosphorylated Smad1, forming an inactive Smad1-Smad6 complex in the cytoplasm. Previously, we identified that Smad6 acts as a transcriptional co-repressor in BMP signaling through interacting with Hoxc-8. Here we report that Smad6 represses gene transcription by directly recruiting histone deacetylases (HDACs). HDACs are involved in chromatin modification and are recruited to specific gene promoters by transcriptional repressors or co-repressors that silence gene expression. Transfection studies demonstrated that the HDAC inhibitor, trichostatin A (TSA) partially blocks Smad6-mediated osteopontin promoter activity, indicating HDAC is involved. In immuno-precipitation assays, both Smad6 and Hoxc-8 were co-precipitated with HDAC-1. Furthermore, overexpression of HDAC-1 and Smad6 completely inhibited the BMP-induced osteopontin promoter activity. We also performed the HDAC activity assay with immunoprecipitated Smad6 protein complexes, in which 3H-labled acetylated histones were incubated with Smad6 complex. The result showed that the histones deacetylation rate with Smad6 complex is 25% comparing with the 1% in the control. In addition, we found that Smad6 MH1 domain directly binds to DNA, and the MH2 domain of Smad6 masks this binding activity, suggesting that Smad6 MH1 and MH2 domains associate reciprocally and inhibit each other's function. Interestingly, the interaction of Hoxc-8 with Smad6 induced Smad6 binding to DNA in gel-shift assay. Our data indicate that Smad6 and Hoxc-8 interact with HDAC-1, and recruit HDAC-1 to the osteopontin promoter, leading to inhibited BMP-induced transcriptional activity as the negative feedback loop in the BMP signaling pathway.

F019

Volumetric Evaluation of Newly Formed Vessels in the Distracted Site of Callotasis in Rabbits Using Micro-CT Scan. K. Inui,[1] Y. Azuma,*[2] A. Matsumura,*[1] A. Shimazaki,*[1] Y. Yamano,*[1] T. Koike.[1] [1]Orthopaedic Surgery, Osaka City University Medical School, Osaka, Japan, [2]Pharmacological Research Department, Teijin Institute for Bio-Medical Research, Tokyo, Japan.

Ilizarov emphasized significance of the corticotomy on the surgical procedure in callotasis, so as to preserve blood supply of the distracted callus. However, the precise process how the vessels are forming within the distracted site during callotasis is not well understood. In this study we performed volumetric evaluation of newly-formed vessels in the distracted site during maturation period using micro-CT scan. Twelve male Japanese white rabbits were used in this study. Transversely osteotomised right tibia was fixed by a unilateral external fixator, followed by callotasis for 14 days after 7-days of waiting period at a rate of 1mm/day. On the next day of ceasing distraction (day 0), after 5 days (day 5), 7 (day 7) and 14 (day 14), 3 animals in each day group were sacrificed. After perfusion of heparinized physiological saline through their femoral artery, 15% of barium sulphate solution was perfused as contrast dye, sufficient to fill the femoral vein with dye. Axially scanning of the elongation site of callotasis was performed 10 mm in length, 50 mm in thickness. Only the images of vessels were obtained by subtracting the other images by setting the density threshold. Two hundred digitized images of each specimen were reconstructed in 3 dimensions. The volume of vessels were calculated from the boxels of these vascular images using the software, 3 dimensional evaluation software of bone structure (3D-TBSAS; Teijin, Tokyo, Japan). In the image on day 0, most of blood flow was supplied from circumferential periosteum and few vessels were observed inside of the new bone. On day 7, many capillary vessels were formed inside of the new bone, which were organized in axial direction on day 14. Volumes (V) and surface area (SA) of the vessels filled with contrast dye was shown in Table 1. The vascular volume of the distracted site was gradually increase until the end of this study, while peak value of the surface area was seen on day 7. The peak value of SA divided by V, representing the complexity of vessels, was seen on day 7. In summary, the newly formed vessels were seen circumferentially in the early stage of bone maturation period, subsequently the capillary-like structures connecting to the peripheral vessels grew inside of the neo-generated bone. Then, those vessels were organized in axial direction.

F024

Identification and Characterization of Cystatin 10, a Novel Chondrocyte-Specific Protein Inducing Endochondral Ossification. Y. Koshizuka,[1] S. Ikegawa,*[2] K. Hoshi,[1] T. Yamada,*[1] H. Kawano,[1] Y. Nakamura,*[1] K. Nakamura,[1] H. Kawaguchi.[1] [1]University of Tokyo, Tokyo, Japan, [2]Institute of Physical & Chemical Research, Tokyo, Japan.

We found that the systemic endochondral ossification of the *ttw* mouse, a model for ectopic ossification, was markedly enhanced by a high-phosphate diet. This study sought to identify genes involved in endochondral ossification *in vivo* by a differential display analysis of the auricular cartilage mRNA from *ttw* mice with high and low phosphate diets. Several genes including osteopontin that were up-regulated with a high-phosphate diet were identified. Among them, we isolated a novel gene whose mRNA expression was specific to cartilage in wild-type mice. The gene encodes a protein of 149 amino acids with a putative signal sequence at its N terminus showing 39% homology to cystatin C, a cysteine

protease inhibitor, and was named cystatin 10 (Cst10). In mouse chondroblastic ATDC5 cell cultures, Cst10 mRNA expression could not be detected without induction of differentiation by insulin; however, in the presence of insulin, it appeared at 3 days followed by the type X collagen expression, and the expression level was increased with differentiation thereafter. In mouse growth plates, Cst10 protein was localized at hypertrophic chondrocytes, but not at lower-differentiated chondrocytes as determined by immunohistochemistry. In COS7 cells transfected with Cst10 cDNA, Cst10 protein was shown to be co-localized with lysosome in the cytoplasm by the double staining of immunocytochemistry. To investigate the function of Cst10, the full-length Cst10 cDNA was stably transfected into ATDC5 cells, and several clones highly expressing Cst10 were selected by Western blot analysis. Although type II collagen expression and Alcian blue staining were similar between cultures of Cst10-expressing clones and control clones stably transfected with the vector alone, cell hypertrophy and type X collagen expression were observed earlier, and stainings of Alizarin red and von Kossa were markedly increased in cultures of Cst10-expressing clones. A flow cytometric analysis revealed an approximately 4-fold increase in the number of cells in the sub-G1 phase of the cell cycle in Cst10-expressing cultures compared with that in control cultures. These cells were frequently accompanied by fragmentation and condensation of nuclei, a loss of cell membrane phospholipid asymmetry, and significant activations of caspase 3, 8 and 9 (3-6 fold vs. control clones). These results demonstrate that a novel chondrocyte-specific protein Cst10 may play an important role in endochondral ossification as an inducer of later differentiation and apoptosis of chondrocytes.

F027

Identification of Two Genes Involved in the Differentiation of Chondrocytes through Gene Trapping in Chondrogenic ATDC5 Cells. M. Watanabe,* M. Yamazaki,* T. Uchihashi,* T. Michigami, K. Ozono. Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan.

The gene trapping method is a genome-wide approach applied to clarify the function of a gene based on the idea that the random insertion of the trap vector into a gene disturbs its function. To identify genes involved in the differentiation of chondrocytes, we used the method in ATDC5 cells, which can be induced to differentiate into mature chondrocytes in vitro. The trap vector pT1geo, which has neither its own promoter nor enhancer but has LacZ and neomycin-resistance (neo) genes driven by the promoter of the inserted gene, was introduced into ATDC5 cells by electroporation. The transfected cells were selected in the culture with G418. Approximately 1000 neomycin-resistant clones were obtained and then screened for the activity of b-galactosidase. Then, 15 trapped clones with high b-gal activity were studied in terms of the capability of chondrogenic differentiation. Under the culture condition allowing the ATDC5 cells to differentiate into chondrocytes, two of the trapped clones exhibited the impaired (clone A) and accelerated (clone B) differentiation into chondrocytes by alcian blue and alizarin red staining. The expression of genes characteristic to chondrocytes was examined by RT-PCR every week in clones A and B and ATDC5 cells cultured in differentiation-promotive media. The expression of collagen type II, X, XI, aggrecan, PTH/PTHrP receptor and ALP genes started late and early in clones A and B, respectively, compared with ATDC5 cells. The trapped genes were identified by 5'RACE. In clone A, the trapping vector was inserted in the intron 1 of the p85alpha subunit gene of the phosphatidylinositol 3-kinase (PI3K). As a result, the decreased level of full-length p85alpha and the fused protein of the N-terminal portion of p85alpha with LacZ and neo products were confirmed by western blot. The phosphorylation of Akt protein, one of the target proteins of PI3K, was reduced in clone A. The function of the gene trapped in clone B has not been reported so far. Interestingly, the alpha1 chain of the collagen type IX was not expressed in clone B. In conclusion, we obtained two clonal cells with altered capacity of chondrocytic differentiation by a gene-trap. One trapped clone showed the delay of chondrocytic differetiation associated with the reduced activity of PI3K. The other clone showed the accerelated chondrocytic differentiation, and helps us to identify the novel gene involved in the process.

F029

Abnormalities in Development of the Growth Plates of Thanatophoric Dysplasia Type II (TD II) Fetuses Result from Enhanced Vascular Invasion and Osteoclastic Activity. N. Amizuka,[1] M. Chen,*[2] T. Sasaki,[1] Y. Asawa,*[1] H. Ozawa,[1] J. E. Henderson.[3] [1]Oral Anatomy, Niigata University, Niigata, Japan, [2]Pathology, McGill University, Montreal, Canada, [3]Medicine, McGill University, Montreal, Canada.

Thanatophoric Dysplasia (TD) is the most common form of lethal osteochondrodysplasia. The molecular basis of TD Type II is a point mutation, which results in a K650E substitution in the second tyrosine kinase domain of the fibroblast growth factor receptor type 3 (FGFR3). The K650E substitution results in constitutive activation of the receptor, which has been shown to mediate an inhibitory influence on chondrocyte proliferation and differentiation in vivo and in vitro. In the present study we examined the cellular and molecular abnormalities of the growth plates of tibiae harvested from medically aborted TD type II fetuses of 18-20 weeks gestation. Irregular and aggressive invasion of connective tissue in the tibial epiphysis was marked by an increase in CD31-positive blood vessels, a lack of columnar organization of immature cells and a decrease in the zone of hypertrophic cells, which was also of uneven thickness. Intramembranous bone extended into the outer aspects of the hypertrophic zone and few bony trabeculae were observed in the medial aspect, adjacent to the region undergoing endochondral ossification. Vascular endothelial growth factor (VEGF) immunostaining was apparent mainly in the hypertrophic region of the epiphyseal cartilage while its cognate receptor Flk-1/KDR was localized primarily in the invading blood vessels, suggesting that the principal VEGF target cell in the growth plate is the vascular endothelial cell. Furthermore, cathepsin K-immmunopositive osteoclasts were abundantly present in the metaphyseal region of the TD type II-tibiae. To further explore the relationship between FGFR3 signaling and VEGF expression, CHO cells were transfected with cDNA encoding either wild-type FGFR3 or FGFR3 carrying the K650E mutation. VEGF expression, as assessed by RT-PCR, was upregulated in CHO cells expressing the mutant receptor compared with those expressing wild type FGFR3. In summary, these studies demonstrate that constitutive activation of FGFR3 in chondrocytes 1) stimulates VEGF expression and enhances vascular invasion in the growth plates of TD Type II fetal bones; 2) augments osteoclast formation in the region of newly formed trabecular bone. The defects in bone development associated with TD type II can therefore be attributed to aggressive vascular invasion of the epiphyseal growth cartilage as well as to a decrease in the proliferation of chondrogenic cells.

F031

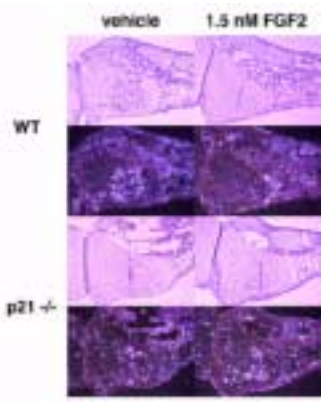
Targeted Overexpression of C-Type Natriuretic Peptide in the Growth Plate Rescued Dwarfism of the Transgenic Mice of Constitutive Active FGF Receptor 3. A. Yasoda,*[1] Y. Komatsu,[1] H. Chusho,*[1] T. Miyazawa,*[1] M. Miura,*[1] M. Suda,*[1] K. Tanaka,[1] Y. Ogawa,*[1] D. M. Ornitz,*[2] K. Nakao.*[1] [1]Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan, [2]Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO, USA.

The natriuretic peptide (NP) family consists of three ligands, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). Although NPs were regarded as regulators of the body-fluid homeostasis, we have recently proposed that NPs, especially CNP is playing important roles in the regulation of the endochondral ossification. We have exhibited that CNP promotes the longitudinal growth of the growth plate cartilage in the organ culture experiments using fetal mouse tibiae. We have also reported the longitudinal overgrowth of the long bones and vertebrae observed in the transgenic mice that overexpress CNP specifically in the growth plate cartilage using the type II collagen promoter (CNP-Tg). In this study, we explore whether CNP acts on the skeletal defect and rescues the dwarfism observed in the transgenic mice of constitutive active FGF receptor 3 (FGFR3). FGFR3 has been identified as a critical regulator of the endochondral bone growth, as autosomal dominant mutations in FGFR3 cause the dwarfing chondrodysplasias in humans. A mouse model for human genetic disease, achondroplasia, has been generated by the targeted overexpression of the activated FGFR3 (containing the G380R mutation responsible for the human disease) in cartilage, using the type II collagen promoter (FGFR3-Tg). We mated FGFR3-Tg with CNP-Tg and analyzed the phenotypes of the F1 progeny. The naso-anal length of the resultant CNP-Tg/ FGFR3-Tg was 14% longer than FGFR3-Tg that were 19% shorter than their non-transgenic littermates. The soft X-ray analysis revealed that the growth retardation of the long bones and the vertebrae observed in FGFR3-Tg was partially recovered in CNP-Tg/ FGFR3-Tg. The shortening of the growth plate cartilage observed in the FGFR3-Tg by the histological analysis was also improved in the CNP-Tg/ FGFR3-Tg. These results suggest that CNP, at least one part, promotes the longitudinal growth of bones independently to the FGFR3 pathway of the regulation of the endochondral ossification, and that local application of CNP may be useful for the treatment of patients with achondroplasia.

F033

FGF Inhibits Chondrocytic Growth through Induction of p21 and Subsequent Inactivation of Cyclin E-Cdk2. T. Aikawa, G. V. Segre, K. Lee. Endocrine Unit, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA.

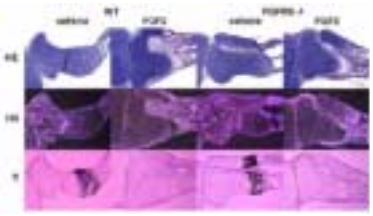
Fibroblast growth factor (FGF) and its receptor (FGFR) are thought to be negative regulators of chondrocytic growth, as exemplified by achondroplasia and related chondrodysplasias, which are caused by constitutively active mutations in FGFR3. To understand the growth-inhibitory mechanisms of FGF, we analyzed the effects of FGF2 on cell-cycle regulating molecules in chondrocytes. FGF2 dramatically inhibited proliferation of RCS (rat chondrosarcoma) cells, and arrested their cell cycle at the G1 phase. FGF2 increased p21 expression in RCS cells, which assembled with the cyclin E-cdk2 complexes, although the expression of neither cyclin E nor cdk2 increased. In addition, the kinase activity of immunoprecipitated cyclin E or cdk2, assessed with retinoblastoma protein (pRb) as substrate, was dramatically reduced by FGF-2. Moreover, FGF2 shifted pRb to its underphosphorylated, active form in RCS cells. FGF2 not only induced p21 protein expression in proliferating chondrocytes in mouse fetal limbs cultured in vitro, but also decreased their proliferation as assessed by the expression of histone H4 mRNA, a marker for cells in S phase (Fig.). Furthermore, inhibitory effects of FGF2 on chondrocytic proliferation were partially reduced in p21-null limbs, compared to those in WT limbs in vitro (Fig.). Taken together, FGF's growth inhibitory effects of chondrocytes appear to be mediated at least partially through p21-induction, and the subsequent inactivation of cyclin E-cdk2 and activation of pRb.



F035

FGF Signaling through FGFR1 or FGFR2 Is a Negative Regulator of Chondrocytic Growth in Fetal Growth Plate. T. Aikawa,[1] T. Koike,[1] G. V. Segre,[1] J. Colvin,*[2] D. Ornitz,*[2] K. Lee.[1] [1]Endocrine Unit, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA, [2]Department of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, MO, USA.

Fibroblast growth factor receptor 3 (FGFR3) has been thought to be a negative regulator of endochondral bone development. Mutations that render FGFR3 constitutively active cause achondroplasia and related dwarfisms in both humans and mice, and inactivation of FGFR3 results in skeletal overgrowth in mice. In contrast, constitutively active mutations in FGFR1 and FGFR2 are known to cause craniosynostosis syndromes in humans. However, although both FGFR1 and FGFR2 have been shown to be widely expressed in developing endochondral bones, their roles in regulating chondrocytic development are yet to be clarified. To test the hypothesis whether FGFR3 is the only FGFR that negatively regulates endochondral bone development, we first examined the expression of transcripts for FGFR1 and FGFR2, and then compared the effects of FGF2 on growth-plate chondrocytes in limbs isolated from FGFR3 (-/-) and Wild-type (WT) mouse fetuses. Transcripts for both FGFR1 and FGR2 were expressed in an indistinguishable manner in the growth plate of WT and FGFR3 (-/-) fetuses. When ED 16.5 fetal limbs isolated from WT mice were treated with 24 nM FGF2 for 2 days, both differentiation and proliferation of chondrocytes were completely suppressed, as evidenced by the disappearance of transcripts for Type X collagen and H4-histone, a marker for cells in S-phase, from the growth plate. In contrast, two-day FGF2 treatment only partially inhibited the expression of these two marker mRNAs in fetal limbs isolated from FGFR3 (-/-) mice; although, FGF2 completely suppressed their expression by 4 days (Fig.). These results demonstrate that not only FGFR3 but also FGFR1 and/or FGFR2 negatively regulates chondrocytic development in developing endochondral bones.



F036

Tissue-specific Knockout of Smoothened Reveals a Critical Role for Hedgehog Signaling in Chondrocyte Proliferation. F. Long,* X. Zhang,* A. P. McMahon.* Harvard University, Cambridge, MA, USA.

Indian hedgehog (Ihh), one of the three mammalian Hedgehog proteins, plays an essential role in endochondral bone development by regulating chondrocyte proliferation, differentiation and osteoblast differentiation. Smoothened (Smo) is a transmembrane protein that transduces Hedgehog signals. In order to investigate further the regulatory functions of Indian Hedgehog (Ihh) during endochondral bone formation, we have used the CRE-LoxP approach to remove Smo activity in chondrocytes. These animals develop shorter long bone when compared to wild-type littermates. In contrast to Ihh-/- mice, chondrocyte differentiation appears to be relatively normal, and osteoblast differentiation does occur. However, like Ihh-/- mice, proliferation of chondrocytes is markedly reduced: Brdu analyses at E14.5 show that proliferation is about 50% of that of the wild type. This result suggests that Ihh directly regulates chondrocyte proliferation. By expressing either Ihh or a constitutively active Smo allele (Smo*) specifically in the cartilage using the bigenic UAS-Gal4 system, we demonstrate that proliferation of chondrocytes is significantly increased. Thus, activation of the Ihh signaling pathway is sufficient to promote chondrocyte proliferation. Taken together, the present study establishes Ihh as a key mitogen in the endochondral skeleton.

F038

POEM: A Novel Pre-osteoblastic Ligand Molecule for α8β1 Integrin. N. Morimura,*[1] Y. Tezuka,*[1] N. Watanabe,*[1] M. Yasuda,*[1] S. Miyatani,*[2] N. Hozumi,*[1] K. Tezuka.[1] [1]Research Institute for Biological Sciences, Science University of Tokyo, Noda, Japan, [2]The Institute of Physical and Chemical Research, Wako, Japan

We have cloned a novel adhesion molecule from the mouse osteoblastic MC3T3-E1 cell line, by RT-PCR using a set of degenerate primers carrying EGF-repeat consensus sequences. This gene encoded a putative protein with 5 EGF-like repeats, an RGD cell binding motif, and a MAM domain. We named this novel protein POEM (**p**re-**o**steoblast **E**GF-repeat protein with **M**AM domain). POEM mRNA was expressed preferably at the pre-osteoblastic stage of MC3T3-E1 cells. By *in situ* hybridization using E16.5 day mouse embryo, strong expression of POEM mRNA was observed in the renal tubules of developing kidney, parathyroid and thyroid glands, developing bone, tooth germ, and midbrain. When expressed in COS-7 cells, POEM accumulated in dot like structures over the plasma membrane. More interestingly, in MC3T3-E1 cells, POEM was accumulated in intercellular space, suggesting that this molecule plays a role in the cell-cell interaction. Recombinant MBP-POEMc protein (MBP fused with POEM's RGD motif and MAM domain) was shown to promote adhesion, spreading, and survival of MC3T3-E1 cells. By mutating the RGD sequence to RGE, the spreading and survival activities were significantly decreased. The tissue distribution and RGD-based cell adhesion activity of POEM led us to hypothesize that POEM may be a target for α8β1 integrin. Therefor, we conducted the cell-binding assay using KA8 cells, a K562 leukemia clone stably expressing α8 integrin (kindly provided by L. F. Reichardt, University of California, San Francisco). KA8 cells bound and spread on MBP-POEMc. On the other hand, parental K562 cells, which express only α5β1 integrin, bound to fibronectin but not to POEM. Thus, POEM is a novel and preferable ligand for α8β1 integrin and may be involved in morphogenesis of kidney, bone, endocrine organs, and nerve systems.

F040

A Novel Mechanism for the Regulation of Osteoblast Differentiation: Transcription of Periostin, a Member of the Fasciclin I Family, Is Regulated by the bHLH Transcription Factor, Twist . A. Oshima,*[1] C. A. Glackin,*[2] S. D. Flanagan,*[2] G. Lowe,*[2] A. Kudo.[1] [1]Tokyo Institute of Technology, Yokohama, Japan, [2]Beckman Research Institute of the City of Hope, Duarte, CA, USA.

Periostin is a secreted protein that is highly expressed early in osteoblastic cells. It has a similar structure to proteins of the fasciclin I family. It is known that periostin supports cell adhesion and spreading in vitro. It is preferentially expressed in periosteum and periodontal ligament. Twist, a basic helix loop helix (bHLH) transcription factor is important for cell type determination and differentiation and has been shown to play an important regulatory role in osteogenesis. In order to examine the target genes that are regulated by Twist during osteogenesis, microarray analysis was performed. Results from microarray and northern analyses reveal that overexpressing Twist up-regulates periostin. Although the mechanisms of periostin transcription are poorly understood, these findings suggest that periostin expression may be regulated by Twist. Here we investigated the 5' flanking region of the periostin gene and the effects of Twist on periostin transcription. To characterize the mechanisms regulating expression of the periostin transcript, primer extension was carried out to identify the transcription start site. DNA sequence analysis of 3kb of the mouse periostin 5' flanking region revealed the presence of two Twist box sequences at -443 to -458 and at -2655 to -2670 respectively. Gel shift and super-shift experiments were performed using oligos, spanning the two Twist box sequences and a Twist polyclonal antibody using MC3T3-E1 nuclear extracts. Results confirmed that both Twist box sites were detected by gel shift and supershifts on the periostin promoter using a Twist antibody. In vivo footprinting experiments were then performed on the mouse periostin promoter surrounding the two twist box sequences. Both undifferentiated and differentiated MC3T3-E1 cells were used to identify the two twist box sites and additional cis-acting binding elements. In vivo footprinting results indicate that the twist box sequences and several additional binding sites are differentially protected in undifferentiated versus differentiated MC3T3-E1 cells. These data suggest that Twist remains bound to the periostin 5' flanking region in undifferentiated osteoblasts, consistent with the up-regulation of periostin expression by Twist as observed in the microarray data. To determine whether Twist actually regulates periostin expression, deletion analyses of the periostin 5' flanking region and overexpression of Twist in MC3T3-E1 cells are now in progress.

F042

Transcriptional Profiling of the Early Phases of Bone Regeneration via cDNA Library Construction and Custom Microarrays. F. Lombardo,*[1] D. White,*[2] A. Dhundale,*[1] C. T. Rubin,*[1] M. Hadjiargyrou.[1] [1]Biomedical Engineering, SUNY, Stony Brook, NY, USA, [2]Millennium Pharmaceuticals, Cambridge, MA, USA.

Bone regeneration occurs as an elaborate series of events that requires temporal and spatial orchestration of numerous cell types and expression of hundreds to thousands of genes. The healing of a fractured bone is, in essence, a recapitulation of embryonic bone development that proceeds through similar processes such as chondrogenesis, ossification and remodeling. In order to be able to influence these biological events and thus the overall bone regeneration process, a more comprehensive molecular understanding is essential. In an effort to identify gene expression patterns that occur during bone regeneration, a cDNA library was constructed. This library consisted of transcriptionally induced genes (pooled from RNA isolated from post fracture (PF) 3, 5, 7 and 10 day callus) that were subtracted following hybridization with RNA derived from intact bone. Following amplification, subtractive hybridization and cloning, 4,183 cDNA clones were identified as up-regulated genes and further characterized. Of these, 3,799 (91%) were successfully sequenced. These genes included 301 (8%) and 60 (1.6%) that showed homology to mitochondrial and ribosomal genes, respectively. In addition, 2,002 (52.7%) had homology to other known genes and represented multiple functional gene families. Further, more than one third of these clones had no functional information in the literature or public databases. Of these, 1,317 (34.7%) showed homology to expressed sequenced tags (EST's) and 119 (3%) were completely novel. To obtain a more comprehensive understanding of temporal gene expression and significance of the genes in the healing process, custom microarrays were constructed that contained all 4,183 clones. PF day 3, 5, 7, 10, 14 and 21 callus RNA samples were used to probe these microarrays and confirm that greater than 80% of cDNAs are up-regulated greater than two fold, on at least one of the PF days, in comparison with intact bone. We are currently investigating the differential expression of these genes as a function of time (i.e. progression of the healing callus), and performing cluster analysis to potentially assign function to the thousands of EST's, novel sequences and known genes that have not as yet been described as involved in the bone regeneration process. Taken together, these data provide a "window" into the molecular events responsible for the early phases of bone regeneration and suggest that many of the genes involved remain uncharacterized. Further experiments utilizing these custom cDNA microarrays should shed more light on the molecular complexity of wound repair.

F046

Development of a Connexin43 (Cx43) Conditional Gene Deletion in Mice. C. H. M. Castro,[1] S. S. Sheikh,*[1] F. Lecanda,[2] R. Civitelli.[1] [1]Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA, [2]School of Medicine, University of Navarra, Navarra, Spain

Osteoblasts are highly coupled by gap junctions formed primarily by Cx43. We have shown that either dominant-negative (Cx45 overexpression) or recessive (Cx43 ablation)

inhibition of Cx43 expression or function significantly alters the development of an osteoblast phenotype. Targeted null mutation of Cx43 in mice leads to developmental defects of the skeleton and cardiovascular malformations incompatible with adult life. To overcome the perinatal lethality of the Cx43 null mutation, and determine its physiologic role for bone mass achievement and maintenance in adult animals, we developed an in vivo model of conditional Cx43 deletion in osteoblasts, using the Cre/loxP method. A mutated Cx43 allele (Cx43 flox) was generated, in which the coding region was flanked by two loxP sites and a reporter Lac-Z cassette inserted between the 3' loxP site and the 3'UTR. These mice were mated with mice expressing bone specific Cre recombinase driven by the OG2 osteocalcin promoter to generate OG2-Cre;Cx 43 flox/ + and OG2-Cre;Cx 43 flox/ flox mice. Cre-mediated recombination removes Cx43 coding region, leaving Lac-Z under the control of the native Cx43 promoter ("knock-in"). These mice were then interbred with Cx43 null heterozygous mice (Cx 43 +/-) to generate a strain in which one Cx 43 allele is null in all cells and the other deleted only in osteoblasts (OG2-Cre;Cx 43 flox/ -). Genotyping was performed by PCR and deletion of the Cx43 flox allele confirmed by X-Gal staining of tail bone sections 8-10 days after birth. Whole body bone mineral density (BMD) was measured by DEXA (precision error= 1.34% CV). All genotype groups were obtained with the expected Mendelian frequency and were viable. At one month of age, BMD was lower in the OG2-Cre;Cx 43 flox/ - (46.8±3.4mg/cm2) than in most other groups (49.1±4.1mg/cm2 for Cx 43 flox/ flox, 48.9±3.4mg/cm2 for OG2-Cre;Cx 43 flox/ flox , 49.2±0.6mg/cm2 for OG2-Cre;Cx 43 flox/ + and 47±3.5mg/cm2 for OG2-Cre;Cx 43 +/+), but the difference was not statistically significant. Cx 43 flox/flox mice appeared to be smaller with a higher percentage of body fat compared to the other groups, probably reflecting a strain background effect. Since the OG2 gene promoter is expressed only at around birth, these results reflect only one month of Cx43 deficiency in differentiated osteoblasts. This Cx43 conditional deletion model will be useful to study the consequences of Cx43 deficiency in skeletal maturation and response to anabolic agents.

F049

Osteoblasts Define the Maintenance of Hematopoiesis and Progenitor Lineage Commitment in the Bone Marrow. D. Visnjic,*[1] Z. Kalajzic,*[2] V. Katavic,*[3] J. Lorenzo,[3] D. W. Rowe,[2] L. H. Aguila.*[4] [1]Department of Physiology, Zagreb University School of Medicine, Zagreb, Croatia, [2]Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA, [3]Department of Medicine, University of Connecticut Health Center, Farmington, CT, USA, [4]Department of Immunology, University of Connecticut Health Center, Farmington, CT, USA.

We have generated a transgenic mouse model expressing a modified herpes virus thymidine kinase (TK) gene under the control of collagen 2.3 promoter. This construct, Col2.3DTK, permits the expression of TK almost exclusively during early osteoblast lineage development. Upon treatment with Gancyclovir (Gcv), these animals are depleted of osteoblasts and present a drastic bone loss. This phenomenon is concurrent with a loss in bone marrow cellularity. Analysis of hematopoietic components during the early phase of treatment evidenced a loss of B cell, erythroid and osteoclast progenitors. However, cells expressing very early hematopoietic markers, similar to hematopoietic stem cells (HSCs), are still present. Interestingly, these bone marrow progenitors can progress normally towards differentiation in vitro.Active extramedular hematopoiesis is also observed, with a dramatic increase in cells with HSC phenotype in liver and spleen. The kinetics of osteoblast disappearance, loss of medullar hematopoiesis and appearance of progenitors and active hematopoiesis in periphery is concerted. Extramedular hematopoiesis seems to be sufficient to maintain the homeostatic hematological values in periphery. When the Gcv treatment was stopped, the osteoblast lineage is recovered and an exacerbated generation of bone and areas of active hematopoiesis are observed in the bone marrow. Gradually the extramedular hematopoiesis decrease to normal levels. This model provides, the first direct indication on the role of osteoblasts during the hematopoietic process, providing an exceptional tool to study interactions between bone remodeling mechanisms and hematopoiesis.

F051

The Osteonectin-null Mutation Compromises Osteoblast Formation and Maturation. A. M. Delany, E. Canalis. Research, Saint Francis Hospital and Medical Center, Hartford, CT, USA.

Osteonectin or SPARC plays a critical role in the maintenance of skeletal integrity. It is necessary for normal bone remodeling and maintenance of bone mass, as osteonectin-null mice develop a low turnover osteopenia that becomes more severe as the animals age. The osteopenia is correlated with a progressive decrease in osteoblast surface and bone formation rate, however the molecular mechanisms involved are not known. To understand how osteonectin supports the osteoblast life-style, we studied primary cultures of osteoblasts and bone marrow stromal cells from control and osteonectin-null mice. In addition, we analyzed immortalized cell lines created by transduction of control and osteonectin-null osteoblasts and bone marrow stromal cells with retrovirus expressing the human papilloma virus E6 and E7 genes. We found that bone marrow stromal cells from osteonectin-null mice contained 15 to 20% fewer fibroblast-colony forming units than those from control mice, although differences in cell proliferation were minimal. DNA laddering assays in primary stromal cell cultures suggest that cells from osteonectin-null mice had increased apoptosis compared to cells from control mice. Osteonectin-null cultures contained fewer mineralized nodules, and PTH stimulation of these stromal cells resulted in decreased accumulation of cAMP compared with controls. Analysis of the stromal cell lines from control and osteonectin-null mice confirmed that mineralized nodule formation was decreased in the absence of osteonectin. Decreased mineralized nodule formation was also observed in cultures of primary osteoblasts from osteonectin-null mice. Compared to controls, these cultures had decreased levels of osteocalcin and alkaline phosphatase transcripts, but type I collagen mRNA was unchanged. Analysis of the osteoblast cell lines confirmed these findings. The ability of the cell lines to differentiate and form mineralized nodules, and the profile of osteocalcin, alkaline phosphatase and collagen mRNAs mimicked that of the primary cultures. In addition, the osteonectin-null osteoblast cell line had decreased accumulation of cAMP in response to PTH stimulation. Osteonectin is thought to modulate the response of cells to certain growth factors and cytokines. However, preliminary data suggest that the osteonectin-null osteoblast cell line is not differentially sensitive to the skeletal cytokines PDGF BB, TGF ß or IGF I. In conclusion, our data indicate that the absence of osteonectin causes multiple defects in osteoblast and stromal cell behavior, resulting in decreased formation of fully functional osteoblasts.

F053

Deletion of the Fibronectin Gene Causes a Dramatic Delay in Incorporation of LTBP1 into the Extracellular Matrix: Rescue With Exogenous Fibronectin. S. L. Dallas,[1] L. F. Bonewald,[2] D. Pesciotta Peters,*[3] C. Barley,*[1] J. L. Rosser,*[2] D. F. Mosher,*[3] C. M. Kielty.*[1] [1]Biochemistry, University of Manchester, Manchester, United Kingdom, [2]Medicine, University of Texas Health Science Center, San Antonio, TX, USA, [3]Medicine, University of Wisconsin, Madison, WI, USA.

Latent transforming growth factor beta binding proteins (LTBPs) are members of the fibrillin superfamily of extracellular matrix (ECM) proteins that bind transforming growth factor betas (TGFβs) and influence their availability. Mutations and deletions in members of this superfamily are associated with Marfan syndrome and related disorders, which often show skeletal abnormalities. LTBP1 regulates TGFβ activity in bone at multiple levels, including its secretion, extracellular matrix storage and its release during osteoclastic resorption. Given these critical functions, an understanding of the structural arrangement of LTBPs in bone ECM and the molecular mechanisms by which they regulate TGFβ availability is of major importance. The goal of these studies was to examine the molecular mechanisms for LTBP1 assembly into bone ECM, identify interacting proteins and determine the domains responsible for these interactions. Double-labeled immunofluorescence studies using primary cultures of fetal rat calvarial cells (FRC) and MG63 osteosarcoma cells showed that LTBP1 was initially deposited into the ECM in association with fibronectin but that by two weeks of culture, a large proportion of the LTBP1 was localized in fibrillin-1-positive microfibrils that lacked fibronectin. Fibrillin-1 was not required for the association of LTBP1 with fibronectin, since overexpression of LTBP1 in UMR-106 osteosarcoma cells, which lack endogenous fibrillin-1, still resulted in the association of LTBP1 with fibronectin. Disruption of fibronectin assembly using either a 70KDa N-terminal fibronectin peptide, or by using cells from fibronectin null mouse embryos, resulted in a dramatic delay in LTBP1 incorporation into the ECM, which was partially recovered with extended culture times (10-14 days). The delay in LTBP1 incorporation in fibronectin-null cells could be rescued by addition of exogenous fibronectin. Transient transfection experiments in 2T3 osteoblast-like cells using LTBP1 deletion constructs indicated that the 60KDa amino-terminus of LTBP1 was sufficient for incorporation into the ECM in association with fibronectin. These studies suggest that fibronectin is important in the initial assembly of LTBP1 into bone ECM. Therefore, in addition to its roles as an ECM protein and in regulation of cell differentiation and apoptosis, fibronectin may regulate TGFβ bioavailability via its interaction with LTBPs.

F055

Acetylcholinesterase: A Secreted Bone Matrix Protein Regulated by Osteogenic Stimuli and Mechanical Loading. C. A. Inkson, T. S. Grewal,* A. C. Brabbs,* T. M. Skerry, P. G. Genever. Biology, University of York, York, United Kingdom.

Acetylcholinesterase (AChE) is best known for its role in cholinergic signaling, however a growing body of evidence indicates that AChE has multiple biological functions with roles in cell adhesion and stress response. Previously we have shown that osteoblasts express and secrete AChE and that AChE is present in osteoid seams and cement lines, implicating AChE in cell-matrix interactions in bone. We therefore investigated the regulatory pathways that control AChE expression during osteogenesis in vitro and in vivo, and its functional role in bone. Using a specific colorimetric assay, we demonstrated that AChE secretion increased significantly during differentiation of primary rat osteoblasts in vitro. Monensin and brefeldin A, blockers of golgi-ER export, inhibited AChE secretion and intracellular AChE co-localised with golgi-specific ceramide dyes, supporting a golgi-ER mediated secretion pathway for AChE in osteoblasts. Western blot analysis of whole cell lysates from primary rat osteoblasts and MG63 cells revealed that TGF-β1 (0.5-2ng/ml) and bFGF (0.5-5ng/ml) markedly increased AChE expression. Furthermore, TGF-β1 induced expression of an additional 55kDa AChE isoform, which was absent in untreated controls. In adhesion assays we demonstrated that specific AChE inhibitors, diisopropyl fluorophosphate and BW284C51 (10^{-7}M–10^{-4}M) and antisense oligonucleotides targeted to AChE, significantly and dose-dependently inhibited attachment of osteoblastic cells ($P<0.001$), compared to appropriate controls. Antisense inhibition of osteoblast adhesion was rescued by plating the cells on exogenous AChE substrates, but not by the addition of soluble AChE to the culture medium. To determine the effects of mechanical loading on AChE expression, TE85 osteoblast-like cells were grown on type I collagen-coated plates and subjected to cyclical strains of 4500με using a FX-3000 Flexercell device (10 min, 1Hz) and cell lysates collected at different time points up to 48h after loading. Using western blot analysis, we demonstrated that AChE expression increased markedly 4 hours after loading which was maintained for 48 hours compared to non-loaded controls. Following in vivo loading, immunolocalisation of AChE on sections of ulnae subjected to mechanical strain of ~4000με (3.3 min, 2Hz) on 5 consecutive days revealed a clear increase in expression of AChE on bone forming surfaces in regions of alkaline phosphatase positivity, which were absent in contralateral control ulnae. These findings indicate that AChE acts as a secreted bone matrix protein and may be instrumental in guiding remodeling events by regulating osteoblast adhesive interactions.

F057

Increased Osteoblastogenesis in Thrombospondin-2-null Mice Protects Against Ovariectomy-induced Bone Loss. K. D. Hankenson,[1] P. Bornstein,*[1] S. Apone,[2] G. B. Stroup,[3] I. E. James,[3] S. M. Blake,[3] X. Liang,[3] M. W. Lark.[3] [1]University of Washington, Seattle, WA, USA, [2]Ostex Intl., Seattle, WA, USA, [3]GlaxoSmithKline Inc., King of Prussia, PA, USA.

Thrombospondin-2 (TSP2) is a 450 kDa ECM protein produced by osteoblast-lineage cells. Mice deficient in TSP2 (TSP2-null) form excess endosteal bone and have an increase in marrow stromal cell (MSC) number. In culture, TSP2-null MSC show an increased proliferation rate, but a delay in osteogenesis. Since cells of the osteoblast lineage, including MSC, are responsible for regulating osteoclasts and TSP2-null MSC are functionally different than WT, we postulated that TSP2-null mice could have an alteration in osteoclast development or function. Geometry of mid-diaphyseal femurs, harvested 5 weeks post-ovariectomy (OVX), was analyzed by pQCT. As previously reported, TSP2-null sham mice had a significantly greater cortical area (10%) relative to WT sham. When ovariectomized, WT mice showed a significant loss of bone relative to sham (14% increase in marrow area), but surprisingly, OVX did not alter TSP2-null bone geometry. To examine whether a decrease in osteoclastic activity was responsible for this observation, a bone-specific, cross-linked NH_2-telopeptide of type I collagen (NTx) that is released during bone resorption was evaluated by ELISA in serum collected 5 weeks post-OVX. Sham-operated WT and TSP2-null mice had equivalent levels of NTx, verifying that the TRAP+ multinucleated cells (MNCs) observed in TSP2-null bone were functional. With OVX there was an increase in both TSP2-null and WT NTx; however, TSP2-null mice had 50% less NTx released into serum than WT mice. This indicates that either there is a reduction in OCL function in TSP2-null mice or that OCL numbers are decreased relative to WT. The number of OCL in OVX mice is currently being determined by histomorphometry; however, we have found that an equal number of OCL develop in culture from TSP2-null and WT marrow-derived mononuclear cells. Although these results provide a partial explanation for the maintenance of bone geometry observed in TSP2-null OVX mice, enhanced endosteal bone formation may also compensate for the resorption that does occur. Evidence for an increase in bone formation in TSP2-null OVX mice is provided by a 3-fold increase in TSP2-null MSC number relative to MSC in WT OVX mice. Numerous studies have shown that the number of MSC is positively correlated with endosteal bone formation. Bone formation is also being evaluated by histomorphometry in sham and OVX samples. In summary, although sham-operated TSP2-null mice have fully functional OCL in vivo, we find that the osteoclastic response to OVX is decreased in TSP2-null mice relative to WT. This effect could be due to an increase in a soluble mediator, such as osteoprotegerin, which is produced by the increased number of MSC, which are also observed. To our knowledge, this is the first study to suggest that an overabundance of osteoblast progenitors is associated with decreased osteoclast formation and/or function.

F059

Matrix Protein Regulation of Collagenase-3 and Osteocalcin Gene Expression in Differentiating Osteoblastic Cells. R. C. D'Alonzo,*[1] N. C. Partridge,[1] A. Kowalski,*[2] D. T. Denhardt.[2] [1]Department of Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA, [2]Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ, USA.

Previously, we demonstrated that expression of collagenase-3 in primary rat osteoblasts is limited to post-proliferative stages of cell differentiation and reaches maximum levels during the mineralization stage. Here we show that clonal osteoblastic MC3T3-E1 cells follow a similar pattern and produce detectable levels of collagenase-3 mRNA one week after reaching confluence and dramatically increase mRNA after two weeks. This increase in collagenase-3 expression is dependent upon the presence of ascorbic acid and is inhibited in the presence of the collagen synthesis inhibitor, 3,4 dehydroproline (DHP). Transient transfection studies using the 148 base pairs upstream of the collagenase-3 promoter show that collagenase-3 promoter activity also increases during differentiation and requires the presence of ascorbic acid. Additionally, this stimulation is reduced by mutation of either the activator protein-1 binding site (*AP-1*) or the runt domain binding site (*RD*). The presence of DHP or the absence of ascorbic acid in the media of differentiating MC3T3-E1 cells also inhibits the expression of osteocalcin, as has been shown in other studies. Additionally, in the absence of β-glycerophosphate supplementation, osteocalcin expression increases, osteopontin expression decreases, and collagenase-3 expression is relatively unchanged. This led us to suspect that osteopontin may negatively regulate osteocalcin expression. In the presence of an RGD mimetic compound, which blocks the binding of the αv integrin subunit to osteopontin and other substrates, osteocalcin mRNA expression increases dose dependently. No significant change in collagenase-3 expression was noted. Based on these results, we conclude that collagenase-3 expression, like osteocalcin expression, increases during MC3T3-E1 cell differentiation and requires collagen synthesis. Further, both the *AP-1* and *RD* sites are required for full activation of the collagenase-3 promoter during cell differentiation. Finally, we observed that an increase in osteocalcin, but not collagenase-3, expression is correlated with a reduction of osteopontin expression and with the blockage of an osteopontin binding integrin. This finding suggests that osteopontin acts as a brake on osteocalcin expression during osteoblastic cell differentiation.

F060

Human Myeloma Cells Upregulate RANKL and Downregulate OPG in Bone Environment: Evidence of OPG/RANKL Imbalance in Multiple Myeloma Patients. N. Giuliani,[1] R. Bataille,*[2] C. Mancini,*[3] M. Lazzaretti,*[3] S. Barille.*[2] [1]Chair of Haematology, Department of Internal Medicine, University, Parma, Italy, [2]INSERM U463, Nantes, France, [3]Pathology, University, Parma, Italy.

Multiple myeloma (MM) is a plasma cells malignancy characterized by the high capacity to induce bone destruction. The tight relationship between myeloma cells and bone environment is critical in the pathogenesis of bone lesions, even if the biological mechanisms are poorly understood. In murine system it was recently hypothesized a possible involvement of OPG and its ligand RANKL in MM-induced osteolysis. The aims of our study were to investigate whether human myeloma cells could directly produced RANKL or affect the physiological OPG/RANKL balance in the bone environment and to characterize this system in MM patients. First, we found that neither human myeloma cells lines (XG-6, XG-1, MDN, U266, RPMI, OPM-2, LP-1, and JJN-3), nor fresh myeloma cells isolated from 26 patients with MM did directly express RANKL and rarely produce a low amount of OPG. In a coculture system we demonstrated that XG-6 and XG-1 upregulate RANKL and downregulate OPG production in primary human bone marrow pre-osteoblast or stromal cells (BMSC) at both mRNA and protein levels. This effect was dependent on the cell-to-cell contact and partially blunted by anti VLA-4 mAb. On the contrary, an anti TNF mAb, or IL-1 mAb or IL-6 mAb have no effect. Human CD 34+ derived osteoclastogenesis was stimulated by XG-6 only in the presence of BMSC and reduced by rhOPG. With a semiquantitative RT-PCR, we have also observed that BMSC isolated by MM patients overexpressed RANKL mRNA in comparison to normal subjects. In addition, using an immunohistochemical staining, performed on bone marrow tissue sections, we confirmed that RANKL is not expressed by myeloma cells being detectable on stromal cells and that OPG is detected on osteoblastic and osteoclastic cells but not on plasmacells. A reduction of OPG expression in trabecular osteoblasts with an increase of RANKL positive stromal cells was observed in MM patients with bone lesions in comparison to patients without osteolysis or to control subjects. (OPG+ cell% ± SE: 3.2±0.7 vs. 6,5±1.6 and 6.7±1.2 respectively, $p < 0.05$; RANKL+/mm2 ± SE: 2.1 ± 0.03 vs. 0.91±0.08 and 0.93±0.18 respectively, $p<0.05$). In line with these observations, OPG serum levels were reduced in MM patients compare to normal subjects (mean±SD: 26.6±4.8 vs. 18.5±6.0 ng/ml;$p=0.009$). Our results clearly indicate that human myeloma cells donot produced RANKL but are able to induce an imbalance in OPG/RANKL ratio in the bone environment in favour to RANKL supporting the hypothesis that this system could be involved in the pathogenesis of MM-induced bone disease.

F062

The Bisphosphonate Zoledronic Acid Inhibits Metastases to Bone and Liver with Suppression of Osteopontin Production in Mouse Mammary Tumor. H. Nobuyuki, T. Hiraga, P. J. Williams,* M. Niewolna,* N. Shimizu, G. R. Mundy, T. Yoneda. Div Endocrinol, Univ TX Hlth Sci Ctr, San Antonio, TX, USA.

Bisphosphonates (BPs) are effective therapeutic agents for bone metastases. Accumulating evidence indicates that BPs inhibit bone metastases through inhibition of osteoclastic bone resorption. On the other hand, recent in vitro studies have shown that BPs reduce cell-to-matrix attachment, inhibit growth and increase apoptosis in a variety of tumor cells. Moreover, the BP clodronate has been shown to suppress visceral metastases and prolong survival in breast cancer patients. These results suggest that BPs have direct actions on tumor behavior. Here, we examined the effects of the BP zoledronic acid (ZOL) on the behavior of breast cancer using the 4T1 mouse mammary tumor cells that spread to bone, lung, liver following orthotopic inoculation in the mammary fat pad in female Balb/c mice. These cells were stably transfected with firefly luciferase for quantitative measurement of tumor burden. Administration of ZOL (0.5 or 5ug/mouse, iv, once every 4 days) was started 7 days after 4T1 cell inoculation when tumor formation became visible and continued until mice were sacrificed at day 28. Radiologic and histomorphometric examination revealed that ZOL reduced metastatic tumor burden in bone with impaired osteoclastic bone resorption in a dose-dependent manner. Surprisingly, metastatic tumor burden in liver as determined by luciferase activity was also significantly decreased in ZOL-treated mice. Lung metastases were also marginally reduced. Since osteopontin (OPN) has been implicated in breast cancer metastasis and our study showed 4T1 cells produced large amounts of OPN, we hypothesized that ZOL suppressed metastases through affecting OPN production. In support of this notion, we found that ZOL inhibited OPN production by western analysis and transcription of the OPN gene using a luciferase reporter construct. Of note, ZOL inhibited the anchorage-independent growth of 4T1 cells in colony formation assay in soft agar. Collectively, these results suggest OPN contributes to 4T1 cell growth. Consistent with this notion, a neutralizing monoclonal antibody to OPN also inhibited the anchorage-independent growth of 4T1 cells and tumor formation in the orthotopic site. In conclusion, these results suggest that ZOL inhibits the growth of 4T1 breast cancer through inhibition of OPN production, thereby suppressing its metastases and that ZOL has direct anti-proliferation effects on 4T1 breast cancer cells. Our results also suggest that OPN is an autocrine growth-stimulating factor and may be a molecular target of ZOL actions in some breast tumors.

F064

Myeloma Bone Disease and Tumor Burden Reversed by Neutralizing Antibodies to Macrophage Inflammatory Protein (MIP)-1α/CCL3 In Vivo. B. O. Oyajobi, P. J. Williams,* B. Story,* G. R. Mundy. Medicine/Endocrinology, UTHSCSA, San Antonio, TX, USA.

Multiple myeloma (MM) is commonly associated with severe bone destruction due to increased osteoclast (OC) activity induced by as yet unidentified factor(s) secreted by the tumor cells. MM cells produce MIP-1α, a CC chemokine whose levels are reportedly elevated in marrow plasma of MM patients compared with other lymphoid malignancies and normal controls. We recently showed that MIP-1α increases OC formation and bone resorption in vivo when expressed adjacent to bone surfaces, consistent with the notion that it plays an important role in the pathogenesis of osteolysis in MM. We therefore hypothesized that neutralizing MIP-1α bioactivity in vivo should limit development and/or progression of osteolytic lesions in a model of human MM. To test this, we used the murine myeloma (5TGM1) cell line which secretes MIP-1α constitutively. Following intravenous injection into syngeneic C57BL/KaLwRij or *bg-nu-xid* mice, 5TGM1 cells home to the bone marrow and spleen and expansion within these sites results in radiographically-detectable osteolytic lesions, greatly elevated titers of the monoclonal IgG2bκ (a marker of tumor load) in serum and splenomegaly. Here, we report that systemic administration of an antibody to MIP-1α was efficacious in ameliorating disease progression in this model.

Mice inoculated with 5TGM1 cells were randomized into groups and treated with a monoclonal rat anti-mouse MIP-1α antibody (R & D Systems) or an isotype rat monoclonal IgG2a (100µg i.p. twice weekly for 4 weeks). A third tumor-bearing group received PBS using the same dosing schedule. Treatment with anti-MIP-1α antibody resulted in a significant reduction in serum IgG2bκ titer in 7 out of 10 tumor-bearing mice (5.45 ± 1.56 mg/ml), below the median for the isotype IgG- and PBS-treated controls pooled (9.26 mg/ml; n=9). Serum paraprotein levels also directly correlated with splenic wet weights (r= 0.83). The mean surface areas of the lytic lesions visible on radiographs, assessed by blinded image analysis, were significantly reduced in mice that received the anti-MIP-1α antibody compared to control IgG- and PBS-treated mice (0.019 vs 0.031 mm^2). Histomorphometric analyses of bones revealed that blockade of MIP-1α function resulted not only in a diminution in tumor volume, but also a reduction in the number and intensity of staining of $TRAP^+$ OC. Together, these data strongly implicate MIP-1α in the pathogenesis of MM-induced bone destruction and possibly myeloma itself. Furthermore, our results suggest that small molecule antagonists of MIP-1α receptors (CCR-1 & CCR-5), which are now available, hold promise as beneficial adjuncts to current standard therapeutic approaches in MM.

F066

Effects of Tumor Secretion of Recombinant Osteoprotegerin (Opg) on Osteolytic Metastases. T. Oba, X. Sun, B. Grubbs,* Y. Cui,* R. Kakonen,* T. A. Guise, J. M. Chirgwin. Medicine, U TX Hlth Sci Ctr, San Antonio, TX, USA.

Osteoprotegerin inhibits bone resorption by binding to and neutralizing RANK ligand, the central physiological stimulator of osteoclast formation and activity. However, it can also protect tumor cells from apoptosis. Thus, the actions of Opg on osteolytic bone metastases may not be beneficial. We expressed osteoprotegerin in the MDA-MB-231 human breast cancer line, which causes osteolytic metastases, and the cells were tested in an animal model.Mouse Opg coding sequence was cloned, from RNA isolated from the ST2 cell line stimulated with 5ng/ml TGFbeta1, by RT-PCR into the expression vector pcDNA3neo. Conditioned medium from transfected cells inhibited osteoclast-like multinucleated cell formation in bone marrow cultures, confirming that the cDNA encoded functional Opg. Mouse Opg-Fc fusion protein and a polyclonal antibody against Opg were used to establish an ELISA for mouse Opg. Clones of MDA-MB-231 cells were selected for resistance to G418 and tested for continuing expression of Opg protein for at least 30 days in the absence of antibiotic selection. Three stable clones were chosen for testing in vivo. These produced 24, 60, and 150ng Opg/ml/105 cells/48hr. An empty vector clone (EV) produced no detectable Opg. All cell lines had the same growth rates. They were inoculated into female nude mice (10/group) via the left cardiac ventricle. Progress of osteolytic metastases was followed by x-ray. At 3 weeks the number of osteolytic lesions was less [number=4.2±1.3 vs. 12.0±2.2] in the three Opg-secreting groups pooled compared to control cells [$p<0.01$]. Area per lesion was not significantly different. Animals were sacrificed when paraplegic or cachectic. Tumor cells in bone continued to express Opg by immunohistochemistry. The two groups of animals with tumors producing moderate amounts of Opg showed delayed time to sacrifice, compared to the EV control [$p<0.05$]. However, the high Opg clone conferred no survival advantage. Tumor cells from this clone showed a 5-fold decrease in number of apoptotic nuclei in bone, relative to the other 3 groups [$p<0.001$ vs. EV].The results suggest that modestly increasing the local concentration of wild-type Opg can slow the progression of osteolytic lesions and provide a survival advantage in an animal model of bone metastasis. However, high concentrations of Opg may increase tumor growth by blocking apoptosis. Thus, reagents designed specifically to inhibit RANK ligand but not to affect apoptosis may be more effective against osteolytic metastases than osteoprotegerin.

F068

Synergistic Suppression of Metastatic Phenotype in Vitro and Metastasis in Vivo by a Bisphosphonate Plus a Farnesyl Transferase Inhibitor. V. Andela,*[1] E. Schwarz,*[2] R. O'Keefe,*[2] J. Rosenblatt,*[2] E. Puzas,[2] R. Rosier.[2] [1]Orthopaedics Research, Univ. of Rochester, Rochester, NY, USA, [2]University of Rochester, Rochester, NY, USA.

Bisphosphonates used in the management of metastatic bone disease have direct anti-tumor properties in addition to preventing osteoclast assisted bone metastasis. Aminobisphosphonates inhibit the mevalonate synthesis pathway, thus the generation of isoprenoid groups, that are enzymatically transferred (by farnesyl or geranyl geranyl transferases) unto such proteins as those of the Ras superfamily, (Ras, Rho, and Rac). Because isoprenoid generation and transfer represent two distinct targets, this study tested the hypothesis that synergistic inhibition of prenylation would result from combining an aminobiphosphonate and a farnesyl transferase inhibitor. Given the extensive implication of Ras proteins in malignancy, and the critical role of Rac and Rho in cytoskeletal architecture and function, it was hypothesized that this regimen would effectively inhibit tumor invasion and metastasis. Methods: Using a murine lung alveolar carcinoma cell line (Line 1), we characterized the sensitivity of the cells to 48 hr exposures of Alendronate and/or an FTI (R115777) in culture. Invasion assays and fluorescent imaging were used to analyze the cellular effects of these agents on basement membrane invasion and cytoskeletal architecture. In addition, Ras, Rho and Rac subcellular compartmentalization, downstream signaling events and MMP 9 secretion were analyzed. Finally the effects of the drug regimen was assessed in vivo using a murine pulmonary metastasis model.Results: Alendronate and FTI both had minimal inhibitory effects on tumor cell invasion, but the combination was synergistic. Western blot analysis of subcellular fractions showed decreased membrane association of Ras and increased membrane association of Rac and Rho, as well as suppression of downstream signaling events (phosphorylation of Erk1 Erk2, and p38). Fluorescent imaging showed a disruption of lamellopodia and membrane ruffles, and demonstrated an organellar distribution of Rho and Rac. As would be expected of the cytoskeletal compromise, MMP 9 secretion and activity was decreased. In vivo, Alendronate minimally suppressed metastasis, while FTI significantly inhibited metastasis and the combination was additive.Conclusion: Aminobisphosphonates and FTIs synergistically inhibit the prenylation and function of proteins involved in tumor cell invasiveness and metastasis thus FTIs could potentiate the antitumor activity of aminobiphosphonates, notably in soft tissue where aminobiphosphonates do not achieve high concentrations.

F070

Examination of Effects of Zoledronic Acid on Prostate Cancer. E. Corey, J. E. Quinn,* L. G. Brown,* M. P. M. Roudier, C. Higano,* R. L. Vessella.* Urology, University of Washington, Seattle, WA, USA.

Zoledronic acid is a third-generation bisphosphonate, which is a very potent inhibitor of bone lysis. Interest has recently arisen in potential direct anti-tumor effects of this compound, and in whether its use would be of benefit to prostate cancer (CaP) patients with bone metastasis. We have recently shown that zoledronic acid affects proliferation and apoptosis of prostate cancer cells, but only at concentrations higher than the peak plasma levels that can be achieved in patients. Here we report observations of the effects of zoledronic acid used in combination with Taxol on tumor cell proliferation and invasiveness *in vitro*. A combination of 10 mM zoledronic acid and 10 nM Taxol was more effective in decreasing proliferation of CaP cells than either of these compounds alone. Moreover, concentrations of zoledronic acid (0.1 µM and 1 µM) that did not affect CaP cell proliferation had significant effects on invasiveness. We also examined the effects of zoledronic acid on prostate cancer cells in subcutaneous (SC) tumors, as well as in an "osseous CaP" model. We detected no significant decrease in volume of subcutaneous PC-3, LNCaP, and LuCaP 23.1 tumors treated with zoledronic acid (5 µg per injection twice a week). The absence of observable effects on SC tumors may well be due to poor bioavailability of zoledronic acid, in part because the compound rapidly accumulates in bone. In contrast to the results with SC tumors, when we used an osseous CaP model consisting of direct injection of CaP cells into tibiae to evaluate the effects of zoledronic acid on CaP bone metastasis, we detected significant differences in bone density in zoledronic acid-treated animals bearing PC-3 tumors in tibiae. Bone histomorphometric analysis confirmed the differences in bone area between untreated and treated animals. LuCaP 23.1 in the osseous CaP model exhibited significant decreases in serum levels of PSA in animals injected with zoledronic acid either simultaneously with tumor cells or after the bone tumors were established (PSA of 5-10 ng/mL). Bone histomorphometric analysis is underway. In conclusion, we have shown that zoledronic acid has significant anti-tumor effects on CaP cells *in vitro* and *in vivo*.

F072

PTHrP Expression Promotes Prostate Cancer Metastases In Vivo. D. W. Burton,[1] P. Jiang,[2] M. Xu,[2] C. M. Lu,*[1] R. M. Hoffman,[2] L. J. Deftos.[1] [1]UC San Diego and Veterans Affairs Medical Center, San Diego, CA, USA, [2]Anticancer, Inc., San Diego, CA, USA.

Parathyroid hormone-related protein (PTHrP) is commonly expressed in prostate cancer. As in other cancers, this oncoprotein has been demonstrated to regulate the growth of prostate cancer cells in vitro. In order to study the effect of PTHrP on tumor pathogenesis, growth, and progression in vivo, we developed a mouse orthotopic prostate cancer model. We initially studied the DU 145 cell line, which was derived from a human prostate carcinoma brain metastasis. This line has a low constitutive PTHrP expression and does not grow well in mouse tumor models. We studied four types of DU 145 cells that were genetically-engineered to constitutively express green fluorescent protein (GFP): 1. Wild type cells, 2. PTHrP1-173 transformed cells, 3. PTHrP1-87 transformed cells and 4. Vector (pCi-neo) transformed cells. The stably transduced cells were injected subcutaneously into immunocomprised mice to form solid tumors, and 1-mm3 pieces of the tumor were then surgically transplanted into the prostate bed of SCID mice. The mice were evaluated by GFP imaging, x-ray, autopsy, and PTHrP studies on sera and tumors. The PTHrP expressing prostate cancer cells demonstrated significant tumor growth and metastases, while no subcutaneous tumor growth was observed in the wild-type and vector transformed groups. At 9 weeks after implantation, primary and metastatic tumor burden were determined and serum PTHrP was measured for each mouse. GFP visualization and radiographic analysis revealed the sites of metastases. The PTHrP transformed groups demonstrated bone and visceral metastases, and no metastases were observed in the two control groups. In the PTHrP-expressing cancers, serum PTHrP levels correlated with tumor volume (r = 0.99). PTHrP levels were not detectable in control mice with no tumor. Primary tumor specimens were analyzed by immunohistology, immunoassay, and westerns and demonstrated robust PTHrP expression. Our results demonstrate that PTHrP expression by prostate cancer cells promotes the development of skeletal as well as non-skeletal metastases by the malignancy and that PTHrP expression in prostate cancer cells was required for the development of skeletal metastases. PTHrP secreted into the blood of tumor-bearing animals served as a tumor biomarker by correlating with the primary prostate tumor volume and the degree of metastastic burden. Further studies can help to define the role of PTHrP in the pathobiology of prostate cancer. These animal models can generate clinical hypotheses designed to elucidate the role of PTHrP in human prostate cancer pathogenesis and to identify specific PTHrP species as diagnostic and therapeutic targets for this malignancy.

F076

Prediction of Fracture Risk in Postmenopausal Caucasian Women With Peripheral Bone Desitometry: Evidence From National Osteoporosis Risk Assessment (NORA). P. Miller,[1] E. Siris,[2] T. Abbott,[3] L. Wehren,[3] Y. Chen,[4] K. Faulkner,[5] E. Barrett-Connor,[6] M. Berger,[3] A. Santora,[3] L. Sherwood.[3] [1]Colorado Center for Bone Research, Lakewood, CO, USA, [2]Columbia Presbyterian Medical Center, New York, NY, USA, [3]Merck & Co., Inc., West Point, NY, USA, [4]Merck & Co., Inc, West Point, NY, USA, [5]GE Medical Systems/Lunar, Madison, WI, USA, [6]University of California, San Diego, CA, USA.

Low bone mineral density (BMD) is a risk factor for fracture. Several new peripheral technologies have been introduced. At present, there are few prospective data supporting these newer technologies. Using data from the National Osteoporosis Risk Assessment

(NORA), we evaluated the association between BMD measurements at peripheral sites using some of the newer technologies and subsequent fracture risk. Each participant had BMD measured at one peripheral site: heel SXA (Osteoanalyzer, Dover), forearm (pDEXA, Norland), finger (AccuDEXA, Schick), or heel ultrasound (US)(Sahara, Hologic). Women were divided into groups based on T-score: t>-1.0; -1.0>t>-2.5, and t<-2.5. Fractures occurring in the subsequent year were identified by self-report. This analysis is limited to 148,649 Caucasian women who had valid baseline BMD data and responded to the follow-up survey. A total of 2259 osteoporosis-related fractures (hip, rib, forearm/ wrist, and spine combined) were reported. Risk ratios for fractures according to site of BMD measurement are shown.

	Heel (SXA)	Forearm	Finger	Heel (US)
# women	79,200	51,949	10,837	7,563
#(%) osteo fractures	1224 (1.6)	781 (1.5)	133 (1.2)	121 (161)
% -1.0> T-score >-2.5	43.6	34.8	27.8	33.4
risk ratio*	1.77	1.86	1.75	2.15
95% CI	1.60-1.96	1.65-2.10	1.34-2.29	1.66-2.76
% T-score < -2.5	4.1	9.4	12.0	2.9
Risk ratio*	3.06	3.02	2.41	5.78
95% CI	2.59-3.63	2.60-3.52	1.77-3.28	3.73-8.96

Risk ratio compared to T-score > -1.0 using proportional hazard model adjusting for age and prior fracture. We conclude that low BMD identified at a peripheral site is a good predictor of fracture risk within one year of the measurement

F078

A Model for Clinical Assessment of Pediatric Bone Mineral Status: Total Body Bone Mineral (TBBM) Adjusted for Total Body Bone Area (TBBA), Body Size, Sex, Black vs. non-Black Ethnicity, Tanner Stage (TS), and Age. M. Horlick,*[1] J. C. Thornton,*[2] R. N. Pierson,*[2] L. S. Levine,*[1] J. Wang.*[2] [1]Pediatrics, Columbia University, New York, NY, USA, [2]Body Composition Unit, St. Luke's - Roosevelt Hospital Center, New York, NY, USA.

Areal bone mineral density (g / cm^2) by dual energy x-ray absorptiometry (DXA) compared to reference standards has established clinical correlates in adults, but the most valid approach for clinical assessment of bone mineral status in children and adolescents is unclear. Our purpose was to develop a predictive model for pediatric bone mineral by DXA that included known relevant variables. Healthy Asian, black, Hispanic, and white subjects from metropolitan New York [n=1125 (535 girls, 590 boys); mean age 12 ± 3.5 years (range 5 – 19)] had measurement of height (cm), weight (kg), TBBM (g) and TBBA (cm^2) by DXA (Lunar DPX), and pubertal status by the criteria of Tanner. TBBM was analyzed as the dependent variable, with TBBA, height, weight, sex, race, age (years), and TS as independent variables. TBBM was transformed to TBBM ^{½} for analysis. The model explained 98% of the variability of TBBM in this population. TBBM ^{½} was related to TBBA, weight, and height (all p<0.001), with the same coefficients for these variables for all subjects. There were main effects of black vs. non-black ethnicity (p<0.001), sex (p<0.001), age (p<0.001), and TS (p=0.03) on TBBM ^{½}. There were also two-way interactions of sex with age and TS with age and sex respectively (p<0.001 – 0.048), and a three-way interaction of TS with sex and age (p<0.001). Prediction Equations for TBBM ^{½}

	TS 1 - 4	TS 5
Girls: non-black	16.77 + 0.34 age + q*	19.39 + 0.19 age + q*
Girls: black	17.39 + 0.34 age + q*	20.01 + 0.19 age + q*
Boys: non-black	18.27 + 0.19 age + q*	14.42 + 0.47 age + q*
Boys: black	18.89 + 0.19 age + q*	15.04 + 0.47 age + q*

*(*q* = 0.018 TBBA + 0.033 weight – 0.071 height)

These equations allow assessment of individual TBBM in relation to peers. The effect of ethnicity is independent of all other variables. When age and TS are included as predictors, the influence of TBBA, weight, and height is uniform across sex and ethnicity. The results imply that TS 5 subjects continue to gain TBBM, with a greater effect of a given age in males than females. Hispanic (largely Dominican) and Asian (Chinese and Korean) subjects in this study did not differ in bone mineral from whites, but this may not be applicable to all Hispanic or Asian groups. These equations are specific for Lunar densitometers, but the principle is applicable to all manufacturers. We suggest that TBBM by DXA adjusted for these variables is more valid than areal bone mineral density for evaluation of bone mineral status in children and adolescents.

F080

Total Hip or Femoral Neck? Differences in WHO Classification in Postmenopausal Women. Influence of Body Weight. J. L. Mansur. Centro de Endoc y Osteop. La Plata, La Plata, Argentina.

Hologic and the last versions of Lunar equipments (IQ,Prodigy) measure the bone mineral density (BMD) of "Total Hip". This region includes femoral neck, trochanter and shaft (or intertrochanter). The ICSBM recommends to use standardized BMD of total hip (TH) instead of femoral neck (FN), since TH correlates with hip fracture in a similar way as FN, but with better precision (as the area of TH is bigger). Actually half of the abstracts presented (ASBMR 2000) are based in TH and the other half in FN, and NOF recommendations use the word "hip" or "proximal femur". Objective: To study characteristics of TH and to know if the WHO suggested classification differs if we choose TH or FN. Patients and methods: 200 postmenopausal women without HRT or treatment were measured with Lunar DPX (software IQ). CV:TH=0.9 %, FN=1.5 % . Results: TH is the addition of 3 regions that measure areas of different sizes: 1) Shaft: 14.37 cm(SD:1.0)=44.6 %; 2) Troch: 12.98 cm(SD: 2.2)=40.3 %; 3) FN: 4.83 cm(SD: 0.4)=15.0 %; (In Hologic systems the size of shaft is bigger and troch is smaller) Total hip: 32.19 cm(SD: 2.7)=100 %. Ward area is included in FN and Troch. We find that 83 % of the patients were in the same category (WHO classification), discordance: 17 %. BMD was lower in FN in 12.5 % and in TH in 4.5 % of the patients. Correlation R of TH: vs FN: 0.93; vs Troc: 0.95; vs Shaft: 0.98; vs Ward: 0.90. The difference in T-Score between FN and TH is 0.21 (-1.03 in FN and -0.82 in TH) and it depends on body weight. Dividing the total of patients in two groups, the difference is 0.14 in thinner group and 0.29 in heavier group. Dividing them in 3 groups, it is 0.06 in the women with less than 60 Kg, 0.20 in the group 60-80 Kg, and 0.40 in women with more than 80 Kg (more body weight = more differences are between FN and TH BMD). Weight and BMD of all regions are correlated (p<0.05): R= Ward: 0.38, FN: 0.40, Shaft: 0.43, TH: 0.48, Troc: 0.58. The R between weight and Troc BMD changes with age: 60 y: 0.70 , > 69 y: 0.77, and also vs TH BMD: < 61 years(y): 0.44, > 60 y: 0.65 , > 69 y: 0.69 Discussion: It is amazing that people use nearly as synonymous the regions FN and TH (or as NOF recommendations "hip" or "femur proximal") when there are regions with a different proportion of trabecular and cortical bone. As when we study a patient the objective is to know the risk of fracture, we propose to observe the T-Score of both regions and to inform the worst. Since 12.5% are in a better category if we use total hip, we suggest not to use this region as a routine. The discordance is bigger in heavier patients, that has a BMD better in Total Hip. The BMD of trochanter correlates with weight and produces this result, and in older women the correlation is higher.

F082

Digital X-Ray Radiogrammetry Predicts Hip Fracture in Elderly Women. R. U. Ashford,[1] A. Dey,*[1] K. Kayan,*[1] H. Delaney,*[1] D. Stabel Nissen,*[2] D. Arnbjerg,*[2] L. Reaney,*[1] C. McGurk,*[1] E. McCloskey.[1] [1]WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, Sheffield, United Kingdom, [2]Pronosco, Vedbaek, Denmark.

We have undertaken a comparative study of Digital X-ray Radiogrammetry (DXR) and DXA measurements to predict incident hip fractures in elderly community-dwelling women in the UK.A case-control analysis was performed within a randomized, double-blind prospective study of hip fracture prevention. The analysis remains blinded to treatment. Cases comprised 44 women aged 75 years or older who sustained an incident hip fracture during a median follow-up of 15 months. 173 women were randomly selected as controls from those who did not suffer a hip fracture. At baseline, DXA measurements of BMD were undertaken at the total non-dominant hip (Hologic QDR4500) and the distal and ultra-distal non-dominant forearm (DTX200, Osteometer, Denmark). Hand radiographs were analysed using the Pronosco X-posure SystemTM to derive DXR-BMD and DXR-MCI (metacarpal cortical index).At baseline, the cases were significantly older and thinner than the non-fracture controls. In addition, the cases had significantly lower forearm BMD (0.29 ± 0.07 vs. 0.36 ± 0.07, p<0.001), total hip BMD (0.64 ± 0.14 vs. 0.78 ± 0.13, p<0.001), DXR BMD (0.44 ± 0.05 vs. 0.41 ± 0.05, p<0.001) and DXR-MCI (0.29 ± 0.05 vs. 0.33 ± 0.05, p<0.001) than controls. All skeletal assessments showed significant gradients of risk (expressed as the odds-ratio per 1SD decrease in measurement) for incident hip fractures (Table). The odds-ratios remained significant after adjusting for age and weight in a forward conditional logistic model. The incidences of fracture in those subjects with DXR BMD and DXR-MCI in the lowest quartile of measurements were 49% and 46% respectively, compared to 12% and 9% in the highest quartiles. For total hip BMD, the incidence in the lowest and highest quartiles were 58% and 5% respectively.

	OR (95%CI)	OR (95%CI) adjusted
Total Hip BMD	3.13 (2.04-4.81)	2.99 (1.95-4.59)
DXR-BMD	1.91 (1.31-2.77)	1.76 (1.21-2.56)
DXR-MCI	2.19 (1.47-3.27)	2.02 (1.34-3.05)
Forearm BMC	2.61 (1.69-4.02)	2.50 (1.60-3.89)
Forearm BMD	2.89 (1.87-4.46)	2.76 (1.78-4.28)

We conclude that hip BMD is the most predictive skeletal assessment for future hip fracture. Measurements of DXR-BMD are also significantly predictive and may have advantages of wider applicability and relatively lower cost. Further analyses are required to compare the clinical utility of techniques with differing costs, sensitivities and specificities

Disclosures: Pronosco,2.

F084

Ten Year Probabilities of Osteoporotic Fractures According to BMD and Diagnostic Thresholds. **J**. A. Kanis,[1] O. Johnell,[2] A. Oden,*[3] A. K. Oglesby,*[4] C. E. DeLaet,*[5] B. Jonsson.*[6] [1]WHO Collaborating Centre for Metabolic Bone Disease, University of Sheffield, Sheffield, United Kingdom, [2]Department of Orthopaedics, Malmo General Hospital, Malmo, Sweden, [3]Consulting Statistician, Gothenberg, Sweden, [4]Global Economic Affairs, Eli Lilly & Company, Indianapolis, IN, USA, [5]Institute for Medical Technology Assessment, Rotterdam, The Netherlands, [6]Centre for Health Economics, Stockholm School of Economics, Stockholm, Sweden.

The objectives of the present study were to estimate ten year probabilities of osteoporotic fracture in men and women according to age and bone mineral density (BMD) at the femoral neck. Probabilities were computed from the hazard of a first hip, distal forearm, proximal humerus and symptomatic vertebral fracture from patient records in Malmo, Sweden and future mortality hazard for each year of age for Sweden using Poisson models.

Fracture probability was computed using the Swedish population and cut-off values for T-scores based on the NHANES III female population. We assumed that the risk of fracture increased with decreasing BMD as assessed by meta-analysis in independent studies. The ten year probability of any fracture was determined from the proportion of individuals fracture free from the age of 45 years. With the exception of forearm fractures in men, 10 year probabilities increased with age and T-score. In the case of hip and spine fractures fracture probabilities for any age and BMD were similar between men and women. Ten year probabilities for hip fracture assessed by BMD at the hip are shown in the Table.

	T-score (men)			T-score (women)		
Age (Years)	**0**	**-1**	**-2**	**0**	**-1**	**-2**
50	0.2	0.6	1.9	0.2	0.4	1.1
60	0.3	0.8	2.2	0.4	1.0	2.7
70	0.6	1.8	4.8	0.7	1.9	5.3
80	1.1	2.9	7.7	0.8	2.4	6.8

The effect of age on risk independently of BMD suggests that intervention thresholds should not be at a fixed T-score but vary according to absolute probabilities. Intervention thresholds based on hip BMD T-scores are similar between sexes.

Disclosures: Eli Lilly & Company Ltd.,2.

F086

Females with Hip Fracture Have Smaller Femora. R. B. Mazess,[1] C. Mautalen.[2] [1]Department of Medical Physics, University of Wisconsin, Madison, WI, USA, [2]Centro de Osteopatias Medicas, Buenos Aires, Argentina.

It has been demonstrated for over a decade that patients with osteoporotic fracture have about 15% (1 SD) lower bone mineral density (BMD in g/cm^2) than age-matched controls at the fracture sites. While fracture patients also are lighter in weight (about 5 kg) than their peers, this accounts for only a few percent of the BMD deficit. Recently studies have shown that patients with spine and/or femur fractures also have smaller bones at these fracture sites. We examined the femur scans by DEXA (Lunar DPX) in 59 females with recent hip fracture (average age 72 years) to ascertain the influence of bone size. These results were compared to controls age 70 to 79 years (n=374). RESULTS: The fracture patients showed about 15% lower BMD than controls. For the femur neck and total femur, the relative deficit in BMC was only slightly greater than that for BMD, but at the trochanter site there was a pronounced BMC deficit (30%) compared to controls. This deficit was obscured using BMD because the bone area was much smaller (15%) in fracture patients. CONCLUSION: The smaller bone size of patients with hip fracture can obscure a larger BMC deficit when clinicians depend on BMD. This is particularly true for the trochanter site. Small size of the trochanter, which is the point of impact on falling, may predispose to fracture. This also suggests that the association of increased hip axis length (i.e., larger trochanter size) with fracture risk may be an epiphenomenon rather than a reflection of inherent bone strength.

	Fracture (n=59)		Controls (n=374)		Fracture % Control	Z-Score
	Mean	**SD**	**Mean**	**SD**		
NECK						
BMD (g/cm^2)	0.646	0.08	0.744	0.10	86.8	-0.95
BMC (g)	2.92	0.56	3.48	0.66	84.0	-0.85
Area (cm^2)	4.54	0.69	4.66	0.45	97.3	-0.28
TROCHANTER						
BMD (g/cm^2)	0.542	0.09	0.658	0.12	82.4	-0.96
BMC (g)	6.42	1.73	9.24	2.81	69.5	-1.01
Area (cm^2)	11.73	1.99	13.88	2.64	84.5	-0.81
TOTAL						
BMD (g/cm^2)	0.666	0.09	0.794	0.12	84.0	-1.03
BMC (g)	20.09	4.31	25.78	5.20	77.9	-1.09
Area (cm^2)	30.50	3.21	32.33	3.02	94.3	-0.61

F088

The Influence of Bone Density Testing and Fractures on Prescriptions of Bone-sparing Medications in Elderly Women in Ontario, Canada: A Population-based Study. S. B. Jaglal,[1] L. Jaakkimainen,*[2] W. McIsaac,*[2] G. Hawker,[2] S. Cadarette,[2] K. Wu.*[3] [1]Rehabilitation Science, University of Toronto, Toronto, ON, Canada, [2]University of Toronto, Toronto, Canada, [3]Institute for Clinical Evaluative Sciences, Toronto, Canada.

The objective of this population-based cohort study was to determine the influence of bone mineral density (BMD) testing and fracture on prescriptions of bone-sparing medications in women 65 years and older in Ontario, Canada. Several studies have reported that having a BMD test influences a woman's decision to begin hormone replacement therapy. Previous studies were not population-based and reported on only one bone sparing medication, estrogen. Rates and types of prescriptions as well as discontinuation rates after the first prescription whether or not a woman had a BMD test in the past year were compared. Whether or not the patient suffered a hip or wrist fracture prior to their prescription of a bone sparing medication was also compared. BMD and non-BMD cohorts and their drug exposure were created by record linkage of the physician claims, drug benefit and hospital discharge abstract databases. In 1996, 23,119 women > 65 years had an incident BMD test (BMD cohort) and 759,361 women did not (non-BMD cohort). Women who had a BMD test were 6 times more likely to fill a prescription for a bone sparing medication (40.5% BMD vs. 6.8% non-BMD). Of those who filled a prescription, women with a BMD were more likely to fill a prescription for a bisphosphonate (58.5%) versus estrogen (29.5%) than were the non-BMD cohort (43.5% bisphosphonate vs. 48.5% estrogen). There was a strong relationship between age and type of bone-sparing drug prescribed. Only 46.7% of women in the 65 to 69 year old age range who had a BMD test were prescribed only a bisphosphonate compared to 86.6% of those over age 85 and older. Similarly for women who did not have a BMD test, the proportion being prescribed a bisphosphonate increased with age from 27.0% to 76.3%. Among women with a BMD test, more than half who were prescribed fluoride (64.0%), salmon calcitonin (62.5%) or an estrogen patch (53.3%) discontinued use after the first prescription compared to 20.2% for a bisphosphonate and 27.7% for oral estrogen. In the subgroup of women who did not have a BMD test (n=51,343) but had a new prescription for a bone-sparing medication only 2,273 (4.4%) had had a previous hip or wrist fracture in the year prior. These data suggest that BMD testing positively influences the decision to initiate drug treatment in older women but not hip or wrist fracture.

F089

Screening Paradox in Osteoporosis. M. van der Klift,*[1] E. Seeman,[2] C. E. D. de Laet,*[1] A. Hofman,*[3] H. A. P. Pols.[4] [1]Institute for Medical Technology Assessment, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands, [2]Austin and Repatriation Med Centre, University of Melbourne, Melbourne, Australia, [3]Department of Epidemiology and Biostatistics, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands, [4]Department of Internal Medicine, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands.

Currently, in fracture prevention only subjects at high risk are identified and treated (the high-risk, case finding approach). The alternative approach for prevention is the population approach, in which the entire population at risk is regarded.The WHO diagnostic criterion of a T-score < -2.5 is often used as a threshold for treatment. To investigate the effectiveness of this high risk versus the population-based approach for fracture prevention, we used the data from the Rotterdam Study. We modelled the effect of a 0.5 and 1.0 SD increase of BMD on fracture incidence. At baseline, bone mineral density was measured at the femoral neck by DEXA (Lunar DPX-L). T-scores were calculated based on reference data of the Dutch population. During an average follow-up of 5.3 years 337 non-vertebral fractures were reported, while during a mean follow-up of 6.3 years 164 subjects suffered at least one incident vertebral fracture (assessed using the McCloskey/Kanis method). Results: see Table.

	Only treat if T-score < -2.5	**Treat all**
N (% of total population)	550 (9.5%)	5794 (100%)
Non-vertebral fractures (% of total)	83 (22.0%)	337 (100%)
Nr of fractures prevented by intervention		
+ 0.5 SD	20 (5.4%)	100 (26.6%)
+ 1.0 SD	25 (6.6%)	169 (44.7%)

These analyses were repeated for subgroups of fractures. For wrist and vertebral fractures, results were in a similar direction. When we analysed hip fractures alone, we found that when we increased the T-score of BMD in the high risk group with 1.0 SD, we would prevent 29 fractures (24.4 % of all hip fractures), whereas if we would increase the BMD with 0.5 SD in the whole population we would prevent 54 fractures (42.9 %).The results of this study clearly indicate that when only a high risk subgroup is treated, only a small reduction of the total burden of fractures within the population will be obtained.

F092

Digital Topological Analysis of in Vivo MR Microimages of Trabecular Bone Reveals Structural Implications of Osteoporosis. F. W. Wehrli,[1] B. R. Gomberg,*[2] P. K. Saha,*[1] H. Song,*[1] S. N. Hwang,*[1] P. J. Snyder.*[3] [1]Radiology, University of Pennsylvania, Philadelphia, PA, USA, [2]Bioengineering, University of Pennsylvania, Philadelphia, PA, USA, [3]Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Osteoporosis is a disease characterized by bone volume loss and architectural deterioration. The majority of work aimed at evaluating the structural implications of the disease has been performed on the basis of stereologic analysis of histomorphometric sections. Only recently noninvasive imaging methods have emerged that provide sufficient resolution to resolve individual trabeculae. Here we apply digital topological analysis (DTA), a technique recently developed in the authors' laboratory, to magnetic resonance (MR) microimages of the radius obtained at 137x137x350µm3 voxel size in a cohort of 79 women of widely varying bone mineral density and vertebral deformity status. DTA allows unambiguous determination of the three-dimensional topology of each voxel in a trabecular bone network. The analysis involves generation of a bone volume fraction map, which is subjected to subvoxel processing to alleviate partial volume blurring, followed by thresholding and skeletonization. The skeletonized images contain only surfaces, profiles, curves and their mutual junctions as the remnants of trabecular plates and rods. DTA parameters were compared with integral bone mineral density in the lumbar spine and femur as well as MR derived bone volume fraction. Vertebral deformities were determined

on the basis of sagittal MR images of the spine with a semiautomatic method and the number of deformities counted. DTA strucural indices were found to be by far the strongest discriminators of subjects with vertebral deformities from those without deformities. Subjects with deformities (N=29) had lower topological surface density ($p<0.0005$) and surface-to-curve ratio (SCR, a measure of the ratio of plate-like to rod-like trabeculae; $p<0.0005$) than those without. Profile interior density,a measure of intact trabecular rods, was also lower in the deformity group ($p<0.0001$). SCR was found to span a range one order of magnitude larger than bone volume fraction, thus greatly amplifying the effect of osteoporotic bone loss. The data in this work provide the first in vivo evidence of the structural implications inherent in postmenopausal osteoporosis accompanying bone loss, i.e. the conversion of trabecular plates to rods and disruption of rods due to repeated osteoclastic resorption.

F095

The Effect of Age on Serum Levels of Osteoprotegerin. S. Kudlacek,*[1] B. Schneider,*[2] P. Pietschmann,[3] W. Woloszczuk,[4] R. Willvonseder.*[5] [1]Medical Department, Barmherzige Brüder, Vienna, Austria, [2]Department of Statistical Medicine, University, Vienna, Austria, [3]Department of Pathophysiology, University, Vienna, Austria, [4]LBI of Experimental Endocrinology, University, Vienna, Austria, [5]Medical Department, Barmherzige Brüder & LBI of Aging Research, Vienna, Austria.

The purpose of the present study was to determine normative serum values of osteoprotegerin (OPG), playing an important role in the negative regultion of osteoclastic bone resorption.Regulation of the balance of osteoblastic and osteoclastic activity is critical for the understanding of normal and pathologic bone cell biology. The recent discovery of osteoprotegerin, a soluble member of the TNF receptor superfamily has provided new insights into the regulation of osteoclastogenesis. We therefore studied serum OPG levels in a large group of healthy Austrian females (n=630) and males (n=394). Participants were selected by chance from a population based register and contacted by a standard written invitation. A questionnaire was used to evaluate predefined exclusion criteria. OPG was detected by a sandwich enzyme immunoassay using a mouse monoclonal antibody and a rabbit polyclonal antibody for detection, and recombinant human OPG as standard material. (Biomedica, Vienna, Austria). The assay detects both monomer and dimeric forms of OPG, including OPG bound to its ligand. All samples were measured in duplicate and averaged, results differing of more than 20% were reassayed. In the whole group of subjects we found a significant increase of serum OPG levels with age. Stepwise regression analysis revealed that gender and age independently influenced OPG levels. Serum OPG levels were higher in females than in males (63,8±45 pg/ml vs. 41,7±30 pg/ml, $p<0.0001$).Our results give evidence of an increase of serum OPG levels with age, moreover OPG was negatively correlated with serum PTH levels ($r = -0.14$, $p<0.001$). We could not find an association of OPG levels with lumbar spine and femoral neck bone mineral density.

F098

The Type I Collagen Fragments ICTP and CTX Reveal Distinct Enzymatic Pathways of Bone Collagen Degradation. M. A. Karsdal,[1] P. Garnero,[2] M. Ferreras,*[1] J. Risteli,[3] P. Qvist,[4] N. Foged,*[1] J. M. Delaissé.[1] [1]OSTEOPRO A/S, Herlev, Denmark, [2]INSERM Research Unit 403 and Synarc, Lyon, France, [3]University of Oulu, Oulu, Finland, [4]Osteometer Biotech, Herlev, Denmark.

Bone resorption generates collagen fragments such as ICTP and CTX, that can be quantified in serum and/or urine by using specific immunoassays. Their concentrations correlate with the levels of bone resorption, but the relative abundance of ICTP and CTX varies according to the type of bone pathology, suggesting that these two fragments are generated through distinct collagenolytic pathways. In this study, we analysed systematically the release of ICTP and CTX from bone collagen by the proteinases believed to participate in the solubilization of bone matrix. First, we incubated collagen isolated from human cortical bone with cathepsin K, MMP-9, MMP-13, MMP-14, or MMP-2, all of which show high collagenolytic activity and are synthesized by osteoclasts or are closely related to resorbing osteoclasts. All incubations were performed for the same time and at the same concentration of active enzyme as determined by active site titration. We found that ICTP was efficiently released by MMP-2 and MMP-13, and to a lesser extent by MMP-14. In contrast, ICTP was not detected upon incubations of collagen with MMP-9 or cathepsin K alone, although large amounts of ICTP were generated after successive incubations with cathepsin K and MMP-9, respectively. CTX was efficiently released by cathepsin K, and to a small extent by MMPs, but the release of CTX was increased 2-fold when incubating cathepsin K-generated fragments with MMP-9. Second, we cultured mouse tibiae and calvariae with PTH to induce resorption, and tested the effect of inhibitors of MMPs or cathepsin K on the release of ICTP, CTX, and Ca in their culture media. In accordance with our test tube assays the release of ICTP was completely prevented by MMP inhibitors, but rather increased in the presence of cathepsin K inhibitor. The latter supports the view that MMPs compensate for lack of cathepsin K in pycnodysostosis and cathespin K knockouts. The release of CTX was partially inhibited when either cathepsin K or MMPs were inactivated, and completely inhibited when both were inactivated. The release of Ca was diminished in the presence of either type of proteinase inhibitors, thus confirming published data. In conclusion, we have shown both in test tubes and in native bone, that ICTP and CTX are generated by different collagenolytic pathways. These findings explain why these two markers may discriminate between different bone pathologies, and strengthen the view that specific antagonists of collagenolysis could be used selectively against given bone diseases.

F101

Comparison of Three Bone Ultrasounds for Determining Hip Fracture Odds-Ratios - Results of the Semof Study. M. Krieg, J. Cornuz,* P. Burckhardt and the Semof Study Group. Internal Medicine, University Hospital, Lausanne, Switzerland.

Background : Bone ultrasound (QUS) is known to discriminate subjects who suffered of fractures and subjects with no fracture history. QUS is predictive of fractures. The Swiss Evaluation of the Methods of Measurement of Osteoporotic Fracture Risk (SEMOF) study is a prospective multicenter study which compares 3 QUS devices for the assessment of hip fracture risk in a population-based sample of elderly Swiss women. The aim of this cross-sectional analysis was to compare the 3 QUS for determining hip fracture odds ratios (OR).Method : Among the 7702 women aged ±75.3 yrs (70 - 87) assessed during the inclusion phase of the SEMOF study. The history of previous hip fractures was obtained with a specific mailed questionnaire. 3696 had no fracture whereas 93 reported a low trauma hip fracture after 50 yrs. All the participants were measured with the 3 QUS: Achilles+ [Stiffness index (SI)], Sahara [Quantitative ultrasound index (QUI)], and DBM sonic 1200 [Amplitude-dependent speed of sound (ADSOS)]. New parameters, such as Speed of sound (SOS), time frame (TF), ultrasound bone profile index (UBPI) were calculated from the DBM data.Results : Mean QUS value (± SD) were adjusted for age and BMI, and ORs were calculated for 1 SD decrease, with 95% CI (***$p<0.001$, **$p<0.01$, *$p<0.05$ compared to non fractured group).Discussion : All the ultrasound parameters could discriminate elderly women with hip fracture from women with no fracture history. In this population of elderly women, discrimination was significantly higher with measurements on the heel than on the phalanges. This could be due to the high prevalence of osteoarthritis of the hands at this age, which could influence the results.

F103

Association of Quantitative Ultrasound Parameters and Bone Density with Osteoporotic Vertebral Deformities in a Population Based Sample: The OPUS Study. C. C. Glüer,[1] R. Eastell,[2] D. M. Reid,[3] F. Alenfeld,[4] S. Kolta,[5] R. Barkmann,[1] J. Clowes,[2] A. Stewart,[3] C. Roux,[5] D. Felsenberg.[4] [1]University Hospital Kiel, Kiel, Germany, [2]University of Sheffield, Sheffield, United Kingdom, [3]University of Aberdeen, Aberdeen, United Kingdom, [4]Free University Berlin, Berlin, Germany, [5]René Descartes University, Paris, France.

It is unclear whether the many different methods for measuring quantitative ultrasound (QUS) of bone have similar associations with osteoporotic fractures. To evaluate this in a systematic approach, we initiated the Osteoporosis & Ultrasound (OPUS) study. In 5 European cities we recruited a population-based sample of 2206 women (ages 55 to 80 years). Spinal radiographs were examined for vertebral deformity at a single centre (using a combined morphometric and visual approach). All subjects had dual-x-ray absorptiometry (DXA) of the lumbar spine and the total hip and QUS of the heel on 4 devices (Lunar Achilles+, Osteometer DTU-one, Quidel/Metra QUS-2, and DMS UBIS 5000) and of the finger on one device (IGEA DBM Sonic BP). We examined the association of these measurements with vertebral deformity by calculating standardised odds ratios (sOR, per one SD decrease of population variance), OR adjusted for age, and compared devices by calculating the area under the receiver operating characteristic (ROC) curve. This last analysis was performed in the 1236 women who had a complete set of measurements. We identified vertebral deformities in 362 women (16.4%).

Variable	n (for OR)	SOR	Age-adj. sOR	Area ROC (n=1236)
DXA spine BMD	2189	1.7	1.6 (1.4-1.8)	0.683
DXA hip BMD	2206	1.9	1.7 (1.5-1.9)	0.680
Achilles BUA	1788	1.6	1.4 (1.3-1.7)	0.660
DTU-one BUA	2170	1.5	1.3 (1.2-1.5)	0.660
UBIS 5000 BUA	2024	1.6	1.4 (1.3-1.6)	0.658
QUS-2 BUA	1477	1.7	1.5 (1.3-1.8)	0.664
Achilles SOS	1788	1.8	1.5 (1.3-1.8)	0.680
DTU-one SOS	2170	1.6	1.4 (1.2-1.6)	0.673
UBIS 5000 SOS	2024	1.7	1.5 (1.3-1.7)	0.673
DBM BP AD-SoS	2144	1.4	1.2 (1.1-1.4)	0.658
DBM BP UBPI	2132	1.5	1.3 (1.1-1.5)	0.658

The SOS measurements of the heel had ROC areas similar to DXA measurements, and larger than BUA of the heel using the same device ($p<0.05$); finger measurements had significantly lower ROC areas compared to DXA. Conclusion: SOS of the calcaneus demonstrated a discriminatory power comparable to DXA. Small but significant differences in performance were observed, but all QUS variables showed significant associations with deformities.

Disclosures: Igea,2; Quidel,2; Osteometer,2; DMS,2.

F107

Genetic Dissection of Breaking Strength. R. F. Klein,[1] M. Shea,[2] M. Serang,*[2] R. J. Turner,*[1] J. K. Belknap,*[1] E. S. Orwoll.[1] [1]Bone and Mineral Unit, Oregon Health Sciences University, Portland, OR, USA, [2]Department of Orthopaedics, Oregon Health Sciences University, Portland, OR, USA.

An osteoporotic fracture represents a structural failure of bone whereby the forces applied to the bone exceed its load-bearing capacity. Quantitative trait loci (QTLs) have been identified that strongly influence bone mineral density (BMD) and femoral size in mice. However, additional properties (e.g., spatial distribution, micro-architecture, collagen content and fiber orientation, etc.) also influence the mechanical behavior of bone. We hypothesized that some of the genes regulating other important aspects of skeletal

integrity would be distinct from those that determine BMD. Using 16-week-old male mice from a panel of 18 BXD recombinant inbred (RI) strains, derived from a cross between C57BL/6 and DBA/2 progenitors, we measured mid-diaphyseal femoral cross-sectional moment of inertia (MOI) and failure load (FL) in 3-point bending and calculated Young's modulus (a measure of the intrinsic stiffness of the femoral bone tissue). The distribution of values among the strains for each of these biomechanical measures clearly indicated the presence of strong genetic influences, with an estimated heritability of 76%, 53% and 28% for femoral MOI, FL and modulus, respectively. Failure load, a summary measure of the ability of the femoral shaft to resist fracture, was strongly related to femoral MOI (r=0.62; p<5x10-20), but there was no correlation between FL and modulus (r=0.06; p=0.44). However, there was a strong inverse relationship between modulus and MOI (r=-0.36; p<2x10-6). Using a p <= 0.01 (two-tailed, single test) alpha level of significance, QTL analysis of the BXD RI strain series provisionally identified 7 chromosomal sites linked to MOI, 8 sites linked to FL and 9 sites linked to modulus. Only one provisional QTL (located on distal chromosome 4) was associated with all three phenotypes, and in each pair-wise comparison two additional distinct loci were shared: MOI and FL (chr 11 & 12); FL and modulus (distal chr 1 & 13); MOI and modulus (proximal chr 1 & 9). Two FL QTLs (chr 5 & 8) were not detected in analyses of either MOI or modulus, suggesting the presence of genetic influences on FL that were independent of these two major indices of femoral geometric and material properties. These results provide evidence of strong genetic contributions to the major determinants of bone strength in this mouse model. The finding of unique FL-related QTLs in addition to distinct QTLs for bone geometry (MOI) and intrinsic material properties (modulus) that appear to independently influence FL suggests the presence of a network of genes that are essential to skeletal integrity.

Disclosures: Merck,8; Lilly,8; Aventis,8; Procter & Gamble,8.

F109

Differential Gene Expression Between a Congenic Strain That Contains a Quantitative Trait Loci (QTL) of High Bone Density from CAST/EiJ and Its Wild Type Strain C57BL/6J. W. Gu,[1] X. Li,[1] K. H. W. Lau,[1] B. Edderkaoui,[1] L. R. Donahue,*[1] C. J. Rosen,*[2] W. G. Beamer,[3] K. L. Shultz,*[3] A. K. Srivastava,[1] S. Mohan,[1] D. J. Baylink.[1] [1]MDC, Pettis VAMC, Loma Linda, CA, USA, [2]St. Joseph's Hospital, Bethesda, ME, USA, [3]The Jackson Lab, Bar Harbor, ME, USA.

Peak bone density is an important determining factor of future osteoporosis risk. We previously identified a QTL that contributes significantly to high bone density (30% of the variation of bone density) on mouse chromosome 1 from a cross between C57BL/6J (B6) and CAST/EiJ (CAST) mouse strains. We then generated a congenic strain, B6.CAST-1, in which this QTL region was transferred from CAST to the B6 background. Prior to this study, we constructed a BAC contig for the QTL region. In this study, cDNA microarray analysis was conducted by Incyte Genomic Systems on 8,734 gene accessions using mRNA from mouse femurs (free of marrow) of both the congenic and B6 strains to test two hypotheses: 1) microarray analysis will identify candidate genes for bone density; and 2) by comparing the gene expression profile outside of the QTL region between B6 and the congenic mice, we can better characterize the molecular phenotypic mechanisms that contribute to the high bone density QTL. Although the 8,734 gene accessions on microarray chips were derived from non-bone tissues, we found that approximately 60% of them were expressed in the femur of B6 mice. Comparison of expression levels of genes between B6 and the congenic mice (ANOVA) indicated that the gene expression of the two mouse strains had a similar pattern (P=0.468). Regarding hypothesis 1, out of a total of 19 known genes and 85 ESTs showing at least a 2-fold (significant) difference between these two strains, we found only one gene and two ESTs (i.e., candidate genes) that are known to be present in the QTL region. Regarding hypothesis 2, by comparing the gene expression profile outside of the QTL region, we found that expression levels of genes related to bone formation were lower in congenic than in B6 mice: Osteoblast specific factor 2 (OSF2) and procollagen, type I, alpha 1, were expressed between 2-3 times lower in congenics than in B6 at 6 and 14 weeks of age. Consistent with these microarray data, the serum alkaline phosphatase activity in the congenic was only 73% of that of B6 mice (P<0.01). Additionally, expression levels ofa potential osteoclast inhibitor (an EST sequence similar to SUR8) were significantly greater in the congenic than in the B6 mice. In conclusion, microarray analysis is useful for: 1) the identification of candidate genes of bone density; and 2) the characterization of molecular mechanisms of the QTL locus that regulate bone density.

F112

Testing In Vivo Effects of a Genetic Determinant of IGF-I Expression on Bone Phenotypes In a New Congenic Mouse. C. Ackert,[1] L. Donahue,*[1] C. H. Turner,[2] R. T. Turner,*[3] M. B. Bouxsein,[4] R. Muller,*[4] W. G. Beamer,*[1] K. L. Schultz,*[1] C. J. Rosen.[5] [1]The Jackson Laboratory, Bar Harbor, ME, USA, [2]Indiana University, Indianopolis, IN, USA, [3]Mayo Clinic, Rochester, MN, USA, [4]Beth Israel Deaconess Medical Center, Boston, MA, USA, [5]St. Joseph Hospital, Bangor, ME, USA.

IGF-I is an important growth factor for the skeleton. However its regulation is complex and, for the most part, heritable. We previously demonstrated that changes in both serum and skeletal IGF-I are related to strain specific differences in mouse BMD. We also identified several quantitative trait loci (QTLs) for serum IGF-I in F2 mice derived from progenitor crosses between C3H (high density high IGF-I) and B6 (low density, low IGF-I) strains. The most powerful QTL (LOD~ 9.0) accounts for >6% of the variance in serum IGF-I (*Igfls1*) and is located on the distal third of Chromosome 6. Paradoxically, IGF-I levels are lower in F_2 mice with C3 alleles in the Chr6 QTL. To test the size effect for *IgflsI* on both serum IGF-I and skeletal morphology, we initially generated a congenic mouse (B6C3H-6 c3/c3) by placing the Chr 6 QTL from C3H on a B6 background over 6 generations(N6F2). These mice are > 98% B6 in respect to their background genome, but maintain C3H alleles from *Igfls1*. We have now completed congenic construction (N10F2) for both males and females of this strain. IGF-I and skeletal phenotypes in N6 and N10F2 congenics were compared with age and gender matched B6. Serum IGF-I levels in male (M) and female (F) N6 and N10F2 congenics at 6 and 8 weeks of age were reduced by ~25%(p<0.01) compared to progenitors. There was a 40 % reduction in secreted IGF-I in conditioned media (CM) of calvarial osteoblasts (OB)from N6F2 congenics vs B6. F but not M congenics showed reduced vertebral and femoral BMD by pQCT compared to B6 (p<0.05). Congenics also had lower trabecular BMD when measured by uCT (vertebrae) and histomorphometry (proximal tibia) (p<0.01). Medial lateral dimensions of the femur were reduced in N6F2 B6C3H-6 c3/c3 compared to B6 (p<0.001).Femoral strength and cross-sectional moment of inertia were also significantly reduced in the congenics(p<0.01)compared to B6. In sum, we identified and tested, in vivo, a major genetic regulatory determinant for serum IGF-I. This factor has a profound effect on several skeletal phenotypes, which may be gender specific. We conclude that the congenic on Chr 6 will be a useful model for defining the regulation of serum and skeletal IGF-I, as well as testing the effects of genetic determinants on parameters such as BMD, skeletal morphology and strength.

F114

Genetic Relationships Among Bone Phenotypes Regulated by Sets of Quantitative Trait Loci (QTL) in B6C3F2 Mice. W. G. Beamer,[1] S. Sen,*[1] G. A. Churchill,*[1] C. J. Rosen,[1] L. R. Donahue,*[1] K. L. Shultz,[1] J. O. Mytar,*[1] C. H. Turner,[2] R. Muller,*[3] T. Uchiyama,*[3] M. L. Bouxsein.[3] [1]The Jackson Laboratory, Bar Harbor, ME, USA, [2]Indiana University, Indianapolis, IN, USA, [3]Beth Israel Medical Center, Boston, MA, USA.

It is axiomatic that skeletal properties of mineral density, size, shape, architecture, and bone quality are functions of both heritable and environmental determinants, however the complexity of the genetic regulation is just beginning to be elucidated. To gain insights about skeletal genetic regulation, we have analyzed data gathered on 36 physical, structural and functional phenotypes from femora and lumbar vertebrae of 4 month old adult B6C3F2 intercross progeny of C57BL/6J (B6) and C3H/HeJ (C3H) progenitor strain mice. Phenotypes were measured by pQCT, MicroCT 20, PIXImus, materials testing machines, and digital caliper instruments. The genome wide scans were based on genotyping of DNA obtained from ~1000 females for 107 markers across all 19 autosomes at average genetic distance of 14-15 centiMorgans. QTLs were identified by regressions analyses of phenotype on genotype using computer programs MapManagerQT (K Manly) and Pseudogene (G Churchill). Chromosomal markers with LOD scores greater than 2.8 (multiple test correction by permutation tests) were considered as suggestive, while LODs of 4.3 or better were considered as significant linkage with a phenotype. We found that every bone phenotype evaluated has complex genetic regulation dependent upon 2 to 9 QTLs, with LOD scores of 3.0 to 20.5. The phenotypic variance accounted for by individual QTLs ranged from 1 to 9%. Assessment of QTL x QTL interactions has been markedly variable, depending on phenotype being evaluated. As would be expected, some of the femoral and vertebral phenotypes revealed both shared and unique QTLs. Within the set of QTLs for each phenotype, the net phenotypic difference between the progenitor strains is the consequence of QTLs with major and minor effects. We also found that for each bone site and phenotype, either progenitor strain could contribute a QTL with alleles that increased the phenotype evaluated in the F2 progeny. The effects of the progenitor alleles for a given QTL on each phenotype proved to be either dominant-recessive or additive in action. Congenic strains developed from backcrossing C3H chromosome segments onto the B6 background has shown that QTL effects on strength, structure, or density are detectable. These data demonstrate that the complex genetic regulation of bone can be decomposed into basic, assessable elements that will be amenable to future molecular medicine diagnostic and therapeutic actions.

F117

Linkage of Albers-Schönberg Disease (Autosomal Dominant Osteopetrosis Type II) to Chromosome 16p13.3. O. D. Bénichou,*[1] E. Cleiren,*[2] J. Gram,[3] J. Bollerslev,[4] M. de Vernejoul,[1] W. Van Hul.[2] [1]INSERM U 349, Hôpital Lariboisière, Paris, France, [2]Medical Genetics, University of Antwerp, Antwerp, Belgium, [3]Medicine, Ribe County Hospital, Esjberg, Denmark, [4]Endocrinology, Rikshospitalet, Oslo, Norway.

The osteopetroses are a heterogeneous group of conditions characterized by an increased bone density due to impaired bone resorption. Besides the at least two autosomal recessive types, two autosomal dominant forms of osteopetrosis are described, differentiated by clinical and radiological signs. Autosomal dominant osteopetrosis type II (ADOII), also known as Albers-Schönberg disease, is characterized by sclerosis, predominantly involving spine (vertebral endplate thickening, « Rugger Jersey spine »), pelvis (« bone within bone » sign), and the skull base. An increased fracture rate can be observed in these patients. By linkage analysis, the presence of a gene causing ADOII was previously suggested for chromosome 1p21. However, analysis of extra ADOII families indicated genetic heterogeneity within ADOII, with the chromosome 1p21 locus only being a minor locus.We now performed a genome linkage scan in an extended French ADOII-family allowing us to localize an ADOII gene in the chromosomal region 16p13.3. Analysis of microsatellite markers in five extra ADO II families could not exclude this chromosomal region in any of these. A maximum LOD score of +12.70 was generated with marker D16S3027 at a recombination fraction of 0. Based on the key recombinants in the families, a candidate region of 8.4 cM could be delineated flanked by markers D16S521 on distal and D16S423 on proximal side. Surprisingly, one of the families analysed is the Danish family previously suggested to be linked to chromosome 1p21. This family can clearly not be excluded from the chromosome 16p13.3 region and generates a maximum LOD score of +4.21 (at q = 0) with marker D16S3027. Since at present no other ADOII family has been proven to be linked to chromosome 1p21, the most likely localization of the disease causing gene in this family is definitely on chromosome 16p13.3 reopening the possibility that ADOII is genetically homogeneous caused by one gene on chromosome 16p13.3.

F119

Identification of Gene Loci Regulating Bone Size and Strength in Mice. J. E. Wergedal,[1] C. Ackert,*[2] M. H. C. Sheng,[1] W. G. Beamer,[2] D. J. Baylink.[1] [1]Musculoskeletal Disease Center, JL Pettis VA Medical Center, Loma Linda, CA, USA, [2]The Jackson Lab, Bar Harbor, ME, USA.

Bone structural properties are a very important determinant of bone strength. To determine if the genes regulating bone size could be determined by genetic studies in mice, we surveyed outer dimensions of the femur in 29 inbred mouse strains by peripheral quantitative computed tomography (pQCT) analysis. Significant variations between strains were found in all femur parameters. These data provided evidence that bone size is a heritable trait in inbred strains of mice (broad-sense heritability, 94%) and that mice are a suitable model for identifying the genes regulating bone size. There were strong correlation's (0.7 to 0.9) between parameters and body weight consistent with the hypothesis that genes regulating body size play an important role in determining bone dimensions. However, correction of midshaft size for body size by dividing by femur length did not remove all of the variation. Therefore some genes regulate bone size independent of overall body size or femur length. To investigate genes regulating bone size, we selected two inbred strains of mice (NZB/B1nJ and RF/J) that differ in femur midshaft size but not femur length. Total midshaft area was 40±2% higher in RF/J than NZB/B1nJ mice. Femur breaking strength was 85% higher in RF/J mice than in NZB/B1nJ mice. To identify genes responsible for regulating bone size, genetic analyses have been conducted in a cohort of F2 mice derived from intercross matings of (NZB/B1nJ x RF/J)F1. Femurs were isolated from 366 10-week-old females. Bone size data were obtained by pQCT and genotype data were obtained by polymerase chain reaction assays for polymorphic markers carried in genomic DNA of each mouse. Genome wide scans for co-segregation of genetic marker data with high or low bone size revealed loci on four different chromosomes. Loci on chromosome 7 and 11 achieved highly significant (LOD score of 7.5 and 8 respectively) linkage with bone size. Secondary loci were found on chromosome 12 and 15. Two strong QTL's were also identified for body weight (Chr 10, LOD 4.6: Chr12, LOD 8.0) These QTL's were different from the bone size QTL's. Femurs are currently being assessed for breaking strength to determine if strength loci overlap with size loci. Candidate genes include PTH and calcitonin on chromosome 7 and growth hormone on chromosome 11. In summary: 1) inbred mice show large variations in bone size independent of body size, 2) genetic analysis has identified four QTL's for bone size.

F122

Partitioning the Genetic Regulation of Bone Mineral Density: Loci Independent of Growth Hormone and IGF-I Regulation. L. R. Donahue,[1] W. G. Beamer,[1] M. L. Bouxsein,[2] R. Muller,[2] C. H. Turner,[3] K. L. Shultz,*[1] C. J. Rosen.*[1] [1]The Jackson Laboratory, Bar Harbor, ME, USA, [2]Beth Israel Medical Center, Boston, MA, USA, [3]Indiana University, Indianapolis, IN, USA.

We have demonstrated that areal BMD (DEXA, PIXImus, GE Lunar) is a polygenic trait and have discovered 4 loci (QTLs on Chrs 1, 4, 14, 18) for vertebral BMD utilizing F2s from C57BL/6J (B6), X C3H/HeJ (C3H) cross. To determine if these BMD QTLs were also QTLs for insulin-like growth factor-I (IGF-I), a growth factor for bone known to differ between B6 and C3H, we did an analysis for IGF-I in these F2s. The 4 IGF-I QTLs (Chrs 6,10,11, 15) identified were different from the BMD QTLs. To decompose the interaction between BMD and IGF-I, we made a model utilizing a spontaneous mutation in the mouse known as *little*. In C57BL/6J-*lit/lit* mice, GH is not detectable in circulation, IGF-I is fixed at low levels, and BMD, skeletal size, and body mass are reduced. We developed a new congenic strain by transferring the *little* mutation from the low BMD B6 to the high BMD C3H background. The new C3.B6-*lit/lit* mice have the same small body and skeletal size, but higher whole body areal BMD than the original B6-*lit/lit* mice. Size and whole body BMD in *lit/lit* strains at 4 months:

Strain	Sex	Body Wt (g)	Femur Lgth (mm)	BMD (g/cm^2)
B6/lit/lit	Male	15.0±1.0	12.0±0.1	.031±.001*
C3.B6-*lit/lit*	Male	16.5±1.2	12.4±0.1	.034±.001
B6-*lit/lit*	Female	13.0±0.6	12.0±0.1	.029±.001*
C3.B6-*lit/lit*	Female	12.0±0.4	12.3±0.1	.035±.001

(n=6-7); *p<.02; comparison between strains within sex

We have developed a model that can be used to locate BMD genes that are independent of GH/IGF-I and size effects. We have preliminary whole body areal BMD data from 457 adult F2 mice using a cross between B6-*lit/lit* and C3.B6-*lit/lit*, where all mice are homozygous *little* and segregating genes from the two background strains. We find a Gausian distribution of BMD values ranging from .027 to .040, indicating that the regulation of BMD in this population is polygenic and that it will be possible to find loci for BMD that are GH/IGF-I independent. By comparing the loci found in this homozygous *little* cross with the loci found in the B6C3F2 cross with normal GH/IGF-I values, we will be able to partition the BMD phenotype into GH/IGF-I dependent and independent loci.

F125

Osteoclast Formation in Transgenic Mice Overexpressing Fibroblast Growth Factor-2. T. Sobue,[1] X. Zhang,[1] L. G. Raisz,[1] J. D. Coffin,*[2] M. M. Hurley.[1] [1]Endocrinology, University of Connecticut Health Center, Farmington, CT, USA, [2]Pharmaceutical Science, University of Montana, Missoula, MT, USA.

Basic fibroblast growth factor (FGF-2) is an important regulator of bone formation and resorption as well as a potent mitogen for osteoblasts and stromal cells. We previously reported that overexpression of FGF-2 in transgenic (TgFGF2) mice caused premature and enhanced apoptosis in chondrocytes. In other studies, we showed that FGF-2 also stimulated osteoclast formation. In the present study, we investigated whether overexpression of FGF-2 would enhance osteoclast (OCL) formation as measured by the formation of multinucleated tartrate resistant acid phosphatase positive cells in response to 1,25(OH)2D3, PTH and PGE2 in bone marrow cultures using 6-8 week old non transgenic (NTg) and TgFGF2 litter mates. Few OCL formed in vehicle treated bone marrow cultures from NTg and TgFGF2 mice. Treatment with 1,25(OH)2D3, PTH or PGE2 stimulated OCL formation in both NTg and TgFGF2 bone marrow cultures but there were significantly fewer OCL formed in response to these agents in bone marrow cultures from TgFGF2 mice. In contrast, treatment with exogenous FGF-2 (0.01-10 nM) caused a significantly greater increase in OCL formation in bone marrow cultures from TgFGF2 mice than NTg mice. Differences were not due to the number of OCL precursors since similar numbers of OCL formed in bone marrow cultures from NTg mice and TgFGF2 mice treated with RANKL (10 ng/ml). Earlier reports indicated that FGF receptor-1 (FGFR1) is important in FGF-2 induced OCL formation. We therefore compared the expression of FGFR1, as well as RANKL and M-CSF, mRNAs in bone marrow stromal cell cultures from both genotypes. Northern blot analysis revealed that basal expression of FGFR1 was decreased by 60 % in TgFGF2 mice relative to NTg mice. Twenty four h of treatment with exogenous FGF-2 increased FGFR1 mRNA expression 280% in TgFGF2 bone marrow stromal cells but only 66% in NTg stromal cells. Basal expression of RANKL, M-CSF and OPG mRNA were similar in bone marrow stromal cell cultures from both genotypes. However, FGF-2 (10 nM) caused a greater increase in mRNAs for RANKL (386% vs 111%) and M-CSF (182% vs 114%) in bone marrow stromal cell cultures from TgFGF2 mice compared to NTg mice. We conclude that overexpression of FGF-2 enhances osteoclast formation in response to exogenous FGF-2 but not to other stimulators of resorption. We speculate that endogenous overexpression of FGF-2 may enhance OCL formation by increasing the sensitivity to exogenous FGF-2 including its ability to increase FGFR1.

F128

In Vivo Administration of Interleukin-1 Enhances Osteoblast Proliferation and Differentiation in Ex-Vivo Bone Marrow Cultures From Fgf-2 Null Mice but not Osteoclast Formation in Calvariae of Fgf2 Null Mice. T. Sobue,[1] X. Zhang,[1] J. D. Coffin,*[2] M. M. Hurley.[1] [1]University of Connecticut Health Center, Farmington, CT, USA, [2]University of Montana, Missoula, MT, USA.

The cytokines Interleukin-1 (IL-1) and fibroblast growth factor (FGF-2) are potent stimulators of bone resorption and formation. We reported that IL-1 increased Fgf2 mRNA and protein expression in normal human osteoblastic cells as well as osteoblasts from patients with osteoarthritis. To assess the physiological relevance of IL-1 regulation of FGF-2, we examined the effect of 3 days of injection of vehicle or IL-1 (2.5 µg) over the calvariae of wild type (Fgf2+/+), heterozygote (Fgf2+/-) and mice with disruption of the Fgf2 gene (Fgf2-/-). Mice were sacrificed one day after the last injection and histomorphometric analysis of calvariae was performed including tartrate resistant acid phosphatase (TRAP) staining for multinucleated osteoclasts (OCLs). IL-1 significantly increased osteoclast surface/bone area (3850 vs 1469 µm/µm^2, p<0.05) and osteoclast number (79 vs 37/ mm^2 bone area, p<0.05) in Fgf2+/+ mice relative to vehicle treated calvariae. The response was similar in heterozygotes. In contrast, there were no differences in osteoclast surface/ bone area (1637 vs 1433 µm/µm^2) or osteoclast number (36 vs 49/ mm^2 bone area) between IL-1 and vehicle in Fgf2-/- mice. In these experiments, long bones were flushed to measure bone formation ex vivo in marrow stromal cell cultures from vehicle and IL-1 treated Fgf2+/+, Fgf2+/- and Fgf2-/- mice. Cells were plated at 3x10^6 cells/well and cultured in differentiation media for 7, 14 and 21 days. Alkaline phosphatase positive (AP) colonies were 60% decreased in cultures from Fgf2-/- mice relative to Fgf2+/+ or Fgf2+/- mice. AP colony number was increased in IL-1 treated marrow cultures from all 3 genotypes at 7, 14 and 21 days. However, the absolute number of AP colonies were lower in cultures from Fgf2-/- mice. At 21 days, there was a greater increase in mineralization as determined by von-Kossa and Alizarin-red staining in bone marrow cultures from IL-1 treated mice from all 3 genotypes relative to vehicle treated mice. On Northern analysis, type1 collagen (COL1A1) and osteocalcin (OC) mRNA levels were reduced in vehicle treated cultures from Fgf2+/- and Fgf2-/- mice. However, IL-1 increased COL1A1 and OC mRNAs in all genotypes. We conclude that endogenous FGF-2 is important in IL-1 induced bone resorption in vivo. However, despite low basal levels in cultures from animals with no FGF-2 expression, IL-1 can increase colony formation and differentiation in vitro. We speculate that the different interactions between IL-1 and FGF-2 may be important in pathologic responses in bone.

F131

Molecular Cloning and Characterization of FGFR2 in Osteoblast-like Cells Derived from an Apert Syndrome Patient. Y. Tanimoto,*[1] M. Yokozeki,*[1] K. Matsumoto,[2] H. Nakanishi,*[2] K. Hiura,[1] K. Moriyama.[1] [1]Department of Orthodontics, School of Dentistry, University of Tokushima, Tokushima, Japan, [2]Section of Plastic and Reconstructive Surgery, University Hospital, University of Tokushima, Tokushima, Japan.

Apert syndrome characterized by craniosynostosis, hypertelorism, ocular proptosis and syndactyly is pathogenically associated with point mutations of fibroblast growth factor receptor (FGFR) 2 (S252W, P253R). We previously reported the high expression of osteoblastic marker genes and the acceleration of in vitro mineralization of osteoblast-like cells derived from two independent Apert syndrome patients (S252W). Between two major isoforms of FGFR2, FGFR2-IIIb and FGFR2-IIIc, that are created by alternative splicing in the third Ig-like domain (IgIII), FGFR2-IIIc is exclusively expressed and play critical roles in differentiation and function of mesenchymal cells. Therefore, in order to verify FGFR2 isoforms expressed in osteoblast-like cells derived from Apert syndrome patient (ApOB), RT-PCR was performed under appropriate informed consent by using a pair of the specific primers including the start and stop codons. It is proved from sequencing results that two clones isolated from ApOB were a complete form of FGFR2-IIIc gene with S252W mutation, and that fourteen clones were a variant of FGFR2-IIIc gene without mutation which lacked exon 3 encoding the first Ig-like domain (FGFR2-IIIcDIgI). Nine clones isolated

from osteoblast-like cells of a non-syndromic polydactyly patient (HOB) were all identified as FGFR2-IIIcDIgI without mutation. These clones contained the sequences relevant to the signal peptide, the acidic box, the second and the third Ig-like domains, the transmembrane domain, and the complete carboxyl termini. FGFR2-IIIcDIgI was expressed in ApOB and HOB in a predominant manner as compared with FGFR2-IIIc. Furthermore, FGFR2-IIIcDIgI was ubiquitously expressed in human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas tissues, whereas FGFR2-IIIc expression was restricted in human liver, skeletal muscle and pancreas tissues. These results suggest FGFR2-IIIcDIgI may be the most commonly expressed variant in human osteoblasts, and may be implicated in the osteoblastic phenotype of ApOB and HOB.

F134

Ovariectomy Induces Bone Loss by Increasing the Number of TNF Producing T cells. Y. Gao,[1] C. Roggia,[2] S. Cenci,[1] G. Toraldo,[3] M. N. Weitzmann,[1] J. Kindle,[1] R. Pacifici.[2] [1]Washington University School of Medicine, Saint Louis, MO, USA, [2]Washington University School of medicine, Saint Louis, MO, USA, [3]Washington university School of medicine, Saint Louis, MO, USA, [2]Washington University School of medicine, Saint louis, MO, USA.

It is now recognized that one of the mechanisms by which ovariectomy (OVX) induces bone loss is by increased TNF production by bone marrow T cells, but the mechanism of this phenomenon is unknown. In the present study, we utilized freshly isolated bone marrow from OVX and estrogen replete mice to investigate how estrogen regulates T cell TNF production in vivo using immunohistochemistry, flow cytometry and microarray (gene chip) analysis. Immunohistochemistry and quantitative flow cytometry analysis showed that average number of T cells (CD90 or CD3 positive cells) was 5 fold higher in OVX than in estrogen replete mice. OVX also caused a 2.5 fold increase in the total number of bone marrow cells, then the number of T cells, expressed as a percentage of total bone marrow cells, was 2 fold higher in OVX than in estrogen replete mice. Further studies revealed that OVX increased T cell numbers by stimulating T cell proliferation by 3 fold, as measured by thymidine incorporation. Double staining studies revealed that OVX increased by 2 fold the number of CD3+/TNF+ and CD90+/TNF+ cells (expressed as a percentage of total bone marrow cells), thus confirming that OVX increases the number of TNF producing T cells in bone marrow. However, the percentage of TNF producing T cells, expressed as a percentage of total T cells, was unchanged. Furthermore, the amount of TNF protein produced by individual T cells from OVX mice was identical to that produced by individual T cells from estrogen replete mice, thus demonstrating that estrogen does not upregulate TNF production per cell. Identical results were found in spleen cells, thus establishing that the regulatory effects of estrogen on cells are not confined to the bone marrow. Microarray (Affymetrix) analysis revealed equal TNF mRNA level in T cells from OVX and estrogen replete mice, a finding which confirms that estrogen does not directly regulate TNF gene expression in T cells. Together, the data demonstrate that OVX increases TNF levels in the bone marrow by increasing the number of TNF producing T cells by a mechanism involving enhanced T cell proliferation. Regulation of TNF production via modulation of the number of TNF producing cells, without changes in TNF production per cell, is a novel regulatory mechanism that contributes to explain the bone loss associated with estrogen deficiency.

F136

Over-Expression of the Cell-Surface Isoform of Colony Stimulating Factor-1 in Osteoblasts Causes Increased Osteoclastogenesis and Bone Loss. G. Yao, J. Wu,* B. Sun, M. Mitnick, K. Insogna. Yale University, New Haven, CT, USA.

Alternative splicing of the CSF-1 gene gives rise to two protein products, soluble (sCSF-1) and cell-surface or membrane bound (mCSF-1) isoforms. Both isoforms are expressed by osteoblasts and osteotropic hormones regulate the expression of both. While sCSF-1 has been shown to stimulate osteoclastogenesis in vitro and in vivo, little data are available on the bioactivity of mCSF-1 in vitro and there are no in vivo data. To help clarify the role of mCSF-1 in bone, a transgenic mouse was developed in which targeted overexpression of human mCSF-1 in osteoblasts was achieved under the control of the 2.4 kb rat alpha I collagen promoter. A tissue survey by Northern analysis demonstrated overexpression of mCSF-1 in bone with no expression in lung, heart, skin, spleen, liver and kidney. Cultured calvarial osteoblasts from transgenic but not wild-type mice produced human CSF-1. Human mCSF-1 was also detected in purified calvarial osteoblast membranes from transgenic mice but not wild-type mice. Bone density determined by pQCT in 33 femurs from transgenic mice was compared to findings in 33 femurs from wild-type animals. Overall bone density was reduced by 8% in the transgenic mice as compared to wild type animals. When analyzed by sex, females were found to have 3-fold greater bone loss than males (-12% vs. -4%, $p < 0.05$). Histomorphometric analysis indicated the numbers of osteoclasts in bone (NOc/BPm, NOc/TAR, OcS/BS) was significantly increased in transgenic mice (1.8-2.0 fold, $p < 0.01$ for each) compared to wild-type animals. Osteoblast number and bone formation rate were no different in the two groups of animals. These data indicate that over-expression of mCSF-1 in osteoblasts increases bone resorption and are consistent with the hypothesis that this isoform plays a role in physiologic and perhaps pathologic states bone remodeling. Further, the female skeleton appears to be more sensitive to over-expression of mCSF-1. Since mCSF-1 has been reported to be selectively upregulated following estrogen withdrawal, this isoform may be a potential target for drug discovery.

F137

Toll-like Receptor 4 (TLR4) Is Essential for LPS-induced, mPGES-mediated PGE Production by Osteoblasts and Inflammatory Bone Loss. C. Miyaura, T. Ohshiba,* C. Matsumoto,* S. Harada,* A. Ito.* Biochemistry, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Toll-like receptors (TLR) are a family of mammalian proteins homologous to Drosophila Toll, and play important roles in the host defense against pathogens. Recently, TLR2 and TLR4 have been shown to be involved in LPS signaling. Bacterial infection causes bone resorption in inflammatory bone disorders including periodontitis, but the role of TLR signaling in bone metabolism is not known. PGE is a typical mediator for inflammation, but the relationship between PGE and TLR-signaling is not known. In this study, we investigated which TLR mediate LPS action in inflammatory bone loss and in PGE production by osteoblasts in vitro and in vivo. LPS induced osteoclast formation in co-cultures of bone marrow cells and osteoblasts collected from C3H/HeN (control) mice. In this culture, PGE2 production was markedly elevated by LPS, and indomethasin completely suppressed osteoclast formation, which suggests that LPS induces osteoclast formation mediated by PGE2. When cells were collected from C3H/HeJ mice that contain a nonfunctional mutation in the TLR4 gene, neither osteoclast formation nor PGE2 production were observed in the co-cultures. Osteoblasts expressed both TLR2 and TLR4 mRNAs in northern blot, and the level of TLR4 mRNA was higher than that of TLR2. LPS markedly induced the expression of COX-2 and membrane PGE synthase (mPGES) mRNAs, and PGE2 production by osteoblasts collected from C3H/HeN mice, but not from C3H/HeJ mice. In vivo, mice were injected with LPS, and we measured the level of PGE2 in bone marrow fluid and femoral bone mineral density (BMD). In C3H/HeN mice, the level of PGE2 in bone marrow fluid was markedly elevated, and BMD was reduced by LPS administration due to the loss of cancellous bone by increased osteoclastic bone resorption. In contrast, LPS induced no PGE2 production and impaired bone loss in C3H/HeJ mice with the mutated TLR4 gene. Thus, LPS acts on osteoblasts to induce COX-2- and mPGES-mediated PGE synthesis via TLR4, and the TLR4-mediated osteoclast formation may contribute to the pathogenesis of bone disorders associated with inflammation.

F140

IL-10 GeneTherapy Inhibits Inflammatory Bone Resorption. E. E. Carmody,* J. E. Puzas, R. N. Rosier, R. J. O'Keefe, E. M. Schwarz. Center for Musculoskeletal Research, University of Rochester, Rochester, NY, USA.

Osteolysis frequently occurs following total joint arthroplasty and results in failure in up to 20% of prostheses. Osteolysis is associated with the generation of microscopic debris particles, leading to localized inflammation and osteoclast activation. This study investigates the ability of adenoviral vIL-10 gene therapy to block these events both in vitro and in vivo. Fibroblasts infected with adenoviruses expressing either vIL-10 or LacZ were cultured on transwell membranes and added to ANA-1 macrophage cultures stimulated with titanium particles. In the presence of LacZ infected fibroblasts, titanium stimulated macrophages exhibited a marked increase in TNFa (6.5-fold), IL-6 (13-fold), and IL-1 (5-fold) secretion. Co-culture with vIL-10 infected fibroblasts supressed cytokine secretion to basal levels, while addition of an IL-10 neutralizing antibody completely blocked this effect. Partial inhibition of cytokine secretion ocurred when vIL-10 infected fibroblasts were added after 12 or 24 hours, demonstrating the ability of vIL-10 to inhibit an established inflammatory response to particles. Osteoclastogenesis was assessed in murine splenocyte cultures treated with RANKL and mCSF in the presence of fibroblast co-cultures. A 10-fold decrease in the number of TRAP+, multinucleated cells was observed in cultures containing Ad-vIL-10 infected fibroblasts. Addition of an IL-10 neutralizing antibody to these cultures resulted in complete recovery in osteoclastogenesis to levels observed in cultures containing Ad-LacZ infected fibroblasts (900 TRAP+ cells/well). These findings indicate that viral IL-10 directly inhibits the ability of osteoclast precursors to undergo differentiation in response to mCSF/RANKL. The effect of viral IL-10 on osteoclastogenesis and bone resorption in vivo was examined using a well established mouse calvaria model of particle induced osteolysis that measures osteoclasts and bone resorption. In control animals, titanium implantation resulted in a 66% increase in osteoclasts ($p<0.05$) and a 55% increase in sagittal suture area ($p<0.05$). The increase over control levels was completely blocked in mice receiving IP injection of Ad-vIL-10, all of whom had measurable serum vIL-10 levels for the duration of the experiment. These findings demonstrate that vIL-10 inhibits both cytokine secretion and osteoclastogenesis in vitro, the two critical events involved in inflammatory bone resorption. Consistent with these findings, Ad-vIL-10 gene therapy completely inhibited particle induced bone resorption in vivo, defining IL-10 as a potential therapeutic candidate for the treatment of this disease.

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Suppression of IGF Signaling Propagation and NF-kB Activation Reduces Bone Metastases in Breast Cancer. T. Hiraga,[1] A. Myoui,[2] P. J. Williams,*[3] G. R. Mundy,[3] T. Yoneda.[3] [1]Dept. Biochemistry, Osaka Univ., Osaka, Japan, [2]Dept. Orthopedics, Osaka Univ., Osaka, Japan, [3]Div. Endocrinology, Univ. TX. Hlth. Sci. Ctr., San Antonio, TX, USA.

Bone abundantly stores growth factors including IGFs and TGF-beta (TGFb). Previous data have demonstrated the blockage of TGFb signaling activation in breast cancer cells inhibits bone metastases, suggesting that the bone-derived TGFb modulates behavior of metastatic breast cancer cells in bone. On the other hand, the effects of the bone-derived IGFs, which are more abundant than TGFb, on bone metastasis in breast cancer still remains unclear. To study this, the MDA-MB-231 human breast cancer cells (MDA-231 cells) were stably transfected with a dominant-negative IGF type-I receptors (MDA-231/IGFIR-486STOP) and examined for the capacity to develop bone metastases following heart inoculation in female nude mice. MDA-231/IGFIR-486STOP exhibited impaired propagation of IGF signaling pathways including IGFIR, IRS-1, PI-3 kinase and AKT/PKB in response to IGF-1. Radiographic and histomorphometric examination showed bone metastases were markedly reduced in MDA-231/IGFIR-486STOP compared with empty vector-transfected MDA-231 cells (MDA-231/EV). In vitro growth and tumorige-

nicity in orthotopic site were not different between MDA-231/IGFIR-486STOP and MDA-231/EV. Since AKT/PKB has been shown to activate the transcription factor NF-kB and NF-kB has been implicated in the malignant behavior of breast cancer, the status of NF-kB activation was next examined in MDA-231 cells overexpressing wild-type IGFIR (MDA-231/IGFIR). We found IGF signaling pathways and NF-kB activation was up-regulated in these cells. Moreover, MDA-231/IGFIR showed increased bone metastases. To further study the role of NF-kB in bone metastasis, MDA-231 cells were stably transfected with a N-terminus-truncated dominant-negative IkB-alpha (MDA-231/IkB-alphaDN) and examined for the capacity to develop bone metastases. As expected, MDA-231/IkB-alphaDN exhibited decreased bone metastases. In addition, consistent with the notion that NF-kB is anti-apoptotic, apoptosis in MDA-231/IkB-alphaDN in bone metastases was significantly increased. There was no change in the production of PTH-rP, a cytokine which plays a central role in bone metastasis, in MDA-231/IkB-alphaDN compared with MDA-231/EV. In conclusion, our results suggest that bone-derived IGFs reduce apoptosis in breast cancer cells through activation of IGF signaling pathways, AKT/PKB and NF-kB, thereby stimulating bone metastases. The results also suggest that IGF signaling molecules and NF-kB are potential targets in design of pharmacological interventions for bone metastasis in breast cancer.

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Insulin-like Growth Factor Binding Protein (IGFBP)-5 Overexpression Decreases Bone Formation in vivo. R. D. Devlin,[1] Z. Du,[1] V. Jorgetti,[2] E. Canalis.[1] [1]Saint Francis Hospital and Medical Center, Hartford, CT, USA, [2]Universidade de Sao Paulo, Sao Paulo, Brazil.

IGFBP-5 is unique among the six IGFBPs synthesized by skeletal cells. IGFBP-5 has been considered to be anabolic, since it enhances bone cell growth in vitro and biochemical markers of osteoblastic function in vivo. However, the true role of IGFBP-5 in bone formation and bone resorption in vivo is not known. To determine in a definitive manner the function of IGFBP-5 in bone, we created transgenic mouse lines overexpressing IGFBP-5 under the control of the osteocalcin promoter so that IGFBP-5 would be expressed in the bone microenvironment. A transgenic line carrying approximately 8 copies of the transgene was analyzed at 4, 8, 12 and 26 weeks of age using contact X-ray radiography, total bone mineral density (BMD) using a GE/Lunar Piximus densitometer, and static and dynamic bone histomorphometry. Transgenic mice were compared to age-matched control wild type mice. At 4 weeks of age, transgenic IGFBP-5 mice had a BMD that was 12% lower than age-matched controls, although X-ray analysis did not reveal the presence of severe osteopenia or fractures. Histomorphometric analysis revealed a 30% reduction in trabecular bone volume in the femurs of IGFBP-5 transgenic when compared to wild type mice. This decrease was due to a marked reduction in trabecular thickness. Although the number of osteoblasts in IGFBP-5 transgenic mice was normal, bone formation rate was 30% lower than in wild type mice, as determined by double calcein labeling, indicating impaired osteoblastic function. Osteoclast number and bone resorption were not altered in transgenic IGFBP-5 mice. To confirm skeletal overexpression of IGFBP-5 and to characterize changes in gene expression associated with this condition, Northern blot analysis was performed on RNA extracted from calvariae and femurs of 4-week-old transgenic and control mice. IGFBP-5 mRNA levels were 30 fold higher in transgenic than in control mice, confirming overexpression of IGFBP-5 in bone. Alkaline phosphatase mRNA levels in IGFBP-5 transgenic mice were decreased by over 50%, confirming impaired osteoblastic function. To further investigate this role of IGFBP-5, stromal cells from 7-week-old IGFBP-5 transgenic and control mice were cultured. Stromal cell cultures from transgenic mice displayed reduced differentiated function, confirming the in vivo observation. In conclusion, in contrast to prior knowledge, IGFBP-5 in excess inhibits the function of the osteoblast in vivo and in vitro. These inhibitory effects are opposite to those observed in transgenic mice overexpressing IGF I, suggesting that IGFBP-5 decreases bone formation by binding IGFs in the bone microenvironment.

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Human *Seven in Absentia* Homologues Regulate TGFβ Inducible Early Gene (TIEG) Stability and Function Through the Ubiquitin-Proteasome Pathway. S. A. Johnsen,* M. Subramaniam, R. Janknecht,* T. C. Spelsberg. Biochemistry & Molecular Biology, Mayo Clinic & Foundation, Rochester, MN, USA.

Transforming growth factor β (TGFb) is an important pleiotropic growth factor for skeletal development and osteoblast (OB) differentiation. The TGFβ inducible early gene (TIEG) was identified in our laboratory as an early downstream component of TGFβ signaling in osteoblasts. We have previously demonstrated that overexpression of TIEG mimics TGFβ action by increasing markers of OB differentiation in MG-63 osteosarcoma cells. More recently we have shown that TIEG functions to enhance TGFβ action by repressing the promoter of the TGFβ/Smad signaling inhibitor Smad7. In order to further understand the regulation of TIEG protein we utilized the yeast two-hybrid system and identified SIAH 1 and 2 as TIEG interacting proteins. Interestingly, *seven in absentia*, the *Drosophila* homologue of SIAH 1 and 2, acts downstream of the receptor tyrosine kinase, *Sevenless*, and serves to relieve repression of specific developmental genes involved in photoreceptor differentiation by targeting a transcriptional repressor for degradation by the ubiquitin-proteasome pathway. In an analogous manner, we show that co-expression of SIAH 1 or 2 with TIEG in mouse OB (MC3T3-E1) cells results in degradation of TIEG through the ubiquitin-proteasome pathway. Using western blot and immunofluorescence localization, we provide evidence that degradation of TIEG by the SIAH proteins requires nuclear localization and the amino terminal region of TIEG which interacts with SIAH1/2. The functional significance of the SIAH-mediated degradation of TIEG was demonstrated by the co-expression of SIAH1/2 along with TIEG completely reversing the repression of Smad7 promoter activity that is induced by overexpression of TIEG alone. These findings suggest a novel mechanism whereby the effects of TGFβ on OB differentiation may be regulated by proteasomal degradation of the downstream transcriptional repressor TIEG. In this manner, turnover of downstream effectors such as TIEG could serve to limit the duration and/or magnitude of TGFβ responses.

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TGFb Stimulates Osteoclast Resorption and Bone Formation Through Different Cells in the Osteoblast Lineage. Z. Mi,*[1] B. Oyajobi,[1] E. Filvaroff,*[2] S. Harris,[1] G. Mundy,[1] D. Chen.[1] [1]Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [2]Endocrinology, Genentech, San Francisco, CA, USA.

Transforming growth factor b (TGFb) has complex effects on bone resorption. From in vitro studies, these effects appear to be mediated by cells in the osteoblast lineage. To determine the cell target for effects of TGFb on osteoclasts in vivo, we examined its effects in transgenic (tg) mice which overexpress a truncated dominant-negative type II TGFb receptor under the control of the osteocalcin promoter, which is expressed late in the osteoblast lineage. In these tg mice, osteoclast numbers were decreased significantly [103 ± 24 OC/mm in wild-type (wt) and 67 ± 14 OC/mm in tg]. Bone mineral densities were increased compared with the wt littermates (0.061 ± 0.003 mg/cm2 in wt and 0.071 ± 0.003 mg/cm2 in tg). Subcutaneous injection of TGFb (125 ug/kg/day, x5d) over the calvariae of one month old tg mice and wt littermates induced new bone formation, although less new bone was formed in the tg mice compared with their wt littermates (new bone width: 0.37 ± 0.05 mm in wt and 0.26 ± 0.04 mm in tg+/-). The formation of the bone marrow cavity in calvarial bones was enhanced by TGFb in wt mice but not in tg mice. These results suggest that osteoclast formation was inhibited in the tg mice and the target cells for effects of TGFb on bone formation are osteoblasts at an early differentiation stage that are not expressing osteocalcin. To further determine the role of osteoblasts in TGFb-induced osteoclast formation, we isolated osteoblasts from the calvariae of wt and tg mice and co-cultured these cells with spleen cells from wt mice in the presence of 10 nM 1,25-dihydroxy vitamin D3. We found that the osteoblasts isolated from tg mice but not from wt mice failed to support osteoclast formation even in the presence of 1,25-dihydroxy vitamin D3 (65 ± 15 MNCs/well in wt, 10 ± 5 MNCs/well in tg). To determine the downstream genes affected by the impairment of TGFb signaling, we examined mRNA expression of RANK ligand and OPG in osteoblasts from these mice using RT-PCR. Although RANK ligand was expressed at similar levels in both wt and tg mice, OPG expression in osteoblasts was significantly increased in the tg mice compared with their wt littermates. These findings demonstrate that TGFb stimulates osteocalcin-expressing (late stage) osteoblasts to activate osteoclast formation, but induces bone formation through non-osteocalcin-expressing osteoblasts earlier in the osteoblast lineage. It also demonstrates that TGFb is a critical mediator of osteoblast-mediated osteoclastic bone resorption in the basal state, since mice with unresponsive osteoblasts formed less normal osteoclasts or marrow cavities.

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α-Melanocyte Stimulating Hormone (α-MSH) - A Novel Factor Affecting Bone Turnover. J. Cornish,*[1] K. E. Callon,[1] K. G. Mountjoy,*[2] C. Lin,*[1] U. Bava,*[1] J. Lin,*[1] I. R. Reid.[1] [1]Department of Medicine, University of Auckland, Auckland, New Zealand, [2]Department of Paediatrics, University of Auckland, Auckland, New Zealand.

Recent evidence suggests that various neuropeptides that influence energy expenditure and body weight, in particular fat mass, also regulate bone mass. Fat mass is one of the principal determinants of bone density but the mechanism underlying this relationship remains controversial. The importance of central melanocortin pathways has been highlighted recently in genetic studies in obese humans and mice. Disruption of either the pro-opiomelanocortin (POMC) gene or the melanocortin-4 receptor (MC4-R) in mice and humans, as well as over-expression of agouti protein, which antagonises MC4-R, all result in obesity. MC4-R deficient human subjects have a marked increase in bone mineral density. The endogenous ligand most probably involved in the control of body weight is α-MSH which binds with high affinity to MC4-R. In addition, the peripheral administration of an α-MSH agonist to POMC knockout mice led to a substantial weight loss in the mutant animals as compared to the wild-type litter mates. Thus, there are data to suggest that peripheral actions of melanocortins, along with central pathways of action, are involved in controlling weight. Melanocortins circulate in the periphery and the receptors are found in peripheral tissues. Whether they act directly on bone is unknown but we have recently isolated MC4-R in primary rat osteoblasts and in the UMR106 osteoblast cell line, so this is a possibility. In the current study, we have investigated the effects of α-MSH on bone cells in vitro and in vivo.In primary cultures of fetal rat osteoblasts, α-MSH dose-dependently stimulated proliferation from nanomolar concentrations and greater. Similarly, α-MSH ($>10^{-10}$M) increased proliferation of cultures of canine chrondrocytes. In mouse bone marrow cultures, α-MSH ($>10^{-9}$M) stimulated osteoclastogenesis, but it had no effect on bone resorption in 2 assays using mature osteoclasts. Systemic administration of α-MSH to male adult mice (20 injections of 4.5 μg/day over 4 weeks) decreased the trabecular bone volume in the proximal tibiae (from 19.5 ± 1.8% to 15.2 ± 1.4%, p=0.03) and reduced trabecular number (p=0.001).It is concluded that there are direct effects of α-MSH on bone that tend to increase bone turnover, and that when α-MSH is administered systemically this leads to decreased bone volume. These results could explain the higher bone density measurements seen in subjects affected by mutations of the MC4-R gene. This data also suggests that α-MSH may have central and peripheral actions in regulating fat mass and bone density.

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Mechanical Strain Increases Nitric Oxide Production in Murine Stromal Cell Culture. X. Fan, T. C. Murphy,* L. Zhu,* M. S. Nanes, W. R. Taylor,* J. Rubin. Department of Medicine, Emory University Medical School/VAMC, Atlanta, GA, USA.

Mechanical loading is an important regulator of skeletal mass and architecture. We have reported previously that mechanical strain decreased 1,25D stimulated osteoclast recruitment, and that this effect was due to down-regulation of receptor activator of NF-kappa B ligand (RANKL) expression. However, the mechanisms by which these adaptive responses are initiated and subsequently coordinated are unclear. Many reports have shown that nitric

oxide (NO) has a role in bone remodeling and that mechanically induced bone formation may require NO production. We have recently shown that NO inhibits RANKL expression. This suggested that the mechanism whereby mechanical strain decreases RANKL expression might involve NO. To investigate this hypothesis, primary bone marrow stromal cells from C57BL/6 mouse tibia and femurs were cultured on collagen-coated 6-well Bioflex plates. On day 6, media were replaced with phenol-red free α-MEM containing 2% FBS and strained at 1.8%, 10 cpm using Bioflex loading station for specified periods. NO was measured in the culture medium using 2,3-Diaminonaphthalene (DAN) to assess nitrite. NO production was significantly increased two-fold after 24 hours of strain application (nitrite in control was 32.8 ± 9 μM). There were no changes in NO production in cells strained ≤ 8 hrs compared to the control group. N_{ω}-nitro-$_L$-arginine methyl ester (L-NAME) at 500 μM, a nitric oxide synthase (NOS) inhibitor, blocked the strain induction of NO. The time required for strain to increase NO levels suggested that NOS protein level rather than activity might be mechanically regulated. Western blots showed that NOS type III (eNOS) expression increased at least three-fold in stromal cells after 24 hrs strain. The mechanical induction of eNOS protein was reflected in a similar increase in eNOS mRNA as assessed by RT-PCR. Our results suggest that increases in NO production by mechanical strain arise through stimulation of eNOS synthesis. These findings raise the interesting possibility that mechanical strain inhibits bone resorption through increasing NO production with a subsequent regulation of RANKL expression.

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Integrins and Src kinases Clustered in Caveolae Are Essential Components of the Signalsome that Mediates Mechanically Induced ERK Activation in Osteocytes. I. Mathov, J. Davis,* L. I. Plotkin, S. C. Manolagas, T. Bellido. Endo/Metab. Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. of Arkansas for Medical Sciences, Little Rock, AR, USA.

Osteocytes are key components of the sensory apparatus by which bone responds to mechanical stimuli. Similar to systemic factors, mechanical stimulation prevents osteocyte apoptosis and activates the extracellular signal regulated kinases (ERKs). ERK activation by mechanical signals involves the actin and tubulin cytoskeletons, and integrins connect the extracellular matrix with cytoskeletal and catalytic proteins. Therefore, we examined whether integrins take part in ERK activation induced by mechanical stimulation. MLO-Y4 osteocytic cells were plated on flexible-bottom wells coated with different substrates and subjected to biaxial stretching using a BioFlex Loading Station. Cells plated on collagen-I responded to stretching by a 2-3 fold increase in ERK phosphorylation, whereas stretching did not induce ERK activation in cells plated on the non-integrin-dependent substrate poly-L-Lys at any time or strain tested. On the other hand, ERK activation in response to serum or EGF was similar in cells plated on either substrate, indicating that integrins are specifically required for mechanically induced ERK activation. We next examined the effect of engagement of particular integrins using activating anti-integrin antibodies. Whereas stretching had no effect in cells plated on non-immune IgG or anti-β2 antibody, it did induce ERK activation in cells plated on anti-β1, or anti-α5, or anti-α2 antibodies. Moreover, stretching-induced ERK activation in cells plated on anti-β1 antibody in combination with either anti-α5 or anti-α2 antibodies was greater than in cells plated on each antibody separately. These results are consistent with the expression of integrin β1 in osteocytes in vivo and suggest that α2β1 (which binds collagen-I) and α5β1 (which binds fibronectin) are involved in osteocyte response to mechanical stimuli. Integrin β1 and signaling molecules that participate in the ERK pathway, such as Src, cluster in caveolae - plasma membrane domains rich in cholesterol and the protein caveolin; and caveolin may facilitate integrin clustering. Consistent with this, cyclodextrin - an agent that disrupts caveolae by sequestering cholesterol - or the specific inhibitor of Src kinases PP1, abolished stretching-induced ERK phosphorylation. These findings suggest that integrins clustered in caveolae transduce mechanical signals into ERK activation by activating Src kinases in osteocytic cells.

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Fluid Shear Stress Inhibits TNF-α-Induced Apoptosis in MC3T3-E1 Osteoblasts. F. M. Pavalko,[1] P. J. Gallagher,*[2] R. L. Gerard,*[2] S. M. Ponik,*[2] Y. Jin,*[2] S. M. Norvell.*[2] [1]Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Indiana University School of Medicine, Indianapolis, IN, USA.

Mechanical loading of bone plays an important role in bone remodeling. Osteoblasts and osteocytes respond to the load-generated flow of interstitial fluid through the spaces inside bone by altering their biochemical and anabolic activities, however the mechanisms of this response are poorly understood. Increased growth and survival of osteoblasts subjected to fluid flow-induced shear stress may promote bone formation. A large proportion of bone-forming osteoblasts normally undergo programmed cell death (apoptosis) and we hypothesize that the absence of loading may further promote this process. We investigated the cellular mechanisms of load-induced bone remodeling by treating MC3T3-E1 osteoblasts with 1-50 ng/ml TNF-α in the presence of 10 μM cyclohexamide (CHX) for up to 4 hours to induce apoptosis and studied the effects of steady fluid shear stress on cell survival using a parallel plate flow chamber. We found that TNF-α/CHX treatment of cells in static culture conditions caused membrane blebbing characteristic of apoptosis and induced a dose dependent activation of caspases resulting in degradation of poly-(ADP ribose)-polymerase (PARP), the cell-cell adhesion molecule N-cadherin, and the cytoskeletal linker/signaling protein, β-catenin. In cells treated with 5 or 10 ng/ml TNF-α/CHX and subjected to fluid shear stress (12 dynes/cm^2), membrane blebbing was reduced and cleavage of PARP, N-cadherin and β-catenin were significantly inhibited compared to cells treated with TNF-α/CHX and maintained in static culture. Fluid shear stress also induces release of prostaglandin E2 (PGE_2) from MC3T3-E1 cells and causes upregulation of cyclooxygenase-2 (COX-2). Furthermore, in many cell types, inhibition of COX-2 blocks shear-induced PGE_2 release and promotes apoptosis suggesting that PGE_2 and COX-2 may contribute to shear-induced osteoblast survival. To test this hypothesis, MC3T3-E1 osteoblasts maintained in static culture were treated with 5 ng/ml TNF-α/CHX in the presence or absence of 10 μM PGE_2. Just as fluid shear stress inhibited apoptosis, the addition of PGE_2 to the cells in static culture inhibited TNF-α/CHX-induced membrane blebbing and cleavage of PARP, N-cadherin and β-catenin. These results suggest that fluid shear stress may enhance bone formation by inhibiting apoptosis and promote the survival of osteoblasts through a mechanism involving PGE_2 autocrine/paracrine signaling.

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Activation of a Ca/ERK/Delta-fosB(AP-1) Cascade by Mechanical Stress in Osteoblasts, Leading to Up-regulation of an Osteogenic Cytokine, Interleukin (IL)-11. S. Kido, D. Inoue, E. Tohjima, S. Kato,* T. Matsumoto. First Department of Internal Medicine, University of Tokushima School of Medicine, Tokushima, Japan.

Mechanical stress to bone plays a critical role in maintaining bone mass and strength. Unloading such as immobilization and space flight results in rapid bone loss mainly due to impaired bone formation. However, the molecular mechanism of mechanical stress-induced bone formation remains to be fully elucidated. We have shown that delta-fosB, a fosB splicing variant which is known to stimulate bone formation in vivo, is rapidly induced by reloading in tail-suspended mouse bone as well as by fluid shear stress (FSS) in primary osteoblast (POB) cultures. As a candidate for AP-1 targets induced by mechanical stress, we identified an anti-adipogenic cytokine, IL-11, which has been shown to prevent aging-related bone loss by stimulating bone formation in transgenic mice. We have also reported that IL-11 transcription is totally dependent on AP-1 and that impaired bone formation in the aged is associated with reduction in activities of AP-1, particularly Jun D, and IL-11 expression in marrow stromal cells. In order to further delineate the mechanical stress-induced signaling pathways, we investigated the mechanism of AP-1/IL-11 induction by FSS in POB. The results indicated that induction of delta-fosB and IL-11 was completely blocked by gadolinium, an intracellular Ca chelator BAPTA, or an ERK1/2 inhibitor U0126, but not by a L-type Ca-channel blocker nifedipine, or a p38 kinase inhibitor SB203580. Conversely, a Ca ionophore A23187 activated ERK1/2 within 15 min and induced delta-fosB/IL-11 expression. We also demonstrated that intermittent FSS reduced stromal cell differentiation into adipocytes induced by troglitazone or indomethacine to less than 50%, consistent with the biological effects of IL-11. Surprisingly, FSS also induced Smad1 phosphorylation within 15 min in POB in an ERK-independent manner. Co-immunoprecipitation experiments revealed induction of a complex of endogenous Delta-FosB and Jun D by 3 hour FSS in POB, and association of over-expressed Delta-FosB/Jun D and Flag-Smad1 in Cos-7 cells. Moreover, EMSA using the IL-11 AP-1 site as a probe showed the presence of over-expressed Flag-Smad in the AP-1 complex only when delta-fosB was over-expressed. These results suggest a critical role of a Ca/ERK/AP-1/IL-11 cascade in mechanical stress-induced bone formation, and further imply participation of Smad in this signaling pathway. We propose that the mechanical stress-induced delta-FosB/Jun D complex may recruit and utilize Smad as a co-factor to efficiently induce IL-11 transcription, thereby stimulating bone formation.

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Fluid Flow Shear Strain Activates Multiple Pathways on Human Osteoblast Proliferation and Competing Pathways on Human Osteoblast Differentiation. S. Kapur,* D. J. Baylink, K. H. W. Lau. J. L. Pettis Memorial VAMC, Loma Linda, CA, USA.

Mechanical strain stimulates osteoblast proliferation and differentiation through multiple signaling pathways. This study sought to assess the role of several pathways in the fluid shear flow-induced proliferation and differentiation of human bone cells. In this regard, we confirmed that a 30-min fluid flow strain produced by the Cytodyne Flow Chamber at 20 dyne/cm^2 increased the proliferation ([^{3}H]thymidine incorporation) (2.3-fold, $P<0.01$) and alkaline phosphatase (ALP) activity (an index of differentiation) (1.6-fold, $P<0.05$) in human osteoblasts. This fluid flow also increased tyrosine phosphorylation of ERK1 and ERK2 (by 3-fold each, $P<0.001$) and expression of integrin b1 (by 1.5-fold, $P<0.001$). We then evaluated if specific inhibitor of the Gi pathway (PTX), the MAPK pathway (PD 98059), the NOS pathway (L-NAME), and the PGE_2 pathway (indomethacin) would inhibit the flow-induced proliferation and differentiation of normal human bone cells. The MAPK pathway inhibitor, PD98059, completely blocked the fluid flow-induced increases in ERK phosphorylation, [^{3}H]thymidine incorporation, and ALP activity ($P<0.001$ for each). Similarly, L-NAME and indomethacin each blocked ($P<0.001$) the fluid flow-induced increases in ERK phosphorylation, [^{3}H]thymidine incorporation and ALP activity, confirming that the MAPK, NOS, and PGE_2 pathways are essential for the flow-induced bone cell proliferation and differentiation. However, none of these three inhibitors had no effect on integrin β1 expression, suggesting that the integrin b1 expression is not essential for bone cell proliferation and differentiation. While PTX completely blocked the flow-induced increase in integrin β1 expression, this inhibitor had no effect on the flow-induced increase in the phosphorylation levels of ERK or in [^{3}H]thymidine incorporation. More intriguingly, PTX not only did not inhibit but also significantly enhanced ($P<0.05$, ANOVA) the stimulatory effect of fluid flow on ALP activity in human bone cells, suggesting that the PTX-sensitive G protein pathway may have an inhibitory role in osteoblast differentiation. In summary, we show that fluid flow shear strain activates multiple signaling pathways to stimulate human bone cell proliferation and that one of these signaling pathways (e.g., the PTX-sensitive G protein pathway) has completing effects with an unknown pathway on human bone cell differentiation.

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IGF-1 and Mechanical Loading Synergistically Enhance Bone Formation. T. S. Gross,*[1] T. L. Clemens,[2] S. Srinivasan.[1] [1]Orthopaedics and Sports Medicine, University of Washington, Seattle, USA, [2]Medicine, University of Cincinnati, Cincinnati, OH, USA.

IGF-1 and mechanical loading are both potential anabolic stimulants of bone formation. We hypothesized that low levels of either stimulus alone would not be sufficient to induce bone formation, but if the two stimuli were coupled bone formation would be synergisti-

cally induced. To examine this hypothesis, we used a recently developed non-invasive device to load the right tibia of transgenic mice selectively over-expressing IGF-1 in osteoblasts (18 wk of age). The wild type (FVB/N background, n=3) and over-expressing mice (n=3) underwent a brief 5 d low magnitude loading protocol (100 cycles per day, 1 Hz sawtooth waveform, 650 με peak strain). The mice freely ambulated within their cages during non-loading periods. Following the 5 d of external loading, the mice were allowed an additional 3 wk of free cage ambulation to facilitate consolidation of osteoblastic activation. Calcein (10 mg/kg, IP) was injected 1 d prior to the protocol and 1 d prior to sacrifice. Single labeled surface, double labeled surface, and interlabel widths were assessed to determine bone formation rate (surface referent) on the endocortical and periosteal surfaces. Wild type and IGF-1 over-expressing mice both demonstrated negligible periosteal bone formation at the mid-diaphysis of the contralateral tibia (0 and 0.0061 ± 0.0061 $\mu m^3/\mu m^2$/d, respectively). Low magnitude loading (inducing strains equivalent to walking) of the wild type mice did not induce periosteal bone formation. In contrast, the IGF-1 over-expressing mice demonstrated a sustained adaptive response to the same low magnitude loading, with periosteal bone formation rate elevated 5-fold versus contralateral bones (.032 ± .024 $\mu m^3/\mu m^2$/d). In this study, neither low magnitude mechanical loading or IGF-1 over-expression alone was sufficient to induce more than negligible bone formation. However, when the same two stimuli were combined, sustained bone formation was induced. One advantage of our approach over one in which locomotion is superimposed upon systemically administered factors (e.g., PTH) is that the synergism we sought to observe was confined to the tissue under examination and was not confounded by systemic influences. Our data highlight the potential for physiologic magnitude loading to diminish the dosage of drugs required to induce bone formation, which should lead to enhanced efficacy and reduced side effects.

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Longitudinal Survey of Trabecular Bone Architecture Deterioration in the Hindlimb Unloaded Female Rat Model Using 3D Micro-Computed Tomography. V. N. David,[*1] R. De Vittoris,[*1] A. Laib,[*2] B. Koller,[*2] S. Haemmerle,[*2] M. Lafage-Proust,[1] C. Alexandre,[1] P. Ruegsegger,[3] L. Vico.[1] [1]LBBTO, INSERM E9901, Saint-Etienne, France, [2]Scanco Medical, Bassersdorf, Switzerland, [3]IBT, Zurich, Switzerland.

Bone mass and microarchitecture are the main determinants of bone strength. Three-dimensional micro-computed tomography (3DμCT) has the potential to examine, in vivo, bones of small laboratory animals with high resolution in a non-invasive way. In this work, the proximal part of the tibiae of hindlimb unloaded (HU) and control (CTR) female rats (12 week-old, 250 ± 10g body weight) were measured with 3DμCT, and the secondary spongiosa of the scanned region was evaluated using direct evaluation techniques that do not require model assumptions. Kinetics of trabecular bone alterations and linear x-ray attenuation coefficient (likened to apparent bone mineral density) was analyzed at three time points during a 14-day tail suspension period.

PERIOD	BV/TV (%)	LIN ATT	TB.N (/mm)	Connectivity Density (/mm^3)
HU baseline	26.7 ± 6.3	1.011 ± 0.061	4.46 ± 0.40	45.4 ± 10.8
HU 1-wk	24.8 ± 7.3 *	0.989 ± 0.079 *	4.20 ± 0.60 *	38.9 ± 14.5 *
HU 2-wk	23.7 ± 7.6**	0.977 ± 0.081 *	4.17 ± 0.58 *	34.5 ± 12.1 **
CTR baseline	26.7 ± 8.8	1.005 ± 0.089	4.40 ± 0.66	44.8 ± 15.5
CTR 1-wk	28.2 ± 10.9	1.019 ± 0.103	4.50 ± 0.67	46.7 ± 14.1
CTR 2-wk	29.0 ± 10.1	1.028 ± 0.096	4.54 ± 0.67	48.7 ± 15.8

$p<0.05$ * vs HU baseline, ** vs HU 1-wk. N=10 per group

In HU animals, "individual" bone loss assessed either by BV/TV or LIN ATT (Linear Attenuation) was significant after a 1-wk period and plateaued at 2 weeks. It was associated to fewer trabeculae that were less connected while the trabecular thickness was not altered. When we compared HU and CTR rats, "group" bone loss was significant only after the second week of suspension. Furthermore, the trabecular structure type in HU rats became more rod-like (assessed by structure model index) and anisotropic than in CTR rats. We concluded that in vivo 3DμCT is a powerful tool allowing longitudinal survey. We showed that longitudinal comparisons have more discriminant power than transversal ones when the groups still greatly overlap.

F167

Pre-osteoblast Proliferation Following in vivo Mechanical Loading. S. J. Tanner,[1] J. A. Yee,[1] D. M. Cullen.[2] [1]Biomedical Sciences, Creighton University, Omaha, NE, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

The appearance of osteoblasts following a brief bout of mechanical loading has been documented, but the proliferation of osteoblast precursors in the cellular periosteum has not yet been investigated. In this study, a single bending load (36 cycles, 2Hz, 2000 μstrain) was applied to the right tibia of adult female rats (351±20g) and the time course of periosteal pre-osteoblast (pOB) proliferation was examined. A single injection of bromodeoxyuridine (40mg/kg) was administered 1 hour prior to collection times at 12, 24, 30, 36, 42, 48, 60, and 72h post-load. Endpoints included surface type (osteoblast, flat cell, or unmeasurable), and pOB labeling index (LI, labeled/total *100). A cell was counted as a pOB if it: 1) resided within the cellular periosteal layer; 2) was not directly upon the bone surface; and 3) had a single round, ovoid, or elongated ovoid nucleus. Differences were tested by ANOVA and are reported as means (SD), $P<0.05$.

Endpoint	Leg	12h	24h	30h	36h	42h	48h	60h	72h
pOB LI (%)	nL	0.40 (0.90)	0.00 (0.00)	3.98 (5.59)	0.00 (0.00)	0.00 (0.00)	2.67 (3.62)	0.00 (0.00)	5.56 (12.4)
	L	6.75 (10.6)	4.79 (5.07)	6.07 (6.34)	17[abc] (8.18)	13.8[a] (5.38)	13.7 (7.36)	15.3[ac] (8.3)	14.2 (5.45)
Ob.S (%)	nL	7.41 (7.29)	4.93 (7.33)	4.22 (5.23)	2.93 (4.37)	3.03 (1.54)	4.26 (3.67)	7.94 (14.6)	3.45 (4.84)
	L	7.87 (6.64)	25.2 (16.4)	6.22 (4.93)	33[ab] (24.5)	32[ab] (25.6)	35[ab] (20.9)	34[ab] (13.6)	43[ab] (15.1)

[a]Loaded different from nonloaded leg; different from loaded leg at [b]12 and 30h, [c]24h

From 36-72h post-load, pOB LI and osteoblast surface in loaded legs were greater than in nonloaded legs, and also greater than in 12-30h loaded legs. Nonloaded legs did not differ with time or loading. The concurrent increases in Ob.S and pOB LI indicate that the early response to loading may be the conversion of non-proliferative surface cells into osteoblasts. Furthermore, these results show that a single mechanical load is sufficient to stimulate DNA synthesis (cell proliferation) among the pOB population within 36h post-load and maintain this response through 72h.

F169

Response of Growing Bones to a Jumping Protocol of Reduced Repetitions: A Randomized Controlled Trial. R. K. Fuchs,* D. P. Williams,* C. M. Snow. Exercise and Sport Science, Oregon State University, Corvallis, OR, USA.

We have previously reported significant (3 to 5%) gains in spine and hip bone mass in pre-pubescent children who performed 300 jump repetitions/week at a magnitude of 8 body weights at the ground. Our aim in this investigation was to examine the bone response at the hip and spine when jump repetitions are reduced by one half to 150 jump repetitions/week at the same load magnitude of 8 body weights at the ground. Ninety pre-pubescent children between the ages of 5 and 9 years were randomized into a jumping (n=23 boys, n=22 girls) or control group (n=24 boys, n=21 girls). The exercise intervention was incorporated into the curriculum during the school day, two times per week for 7-months. The jumping group performed 75, two footed jumps off 61cm boxes each session, while the control group performed non-impact stretching exercises during the same time period. Bone mineral content (BMC; g), bone area (BA; cm2) and bone mineral density (BMD; g/cm2) of the left proximal femoral neck and lumbar spine (L1-4) were assessed by DXA (Hologic QDR/4500-A). In addition, anthropometric characteristics (height, weight and body fat), Tanner staging, physical activity and dietary calcium intake were assessed. At baseline there were no differences between groups for height, weight, body fat, physical activity and bone variables. After 7-months, jumpers and controls had similar increases in height, weight and body fat. Both groups had similar intakes of calcium at baseline and 7-months (mean intake = 1,200 mg). Using repeated measures ANCOVA (dependent variable: absolute changes in bone; covariates: initial age and bone values and changes in height and weight) for bone mineral content, the primary outcome variable, jumpers and controls had similar increases at the femoral neck (jumpers: 0.157 ± 0.016 g vs. controls: 0.154 ± 0.016 g) and lumbar spine (jumpers: 1.43 ± 0.15 g vs controls: 1.68 ± 0.15 g). In repeated measures ANCOVA of secondary outcomes (BMD and BA), jumpers and controls had similar increases in BMD of the femoral neck (jumpers: 0.023 ± 0.004 g/cm2 vs. controls: 0.019 ± 0.004 g/cm2) and lumbar spine (jumpers: 0.016 ± 0.003 g/cm2 vs. controls: 0.012 ± 0.003 g/cm2), and similar increases in BA of the femoral neck (jumpers: 0.116 ± 0.020 cm2 vs. controls: 0.152 ± 0.020 cm2) and lumbar spine (jumpers: 1.52 ± 0.15 cm2 vs. controls: 2.16 ± 0.15 cm2). In conclusion, a jumping protocol previously shown to increase bone mass had no effect when repetitions were reduced by one half. This is the first report to examine the effect of varying jump repetitions while holding magnitude constant in the growing skeleton. The apparent synergy between load magnitude and repetitions warrants further investigation.

F172

Protein Kinase A-Dependent Phosphorylation and Inactivation of the Pro-apoptotic Protein Bad Mediates the Anti-Apoptotic Effect of PTH on Osteoblastic Cells. T. Bellido, L. I. Plotkin, J. Davis,* S. C. Manolagas, R. L. Jilka. Div. Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. of Arkansas for Med. Sci., Little Rock, AR, USA.

Inhibition of osteoblast apoptosis may account for the anabolic effect of intermittent PTH administration on the skeleton. Based on evidence that the anti-apoptotic effect of PTH is mediated by cAMP, and the role of intracellular kinase cascades in the regulation of cell survival, we carried out studies to elucidate the cAMP-regulated pathways and target proteins involved in apoptosis suppression. PTH (50 nM) prevented apoptosis of osteoblastic OB-6 cells induced by 6 hour treatment with 50 μM etoposide, as assessed by nuclear fragmentation, trypan blue uptake and caspase-3 activity. Sphingosine-1-phosphate, the product of sphingosine kinase, did not inhibit apoptosis, eliminating a role for this cAMP-activated kinase. Inhibition of extracellular signal regulated kinases (ERKs) with PD98059, or transfection with a dominant negative ERK kinase, also failed to block the anti-apoptotic effect of PTH; thus, ERKs are not involved either. However, the protein kinase A (PKA) inhibitors RpcAMP and H89 did prevent suppression of apoptosis by PTH, indicating a requirement for this cAMP-activated kinase. The pro-apoptotic protein Bad is a likely substrate of survival kinases because when phosphorylated at Ser136 and Ser155, it is inactivated, thus resulting in cell survival even in the face of a pro-apoptotic stimulus. Western blotting revealed that PTH induced a 3 to 5-fold increase in phospho-Ser155-Bad within 30-60 minutes, followed by a slow decline to basal levels at 6 hours. RpcAMP and H89 blocked PTH-stimulated phospho-Ser155-Bad production, confirming the role of PKA in this process. Bad phosphorylation was evidently essential for anti-apoptosis, as PTH did not promote survival in cells expressing a Bad mutant that cannot be phosphorylated at S155 or S136 (because of substitution by Ala). Moreover, apoptosis suppression was not sustained after phospho-Ser155-Bad had declined to basal levels. We conclude that cAMP-dependent PKA-mediated Bad phosphorylation is responsible, at least in part, for the anti-apoptotic effect of PTH, and thereby the stimulatory effect of intermittent PTH administration on bone formation. The transient nature of Bad phospho-

rylation may explain the need to give repeated injections of PTH to obtain an anabolic effect. It may also explain the failure (reported elsewhere at this meeting) of sustained PTH elevation to suppress osteoblast apoptosis in vivo, as only one transient episode of apoptosis suppression can be induced in this condition.

F175

Osteoblast Differentiation by BMP-2 Is Mediated by Down-Regulation of Cyclin-Dependent Kinase 6 (CDK6). T. Ogasawara, M. Katagiri, T. Miura,* T. Susami,* T. Takato,* K. Nakamura, H. Okayama,* H. Kawaguchi. Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

Cell cycle control proteins such as cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors (CKIs) are known to regulate not only proliferation but also differentiation of cells, and cell cycle arrest at the G1 phase is a prerequisite for cell differentiation. To elucidate the possible regulatory mechanism of osteoblast differentiation by cell cycle control proteins, this study investigated the involvement of these proteins in the action of bone morphogenetic protein-2 (BMP-2), a potent inducer of osteoblast differentiation, on mouse osteoblastic MC3T3-E1 cells. Initially, we analyzed the expression of cell cycle control proteins that are known to affect the G1 phase: cyclin D1, D2, D3, E, CDK2, 4, 6, and CKIs (p15, p16, p18, p19, p21 & p27). Western blot analysis revealed that only the CDK6 level was markedly decreased by BMP-2 (300 ng/ml) at 24 h of culture and thereafter, while those of other proteins were not affected by BMP-2 throughout the culture period (up to 55 h). This effect of BMP-2 was not due to the stimulation of CDK6 protein degradation but to the inhibition of its expression because MG132, a potent proteasome inhibitor, did not change the effect. This inhibition, however, was not seen in MC3T3-E1 cells transfected with an inhibitory Smad, Smad6, using an adenovirus vector, indicating that this effect is mediated by the Smad signaling. We further established stable clones of MC3T3-E1 cells transfected with CDK6 cDNA, and selected several clones with high expression and low expression on Western blotting. Flow cytometric analysis showed that BMP-2 increased the number of cells in the G0/G1 phase of the cell cycle in cultures of low expressing clones, but not in those of high expressing clones. BMP-2 stimulated alkaline phosphatase activity and osteocalcin mRNA level in low expressing clones, whereas these effects were markedly reduced in high expressing clones. Since there was no significant difference in the doubling time of cells between low and high expressing clones, the inhibitory effect of CDK6 on osteoblast differentiation was shown to be independent of its effect on osteoblast proliferation. These results demonstrate that CDK6 is a critical mediator of the BMP-2 effect on osteoblast differentiation as a downstream effector of Smad signaling. Because we also found that fibroblast growth factor-2, a potent inhibitor of osteoblast differentiation, up-regulated the CDK6 protein level in this culture system, it is postulated that CDK6 is a key molecule determining the differentiation rate in osteoblasts.

F180

Withdrawn

F187

The Orphan Receptor, Estrogen Related Receptor ERRα, Is Regulated by Estrogens in Bone and Is Involved in Not Only Osteoblast But Also Adipocyte Development. E. Bonnelye,[1] V. Kung,*[1] A. Samuels,*[2] J. H. Tobias,[2] J. E. Aubin.[1] [1]Department of Anatomy and Cell Biology, University of Toronto, Toronto, ON, Canada, [2]Rheumatology Unit, Division of Medicine, University of Bristol, Bristol, United Kingdom.

The orphan estrogen receptor related receptor, ERRα, is expressed by osteoblastic cells during development and modulation of its levels by over- and underexpression strategies alters osteoblast differentiation and bone formation in the 21 d. fetal rat calvaria (RC) cell model in vitro. ERRα is also expressed in adult osteocytes in calvariae and long bones, suggesting a function for ERRα in adult bone and diseases of bone, such as osteoporosis where bone loss is accelerated by a decrease in secretion of estrogens in post-menopausal women. We have therefore investigated the effects of estrogen (E2) on ERRα both in vitro and in vivo. Chronic treatment of 21 d. fetal rat calvaria (RC) cells by estrogen (E2) upregulates ERRα mRNA expression at early times in culture (day 6), suggesting a link between ERRα and E2 during osteoprogenitor proliferation. To determine whether E2 may elicit similar regulatory activity in vivo, we analysed ERRα expression levels in samples of mRNA extracted from femurs, from which bone marrow had been flushed, of mice treated with 17b-estradiol at doses known to elicit a large anabolic effect on endosteal bone. A marked stimulatory effect of E2 on ERRα expression levels was evident at day 1, 2 and 4 post-E2 treatment. Four weeks after ovariectomy (OVX) of rats, we found that ERRα remained highly expressed in osteocytes in the secondary ossification zone and cortical bone and osteoblasts associated with trabecular bone. The fact that ERRα is highly expressed in the osteoblastic cells present in the high turnover bone of the OVX rat model may indicate that ERRα expression is essential for osteoblast function in osteoporosis as in normal turnover. Given these observations, we next blocked ERRα in differentiating RC cell cultures using phosphorothioate-modified antisense oligonucleotides; sense oligonucleotides were used as control. As we observed previously, antisense but not sense oligonucleotides completely inhibited bone nodule formation. Interestingly, we observed a concomitant increase in adipocyte colony formation as well as an increase in PPARγ, CEBPα and PLA_2 mRNA expression levels. These results suggest that ERRα is important not only in osteoblast, but also in adipocyte development, suggesting that it may play a role in balancing stromal cell fate choices in the normal or OVX situation. They also suggest that agonists and antagonists of ERRα may be useful as therapeutic agents in a wide variety of bone metabolic and other diseases affecting bone.

F190

Translational Regulation Is a Control Point in RUNX2 (Cbfa1) Gene Expression. S. Sivasubramaniam,* Y. Li,* M. S. Katz, N. Elango.* Univ Texas Hlth Sci Center and GRECC, VAMC, San Antonio, TX, USA.

RUNX2/Cbfa1, a transcription factor implicated in the regulation of osteoblast differentiation and osteoblast specific gene expression, is expressed as two isoforms. The two isoforms, type-l and type-ll, are encoded by two different mRNAs with distinct 5'untranslated regions (type-1 5'UTR: 1015 nucleotides and type-ll: 210 nucleotides). In addition, type-l mRNA differs from type-ll mRNA by having 15 nucleotides encoding the amino terminal 5 amino acids MRIPV in place of 57 nucleotides encoding the amino terminal 19 amino acids MASNSLFSAVTPCQQSFFW of type-ll; the remaining 508 amino acids of the two isoforms are identical. Undifferentiated osteoblast precursor cells (MC3T3-E1 and ST2 cells) express RUNX2 mRNAs but not the osteoblast markers bone sialoprotein and osteocalcin, indicating that the expression of RUNX2 mRNAs is not sufficient for osteoblast differentiation. In this study we investigated expression of the RUNX2 gene at mRNA and protein levels in osteoblastic and non-osteoblastic cells, to determine whether translational regulation is a control point in RUNX2 gene expression. Total RNA from osteoblastic cells (fetal rat calvarial cells [FRC]; ROS 17/2.8, UMR-106, SaOS-2 and U2OS cells), osteoblast precursors (MC3T3-E1 and ST2 cells), and non-osteoblastic fibroblasts (COS-7, C3H10T1/2 and NIH3T3 cells) was subjected to Northern analysis using cDNAs of the 5' UTRs of type-l and type-ll mRNAs as probes. Northern blots demonstrated the presence of type-l mRNA in all cell types tested, and type-ll mRNA in osteoblasts and osteoblast precursors; type-ll mRNA was demonstrable in non-osteoblastic fibroblasts by RT-PCR analysis. Immunoblot analysis was performed using type-l specific antiserum raised against the synthetic peptide MRIPV, and type-ll specific antiserum generated against the synthetic peptide MASNSLFSAVTPCQQSFFW. Immunoblots revealed expression of both type-l and type-ll proteins in cells with the mature osteoblast phenotype (FRC; ROS 17/2.8, SaOS-2 and U2OS cells) but only type-l protein in immature osteoblastic cells (UMR-106); neither protein was detected in osteoblast precursors and non-osteoblastic fibroblasts. These results suggest that type-l protein is expressed earlier than type-ll protein in cells differentiating along the osteoblast lineage. Accordingly, type-l and type-ll proteins may respectively control the expression of early and late markers of osteoblast differentiation. In conclusion, the demonstration of RUNX2 mRNAs in differentiated and undifferentiated cells, but type-l and type-ll proteins only in differentiated osteoblasts, implicates control of RUNX2 expression at the translational level.

F192

Factors Possessing Histone Acetyltransferase and Deacetylase Activity Interact With Runx2 (Cbfa1) and Modify Its Transcriptional Activity. J. J. Westendorf,[1] X. Li,*[1] B. Lutterbach.*[2] [1]Orthopaedic Surgery and Cancer Center, University of Minnesota, Minneapolis, MN, USA, [2]Vanderbilt University, Nashville, TN, USA.

Runx2 (Cbfa1) is a transcription factor required for osteoblast differentiation and chondrocyte hypertrophy. Like other Runt-domain proteins, Runx2 is an activator and repressor of transcription. Multiple repression and transactivation domains have been previously identified in Runx2. Thus far, no chromatin-altering enzymatic activities have been directly linked to Runx2, therefore, the molecular mechanisms by which Runx2 regulates tissue-specific gene transcription are not clear. Associations between other lineage-required transcription factors and counteracting proteins possessing either acetyltransferase or deacetylase activity are crucial for tissue-specific gene expression and cell differentiation. Thus, histone acetyltransferase (HAT) activity is associated with relaxed chromatin structure and enhanced gene transcription, while histone deacetylase (HDAC) activity is linked to condensed chromatin and transcriptional repression. The purpose of this study was to determine if proteins possessing HAT or HDAC activity interact with Runx2 and modify its activity. In activation studies, we have found that the HATs, p300 and CBP, but not P/CAF, cooperate with Runx2 to synergistically activate the osteocalcin promoter. In repression studies, we found that Runx2 represses the p21(CIP1/WAF1) promoter in a manner that is partially dependent on the carboxy-terminus of Runx2. This repression is sensitive to the HDAC inhibitor, trichostatin A. In co-immunoprecipitation-immunoblotting experiments, the penultimate carboxy-terminal repression domain of Runx2 interacted with HDAC-6. To begin to understand how Runx2-interacting co-activators and co-repressors affect Runx2 activity during osteoblast differentiation, we examined the expression of several co-factors in differentiating MC-3T3-E1 cells. Interestingly, p300 and HDAC6 are differentially expressed during osteoblast differentiation. p300 is expressed at high levels during the first twelve days of differentiation, but at markedly lower levels during days 14-28. In contrast, HDAC6 is transiently expressed during early stages of differentiation (days 2-14) but at steady levels thereafter. We conclude that interactions between Runx2 and cofactors possessing either HAT or HDAC activity are crucial for regulating Runx2 target gene expression. Moreover, differential expression of the co-factors may be one level of regulation affecting Runx2 activity during osteoblast differentiation.

F194

The Osteoblast Differentiation Factor RUNX2/Cbfa1 Is Suppressed by Tumor Necrosis Factor-alpha. L. C. Gilbert,*[1] X. He,*[1] J. Rubin,[1] H. Drissi,*[2] A. J. van Wijnen,[2] J. B. Lian,[2] G. S. Stein,[2] M. S. Nanes.[1] [1]Division of Endocrinology and Metabolism, Department of Medicine, Emory University and VAMC, Atlanta, GA, USA, [2]Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA.

The transcription factor RUNX2 (Cbfa1/AML3/Pebp2alphaA) is critical for determination of osteoblastic lineage during bone formation. We investigated the effect of the inflammatory cytokine tumor necrosis factor alpha (TNF) on the regulation of RUNX2 expression since TNF is an important contributor to bone pathology after menopause and RUNX2 levels in bone diminish with age. TNF inhibits the differentiation of fetal calvaria pre-osteoblasts and of the clonal preosteoblast cell line MC3T3-E1 (Gilbert et al Endo-

crinol 141:3956, 2000). TNF treatment of fetal calvaria cells on culture day 7 (just prior to expected nodule formation) or MC3T3-E1 cells on day 2 (pre-differentiation) caused a dose-dependent suppression of RUNX2 steady-state mRNA as measured by RT-PCR using primers flanking the conserved RUNT homology domain. The IC50 for TNF inhibition was 0.6 ng/ml. The effect of TNF was also studied using RUNX2 isoform-specific primers. TNF suppressed expression of the type 1 isoform mRNA 95% (p56/MRIPV) while inhibiting expression of the type 2 isoform mRNA 50% (p57/MANSN). RUNX2 nuclear content was evaluated by EMSA using a rat osteocalcin promoter 32P-RUNX2 binding sequence as probe and by western analysis. Antibody-specific supershift confirmed RUNX2 identity in the EMSA. TNF reduced nuclear RUNX2 binding to the probe by >90%. Western analysis showed that TNF decreased nuclear RUNX2 protein by >90%. Inhibition of new protein synthesis with cycloheximide failed to prevent TNF inhibition of RUNX2 mRNA. The half-life of RUNX2 mRNA is 2.3 HR as measured using actinomycin D, a value not significantly changed by TNF treatment. The effect of TNF on RUNX2 gene transcription was evaluated using a 0.6 kB RUNX2 promoter-luciferase reporter in transient transfected MC3T3-E1 cells. TNF caused a dose-dependent inhibition of RUNX2 promoter transcription to 50% of control values, similar to the inhibition of mRNA. Deletion analysis of the RUNX2 promoter suggested localization of a TNF inhibitory region downstream of -108; however, further localization was obscured by loss of basal promoter activity with progressive deletion. Our results suggest that TNF regulates RUNX2 expression at the transcriptional level but may have additional post-transcriptional inhibitory effects on RUNX2 nuclear content. Suppression of RUNX2 by TNF may decrease osteoblast differentiation and inhibit bone formation in TNF excess states.

F196

CNBP Enhances Bone Formation by Promoting Osteoblast Cell Proliferation and Inhibiting Osteocalcin Expression. K. Shimizu,* W. Chen,* Y. Liang,* R. Isoda,* W. Deng,* Y. Li. Department of Cytokine Biology, Forsyth Institute, Boston, MA, USA.

There is clear clinical evidence that genetic modulated osteoporosis results from functional defects of the osteoblast. However, the genes underlying these genetic syndromes of osteoporosis remain largely unknown. The purpose of this study was to characterize osteoblast transcription factors that regulate bone formation. Subtractive-differential screening was used to clone the transcription factors selectively expressed in osteoblasts. We identified Cellular Nucleic Acid Binding Protein (CNBP) that is predominately expressed in mature osteoblasts. We confirmed that CNBP exhibits similar up-regulated expression as osteocalcin during osteoblast differentiation by Northern Blot and In Situ Hybridization. To study in vivo function of CNBP in bone formation, we generated a mutant mouse strain with null mutation of CNBP. Homozygous mutants died in the prenatal period, while about 40% heterozygous survive to develop postnatal abnormalities associated with altered bone formation and severe osteoporosis. Northern blot showed that expression of CNBP was largely reduced in the heterozygous when compared with that in wild type mice. Forced expression of CNBP in osteoblastic cells resulted in the cells grew significantly more rapidly. The number of cells was about 2.5 fold when compared with that in the control group. Cotranfection study demonstrated that osteocalcin promoter activity was reduced to 20% of that of the control group without CNBP expression. Previous study in other Laboratory indicated that osteocalcin is an inhibitor of bone formation. Our results suggested that CNBP is a key regulator of bone formation. CNBP enhances bone formation by promoting osteoblast cell proliferation and inhibiting osteocalcin expression. Mutation of CNBP may be responsible for one of syndromes of osteoporosis.

F198

The Yes-Associated Protein (YAP) Is A Suppressor of Runx2/Cbfa1 Dependent Activation of the Bone Specific Osteocalcin Gene. S. K. Zaidi,[1] A. J. Sullivan,*[1] Y. Ito,*[2] A. J. van Wijnen,[1] J. L. Stein,[1] G. S. Stein,[1] J. B. Lian.[1] [1]Cell Biology, UMass Medical School, Worcester, MA, USA, [2]Viral Oncology, Kyoto, Japan.

The Yes-associated protein (YAP) transduces signals from receptor tyrosine kinases to the nucleus and interacts with members of the Runx family of transcription factors. This interaction is mediated through the WW domain of YAP and the highly conserved PY motif (PPPY) of Runx proteins. Runx2, which is essential for bone development, is a strong activator of the bone-specific osteocalcin promoter. Here we addressed the functional role of YAP in Runx2 mediated regulation of osteocalcin. In transient transfection assays, YAP had no effect on the basal osteocalcin promoter activity. However, when YAP was co-expressed with Runx2, a dose-dependent repression of osteocalcin was observed. When expression constructs encoding differently tagged Runx2 and YAP proteins were co-transfected, a strong interaction of YAP with Runx2 was observed by co-immunoprecipitation and immunofluorescence microscopy. This interaction was abolished when the PY motif of Runx2 was mutated (Runx2 Y433A). Functional analysis of Runx2 Y433A mutant showed 3-4 fold higher activation of osteocalcin promoter activity compared to the wild type Runx2, suggesting that the Y433A mutation relieves the suppression contributed by the endogenous YAP. To confirm these findings, we co-expressed wild type Runx2 with a dominant negative inhibitor of YAP function (YAP 1-301) and observed a similar activation of the osteocalcin promoter as that of the Runx2 Y433A mutant protein with the wild type YAP. Thus, interfering with function of the endogenous YAP inhibits YAP mediated suppression of the osteocalcin promoter. Taken together, these findings demonstrate that YAP is a potent suppressor of Runx2 dependent activation of the bone specific osteocalcin gene and suggest that YAP may contribute to physiological regulation of osteocalcin expression osteoblast differentiation.

F200

Developmental Utilization of Distinct Promoters Regulating the Type I and Type II Isoforms of the Bone Runx2/Cbfa1 Transcription Factor. H. Drissi, A. Javed, E. Balint, C. Banerjee,* F. Adamidou,* A. J. Van Wijnen, J. L. Stein,* G. S. Stein, J. B. Lian. Cell Biology, Umass Med School, Worcester, MA, USA.

The importance of the Runx2/Cbfa1 transcription factor during osteoblast differentiation and skeletal development has been demonstrated both in vitro and in vivo. Runx2/Cbfa1 encodes two major N-terminal isoforms (type I/MRIPV and type II/MASNS) which originate from two distinct promoters and are functionally equivalent in the activation and repression of bone-specific genes. The promoter dependent mechanisms by which the levels of these isoforms are regulated in vivo remain to be established. In this study, we examined developmental expression of the type I and type II isoforms in mouse embryos, mesenchymal cells and osteoblasts. Northern blot analysis shows expression of at least four Runx2 splice variants during osteoblast differentiation of mouse osteoblastic MC3T3-E1 cells and rat primary osteoblasts (ROB). RT-PCR using primers specific for mRNA encoding the type I and the type II mRNA of Runx2 shows that both transcripts are expressed in ROB cells at the late proliferation stage. The type I mRNA is constitutively expressed during osteoblast differentiation, whereas the type II mRNA is up-regulated along with osteocalcin transcripts during matrix maturation and mineralization stages. Expression of each isoform in specific mouse tissues was examined. While type II mRNA is restricted to bone, the type I isoform is present at high levels in several soft tissues, suggesting that this isoform is ubiquitously expressed. During mouse embryogenesis Runx2 type I mRNA is detected as early as 9.5 dpc, prior to appearance of the type II isoform, which is significantly expressed at 11.5 dpc in early mesenchymal condensations. Thus, there is differential induction of the two Runx2 promoters. The type I mRNA remains constitutively expressed throughout development until birth. In contrast the type II transcript is up-regulated between 12.5 and 14.5 dpc during cartilage formation and then down regulated between 15.5 dpc and birth, suggesting in vivo responsiveness of the Runx2 upstream promoter driving expression of the type II isoform to osteogenic signals. The regulation of these two isoforms in mesenchymal cell lines was assessed upon treatment with the osteogenic factor BMP2. Our findings indicate that BMP2 specifically up-regulates type II transcript levels in preosteoblastic and prechondrocytic clonal cell lines whereas the type I isoform is not regulated. Therefore our results provide insight into the developmental induction and tissue specific utilization of the two promoters regulating Runx2 activity during osteogenesis.

F202

Identification of Novel Protein/DNA Interactions within the Proximal Promoter of the Bone-Related Transcription Factor Runx2/Cbfa1. H. Drissi, A. Pouliot,* J. L. Stein,* J. B. Lian, G. S. Stein, A. J. Van Wijnen. Cell Biology, Umass Med School, Worcester, MA, USA.

Runx2/Cbfa1, which belongs to the runt homology family of transcription factors, is essential during bone development and regulates osteoblast differentiation through control of osteoblast phenotypic genes. Defining the regulatory mechanisms that govern expression of Runx2 transcripts is necessary to understand the precise role of this transcription factor in modulating the events that control skeletal development. We have previously reported cloning and characterization of the bone related Runx2 promoter from both rat and mouse. Deletion analysis showed that the proximal segment of the rat promoter contains several activating domains between nt -288 and nt -92 which are flanked upstream by a purine rich region and downstream by a negative Vitamin D responsive element. We systematically examined transcription factor binding within this region using a series of overlapping oligonucleotides for gel shift assays. We found two novel protein/DNA interaction sites within the very proximal promoter that are mediated by sequence specific factors based on cross-competition experiments and point mutations. Gel shift immunoassays show that the first complex recognizes a non-canonical Runx2 site, whereas the other factor binds to a palindromic sequence. Site directed mutagenesis of the novel Runx2 motif reduces promoter activity (2 fold), suggesting that this site mediates enhancement of Runx2 promoter activity. However, point mutation of the palindromic motif induced a 2 to 3 fold activation of the Runx2 promoter indicating that the wild type sequence mediates strong repression. The Runx2 site and the palindromic element function in the context of other protein/DNA interactions within the initial 300 base pairs of the Runx2 promoter, which are currently under investigation. These studies, together with our previous findings of auto-suppression of the Runx2 promoter and negative regulation by the steroid hormone 1-25 (OH)2 Vitamin D3, suggest considerable complexity in the Runx2 promoter to accommodate physiological control of Runx2 gene expression.

F204

The Human Protein A Kinase Adaptor Protein 2 Is a TNFalpha Regulated Gene in Human Osteoblasts and Localizes to the Actin Cytoskeleton. N. Schuetze,[1] S. Reichel,*[2] U. Barthelmes,*[2] N. Ruecker,*[2] J. Pfeufer,*[3] A. Lechner,*[2] F. Jakob.*[2] [1]Labor fuer Molekulare Experimentelle Orthopaedie, Orthopaedische Universitaetsklinik, Wuerzburg, Germany, [2]Medizinische Poliklinik, Wuerzburg, Germany, [3]Orthopaedische Universitaetsklinik, Wuerzburg, Germany.

Protein kinase A adaptor proteins (AKAP´s) associate with discrete cellular structures such as the centrosome as well as other organelles and are able to bind to the regulatory subunits of type II of protein kinase A (PKA). Thereby, a specific localization of the PKA holoenzyme is achieved. Applying differential display PCR to immortalized human osteoblasts we identified the novel human protein kinase adaptor protein 2 (AKAP-2) as an early regulated gene. No RNA or protein data except for a partial sequence are available in the literature to date for AKAP-2.The 7.5 kbp cDNA sequence was obtained by sequencing the ddPCR product, IMAGE clones, cDNA array-filter derived clones and gene specific RT-PCR products. RNA levels were measured by Northern hybridization. Intracellular localization was determined by transient transfection of an AKAP-2-GFP fusion protein

and control immunohistochemistry analyses were performed with organelle-specific antibodies.The AKAP-2 mRNA was found to be transiently regulated by TNFalpha 5-fold with a maximum after 5 hours of treatment (3.5 ng/ml). Actinomycin D as well as cycloheximide cotreatments indicate a transcriptional mechanism of regulation. Further, phorbol ester (TPA) also markedly enhanced AKAP-2 mRNA levels via a transcriptional regulation. The AKAP-2 sequence obtained from human osteoblasts differs from the existing database entry by additional 2.5 kbp at the 3´-end. An AKAP-2-GFP fusion protein localized to an unpaired structure within the cytoplasm. The protein contains three regions of coiled coil structures often observed for centrosomal proteins. Control immunohistochemistry analyses with a centrosomal specific antiserum applied to AKAP-2-GFP transfected osteoblasts indicated no colocalization of AKAP-2 to the centrosome. However, cytochalasin D cotreatments showed an association of AKAP-2 with the actin cytoskeleton. Since expression of AKAP´s is the prerequisite for subcellular distribution of PKA enzyme activity and subsequently numerous signal transductions events occur in osteoblasts, these findings suggest an important link between bone relevant growth factor function and intracellular PKA-dependent signaling pathways in human osteoblasts.

F206

The Ontogeny of Phex Protein in the Developing Mouse Skeleton and Studies on the Subcellular Distribution of Phex Protein in Osteoblasts. D. L. Thompson,*[1] Y. Sabbagh,*[2] H. S. Tenenhouse,[2] P. C. Roche,*[1] M. K. Drezner,[3] J. L. Salisbury,*[1] J. P. Grande,*[1] E. M. Poeschla,*[1] R. Kumar.[1] [1]Mayo Clinic/Foundation, Rochester, MN, USA, [2]McGill University, Montreal, PQ, Canada, [3]University of Wisconsin, Madison, Madison, WI, USA.

PHEX, a phosphate regulating gene with homologies to endopeptidases on the X chromosome, is mutated in X-linked hypophosphatemia in humans and mice (*Hyp).* Although recent observations indicate that Phex protein is expressed primarily in bone and may play an important role in osteoblast function and bone mineralization, the expression patterns of the Phex protein in the developing skeleton and the subcellular localization of the protein in osteoblasts remain unknown. We examined the ontogeny of the Phex protein in the developing mouse embryo and its subcellular localization in mouse osteoblast cultures using a specific antibody to the protein. Immunohistochemical staining of mouse embryos revealed expression of Phex in osteogenic precursors in developing vertebral bodies and developing long bones on day 16 p.c. and thereafter. Calvaria from day 18 p.c. mice showed Phex epitopes in osteoblasts. No Phex immunoreactivity was detected in lung, heart, hepatocytes, kidney, intestine, skeletal muscle, or white adipose tissue of mouse embryos. Interestingly, embryonic mouse brown adipose tissue showed moderate amounts of Phex immunostaining. In post-natal mice, Phex expression was observed in osteoblasts and osteocytes. Moderate expression of Phex was seen in odontoblasts and slight immunoreactivity was observed in ameloblasts. In permeabilized osteoblast cultures derived from normal and *Hyp* mice, confocal microscopy revealed the presence of immunoreactive Phex protein in the Golgi apparatus and endoplasmic reticulum in normal osteoblasts but not in *Hyp* osteoblasts. However, following transduction of *Hyp* osteoblasts with a human Phex viral expression vector, Phex protein was detected in the same subcellular compartments as normal osteoblasts. Our data indicate that Phex protein is expressed in normal osteoblasts and osteocytes during the embryonic and post-natal periods and that Phex may be a unique marker for cells of the osteoblast/osteocyte lineage.

F210

Lovastatin Stimulates, Rather than Inhibits, Proteasome Activity in vitro and in Osteoblasts. E. J. B. Murray,* S. S. Murray. GRECC/Medicine, VAGLAHS/UCLA, Sepulveda, CA, USA.

Others have hypothesized that 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitors ("statins") are anabolic with respect to bone through a mechanism involving inhibition of the chymotryptic activity of the proteasome. This hypothesis was based on structural similarities between some statins and some proteasome inhibitors. The aim of these studies was to determine the direct effects of lovastatin on proteasome activities in vitro and in MC3T3-E1 osteoblastic cells. The chymotryptic activity of the purified rabbit 20S proteasome was tested after preincubation with 0.0 to 5.0 uM lovastatin. The chymotryptic, tryptic, and peptidylglutamyl-peptide bond hydrolase (PGPH)-like activities of the proteasome were assayed in MC3T3-E1 osteoblastic cells after 4 hours to 4 days of treatment with lovastatin. Total protein and DNA were also measured in these cells in order to assess the possible anabolic effects of lovastatin on osteoblastic cells in culture. Lovastatin increased chymotryptic activity up to 20 fold in vitro in a dose dependent manner. Lovastatin increased DNA and protein content to a very modest degree in low density, but not high density, MC3T3-E1 cells. Lovastatin increased chymotryptic activity in low density cells. In high density cells, lovastatin increased chymotryptic and tryptic activities, but had no effect on PGPH-like activity. We conclude that lovastatin stimulates, rather than inhibits, proteasome activities in vitro and in cultured osteoblastic cells. Our studies do not support the hypothesis that lovastatin has an anabolic effect on bone that is mediated through an inhibition of the proteasome. Our results also suggest that other cell types in bone, such as osteoclasts, rather than osteoblasts, may be affected by lovastatin. Our conclusions are consistent with previous findings that indicate that proteasome activity is necessary for proliferation of osteoblastic cells.

F212

Wnt1, 2 and 3a Induce Osteoblast Commitment of Mesenchymal Pluripotent Cells C3H10T1/2 by a Beta-catenin Pathaway Dependent Signaling. G. Rawadi ,*[1] T. Garcia,[1] S. Spinella-Jaegle,*[1] S. Gallea,*[1] C. Faucheu,*[1] S. Kawai,*[1] R. Baron,[1] S. Roman-Roman.[1] [1]Aventis Pharma, Romainville, France.

Mammalian Wnt genes encode secreted glycoproteins that participate in normal embryonic development, tissue differentiation and cell proliferation. Several members of the Wnt family have been implicated in regulating chondrocyte differentiation and very recently Wnt3a has been shown to inhibit adipogenesis. Frizzled (Fz) genes encode seven membrane spanning proteins which act as receptors for Wnts. Data obtained by genome-wide expression analysis show the expression of Wnt and Fz genes in mouse calvaria and human trabecular osteoblasts. The involvement of Wnt signaling in osteoblast differentiation has not been so far addressed. We have investigated the effect of Wnt1, 2, 3a, 4 and 5a overexpression in the pluripotent mesenchymal cell line C3H10T1/2. Wnt1, 2, and 3a induced alkaline phosphatase (ALP) activity in this cell line whereas Wnt4 and 5a did not exhibit the same activity. In addition, Wnt 1, 2, and 3a synergized with BMP-2 to induce ALP activity in C3H10T1/2 cells. Molecular events leading to this synergy seems independent of Smad transcriptional activity. While Wnt1 and 3a signaling relays on beta-catenin, Wnt4 and 5a act independently of beta-catenin pathway. We have therefore examined the effect of beta-catenin in C3H10T1/2 cells. Interestingly, overexpression of a beta-catenin stable mutant mimicked the effect of Wnt1, 2, and 3a, strongly suggesting that these Wnt proteins induce ALP via beta-catenin signaling. Expression profiling of C3H10T1/2 cells treated with BMP-2 showed an increase in secreted related-frizzled protein 2 (SRFP-2) mRNA. SFRP proteins are known to act as a Wnt antagonist. Interestingly, overexpression of SRFP-2 significantly inhibited Wnt3a-induced ALP activity. On the contrary no inhibitory effect could be observed when cells were co-transfected with SRFP-2 and Wnt1 or 2. Altogether, our data suggest that Wnt/beta-catenin signaling play a role in osteoblast differentiation. The analysis of the activity displayed by different members of Wnt, Fz and SRFP families on osteoblast precursors and mature osteoblasts and the comprehension of the cellular events responsible for the activity described herein could help to define new approaches for treat osteopenia disorders.

F214

PGE2 Stimulates Bone Formation via EP4 Subtype of Prostaglandin E Receptor. K. Yoshida,*[1] T. Kobayashi,*[1] T. Tsuboyama,*[2] M. Matsushita,*[2] T. Nakamura,[2] S. Narumiya.*[1] [1]Department of Pharmacology, Kyoto University Faculty of Medicine, Kyoto, Japan, [2]Department of Orthopaedic Surgery, Kyoto University Faculty of Medicine, Kyoto, Japan.

Prostaglandins are proposed as important local factors that regulate both bone formation and bone resorption. Little has been known about their receptor mechanism in bone, though a receptor subtype EP4 was recently suggested to be the main factor in PGE2-induced osteoblast differentiation in vitro. In addition, PGE2 is known to induce a significant bone-forming effect when administered systemically or locally.To understand which of the four subtypes of PGE receptors (EP1-EP4) is involved in this process, we used mice deficient in each subtype of PGE receptors as well as subtype-specific agonists and antagonists. These compounds were examined by measuring the volume of newly formed callus after local infusion in vivo, and quantificaton of mineralized nodules in primary cultures of bone marrow cells in vitro.Each compound was infused locally into femoral periosteal tissue in the leg of mice with an implanted osmotic pump for 6 weeks, and the bone formation was investigated roentogenographically and histologically. PGE2 and a selective EP4 agonist caused a massive new bone formation on the cortices of femora in a dose-dependent manner, while agonists for other EP subtypes and two selective EP1 antagonists had little effect on bone. Infused PGE2 in mice lacking EP4 showed no bone-forming effect.On the other hand, each compound was added into primary bone marrow cell cultures from mouse femora. At the end of the 3-week culture period, the cells were stained by von Kossa method and the area of mineralized nodules was measured using an image analysis system. In these cultures, PGE2 and a selective EP4 agonist caused mineralized nodule formation in a concentration-dependent manner, while agonists for other EP subtypes and two selective EP1 antagonists had little effect. Bone marrow cells from EP4-deficient mice showed no increase in the area of mineralized nodules with PGE2 at all.In this study, we show that PGE2 and selective EP4 agonist stimulated new bone formation when administered in vivo, and induced mineralized nodule formation when added into the primary cultures in vitro. Consistent with these findings, PGE2 had neither osteogenic effect to EP4-deficient mice in vivo nor in vitro. These results suggest that the anabolic effect of PGE2 on bone formation is mediated via EP4 in vivo and in vitro.Recently, EP4 was also reported to be the main receptor in PGE2-induced osteoclast formation. The molecular cascade subsequent to the activation of EP4 in osteoblastic cells determining the two actions of PGE2 should be investigated in future studies.

F216

Loss of Pi Handling via Pit1, the Type III NaPi Cotransporter, Interferes with the Proliferation-Osteogenic Differentiation-Mineralization Sequence in Fetal Rat Calvaria Cell Populations. Y. Yoshiko,[1] K. Oizumi,[1] N. Maeda,*[2] J. E. Aubin.[1] [1]Anatomy and Cell Biology, University of Toronto, Toronto, ON, Canada, [2]Oral Anatomy, Hiroshima University, Hiroshima, Japan.

The ability of osteogenic cells to transport inorganic phosphate (Pi) appears to be an essential requirement for the onset of mineralization. Pi handling by osteogenic cells is thought to proceed via sodium-dependent phosphate (NaPi) cotransporters different from the kidney transporters. Recently, expression of type III NaPi cotransporters/retroviral receptor genes was determined in osteogenic cell lines and hypertrophic chondrocytes in vivo, but their functional roles have not yet been elucidated. We now report the molecular identity of NaPi cotransporters and their functional activities during the proliferation-osteogenic differentiation-mineralization sequence in fetal rat calvaria (RC) cell cultures. By RT-PCR of both RC cell populations and discrete single cell-derived RC osteogenic colonies, the type III (Pit1 and Pit2) and type Ib NaPi cotransporters were identified, but not the type Ia, type IIa and type IIb. Pit1 mRNA appears to be expressed at much higher level than Pit2 mRNA and the type Ib mRNA level is very low in RC cells. Unexpectedly, although NaPi uptake increased during the development sequence in RC cultures, Pit1 and Pit2 mRNA levels decreased. To investigate the possible involvement of Pi handling by osteogenic cells, the effects of phosphonoformic acid (PFA), an inhibitor of NaPi cotransport, were determined. Treatment of either monolyer or nodule-forming cells with PFA inhibited NaPi uptake. PFA treatment at different development time-windows, i.e., during proliferation, differentiation/nodule formation, or nodule maturation/mineralization stages,

caused distinct inhibitory effects on nodule formation and mineralization as well as osteoblast marker mRNA expression. An acute treatment for only 12-48 h before culture termination completely inhibited mineralization concomitant with a slight decrease in osteocalcin mRNA levels. Analysis of the early molecular consequences of PFA treatment also suggested that stanniocalcin 1, a newly identified calcium/phosphate regulator, rapidly increased. Treatment of cultures with antisense oligonucleotides to Pit1 mimicked PFA treatment. Taken together, these observations suggest that Pi handling via Pit1 activity in osteogenic cells plays a pivotal role not only in matrix mineralization but also in osteoblast development.

F219

FGD1, an Osteoblast Specific Cdc42GEF, Directly Interacts with mAbp1 and Cortactin, Actin-binding Src Kinase Targets. P. Hou,*[1] A. B. Vojtek,*[2] J. L. Gorski.*[1] [1]Pediatrics and Human Genetics, The University of Michigan Medical School, Ann Arbor, MI, USA, [2]Biological Chemistry, The University of Michigan Medical School, Ann Arbor, MI, USA.

Mutations in FGD1 result in faciogenital dysplasia, an inherited disease of skeletal formation. Consistent with its role in skeletal formation, FGD1 has a restricted pattern of expression that is limited to regions of incipient and active ossification including osteoblasts and osteosarcoma cell lines. FGD1 encodes a guanine nucleotide exchange factor (GEF) that specifically activates the p21 GTPase, Cdc42. By activating Cdc42, FGD1 stimulates actin cytoskeletal reorganization and filopodia formation. The FGD1 protein is composed of (in order) a proline-rich N-terminal region, a GEF domain, a pleckstrin homology (PH) domain, a FYVE domain, and a second PH domain (PH2) that are involved in signaling and subcellular localization; however, mechanisms by which FGD1 participates in osteoblast signaling remain unclear.Here we report the use of yeast two-hybrid analysis with an osteoblast derived cDNA library to identify two FGD1-interacting proteins, actin-binding protein 1 (mAbp1) and cortactin. Cortactin and mAbp1 are both Src kinase targets and contain similar functional domains including (in order) N-terminal actin-binding domains, a-helical regions, phosphorylation-targeted serine-/threonine-rich domains, and C-terminal Src homology 3 (SH3) domains. In yeast, FGD1 interacts with mAbp1 and cortactin SH3 domains via its N-terminal proline-rich region; studies show that the FGD1 SH3-binding domain (PQVPPKP) is responsible for the interactions. Far western blot analysis using purified FGD1 peptides show that FGD1 directly binds to purified mAbp1 and cortactin SH3 domains; in contrast, mutant FGD1 peptide fails to interact with these SH3 domains. Used to perform co-immunoprecipitation studies, cells overexpressing FGD1/mAbp1 or FGD1/cortactin show that FGD1 interacts with both mAbp1 and cortactin in vivo. These data indicate that FGD1 binds to mAbp1 and cortactin both in vitro and in vivo. Moreover, mAbp1 and cortactin colocalize with FGD1 to the subcortical actin cytoskeleton. This data indicates that the osteoblast specific Cdc42GEF FGD1 directly interacts with mAbp1 and cortactin. Accumulated data strongly suggest that FGD1 signaling plays an important role in osteoblast signaling and actin cytoskeletal reorganization.

F223

The Effect of IGF-I to Promote the Production of Osteoblast Progenitors Is Estrogen Dependent. Y. Kasukawa, L. Stabnov, N. Miyakoshi, D. J. Baylink, S. Mohan. Musculoskeletal Disease Center, JL Pettis VAMC, Loma Linda, CA, USA.

The pathogenesis of senile osteoporosis involves increased bone resorption that is not compensated by a corresponding increase in bone formation (BF). The age-related impairment in BF may be caused by a deficiency in the number of osteoblast progenitor cells (colony forming unit osteoblast, CFU-OB), which has been shown to decline with age. Based on the findings that the IGF-I level in both serum and bone decline with age and that IGF-I is an important regulator of BF, we proposed the hypothesis that a deficiency in the production and/or actions of IGF-I could contribute to the decline in the number of CFU-OBs and consequently impaired BF in old age. As a means of testing this hypothesis, we first tested if the number of CFU-OBs is decreased in the bone marrow cells (BMC) of growth hormone (GH) deficient lit/lit mice. We found that the number of CFU-OBs was decreased by 20% (361±50 vs 451±56 CFU-OB/106 BMC, P<0.01, n=7-9) in GH-deficient mice compared to age-matched control mice. Consistent with this data, we found that in vitro treatment of BMC derived from 8-week-old C3H mice with 30 ng/ml IGF-I for 10 days increased the number of CFU-OBs by 44% compared to vehicle-treated control cultures (P<0.01). We next evaluated if IGF-I treatment increases the number of CFU-OBs in vivo. Because recent findings demonstrate that estrogen activates the IGF-I signaling pathway by upregulating the expression of IGF-I downstream signaling molecules, we tested IGF-I effects on CFU-OBs in ovariectomized (OVX) and sham-operated C57BL mice at 12 months of age. Four weeks after surgery, IGF-I (2 ug/g body wt) or vehicle was administered twice on day 1, and 5 days later BMC were removed from femur and cultured for 10 days for CFU-OB assay (n=14-16 per group). IGF-I administration increased the number of CFU-OBs by 56% (P<0.001) and 28% (P<0.05), respectively, in sham and OVX mice compared to vehicle-treated control group. IGF-I treatment increased the total colony number by 28% (P<0.05) in the sham but not in the OVX mice (8%, P=0.44). As previously shown, OVX increased CFU-OBs and bone turnover markers significantly compared to sham mice. Serum IGF-I level was similar in OVX mice compared to sham mice, a finding which is different from that of rats in which OVX increases the serum IGF-I level. Conclusions: 1) The number of CFU-OBs was decreased in mice lacking GH. 2) IGF-I treatment increased the number of CFU-OBs both in vitro and in vivo. 3) IGF-I response on CFU-OBs was impaired in OVX mice compared to sham mice. 4) During estrogen deficiency, a decrease in the production and/or action of IGF-I could decrease the number of CFU-OBs and contribute to the age-related impairment in BF.

F225

Stromal Cells from Human Subcutaneous Adipose Tissue Support Hematopoiesis. J. M. Gimble,*[1] H. A. Leddy,*[2] S. Potiny,*[2] D. M. Franklin,*[3] K. Hicok,*[4] A. L. Kloster,*[4] R. W. Storms.[2] [1]Tissue Engineering, Artecel Sciences, Inc., Durham, NC, USA, [2]Duke University Medical Center, Durham, NC, USA, [3]Zen-Bio, Inc., Research Triangle Park, NC, USA, [4]Artecel Sciences, Inc., Durham, USA.

It is well established that bone marrow stromal cells capable of supporting hematopoiesis can also undergo adipocyte differentiation. The current study evaluated the converse hypothesis; can stromal cells derived from human subcutaneous adipose tissue support hematopoiesis? Flow cytometry revealed that adipose-derived stromal cells expressed cell surface proteins associated with hematopoietic support, including CD9, CD10, CD13, hyaluronate receptor (CD44), VLA4 (CD29/CD49d), endoglin (CD105) and ALCAM (CD166). In response to inducing agents, the adipose stromal cells expressed the following cytokines based on PCR and ELISA assays: M-CSF, GM-CSF, Flt 3 ligand, Kit ligand, LIF, TNFa, IL-6, IL-7, IL-8 and IL-11. To assess the stromal cells capacity to support hematopoiesis, human umbilical cord blood CD34+ cells were co-cultured with adipose stromal cells in vitro. The stromal cells promoted the growth of CD13+,CD14+ and/or CD15+ myeloid cells; myeloid colony forming units were present at a frequency of 40 per 100 CD34+ cells after 3 weeks and 82 per 1000 CD34+ cells after 6 weeks. After 12 days in co-culture, the total hematopoietic cell number increased by 5.1 ± 3.2-fold; 57.6% ± 9.7% of these cells were CD34+, reflecting a 2.7 ± 1.3-fold expansion. Over a 3 week period, the number of CD34+ cells in the co-cultures expanded by approximately 7-fold. In both the absence and presence of exogenous supplemental cytokines (KL, Flt3 L, IL-2, IL-7 and IL15), the adipose-derived stroma promoted the development of lymphoid lineage cells (CD7+, CD10+) within a 12 day period; the presence of lymphoid cells was maintained for up to 6 weeks in the co-culture conditions. This novel work shows that adipose tissue provides an alternative to bone marrow as a source of stromal cells for the ex vivo expansion of hematopoietic progenitors from umbilical cord blood or other sources. Since adipose tissue is accessible, abundant and replenishable, it has significant potential for tissue engineering applications in the clinical arena.

Disclosures: Artecel Sciences,1,2,3,5.

F228

Regulation of Cathepsin K Promoter Activity. M. Gyda,* B. R. Troen. Mount Sinai School of Medicine, Bronx VAMC, Bronx, NY, USA.

Osteoporotic fractures lead to significant morbidity and mortality in the elderly. An essential component of bone resorption is the synthesis and secretion of cathepsin K (CTSK), a cysteine proteinase, by osteoclasts. In order to characterize the molecular mechanisms of bone resorption, we are studying the regulation of cathepsin K gene expression. We have isolated and cloned the rat cathepsin K gene and characterized its structure using a combination of sequencing and PCR. It has 8 exons and 7 introns, and exons 2 through 7 are the same size as in the mouse CTSK gene. The transcribed portion of the gene is 92% similar to the mouse gene, and the first, second and third introns of both rat and mouse genes display 73 to 82% similarity. There are no classical TATA or CAAT boxes in the 5' flanking region of the rat cathepsin K gene. The rat cathepsin K gene contains canonical binding sites in both the promoter and the first intron for nuclear factors that play critical roles in osteoclast development and activation; these include AP-1, PU.1, micropthalmia (Mitf), and interferon-γ (IFN-γ) activated (GAS) motifs. To investigate transcriptional regulation of the CTSK gene, we have transiently transfected RAW 264.7 cells with CTSK promoter-firefly luciferase reporter gene (CTSK-Luc) plasmids and normalized activity with a co-transfected renilla luciferase control vector. Cells transfected with rKLPE1 (containing 1628 bp of the promoter region of the cathepsin K gene) exhibit 50 fold greater activity than cells transfected with the promoterless vector (GL3B). Cells transfected with the rKSPE1 vector containing only 74 bp of the 5' flanking region of the gene exhibit no more luciferase activity than those transfected with GL3B. Nested deletion mutation analysis reveals a reproducible pattern of marked enhancement and reduction in CTSK-luciferase activity based upon the length of the promoter in the plasmid. IFN-γ treatment (25 ng/ml) of the cells transfected with rKLPE1 suppresses luciferase activity by 75%. In order to study the role of members of the ETS nuclear transcription factor family in regulating CTSK gene expression, we co-transfected SV40-PU.1 and SV40-ETS2 expression vectors with CTSK-Luc plasmids and observed increases in promoter activity of 2.5 to 5-fold. Therefore, IFN-γ inhibits transcription, whereas PU.1 and ETS2 stimulate transcription of the CTSK gene. Additional transfection and gel mobility shift analyses will allow us to identify critical motifs in the promoter that regulate CTSK gene expression in response to IFN-γ, PU.1, and ETS2, to characterize nuclear binding factors that mediate the responses to IFN-γ, and to further elucidate the molecular basis of osteoporosis.

F233

Dynamin Plays a Role in Osteoclast Motility and its Interaction with Cbl is Differentially Modulated by the Expression of Src or Pyk2. A. Bruzzaniti,*[1] A. Sanjay,[1] L. Neff,[1] C. Bardelay,*[2] E. Antoine,*[2] K. Lee,*[1] P. DeCamilli,[1] R. Baron.[1] [1]Yale University School of Medicine, New Haven, CT, USA, [2]Aventis Pharma, Paris, France.

Osteoclast motility requires attachment to integrins, recruitment of signaling proteins and subsequent cystoskeletal reorganization resulting in the formation of podosomes. Following engagement of the vitronectin receptor (VnR), Cbl, Src and Pyk2 interact to form a trimolecular signaling complex which plays a role in osteoclast attachment and motility. The absence of this signaling complex most likely contributes to the osteopetrotic phenotype observed in Src-/- mice (Sanjay et al., JCB, 152:181-195, 2001). Since podosome formation requires rapid actin turnover, we examined whether dynamin, a large GTPase involvement in endocytosis and remodeling of the cell membrane, interacts with Cbl and other signaling molecules downstream of integrins and if so, whether it plays a role in osteoclast motility. Previously, we demonstrated that dynamin II co-localized with podosomes in osteoclasts and in v-Src transformed cells, and that mutation within the GTPase domain of dynamin abolished podosome formation (Ochoa et al., JCB, 150:377-389, 2000). In the current study, we used co-immunoprecipitation followed by Western blot analyses to demonstrate that Cbl constitutively interacts with endogenous dynamin in osteoclast-like cells. Cbl and dynamin were also co-immunoprecipitated when overexpressed in HEK-293 cells stably expressing the VnR (293-VnR). Formation of the Cbl-

dynamin complex was adhesion-independent, but was differentially modulated by the expression of Src or Pyk2. Interestingly, over-expression of c-Src had a dominant-negative effect on Cbl-dynamin association, while inhibition of c-Src kinase activity with PP1, increased their association. Unlike c-Src, over-expression of Pyk2 enhanced Cbl-dynamin complex formation, perhaps by sequestering c-Src to the cell membrane. We next examined the effect of cytokine-induced phosphorylation on Cbl-dynamin association. EGF treatment decreased Cbl-dynamin complex formation in 293-VnR cells, and disrupted podosome and actin ring formation in osteoclasts. Finally, cyclosporin A and cypermethrin, specific inhibitors of the Ca-dependent phosphatase calcineurin, prevented the dephosphorylation of dynamin, and also disrupted podosome formation and decreased osteoclast motility. Overall, these studies suggest that dynamin II plays a role in osteoclast attachment and motility, possibly by interacting with Cbl and thereby affecting the formation of the Src/Cbl/Pyk2 trimolecular complex.

F236

Interleukin-7 Is a Direct Inhibitor of Osteoclastogenesis. S. Lee, J. Kalinowski,* S. Jastrzebski,* V. Katavic,* L. Puddington,* J. A. Lorenzo. University of Connecticut Health Center, Farmington, CT, USA.

Some cells expressing B220, a B lymphocyte antigen, can differentiate *in vitro* into osteoclast-like cells (OCL). Interleukin-7 (IL-7) is a major regulator of B-lymphopoiesis that is produced in bone marrow stromal cells. Previous studies found that systemic IL-7 administration enhanced osteoclastogenesis *in vivo* and interleukin-7 receptor deficient mice had increased trabecular bone mass. We examined the direct effects of IL-7 on osteoclastogenesis in bone marrow cultures. We also examined *in vitro* OCL formation in cells from IL-7 deficient mice and their bone mass *in vivo*. OCL were measured as TRAP(+) multinucleated cells. Bone mass in the humerus was measured by μ computerized tomography (SCANCO μCT-20).IL-7 inhibited OCL formation in M-CSF and RANKL (both at 30 ng/ml)-stimulated bone marrow cultures. Significant inhibitory effects were seen at 1 (57%) and 10 ng/ml (86%). IL-7 (10 ng/ml) treatment for the first 4 days of a 6-day culture was as effective as treatment for the entire period (77% inhibition). IL-7 also inhibited ($p<0.05$) OCL formation in bone marrow cultures that were treated with vitamin D_3 (10^{-8}M) (60%) or PTH (100 ng/ml) (54%). We found no significant effect of IL-7 treatment on the expression of RANKL or OPG mRNA as measured by RT-PCR in bone marrow cultures (treated with or without vitamin D_3, PTH or RANKL). IL-7 (10 ng/ml) increased the number of $B220^+$ cells (from 47% to 85%) in bone marrow cultures by flow cytometry.Bone marrow cells from IL-7 -/- mice showed a significant ($p<0.05$) increase in TRAP(+) OCL numbers in cultures that were stimulated with vitamin D_3 (136±13.3%) or PTH (196±18.8%) compared to cells from IL-7+/-. In addition, the number of granulocyte-macrophage colony-forming units (CFU-GM) in bone marrow of IL-7 -/- mice was 3 fold greater than in +/- controls. $B220^+$ bone marrow cells from IL-7 -/- mice demonstrated a 9 fold increase in TRAP (+) OCL numbers compared to cells from IL-7 +/- ($p<0.05$). IL-7 -/- animals also showed a 37% reduction in trabecular bone volume (BV/TV) and a 66% increase in trabecular separation (Tb.Sp) compared to IL-7 +/- animals ($p<0.05$).These results demonstrate a direct inhibitory effect of IL-7 on OCL formation *in vitro*. We also found that bone marrow cell cultures from IL-7 KO mice have a markedly increased capacity to form osteoclasts in unfractionated and $B220^+$ cell cultures. Furthermore, IL-7 -/- animals had significant trabecular bone loss suggestive of increased osteoclast activity. These findings are in contrast to previous reports that examined *in vivo* IL-7 administration or IL-7 receptor deficient mice. We conclude that in the present mouse models IL-7 is a direct inhibitor of osteoclastogenesis, which affects bone turnover *in vivo*.

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RANKL or TNF-alpha-Induced Osteoclastogenesis Requires TRAF5. K. Kanazawa,*[1] Y. Azuma,*[2] H. Nakano,*[3] A. Kudo.*[1] [1]Tokyo Institute of Technology, Yokohama, Japan, [2]Teijin Institute for Biomedical Research, Teijin Limited, Tokyo, Japan, [3]Department of Immunology Juntendo University School of Medicine, Tokyo, Japan.

TRAF families are intracellular adapter proteins that are involved in signal transduction pathways initiated by cell surface receptors, mainly TNF receptor family. TRAF2,-5,-6 activate NF-kB and JNK that are essential for osteoclast differentiation. We investigated TRAF5 function by using TRAF5 deficient mice in-vitro and in-vivo. Osteoclast progenitors were obtained by culturing bone marrow cells in the presence of M-CSF. To examine the ability of osteoclast differentiation, TRAF5+/+ and -/- osteoclast progenitors were differentiated into TRAP positive multinucleated cells (MNCs) by treating with sRANKL (50ng/mL) or TNF-alpha (50ng/mL) in the presence of M-CSF. The number of TRAP(+)MNCs from TRAF5-/- compared to TRAF5+/+ was 75% with RANKL, or 50% with TNF-alpha. Resorption pits on the osteologics were detected in mature osteoclasts differentiated from TRAF5-/- osteoclast progenitors. As in-vivo system, we employed hypercalcemia model mice by the continuous injection of PTH, and serum Ca concentration was measured. Although the peak of the Ca concentration was observed at the third day after injection in TRAF5+/+ mice, however, it was detected at the fifth day in -/- mice, demonstrating the delay of osteoclast differentiation in -/- mice. Because the delay of osteoclast differentiation was thought as the functional disorder of stromal cells, we examined calvarial cells that expressed TRAF5. In the presence of VtD3, osteoclast progenitors were co-cultured with calvarial cells from TRAF5+/+ or -/-. Because the number of TRAP(+)MNCs from TRAF5-/- was similar to that from TRAF5+/+, we conclude that TRAF5-/- stromal cells didn't influence the defect of osteoclast differentiation. After stimulation with RANKL or TNF-alpha, NF-kB and JNK were activated in osteoclast progenitors from TRAF5-/- as well as +/+, suggesting the unknown signal pathways in osteoclast differentiation. The decline of osteoclast differentiation in TRAF5-/- in vitro reflects the delay of osteoclast differentiation in vivo. We speculate that in -/- mice, unknown signal pathways via TRAF5 are disrupted. Here, we have provided the first evidence demonstrating TRAF5 function in osteoclast differentiation.

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Regulation of Mouse Osteoprotegerin Gene Expression by 1,25 Dihydroxyvitamin D_3. T. Kondo,* R. Kitazawa, S. Maeda,* S. Kitazawa. Division of Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan.

Vitamin D_3 ($1,25(OH)_2D_3$) promotes osteoclastogenesis by upregulating the expression of receptor activator of NF-κB ligand (RANKL) gene and by downregulating that of osteoprotegerin (OPG) gene. To investigate the mechanism of the latter process, we analysed the effect of $1,25(OH)_2D_3$ on cis-acting elements of the mouse OPG gene and on the posttranscriptional modification process. The 5'-flanking region of the mouse OPG gene was cloned, and a series of deletion constructs ligated with a pGL3-Basic vector plasmid (Luc -116, Luc -697, Luc -1125, Luc -1487) were transfected into ROS17/2.8 and ST2 cells and subjected to luciferase assay. Transfected cells were treated with $1,25(OH)_2D_3$ (10nM) for 12h to assess its effect on the promoter activity. A DNA construct containing 116bp upstream of the transcription start site demonstrated basic promoter activity. Vitamin D_3 reduced the promoter activity of Luc -697, Luc -1125, and Luc -1487 to 70%. Electrophoretic gel motility shift assay (EMSA) was then carried out to determine the protein-DNA binding on the putative AP-1 binding site (-293/-287, TGACTGA). The nuclear extract from ROS17/2.8 cells was subjected to the binding reaction with ^{32}P-labeled oligonucleotide spanning the putative AP-1 binding site, and antibodies against c-Jun, Jun-D, Jun-B, c-Fos, Fos-B, Fra-1, and Fra-2 were used for the supershift reaction. EMSA showed specific binding to the putative AP-1 site and the supershift with an anti c-Jun antibody; the mutation of the putative AP-1 site (TGACTGA to CTCCCTC) nullified the protein-DNA binding. A construct containing the mutated AP-1 binding site (Luc -1487m) showed reduced promoter activity. Moreover, the Luc -1487m construct was resistant to $1,25(OH)_2D_3$-driven suppression. Since the OPG promoter has no consensus negative VDREs, the negative effect of $1,25(OH)_2D_3$ on promoter activity may be mediated by an indirect mechanism, probably through the AP-1 binding site (-293/-287). To assess the effect of $1,25(OH)_2D_3$ on OPG mRNA stability, ST2 cells were treated with $1,25(OH)_2D_3$ (10nM) in the presence of actinomycin D (10nM) and subjected to Northern blotting. OPG mRNA decreased significantly in $1,25(OH)_2D_3$-treated ST2 cells, suggesting that $1,25(OH)_2D_3$ shortens the halflife of OPG mRNA. It is therefore speculated that $1,25(OH)_2D_3$ negatively regulates the OPG gene by accelerating degradation of OPG mRNA as a main pathway and, additionally, by suppressing the OPG promoter activity through the AP-1 site.

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Importance of IL-1 and TNF-a in Physiological Bone Remodeling. H. Yasuda,[1] Y. Lee,*[1] S. Nakae,*[1] R. Horai,*[1] K. Sekikawa,*[2] M. Asano,*[3] Y. Iwakura.*[1] [1]Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Department of Immunology, National Institute of Animal Health, Tsukuba, Japan, [3]Institute of Experimental Animals School of Medicine, Kanazawa University, Kanazawa, Japan.

Inflammatory cytokines such as IL-1 and TNF-a play a major role in bone resorption in pathological conditions (e.g. Rheumatoid arthritis and Periodontal diseases). However, the roles of these cytokines in bone remodeling in physiological conditions are unknown. Previous studies demonstrated that no obvious abnormality in bone in IL-1 receptor type I (IL-1R1) KO mice and TNF-a receptor type I (TNFR1) KO mice whose genetic background were C57BL/6 x 129/SV. In the present study we addressed the role of IL-1 and TNF-a in physiological bone remodeling using IL-1a,b double KO mice, TNF-a KO mice, and IL-1a,b, TNF-a triple KO mice, all of which were backcrossed to BALB/cA strain mice for 8 generations. Measurement of Bone Mineral Density (BMD) of femur with dual energy X-ray absorptiometry revealed significant increases in 8-week old mice with each genotype; IL-1a,b KO, 12.1%; TNF-a KO, 9.9%; IL-1a,b, TNF-a KO, 24.8% relative to wild type. Radiographs showed massive increase in bone density especially in the epiphysis and metaphysis of femur of these KO mice. Histological analysis also showed that marked increase of bone volume in trabecular bone of these KO mice. The thickness of cortical bone also was increased in these KO mice. The morphology of the growth plate and the columnar organization of chondrocytes were normal, but cartilaginous remnants were markedly observed in the cortical bone of these KO mice, which suggests a decrease in osteoclastic activity in resorption of bone and cartridge. Preliminary results of in vitro culture showed that osteoclast differentiation and/or activation is partially impaired in bone marrow of these KO mice. Taken together these results indicate that IL-1 and TNF-a have an important role in physiological bone remodeling and their effects are synergistic.

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A Peptide Antagonist Mimicking the 3rd TNF-like Cysteine-Rich Domain of Mouse OPG Inhibits RANKL/M-CSF Induced Osteoclastogenesis. S. Li,*[1] E. Lee,*[1] Y. Jin,*[1] R. Namgung,[2] S. Lim.[1] [1]Internal Medicine, Medical School of Yonsei University, Seoul, Republic of Korea, [2]Department of Pediatrics, Medical School of Yonsei University, Seoul, Republic of Korea.

OPG, as a decoy receptor, is well known to inhibit osteoclastogenesis by preventing RANKL from binding to RANK. To study which region of OPG is important for its biological activity and to develop the therapeutic peptidomimetics, we deleted the extracellular domains of OPG and synthesized oligopeptides according to the sequence of the 3rd TNF-like cysteine-rich domain of mouse OPG. Several deleted forms of mouse OPG DNAs, encoding 401(OPG1)-, 194(OPG2)-, 123(OPG3)-, 110(OPG4)-, 73(OPG5)-, and 78(OPG6)-amino acid residues, were constructed by using PCR method. They were then subcloned into pMT/V5-His, an inducible expression vector for insect cell (S2 cell). After stable transfection to the S2 cell with the constructed DNAs, we obtained several S2 cell lines stably over-expressing different OPGs in the secreted forms, followed by identification with western blotting analysis. Osteoclasts were successfully induced from mouse bone marrow cells with 3~5 days treatment of M-CSF and RANKL, and were identified using TRAP assay and acid phosphatase assay. The CuSO4-induced media obtained from each cell lines were applied to the bone marrow cells together with M-CSF and RANKL at

the beginning of culture. Compared to the positive control that were treated only by M-CSF and RANKL, the OPG1 and OPG2 showed strong inhibitory effect on the osteoclast formation and the acid phosphatase activity after 3~5 days co-treatment. However, the induced media containing OPG3, OPG4, OPG5 and OPG6, respectively, failed to show any inhibitory activity in the TRAP assay and the acid phosphatase assay. In addition, three oligopeptides, which were designed according to the sequence of the 3rd TNF-like cysteine-rich domain of mouse OPG, were also tested to study if short oligopeptide could produce a similar inhibitory action as the fragmented OPGs. Interestingly, two of three oligopeptides showed the significantly inhibitory effect on osteoclastogenesis only at high concentration (10-4M). These results suggest that not only the domain 3 and 4, but also domain 1 and 2 do be necessary for full biological activity of OPG, and the short oligopeptide being capable of interfering RANK ligand and RANK interaction, thereby decreasing the osteoclastogenic potential of this cytokine, could be developed.

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Estrogen Regulates the Production of VEGF for Osteoclast Formation and Activity in Osteopetrotic *op/op* Mice Lacking Functional M-CSF. I. Kodama,[1] S. Niida,[2] M. Sanada,[1] Y. Yoshiko,[2] M. Tsuda,[1] N. Maeda,[2] K. Ohama.[1] [1]Obstetrics and Gynecology, Faculty of Medicine, Hiroshima University, Hiroshima, Japan, [2]Anatomy, Faculty of Dentistry, Hiroshima University, Hiroshima, Japan.

Purpose: It is recognized that the estrogen deficiency increases the macrophage colony- stimulating factor (M-CSF) production in stromal cells to support osteoclastic bone resorption. We recently demonstrated that angiogenetic cytokine, vascular endothelial growth factor (VEGF), could substitute for M-CSF in the osteoclast recruitment in the osteopetrotic *op/op* mice lacking functional M-CSF. Furthermore, we found that estrogen deficiency induced by ovariectomy (OVX) up-regulated osteoclastic bone resorption in these mice. We therefore assessed the effects of VEGF on the bone loss induced by estrogen deficiency in these mice. **Design and methods:** *Op/op* mice 8 weeks of age were bilateral OVX or sham-operated. Mice were sacrificed at 8, 10, and 12 weeks of age and femurs were removed for preparations. Some OVX mice were treated with the three consecutive injections of 120 micro liters per body of VEGF neutralizing antibody at 12 hours intervals starting from 36 hours before sacrificing at 4 weeks after OVX. VEGFR-1/Fc chimeric protein (600 micrograms / kg per day) setting in the implanting Alzet 1002 osmotic pumps or the implanting slow release subcutaneous pellets of 17beta-estradiol were administrated in a dorsal subcutaneous pocket of the mice at the time of OVX. These mice were sacrificed at 2 weeks after surgery. Changes of serum levels of VEGF were measured by enzyme linked immunosorbent assay (ELISA). Changes of messenger RNA (mRNA) levels of VEGF, VEGF receptor-1/Flt-1, and osteoclast differentiation factor (ODF/TRANCE/RANKL) in bone tissue were measured by RT-PCR. **Results:** Trabecular bone volume of femur decreased significantly in OVX-*op/op* mice. The number of TRAP-positive osteoclasts increased significantly in OVX mice. Osteoclasts induced by OVX predominantly expressed Flt-1. But we could not detect Flk-1/KDR in these osteoclasts. Osteoblasts in OVX mice expressed VEGF stronger than osteoblasts in Sham-operated mice. Increase of TRAP-positive cell number was inhibited by not only 17beta-estradiol treatment but also VEGF antagonistic treatment. Serum levels of VEGF demonstrated higher in OVX mice than Sham-operated mice. VEGF mRNA, Flt-1 mRNA, and ODF mRNA levels in bone tissue, demonstrated higher in OVX mice than Sham-operated mice. **Conclusions:** The production of VEGF stimulated by estrogen deficiency resulted in increase of the osteoclastic bone resorption in M-CSF deficient *op/op* mice. Furthermore, our results demonstrated that VEGF signal was predominantly mediated through Flt-1.

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The Novel Molecule on Mouse Stromal Cells, OCRA, Regulates Osteoclastogenesis. S. Arai,*[1] N. Amizuka,[2] Y. Azuma,[3] A. Kudo.[4] [1]Tokyo Institute of Tecnology, Kanagawa, Japan, [2]Niigata University, Niigata, Japan, [3]Teijin institute for Biomedical Research, Tokyo, Japan, [4]Tokyo Institute of Technology, Kanagawa, Japan.

Osteoclastogenesis is regulated by RANKL expressed on stromal cells. We have observed that RANKL expressed on the mouse pre-B cell lines stimulated none or less osteoclastogenesis, suggesting that RANKL alone on the cells hardly regulates osteoclastogenesis, and osteoclastogenesis requires another molecule or system on stromal cells for effective activation. Here, we tried to isolate new surface molecules on stromal cells by establishing monoclonal antibodies. The mouse stromal cell line, TSB13, which can support osteoclastogenesis, was immunized into a rat, and a monoclonal antibody, A15-1, was chosen. A15-1 bound to the surface antigen of TSB13, termed OCRA (osteoclastogenesis related antigen). A15-1 was positive for mesenchymal cell lines, but negative for hematopoietic cell lines analyzed by flow cytometry. The expression level of OCRA was slightly induced by the treatment of VtD3. Immunoprecipitation revealed that OCRA is 55 kDa. When A15-1 was incubated in the co-culture of osteoclast progenitors and TSB13 in the presence of VtD3, the osteoclast cell number was decreased in the antibody dose-dependent manner although no significant changes were observed in the RANKL-induced osteoclastogenesis in the same antibody conditions. The result showed that A15-1 inhibited only stromal dependent osteoclastogenesis. Using other osteotropic factors, PTH and IL-1β, the inhibition of A15-1 for osteoclastogenesis was also observed. Next, we examined the stage of A15-1 inhibition in the osteoclast differentiation system in various time courses, indicating that the initial 2 days treatment of A15-1 was good enough for inhibition, therefore A15-1 inhibits the early stage of osteoclast differentiation. The inhibitory effect of A15-1 was tested in the primary stromal cells derived from bone marrow. Bone marrow cells cultured in the presence of VtD3 were treated with A15-1, resulting that A15-1 inhibited osteoclastogenesis dose-dependently. The result showed that A15-1 acts on bone marrow stromal cells, which have the supportive activity for osteoclastogenesis. In in-vivo, mice for PTH-induced hypercalcemia were treated with A15-1. The enhanced number of osteoclasts was significantly decreased in the treatment of A15-1, and A15-1 restored the severe level of the osteoclast related parameters, ES/BS and N.Oc/B.Pm to the normal, although osteoblast related parameters were not significantly altered. This study revealed that A15-1 antigen, OCRA, regulates osteoclastogenesis on stromal cells.

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Ovariectomy-Induced Increases in Osteoclastogenesis in Mice Are IL-1 R1 Dependent and Mediated by B220$^+$ Bone Marrow Cells. V. Katavic,* S. K. Lee, H. L. Aguila,* L. Puddington,* J. A. Lorenzo. University of Connecticut Health Center, Farmington, CT, USA.

Ovariectomy (OVX) increases the number of osteoclast (OCL) and lymphocyte precursor cells in the bone marrow of mice. As a result, OCL formation increases and bone loss occurs. We suspect that osteoclasts and B-lymphocytes share a common progenitor within the bone marrow. This OCL progenitor appears to have a B220$^+$/RANK$^-$ phenotype. We previously showed that the number of OCL formed in B220$^+$ cell cultures from OVX mice is 2-fold greater than from sham operated (SHAM) mice. We also showed that type I interleukin-1 receptor (IL-1 R1) deficient (KO) mice do not lose bone mass after ovariectomy. In this study, we examined differences in OCL formation in bone marrow cell cultures from SHAM and OVX wild type (WT) and KO mice, 3 weeks after surgery. OCL formation was stimulated by treatment with M-CSF and RANKL (30 ng/mL for each, $1x10^5$ cells/well of a 96 well plate for 6 days). OCL were identified as TRAP$^+$ multinucleated cells. These cells expressed abundant calcitonin receptors and resorbed bone.The total number of bone marrow cells increased with OVX by 51.9±3.8% in WT ($p<.001$) and 22.8±2.6% in KO mice ($p<.001$). The percentage of B220$^+$ bone marrow cells increased 1.9-fold in WT mice (SHAM=24.7±2.0%, OVX=47.1±0.8%, $p<.001$), and 1.6-fold in KO mice (SHAM=25.3±1.9%, OVX=41.2±3.1%, $p<.001$). There were no differences between WT and KO mice in the ability of OVX to increase the number of pro-B or pre-B-lymphocytes (HSA^{low} or HSA^{high} respectively, both B220$^+$/IgM$^-$) or the percentage of cells expressing c-Fms (the M-CSF receptor) in bone marrow. Unfractionated bone marrow cultures from WT OVX mice showed a 1.8-fold increase in OCL formation compared to SHAM ($p<.05$). However, in cultures from KO mice, there was no change in OCL formation with OVX. Using FACS, we separated bone marrow cells into B220$^+$ (purity≥98.5%) and B220$^-$ populations (purity≥99.8%). In both WT and KO B220$^-$ cell cultures OCL formation was not affected by OVX. In B220$^+$ WT cells OVX increased the number of OCL by 2-fold ($p<.05$). In contrast, in B220$^+$ KO cells there was no effect of OVX on OCL formation.These results demonstrate that OVX increases the number of B-lymphocyte precursors in both WT and KO mice. However, OVX increases OCL formation in cultures of whole bone marrow and B220$^+$ cells only from WT and not KO mice, suggesting that OVX-induced increases in OCL formation are dependent on the presence of IL-1 R1. We conclude that the ability of estrogen withdrawal to increase osteoclastogenesis in bone marrow cells is mediated by changes in the B220$^+$ bone marrow population through an IL-1 R1 dependent pathway.

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Phagocytosis Determines Lineage Destiny of Macrophage-Osteoclast Precursors Incubated in TNF-α/RANKL Through Smad7 Induction and Suppression of TGF-β Signaling. K. Fuller, K. E. Bayley,* T. J. Chambers. Cellular Pathology, St George's Hospital Medical School, London, United Kingdom.

It has recently been found that TNF-α, like RANKL, can induce osteoclast formation in vitro from cells of the mononuclear phagocyte system. This is unexpected, since TNF-α is considered crucial to macrophage activation in host defense, yet osteoclasts are not seen in inflammatory tissue. Presumably, there are additional factors in vivo that determine the lineage response of macrophage-osteoclast precursors to TNF-α. It has been suggested that osteoclast-induction by TNF-α in vitro is due to 'priming' by RANKL in stromal cell-contaminated cultures, but we could find no evidence for this. One of the characteristics of all inflammatory tissues is that they contain endocytogenic material. We therefore tested the response of osteoclast-macrophage precursors to TNF-α in the presence vs the absence of phagocytic targets. We found that formation of tartrate-resistant acid phosphatase positive multinuclear cells (TRAP cells) was strongly suppressed in cultures to which latex particles, glutaraldehyde-fixed red cells, or necrotic thymocytes were added. Inhibition was observed even when TGF-β was added to cultures, or when precursors were incubated on bone slices, conditions which otherwise strongly enhance osteoclast-induction. Importantly, incubation of precursors with powdered bone also strongly suppressed TRAP cell formation, suggesting that it is the act of phagocytosis, rather than the nature of the material ingested, that determines lineage. Induction of TRAP cells and bone resorption by RANKL was similarly inhibited by phagocytosis. The inhibition of TRAP cell formation was not attributable to an autocrine effect of release into the supernatant of osteoclast-inhibitory material by phagocytosing cells, or to changes in TRAF6 expression. It is known that TGF-β is essential for osteoclast formation. We therefore tested the effect of phagocytosis on components of the TGF-β signaling pathway. Phagocytosis increased levels of protein expression of the antismad Smad7, and suppression of TGF-β signaling, induced by overexpression of Smad7, suppressed TRAP cell formation. This may account for absence of osteoclasts in sites of inflammation. It also raises the possibility that RANKL might have actions on macrophages other than osteoclast-induction.

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The p38 Specific Inhibitor SB203580 Blocks Osteoclast Formation in RAW 264.7 Cells Through Down Regulation of cFos mRNA Expression and Reduced AP-1 Binding. S. Srivastava, N. K. Shevde, A. C. Bendixen, K. M. Dienger,* J. W. Pike. Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA.

RANKL and its receptor RANK are critical regulators of osteoclast (OC) formation. It is now well documented that RANKL signaling engages TRAF6 which in turn activates downstream MAPKs, NF-kB and the PI3 pathways that lead to activation of the transcription factors complex AP-1 (Fos and Jun) and NF-kB. We and others have previously shown that RANKL treatment simultaneously stimulates JNK and p38 pathways and that the p38 specific inhibitor SB203580 blocks osteoclast formation in a dose-dependent manner in both RAW264.7 cells and bone marrow monocytes. The exact molecular mechanism through which SB203580 blocks osteoclast formation is not known, however. To explore

this inhibitory effect, we transfected RAW264.7 cells with luciferase reporter genes under the control of either AP-1 or NF-kB transcription factors and then treated the cells with RANKL in the absence or presence of SB203580. AP-1 dependent luciferase activity was strongly induced by RANKL; inclusion of SB203580 (5uM), however, suppressed this luciferase activation. These results suggest that the p38 kinase might regulate either directly or indirectly the activity of either c-Jun or c-Fos. To further investigate whether decreased transcription activity was due to decreased DNA binding, we conducted EMSA using both AP-1 and NF-kB consensus DNA binding sequence as probes. Our results clearly demonstrate that SB203580 pretreatment suppressed AP-1 DNA binding in a dose-dependent manner while it had no effect on NF-kB DNA binding. SB203580 pretreatment had no effect on JNK1 activity and did not suppress the expression of c-Jun protein. We therefore hypothesized that SB203580 pretreatment might affect the expression of c-Fos. To determine whether the expression of c-Fos mRNA was altered by SB203580, we treated RAW264.7 cells with RANKL in absence or presence of SB203580 (5uM) for 3 hours, extracted total RNA using the Trizol reagent, prepared cDNA, and then subjected the samples to semi-quantitative cycle-dependent RT-PCR using primers to b-actin and c-Fos as well as NF-kB dependent IL-6 and AP-1 dependent TNF-a. SB203580 treatment significantly suppressed the expression of both c-Fos and TNF-a, but had no effect on the expression of mRNA for IL-6 . We conclude that activation of p38 by RANKL is essential for osteoclast formation in RAW264.7 cells and that at least one target of p38 action is c-Fos.

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TNF-alpfa and RANKL Synergistically Stimulate Murine Osteoclast Differentiation from TRAF6-deficient Osteoclast Progenitor Cells. Y. Azuma,[1] K. Kaji,*[2] A. Naito,*[3] T. Kamimura,*[1] J. Inoue,*[4] A. Kudo.[2] [1]Department of Pharmacology, Teijin Institute for Biomedical Research, Tokyo, Japan, [2]Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan, [3]Department of Oncology, Institute of Medical Science, Tokyo, Japan, [4]Faculty of Science and Technology, Keio University, Yokohama, Japan.

Although tumor necrosis factor receptor-associated factor(TRAF)6 is required in RANK-RANKL signaling for osteoclastogenesis, it has been remained unclear whether TRAF6 is crucial in tumor necrosis factor-alpha(TNF)-induced osteoclastogenesis. We examined TRAF6 function in the TNF-induced osteoclastogenesis by using osteoclast progenitor cells (MDS-/- cells) from TRAF6-deficient mice. The results demonstrated that TNF did not effectively induce osteoclast differentiation from MDS-/- cells. These results demonstrated that TRAF6 is essentially involved in the TNF receptor(TNFR) signaling in osteoclast differentiation. However, a few mature multinucleated osteoclasts were left in TNF-treated MDS-/- cells and they have been resorption activity, suggesting that the some other signaling mechanism besides the TRAF6 pathway. In order to reveal how TRAF6 is involved in the TNF-induced osteoclast differentiation from osteoclast progenitor cells, we examined the expression of TRAF6 protein in the TNF-treated osteoclast progenitor cells. In the process of TNF-induced osteoclastogenesis, the expression of TRAF6 protein increased. TNF synergistically stimulated RANKL-induced osteoclast differentiation from osteoclast progenito cells, suggesting the relationship between TRAF6 up-regulation by TNF-treatment and RANK-RANKL signaling. To demonstrate that the role of TRAF6 in the synergy between TNF and RANKL, MDS-/- cells were treated with various combinations of TNF and RANKL. Surprigingly, co-treatment with TNF and RANKL rescued the failure of RANKL-induced osteoclast differntiation from MDS-/- cells. These results suggest that TNF and RANKL synergistic induction of osteoclastogenesis involves the cross-talk between TNF-TNFR and RANK-RANKL signaling pathways besides the TRAF6.

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Multiple RANKL-Induced Programs of Gene Expression are Suppressed by Estrogen During Osteoclast Differentiation. N. K. Shevde,[1] A. C. Bendixen,[1] D. E. Murphy,*[1] R. Sladek,*[2] T. J. Hudson,*[2] J. W. Pike.[1] [1]Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA, [2]Human Genome Center, McGill University, Montreal, Quebec, Canada.

Steroid deficiency leads to a substantial increase in bone turnover and a critical imbalance between bone formation and resorption. This imbalance results in a progressive loss of trabecular bone mass, due in part to an increase in osteoclast formation from monocytic precursors in bone marrow. We have used bone marrow monocytes and RAW264.7 cells as models of osteoclast (OC) differentiation to examine the ability of estrogens (E) to negatively regulate this process. Accordingly, we have found that E suppresses OC formation, largely through a downregulation of JNK1 activity and suppression of both c-Jun activation and expression. To examine E's actions more globally, we contrasted RNA expression profiles from RAW264.7 cells that had been treated with RANKL for periods up to 120 hr in the absence or presence of E using DNA microarrays. All changes in gene expression were confirmed using Realtime PCR analysis. RANKL induced rapid (1 to 4 hr) changes in the expression of members of several classes of genes including immediate early gene transcription factors such as c-Fos, c-Jun, JunB, EGR1 and 2 and others. In addition, RANKL prompted a massive induction of proinflammatory cytokine genes such as IL-1a, IL-1b, TNFa, and to a lesser extent IL-6, as well as numerous other cytokines, chemokines and inflammatory factors. These responses returned to baseline within 12 hr of RANKL treatment. While multinucleated OC were not detectable until 48 hours post RANKL treatment, transcripts for TRAP, MMP-9, cathepsin K, carbonic anhydrase II and others inherent to the osteoclast phenotype emerged beginning at 24 hr and accumulated significantly over the next 3 days. Transcripts responsible for the macrophage phenotype were simultaneously extinguished. The addition of E and RANKL together at 0 hr exerted a profound suppressive effect on RANKL- induced gene expression. Accordingly, numerous immediate early genes were strongly (50%) suppressed. E also suppressed transcripts for the proinflammatory cytokines, including those for IL-1a, IL-1b, IL-6 and TNF. E exerted numerous additional effects, acting to suppress the expression of transcription factors that may play a role in OC formation as well as the induction of genes representative of the OC phenotype. These and other observations suggest that E may play a dominant and pervasive role in regulating multiple facets of OC differentiation in RAW264.7 cells. Current studies are focused upon the mechanism through which small numbers of E receptors can mediate such profound effects on the formation of OCs in vitro.

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Use of Phage Display to Identify Peptides that Inhibit Osteoclast Differentiation. P. He,*[1] N. Islam,*[1] B. Wang,*[2] E. M. Greenfield.[1] [1]Orthopaedics, Case Western Reserve University, Cleveland, OH, USA, [2]Medicine, Case Western Reserve University, Cleveland, OH, USA.

Stromal cells regulate differentiation and activity of osteoclasts through interactions that require direct cell-cell contact. Osteoprotegerin blocks these interactions by binding to RANKL expressed on the stromal cell plasma membrane. We employed peptide phage display to identify novel molecules that inhibit interactions between stromal cells and osteoclast precursors. For this purpose, a mixture of two peptide libraries ($X_2CX_{14}CX_2$ and X_2CX_{18}) was initially screened by four rounds of positive/negative selection. Positive selection depended on binding to stromal cells that had been cultured with 10 nM 1,25-dihydroxyvitamin D_3 (1,25-D_3) for six days, while negative selection depended on the absence of binding to cells cultured without 1,25-D_3. ST2 stromal cells were used for the first and third rounds of selection and MC3T3-G2/PA6 stromal cells were used for the second and fourth rounds. Thirty phage clones were isolated and DNA encoding the inserted peptides was sequenced. Three families of peptides were identified, none of which exhibit significant homology with known proteins or genes. This result is not surprising since peptide phage display often selects for "mimotopes" that mimic the three dimensional structure of endogenous proteins rather than their linear amino acid sequence. The ability of the isolated phage clones to bind to ST2 cells was measured. All 30 clones exhibited high affinity binding to ST2 cells that had been cultured with 10 nM 1,25-$D_3$for six days. In contrast, little or no binding was found using ST2 cells cultured without 1,25-D_3. Peptides corresponding to the consensus sequences of the phage families were synthesized. The effect of the peptides on osteoclast differentiation induced by 1,25-D_3 was assessed in co-cultures of murine spleen cells and ST2 cells. Peptide #3 inhibited osteoclast differentiation in a dose-dependent manner. Maximal effects were induced by 140 nM peptide #3, which inhibited formation of TRAP+ multinucleated cells by 81±4.5% (p=0.0001). Half-maximal inhibition was induced by 1.4 nM peptide #3 (p=0.005). In contrast, 140 nM of peptide #1, peptide #2, or a scrambled form of peptide #3 had little or no effect on osteoclast differentiation. Despite potently inhibiting formation of TRAP+ multinucleated cells, peptide #3 had little or no effect on formation of TRAP+ mononucleated cells. Thus, peptide #3 blocks a relatively late step in osteoclast differentiation through a mechanism of action that is distinct from that of osteoprotegerin, which blocks formation of both TRAP+ multi- and mono-nucleated cells. Peptide #3 should be a useful starting point for a peptidomimetic drug discovery program aimed at blocking osteoclast differentiation.

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Melatonin Increases Bone Mass by Suppressing Bone Resorption Through Down-Regulation of the RANKL-Mediated Osteoclastogenesis in Mice. K. H. W. Lau,[1] H. Koyama,*[1] O. Nakade,[2] Y. Takada,*[2] T. Kaku.[2] [1]Pettis Mem VAMC, Loma Linda, CA, USA, [2]Health Sciences University of Hokkaido, Hokkaido, Japan.

We and others recently reported that melatonin stimulates osteoblast activity in vitro. In this study, we sought to test if daily melatonin administration in mice would increase bone mass in vivo. Four groups of 4-week-old male ddy mice (n = 6 per group) received daily injections of solvent, 1, 5, or 50 mg/kg/day melatonin, respectively, for 4 weeks. The melatonin treatment significantly increased BMD (by DEXA) (by 36%, P<0.005), TV (by 49%, P<0.01), and Tb.Th (by 19%, P<0.05) in the tibia, supporting the premise that melatonin increases bone mass in mice. There was no significant increase in any of the test bone formation parameters (BFR/BS, MAR, OV/TV, OS/BS, Ob.S/BS) between melatonin- and vehicle-treated mice. In contrast, the bone resorption parameters were markedly reduced in a dose-dependent manner in melatonin-treated mice compared to vehicle-treated mice [i.e., Oc.S/BS (by 74%, P<0.05) and N.Oc/BS (by 76%, P<0.005)], indicating that melatonin increases bone mass through a suppression of bone resorption rather than a stimulation of bone formation. Further assessment of the effects of melatonin on bone resorption revealed that melatonin (up to 500 μM) caused a significant (P<0.001) dose-dependent inhibition of bone resorption activity (assayed with the pit formation assay) of osteoclasts in vitro, but only in the presence of osteoblasts. Melatonin had no effect on the bone resorption activity of isolated rabbit osteoclasts, suggesting that melatonin inhibits osteoclastic resorption through osteoblasts. Two osteoblastic proteins (RANKL and OPG) are potent regulators of bone resorption, in that RANKL increases and OPG inhibits osteoclastogenesis. Thus, we tested if melantonin treatment would affect the production of RANKL and/or OPG in the mouse MC-3T3-E1 osteoblastic cells. Melatonin (from 1 to 500 μM) significantly (P<0.001 for each) increased, in a dose-dependent manner, both the mRNA (up to 2-fold) and protein (up to 4-fold) levels of OPG. Conversely, melatonin at the same doses markedly (P<0.001) reduced the mRNA level of RANKL (to <20% of that of the vehicle-treated control). Thus, these findings support our contention that melatonin may inhibit osteoclastic resorption through an increased OPG expression as well as a decreased RANKL expression in osteoblasts. In conclusion, we have demonstrated for the first time that melatonin inhibits bone resorption through downregulation of the RANKL-mediated osteoclastogenesis, which subsequently leads to an increase in bone mass.

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A Signaling Complex Containing TAK1, TAB2, and TRAF6 Is Recruited to RANK in Response to the Ligand Stimulation. N. Sakurai,[1] J. Mizukami,*[1] N. Sato,*[1] M. Tsuda,*[1] H. Sakurai,*[1] G. Takaesu,*[2] J. Ninomiya-Tsuji,*[2] K. Matsumoto,*[2] H. Akatsuka.*[1] [1]Discovery Research Laboratory, Tanabe Seiyaku Co., Ltd., Osaka, Japan, [2]Dept. of Molecular Biology, Nagoya University, Nagoya, Japan.

Receptor activator of NF-κB(RANK) and its ligand, RANKL, are key molecules for differentiation and activation of osteoclasts. Recent studies have demonstrated that TNF

receptor-associated factor 6 (TRAF6) is a pivotal component of RANK signaling pathway. Previously, we showed that dominant negative forms of TGF-β activated kinase 1 (TAK1) and TAK1 binding protein 2 (TAB2), components in the TRAF6-mediated IL-1 signaling, suppressed NF-κB activation induced by overexpression of full-length RANK in HEK293T cells. However, the intracellular behavior of these proteins in the RANKL-induced signaling pathway under physiological conditions has been poorly characterized. Here, we investigate the mechanism of endogenous signal transduction induced by RANKL.To investigate the ligand-stimulated RANK signaling, we generated a clonal cell line of HEK293, 293RANK, stably expressing full-length RANK. Activations of NF-κB, JNK, and p38MAPK that are activated by RANKL in osteoclasts were induced in response to RANKL in 293RANK cells. Therefore, the 293RANK cells are useful for studying endogenous signaling events initiated by RANKL. RANKL induced the activation of endogenous TAK1 in 293RANK cells, with maximal activation at 2-5 min after stimulation. TAK1 activation was followed by activations of IKK and JNK that are situated downstream of TAK1 in IL-1 signaling. Furthermore, RANKL stimulation induced an interaction of TAK1 with TRAF6 at the comparable time of the TAK1 activation and then TAK1 was rapidly released from TRAF6. In IL-1 signaling pathway, TRAF6 and TAK1 are known to associate via TAB2. To examine the complex formation of these signaling components with RANK, immunoprecipitation experiments were performed. In the RANKL-treated 293RANK cells, RANK was coimmunoprecipitated as well as TRAF6 with anti-TAB2 antibody, indicating that associations of TAB2 with RANK and with TRAF6 also depend on the ligand stimulation. Surprisingly, the immunopricipitates of the RANKL-treated extracts with anti-TAK1 antibody also contained both RANK and TRAF6, suggesting direct and transient association of TAK1-TAB2-TRAF6 to RANK.These results suggest that the formation of a complex containing RANK, TRAF6, TAB2, and TAK1 is involved in the RANK signaling pathway and may regulate development and function of osteoclasts.

Disclosures: Tanabe Seiyaku Co., Ltd.,3.

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Identification of the Src SH3 Binding Site Within the Proline-Rich Region of Cbl. A. Sanjay, T. Miyazaki,* W. C. Horne, R. Baron. Yale University, New Haven, CT, USA.

Cbl is a substrate of the non-receptor tyrosine kinase Src which is phosphorylated downstream of integrins in osteoclasts and, like Src, is involved in the regulation of osteoclast motility. We and others have found two distinct interactions between Src and Cbl, the first between the SH3 domain of Src and the proline rich region (PR) of Cbl (residues 481-690) and the second between the phosphorylated Y416 of Src and the phosphotyrosine binding domain (PTB) of Cbl. The latter interaction both inhibited Src kinase activity and reduced cell adhesion of vitronectin receptor-expressing 293 cells to vitronectin. Mutation in the PTB domain of Cbl, however, did not prevent the Src-Cbl interaction, indicating that the primary interaction is between the Src SH3 and Cbl PR regions. Here, we have identified the specific sequence within the Cbl PR domain that associates with the Src SH3 domain.Analysis of the PR sequence revealed that two sites, RDLPPPPPPDR (Cbl540) and RPIPKVPV (Cbl592), matched the consensus sequence RxxPxxP for binding to the SH3 domain of Src. To determine which of these regions interact with Src, we performed a competitive binding assay, incubating 293 VnR cell lysates which contain endogenous Cbl with GST-conjugated SrcSH2, SrcSH3 and Src SH3SH2 domains in the absence or presence of either a positive control peptide (RALPPLPRA), Cbl540 or Cbl592. Western blot analysis revealed that Cbl bound to both GST-SrcSH3 and GST-SrcSH3SH2, but not to GST or GST-SrcSH2. Peptide Cbl540 prevented endogenous Cbl binding to both GST-SrcSH3 and GST-SrcSH3SH2, whereas peptide Cbl592 failed to compete with Cbl even at high concentrations, indicating that it is Cbl540 that contains the primary Src-binding motif within the PR region of Cbl. Theoretically, Cbl 540 might bind the SH3 domain in either the N-to-C (RDLPPPP) or the C-to-N (PPPPDR) direction. To determine in which orientation the SH3 domain binds to Cbl PR, we replaced prolines with alanines [RDLAPPA (Cbl540F) and APAPDR (Cbl540R)] and used the mutated peptides in the assay. Peptide Cbl540F had no effect whereas Cbl540R completely abolished the binding of Cbl to the SH3 domain of Src, indicating that it is the reverse orientation of the peptide Cbl540 which binds to Src. Expression of these mutated proteins either in the 293-VnR cells or in osteoclasts will help elucidate whether initial docking of Src at the Cbl PR region is required for the subsequent down regulation of Src kinase activity by Cbl, and/or for the Cbl-mediated ubiquitination of Src events that participate in the regulation of osteoclast adhesion and migration.

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PYK2 Autophosphorylation Site Y402, but Not Kinase Activity, Is Necessary for Adhesion-induced Phosphorylation of Yyrosines in PYK2 C-terminal Domain and Osteoclast Spreading. P. T. Lakkakorpi,*[1] A. J. Bett,*[2] L. Lipfert,*[3] G. A. Rodan,[3] L. T. Duong.[3] [1]Anatomy, Inst. of Biomed., Univ. of Turku, Turku, Finland, [2]Virus and Cell Biol., Merck Res. Labs., West Point, PA, USA, [3]Bone Biol. & Osteoporosis, Merck Res. Labs., West Point, PA, USA.

Ligand engagement of alpha v beta 3 integrins in osteoclasts induces PYK2 tyrosine phosphorylation, activation of its tyrosine kinase, its association with c-Src, and cytoskeletal reorganisation. PYK2 localises to podosomes, and in bone resorbing osteoclasts, to the sealing zone. Using adenovirus expressing PYK2 antisense we have recently shown that PYK2 is necessary for osteoclastic bone resorption. To further study the function of PYK2 in osteoclasts we examined the effects of overexpressing (using adenovirus) a PYK2 kinase-dead (K457A) mutant and an autophosphorylation site (Y402F) mutant on adhesion-mediated signalling, cell spreading and migration. Infection of co-cultures for 3-days, resulted in 3-4 fold higher expression, in purified pre-fusion osteoclasts (pOCs) or osteoclast-like cells, of exogenous wild type PYK2(wt) or its mutants, relative to endogenous PYK2. In adhered kinase-dead PYK2(K457A) cells, tyrosines Y402, Y579/Y580 and Y881 were phosphorylated to the same extent as PYK2(wt), whereas phosphorylation of these tyrosines was severely reduced in PYK2(Y402F) cells. Similarly, expression of kinase-dead PYK2(K457A) did not reduce pOC spreading, while expression of PYK2(Y402F) abolished cell spreading on vitronectin. Co-immunoprecipitation experiments showed that PYK2(wt) and (K457A) associate with c-Src, whereas PYK2(Y402F) does not. On the other hand, neither PYK2(wt) nor its mutants inhibited pOC chemotactic migration towards M-CSF. These observations are in agreement with previous findings, showing that diminished cell spreading and migration in Src-/- pOCs, was fully occurring with M-CSF. Interestingly, both PYK2 mutants localised to podosomes in osteoclasts seeded on glass, suggesting that podosomal targeting is independent of the ability to recruit c-Src. Taken together, PYK2 phosphorylation at Y402, but not its kinase activity, appears to be important for its adhesion-induced association with c-Src, which in turn phosphorylates additional tyrosines in the C-terminal domain of PYK2. This leads to further recruitment of downstream signalling molecules, important for the cytoskeletal reorganisation of osteoclasts. Furthermore, phosphorylation of kinase-dead PYK2(K457A) at Y402 suggests that a separate kinase can activate PYK2, which thus functions as an adaptor, associates with c-Src and allows normal osteoclast spreading.

Disclosures: Merck & Co., Inc..

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Body Composition Contributes to Higher Bone Mass in Older Diabetic White & Black Women, but Not Men. **E**. Strotmeyer,[1] J. Cauley,[1] A. Schwartz,[2] M. Nevitt,[2] H. Resnick,*[2] J. Zmuda,[1] D. Bauer,[2] A. Newman.*[1] [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of California, San Francisco, CA, USA.

Older white women with type 2 diabetes have higher bone mineral density (BMD) but little information exists for men and older black adults. Higher BMD in type 2 diabetics may be due in part to greater obesity compared to non-diabetics. The purpose of the study was to determine if higher BMD in diabetics is accounted for by measures of body composition and fasting insulin. The Health, Aging, and Body Composition Study, which included white and black, physically able, men and women age 70-79 years, collected data on total fat, lean mass, and visceral fat from DXA and CT scans. BMD was measured by DXA (QDR 4500A, Hologic Inc, Bedford, MA). Those reporting diabetes, on diabetes medications, or with fasting glucose of $\geq$126 mg/dl were considered diabetic. Exclusion criteria were oral steroid use (n=69), diabetes diagnosis at age <20 years (n=5), or missing diabetes status (n=22). Of the remaining 2979 participants, 19% had diabetes at the Year 1 visit. Men were 57% of the diabetic and 47% of the non-diabetic group ($p<0.001$). Whites were 43% of the diabetic and 62% of the non-diabetic group ($p<0.001$). For both groups, mean age was 74$\pm$3 years. Mean fasting insulin (12$\pm$16 vs. 8$\pm$5 IU/ml; $p<0.001$), visceral fat (169$\pm$74 vs. 137$\pm$64 cm^2; $p<0.001$), total fat (26.0$\pm$9.1 vs. 23.8$\pm$8.6 kg; $p<0.001$), and bone-free lean mass (54.0$\pm$10.3 vs. 48.9$\pm$10.5 kg; $p<0.001$) were significantly higher in diabetics compared to non-diabetics. Higher hip BMD was found for diabetics vs. non-diabetics (0.96$\pm$0.17 vs. 0.73$\pm$0.14 g/cm^2, $p<0.001$). Multiple linear regression was performed to determine if diabetes was related to differences in BMD, independent of body composition measures, adjusting for age, race, and gender. Visceral fat ($p=0.25$) with adjustment for total fat, did not explain differences in hip BMD and diabetes remained a significant predictor of BMD ($p<0.001$). Total fat ($p<0.001$) and lean mass ($p<0.001$) were significantly related to BMD, with diabetes also remaining a significant predictor of hip BMD ($p<0.001$). Furthermore, diabetes remained an independent predictor of hip BMD ($p<0.001$) adjusting for all body composition measures and fasting insulin. The effect of diabetes was similar for whole body and femoral neck BMD. However, while diabetes was a significant and independent predictor of hip BMD in both white and black men after adjusting for all body composition measures, it was not among white or black women. In conclusion, diabetes is associated with higher BMD in men and women, both black and white. Body composition measures did not account for higher BMD in this diabetic population as a whole but may play a larger role in women than men.

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Dietary Contributions to Serum 25(OH) Vitamin D Levels [25(OH) D] Differ in Black and White Adults in the United States: Results from NHANES III. Y. K. Park,* C. N. Barton,* M. S. Calvo. Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA.

The relative importance of diet and sunlight to serum 25(OH) D levels varies with season, latitude, age and race. Our goal is to evaluate the contributions of vitamin D intake to serum 25(OH) D concentrations for data that reflect varied conditions of exposure to sunlight due to the variability in season or latitude when blood samples were taken. We explored the relationship between vitamin D intake and 25(OH) D levels in adult (> 20 y) Non-Hispanic White (n=5822) and Black (n=3617) men and women who participated in NHANES III (1988-94). Vitamin D intake, both 24 hr and usual, were estimated from food only and from food plus supplements. We used simple regression analysis to examine the relationship by age (<50, >50y), gender and race. For all four methods of estimating vitamin D intake, the intake showed a consistent significant association with 25(OH) D level in all age/gender groups of both races. For all age and gender groups, Black men and women had lower mean 25(OH) D levels and lower mean dietary intakes and reported a lower use of dietary supplements than Whites. Milk and ready-to-eat cereals are major sources of dietary vitamin D by fortification in the United States. Consumption of both of these foods was much lower in Blacks than in Whites. Despite their lower vitamin D intakes, the association between dietary intake and serum 25(OH) D was stronger for Blacks than for Whites. In addition, the ratio of vitamin D intake to serum 25(OH) D level was consistently higher in Blacks compared to Whites. This suggests that Blacks may need more dietary vitamin D than Whites to maintain comparable serum levels. Our findings in adults suggest that dietary contributions to 25(OH) D levels appear to be more critical in Blacks than in Whites and emphasize the need to improve vitamin D intakes of the Black population in the United States.

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Nulliparity and Fracture Risk in Older Women: The Study of Osteoporotic Fractures. T. A. Hillier,[1] J. Rizzo,*[1] K. L. Pedula,*[1] K. L. Stone,[2] J. A. Cauley,[3] D. C. Bauer,[2] S. R. Cummings.[2] [1]Kaiser Center for Health Research, Portland, OR, USA, [2]UCSF, San Francisco, CA, USA, [3]U of Pittsburgh, Pittsburgh, PA, USA.

Nulliparity is an established risk factor for breast cancer, but prior studies are conflicting on the relationship of nulliparity with bone mineral density (BMD) and risk of subsequent fractures. It is also unknown if parity may have a greater effect on weight-bearing skeletal sites. During the baseline examination, we assessed self-reported parity, number of live births, and other fracture risk factors in 9,699 postmenopausal women aged 65 years and older. Incident hip and wrist fractures were subsequently ascertained by contact of participants every 4 months after baseline, and verified by physician review of medical records, over an average follow-up of 9.8 years. Incident vertebral fractures were determined by morphometric criteria on serial thoracic and lumbar radiographs (mean follow-up=3.7 years). We used proportional hazards models to assess the independent contribution of nulliparity on hip and wrist fractures and multiple logistic regression for vertebral fractures. Nulliparous women had a greater risk of hip and vertebral, but not wrist fractures (Fx) on univariate analysis (see Table). This relationship remained significant on multivariate analysis for hip fracture. Among parous women, each additional birth resulted in a 14% risk reduction in hip fracture (HR=0.86, p=.007). Nulliparity was not associated with decreased BMD at the hip, spine, or, radius.

Parity		**Hip Fx**	**Vertebral Fx**	**Wrist Fx**
	n	***n(%)***	***n(%)***	***n(%)***
Parous	7864	435(5.5)	291(4.9)	520(6.6)
Nulliparous	1835	166(9.1)	98(7.3)	114(6.2)
Unadjusted risk+		1.72(1.43,2.05)	1.52(1.19,1.92)	0.96(0.78,1.18)
Adjusted risk+*		1.47(1.18,1.84)	1.30(0.99,1.73)	0.85(0.68,1.08)

+Risk is reported as hazard ratios for hip and wrist fractures and odds ratios for spine fractures, with 95% confidence intervals

*Adjusted for age, weight, BMD, family history of osteoporosis, age at menarche, years of menstruation, postmenopausal oral estrogen use, postmenopausal daily calcium intake (dietary+supplement), milk intake age 12-50, and physical activity at age 30

Nulliparous women were more likely to experience postmenopausal hip fractures independent of BMD. Additionally, among parous women, there was a strong relationship with each additional birth in reducing the risk of future postmenopausal hip fractures. More research is needed to elucidate possible mechanisms for this relationship such as altered hip geometry associated with pregnancy. Nulliparous women may also represent a unique risk group to target postmenopausal prevention strategies given their increased risk of breast cancer and potentially osteoporotic fractures

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BMD Independent Risk Factors for Hip Fractures in Elderly Women in England. K. Kayan,*[1] R. U. Ashford,[1] A. Dey,*[1] B. Kohler,*[1] J. Cliffe,*[1] A. Hinch,*[1] A. Ball,*[1] M. Marples,*[1] T. Jalava,*[2] J. A. Kanis,[1] E. V. McCloskey.[1] [1]WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, Sheffield, United Kingdom, [2]Leiras Oy, Helsinki, Finland.

In a prospective study of women aged at least 75 years, we examined BMD independent risk factors for hip fractures.4347 women were enrolled to a placebo-controlled study of the effect of clodronate(BONEFOS)on hip fracture risk. At entry, in addition to hip BMD, all women underwent assessments including quality of life (Euroqol), disability (OPCS disability survey), cardiovascular health, postural stability and muscle strength. Smoking and family fracture history aren't included in this analysis. All incident reported fragility fractures were verified from hospital and/or GP records.During a median follow-up period of 3.1 years, 91 (2.1%) women sustained a low energy hip fracture. In univariate analysis, an increased risk of hip fracture was noted in women with mobility problems (59.3%, OR 1.97; 95% CI 1.23-3.16), problems with usual daily activities (40.2%, OR 1.64; 95% CI 1.08-2.48), walking difficulty (67.8%, OR 1.95; 1.19-3.18), less than 2 child births (3.7%, OR 1.70; CI 1.12-2.57), self-reported prior hip (2.2%, OR 2.69; 1.07-6.79), prior vertebral fracture (1.1%, OR 3.15; 0.96-10.34), increased body sway (OR per 1SD increase in path length standing with eyes open on a firm surface, OR 1.27; 95% CI 1.03-1.55) and a decrease in quadriceps strength in right side dominant women (OR per 1SD decrease in the maximum or average right quadriceps strength, OR 1.32; 1.06-1.64) After adjustment for age, weight and total hip BMD, an increased risk of hip fracture was observed in women with a history of amenorrhoea (2.7%, OR 3.29; 1.45-7.48), those with self-care problems (14.4%, OR 1.91; 1.17-3.11), women who did not do any exercise at the time of assessment (35.0 %, OR 1.74; 1.12-2.73), hospital admission in the preceding year (17.5%, OR 1.64; 1.02-2.64), hospital outpatient /A&E clinic visit in the 3 months prior to study entry (28.9 %, OR 1.60; 1.03-2.48 and 4.7%, OR 2.02, 1.59-2.56 respectively) or aggressive behaviour (0.3%, OR 8.83;1.87-41.64). Difficulty in rising from a chair showed a borderline significant association with the risk of hip fracture (31.8%, OR 1.55; 0.98-2.43).Finally, in a forward conditional multivariate logistic model including hip BMD and all of the significant factors from the adjusted analysis, an increased risk of hip fracture continued to be significantly and independently associated with increasing age, prior amenorrhoea, no current exercise, prior hospital admission and aggressive behaviour.We conclude that relatively simple BMD-independent assessments can predict future risk of hip fractures in elderly women.

Disclosures: Leiras Oy,2.

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International Variations in Hip Fracture Probabilities: Implications for Risk Assessment. A. Oden,*[1] J. A. Kanis,[2] O. Johnell,[3] C. E. DeLaet,*[4] B. Jonsson,*[5] A. K. Oglesby.*[6] [1]Consulting statistician, Gothenberg, Sweden, [2]WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, Sheffield, United Kingdom, [3]Department of Orthopaedics, Malmo General Hospital, Malmo, Sweden, [4]Institute for Medical Technology Assessment, Rotterdam, The Netherlands, [5]Centre for Health Economics, Stockholm School of Economics, Stockholm, Sweden, [6]Global Economic Affairs, Eli Lilly & Company, Indianapolis, IN, USA.

It is recommended that intervention thresholds in the future should be based on absolute risk, but there is a large variation in hip fracture incidence in different regions of the world. The aim of this study was to examine heterogeneity of hip fracture probability in different regions from recent estimates of hip fracture incidence and mortality. Ten year probabilities of hip fracture were computed in men and women at 10 year intervals from the age of 50 years, and lifetime risks at the age of 50 years from the hazard functions of hip fracture and death. Lifetime risk at the age of 50 years varied from 1% in women from Turkey to 28.5% in women from Sweden. High lifetime risks in women were associated with high lifetime risks in men (r=0.83). There were also significant correlations of 10 year risk at any age between men and women. Ten year probability was standardised to that of men and women from Sweden (set at 1.0). There was a 13-fold range in 10 year probability from 1.07 in Norway to 0.08 in Chile and Korea. Countries were categorised by 10 year probabilities comprising very high risk (Norway, Sweden, Iceland and Denmark), high risk (Singapore, China (Taiwan), USA, Switzerland, Canada, Australia, Germany, Netherlands and the UK), medium risk (Greece, Kuwait, Argentina, Spain, Hungary, Portugal, Japan, China (Hong Kong) and France), and low risk (Venezuela, China, Turkey, Korea and Chile). The categorisation of hip fracture probabilities can be used to adjust intervention thresholds that are based on age, sex and relative risk from a reference population such as Sweden.

Disclosures: Eli Lilly & Company Ltd.,2.

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Osteocytic Expression of eNOS in the Femoral Neck Cortex: A Possible Role in Bone Fragility. N. Loveridge,[1] S. Fletcher,*[2] M. Parker,*[3] N. Rushton,*[1] V. Das-Gupta,*[2] J. Power,*[1] A. Caballero Alías,[1] J. Reeve,[1] A. Pitsillides.*[2] [1]University of Cambridge, Cambridge, United Kingdom, [2]Royal Veterinary College, London, United Kingdom, [3]Peterborough District Hospital, Peterborough, United Kingdom.

Intracapsular hip fracture is linked to increased porosity of the anterior and inferior femoral neck cortices with little change elsewhere. We showed previously that this results from the merging of haversian canals, through excessive resorption within spatially clustered remodelling osteons. As the inferior cortex is heavily loaded in compression during (normal) gait we have examined the possibility that hip fracture is associated with relative disuse or a defect in components of the adaptive response to load. Nitric oxide (NO) inhibits bone resorption and stimulates bone formation and its release from bone cells is increased by both mechanical strain and estrogen.Endothelial nitric oxide synthase (eNOS) is the principal isoform present in bone cells, particularly osteocytes. We have used immunocytochemistry to determine the location and density of osteocytes expressing eNOS (eNOS+ve) in cortical BMUs in sections from the femoral neck of 7 cases (female, 70-96y) and 7 controls (female, 68-96y). Their number and location in the superior and inferior regions was measured by image analysis. Osteocytes generally, were identified with propidium iodide (PI+ve). The median, and minimum distances of eNOS+ve and PI+ve osteocytes from the nearest canal surface was calculated for individual BMUs and averaged for both regions of each biopsy.The median distance of eNOS+ve osteocytes to the canal surface was higher than that for PI+ve osteocytes (eNOS: 66.6±2.0µm; PI: 54.7±1.2µm, $p<0.001$); this was independent of region and biopsy type. Also, the nearest eNOS+ve osteocyte was 57% further away from the canal surface than the nearest PI+ve osteocyte ($p<0.001$); this was more marked in cases than controls (+12.0±2.2µm, vs +17.5±2.9µm $p<0.001$) but independent of region. In controls, the density of eNOS+ve osteocytes was 93% higher in the inferior region ($p=0.001$). Cases differed substantially from controls with a 53% lower density of eNOS+ve osteocytes inferiorly ($p<0.001$), but not superiorly.In conclusion eNOS+ve osteocytes are peripherally located in cortical BMUs. They are more numerous in a region subjected to habitual compressive load. In cases of hip fracture, their density in that region, but not that loaded in tension, is substantially reduced. As NO inhibits osteoclast activity, eNOS+ve osteocytes may act as sentinels to confine resorption within cortical BMUs. eNOS+ve osteocytes may be key regulators of bone conservation and the regulation of eNOS expression requires further investigation.

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Repeat Hip Fractures in a Population-based Sample of Medicare Recipients in the US: Rates, Timing and Gender Differences. H. A. Bischoff,[1] D. H. Solomon,[2] B. Dawson-Hughes,[3] P. S. Wang,*[2] J. Avorn.*[2] [1]Div. of Rheumatology, Immunology and Allergy; the Robert B. Brigham Multipurpose Arthritis Center, Brigham and Women's Hospital, Boston, MA, USA, [2]Div. of Pharmacoepidemiology, Brigham and Women's Hospital, Boston, MA, USA, [3]Jean Meyer US Department of Agriculture; Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA.

To investigate rates, timing and gender differences of repeat hip fractures in subjects 65 years or older. All patients 65 years or older enrolled in Medicaid or Medicare and the Pharmacy Assistance for the Aged and Disabled program who underwent surgical repair of a hip fracture in 1994 in one large US State were part of the study cohort. Subjects with a high- or multi-trauma hip fracture or a hip fracture within 3 years prior to the index date were excluded. Over a 3 year follow-up period rates of repeat hip fractures and the time to

repeat hip fractures were assessed. Adjustments were performed for clinical characteristics (Charlson Comorbidity Index, number of prescribed drugs over 180 days prior to the index hip fracture) and age. Rates were compared between women and men. 1222 eligible patients underwent surgical repair of a hip fracture in 1994. 1014 women and 208 men. Mean age of women was 82.8 ± 7.4 and for men 80.0 ± 7.4. 10.3 % of these subjects (n = 126) suffered a repeat hip fractures within 3 years from their index hip fracture. Men had a significantly higher rate of repeat hip fractures (14.4%) compared to women (9.5%,chi-square p = .032). This difference remained significant after adjustment. Mean time to repeat hip fracture was 243.0 ± 240.0 days and did not differ by gender. Eighteen percent of repeat hip fractures occurred within 1 month, 51% within 6 months and 75% within 12 months of the index hip fracture. These data suggest that over a 3-year follow-up period repeat hip fractures occur at a rate of 10.3%. Rates are slightly higher in men than women. The majority of repeat hip fractures occur within one year following the index fracture.

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Incident Vertebral Fractures and Mortality in Older Women: A Prospective Study. D. M. Kado,[1] T. Duong,[2] K. L. Stone,[3] K. E. Ensrud,[4] M. C. Nevitt,[2] M. C. Hochberg,[5] G. A. Greendale,[1] S. R. Cummings.[3] [1]Medicine, University of California, Los Angeles, Los Angeles, CA, USA, [2]Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA, [3]Medicine, University of California, San Francisco, San Francisco, CA, USA, [4]Medicine, VA Medical Center, Minneapolis, MN, USA, [5]Medicine, University of Maryland, Baltimore, MD, USA.

Older persons with prevalent vertebral fractures have an increased risk of mortality. To test whether incident vertebral fractures are also associated with mortality, we prospectively followed 7,238 white women age 65 or older in the Study of Osteoporotic Fractures. Incident vertebral fractures were assessed by morphometry of paired lateral spine x-rays taken an average of 3.7 years apart. 389 (5.4%) women were identified as having a new vertebral fracture (≥ 20% and ≥ 4 mm decrease in at least one vertebral height dimension). During an average of 11.6 years additional follow-up, 1,617 (22%) died. Relative hazards were adjusted for age and 12 other predictors of mortality. Women with at least one new fracture had an age-adjusted 31% increased risk of mortality (R.H.= 1.3; 95% C.I.: 1.1-1.6; p = 0.003). After adjustment for age and baseline prevalent vertebral fracture, women had a 22% increased risk of mortality (R.H. = 1.2; 95% C.I.: 1.0 -1.5; p = 0.035). However, adjustment for age, weight change, and an inability to rise from a chair accounted for most of the association (R.H.= 1.1; 95% C.I.: 0.94-1.4; p = 0.20). Among women without a baseline prevalent fracture, those who had suffered 2 or more incident fractures were at the greatest age-adjusted risk of subsequent mortality (R.H.= 2.0; 95% C.I.: 1.2-3.3), but this, too, was accounted for by adjustment for other predictors of mortality (R.H.= 1.2; 95% C.I.: 0.88-1.7). We conclude that older women with incident vertebral fractures have an increased risk of subsequent mortality that is explained to a large degree by weight loss and physical frailty.

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Osteoporosis Diagnosis and Management Following Hip Fracture. J. N. Black,*[1] S. L. Follin,Pharm D,,*[1] M. T. McDermott,MD,.[1] [1]University of Colorado Health Sciences Center, Denver, CO, USA.

To evaluate the management of osteoporosis in patients with hip fractures, we reviewed the inpatient charts of 120 patients who were admitted to our teaching hospital for a diagnosis of hip fracture from 1993-1998. The median age of the patients was 72 years; 62% were female. Potential risk factors included tobacco use (43%), hypertension (43%), alcohol use (38%), diabetes mellitus (26%), and alcoholism (11%). A fall from the standing position preceded the fracture in 97% of the patients (3% couldn't recall). Numerous patients reported a history of previous fragility fractures (hip fracture, 18%; vertebral fracture, 4%; wrist fracture, 6%). The diagnosis of osteoporosis was noted in the chart in 14% of the patients during their hospitalization or in the discharge summary. Bone densitometry testing was performed in 3% of patients, chest x-rays were done in 68%, and lateral spine x-rays were done in 9%. Osteoporosis related blood and urine testing (serum PTH or vitamin D metabolites; urine calcium or bone resorption markers), was performed in < 2% of patients. Medications for the treatment of osteoporosis could be documented in only a small minority of patients at discharge (calcium, 10%; multivitamins, 10%; vitamin D, 3%; estrogen, 8%; alendronate, 2%; calcitonin, 1%). We then reviewed the outpatient records for the 95 patients who had follow-up visits (median, 2 visits) by physicians in our system in the year following their hip fracture. During this time, the diagnosis of osteoporosis was recorded in the charts of 24% of the patients. Bone densitometry testing was performed in 6% of the patients. Osteoporosis related blood and urine testing was performed in < 4% of patients. Documented treatment for osteoporosis was uncommon (calcium, 16%; multivitamins, 8%; vitamin D, 4%; estrogen, 9%; alendronate, 5%; calcitonin, 4%). Fall prevention education was recorded for only 3% of patients. New falls were reported in 13% of the patients (mean, 1.4 falls). New fractures occurred in 11 patients (21%) of the group (hip, 6%; vertebral, 2%; other sites, 5%). Of those patients who had new fractures, 5 (45%) were taking estrogens and none were on a bisphosphonate. Conclusion: In patients with hip fractures, osteoporosis is commonly not diagnosed or treated appropriately.

Disclosures: Merck,8; Procter and Gamble,8.

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Circulating Levels of Osteoprotegerin Are Inversely Related to Biochemical Markers of Bone Turnover Measured in Serum in a Population Based Cohort of Postmenopausal Women. A. Rogers, G. Saleh,* R. A. Hannon, D. Greenfield,* R. Eastell. Bone Metabolism Group, Division of Clinical Sciences (North), University of Sheffield, Sheffield, United Kingdom.

Osteoprotegerin (OPG) is recently identified cytokine, which has been shown to be an important inhibitor of osteoclast differentiation and activation in rodent models. The aim of this study was to investigate the relationship between biochemical markers of bone turnover and bone density and circulating OPG in a population based cohort of postmenopausal women.Subjects were 180 women ages 50 to 85 years (mean 63 years). Markers of bone formation (bone alkaline phosphatase (Bone ALP), immuno-reactive bone alkaline phosphatase (IBone ALP), type I procollagen carboxy terminal peptide (PICP), osteocalcin (OC)) and bone resorption (immuno-reactive free deoxypyridinoline, pyridinoline, cross-linked N-telopeptides of type I collagen and serum cross-linked C-telopeptides of type I collagen (SCTX)) were measured by standard methods. Serum concentrations of OPG were determined by ELISA (Immundiagnostik, Germany), a sandwich assay designed to detect monomeric, dimeric and ligand bound forms of human OPG. Bone mineral density at total body (TBBMD), total hip (THBMD), femoral neck (FNBMD) and lumbar spine (LSBMD) was measured by DXA (Lunar DPX). The relationship between biochemical markers of bone turnover (after log transformation) and bone density and serum OPG was determined by Pearson's product moment correlation. Multiple regression analysis was used to determine possible confounders and results were adjusted for age (biochemical markers) and age and body mass index (bone density).We saw significant inverse relationships between OPG and serum bone turnover markers before and after adjustment for age. (Table)

Variable	r	p	r (adjusted)	p
Bone ALP	-0.22	0.003	-0.20	0.006
IBone ALP	-0.20	0.01	-0.19	0.02
PICP	-0.23	0.002	-0.21	0.006
OC	-0.16	0.04	-0.15	0.05
SCTX	-0.18	0.05	-0.17	0.05

There was a significant positive relationship between OPG and bone density at TBBMD, THBMD and FNBMD (r values from 0.17 to 0.2, p<0.05) which was lost after adjustment for age and body mass index. We saw no relationships between OPG and urine bone turnover markers.We conclude that circulating levels of OPG may reflect OPG activity in bone and that higher levels of OPG lead to lower rates of bone turnover and higher bone density in these postmenopausal women. We have previously reported high levels of variability in urine markers of bone resorption and we suggest that this could account for the absence of an association between these markers and circulating OPG

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Higher BMDs in NSAID Users Compared to Non-users: The Framingham Study. M. T. Hannan,*[1] K. Broe,*[2] S. Ferrari,[3] D. R. Gagnon,*[4] L. A. Cupples,*[4] D. P. Kiel.[1] [1]Hebrew Rehab Center & Harvard Medical Sch, Boston, MA, USA, [2]Hebrew Rehab Center, Boston, MA, USA, [3]BI Hospital, Boston, MA, USA, [4]BU Sch Public Health, Boston, MA, USA.

Non-steroidal anti-inflammatory drugs (NSAID) may have direct and indirect effects on bone metabolism inhibiting bone loss and preserving BMD; thus, we investigated the relation between NSAID and bone density (BMD) in the Framingham Offspring cohort. BMD was measured in g/cm^2 at femoral neck, Ward's area, trochanter and lumbar spine in 1996-00 using a Lunar DPX-L densitometer. NSAID use (current, former, never) including regular aspirin use was determined by physician query, while osteoarthritis (OA) was based on physician exam. Including aspirin as an NSAID was done to take into account possible confounding by indication for other NSAID use. We conducted sex-specific linear regression models to examine the relation between each BMD site and NSAID use first adjusting for age and weight and then further adjusting for height, OA and in women, current estrogen use and menopausal status.In the 2,903 Offspring (1276 M, 1627 F) with BMD data, 38% currently used NSAIDs (564 M, 531F). The mean age was 59 (sd 9.6, range: 29-86 years). After adjusting for age, current NSAID users had significantly higher BMDs (range 2-4%) at all sites compared to non-users(all 0.0001 < p < 0.05) for both men and women. Adding weight to the model attenuated this effect at all sites for women, but men continued to have higher BMDs in NSAID users at all bone sites. Adjustment for other covariates, including OA, did not change these results for men: NSAID users had 2-3% higher BMDs than nonusers (0.005 < p <0.06). For women, there were no differences in BMDs between current NSAID users compared to never users (all p<0.63). The mean BMD for former NSAID users was not statistically significantly different from either group for any analysis.In sum, these findings indicate the NSAID use is associated with higher BMDs for men but not women in the Framingham Offspring Study. For middle-aged women, the effect of NSAIDs upon BMD is attenuated by other major risk factors with an effect on bone, notably weight, menopause and estrogen use. These findings extend work with similar results from other cohort studies. Randomized studies are needed to test the hypothesis that NSAIDs favorably influence BMD as observational studies may still be affected by biases for which study design or analysis has not completely accounted.

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Association Between Lipid Profile and Bone Mass in Healthy Men. V. Braga, D. Gatti, M. Rossini,* S. Adami. Rheumatology Unit, Valeggio S/M, Italy.

It has been suggested that the cholesterol-lowering drug statins increase bone formation. In three case-control studies an association between statin use and lower risk of osteoporotic fractures was found. However, the results of other studies did not support this early finding and in the first and only prospective study so far published no evidence of an effect of statins on fracture risk was found. This might raise the suspicion that some positive results were driven by biases in the study population. From the data-set of an ongoing longitudinal study on the incidence of osteoporosis among healthy male subjects aged 40 to 70 years of our Health District (n. 427, mean age 57,61) we evaluated the relationship between lipid profile and bone mineral density (BMD) [QDR 4500, Hologic, USA] at the spine, femoral neck and total hip. The individuals with conditions or on treatment with drugs known to interfere with bone metabolism and those on statins or other lipid lowering

agents were excluded. The subjects were divided in three groups according to tertiles for values of LDL cholesterol, HDL cholesterol and LDL/HDL ratio respectively. The subjects with the most favorable lipid profile had consistently the lowest bone mass values expressed in terms of Z-score (Table: mean±SD BMD Z-scores; *p<0.05; †p<0.01 most versus less favorable lipid profile; °p>0.05 less versus mid favorable lipid profile).

Lipid profile subgroups	Lumbar spine BMD	Femoral neck BMD	Total hip BMD
LDL cholesterol (≤2.80 mmol/L)	-0.91 ± 1.46	-0.11 ± 1.37	-0.24 ± 1.01
LDL cholesterol (2.90-3.66 mmol/L)	-0.23 ± 1.91†	0.24 ± 1.26*	0.07 ± 1.13*
LDL cholesterol (3.67-5.62 mmol/L)	-0.35 ± 2.06†	0.18 ± 1.21*	0.06 ± 1.02†
HDL cholesterol (≤1.21 mmol/L)	-0.17 ± 2.12	0.28 ± 1.40	0.12 ± 1.07
HDL cholesterol (1.22-1.44 mmol/L)	-0.71 ± 1.84*	0.12 ± 1.28	-0.03 ± 1.08
HDL cholesterol (1.45-2.51 mmol/L)	-0.70 ± 1.49*	-0.09 ± 1.18†	-0.21 ± 1.02†
LDL/HDL (≤2.07)	-0.75 ± 1.50	-0.06 ± 1.31	-0.19 ± 1.05
LDL/HDL (2.08-2.86)	-0.59 ± 1.64°	0.03 ± 1.21°	-0.06 ± 1.10°
LDL/HDL (2.87-5.28)	-0.14 ± 2.32†	0.31 ± 1.32†	0.13 ± 1.05†

Body weight, BMI, age, smoking habit, dietary calcium intake were not different in the three groups of subjects and the level of significance in bone mass did not change after "adjusting" for all these variables. These results indicate that the lower incidence of osteoporotic fracture among statin users might be explained more by their prevailing higher bone mass rather than by a pharmacological effect of statins on bone metabolism.

F309

Serum Osteoprotegerin (OPG) Levels in Humans. N. A. Sarikaya,*[1] S. W. Martin,[1] D. Chen,*[1] H. Gunn,*[1] M. Arrighi,[2] D. Holloway,*[3] P. Bekker,[3] A. Nakanishi,*[4] C. Dunstan.[5] [1]Pharmacokinetics and Drug Metabolism, Amgen Inc., Thousand Oaks, CA, USA, [2]Health Economics and Epidemiology, Amgen Inc., Thousand Oaks, CA, USA, [3]Clinical Research, Amgen Inc., Thousand Oaks, CA, USA, [4]Biostatistics, Amgen Inc., Thousand Oaks, CA, USA, [5]Development, Amgen Inc., Thousand Oaks, CA, USA.

The objective of this study was to determine human serum OPG levels, and determine the relationship of OPG with age, gender, pre/post menopause, and bone mineral density.We collected serum samples from 311 normal volunteers in a single center, cross-sectional study. The subjects were women (n=154) and men (n=157), ranging in age from 20 to 80 years. Sera were assayed for OPG with two different sandwich ELISA formats. The first format included OPG ligand (OPGL) capture and HRP conjugated mouse anti-human OPG monoclonal antibody for detection (OPGL-Ab). The second format included mouse anti-human OPG monoclonal antibody for OPG capture and HRP conjugated rabbit anti-human OPG polyclonal antibody for detection (Ab-Ab). The assay range and quantification limit were 38.992 – 2495.5 pg/mL and 49.910 pg/mL, respectively, for OPGL-Ab format. For the Ab-Ab format, assay range and quantification limit were 19.496 – 2495.5 pg/mL and 30.380 pg/mL, respectively. Values of 19.496 and 38.992 pg/mL were imputed for the Ab-Ab and OPGL-Ab assays, respectively, for samples that were below quantification limit.Mean serum OPG levels were determined as 40.914 ± 17.2 pg/mL for Ab-Ab assay and 54.462 ± 20.5 pg/mL for OPGL-Ab assay. Although the percentage of samples below quantification limit is different, the two assay formats are highly correlated (Spearman rank, *P*<.0001). In both assay formats, mean serum OPG levels in females were greater than males (*t*-test, *P*<.001). Comparison of pre/post menopausal women, men -50 years showed that mean OPG levels were higher in post-menopausal women and men>50, with both assay formats. One-way ANOVA and categorical analyses showed statistically significant differences among the four groups (*P*<.001). In men, age and OPG levels are positively correlated in both assay formats. In women, correlation between age and OPG levels was observed with both assay formats. With Ab-Ab assay format, a weak association was observed between OPG levels and bone mineral density of spine, total body, and hip. Linear regression analysis of age, as a function of assay, gender, and bone mineral density, showed positive correlation with serum OPG levels.We conclude that the two assay formats provided different serum OPG levels yet with similar relationships with age, gender, and bone mineral density status. Serum OPG levels positively correlate with age, and women have higher serum OPG levels than men.

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Calcaneus Microarchitectural Assessment by Magnetic Resonance Imaging in Male Osteoporosis. B. Cortet,[1] N. Boutry,*[2] P. Dubois,*[2] A. Cotten,*[2] X. Marchandise.*[2] [1]Rheumatology, University-Hospital of Lille, Lille, France, [2]University-Hospital of Lille, Lille, France.

The present study aimed to characterize bone structure assessed by magnetic resonance imaging (MRI, Vision®, Siemens; Erlangen, Germany) at the calcaneus in male subjects suffering from osteoporosis. Fifty subjects were assessed (26 with osteoporosis and 24 control subjects matched for age). Osteoporosis was defined according to the World Health Organization classification (T-score < -2.5) either at the lumbar spine or hip. Seventeen subjects (65%) had a past history of low energy fracture mainly represented by vertebral fractures (11/26, 42%). A three-dimensional gradient-echo sequence was used with a slice thickness of 700 microns and in plane resolution of 172x172 microns. Ten consecutive median sagittal slices were selected for each subject and the mean of the results was taken into account. Bone structure analysis was performed using structural (binary and skeletonized images) and fractal analyses. Bone densitometry by DXA at the calcaneus was also performed. Bone mineral density (BMD) was decreased in osteoporotic patients compared with controls both at the lumbar spine and hip (p<0.01) but also calcaneus (p<0.05). Also thirteen microarchitectural features among 22 measured were significantly different between the 2 groups (p<0.05). The odds ratios for fracture per one control group standard deviation alteration after adjustment for body mass index and BMD at the calcaneus were significant for the following features (p<0.05): apparent BV/TV, 3.1 (1.5-6.5); marrow space star volume, 2.1 (1.2-4.0); apparent trabecular spacing, 1.7 (1.1-2.9); trabecular bone pattern factor, 2.4 (1.2-4.7); total skeleton length (trabecular network), 2.1 (1.1-3.9); interconnectivity index, 2.6 (1.3-5.3); node count, 3.0 (1.4-6.4); terminus count, 2.1 (1.0-4.1); node-to-node strut count, 3.7 (1.5-9.3); terminus-to-terminus strut count, 1.8 (1.2-2.8); node-to-node strut length, 2.7 (1.2-5.9); node-to-terminus strut length, 3.0 (1.4-6.7) and fractal dimension, 2.2 (1.0-4.9). Few and weak correlations (r<0.5) were found between BMD at the calcaneus measured with DXA and features obtained from MRI suggesting that these 2 methods give different informations about bone status.In conclusion male osteoporosis is a disease characterized by decreased bone mass but also microarchitectural deteriorations of bone tissue which are partly independent of BMD.

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Potassium Citrate Prevents Increased Urine Calcium Excretion and Bone Resorption Induced by a High Sodium Chloride Diet. D. E. Sellmeyer,*[1] M. Schloetter,*[2] A. Sebastian.*[2] [1]Medicine/Endocrine, University of California, San Francisco, San Francisco, CA, USA, [2]General Clinical Research Center, University of California, San Francisco, San Francisco, CA, USA.

The amount of sodium chloride in the diet of industrialized nations far exceeds physiological requirements. The impact of abundant dietary salt on skeletal health has yet to be established, but is potentially detrimental through increased urinary calcium losses. We examined the effect of increased dietary sodium chloride on urine calcium excretion and bone turnover in postmenopausal women and, further, whether oral potassium citrate attenuated the effects of increased dietary salt. Women were excluded if they were less than 2 years past menopause, on medications known to affect bone metabolism, had evidence of metabolic bone disease, renal insufficiency or medical conditions potentially worsened by increased dietary sodium or potassium. Study subjects (n=63) were placed on a low salt (87 meq/d sodium) diet for three weeks then randomized to a high salt (225 meq/d sodium) diet + potassium citrate (90 meq/d) or a high salt (225 meq/d sodium) diet + placebo. Fifty-two women (83%) completed the study. The primary reason for withdrawl was nausea from the salt supplements. The table below shows the mean ± SD for urine calcium, N-telopeptide (NTX), and serum osteocalcin measured at the end of the low salt and high salt periods.

	Low salt	High salt + Intervention	p-value vs Low salt
Intervention=Placebo			
Urine calcium (mg/d)	200 ± 14	242 ± 16	0.002
Urine NTX (nBCE/mmol Cr)	38.7 ± 4.2	45.1 ± 3.8	0.001
Osteocalcin (ng/ml)	11.2 ± 0.6	10.6 ± 0.6	0.01
Intervention=Potassium citrate			
Urine calcium (mg/d)	200 ± 14	192 ± 19	0.5
Urine NTX (nBCE/mmol Cr)	41.3 ± 3.6	43.3 ± 3.7	0.3
Osteocalcin (ng/ml)	10.9 ± 0.7	10.7 ± 0.6	0.3

The changes in urine calcium and NTX between the low and high salt periods were also significant between the placebo and potassium citrate groups (p=0.008 and 0.05 respectively, unpaired t-test). There were no significant differences in PTH or cyclic AMP between the low and high salt diets or between the potassium citrate and placebo groups. Increased dietary salt enhanced urinary calcium excretion and bone resorption without changes in PTH. The addition of oral potassium citrate to a high salt diet prevented the increased calcium losses and bone resorption caused by a high dietary salt intake. Diets containing generous amounts of sodium, typical of industrialized nations, may be detrimental to bone health. Reducing sodium chloride intake and increasing intake of dietary sources of alkaline potassium salts may be beneficial for postmenopausal women at risk for osteoporosis.

F322

Continuous Oral Glucocorticoid Therapy Is Associated with an Increased Rate of Hip Bone Loss in Older Women: The Study of Osteoporotic Fractures. M. C. Hochberg,[1] J. K. Tracy,*[1] M. Vogt,[2] K. Stone,[3] K. Ensrud.[4] [1]University of Maryland School of Medicine, Baltimore, MD, USA, [2]University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [3]University of California, San Francisco, CA, USA, [4]University of Minnesota School of Medicine, Minneapolis, MN, USA.

Oral glucocorticoid therapy is the most common cause of secondary osteoporosis and is associated with an increased risk of fracture. Data from short-term placebo-controlled clinical trials have documented bone loss in patients receiving oral glucocorticoids; however, no data are published on rates of bone loss in community-dwelling individuals treated with chronic oral glucocorticoid therapy. Longitudinal dual energy xray absorptiometry scans obtained a mean of 3.5 years apart on a Hologic QDR 1000 (Waltham MA) were analyzed from a total of 4,258 elderly white women (mean [SD] age 77.1 [5.1] years) participants in the Study of Osteoporotic Fractures who had complete data on use of glucocorticoids at Visits 1, 2 and 4. Rates of change in total hip and femoral neck bone mineral density (BMD), adjusted for age, weight, physical activity, smoking and self-reported health status at Visit 4, and weight change between Visit 2 and Visit 4, were compared among classes of oral glucocorticoid users: continuous users at all three visits (N=125), former users [use at either Visit 1 or 2 but no use at Visit 4] (N=202), and never users (N=3931). All groups lost BMD at both the total hip and femoral neck. The adjusted rate of bone loss among current

oral glucocorticoid users was significantly higher than in both the former users and the never users at both sites (see Table) (*P <.01 for all prespecified pair-wise comparisons). The differences in the adjusted rate of bone loss between former users and never users did not reach statistical significance at either site.

Adjusted Rate of Bone Loss (mean [SE]) by Class of Glucocorticoid Usage

	Continuous Users	Former Users	Never Users
Total Hip, mg/cm2/yr	8.77 (1)*	2.88 (1)	3.92 (0.2)
Total Hip, %/yr	1.30 (0.13)*	0.42 (0.10)	0.55 (0.02)
Femoral Neck, mg/cm2/yr	5.52 (1)*	1.63 (1)	2.57 (0.2)
Femoral Neck, %/yr	0.89 (0.17)*	0.25 (0.13)	0.39 (0.03)

These data demonstrate that elderly women taking chronic oral glucocorticoid therapy lose bone at a greater rate than those not on such therapy. Based on these and other data, we suggest that elderly women taking chronic oral glucocorticoid therapy should receive treatment to prevent bone loss as well as to reduce the increased risk of fractures.

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Ovariectomy Fails to Induce Further Bone Loss in Mice Transgenic for Granulocyte Colony-Stimulating Factor. T. Oda,[*1] T. Wada,[1] Y. Kokai,[*1] H. Kuwabara,[*1] N. Sawada,[*1] S. Ishii.[*1 1]Sapporo Medical University, Sapporo, Japan.

Both mice transgenic for granulocyte colony-stimulating factor (G-CSF) (G-Tg mice) and ovarietomized mice exhibit severe osteopenia with increased bone resorption and up-regulated hemopoiesis. To elucidate the relation between abnormal hemopoiesis and bone loss, we performed an ovariectomy (OVX) on G-Tg mice. The G-Tg mice were established using the human G-CSF gene under an SRα promoter. An OVX or sham operation was performed on 12-week-old G-Tg mice or littermates (LMs). Uterine weight, bone mineral density (BMD) of the tibia (by the Dual-energy X-ray Absorptiometry method) and urinary deoxypyridinoline (Dpyr) concentration were measured 8 weeks after the operation. The number of colony-forming units of granulocytes/macrophages (CFU-GMs) and B220-positive cells in the bone marrow were counted 3 weeks after the operation. The OVX did not significantly change the BMD or urinary Dpyr content in G-Tg mice, though a more than 70% decrease in uterine weight was observed irrespective of G-CSF transgene expression. The OVX up-regulated the number of CFU-GMs two-fold in the bone marrow of LMs, which was associated with a 3.6 fold increase in B220-positive cells. The OVX did not alter the number of either CFU-GMs or B220-positive cells in the marrow of G-Tg mice. Taken together, G-Tg mice appeared to be resistant to further bone mass loss via acute estrogen deficiency. This may reflect that the up-regulation of hemopoiesis by G-CSF changed the responsiveness of bone mass to estrogen regulation, suggesting a strong link between bone phenotype and hemopoiesis. There was an increase in CFU-GMs in the bone marrow in ovariectomized mice. No such increase occurred in the G-Tg mice, partly because of the enhancement of CFU-GMs. The changes in number of CFU-GMs in these animal models may be central to bone reduction as an up-regulation of the osteoclast pool in the marrow. Furthermore, progenitors of osteoclasts, including hemopoietic stem cells, may play a role in bone phenotypes, such as osteoporosis. An approach to counteract the G-CSF pathway may prove effective in suppressing osteoporosis, since the OVX did not induce bone loss or bone resorption in G-Tg mice.

Mice	Uterine Weight[a]	BMD[b]	Dpyr content[c]	CFU-GMs[d]	B220s[e]
LM sham	104.3±13.1	471±51	7.84±1.46	1170±120	0.9±0.2
LM OVX	17.6±5.6*	277±49*	12.29±4.77***	2420±210*	3.6±1.1*
G-Tg sham	86.4±4.6	240±42	12.92±3.33	2270±160	0.2±0.1
G-TG OVX	23.1±6.3**	222±46	13.81±3.88	2120±180	0.2±0.1

a: mg, b: x0.0001 g/cm^2, c: nmol/mmol, d: per bilateral tibiae, e: x10^7 per bilateral tibiae *: p<0.01 vs LM sham, **: p<0.01 vs G-Tg sham, ***: p<0.05 vs LM sham

F330

Expression of Interleukin-6 (IL-6) and IL-6 Receptor mRNA in Human Bone Samples from Pre- and Postmenopausal Women. T. Seck,[1] I. Diel,[*2] H. Bismar,[*3] R. Ziegler,[3] J. Pfeilschifter.[4] [1]Orthopedics, Yale University, School of Medicine, New Haven, CT, USA, [2]Gynaecology, University of Heidelberg, Heidelberg, Germany, [3]Internal Medicine, University of Heidelberg, Heidelberg, Germany, [4]Internal Medicine, University of Bochum, Bochum, Germany.

Interleukin-6 (IL-6) has been attributed with induction of osteoclastogenic-precursor cell proliferation and maturation. Estrogens suppress IL-6 production in stromal/osteoblastic cells in vitro. Conversely, estrogen withdrawal is associated with increased IL-6 production. IL-6 is therefore thought to be an important mediator of the increased bone resorption following menopause. However, evidence supporting a rise in the expression of IL-6 or the IL-6 receptor in human bone tissue with menopause is still lacking. To address this question we established a 5'-nuclease-assay to quantitate the expression of human IL-6 and the gp80 subunit of the IL-6 receptor in human bone samples. The number of mRNA copies was normalized to the number of copies of beta actin mRNA. Osteocalcin expression served as an independent control. The study population consisted of 169 women (mean age: 52.4 + 11.6 years) who underwent surgery for early breast cancer. Serum IL-6 was measured by ELISA, serum crosslaps as a marker of bone resorption were measured by ECLIA, serum osteocalcin was measured by chemoluminescence assays.RNA expression of osteocalcin in bone tissue from early postmenopausal women was higher compared with premenopausal women. Local osteocalcin expression was positively associated with circulating osteocalcin (r=0.24, P=0.05) and crosslaps (0.30, P=0.005) concentrations. Postmenopausal women also had higher circulating IL-6 levels, compared with premenopausal women, but circulating IL-6 levels were not associated with IL-6 expression in bone samples. There was no significant difference in the expression of either IL-6 or gp80 in postmenopausal women compared to bone samples from premenopausal women. In conclusion, in contrast to the higher expression of osteocalcin in bone samples of postmenopausal women, we did not observe a local increase in IL-6 or IL-6 receptor expression in human bone tissue with menopause. We can not exclude a small increase below the detection limit of our assay, however our data support the hypothesis, that the involvement of IL-6 in postmenopausal bone loss , could be better reflected by circulating IL-6 levels than by IL-6 production in bone.

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Attenuation of Post-Ovariectomy Bone Loss in Cathepsin K Null Mice. M. A. Gentile,[1] P. Saftig,[*2] G. A. Rodan,[1] D. B. Kimmel.[1 1]Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA, USA, [2]Universitat Gottingen, Gottingen, Germany.

Cathepsin-K (CatK) is a collagen-degrading lysosomal cysteine protease important in osteoclastic bone resorption. CatK null (CatK KO) mice are osteopetrotic and model human pycnodysostosis, caused by mutations in the CatK gene. The purpose of this study was to examine the extent of bone loss caused by ovariectomy (OVX) in CatK KO mice. CatK KO mice were generated by targeted disruption of exon-7 via neo cassette insertion in L129SVJ embryonic stem (ES) cells. Two age groups (23 and 30 weeks; N=20 each) of wild type (WT) and CatK KO mice were OVXd or Sham-OVX. 8 weeks later bone mineral density (mg/cm2) was measured ex vivo by pDXA (Norland) in L3-L6 vertebrae (LV-BMD), proximal 25% (PF-BMD) and distal 25% (DF-BMD) of femora. Results were analyzed by Kruskal-Wallis ANOVA with Student Neuman-Keuls post-hoc analysis. Femur length (F-Lgt, mm) was measured.

Geno/Surg	Age (wks)	DF-BMD	LV-BMD	PF-BMD	F-Lgt
WT/Sham	23	64±4#	57±5#	64±3#	16.5±0.3
WT/OVX	23	54±6	47±4	59±3	17.1±0.6
CatKKO/Sham	23	72±14	62±8	65±8	15.8±0.6
CatKKO/OVX	23	71±8	63±5	66±4	16.1±0.5
WT/Sham	30	66±8#	64±7#	68±6#	16.9±0.3
WT/OVX	30	59±3	55±5	64±3	17.0±0.2
CatKKO/Sham	30	84±7#	68±5	72±5	15.7±0.3#
CatKKO/OVX	30	76±10	66±7	72±7	16.4±0.4

Mean±SD; #different from OVX/same genotype (P<.04)

At all sites CatK KO/Sh mice had higher BMD than WT/Sh (P<.001). CatK KO mice had shorter femurs than WT mice (P<.005). In WT mice, OVX induced significant bone loss at all sites at both ages. In CatK KO mice, OVX caused bone loss only in the distal femur of 30 week old mice; no other bone loss was seen. These data confirm that lack of cathepsin K increases BMD and indicate that OVX-induced bone loss is markedly attenuated in the absence of cathepsin K. These data make cathepsin K inhibition an attractive target for suppression of estrogen-deficiency bone loss in humans

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LHRH Analog Therapy Causes Early Bone Loss in Men Receiving Treatment for Prostate Cancer, P. Taxel,[1] P. C. Albertsen,[*2] R. Dowsett,[*3] P. M. Fall,[4] M. Trahiotis,[*4] J. Zimmerman,[*4] L. G. Raisz.[1 1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Surgery, University of Connecticut Health Center, Farmington, CT, USA, [3]Radiation Oncology, University of Connecticut Health Center, Farmington, CT, USA, [4]General Clinical Research Center, University of Connecticut Health Center, Farmington, CT, USA.

Prostate Carcinoma (Ca) is the most commonly diagnosed Ca in U.S. men, and the second leading cause of Ca death. Recently, more men are receiving hormonal suppression with Luteinizing Hormone Releasing Hormone (LHRH) analogs for locally advanced disease. Some studies have shown that this therapy can lead to bone loss. As hip fractures are a major cause of morbidity and mortality in older men, the potential for compounding this problem with LHRH analogs is the rationale for this study. Bone mineral density (BMD) was measured by dual energy x-ray absorptiometry (DXA) at the lumbar spine (LS), femoral neck (FN), total hip (TH) and total body (TB) at baseline, prior to or within the first month of LHRH analog injection and every 6 months thereafter. Markers of bone resorption, including urinary cross-linked N and C-telopeptides of type 1 collagen (NTX and CTX), and markers of bone formation, including bone specific alkaline phosphatase (BSAP) and osteocalcin (OC) were measured at baseline and every 6 months. Data on 13 men, mean age 68 years (range 56-75), without bony metastases, who received 6 months of neoadjuvant therapy with LHRH analogs for prostate Ca are reported. At baseline, the mean (± standard deviation) BMD of the LS was 1.26 ± .17 gm/cm^2; FN .91 ± .19 gm/cm^2; TH 1.00 ± .13 gm/cm^2 and TB 1.21 ± .08 gm/cm^2. After 6 months of treatment, all 13 men lost more than 1.1% of BMD at at least one site. At the LS, 7 of 13 men lost up to 4.9% (mean loss 3%); at the FN, 6 of 13 men lost between 1 and 4.5% (mean loss 3%); at the TH 8 of 13 men lost between 1 and 6.7% (mean loss 3%). Moreover, men with the lowest baseline hip BMD showed the greatest % bone loss at this site. At 6 months there was a significant increase in NTX and CTX of 63 and 139% (p= .003 and .013), respectively. Nine of 13 men had repeat BMD measurements at 1 year. None regained BMD to pretreatment val-

ues despite discontinuation of LHRH analog therapy. Of these men, only 5 had testosterone levels that were restored to normal. NTX and CTX declined by 21 and 34%, respectively, from 6 to 12 months. We conclude that men treated with LHRH analogs are at risk for bone loss in the first 6 months of treatment, and may not regain bone mass within 6 months of discontinuation of this medication. This loss is associated with an increase in markers of bone resorption. We suggest that preventive measures to slow bone loss are indicated in men receiving LHRH analog therapy.

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Trabecular Bone Microarchitecture Is Related to Clinical Risk Factors in Osteoporosis in Men. É. R. Legrand, D. Chappard, M. Basle, M. Audran. CHU, Angers, Angers, France.

We have shown that trabecular bone connectivity is a major and independant determinant of vertebral fracture in men with mild osteoporosis (1) . To examine the relationships between risk factors for osteoporosis and trabecular bone microarchitecture (evaluated on transiliac bone biopsy) we included 152 men with low BMD (lumbar T-score < -2.5) in a prospective study. The risk factors were checked: age, BMI (80 gr/day), use of corticosteroids (> 10 mg of prednisone for at least 1 year), hypogonadism, chronic disease associated with bone loss. The histomorphometric analysis was done on a Leica quantimet image processor and the followings measures were performed : trabecular bone volume (BV/TV), trabecular thickness (Tb Th) and number (Tb N), Interconnectivity Index (ICI), Star Volume of the bone marrow, Characterization of the trabecular network (node and free-end count).table;The first results are in the TableI: the 36 men with at least 3 clinical risk factors had a marked disorganisation of the trabecular network with an increase in ICI, star volume and free-end and a decrease in trabecualr number and node. Furthermore, to evaluate the role of the different risk factors, logistic regression analysis was performed. Age, alcohol abuse and steroid were not associated with disorganisation of the bone microarchitecture. In contrast, smoking (OR 2.4, $p < 0.05$), low BMI (OR 1.6, $p< 0.05$), hypogonadism (OR 11.9 $p < 0.05$) and chronic disease (OR 1.4 $p < 0.05$) were significantly associated with a low trabecular bone connectivity. These results strongly suggest that, in men with low BMD, higher the number of clinical risk factors, lower the connectivity of the trabecular network and probably higher the fracture' risk.(1) E Legrand et al. Trabecular bone microarchitecture, bone mineral density and vertebral fractures in male osteoporosis. JBMR 2000; 15: 13-19. Table I Comparison of bone parameters in men with no clinical risk factors (RF), 1 or 2 RF or at least 3 RF for osteoporosis.*$p < 0.05$, ** $p <0.01$ versus men with 0 to 2 risk factors

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Smoking and Intestinal Calcium Absorption: Relationships with Vitamin D Metabolites in Postmenopausal Women. A. G. Need,*[1] A. Kemp,*[2] M. Horowitz,*[2] H. A. Morris.*[1] [1]Clinical Biochemistry, Institute of Med and Vet Science, Adelaide, Australia, [2]Department of Medicine, Royal Adelaide Hospital, Adelaide, Australia.

Smoking has been associated with poor intestinal calcium absorption, low bone density and fractures in postmenopausal women, but there are conflicting reports on how it affects the vitamin D endocrine system.In a group of 405 postmenopausal women attending our osteoporosis clinics we took a fracture history and measured intestinal calcium absorption efficiency (with 45Ca using a 20 mg calcium carrier and a single blood sample taken one hour after the dose), serum vitamin D metabolites (25 hydroxyvitamin D by competitive protein binding and calcitriol by HPLC and radioimmunoassay) and parathyroid hormone (PTH)(by immunoradiometric assay). We compared mean values in the 74 smokers and the 331 non-smokers and examined the relationships between the variables in each group. Vertebral fractures were more common in smokers than non-smokers (1.5(2.5SD) vs 0.7(1.4);P=0.013) as were peripheral fractures (1.2(1.8) vs 0.6(1.0);P=0.016). The hourly fractional rate of calcium absorption was less in smokers than non-smokers (0.56(0.29SD) vs 0.68(0.27) fx/h;P=0.001) as were serum calcitriol (95(32) vs 122(37) pmol/L;P<0.001) and PTH (3.8(1.6) vs 4.9(2.6) pmol/L;P<0.001). Serum 25 hydroxyvitamin D was similar in each group (60(25)nmol/L in the smokers vs 62(26) in the non-smokers;P=0.57). In the smokers, calcium absorption was 0.23+0.0034 x serum calcitriol fx/h (R=0.38;P=0.001) and in the non-smokers it was 0.42+0.0021 x serum calcitriol fx/h (R=0.28;P<0.001). There was no difference in this relationship between smokers and non-smokers. In the smokers serum calcitriol, in turn, was significantly related to serum PTH (P=0.001) and 25 hydroxyvitamin D (P=0.027). Hence the poor calcium absorption in the smokers was due to suppression of the PTH-calcitriol axis.We conclude that calcium absorption is decreased in smokers due to suppression of the PTH-calcitriol endocrine axis. This may aggravate their postmenopausal bone loss and also limit the effectiveness of calcium supplements.

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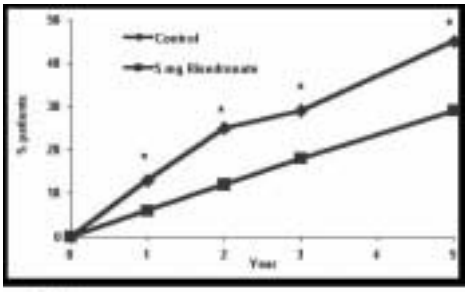
Sustained Fracture Risk Reduction Over 5 Years With Risedronate Therapy. N. B. Watts,[1] J. Brown,[2] D. Hosking,[3] S. Adami,[4] H. Mulder,*[5] J. Y. Reginster,[6] C. Kasibhatla,*[7] A. Chines.[7] [1]Emory University, Atlanta, GA, USA, [2]Le Centre Hospitalier Universitaire de Quebec, Quebec, Canada, [3]Nottingham City Hospital, Nottingham, United Kingdom, [4]Clinicizzato di Valeggio, Valeggio, Italy, [5]Research Center Good Clinical Practice, Rotterdam, The Netherlands, [6]University of Liege, Liege, Belgium, [7]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Clinical extension trials involving bisphosphonates are usually open-labeled continuation of three year double blind studies. In the first placebo-controlled extension studies of a bisphosphonate, safety and efficacy of long term risedronate use were evaluated over 5 years by extending two large 3 year fracture efficacy studies (VERT-MN and VERT-NA) for two years. In VERT-MN, a total of 265 patients (placebo=130 and risedronate 5 mg=135) continued their originally assigned treatment in years 4 and 5. Overall, there was a statistically significant 49% vertebral fracture risk reduction. Non-vertebral fracture risk reduction with risedronate over this period was 37% (p=0.022). Adverse events including upper GI were comparable between the two groups. In VERT-NA, in the extension period (placebo=42, risedronate=44), BMD either was maintained or continued to increase. On biopsy, normal lamellar bone was observed after 5 years of risedronate treatment without any pathological findings. Bone turnover was reduced by 50%. In conclusion, risedronate treatment over 5 years provides sustained efficacy and safety as evidenced by histology showing no pathological abnormalities. Risedronate was well tolerated over the treatment period.



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Risedronate (Ris) Preserves Bone Architecture in Osteoporotic Postmenopausal Women as Measured by 3-D Micro-Computed Tomography: Effects of Bone Turnover. B. Borah,*[1] T. E. Dufresne,*[1] P. A. Chmielewski,*[1] M. C. Prenger,*[1] E. F. Eriksen.[2] [1]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [2]Aarhus Artssygehus, Aarhus, Denmark.

It is well recognized that a combination of low bone mass and the deterioration of trabecular architecture underlies osteoporotic fractures. We present data that indicate for the first time that RIS preserves trabecular architecture in osteoporotic women. We investigated the effects of RIS on trabecular architecture in the iliac crest biopsies using 3-D micro-computed tomography (mCT) in a cohort of 38 postmenopausal osteoporotic women treated with 5 mg RIS (n = 21) or placebo (n =17) for 3 years. Each patient had a biopsy taken at baseline and at 3 yrs. Bone turnover parameters were collected using conventional histological techniques (Eriksen et. al., JBMR 14(suppl. 1):S404, 1999). The biopsies were then imaged using a Scanco Medical mCT scanner at 30 micron isotropic voxel resolution. The analysts were blinded to treatment assignment during image acquisition and architectural analysis. One-way ANOVA was performed to examine architectural changes from baseline and across treatment groups. When analyzing the entire dataset, no significant differences in trabecular architecture were observed between the placebo and RIS groups. However, we found a significant negative correlation ($p = 0.01$) between pairwise change in bone volume (BV/TV) and baseline Mineralizing Surface/Bone Surface (MS/BS) in the placebo group. In the RIS group, no such correlation existed ($p = 0.79$). This provided evidence that higher turnover induces bone loss leading to a higher risk of architectural degradation in osteoporotic patients. With this rationale, we performed subgroup analysis of patients who had baseline biopsies with MS/BS in the upper 50% of the entire cohort (range of 6.4% - 19.4%). In this subgroup, several architectural parameters differed in the placebo group relative to the RIS group, including a significant decrease of BV/TV ($p = 0.016$), a significant decrease of Trabecular Thickness ($p = 0.024$), a significant shift toward more rod-like structure (shown by an increase in Structural Measure Index ($p = 0.014$) and Bone Surface/Bone Volume ($p= 0.008$)), and a marginally significant increase in Marrow Star Volume ($p = 0.086$). In the RIS group, the bone volume and the architectural parameters did not alter significantly from baseline. However, the average gray level in the mCT images, which is proportional to bone density, showed marginal increase ($p = 0.061$), indicating a shift toward increased mineralization with RIS. In conclusion, these data indicate that among women with higher bone turnover, RIS preserves trabecular bone architecture.

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The Effect of 5-Year Risedronate Therapy on Bone Histology and Histomorphometry. E. F. Eriksen,[1] F. Melson,[1] E. Sod,[2] A. Chines,[2] L. Law.*[2] [1]Aarhus Artssygehus, Aarhus, Denmark, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

A recent 3-year double blind, placebo-controlled study demonstrated that 5 mg Risedronate (Ris) significantly decreased the incidence of vertebral and nonvertebral fractures in women with postmenopausal osteoporosis (JAMA 1999; 282:1344). All patients were supplemented with calcium (1000mg/d) and Vit D supplementation was provided for those with low levels. This study was extended to 5 years for a subset of women who had bone biopsies at baseline. There were 42 placebo women (mean 69.2 {S.D. 9.2} y, femoral neck T-score -2.3) and 44 Ris women (mean age 69.3 {S.D. 8.0} y, femoral neck T-score-2.8) in the extension. We now present biopsy data from 21 placebo and 27 risedronate patients who where evaluable for histomorphometric and/or histological analyses at baseline and 5 years. The table shows changes in selected parameters in patients with paired biopsies (baseline and post-treatment). For Ris patients, histology showed normal lamellar bone without pathological findings such as marrow fibrosis, osteomalacia, or woven bone. Bone mineralization was normal as assessed by Osteoid Thickness, Mineral Apposition Rate and Mineralization Lag Time. Bone turnover decreased in both treatment arms, but the change was statistically significant in the Ris arm only. Bone formation at the BMU level (wall thickness) was normal and did not change over time. Double tetracycline labels were present in all biopsy specimens indicating continuous bone turnover. Although cortical thickness decreased in both groups, the decrease was about 3-fold greater in the placebo group indicating that risedronate reduces cortical bone loss. These results are in general similar to those reported after 3 years of risedronate treatment (JBMR 1999;14: S404) and

show no further reduction in bone turnover.

	Placebo		Risedronate 5 mg	
	Baseline	**5 years**	**Baseline**	**5 years**
Osteoid Thickness (mm)	8.6	10	8.9	9.4
Mineralization lab time (days)	28	27	19	31
Mineral apposition rate (mm/d)	0.55	0.6	0.533	0.6
Mineralizing Surface (%)	6.8	4	6.3	1.2*
Activation Frequency (1/yr)	0.44	0.2	0.42	0.09*
Trabecular bone volume (%)	19	19	17	15
Wall Thickness (mm)	39	42	41.5	43
Cortical Thickness (mm)	1321	915*	1017	885*

In conclusion, these unique data from paired biopsy samples show normal bone formation and moderate and sustained decrease in bone turnover without negative effects on bone mineralization or structure after 5 years of risedronate treatment. These data demonstrate the long-term bone safety of risedronate.

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Zoledronic Acid, at a Total Annual Dose of 4 mg, Increases Bone Density at All Sites and Stably Reduces Bone Turnover for One Year when Administered as a Single or a 3-Monthly Intravenous Injection in Postmenopausal Osteoporosis. J. P. Brown,[1] P. Burckhardt,[2] I. R. Reid,[3] Z. D. Horowitz,[4] P. C. Richardson,[4] U. Trechsel.[5] [1]Centre de recherche du CHUL, Université Laval, Québec, Canada, [2]CHUV, Lausanne, Switzerland, [3]University of Auckland, Auckland, New Zealand, [4]Novartis, East Hanover, NJ, USA, [5]Novartis, Basel, Switzerland.

For the Zoledronic Acid Study GroupOral bisphosphonates have become an important therapeutic option for the treatment of postmenopausal osteoporosis but have limitations in terms of low bioavailablility and dosing instructions. Although intravenous (IV) use every 3 months has been studied, the use of very infrequent infusions has not been previously explored. In this study we explore whether the use of zoledronic acid allows very infrequent administration and results in a sustained suppression of bone turnover, whilst avoiding the limitations of regular oral dosing. In this 1-yr RCT, 351 postmenopausal women (age 45 to 80 yrs) with lumbar spine T-score < -2 (mean -2.9) and no prevalent vertebral fracture, zoledronic acid was given as an IV injection over 5 min. in doses of 0.25mg, 0.5mg and 1mg at 3 month (m) intervals. The total annual dose of 4mg was also given as a single 4 mg dose, or two 6-monthly doses of 2mg. All women received a calcium supplement (1g/d). BMD of the spine (LS), femoral neck (FN), total body (TB) and distal radius (DR) were measured by DXA. All BMD values were converted to a Hologic base. BMD results (ITT analysis) at 12 m are shown in the table below as mean % changes (± SEM) from baseline.

	Placebo	**4x0.25mg**	**4x0.5mg**	**4x1mg0**	**2x2mg**	**1x4mg**
LS	+0.4 (0.4)	+5.8* (0.4)	+5.6* (0.5)	+4.8* (0.4)	+4.9* (0.5)	+5.0* (0.5)
FN	-0.5 (0.4)	+2.7* (0.5)	+2.7* (0.3)	+2.5* (0.4)	+3* (0.4)	+2.6* (0.3)
TB	+0.6 (0.7)	+1.7§ (0.6)	+1.5 (0.6)	+1.4¥ (0.3)	+1.4¥ (0.4)	+1.7§ (0.3)
DR	-0.8 (0.4)	+0.1 (0.6)	+0.6¥ (0.4)	+0.7§ (0.4)	+0.5‡ (0.5)	+0.3‡ (0.4)

* p<0.001, § p<0.01, ¥ p<0.05, ‡ p=0.05 compared to placebo. Resorption markers dropped to a nadir at 1 m (median falls of 65-83% for serum CTX and 50-69% for urinary NTX). Suppression was maintained at 12 m in all groups and was significantly different from placebo (p<0.001). BSAP and osteocalcin showed similar responses with a nadir at 3 m and stable suppression until 12 m for all doses (p<0.001). Myalgia, pyrexia and transient bone pain occurred more commonly in the zoledronic acid groups. We conclude that zoledronic acid given at intervals of up to 1 year between injections significantly increases BMD at all sites and stably reduces bone turnover, and therefore should be considered for evaluation in the treatment of postmenopausal osteoporosis.

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Evidence for Mechanism-Based Irritant Effects of Nitrogen-Bisphosphonates on Soft Tissues. J. E. Fisher, G. A. Rodan, A. A. Reszka. Bone Biology and Osteoporosis Research, Merck Research Laboratories, West Point, PA, USA.

Nitrogen-bisphosphonates (N-BPs) act directly on the osteoclast by inhibiting the cholesterol pathway enzyme, farnesyl diphosphate (FPP) synthase. Though bisphosphonates have the potential to irritate the esophagus and skin, the mechanism for these effects has not been elucidated. Recently, in vitro mechanism-based N-BP suppression of cell growth has been demonstrated in keratinocytes, and has been associated with suppression of cdk phosphorylation of the growth-regulatory protein, retinoblastoma (Reszka et al. 2001. Mol. Pharmacol., 59:193-202). In the studies described here, we examine two new models of local irritation. In vitro, Ch1.Es esophageal fibroblasts were used to model esophageal tissues. Twenty four hour treatment with the N-BPs alendronate (ALN) or risedronate (RIS), at 300 micromolar, a concentration that might be present in the esophagus after reflux, induced apoptosis. This effect was associated with caspase cleavage of the pro-apoptotic kinase Mst1 and activation of p38alpha- and Jnk kinases. A loss of Erk1 and Erk2 kinase activities was also observed. Consistent with mechanism-based action, all N-BP-induced apoptotic and growth effects were mimicked by a selective inhibitor of protein geranylgeranylation, and completely blocked by co-incubation with the mevalonate pathway metabolite, geranylgeraniol. Modification of culture conditions or pharmacological intervention by inhibiting squalene synthase, an enzyme immediately downstream of FPP synthase, augmented intracellular levels of both FPP and geranylgeranyl diphosphate (GGPP), and increased the expression of HMG-CoA reductase. These changes completely blocked apoptosis induced by the N-BPs. In vivo we examined localized skin irritation produced, in growing male Sprague Dawley rats, by subcutaneous bolus injections of ALN or RIS. N-BPs were administered at 0.5 mg/kg on each of five consecutive days. Approximately twenty four hours after the last dose, animals were euthanized and skin from injection sites was dissected out, fixed in 10% neutral-buffered formalin and measured. N-BP treatments caused significant (volumetric) skin thickening characterized by disruption of cell-cell contacts and cellular infiltration into the stratum basale. Co-administration of an inhibitor of squalene synthase, to potentiate intracellular accumulation of FPP and GGPP, significantly suppressed skin thickening induced by ALN (46%, p<0.01) or RIS (45%, p<0.02). Taken together, these data suggest that local irritation produced by N-BPs, in esophagus or skin, occurs through suppression of FPP synthase, and provide in vivo evidence for this mechanism-based action.

Disclosures: Merck and Company,1,3.

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Alendronate in the Treatment of Bone Loss after Spinal Cord Injury (SCI): Preliminary Data of a 2-Years Randomised Controlled Trial in 60 Paraplegic Men. M. Luethi,[1] Y. Zehnder,*[2] D. Michel,*[2] H. Knecht,*[2] R. Perrelet,*[1] M. Kraenzlin,*[3] G. A. Zaech,*[2] K. Lippuner.[1] [1]Osteoporosis Unit, University Hospital, Berne, Switzerland, [2]Swiss Paraplegic Center, Nottwil, Switzerland, [3]Endocrine Unit, University Hospital, Basle, Switzerland.

Bone loss appears early after SCI and leads to a high incidence of long bone fractures in paraplegics. To date, only few prospective studies evaluating therapeutic modalities for prevention or treatment of disuse osteoporosis have been published. Alendronate is effective in preventing and treating postmenopausal and male osteoporosis as well as glucocorticoid-induced osteoporosis. To investigate if alendronate is also effective in the management of disuse osteoporosis we performed the following trial.Sixty paraplegic men with posttraumatic SCI (Frankel A or B) were randomly allocated to one of the following treatment groups: alendronate, 10mg/d p.o. plus calcium, 500mg/d (ALN+Ca), or calcium 500mg/d alone (Ca). Subjects were stratified according to time since SCI. Bone mineral density (BMD) was measured at lumbar spine (LS), proximal femur (HIP), tibial diaphysis (T-DIA) and distal tibial epiphysis (T-EPI) at baseline and then every 6 months using dual energy X-ray absorptiometry (Hologic QDR 4500A). Today 51 paraplegics (26 ALN+Ca, 25 Ca) have completed their 18 months visit. Baseline characteristics (mean±SEM) are given in the table:

	ALN+Ca	**Ca**	**P (unpaired t-test)**
Age (years)	**38.4±1.5**	**37.6±2.1**	**0.8**
BMI (kg/m2)	23.7±0.6	22.5±0.5	0.1
Time since SCI (years)	10.6±1.4	9.5±1.5	0.6
BMD LS (g/cm2)	1.145±0.0393	1.119±0.031	0.6
BMD HIP (g/cm2)	0.717±0.0334	0.676±0032	0.4
BMD T-DIA (g/cm2)	1.163±0.041	1.163±0.035	1.0
BMD T-EPI (g/cm2)	0.464±0.035	0.449±0.029	0.8

BMD of LS increased in both groups over the 18 months, however with a significantly larger increase in the ALN+Ca (5.2±0.7%; mean±SEM) compared with the Ca group (2.0±0.8%; p<0.01, 2-way ANOVA). At HIP, BMD significantly decreased in Ca (-3.6±1.8%) whereas it remained stable in ALN+Ca (0.7±0.9%; p<0.05 between groups). Similarly, at T-EPI, BMD in Ca significantly decreased over time (-8.3±2.6%) but remained stable in ALN+Ca (-1.3±2.0%; p<0.05 between groups). The respective changes for T-DIA were: -3.0±0.9% in Ca (p<0.001 vs. baseline) versus –0.7±0.9% in ALN+Ca (p=n.s. vs. baseline).We conclude from these data that alendronate in combination with calcium is effective in preventing bone loss at distal limbs in paraplegic men, whereas calcium alone is not

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Efficacy of Risedronate in Decreasing the Incidence of Femoral Neck and Intertrochanteric Fractures in Older Women with Osteoporosis. R. Eastell,[1] M. R. McClung,[2] J. Y. Reginster,[3] W. G. Bensen,*[4] E. Seeman,[5] G. Cline,*[6] L. Law,*[6] A. Chines.[6] [1]University of Sheffield, Sheffield, United Kingdom, [2]Oregon Osteoporosis Center, Portland, OR, USA, [3]University of Liege, Liege, Belgium, [4]McMaster University, Hamilton, Canada, [5]University of Melbourne, Melbourne, Australia, [6]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Epidemiological studies suggest a difference in pathophysiology between femoral neck (FN) and intertrochanteric (IT) fractures. Above age 80, there is a much stronger relationship between low BMD and the risk of IT than FN fractures (Vega et. al., OI 1:81, 1991). The relative risk (RR) for 1 SD decrease in femoral neck BMD for IT fractures is independent of age (RR=2.5) while the RR for FN fracture decreases significantly from 3.3 (CI: 2.3-4.8) for women between 65-79 years to 1.3 (CI:0.6-2.8) for women over 80 years (Nevitt et. al., OI 4:325, 1994). This suggests that nonskeletal factors such as falls contribute proportionately more to FN fractures in older patients. The effect of antiresorptive treatment on these two types of fractures is mostly unknown.

In a 3-year study of 9331 patients, risedronate decreased the risk of all hip fractures in women with osteoporosis by 40-60% (McClung et al, NEJM 344:333, 2001). We examined the effect of risedronate on the incidence of the two most commonly observed types of hip fractures from the above study. Results from this analysis are presented in the table below.

	Women 70-79 yr of Age with Osteoporosis				Women ≥ 80 yr of Age with ≥ 1 Clinical Risk Factors for Hip Fracture			
(# Subjects)	Control (1821)	Ris (3624)	RR	p-value	Control (1313)	Ris (2573)	RR	p-value
IT Fractures	14 (1.0)	15 (.5)	0.54	0.089	22 (2.2)	26 (1.4)	0.60	0.071
FN Fractures	28 (1.9)	34 (1.2)	0.61	0.048	25 (2.7)	53 (2.7)	1.1	0.772

RR=Relative risk; data are number of fractures (%)- incidence based on Kaplan-Meier estimates

For women who are 80 years of age or older and have at least one clinical risk factor for hip fracture, there is a trend towards reduction of risk for IT fractures while FN fractures are unaffected. For women who are 70-79 years of age with confirmed low femoral neck BMD, both IT and FN fractures show a trend toward risk reduction with FN achieving statistical significance.

In conclusion, the beneficial effect of risedronate on IT and FN fractures among women with confirmed osteoporosis and on IT fractures in older women (with at least 1 clinical risk factor for hip fractures), a fracture type closely associated with low bone mass, supports the idea that these fractures could have different pathophysiology as they appear to respond differently to bisphosphonate therapy.

F363

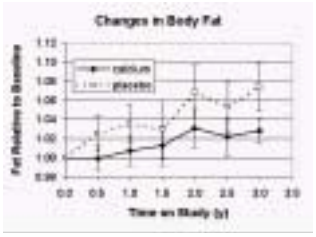
The Post-PTH Experience in Men with Idiopathic Osteoporosis: Bisphosphonates vs. Non-pharmacologic Therapy. E. S. Kurland,[1] S. L. Heller,[1] F. Cosman,[2] B. Diamond,[1] R. Lindsay,[2] J. P. Bilezikian.[1] [1]College of P&S, Columbia University, New York, NY, USA, [2]Helen Hayes Hospital, West Haverstraw, NY, USA.

We have previously reported that parathyroid hormone (PTH 1-34) has shown promise as an effective anabolic agent for the treatment of idiopathic osteoporosis (IO) in men. Important questions remain, however, about the preferred clinical strategy for managing patients beyond a 12-18 month course of PTH exposure. Are anti-resorptive agents useful or necessary after completing PTH therapy? Alternatively, might the cessation of PTH alone result in further gains in bone density, as has been observed after parathyroidectomy in patients with primary hyperparathyroidism? We followed 22 men with IO (mean age 50 +/- 1.9) who had been treated with PTH (1-34) for a mean duration of 23 +/- 7 months. 14/22 men (64%) began a bisphosphonate (BisP) immediately upon completing PTH, while 8/22 men (36%) took no medication. All patients continued to take 1200 mg of calcium and 400 units of vitamin D daily. During 2 years of follow-up, bone densitometry of the lumbar spine (LS), hip and forearm was performed at 6 month intervals with results for LS as follows: During the initial 6 months post-PTH the men on BisP had a mean further increase in LS bone density of 3.0 +/- 2.0% while the men on no additional treatment increased by 0.1 +/- 0.1%. One year post-PTH, men on BisP had no further increase (+0.2 +/- 0.1%) while the men on no treatment had declined minimally (-0.2 +/- 0.1%) (p =NS). The incremental gain in LS density in men on BisP was greatest in the initial 6 months of treatment and plateaued by 1 year. Six men (43%) who took PTH for a mean of 28 +/- 2 months had an increase in LS of 3.0 +/- 1.0% while 8 (57%) men who took PTH for a mean of 16 +/- 2 months experienced an increase of 7 +/- 2%. Six of the 8 men (75%) who initially chose no treatment began BisP within 1 year of discontinuing PTH. Mean change in LS density was -0.1 +/- 0.1% for this group at 6 months, in contrast to the 3.0 +/- 2.0% increase observed in men who started BisP immediately. Two men opted to take no additional treatment. Both experienced declines in LS bone density of 6.0% and 5.0% respectively, from maximal values achieved. We conclude that not only is there an additional gain in LS bone density that can be achieved when BisP immediately follows PTH therapy, but in the absence of such treatment, much of the bone mass accrued during PTH therapy is eventually lost. BisP appears to be most effective when administered sequentially after PTH treatment, with no further gains noted when BisP treatment is delayed. These data suggest that sequential administration of BisP should be an important component of any treatment strategy utilizing PTH as a therapy for IO in men.

F370

Calcium Supplementation May Attenuate Accumulation of Fat in Young Women. M. J. Barger-Lux,[1] K. M. Davies,[1] R. P. Heaney,[2] B. K. Chin,*[1] K. Rafferty.*[1] [1]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [2]University Professor, Creighton University, Omaha, NE, USA.

In earlier work, we found an inverse relationship between dietary calcium-to-protein ratio and fat mass in baseline data from several studies of female subjects. Here we report longitudinal data on body fat in young women who completed a 3-year, double-blind placebo-controlled trial of Ca supplementation (1500 mg/d) that included DXA scanning at 6-month intervals. For each subject, we expressed each DXA-generated value for total-body fat mass as a fraction of her baseline value. We generated dietary Ca:protein ratios (mg:g) from analyses of 7-day food diaries that the subjects kept prior to entry and at mid-study. For the present analysis, we included all subjects (n=52) with a full 36 months' of DXA data on fat mass. At entry, these subjects were aged 23.6 ± 2.8 years. They had BMIs of 22.3 ± 2.8 kg/m2, daily Ca intakes 591 ± 169 mg, and dietary Ca:protein ratios of 10.0 ± 2.0 mg:g. The figure shows timecourse of changes in total-body fat for the Ca and placebo groups (data as mean ± SEM). The curve of the placebo group is consistently above that of the Ca-supplemented group. The aggregate slopes were 2.2 %/yr for the placebo group and 1.1 %/yr for the Ca group; the slope of the Ca group falls below the 95% CI of the placebo group. We conclude that the benefits of Ca supplementation in young women may include attenuation of gain in total-body fat mass.



F374

Effects of Two Years of Exercise and Hormone Replacement Therapy on Bone Mineral Density in Postmenopausal Women. S. B. Going,[1] T. G. Lohman,[2] E. C. Cussler,*[2] R. M. Blew,*[2] L. B. Houtkooper,*[2] L. M. Metcalfe,*[2] H. G. Flint-Wagner,*[2] V. Stanford.*[2] [1]Physiology, University of Arizona, Tucson, AZ, USA, [2]University of Arizona, Tucson, AZ, USA.

Combination therapies are receiving increasing attention in the search for effective regimens to counter bone loss. The Bone, Estrogen and Strength Training (BEST) Study was designed to test the effects of exercise with and without hormone replacement therapy (HRT) on bone mineral density (BMD). Early postmenopausal women (age, 55.8 ± 4.8y) who used HRT (1 - 4 years; n = 108) or who did not use HRT (NHRT, ≥ 1 year, n = 105) were randomized to 24 months of exercise (EX) or no exercise (NEX). BMD and soft tissue composition was measured twice (averaged for analyses) at baseline, 12 months and 24 months by dual energy x-ray absorptiometry. All subjects received 800 mg/day calcium supplements. Exercise (3 days per week) entailed supervised high load, low rep weight lifting (70-80% 1 RM, 2 sets of 6-8 reps, 8 exercises), moderate impact aerobic weight-bearing (25 min), and steps/stair climbing with weighted vests. Baseline height (163.1 ± 6.4 cm), weight (67.1 ± 10.9 kg), percent fat (38.1 ± 6.5%) and BMD's at femur neck (FN, 0.87 ± 0.12 g/cm2), trochanter (FT, 0.74 ± 0.11 g/cm2), and lumbar spine (LS, 1.12 ± 0.15 g/cm2) were similar (p>0.05) among groups. BMD changes were examined using multiple regression to test contrasts of EX versus NEX within HRT and NHRT groups across sites. BMD adjusted for baseline BMD and years past menopause increased in women who exercised or used HRT by 1.0 - 1.5% and decreased in women who did not EX and did not use HRT. Trochanteric BMD was significantly (p<.05) increased at two years. Women who exercised and used HRT gained BMD (+1.24%) compared to women who used HRT and did not exercise (-1.70%) and controls (-1.6%), who lost BMD. We conclude the combination of exercise and HRT counters trochanteric bone loss in postmenopausal women. Supported by: NIH AR39559 and Mission Pharmacal

F380

Are Placebo Controlled Trials Ethical for Women With Osteoporosis? S. R. Cummings,[1] K. Modelska,*[2] M. C. Nevitt,[3] D. M. Black.[4] [1]Medicine, University of California, San Francisco, CA, USA, [2]UCSF Coordinating Center, San Francisco, USA, [3]Epidemiology and Biostatistics, University of California, San Francisco, USA, [4]University of California, San Francisco, USA.

Several treatments reduce fracture risk in women with osteoporosis and guidelines recommend these treatments for women with osteoporosis. Is it still 'ethical' to enroll women with osteoporosis in placebo-controlled trials? We addressed this question by reviewing principles of Western medical ethics and systematically reviewing data about how much benefit a woman with osteoporosis may forfeit if she were assigned to placebo. The principle of beneficence requires an acceptable balance of benefits and risks; the principle of individual autonomy indicates that the individual woman has the right to balance the benefits and risks and decide whether to participate. Although hip or spine fracture have been associated with increased mortality rates in epidemiologic studies, a systematic review of randomized trials indicates that these treatments have not reduced mortality. Data from the Fracture Intervention Trial show that, on average, women with osteoporosis assigned to placebo (plus calcium & vitamin D) had 2.6 to 3.2 more days of fracture-related disability and 1.1 more days of bedrest due to back pain per year than those assigned to alendronate (P<.01). Women with a vertebral fracture within 2 years had a 4.8-fold higher risk of a subsequent vertebral fracture (16.3 % / yr) than did others (3.4% / yr) (P<.01).We conclude that among women with osteoporosis, the risk of mortality and morbidity from assignment to placebo is not sufficient to override an individual woman's right to make an informed decision to participate in a placebo-controlled trial. Women with recent vertebral fractures should be strongly advised to start approved treatment and not enroll in a placebo-controlled trial.

F382

Treatment of Osteoporosis Following a Hip Fracture: Sending Results of Bone Densitometry to Primary Care Physicians Does Not Increase Use of Pharmacologic Therapy. D. L. Orwig,*[1] L. Wehren,[1] J. YuYahiro,*[2] M. Hochberg,*[1] J. Magaziner.[1] [1]University of Maryland Baltimore, Baltimore, MD, USA, [2]Union Memorial Hospital, Baltimore, MD, USA.

Approximately 350,000 hip fractures occur annually in the United States. Hip fracture is associated with substantial excess morbidity and mortality, and people who have fractured are at increased risk of subsequent fractures. Almost all elders who sustain a hip fracture have low bone mineral density (BMD) at the time of the fracture and continue to lose up to 6% BMD in the femoral neck during the first year after the fracture. Although several pharmacologic agents are approved for the treatment of osteoporosis that have demonstrated anti-fracture efficacy, post-fracture management rarely includes bone-strengthening medication. This analysis examined the change over time in use of bone-strengthening

medications in patients who sustained a hip facture. Outcomes of hip fracture have been observed in more than 2000 participants in the Baltimore Hip Studies (BHS). Among a cohort of 205 women enrolled in the BHS,1992-95 (mean age of 81; mean baseline total hip BMD=0.588 gm/cm2), over 87% had osteoporosis as defined by the World Health Organization (femoral neck T score < -2.5) and met the National Osteoporosis Foundation (NOF) treatment guidelines. Among these women, fewer than 10% were taking bone-active medications at the time of fracture (5% estrogen; 5% etidronate), and only 10% received any bone-active medication within the year after fracture. In addition, only 15% were taking calcium while 75% were vitamin D deficient, as defined by low serum levels of 25 (OH) vitamin D. In a current BHS cohort (enrollment began in 1998), results of the baseline BMD measurement and a copy of the NOF's treatment guidelines are sent to participants and their primary care physician (PCP). Participants are strongly encouraged to discuss treatment options with their doctor. While 50% of women were taking calcium and/or vitamin D at the time of the fracture, only 13% received bone-active medication during the year following their fracture. These results suggest that providing BMD results with guidelines to PCPs does not increase the use of bone-strengthening medications in women who have a hip fracture. This work has demonstrated a need to examine the management and treatment of osteoporosis among this at-risk population in order to identify additional communication and intervention strategies to promote treatment.

F384

Dose-related Increases in Bone Mineral Density Due to Norethindrone Acetate in Postmenopausal Women Treated with Ethinyl Estradiol. J. C. Gallagher,[1] M. Greenwald,[2] R. Wasnich,[3] J. Symons.[4] [1]Bone Metabolism Section, Creighton University Medical Center, Omaha, NE, USA, [2]Osteoporosis Medical Center, Rancho Mirage, CA, USA, [3]Hawaii Osteoporosis Center, Honolulu, HI, USA, [4]Pfizer Global Research and Development, Ann Arbor, MI, USA.

Traditionally, the only benefit of the progestin component of hormone replacement therapy was thought to be prevention of endometrial carcinoma in women with intact uteri. However, recent evidence indicates that progestins can positively affect bone mineral density (BMD). This study was undertaken to examine the potential additive effect of norethindrone acetate (NA), when given in combination with ethinyl estradiol (EE), on BMD in postmenopausal women.This was a one-year, double-blind, placebo-controlled, parallel group, multicenter study. Female subjects with intact uteri who had undergone either spontaneous or surgical menopause within five years of the study start were eligible for enrollment. 945 subjects were randomized to one of eight treatment groups (NA in mg; EE in mcg): placebo; 0/5 NA/EE; 0.25/5 NA/EE; 1/5 NA/EE; 0/10 NA/EE; 0.5/10 NA/EE; 1/10 NA/EE; and an open-label arm of 0.625 mg conjugated equine estrogen/2.5 mg medroxyprogesterone acetate. All subjects received a supplement of 1000 mg of calcium carbonate daily. Dual-energy x-ray absorptiometry (DEXA) was used to determine BMD at four skeletal sites: femoral neck, total femur, lumbar spine (L1-L4), and total body. A central laboratory provided analysis of all DEXA scans and quality assurance of BMD data. Only data from the double-blind treatment groups are reported.At month 12, both doses of unopposed EE and all combination NA/EE doses demonstrated a mean increase in BMD at all skeletal sites. The difference was statistically significant compared with placebo at the lumbar spine, total femur, and total body. For both the 5- and 10-mcg doses of EE, there was a linear dose-response trend, with increasing NA dose resulting in a greater percent change in BMD from baseline. The largest increases in BMD were obtained in combination with 1 mg NA; a significant difference (P = 0.008) between the 0.5/10 and 1/10 dose groups was obtained at the lumbar spine.The effect of EE on BMD is dose-related and is potentiated with the addition of 1 mg NA. At all skeletal sites assessed, the combination of EE with 1 mg NA resulted in a greater percent change in BMD than unopposed EE. This confirms an independent effect of NA on bone metabolism.

Disclosures: Pfizer,2,5.

F386

Effect of Discontinuation of Estrogen, Calcitriol, and the Combination of Estrogen and Calcitriol on Bone Mineral Density and Bone markers. J. C. Gallagher,*[1] P. B. Rapuri,[1] G. Haynatzki,[2] J. R. Detter.*[1] [1]Bone Metabolism unit, Creighton University, Omaha, NE, USA, [2]Medicine, Creighton University, Omaha, NE, USA.

489 elderly women were randomized to ERT(hysterectomized) or HRT (CEE.625 mg +MPA2.5mg daily), calcitriol (0.25 mcg. b.i.d.), HRT+calcitriol, or placebo in a double blind, randomized osteoporosis prevention study. At the end of 3 years, treatment was discontinued and 358 subjects were followed for 2 more years. There were annual measurements of bone mineral density (BMD- DEXA), serum PTH, serum osteocalcin and urine crosslinks for 5 years. Calcium absorption (Ca45) and serum 25OHD were measured at baseline, 3 and 5 years. Statistical analysis was done by repeated measures and intention to treat (ITT). Spine BMD increased significantly on HRT and HRT + calcitriol (p<0.001), and calcitriol (p<0.01) compared to placebo. Femoral neck BMD increased significantly on HRT and HRT + calcitriol(p<. 001),but not on calcitriol compared to placebo. After discontinuation of treatment, there was more rapid bone loss in the two groups treated with HRT compared to the calcitriol and placebo groups, however, BMD remained above baseline (table).Most of the loss occurred in the first year after discontinuation. Calcium absorption which had increased in both calcitriol groups (p<.001)returned to baseline. Serum PTH, which decreased in both calcitriol groups(p<. 05), returned to baseline. Serum osteocalcin and urine crosslinks, which had decreased in both estrogen groups, returned to baseline. In conclusion, after discontinuing therapy, there was an increase in bone resorption and a decrease in BMD in all treatment groups. The loss in BMD was greatest in the groups receiving HRT and occurred more rapidly in the first year.Thus,prevention of osteoporosis needs to be long term.

BMD	Time (months)	HRT/ERT	Calcitriol	HRT/ERGT + Calcitriol	Placebo
Spine	36	4.5**	1.8*	5.1**	-0.7
Spine	60	2.3	1.2	1.8	-0.5
Femoral neck	36	3.0**	0.0	3.7**	-0.4
Femoral neck	60	1.7	-0.5	0.7	-0.4

*p<0.05 **p<0.001 compared to placeb

Disclosures: Wyeth Ayerst,2,5; Roche,2,5.

F388

Effects of Raloxifene 60 mg/day on Risk of New Vertebral Fractures in Postmenopausal Women are Independent of Baseline Femoral Neck BMD. O. Johnell,[1] W. Wu,*[2] I. Pavo,*[3] J. L. Stock,[2] S. Sarkar,*[2] M. Wong,[2] K. D. Harper.[2] [1]Dept. of Orthopedics, Universitetssjukhuset MAS, Malmo, Sweden, [2]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA, [3]Lilly Area Medical Center, Eli Lilly and Company, Vienna, Austria.

The aim of this study is to assess the efficacy of raloxifene 60 mg/day (RLX) at 3 years on the risk of new vertebral fractures (VF) in postmenopausal women at different baseline BMD values and in women with osteopenia defined by no prevalent VF and baseline BMD T-score >-2.5. The MORE (Multiple Outcomes of Raloxifene Evaluation) trial randomized ≤ 80 years old, mean age 66.5 years) to placebo (PL, N=2576) or RLX (N=2557) [JAMA 282(1999): 637]. VF were identified in adjudicated radiographs taken at baseline and 3 years. Clinical VF were defined as new VF associated with signs or symptoms suggestive of VF reported at any post-baseline visit, and confirmed with radiographs. Femoral neck (FN) BMD T-scores were defined according to NHANES criteria. RLX had similar fracture efficacy irrespective of baseline femoral neck BMD (interaction term p=0.452). At 3 years, the relative risk (RR) of new VF in women without prevalent VF was 0.45 (95% CI 0.29, 0.71) in the RLX group compared to placebo. In the 1032 women without prevalent VF who had a baseline FN T-score >-2.5, 25 (4.8%) in the placebo group and 7 (1.4%) in the RLX group had new VF at 3 years [RR 0.29 (95% CI 0.09, 0.61)]. The numbers of women needed to be treated to prevent 1 new VF were 42 for women without prevalent VF and 29 for women without prevalent VF who had a baseline FN T-score >-2.5. The RR of new clinical VF was 0.13 (95% CI 0, 0.43) for the RLX group compared to placebo. In the women without prevalent VF and with both FN and lumbar spine T-scores >-2.5, only 7 (2.6%) in the placebo group and 4 (1.5%) in the RLX group experienced new VF, while 3 (1.0%) in the placebo group and none in the RLX group had new clinical VF. These reductions were not significant due to the small numbers of women in this subgroup. In conclusion, raloxifene 60 mg/day decreases the risk of at least 1 new VF at 3 years independently of baseline femoral neck BMD, and also in women with osteopenia defined by no prevalent VF with a baseline femoral neck BMD T-score of >-2.5.

Disclosures: Eli Lilly and Company,1,2,3,5,8.

F390

Bone Density in Long-Term DMPA Users: A Double-Blind Randomised Controlled Trial of Estrogen Replacement Therapy. T. Cundy, R. Ames,* J. Clearwater,* A. Horne,* G. Gamble,* H. Roberts,* I. R. Reid. Division of Medicine, University of Auckland, Auckland, New Zealand.

A reduction in bone mineral density (BMD), particularly in the axial skeleton, is recognised to occur with long-term use of the injectable contraceptive depot medroxyprogesterone acetate (DMPA). The relative estrogen deficiency induced by DMPA is believed to be the likely mechanism underlying bone loss, but there are no data to prove this. We recruited 41 women who were long term DMPA users and had a lumbar spine BMD below the mean for age, and randomised them to receive either conjugated equine estrogens 0.625mg (CE, n=21) or matching placebo (PL, n= 20), once daily by mouth, for two years. All subjects continued their regular 12 weekly DMPA injections throughout the study. Areal BMD was measured 6 monthly at the lumbar spine, the femoral neck and total body sites (Lunar DPX). The primary outcome measure was the change in lumbar spine BMD. At randomisation the two groups were well matched in mean (SD) age (CE 37(7), PL 39(8) years), body mass index (CE 24.6 (5.0), PL 23.7 (3.1) kg/m^2), lumbar spine BMD (CE 1.068(0.087), PL 1.105 (0.069) g/cm^2), median (range) duration of DMPA use (CE 11 (2-22), PL 14 (2-26) years), and theproportion that were smokers (CE 38%, PL 25%). Twelve subjects dropped out of the study before completion(6 from the CE group and 5 from the PL group), mainly for social reasons. Adverse events were no more common in the CE group than the PL group, in particular the numbers reporting moderate or severe breast tenderness (4 CE vs 3 PL) and vaginal bleeding (3 CE vs 2 PL) were similar. Over the two year period lumbar spine BMD was unchanged in women taking CE (mean change +0.9%, 95% CI -0.6 to +2.4), but declined significantly in women taking PL (mean change -3.1%, 95% CI -4.7 to -1.5), p<0.001 for between group difference. Changes in the same direction, but of smaller magnitude, were seen in femoral neck BMD (p=0.037) and total body BMD (p=0.027). Adhesion to the medication was only fair. Of the 15 subjects allocated to CE who completed the study, the mean change in lumbar spine BMD was +2.3%, in the third with the best compliance, compared to +0.9% in the third with moderate compliance and -0.5 % for the third with the poorest compliance. This study confirms that induction of estrogen deficiency is the likely cause of DMPA-associated bone loss. Whilst oral estrogen replacement for women who wish to continue DMPA contraception can arrest bone loss, this may not be the best route of administration, given the relatively poor compliance.

F393

Brief Therapy with Recombinant Human Parathyroid Hormone (1-34) Increases Lumbar Spine Bone Mineral Density in Men with Idiopathic or Hypogonadal Osteopenia. E. Orwoll,[1] W. H. Scheele,*[2] S. Paul,*[2] S.

Adami,*[3] U. Syversen,*[4] A. Diez-Perez,*[5] G. A. Gaich.[2] [1]Oregon Health Sciences University, Portland, OR, USA, [2]Eli Lilly and Company, Indianapolis, IN, USA, [3]University of Verona, Valeggio sul Mincio (VR), Italy, [4]University Hospital, Trondheim, Norway, [5]Hospital del Mar, Barcelona, Spain.

Osteoporosis in men is common but there have been few large trials of potential therapies. Parathyroid hormone, given once daily, stimulates new bone formation and increases bone mass in patients with low bone mass. A randomized, double-blind, placebo-controlled clinical trial was conducted to assess the effects of recombinant human parathyroid hormone [rhPTH(1-34)] on bone mineral density (BMD) in 437 men with spine or hip BMD >2 SD below male young adult mean. The average age was 59 years; 59% had prevalent nonvertebral fractures and 49% had low testosterone (T) levels. No other causes of low BMD were present. Men were randomly assigned to receive either placebo (n=147), PTH 20 µg/day (n=151) or PTH 40 µg/day (n=139) by subcutaneous injections. The median study drug exposure was 11 months. All volunteers were provided with daily calcium (1000 mg) and vitamin D (400-1000 IU). BMD was measured by DXA at the lumbar spine, proximal femur, and total body. The treatment, rhPTH(1-34), increased BMD at the lumbar spine at months 3, 6, 12, and at the proximal femur and total body at month 12. At endpoint, BMD had declined in 57 (40%) men in the placebo group. In the PTH20 and PTH40 groups, 10 (7%) and 8 (6%) of the men did not increase lumbar BMD. More men in the PTH20 (55%) and the PTH40 groups (71%) had spine BMD increases ≥5% at endpoint, compared to the placebo group (10%) (P<0.001). rhPTH(1-34) increased BMD similarly in men with normal and low T levels. The proportion of responders in those with normal T levels was 68%, 91%, 94%, and 52%, 96%, 94% in those with low T levels for the placebo, PTH20, and PTH40 groups, respectively. In summary, therapy with rhPTH(1-34) rapidly and substantially increased BMD in men with osteoporosis, regardless of gonadal status.

Skeletal site (% change from baseline)	Placebo mean ± SD (n)	PTH20 mean ± SD (n)	PTH40 mean ± SD (n)	PTH20 or PTH40 vs Placebo
Lumbar spine BMD	0.5±3.9 (143)	5.9±4.5 (141)	9.0±6.5 (129)	P<0.001
Femoral neck BMD	0.3±4.1 (137)	1.5±4.0 (135)	2.9±6.3 (125)	P=0.029 (PTH20) P<0.001 (PTH40)
Total body BMC	-0.5±2.8 (87)	0.6±3.7 (84)	0.9 ± 3.7 (83)	P=0.021 (PTH20) P=0.005 (PTH40)

Disclosures: Eli Lilly and Company,2,5; Merck,2,5; Proctor & Gamble,2,5; Roche,5.

F396

Twice-Weekly Injection of a Sustained-Duration PTH Construct Increases Cortical and Trabecular Bone Density in Adult Mice. P. J. Kostenuik,[1] S. Morony,[1] K. S. Warmington,*[1] M. V. Porkess,*[1] Z. Geng,[1] T. Boone,*[2] J. Delaney,*[2] D. L. Lacey.[1] [1]Pharmacology/Pathology, Amgen, Inc., Thousand Oaks, CA, USA, [2]Process Science, Amgen, Inc., Thousand Oaks, CA, USA.

PTH increases bone mass when injected daily (SC) but not when infused continuously. We hypothesized that SC delivery of a sustained-duration PTH construct might also be anabolic without the inconvenience of daily injections. We produced a sustained-duration PTH construct (SD-PTH) which has a significantly longer serum half-life in rats compared to PTH-(1-34). Male BDF1 mice (19 weeks old) were injected SC with daily PTH-(1-34) (80 ug/kg) or with twice-weekly SD-PTH (15, 50 or 150 nmol/kg), for 3 weeks (total weekly dose of PTH-(1-34) = 150 nmol/kg; SD-PTH = 30, 100 and 300 nmol/kg). PTH-(1-34) caused significant increases in total and trabecular BMD in the tibia (by pQCT), but not cortical BMD. SD-PTH caused significant and dose-dependent increases in total, trabecular, and cortical BMD in the tibia. The gains in BMD with SD-PTH were significantly greater than those obtained with daily PTH-(1-34). PTH-(1-34) increased trabecular bone volume (BV%TV) in the tibia by a non-significant 74%. SD-PTH caused dose-dependent increases in BV%TV which were significantly greater than those obtained with daily PTH-(1-34). At the highest dose, SD-PTH caused a 290% increase in BV%TV compared to vehicle. PTH-(1-34) caused a significant increase in osteoclast number and a non-significant increase in osteoblast number. SD-PTH caused significantly greater increases in both osteoblast and osteoclast numbers compared to vehicle or daily PTH-(1-34). Daily PTH-(1-34) had no significant effect on serum calcium, alkaline phosphatase (an osteoblast marker), or TRAP (an osteoclast marker). SD-PTH caused significant and dose-dependent increases in each of these parameters. The antiresorptive agent OPG blocked the hypercalcemic response of mice to SD-PTH, indicating that hypercalcemia was largely mediated by osteoclasts. The apparently greater efficacy and potency of SD-PTH compared to PTH-(1-34) could not be explained by increased bioactivity, as SD-PTH was 10-fold less potent than PTH-(1-34) in cAMP assays with murine osteoblasts. In summary, we have demonstrated potent bone anabolic activity for a sustained-duration PTH construct. Twice-weekly administration of SD-PTH caused greater increases in BMD and BV%TV compared to daily PTH-(1-34), suggesting that anabolic activity can be obtained with a sustained-duration PTH construct without the need for daily injections.

F400

Prevention of Early Postmenopausal Bone Loss by Strontium Ranelate: A Randomised, Two-Year, Double-Blind, Placebo-Controlled Trial. J. L. Reginster,*[1] R. Deroisy,*[1] Y. Tsouderos,[2] I. Jupsin,*[1] C. Roux.[3] [1]Bone and Cartilage Metabolism Unit, University of Liège, Liège, Belgium, [2]Institut de Recherches Internationales Servier, Courbevoie, France, [3]Rhumatology, Hôpital Cochin, Paris, France.

Postmenopausal bone loss remains a major public health problem because two-thirds of women do not want to receive, or cannot be treated with, hormone replacement therapy. Strontium ranelate is a novel agent that increases bone formation and appears to uncouple the processes of bone formation and resorption. One hundred and sixty healthy, early postmenopausal women were randomised to receive placebo or strontium ranelate (PROTOS®)125 mg/day, 500 mg/day or 1 g/day for 2 years (40 participants per group). All participants received a calcium supplement of 500 mg daily. The primary efficacy parameter was annual increase in lumbar bone mineral density (BMD), measured using dual-energy X-ray absorptiometry. Secondary efficacy parameters included biochemical markers of bone turnover. At month 24, only strontium ranelate 1 g/day demonstrated a significantly greater increase in adjusted lumbar BMD than placebo (p<0.05). The annual increase was 0.7%, compared with -0.5% with placebo, with an overall beneficial effect after 2 years of about 2.4% in the strontium ranelate 1 g/day group compared with the placebo group. There were no other significant between-group differences in adjusted lumbar BMD. During treatment with strontium ranelate 1 g/day, a trend toward increased levels of bone formation markers was observed, with no effect on markers of bone resorption. Strontium ranelate displayed a tolerability profile similar to that of placebo. The minimum dose at which strontium ranelate is effective in preventing bone loss in early postmenopausal women is 1 g/day. This dose produced significantly greater increases in BMD than placebo, and changes in bone turnover markers were consistent with the exertion of an uncoupling effect on bone turnover. Further studies are now required to assess the efficacy of strontium ranelate in patients with established osteoporosis.

F404

Growth Hormone Increases Bone Mineral Content in Postmenopausal Osteoporosis. K. L. L. Landin--Wilhelmsen, A. Nilsson. Endocrine Division, Dept. of Medicine, Göteborg, Sweden.

Growth hormone (GH) is an important regulator of bone. The aim was to study the effect of GH in osteoporotic, postmenopausal women. Eighty women, 50-70 years, ≥ 5 years after the menopause and ongoing estrogen therapy since ≥ 9 months were randomized to GH (Genotropin®), 1.0 U or 2.5 U/day vs similar amounts of placebo, subcutaneously, double-blind during 18 months. The placebo group then stopped the injections, but both GH groups continued another 18 months, in total 3 years with GH. Calcium 750 mg/day and vitamin D 400 U/day were given to all. Both the placebo group and the 2 GH groups are now followed-up until 4 years, i.e. one year after the GH treatment was terminated, but with continued estrogen and calcium/vitamin D supplementation. The 5-year follow-up will be completed in June 2001. Insulin-like growth factor-1 (IGF-1) and lean body mass increased dose dependently in both GH groups at 3 years (p<0.001) and decreased to pre-treatment levels at 4 years. Lumbar, femoral and areal bone mineral density and bone mineral content (BMC) increased 1-8% in all women, but no differences between the groups were seen after 3 years. Lumbar spine BMC increased 14% on 2.5 U GH and was higher than placebo (p=0.0006) at 4 years. Femur neck BMC increased 13% on 2.5 U GH and was higher than on 1.0 U GH (p=0.01). Total body BMC was higher on 2.5 U GH than on 1.0 U GH (p=0.05) and placebo (p=0.01), respectively. Bone formation in the tetracycline labelled iliac crest bone biopsy increased in the 1.0 U GH group at 3 years, but no differences were seen between the 3 groups. Body weight, height, quality of life, vertebral height on X-ray, handgrip strength and bone markers were unaltered after 3 years. One radius fracture occurred in the 1.0 U GH group. Compliance was good according to the IGF-1 levels, which were blind for the investigator during the double-blind phase. None of the 80 women dropped out. Side effects were rare. In conclusion, BMC increased up to 14% by treatment with 2.5 U GH/day during 3 years and further differentiated from the group treated with 1.0 U GH/day and the placebo group, respectively, at 4-years follow-up, in postmenopausal osteoporosis. There seems to be a delayed, extended and dose dependent effect of GH treatment on bone.

F414

Dermal Application of Lovastatin for 5 days Stimulates Bone Formation in Ovariectomized Rats by 160%. G. Gutierrez,[1] I. R. Garrett,[1] G. Rossini,*[1] A. Escobedo,*[1] D. Horn,*[1] M. Qiao,*[1] J. Esparza,*[1] D. Lalka,*[2] G. R. Mundy.[1] [1]OsteoScreen Inc., San Antonio, TX, USA, [2]West Virginia University, School of Pharmacy, Morgantown, WV, USA.

In recent years, it has been determined that statins, drugs which lower cholesterol by inhibiting HMG-CoA reductase, are also bone anabolic agents, causing substantial increases in bone formation in vitro and in vivo in rodents. Their effects in vitro on bone and when applied locally in vivo are more impressive than when they are administered systemically by oral gavage. Since statins are subject to first-pass metabolism by cytochrome P450 enzymes in the liver, we reasoned that dermal application may produce more sustained blood levels in the peripheral tissues such as bone, and cause more substantial effects on bone than those resulting from oral administration. We determined therefore if topical application of lovastatin to the skin of ovariectomized rats would lead to more sustained circulating blood statin concentrations and increases in bone formation rates. We used this model since it can produce alterations in the cancellous network similar to those seen in the human skeleton during aging and menopause. We measured statins in plasma by HMG-CoA reductase activity. We found that blood statin concentrations following a single dermal dose were higher and maintained longer than following an equivalent oral dose, indicating a greater AUC after dermal application. Rats were ovariectomized and treatment with oral or topical lovastatin was started the day following surgery. In the group treated orally, lovastatin was given daily by gavage for 35 days. In the group treated dermally, the statin was applied topically (upper back after shaving) for 5 days only. Bones were analyzed after 5 weeks for both groups. Sham controls were included in both experiments. Topical lovastatin (1 mg/kg/day) caused 57% increase in trabecular bone after only 5 days of treatment. There was >150% increase in bone formation rate, which persisted for 3 weeks without further treatment in the group treated topically. Oral lovastatin caused lesser but detectable increases in bone formation rates at a)10 times the dose, b)after more prolonged (35 days) treatment. When Lovastatin was given orally only for 5 days, there was no effect. These results suggest that topical application of lovastatin produces greater anabolic effects on bone than oral administration and that alternative modes of administration of the statins, such as topical application through a skin patch, improves biodistribution to bone. They also show that both in vitro and in vivo, intermittent statin treatment is more effective than continuous treatment.

Disclosures: OsteoScreen, Inc.,1,3.

F419

The PPAR-alpha Agonist Wyeth 14643 Increases Bone Mineral Density in Female Rats. U. Syversen,*[1] I. Bakke,*[1] K. W. Slørdal,*[2] H. L. Waldum.*[1] [1]Department of Intra-abdominal Diseases, Norwegian University of Science and Technology, Faculty of Medicine, Trondheim, Norway, [2]Department of Physiology and Bioengineering Techniques, Norwegian University of Science and Technology, Trondheim, Norway.

Peroxisome proliferator-activated receptors (PPARs) are members of the steroid nuclear superfamily of receptors that have been shown to modulate the expression of genes involved in lipid metabolism and fat storage. Recently, the presence of PPARs has also been demonstrated in bone cells and a role in bone metabolism has been postulated.In the present study we have examined the effect of the PPAR alpha agonists Wyeth 14643 and ciprofibrate on bone mineral density (BMD) in female rats. Thirty female Fischer rats were divided into 3 groups and were given methocel (control group), Wyeth 14643 (50 mg/kg body weight) and ciprofibrate (50 mg/kg body weight) for 2 months. BMD in femur and total body in intact animals was measured using a Hologic QDR 4500A. Body weight was registered throughout the study. Blood samples were drawn for measurement of gastrin.There was no difference in body weight between control rats and Wyeth 14643-treated rats, while the body weight was significantly reduced in ciprofibrate treated rats. Serum gastrin levels were significantly increased after 2 months in rats receiving ciprofibrate (x3.3) and Wyeth 14643 (x2), compared to controls. There was a significant increase in total body BMD (0.154 ± 0.007 g/cm^2) in Wyeth 14643 treated rats compared to control rats (0.146 ± 0.004 g/cm^2), $p < 0.05$, while in rats receiving ciprofibrate, total body BMD (0.144 ± 0.004 g/cm^2) was significantly lower than in Wyeth 14643 treated rats. Femur BMD tended to be higher in Wyeth 14643 treated rats (0.263 ± 0.011 g/cm^2) than in control rats (0.251 ± 0.007 g/cm^2), and was significantly higher than in ciprofibrate treated rats (0.225 ± 0.014 g/cm^2), $p < 0.001$.In conclusion, treatment with the PPAR-alpha agonist Wyeth 14643 significantly increased BMD in female rats, while treatment with ciprofibrate resulted in a significantly decrease in BMD.

F422

A Therapeutic RANKL Vaccine Induces Neutralizing Anti-RANKL Antibodies and Prevents Bone Loss in Ovariectomized Mice. M. Hertz,*[1] T. Juji,*[2] S. Tanaka,*[2] S. Mouritsen.*[1] [1]M&E Biotech A/S, Horsholm, Denmark, [2]Department of Orthopaedic Surgery, University of Tokyo, Tokyo, Japan.

Receptor activator of NF-kB ligand (RANKL) and the soluble decoy receptor osteoprotegerin (OPG) are the critical regulators of osteoclast activity. Overexpression of RANKL has been shown to be involved in the pathogenesis of resorptive bone diseases, such as osteoporosis. A therapeutic RANKL vaccine was developed by modifying the soluble TNF-like domain of murine RANKL (amino acids 158-316) to incorporate a promiscuous T helper (Th) epitope. The modified RANKL vaccine was well tolerated and able to bypass immunological tolerance and induce antibodies neutralizing native murine RANKL. Lymphoid organogenesis, lymphocyte development and activation were normal in RANKL-vaccinated mice in contrast to RANKL-deficient mice. Antiserum from vaccinated mice inhibited osteoclastogenesis in vitro. More importantly, the RANKL vaccine protected mice from ovariectomy-induced bone loss. Osteoclast numbers as well as bone resorptive surfaces were significantly reduced in RANKL vaccinated mice. Thus, vaccination against RANKL represents a novel approach for the treatment of osteoporosis, rheumatoid arthritis and other diseases associated with increased osteoclast activity and bone loss.

Disclosures: M&E Biotech A/S,1,3.

F425

Novel Src Tyrosine Kinase Inhibitors Prevent Ovariectomy-Induced Bone Loss in a Swiss-Webster Mouse Model of Post-menopausal Osteoporosis. D. VonStechow,[1] J. M. Alexander,[1] S. Fish,*[1] M. Chorev,[1] R. Mueller,[1] M. Rosenblatt,[1] J. Illiucci,[2] Y. Wang,*[2] C. Metcalf,*[2] T. Keenan,*[2] R. Sundaramoorthi,*[2] W. Shakespeare,*[2] M. R. van Schravendijk,*[2] D. Dalgarno,[2] T. Sawyer.[2] [1]BIDMC/Harvard Medical School, Boston, MA, USA, [2]ARIAD Pharmaceuticals, Inc, Cambridge, MA, USA.

In this investigation, several novel Src tyrosine kinase inhibitors were evaluated in vivo to determine their ability to prevent ovariectomy-induced (OVX) bone loss in Swiss-Webster female mice. Three novel compounds (AP23236, AP23286, and AP23310) were assessed (10 and 25 mg/kg sc BID) and alendronate (0.1 and 1 mg/kg sc BID) was used as a positive control in 12-week old OVX Swiss-Webster mice following 4 weeks of treatment initiated immediately after OVX. The endpoints of this study included: 1) physiologic markers demonstrating estrogen-deficiency in this in vivo model system, and 2) bone mineral content and structure determinations by mCT evaluation of mouse femurs. MicroCT demonstrated a greater than 65% loss of metaphyseal trabecular bone volume (Tb BV/TV) in control vehicle-treated animals 4 weeks post-OVX, a value consistent with earlier findings in the Swiss-Webster mouse strain. This loss was associated with marked decreases in trabecular number and connectivity. Alendronate administration completely preserved Tb BV/TV during the 4-week study. AP23236 and AP23286 prevented OVX-induced trabecular bone loss (98% and 85% preservation of trabecular bone volume compared to OVX vehicle-treated control mice). Conversely, AP23310 (a less potent Src tyrosine kinase inhibitor) did not prevent OVX-induced trabecular bone loss (Tb BV/TV values between 19-41% of controls). These data demonstrate that Src tyrosine kinase inhibitors prevent OVX-induced bone loss in a mouse model of post-menopausal osteoporosis.

Disclosures: Ariad Pharmaceuticals Inc,1,2.

F430

Short-Term Calcitriol Administration Improves Calcium Homeostasis in Adults with Cystic Fibrosis. S. A. Brown,*[1] D. A. Ontjes,[1] R. M. Aris,*[1] G. E. Lester,[2] R. K. Lark,*[1] M. B. Hensler,*[1] A. D. Blackwood,*[1] M. J. Caminiti.*[1] [1]Depts. of Endocrinology and Pulmonology, University of North Carolina, Chapel Hill, NC, USA, [2]National Institutes of Health, Bethesda, MD, USA.

Decreased bone mineral density (BMD) is a well-defined health risk for individuals with Cystic Fibrosis (CF). Among the many factors that contribute to low BMD, we have focused on the altered calcium homeostasis in individuals with CF. Patients with CF tend to have lower 25 hydroxyvitamin D (25(OH)D) levels and increased parathyroid hormone (PTH) values compared to controls suggesting that inadequate vitamin D due to malabsorption contributes to secondary hyperparathyroidism. We conducted a short-term pilot study to measure the effects of calcitriol (0.5mcg po bid for 14 days) on markers of calcium balance including fractional absorption (FA) of ^{45}Ca, urinary calcium:creatinine ratios, PTH, ionized calcium, 25(OH)D, 1,25 dihydroxyvitamin D ($1,25(OH)_2$ D) and urinary N-telopeptide (NTx) levels. Ten adults with CF (18-40 years old) and 10 healthy controls matched for age, sex and body mass index were studied at baseline and 2 weeks after taking calcitriol. At both visits, variables were measured before and after a high calcium meal containing ^{45}Ca for determination of FA. Calcitriol administration resulted in a significant increase in the FA of ^{45}Ca in both groups ($p<0.01$), although the FA in CF subjects remained below controls both before and after calcitriol. In both groups, the usual suppression of PTH levels in response to a high calcium meal was enhanced after treatment with calcitriol (p=0.003 CF, p=0.04 controls). Both groups also had increases in urinary calcium:creatinine ratios. Fasting urinary NTx levels, a marker for bone resorption, fell by 34% in CF subjects in response to calcitriol (96.0 ± 16.0 vs. 63.9 ± 12.7 SE BCE/mmol Cr, p=0.01) while controls had no significant change. Controls had an increase in $1,25(OH)_2$ D levels (29.1 ± 1.8 vs. 37.8 ± 4.0 pg/ml, p=0.02) following calcitriol; however, there was no statistically significant change in $1,25(OH)_2$ D levels in the CF group (25.9 ± 2.4 vs. 29.4 ± 3.0 pg/ml, p=0.19). There were no significant changes in either group in baseline ionized calcium or 25(OH)D levels. Calcitriol administration in this short-term pilot study appears to improve calcium homeostasis in adults with CF by improving fractional absorption of ^{45}Ca and lowering PTH levels while suppressing bone resorption as reflected by urinary NTx measurements. These data suggest that oral calcitriol may be an effective means of improving BMD. The impact of such treatment on bone accrual is being studied in a 2 year trial in children and young adults with CF.

F436

Oral and IV Bisphosphonate are Efficacious in Children with Osteogenesis Imperfecta. L. A. DiMeglio,[1] L. Ford,*[2] G. Liu,*[2] C. McClintock,*[2] M. Peacock.[2] [1]Pediatric Endocrinology, Indiana University, Indianapolis, IN, USA, [2]Medicine, Indiana University, Indianapolis, IN, USA.

Bone mineral density (BMD) and fracture rates in children with osteogenesis imperfecta (OI) improve with intravenous (IV) bisphosphonates. The efficacy of oral bisphosphonates has not been established. We have instituted an open-label, prospective, randomized clinical trial of oral compared to IV bisphosphonate in children with OI. Children are stratified into groups according to bone age, pubertal stage, and type of OI. They then are randomized to receive either IV pamidronate, 3 mg/kg over 3 days every 4 months or oral alendronate 1 mg/kg, from a minimum of 10 mg to a maximum of 40 mg daily. The primary efficacy outcome is BMD in g/cm2 and in SD units, measured at 4-month intervals. Secondary outcomes include: biomarkers of bone turnover, os calcis ultrasound characteristics, fracture incidence, calcium biochemistry, anthropometric measures, effect on dental anomalies, audiologic evaluations, assessment of quality of life, pain measures, and evaluations of gross motor function.Characteristics of eight randomized children (5 oral/3 IV) and two other children who were assigned to IV treatment due to chronic abdominal pain are presented below. In each group, 3 patients have type III/IV OI, while 2 have type I. The response in BMD in both groups is comparable and beyond what would be expected with normal growth. All children have experienced an increase in BMD on therapy.

	Oral (n=5)	**IV (n=5)**
Average age (range)	6.83 yr (3.8-9.5)	9.47 yr (3.4-13.6)
Ave Rx Time (range)	1.07 yr (0.38-1.40)	0.71 yr (.32-1.36)
Annualized % change L2-L4 BMD (range)	30.8% (7%-54%)	51.2% (31%-90%)**
Absolute change L2-L4 Z-score	=+1.0 SD	+0.3 SD**
Annualized % change Total Body BMD (range)	8.6% (2%-20%)	3.6% (1%-13%)
Absolute change Total Body Z-score	+0.4 SD	+0.4 SD

**n=4

Also, all have a concomitant decrease in bone resorption, measured by urine NTX/Cr ratios, which declined on average approximately 50% from baseline. There was no correlation between change in speed of sound by ultrasound at the os calcis and change in total body BMD (n = 7, r = -0.5, p= 0.5) or L2-L4 BMD (n = 7, r = -0.3, p = 0.5). Radiographs of patients on IV bisphosphonate demonstrated multiple, discrete dense bands under growth plates corresponding to treatment courses, whereas those of children on oral bisphosphonate demonstrated a uniform subepiphyseal band corresponding to the length of therapy. Other than fevers following the initial dose of IV bisphosphonate, no adverse effects of therapy have been noted. These data suggest that both oral and IV bisphosphonate therapy are safe and effective for children with OI.

F438

Camurati-Engelmann Disease: New Mutations in the Latency-Associated Peptide of the Transforming Growth Factor β-1 Gene. S. R. Mumm,[1] S. Obrecht,*[1] M. N. Podgornik,*[2] M. P. Whyte.[2] [1]Division of Bone and Mineral Diseases, Washington University School of Medicine and Barnes-Jewish Hospital Research Institute, St. Louis, MO, USA, [2]Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children, St. Louis, MO, USA.

Camurati-Engelmann Disease (CED), also called progressive diaphyseal dysplasia, is an autosomal dominant disorder characterized by painful spreading hyperostosis of the diaphyses of major long bones beginning in childhood. Mutations in the latency-associated peptide (LAP)/transforming growth factor β-1 (TGFβ-1) gene have been implicated as the cause of CED in reports of several Japanese and European patients. The LAP and TGFβ-1 are co-expressed from the same gene as a single peptide, which is subsequently cleaved into separate LAP and TGFβ-1 proteins. A dimerized LAP remains noncovalently bound to a dimerized secreted TGFβ-1, presumably keeping the latter inactive. We have sequenced the coding region of the LAP/TGFβ-1 gene in 8 unrelated CED patients (3 sporadic, 5 familial), identifying mutations in all but one sporadic case diagnosed elsewhere. In 4 of these probands, previously reported mutations were documented in exon 1 (9 bp insertion causing a 3 Leu insertion in a poly-Leu region) and in exon 4 (Arg218His, Arg218Cys). In the remaining 3 cases, new mutations were identified. The novel mutations included a dinucleotide change in exon 4 (Cys223Ser) at a critical residue involved in disulfide bonding and tertiary structure of the LAP; a previously reported single nucleotide mutation results in the same amino acid change (Cys223Ser). The second was a C to T transition in exon 2 (C463T) causing an arginine to cysteine change (Arg156Cys); one may speculate that this newly created Cys residue could also interfere with disulfide bonding and tertiary structure. The two new mutations were not detected in 168 LAP/TGFβ-1 alleles from unaffected individuals, demonstrating the changes are mutations and not polymorphisms. Hence, all 7 of the mutations identified to date causing CED are located in the LAP; none are found within TGFβ-1. This finding suggests the underlying defect in CED is control, at the protein level, of an intact TGFβ-1.

F440

Lifelong Hypopituitarism and Growth Hormone Deficiency Decreases Bone Size but not Volumetric Density yet Increases Prevalence of Fractures. R. Bouillon,[1] K. Koledova,*[2] O. Bezlepkina,*[3] E. Chernikhova,*[4] E. Nagajeva,*[3] A. Bakulin,*[4] J. Nijs,*[5] V. Peterkova,*[3] I. Dedov,*[3] O. Oganov,*[4] A. Attanasio.*[6] [1]Endocrinology, Catholic University of Leuven, Leuven, Belgium, [2]Eli Lilly, Moscow, Russian Federation, [3]Endocrinological Research Center, Moscow, Russian Federation, [4]Space Phisiology Department, Moscow, Russian Federation, [5]Universitary Hospital, Leuven, Belgium, [6]Eli Lilly, Torino, Italy.

Growth hormone (GH) has major effects on bone modelling. Accordingly, lifelong growth hormone deficiency (GHD) will affect bone development and is therefore a good model to study the relationship between BMC, BMD and fracture risk. We studied 66 adults, all with idiopathic hypopituitarism of childhood onset: 18 isolated GHD and 48 GHD with multiple pituitary hormone deficiencies, MPHD. Replacement therapy with missing hormones had not been continuous in all subjects and hGH treatment had (virtually) never been given. As control group Hologic DEXA measurements obtained in more than 700 normal Russians (age 10-92) were used.Patients with isolated GHD (age 24 ± 10 years, height SDS -3.73 ± 1.5) had BMC values for lumbar spine, femoral neck or total body regions which were 54, 71 and 59% of that of age and sex matched controls (all p <. 001). Patients with GHD and MPHD had either open growth plates (GP) (n=28, age 25 ± 8 years; height SDS -4.00 ± 1.4) or closed growth plates (n=20;age 55 ± 12 years; height SDS-5.05 ± 1.4) and similarly decreased BMC at all sites (between 42 and 69 of expected values). The decrease in BMC in all regions was substantially greater in all subgroups than expected from their height differences (being 84, 83, 80% of matched Russian controls).The areal BMD Z-score for all DEXA measurements varied between -2 Z (isolated GHD) and -3 Z (MPHD + open GP) and -1.8 Z (MPHD + closed GP). The calculated volumetric bone density (BMAD) of adult patients with isolated GHD or MPHD + closed GP (Z-score of about +0.3) was however perfectly normal and only slightly decreased (-0.8 Z) in MPHD + open GP.The non-traumatic fracture prevalence was 7% (isolated GHD), 41% (MPHD open GP) and 75% (MPHD and closed GP), which is significantly increased when compared to Russian as well as Caucasian control populations.In conclusion: isolated as well as MPHD GHD of childhood onset very substantially affect adult height and BMC if poorly treated. While areal BMD is frankly decreased, volumetric bone density is unaffected. The recorded fracture incidence is, however, markedly increased indicating that bone size and shape rather than volumetric density determines bone strength.

F442

Blocking Macrophage Inflammatory Protein-1-Alpha in Myeloma Cells Decreases Bone Destruction and Tumor Burden by Decreasing Their Homing Capacity and Growth In Vivo. S. J. Choi, Y. Oba, J. L. Anderson,* G. D. Roodman. Medicine/Hematology, UTHSCSA/VAMC, San Antonio, TX, USA.

We reported that macrophage inflammatory protein-1-alpha (MIP-1-alpha) may be responsible for the bone destruction in multiple myeloma (MM). When the human MM-derived ARH-77 cell line was stably transfected with an antisense construct to MIP-1-alpha (AS-ARH) or an empty vector (EV-ARH) and transplanted into SCID mice, the levels of human IgG that indicate tumor burden in these mice were dramatically decreased in AS-ARH mice compared to EV-ARH mice (0.1-10 ug/ml vs. 80-120 ug/ml). AS-ARH mice lived longer than EV-ARH mice, and had no radiologically identifiable lytic lesions. In contrast, EV-ARH mice had extensive bone disease. Histomorphometric analysis demonstrated that the number of osteoclasts per mm2 bone area and per mm bone surface for AS-ARH mice was significantly less than EV-ARH mice (248 +/- 27.6 vs. 416 +/- 43.1; $p < 0.01$ and 4.4 +/- 0.8 vs. 7.2 +/- 0.8; $p < 0.05$). The percent tumor per total bone area was also significantly decreased in AS-ARH mice compared to EV-ARH mice. To determine if the decreased tumor burden and bone destruction in AS-ARH mice was due to decreased homing, engraftment or survival of the AS-ARH cells, mice were sacrificed at 3-day intervals after transplantation and mRNA expression levels of human specific GAPDH (hsGAPDH) were measured. hsGAPDH levels in AS-ARH mice were decreased by day 3 compared to EV-ARH mice, but were detectable, and decreased further by days 9 and 15. Since MIP-1-alpha enhances expression of adhesion molecules on cells, we then assessed if the decreased homing and survival of AS-ARH cells in AS-ARH mice may result from decreased adherence to marrow stromal cells. Adherence of AS-ARH cells to ST2 stromal cells was significantly decreased compared to EV-ARH cells. The adherence of myeloma cells to stromal cells is in part mediated by alpha4beta1 integrin expressed on myeloma cells. Interestingly, mRNA expression levels of human alpha4 integrin were similar in AS-ARH and EV-ARH cells, regardless of treatment with an anti-MIP-1-alpha neutralizing antibody or rhMIP-1-alpha. In contrast, mRNA expression levels of human beta1 integrin were upregulated by rhMIP-1-alpha in AS-ARH cells and downregulated by an anti-MIP-1-alpha neutralizing antibody in EV-ARH cells. Thus, blocking MIP-1-alpha activity has profound effects on MM cell growth, homing and bone destruction in this in vivo model of MM. These data suggest that the antagonists that block MIP-1-alpha activity in vivo may be useful agents for treatment of MM patients to decrease both their tumor burden and bone destruction.

F444

RANK (Receptor Activator of NF-KB) and RANK Ligand Expression in Multiple Myeloma. S. Roux,[1] V. Meignin,*[2] J. Quillard,*[3] A. Guiochon-Mantel,*[4] E. Milgrom,*[4] X. Mariette.*[1] [1]Rheumatology and Inserm U135, Bicêtre Hospital, Le Kremlin Bicêtre, France, [2]Pathology, Saint-Louis Hospital, Paris, France, [3]Pathology, Bicêtre Hospital, Le Kremlin Bicêtre, France, [4]Inserm U135, Bicêtre Hospital, Le Kremlin Bicêtre, France.

Recent discovery of new members of the TNF receptor-ligand family have pointed out the crucial role of RANKL and its receptor RANK in the osteoclast differentiation and activation. We wondered wether an increased expression of RANKL and RANK may be involved in the excessive bone resorption observed in myeloma. Our objective was to evaluate RANK and RANKL expression in human multiple myeloma by immunohistochemistry performed on bone marrow biopsies. To standardize the immunostaining we used Cos7 cells transiently transfected with a vector expressing human RANK or RANKL cDNA. Bone marrow biopsies (BM) were obtained at diagnosis in 15 patients with myeloma and 5 with monoclonal gammopathy of undetermined significance (MGUS), in addition to 10 normal BM. Plasma cells were not labeled with anti- RANKL nor anti-RANK antibodies in any biopsy specimens. In all biopsies, RANKL was expressed in endosteal bone surface, around vessels and predominantly in cells characterized by cytoplasmic expansions. These latter cells did not express CD45, a marker of the hematopoietic lineage, and were vimentine positive, corresponding therefore to bone marrow stromal cells. We found a greater expression of RANKL in myeloma specimens compared to MGUS and normal BM. In 5 myeloma specimens, characterized by a major plasma cell infiltration, the staining with anti-RANKL antibody was more pronounced in tumoral rather than non tumoral areas, leading sometimes to a reticular staining. With the anti-RANK antibody, a strong staining was observed as expected close to bone in osteoclasts. In addition, small mononuclear cells in the bone microenvironment were positive. These cells did not express CD3 nor CD20 antigens, were distributed in clusters, and had the typical morphologic aspect of erythroblast cells. There was no difference in the pattern of RANK expression in myeloma, MGUS and normal BM. In conclusion, we showed that malignant plasma cells did not express RANKL nor RANK. RANKL was expressed in dendritic stromal cells, with a greater intensity in myeloma specimens. These results suggest that RANKL may be overexpressed by bone marrow stromal cells in myeloma and therefore may participate to the high rate of bone resorption. Conversely, we did not find any changes of RANK expression in this disease.

F446

Shouldn't Ischemic Necrosis of Femoral Head Be Called Ischemic Necrosis and Apoptosis of Femoral Head? H. K. W. Kim,* D. J. Popp,* D. D. Hunter.* Shriner's Hospital for Children, Tampa, FL, USA.

Ischemic necrosis of the femoral head is one of the most serious complications that can arise following an injury or treatment of the pediatric hip. Although the hallmark of ischemic necrosis is cell death that occurs in the marrow and the trabecular bone of the femoral head, the mechanism of cell death following ischemic injury has not been investigated. It is assumed that cells in the femoral head die by necrosis. The purpose of this investigation was to determine whether apoptosis is one of the mechanisms of cell death that occurs in the femoral head following the induction of ischemic necrosis. Fourteen male piglets were used. A suture ligature was placed tightly around the right femoral neck to disrupt the blood supply to the femoral head. The animals were sacrificed 2, 6, 7, 10, 14, 21, and 28 days after disrupting the blood supply to the femoral head. Femoral heads were removed and divided into two halves. One half was fixed, decalcificed, embedded, and sectioned for TUNEL and caspase-3 immunostaining. The other half was used to isolate genomic DNA from the femoral head bone. To assay for the presence of nucleosomal ladders generated during apoptosis, the Ligation Mediated-PCR (LM-PCR) ladder assay kit was used. Since LM-PCR produces semiquantitative results, the femoral head samples were first subjected to 15 to 25 cycles of LM-PCR to determine which number of PCR cycles would be optimal for analysis.The operated femoral head samples clearly contained more nucleosomal ladders than the non-operated femoral head samples and the difference was most evident at 19 cycles. When all the DNA samples were analyzed after performing 19 cycles of LM-PCR, a dominant presence of nucleosomal ladders in the operated femoral head samples were clearly evident in the piglets sacrificed 6, 7, 10 and 14 days following the induction of ischemic injury. In contrast, the non-operated femoral head samples either failed to amplify nucleosomal ladders at all or they were significantly underrepresented as compared to the operated samples. The results of the femoral head samples from 2, 21, and

28 days were very different. The DNA samples failed to amplify nucleosomal ladders in both the operated and the non-operated femoral head samples. Increased TUNEL and caspase-3 staining were observed in the sections from the operated side compared to non-operated side. In conclusion, our results clearly demonstrate that cell necrosis is not the sole mechanism of cell death following ischemic necrosis of the femoral head. Both apoptosis and necrosis are involved. Our results open up the possibility of therapeutically modulating the apoptotic process to improve the clinical outcome following ischemic necrosis of femoral head.

F447

Novel Mutations in the Vitamin D 1α-hydroxylase Gene in Patients with 1α-hydroxylase Deficiency Confer Partial Enzymatic Activity in vitro. X. Wang, M. Y. H. Zhang,* W. L. Miller,* A. A. Portale. Pediatrics, University of California, San Francisco, San Francisco, CA, USA.

The rate-limiting, hormonally regulated step in the biological activation of vitamin D is its 1α-hydroxylation to 1,25(OH)$_2$D in the kidney, catalyzed by the mitochondrial P450 enzyme, P450c1α. We previously cloned human P450c1α (Mol Endocrinol 11:1961, 1997) and identified 14 different mutations, including 7 mis-sense, in 19 patients with 1α-hydroxylase deficiency (Am J Hum Genet 63:1694,1998), also known as vitamin D-dependent rickets type 1. None of the mis-sense mutations encoded a protein with significant enzyme activity in vitro. Affected patients show phenotypic variation, although the molecular basis is unknown. We analyzed 6 additional patients with clinical and radiographic features of rickets; in 4 patients the laboratory abnormalities were typical of 1α-hydroxylase deficiency, but in 2 they were unusually mild (mild hypocalcemia, normal serum 1,25(OH)$_2$D). Direct sequencing revealed that all patients had P450c1α mutations on both alleles. Five new and 2 known mutations were identified. The new mutations included a 5bp deletion with 6bp novel insertion causing a frameshift in exon 2, and a G to A change at +1 of intron 2 which prevented proper splicing; both led to premature stop codons. The 3 new mis-sense mutations identified were tested by expressing the mutant cDNA in mouse Leydig MA-10 cells. Enzyme activity of mutants R389G, L343F and E189G was 0%, 2.5% and 36%, respectively, compared to that of the wild-type control. The L343F and E189G mutations that confer partial enzyme activity in vitro, were found in the 2 patients with mild laboratory abnormalities, thus suggesting that such mutations contribute to the phenotypic variation observed in affected patients.

F449

Characterization of PHEX Catalytic Activity, Regulation and Role in Bone Matrix Mineralization and Phosphate Homeostasis. G. Boileau,[1] I. Lemire,*[2] N. Zhao,*[2] H. S. Tenenhouse,[3] L. DesGroseillers,*[1] P. Crine.*[2] [1]Biochemistry, University of Montreal, Montreal, PQ, Canada, [2]BioMep Inc., Montreal, PQ, Canada, [3]Pediatrics and Human Genetics, McGill University-Montreal Children's Hospital Research Institute, Montreal, PQ, Canada.

Mutations in the PHEX gene (Phosphate-regulating gene with homologies to Endopeptidases on the X chromosome) are responsible for X-linked hypophosphatemia and studies in the Hyp mouse model of the human disease implicate the gene product in the regulation of renal phosphate (Pi) reabsorption and bone mineralization. Although the mechanism for PHEX action is unknown, structural homologies with members of the M13 family of endopeptidases suggest a function for PHEX protein in the activation or degradation of peptide factors involved in the control of renal Pi transport and bone matrix mineralization. To understand the function of PHEX, we used site-directed mutagenesis of the transmembrane domain to generate a recombinant secreted and soluble form of human PHEX (secPHEX) by transfection of mammalian cells. We showed that purified secPHEX has peptidase activity by incubating the enzyme with several peptide substrates, including a variety of bone-related peptides, and analyzing the digest by HPLC. We found that PTHrP107-139 is a substrate for secPHEX. Mass spectrometry of the digestion products revealed that the enzyme cleaves at three positions within the peptide, all located at the amino terminus of aspartate residues. Furthermore, we showed that osteocalcin inorganic pyrophosphate and Pi, all of which are abundant in bone, are inhibitors of secPHEX activity. Inhibition of secPHEX activity by osteocalcin was abolished in the presence of mM Ca2+ with an IC50 of 2.5 nM. Moreover, the use of recombinant osteocalcin produced in E. coli showed that gamma-carboxylation of specific glutamate residues are important for osteocalcin inhibitory activity. The physiological role of PHEX was studied by daily intravenous injections to Hyp mice over a period of 4 days. Injection in Hyp animals resulted in further lowering of serum phosphate and decrease in alkaline phosphatase activity. We suggest that PHEX is involved in the control of the mineralization process and that its activity may be controlled in vivo by pyrophosphate/Pi and Ca2+.Ca 2+ regulation requires the participation of osteocalcin.

Disclosures: BioMep Inc.,1.

F450

ADHR-Associated FGF 23 Mutation Interferes With Protein Processing and PHEX Cleavage. R. B. Finnegan,*[1] A. E. Bowe,*[1] R. Kumar,[2] S. M. Jan de Beur,[3] S. C. Schiavi.*[1] [1]Applied Genomics, Genzyme, Framingham, MA, USA, [2]Nephrology, Mayo Clinic, Rochester, MN, USA, [3]Endocrinology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Experimental and clinical data derived from three distinct diseases, oncogenic osteomalacia (OOM), X-linked hypophosphatemia (XLH), and autosomal dominant hypophosphatemic rickets (ADHR), provide evidence for the existence of a novel hormone termed "phosphatonin" that regulates phosphate homeostasis through a parathyroid hormone-independent mechanism. XLH results from mutation in a membrane bound endopeptidase, PHEX, whereas ADHR is associated with activating mutations of the FGF 23 gene that encodes a novel member of the fibroblast growth factor family. Recent data suggest that tumors from OOM patients synthesize significant levels of FGF 23, providing evidence that over-expression of FGF 23 may be involved in the pathogenesis of OOM. To evaluate a possible relationship between FGF 23 and PHEX, we tested the hypothesis that FGF 23 is a PHEX substrate. Accordingly, wild type FGF23 and an ADHR mutant form, FGF 23, R179Q, were cloned into a mammalian expression vectors containing a carboxy-terminal V5 epitope tag. Two V5-immunoreactive bands of approximately 35- and 15-kDa were detected in medium collected from COS cells expressing wild-type FGF 23 whereas only a 35-kDa immunoreactive band was detected in medium collected from COS cells expressing FGF23 (R179Q). These results are consistent with differential processing of the wild-type and mutant forms of FGF 23. The R179Q mutation occurs within a consensus furin site, but we were unable to demonstrate cleavage of FGF23 by purified furin. To test whether FGF23 is a phex substrate, PHEX, wild-type FGF 23, FGF 23 (R179Q) and several additional genes previously found to be over-expressed in OOM tumors were independently expressed in an in vitro rabbit reticulocyte lysate system. In vitro synthesized proteins were incubated with PHEX containing- or control lysates and the relative amounts of V5 tagged proteins were monitored by immunoblot analysis blot using anti-V5 antibody. After incubation with PHEX, there was a significant reduction in the wild type FGF 23 signal, but no change in signal of the other potential PHEX substrates, including mutant FGF 23. These results suggest that FGF 23 is a PHEX substrate and that the ADHR R176Q mutation reduces susceptibility of FGF 23 to PHEX proteolysis.

F451

Frizzled Related Protein 4 Expression Is Elevated in Tumors Associated With Oncogenic Osteomalacia and Inhibits Phosphate Transport in Vitro. J. Vassiliadis,*[1] S. M. Jan de Beur,[2] A. E. Bowe,*[1] R. B. Finnegan,*[1] M. A. Levine,[3] R. Kumar,[4] S. C. Schiavi.*[1] [1]Applied Genomics, Genzyme, Framingham, MA, USA, [2]Endocrinology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, [3]Pediatric Endocrinology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, [4]Nephrology, Mayo Clinic, Rochester, MN, USA.

Oncogenic osteomalacia (OOM) is a paraneoplastic syndrome characterized by hypophosphatemia, reduced renal tubular phosphate reabsorption, low to normal calcitriol concentrations, normal calcium, normal parathyroid hormone levels and defects in bone mineralization. Several lines of evidence suggest that oncogenic osteomalacia is due to secretion by the tumor(s) of one or more factor(s) termed phosphatonin that inhibit phosphate re-absorption and the formation of calcitriol. Using Serial Analysis of Gene Expression (SAGE), we previously identified 16 genes from OOM tumors that are consistently induced or over-expressed compared to profiles from histologically matched tumors from patients that do not have OOM. To determine whether any of these genes encoded a protein that inhibited phosphate transport, consistently over-expressed genes were isolated and transiently expressed in COS cells. Conditioned medium from cells transfected with secreted Frizzled Related Protein 4 (FRP-4) inhibited uptake of radiolabeled phosphate by opossum kidney (OK) cells and human osteoblast-like SaOS cells by approximately 30 to 80%. The addition of 10 ng/ml sodium heparin had no effect on phosphate uptake. Partially purified carboxy-terminal histidine tagged FRP-4 also inhibited phosphate uptake in OK cells. To assess whether FRP-4 protein possesses additional characteristics of phosphatonin, we evaluated it as a substrate for the endopeptidase, PHEX. Defective PHEX is responsible for X-linked hypophosphatemic rickets, an inherited form of renal phosphate wasting. V5-his-tagged FRP-4 was expressed in an in vitro transcription/translation system in the presence or absence of PHEX and assessed by immunoblot with an anti-V5 antibody. The signal intensity of immunoreactive FRP-4 was reduced when co-expressed with PHEX. By contrast, PHEX treatment of several additional candidate genes had no effect on the integrity of these proteins, suggesting that the apparent degradation of FRP-4 by PHEX is specific. Taken together these results suggest that FRP-4 over-expression contributes to the pathogenesis of OOM and may be important in phosphate homeostasis.

F452

Internalization Pathway of Renal Phosphate Transporter Mediated Chloride Channel. M. Iida,* K. Morita,* H. Yamamoto, Y. Taketani, E. Takeda. Clinical Nutrition, University of Tokushima School of Medicine, Tokushima, Japan.

Physiological/pathophysiological alterations in proximal tubular phosphate (Pi) reabsorption are associated with an altered brush-border membrane (BBM) expression of type IIa Na+/Pi co-transporter molecules (NPT2). We have studied that inhibition of proximal tubular Pi reabsorption by Parathyroid hormone (PTH) or calcitonin (CT) is achieved by an internalization of NPT2 from the BBM. In addition, our previous studies indicated that both PTH and CT induced NPT2 internalization through either protein kinase A and C. Although protein kinases are activated upon the binding of PTH or CT to its receptor, the target for activated kinases relevant for the internalization of NPT2 has not yet been identified. Recently, several investigators have identified chloride channel (ClC-5) activated by PTH in the proximal tubule. In addition, loss of function mutation of ClC-5 affected the internalization of the apical transporter NPT2. Therefore, in the present study we investigated the role of ClC-5 in the NPT2 endocytotic pathway. When PTH or CT was injected into TPTX rats, both hormones increased ClC-5 mRNA levels and decreased the amount of NPT2 protein. In the proximal tubule, ClC-5 protein is located in early endosomes co-localizing with rab5. Therefore, we next analyzed interaction of NPT2 protein with rab5. Rat renal BBM extracts were immunoprecipitated with NPT2 antibody and then immunoblotted with rab5 antibody. We found that NPT2 interacts with rab5 response to PTH or CT. In addition, mammalian two-hybrid experiments showed that the C-terminus 73 amino acid residues of NPT2 are necessary for the interaction with rab5. Our results suggest that both PTH and CT regulate ClC-5 expression for the efficient acidification of endosome vesicles for the degradation of NPT2 and that rab5 represent early endosomes is essential for the interaction of NPT2.

F456

Paget's Disease of Bone: Linkage to a Novel Region on Human Chromosome 18q23. D. Good,*[1] F. Busfield,*[1] B. Fletcher,*[1] D. Duffy,*[2] J. Kesting,*[1] J. Shaw.*[1] [1]Dept of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, QLD, Australia, [2]Queensland Institute of Medical Research, Brisbane, QLD, Australia.

Paget's disease of bone (PDB) is a common metabolic bone disorder characterised by localised areas of increased osteoclast activity and abnormal bone remodelling. Bone pain, deformity, pathological fracture, and an increased incidence of osteosarcoma are associated with PDB. A significant genetic component contributes to the etiology of the disease. Previous studies have demonstrated linkage between PDB and the HLA region on chromosome 6q21.3 (PDB1), and with chromosome 18q21.2-21.3 (PDB2) in some pedigrees. Mutations in TNFRSF11A on 18q21.2-21.3 have been identified segregating in two PDB pedigrees but evidence of genetic heterogeneity exists with other pedigrees showing negative linkage to PDB1 and PDB2 and absence of mutations in TNFRSF11A. We have ascertained a large multigenerational pedigree in which multiple family members are affected by PDB. The disease is inherited as an autosomal dominant trait in the pedigree with high penetrance by the sixth decade. Linkage analysis has excluded the PDB1 and PDB2 regions in this family, whilst sequencing has excluded mutations in TNFRSF11A. We proceeded to a genome-wide microsatellite scan, with 61 individuals typed for 474 markers in a 10cM scan. Initial linkage analysis of the data by FASTLINK v4.1 indicated evidence suggestive for linkage to chromosome 7p and chromosome 18q. Fine mapping, followed by further two point linkage analysis was performed in regions of interest on chromosomes 7p and 18q, including 88 subjects of whom 30 were affected. Peak LOD scores obtained were LOD = +2.75 at D7S507 and LOD +1.76 at D18S70. Multipoint analysis of markers flanking D7S507 did not support linkage to this region of chromosome 7 and haplotype analysis provided further evidence excluding linkage to this region. Haplotype analysis of markers flanking the region of interest on chromosome 18 showed a consistent haplotype associated with PDB in a large subfamily within the pedigree. This subfamily consists of 40 genotyped subjects of whom 13 are affected. The age at diagnosis is significantly lower in this sub-family than in the rest of the pedigree (51.2 + 8.5 vs 64.2 + 9.7 years, p=0.0012). Multipoint linkage analysis of this subfamily gave a peak LOD score of 3.23 at marker D18S1390 (theta = 0). This marker is located approximately 20 Mb telomeric of TNFRSF11A (draft genome browser v4, UCSC). Our data suggest that this locus on chromosome 18 contains a gene important in the development of Paget's disease of bone in our family, and we are continuing with fine mapping, haplotype analysis and candidate gene sequencing in this region.

F458

Expansile Skeletal Hyperphosphatasia is Caused by a 15-Base Pair Tandem Duplication in *TNFRSF11A* Encoding RANK and is Allelic to Familial Expansile Osteolysis. M. P. Whyte,[1] A. E. Hughes.[2] [1]Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children, St. Louis, MO, USA, [2]Department of Medical Genetics, The Queen's University of Belfast, Belfast, United Kingdom.

Expansile skeletal hyperphosphatasia (ESH) is a familial metabolic bone disease characterized in 2000 in a mother and daughter featuring early-onset deafness, premature loss of teeth, progressive hyperostotic widening of long bones causing painful phalanges in the hands, accelerated bone remodeling, and episodic hypercalcemia likely inherited as a highly penetrant, autosomal dominant trait (JBMR 15: 2330-2344, 2000). Absence of large osteolytic lesions with cortical thinning in major long bones and the episodic hypercalcemia indicated that ESH is not a variant of *familial expansile osteolysis* (FEO). FEO is a rare autosomal dominant skeletal disorder characterized by early-onset hearing loss due to degeneration of middle ear ossicles, destruction of teeth, and painful focal bony expansion with cortical thinning from osteolytic lesions within major long bones. The histopathologic findings of the active osteolysis in FEO resemble the changes of *Paget disease of bone*. Here, we investigated the molecular basis of ESH after three families with FEO were reported to have an identical 18-base pair tandem duplication (84dup18) in the signal peptide sequence of the TNFRSF11A gene that encodes receptor activator of nuclear factor-κB (RANK) (Nat Genet 24: 45-48, 2000). We find that ESH is caused by a remarkably similar 15-base pair tandem duplication (84dup15) in *TNFRSF11A.* Sequencing of *TNFRSF11A* in both affected members of the ESH family revealed heterozygosity for this insertion mutation within exon 1. Cloning confirmed the presence of 84dup15. The in-frame insertion is predicted to elongate the signal peptide of RANK by five non-polar amino acids (LLCAL). This duplication overlaps with the reported duplications of six amino acids found in three FEO families and of nine amino acids found in a single family with PDB. The mutation was not identified in DNA from 70 patients with sporadic PDB or a similar number of controls. Hence, ESH and FEO are allelic diseases and ESH, like FEO, probably reflects increased activity in the skeleton of the RANK target, nuclear factor-κB (NF-κB).

F464

A Double Blind Randomized Placebo Controlled Trial of Alendronate in Primary Hyperparathyroidism. A. A. Khan,*[1] J. P. Bilezikian,[2] A. W. C. Kung,*[3] M. M. Ahmed,*[1] S. J. Dubois,*[1] A. Y. Y. Ho,*[3] Z. Motagally,*[1] M. Rubin,*[2] S. J. Silverberg,[2] T. I. M. Standish,*[1] Z. A. Syed.*[1] [1]McMaster University, Hamilton, ON, Canada, [2]Columbia University College of Physicians & Surgeons, New York, NY, USA, [3]The University of Hong Kong, Hong Kong, China.

This study was conducted to determine if alendronate maintains or improves bone mineral density in patients with primary hyperparathyroidism (PHPT). A randomized double blind controlled trial of placebo versus alendronate (10 mg daily) was conducted. Patients with PHPT were eligible if they did not meet surgical guidelines. Patients were randomized to placebo or active treatment arms and stratified for gender. Patients were assessed at baseline, 3, 6, 9 and 12 months. The primary outcome index, bone mineral density (BMD) in the lumbar spine (LS), femoral neck, total hip and distal one-third radius, was measured at baseline, 6 and 12 months with DEXA. Secondary indices of bone metabolism (calcium, phosphate, PTH (IRMA), bone spec. alk phos. urinary calcium, urineNTX, BHCG) were obtained at each visit. The data are provided for 34 patients who have completed 12 months of the protocol. A total of 44 patients are currently involved in the study. Two-tailed independent t-tests were conducted to compare treatment vs. control group 12 month minus baseline mean differences for the following: total hip, lumbar spine, and distal 1/3 radius BMDs; total calcium, ionized calcium, and phosphorus levels. The mean treatment group lumbar spine BMD increased by 5.3 % (0.03 grams/cm2, 95% CI: 3.6,7.0) from baseline, and total hip increased by 3.7 % (0.02 grams/cm2, 95% CI: 2.0,5.4). Radial BMD showed no statistically significant change (-0.002 grams/cm2, 95% CI: -2.2,0.8) . The mean control group BMD showed no statistically significant change at LS, total hip or radial sites.Change of +0.5 % (0.01 grams/cm2, 95% CI: -1.2,2.1) at lumbar spine, -1 % (-0.004 grams/cm2, 95% CI: -2.6,1.2) at total hip, and -1 % (-0.008 grams/cm2, 95% CI: -2.6,0.4) at distal radius.Mean differences were significantly greater for the treatment group BMD lumbar spine (t = 3.5, P=0.01) and BMD total hip (t = 3.37, P<0.01) when compared with the control group. BMD distal radius mean differences were not significant (t = 0.59, P=0.56) nor were total calcium (t = 0.07, P = 0.95), ionized calcium (t = 0.42, P = 0.67), or phosphorus levels (t = -1.97, P = 0.06). This is the first randomized placebo-controlled trial evaluating the effect of Alendronate on BMD in men and women with PHPT. Alendronate is effective in this population in significantly increasing BMD at the lumbar spine and total hip after only 12 months of therapy.

Disclosures: Merck and Company,2.

F466

Somatic Mutation of Mitochondrial DNA in Parathyroid Adenomas. T. Tokura, A. Arnold. Center for Molecular Medicine, University of Connecticut School of Medicine, Farmington, CT, USA.

Much remains to be learned about the molecular pathogenesis of nonfamilial parathyroid adenomas, in addition to the already established roles of the cyclin D1/PRAD1 oncogene and the MEN1 tumor suppressor gene. The mitochondrial genome is an attractive target for mutations that could drive tumorigenesis, especially in a low-replication tissue like the parathyroid, because mitochondrial DNA replicates frequently and independently of the nuclear genome, and also has heightened potential to accumulate damage throughout life due to locally generated mutagenic reactive oxygen species. Furthermore, homoplasmic and clonal mutations of the mitochondrial genome have recently been reported in several human tumors and cell lines. Therefore, we rigorously sought possible acquired mitochondrial DNA mutations by amplifying and completely sequencing the entire 16.6 kb mitochondrial genome of each of ten sporadic parathyroid adenomas plus corresponding normal peripheral blood leukocyte control DNA from the same patients. Resulting sequences were analyzed and compared with the standard Anderson public mitochondrial DNA sequence and with the online Mitomap database of previously published mitochondrial DNA mutations and polymorphisms. A total of 128 homoplasmic sequence variants were detected in the parathyroid adenomas, most of which were previously reported or new polymorphisms found simultaneously in non-tumor control DNA, as predicted from the high level of variability in mitochondrial DNA in human populations. Notably, 8 tumor-specific homoplasmic mutations, 6 being novel, were identified in mitochondrial DNA from three parathyroid adenomas (30%). One mutation altered a noncoding transcriptional binding site, while the other 7 mutations were clustered within the coding regions for subunits ofthe NADH dehydrogenase complex, supporting their functional significance. Five of the 8 mutations were base transitions consistent with damage by reactive oxygen species, and most mutations affected residues that are highly conserved across multiple species. One mutation was a frameshift predicted to prematurely truncate one of the NADH dehydrogenase subunits, again suggesting its functional relevance. Two somatic mutations were silent, had been previously described as constitutional polymorphisms, and were more likely to have arisen through a random drift to homoplasmy. In summary, we detected clonal homoplasmic mutations of mitochondrial DNA in 30% of parathyroid adenomas. Several features of the mutations, including their nonrandom clustering, suggest they may confer a selective advantage and may contribute to the development of parathyroid adenomas.

F468

Turnover and Rescue of Mutant PHEX Proteins Sequestered in the Endoplasmic Reticulum. Y. Sabbagh,*[1] G. Boileau,*[2] L. DesGroseillers,*[2] H. S. Tenenhouse.[3] [1]Biology, McGill University, Montreal, PQ, Canada, [2]Biochemistry, Université de Montréal, Montreal, PQ, Canada, [3]Biology, Pediatrics and Human Genetics, McGill University, Montreal, PQ, Canada.

Mutations in the *PHEX* gene are responsible for X-linked hypophosphatemia (XLH), the most prevalent form of inherited rickets in humans. The *PHEX* gene, which is predominantly expressed in bone and teeth, encodes a protein with high homology to the M13 family of zinc metallopeptidases. Of the 141 *PHEX* mutations that have been identified in XLH patients (http://data.mch.mcgill.ca/phexdb), missense mutations account for 24%. We undertook to examine the effects of three missense mutations, C85R, G579R, and S711R, on synthesis, glycosylation and cellular trafficking of the recombinant proteins. The mutant PHEX cDNAs were generated by PCR mutagenesis and expressed in HEK(293) cells. In contrast to wild-type (WT) PHEX protein, the three mutant proteins were sensitive to endoglycosidase digestion with endoH, indicating that they are not terminally glycosylated. Immunofluorescence of nonpermeabilized and permeabilized cells expressing WT and mutant PHEX proteins demonstrated that the WT protein is localized to the plasma membrane whereas all three mutant proteins are sequestered in the endoplasmic reticulum (ER). Co-immunoprecipitation studies showed that the ER sequestered mutant proteins interact with calnexin whereas only the core glycosylated form of the WT protein is associated with the molecular chaperone. Pulse-chase studies, conducted by labeling transfected cells with ^{35}S-met followed by a cold chase, demonstrated that all three mutant proteins are degraded in the ER, with half-lives of ~95 min for the C85R and G579R mutant proteins and ~55 min for the S711R mutant. Maturation of WT PHEX pro-

tein to a fully glycosylated species was evident after a 60 min chase. To determine whether the mutant PHEX proteins could be rescued from the ER to the plasma membrane, transfected cells were grown either in the presence of the chemical chaperone glycerol or at 26^{o}C. Only the S711R mutant protein achieved partial endo H resistance and cell surface expression in cells grown at the lower temperature. Our study represents the first characterization of WT and mutant PHEX protein expression, glycosylation and cellular targeting and provides a mechanism for loss of PHEX function in XLH patients expressing the C85R, G579R, and S711R mutations.

F474

High 1,25-(OH)2D Levels in Patients with Active Crohn's Disease are Caused by Local Overexpression of 1-alpha Hydroxylase in Macrophages. V. Kantorovich,[1] E. Vasiliauskas,*[1] U. Gruntmanis,*[1] K. Papadakis,*[1] D. Zehnder,[2] M. Abreu,*[1] S. R. Targan,*[1] M. Hewison,[2] J. S. Adams.[1] [1]Burns and Allen Research Institute, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA, [2]Medical Sciences, University of Birmingham, Birmingham, United Kingdom.

Patients with Crohn's disease (CD) have a high incidence of low bone mass for age. We have previously shown that a significant proportion of patients with active CD has increased levels of 1,25-dihydroxyvitamin D (1,25-D; Kantorovich et al. J Bone Miner Res. 2000;15:S498). Here we expand our initial observations to 98 adult patients with active CD. 48% (n=47) of patients with active CD possessed elevated levels of serum 1,25-D (mean [±SE] 76.5±3.8), while in another 30% (n=29) of patients 1,25-D levels resided inappropriately in the upper third of the normal range (51.2±0.8). When compared to the group of CD patients with normal 1,25-D levels (n=22), CD patients with increased 1,25-D had significantly lower age- and sex-adjusted bone density at the lumbar spine (p=0.03) and hip (p=0.02), despite similar CD treatment protocols. Among CD patients 1,25-D levels correlated with the disease activity (Harvey-Bradshaw score, p=0.02). The mean Ca/Creat excretion ratio in CD patients was significantly elevated (p<0.02) compared to an age-matched normal control population without CD (n=59). We theorized that the increased circulating levels of 1,25-D resulted from extrarenal overproduction of the hormone by macrophages in the submucosa of CD-involved gut. Preliminary results indicate that primary cultures of affected gut inflammatory cells convert [3H]25-OHD3 to [3H]1,25-(OH)2D3 with a specific activity 11-fold greater than that of 1,25-(OH)2D3-producing HD-11 monocyte/macrophage cells. In addition, we compared 25-OHD-1alpha-hydroxylase immunostaining in the gut of normal subjects and patients with CD. Compared to normal, colon from patients with quiescent CD exhibited moderate staining of submucosal macrophages while colon from patients with active CD displayed intense staining of macrophages with 1-hydroxylase greatest in giant cells found in the center of submucosal granulomas. Based on these in vivo and in vitro studies, we conclude that: 1] active CD is associated with overexpression of 1 alpha-hydroxylase in intestinal macrophages and 2] increased local production of 1,25-D can "spill" over into the systemic circulation resulting in relative hypercalciuria and accelerated bone loss.

F476

Osteoprotegerin (OPG) and RANKL Expression in Tissues Near Bone in Diseases of Pathological Bone Loss: A Comparison of Rheumatoid Arthritis, Prosthetic Joint Failure and Periodontal Disease. T. N. Crotti,[1] M. D. Smith,*[2] H. Weedon,*[2] M. Capone,*[1] C. Holding,*[1] D. M. Findlay,[3] D. R. Haynes.*[1] [1]Pathology, University of Adelaide, Adelaide, Australia, [2]Rheumatology, Repatriation General Hospital, Adelaide, Australia, [3]Orthopaedics and Trauma, University of Adelaide, Adelaide, Australia.

Osteoprotegerin (OPG) inhibits bone resorption by blocking RANKL, the key regulator of osteoclast differentiation. There is increasing evidence that the relative levels of OPG and RANKL are important in determining osteolysis in disease. The aim of this study was to compare the expression of OPG and RANKL in tissues surrounding bone erosion from patients with rheumatoid arthritis, prosthetic joint loosening and periodontal disease and determine the cell lineage expressing the mediators in these tissues. We also compared patients with active and inactive RA.Immunohistologic analysis of frozen tissue sections was performed using monoclonal antibodies to detect OPG and RANKL expression. Synovial tissue samples were obtained from 30 patients with rheumatoid arthritis. 21 of these patients were classified as having active synovitis. Tissue from 10 patients undergoing revision and 6 patients with periodontal disease was also assessed. Control groups consisted of synovial tissue samples taken from patients with spondyloarthropathy (12), osteoarthritis (10) and normal individuals (14). Dual immunohistochemical evaluation was performed with lineage specific antibodies (macrophages [CD68], fibroblasts [CD55], lymphocytes [CD45Ro] and endothelial cells [Factor 8]) and RANKL or OPG, using commercial antibodies, to determine the cell lineage associated with the proteins. Sections were evaluated by computer-assisted image analysis and semi-quantitative analysis.RANKL was strongly expressed in tissues from the rheumatoid, prosthetic joint failure and periodontal disease patients. RANKL was associated with lymphoid aggregates in periodontal and rheumatoid tissues but appeared to be associated with macrophage and fibroblastic cells in peri-prosthetic tissue. Two different patterns of OPG expression were seen, one exclusively by endothelial cells in all tissues and one predominantly by macrophages in the synovial lining cells of the rheumatoid tissues. OPG was remarkably absent in tissues from patients with active rheumatoid arthritis.The findings support the contention that RANKL and OPG regulate bone lysis in these pathologies. In particular, the deficiency in OPG expression in the inflamed synovium of active RA patients may indicate that OPG expression regulates the joint destruction seen in this pathology.

F482

Analysis of Gene Expression by DNA Microarray Reveals Novel Clues to the Mechanism of the Catabolic and Anabolic Actions of PTH in Bone. J. E. Onyia,[1] L. Gelbert,*[1] M. Zhang,[2] K. Bemis,*[1] A. Maran,[2] X. Lin,*[1] S. Chandrasekhar,[1] C. Frolik,[1] M. Sato,[1] H. Bryant,[1] R. T. Turner.[2] [1]Eli Lilly & Company, Indianapolis, IN, USA, [2]Mayo Clinic, Rochester, MN, USA.

Parathyroid hormone (PTH) has complex effects on bone depending on the mode of administration. PTH given intermittently increases bone mass by stimulating osteoblast differentiation to increase bone formation, while continuously infused PTH decreases bone mass by stimulating a net increase in bone resorption. The molecular events that mediate these markedly different biological responses in bone are unknown. The present investigation was designed to delineate the genes and pathways that are regulated by intermittent and continuous PTH using DNA microarrays. rhPTH (1-34) was administered intermittently (once daily sc injection (8ug/100 g)) or continuously (sc infusion of 4 ug/100 g by osmotic mini pump) to 6 month-old female rats for 1 week to stimulate bone formation and resorption, respectively. Total RNA, isolated from femoral metaphyseal bone, was labeled by in vitro transcription and hybridized to an Affymetrix microarray containing 8500 rat genes. Gene expression analysis (P<0.05) demonstrated that 316 genes (3.6%) were regulated by intermittent PTH. In contrast, 917 genes (10.4%), a 3-fold greater number of genes, were regulated with continuous PTH. Of these regulated genes, 158 were unique to intermittent and 759 were unique to continuous PTH, while the remaining 158 genes were common to both treatments. The regulated genes included 55-60% known genes and 35-40% ESTs. Classification of the genes into pathways and functional hierarchy identified similarities and novel potential differences in pathways affected by the specific PTH treatment. Continuous PTH affected 3-4 fold more pathways and functional platforms than intermittent PTH. The pathway most represented in genes unique to continuous PTH was "protein cleavage and degradation" and included components of the ubiquitin-proteosome degradation complex, metalloproteases and their inhibitors, serine, threonine, aspartic, cysteine, and amino proteases. Based on functional classification, genes unique to continuous PTH encoded many 7 transmembrane/G protein-coupled receptors, and integral membrane proteins. Genes common to both intermittent and continuous PTH include many integral membrane proteins and extracellular matrix proteins. These results demonstrate that the bone anabolic and catabolic effects of PTH regulate both common and unique subsets of genes and pathways. We speculate that the common subset of genes leads to the similarities in the pathways while the unique subsets of genes account for the differences in the anabolic and catabolic effects of PTH.

F484

The High Bone Mass Phenotype of Gcm2-Deficient Mice Is Caused by Hypocalcemia Not by PTH Deregulation. M. Priemel,[1] T. Günther,[2] J. M. Rueger,[1] G. Karsenty,[2] M. Amling.[1] [1]Trauma Surgery, Hamburg University, Hamburg, Germany, [2]Department of Human and Molecular Genetics, Baylor College, Houston, TX, USA.

Glial cells missing2 (Gcm2) is a cell specific transcriptional factor required for parathyroid gland development in mouse and humans. Gcm2-deficient mice lack parathyroid glands, exhibit a biologic hypoparathyroidism, but have a normal PTH serum concentration and a normal lifespan due to an auxiliary thymic source of parathyroid hormone (PTH). As a result of their chronic biologic hypoparathyroidism the Gcm2-deficient mice develop a low turnover high bone mass phenotype, that worsens over time. Here we compared the efficiency of 1,25-dihydroxyvitamin D3 (1,25-(OH)2 D3) and PTH to rescue this phenotype. Long term treatment with 1,25-(OH)2D3 normalizes serum calcium concentration and reduces serum PTH levels significantly. After 1,25-(OH)2D3 treatment urinary crosslink excretion was significantly decreased indicating that 1,25-(OH)2D3 significantly reduced osteoclast activity in Gcm2-deficient mice and histomorphometry confirmed that osteoclast number was also decreased. Thus, 1,25-(OH)2D3 treatment of Gcm2-deficient mice leads to an amplification of their low turnover state. In contrast, treatment with PTH lead to an increase in urinary crosslink excretion to normal levels, indicating an activation of osteoclast activity, and histomorphometric analysis of static and dynamic parameters confirmed that total bone turnover was activated. Regardless of the differences in mechanism of action between the two hormones, treatment with 1,25-(OH)2D3 or infusion of PTH resulted in a normal skeleton as assessed by histological and biomechanical analysis. Thus, normalization of calcium ion homeostasis overcomes the effects of hypoparathyroidism on bone mass irrespective of the mean used to control serum calcium concentration. Our results indicate that low serum calcium concentration alone causes the high bone mass phenotype of the Gcm2-deficient mice.

F487

Roles of PTH/PTHrP Receptor Generated-Signals in RANKL-Induced Osteoclastogenesis. H. Kondo, J. Guo, U. Chung, H. M. Kronenberg, F. R. Bringhurst. Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

Parathyroid hormone (PTH) is a major regulator of osteoclast formation and activation. In several systems, PTH-dependent osteoclastogenesis is accompanied by reciprocal up- and down-regulation of receptor activator of NFkB ligand (RANKL) and osteoprotegerin (OPG), respectively. The roles of specific downstream signals generated by the activated PTH/PTHrP receptor (PTH1R) - i.e., cAMP/PKA and PLC/PKC- in controlling osteoclastogenesis remain uncertain. To address this, we studied PTH regulation of RANKL, OPG, and M-CSF mRNA expression in, and support of osteoclast formation by, an established conditionally transformed clonal murine marrow stromal cell line (MS1). In the presence of dexamethasone, MS1 cells supported osteoclast (TRAP(+)MNC) formation from spleen-cell progenitors in response to PTH(1-34) or 1,25$(OH)_2D_3$. PTH-dependent cAMP generation in MS1 cells was approximately 1-, 10-, 80-, and 70-fold controls when assessed at 1, 3, 7 and 10 days, respectively, following transfer to nontransforming culture conditions. By Northern blot analysis, RANKL mRNA expression was stimulated (whereas OPG mRNA was suppressed) by 7 days of continuous hPTH(1-34) or by 6-24

hours of pulse treatment on day 7. PKA stimulators (8Br-cAMP or forskolin) exerted similar reciprocal up- and down- regulation of RANKL and OPG expression. These effects of PTH were blocked by the PKA inhibitor Rp-CAMPS but not by the PKC inhibitor GF109203X. [G^{1},R^{19}]hPTH(1-28), a PKA-selective PTH analog, exerted effects similar to PTH(1-34) on RANKL and OPG mRNAs and on TRAP(+)MNC formation, with a lowered potency predicted by cAMP responses measured in MS1 cells. Like PTH, forskolin and 8Br-cAMP induced TRAP(+)MNC formation in spleen-cell/MS1 co-cultures, whereas the direct PKC stimulator PMA did not induce MS1-cell RANKL mRNA, up-regulated OPG mRNA and antagonized osteoclast formation induced by 8Br-cAMP. Neither PTH(5-34) nor PTH(7-34) regulated MS1-cell RANKL or OPG expression. These results in spleen-cell/MS1 co-cultures were confirmed using primary bone marrow cell cultures. Finally, we generated PLC-defective PTH1R knock-in mice expressing only a mutant PTH1R(DSEL), that cannot activate PLC but normally stimulates adenylate cyclase. In bone marrow cultured from homozygous DSEL mice, hPTH(1-34) induced more TRAP(+)MNCs than in marrow cultured from normal littermates. Moreover, the tibial marrow space was enlarged in 8-week old DSEL mutant mice. These results strongly suggest that (a) cAMP/PKA signaling via the PTH1R controls RANKL-induced osteoclastogenesis and (b) PLC/PKC signaling may negatively regulate PTH/PKA-dependent osteoclastogenesis.

F490

Phospholipase C Signaling via the PTH/PTHrP Receptor Is Required for Normal Hypertrophic Differentiation During Endochondral Bone Formation. J. Guo, U. Chung, F. R. Bringhurst, H. M. Kronenberg. Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

Mice lacking the PTH/PTHrP receptor (PTH1R) show accelerated chondrocyte differentiation, whereas constitutive activation of this receptor delays chondrocyte differentiation. To determine if phospholipase C (PLC) signaling via the PTH1R regulates chondrocyte differentiation, we created mice expressing a mutant form (DSEL) of the PTH1R that activates adenylyl cyclase but not PLC. Mice expressing only DSEL PTH1R show both delayed chondrocyte differentiation and an accumulation of columnar proliferating chondrocytes in the endochondral growth plate. Two hours after administration of BrdU in vivo at E16.5 or E18.5, the labeling index of cells in the proliferating chondrocyte layer of DSEL mutant embryos was increased by more than 50%, vs. wild type littermates. To assess the rate of cellular transition from the proliferating to the hypertrophic stage, we measured the delayed appearance of previously-labeled cells in the zone of (nonproliferative) hypertrophic chondrocytes. When BrdU labeling was analyzed 24-48 hours after in vivo BrdU administration to E16.5 or E18.5d animals, the labeling index in the mutant hypertrophic zone was decreased by approximately 40% (vs. wild type). Collectively, these observations indicate that PTH1R PLC/PKC signaling slows proliferation and hastens progression toward hypertrophic differentiation in proliferating columnar chondrocytes. Defects in both processes could contribute to the expansion of the proliferating columnar cell layer observed in DSEL mutant mice.To more directly examine the role of the PTH1R PLC/PKC pathway in chondrocyte proliferation and differentiation, we performed organ culture of intact metatarsal and tibia rudiments from E15.5 embryos. In 3-day cultures, 100 nM PTH(1-34) dramatically slowed chondrocyte hypertrophy in both mutant and wild type metatarsals, as shown by decreased or absent expression of collagen X, osteopontin and alkaline phosphatase mRNAs. PTH also increased proliferation comparably in explants of both genotypes, as shown by BrdU labeling in vitro. Treatment with active phorbol ester greatly enhanced generation of cells expressing collagen X mRNA. These results indicate that PLC/PKC activation via PTH1Rs is important for the normal rate of transition from a proliferative to a post-proliferative, more terminally differentiated phenotype in growth plate chondrocytes. Further, these PTH1R-inducd PLC/PKC actions are opposite to those of PTH1R-dependent PKA activation, the loss of which dominates the endochondral phenotype of PTH1R-null animals.

F492

Parathyroid Hormone Receptor Recycling: Regulation by Specific Structural Features of the Receptor. S. Chauvin,*[1] J. Vilardaga,*[2] M. Bencsik,*[1] T. Bambino,*[1] R. A. Nissenson.[1] [1]Endocrine Unit, VAMC, San Francisco, CA, USA, [2]Pharmacology and Toxicology, Wuerzburg, Germany.

Agonist binding to the parathyroid hormone receptor (PTHR) results in signaling that is terminated by desensitization and internalization of the receptor. Internalization of the receptor is thought to result either in routing of the receptor to lysosomes for degradation or resensitization and recycling of the receptor back to the plasma membrane. The present study was designed to investigate the role of specific structural features of the PTHR on receptor recycling. Confocal microscopy of recombinant PTHRs in HEK293 cells revealed that the wt PTHR was rapidly internalized into endocytic vesicles following treatment with PTH (t1/2= 3-5 minutes). Following removal of PTH, receptors recycled back to the plasma membrane with much slower kinetics (t1/2= 2-4 hours). Truncation of all but 16 amino acids of the cytoplasmic tail of the receptor resulted in a slowing of the rate of PTHR endocytosis, and resulted in marked impairment of receptor recycling. Analysis of a series of truncation mutants demonstrated that the region of the cytoplasmic tail required for efficient receptor recycling differs from that previously shown to be important for receptor endocytosis, with the recycling domain localized more distally in the cytoplasmic tail. For some G protein-coupled receptors, phosphorylation is required for efficient receptor recycling. However, a mutated PTHR lacking all of the sites of PTH-stimulated phosphorylation was recycled with kinetics similar to that of the wt PTHR. Activation of the PTHR induced rapid translocation of ß-arrestin2-GFP to the plasma membrane. After longer treatment with PTH (up to 1 hour), the PTHR and ß-arrestin2-GFP co-localized in endocytic vesicles. After removal of PTH, the PTHR recycled whereas arrestin became cytoplasmic. Biochemical studies demonstrated that the PTHR became dephosphorylated during the recycling process, with kinetics similar to those observed for receptor recycling to the plasma membrane. These studies indicate that: 1) withdrawal of PTH results in slow recycling of the PTHR to the plasma membrane; 2) determinants of efficient PTHR recycling are present in the cytoplasmic tail, and are distinct from the known endocytic motif; 3) neither phosphorylation nor persistent arrestin binding is required for PTHR recycling. In addition, the results suggest that dephosphorylation of the PTHR during recycling may facilitate functional resensitization of the receptor.

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Deletion of DNA Binding Domain of the Vitamin D Receptor Abrogates Genomic and Nongenomic Functions of Vitamin D, and Induces an Atypical Chronic Myeloid Leukemia-Like Disease in Aged Mice. R. G. Erben,[1] D. W. Soegiarto,*[2] K. Weber,*[1] U. Zeitz,*[1] M. Lieberherr,[3] R. Gniadecki,*[4] G. Möller,*[5] L. Quintanilla-Martinez,*[6] J. Adamski,*[5] R. Balling.*[2] [1]Institute of Animal Physiology, University of Munich, Munich, Germany, [2]Institute of Mammalian Genetics, GSF National Research Center for Environment and Health, Neuherberg, Germany, [3]INRA, Jouy-en-Josas, France, [4]Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark, [5]Institute of Experimental Genetics, GSF National Research Center for Environment and Health, Neuherberg, Germany, [6]Institute of Pathology, GSF National Research Center for Environment and Health, Neuherberg, Germany.

The vitamin D hormone 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], the biologically active form of vitamin D, is not only essential for mineral metabolism but may have important functions beyond calcium homeostasis. Using gene targeting, we sought to generate vitamin D receptor (VDR) null mutant mice carrying the reporter gene lacZ driven by the endogenous VDR promoter. Here we show that our gene-targeted mutant mice express a VDR with an intact hormone binding domain but lacking the first zinc finger necessary for DNA binding. Homozygous mice are a phenocopy of mice totally lacking the VDR protein, and showed growth retardation, rickets, secondary hyperparathyroidism, and alopecia. Responses to vitamin D metabolites in skin, bone, intestine, parathyroid glands, and kidney were absent in homozygous mice, indicating that the mutant receptor is non-functioning and that vitamin D signaling pathways other than that mediated through the classical nuclear receptor are of minor physiological importance. Furthermore, rapid, nongenomic responses to 1,25(OH)2D3 in osteoblasts were abrogated in homozygous mice, identifying the nuclear VDR as the receptor mediating nongenomic actions of 1,25(OH)2D3. Beyond the age of 6 months, homozygous mutant mice showed an age-dependent increase in the incidence of an atypical chronic myeloid leukemia-like disease characterized by splenomegaly, granulocytosis, thrombocytosis, and myelodysplasia with displacement of erythropoiesis in bone marrow. These findings uncover that the VDR is crucial for the long-term control of myeloid cell proliferation and differentiation in vivo. Thus, 1,25(OH)2D3 may be an important inhibitory factor in the onset and progression of myeloproliferative and myelodysplastic diseases.

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Molecular Analysis of Helix-1 and Helix-3 in the Human Vitamin D Receptor Reveals Functional Subdomains Mediating Hormone Binding, Protein Stabilization, Heterodimerization and Interaction with Coactivators. P. W. Jurutka, T. L. Lamb,* C. E. Dominguez,* G. K. Whitfield, J. C. Hsieh, P. D. Thompson, M. A. Galligan,* C. A. Haussler,* M. R. Haussler. Department of Biochemistry & Molecular Biophysics, College of Medicine, University of Arizona, Tucson, AZ, USA.

The biologic effects of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) are mediated by the nuclear vitamin D receptor (VDR) which acts as a ligand-dependent transcription factor. The published crystal structure of the human (h)VDR ligand binding domain (LBD) (Rochel, et al., Mol Cell 5:173, 2000) reveals the positioning of helix-1 (H1) along one axis of the LBD with several charged amino acids exposed to solvent. In contrast, helix-3 (H3) in hVDR lies on the opposite side of the LBD and is slightly recessed within the ligand pocket in close proximity to helix-12/AF-2 (H12). Using the crystal structure as a guide, several H1 and H3 residues were selected for alteration via site-directed mutagenesis. The mutant VDRs were evaluated for transcriptional potency in transfected COS-7 and osteoblast-like ROS 2/3 cells, utilizing reporter constructs containing either the natural rat osteocalcin promoter or a synthetic vitamin D responsive element. H3 hVDR mutants (D232K, S235K, I238D, and I242D) all displayed significantly reduced transcriptional activity similar to that observed previously with hVDR H12 mutants. Because H12 and H3 form a molecular cleft for interaction with p160 coactivators in nuclear receptors, loss of activity in the hVDR H3 mutants likely results from impaired association of VDR with these coactivator proteins. Most mutations in H1 elicited more modest, yet significant attenuations in transcriptional activation. Specifically, δ123-130 and δ131-141 hVDR were reduced by 80% and 95% in transactivation, respectively, while E126K, E127K/Q128L, D137R and H139A displayed a 30-50% decrease in activity. Further analysis of mutants containing E126K or D137R alterations revealed an impaired ability to heterodimerize with RXR. In contrast, the E127K/Q128L mutant was rapidly degraded in COS-7 cells. We postulate that these latter two residues, which are required for corepressor interactions in other nuclear receptors, are essential for corepressor-mediated receptor stabilization of unliganded hVDR. Finally, H139A displayed a $1,25(OH)_2D_3$ dose-dependent rescue of the mutant phenotype, implicating H139, whose side-chain penetrates the LBD cavity, as pivotal in $1,25(OH)_2D_3$ binding/retention. Thus, distinct helical domains located on opposite faces of the hVDR LBD perform functionally discrete roles, which involve both hormone binding and interaction of VDR with accessory proteins that contribute to receptor activity.

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A Novel Mechanism of the Vitamin D Dependent Transcriptional Repression Through the Human 25-Hydroxyvitamin D3 1a-Hydroxylase nVDRE. A. Murayama, M. Kim,* K. Unno, K. Takeyama,* S. Kato. The Institute of Molecular and Cellular Biosciences, The University of Tokyo/ CREST, Tokyo, Japan.

25-Hydroxyvitamin D3 1a-hydroxylase (1a-hydroxylase) in kidney primarily hydroxylates 25(OH)D3 into 1a,25(OH)2D3, one of hormonal forms of vitamin D. Thus, it hence acts as a key enzyme in vitamin D biosynthesis. We have demonstrated that the negative regulation of renal 1a-hydroxylase by 1a,25(OH)2D3 and the positive regulations by PTH and calcitonin occur at transcriptional levels in intact animals and cultured cells. Subsequently, we identified a region responsible for negative regulation by 1a,25(OH)2D3 (1a-nVDRE) in the human 1a-hydroxylase 5'-flanking region. This ligand-dependent repression in transcription requires VDR/RXR. However, VDR/RXR heterodimer exhibits no direct DNA binding to 1a-nVDRE. In last ASBMR annual meeting, we reported identification of a transcriptional factor(VDIR), which binds directly to the 1a-nVDRE. The present study aimes to clarify the relationship between VDIR and VDR for the transcriptional repression by 1a,25(OH)2D3. In the absense of 1a,25(OH)2D3, VDIR activated transcription to 1a-nVDRE. The transactivation function of VDIR was further enhanced by PKA, which mimicked PTH action. In the presense of PKA, VDIR recruited a co-activator, p300/ CBP. On the contrary, in the presense of 1a,25(OH)2D3, VDR/RXR heterodimer exhibited a 'protein-protein' interaction with VDIR, and recruited co-repressors, HDAC2/Sin3A to repress the transcriptional activity. These results suggested that VDIR may be a key molecule related for both positive and negative regulations of 1a-hydroxylase gene expression. Moreover, this mechanism of the vitamin D-dependent transcriptional repression for 1a-hydroxylase may be different from the known repression mechanisms for PTH or PTHrP by 1a,25(OH)2D3.

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The Vitamin D Receptor Is not Required for the Rapid Activation of PKC and Rise in Intracellular Calcium Induced by 1,25-Dihydroxyvitamin D3 in Mouse Osteoblasts. R. Wali,*[1] J. Kong,*[1] M. B. Demay,[2] T. A. Brasitus,*[1] M. Bissonnette,*[1] Y. C. Li.[1] [1]The University of Chicago, Chicago, IL, USA, [2]Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

The rapid non-genomic actions of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] have been well described. The role of the nuclear vitamin D receptor (VDR) in these effects is, however, unknown. We have previously shown that $1,25(OH)_2D_3$ rapidly stimulates polyphosphoinositide hydrolysis, raises the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and activates protein kinase C (PKC) in rat colonic epithelial cells. To directly determine the role of the VDR in the rapid activation of PKC and the increase in $[Ca^{2+}]_i$ induced by this secosteroid, we studied these responses in primary osteoblasts isolated from VDR null (-/-) and VDR wildtype (+/+) mice. The osteoblast cultures were established from calvaria of 3 day-old mice and cells were then treated with $1,25(OH)_2D_3$ or vehicle (ethanol). After plating on coverslips, cells were loaded with Fura-2 AM for 30 min and then treated with $1,25(OH)_2D_3$ or ethanol. The $[Ca]_i^{2+}$ was calculated from the ratio of the measured fluorescence intensity at 340 and 380 nm. The PKC kinase activity was determined by measuring the phosphorylation of a PKC substrate peptide in the presence of [g^{32}P] ATP. Within 1-3 min of $1,25(OH)_2D_3$ (100 nM) treatment, $[Ca]_i^{2+}$ was increased from 61±25 nM to 119±45 nM in VDR (+/+) cells, and from 49±15 nM to 102±41 nM in VDR (-/-) cells. By 5 min, $1,25(OH)_2D_3$ (100 nM) caused a 2.6- and 2.4-fold increase in PKC enzyme activity in VDR (+/+) and VDR (-/-) osteoblasts, respectively. Gö-6976, an inhibitor of Ca^{2+}-dependent PKC isoforms, significantly reduced PKC enzyme activity in both cell types. By quantitative Western blotting, $1,25(OH)_2D_3$ treatment was found to cause translocations of similar magnitude for PKC-α and -δ, but not PKC-ζ, from the cytosol to the membrane in both VDR (+/+) and VDR (-/-) cells. These experiments demonstrate conclusively in mouse osteoblasts that the $1,25(OH)_2D_3$-induced rapid increase in PKC activity and the rise in intracellular calcium are neither mediated by, nor dependent upon, a functional nuclear VDR.

F501

Estrogen Exerts Both Estrogen Receptor α/β Dependent and Independent Effects. M. K. Lindberg,*[1] N. Andersson,*[1] M. Erlandsson,*[2] S. H. Windahl,*[3] G. Andersson,[3] D. B. Lubahn,*[4] H. Carlsten,*[2] J. Å. Gustafsson,*[5] C. Ohlsson.[1] [1]Dept. of Internal Medicine/Endocrinology, Sahlgrenska University Hospital, Gothenburg, Sweden, [2]Dept. of Internal Medicine/Rheumatology, Sahlgrenska University Hospital, Gothenburg, Sweden, [3]Dept. of Biosciences, Karolinska Institute, NOVUM, Huddinge, Sweden, [4]Dept. of Biochemistry and Child Health, University of Missouri-Columbia, Columbia, MO, USA, [5]Dept. of Medical Nutrition, Karolinska Institute, NOVUM, Huddinge, Sweden.

Estrogen exerts a variety of important physiological effects, which have been suggested to be mediated via the two known estrogen receptors (ERs), ERα and ERβ. Three months old ovariectomized (ovx) mice, lacking either ERα (ERKO), ERβ (BERKO) or both receptors (DERKO), were given estrogen subcutaneously (2.3 mg/mouse/day for three weeks) and the effects on different estrogen responsive parameters, including skeletal effects, were studied. The amount of trabecular bone, as measured by peripheral Quantitative Computerized Tomography (pQCT) at the distal femur metaphysis, was increased in WT (204% over control) and BERKO (168% over control) while no effect was seen in ERKO and DERKO mice, demonstrating that the effect on trabecular bone was ERα-mediated. Similar results were seen using histomorphometry. Furthermore, some other well known effects of estrogen, including thymic involution, fat reduction and increased uterine weight, were also found to be ERα-mediated. Interestingly, estrogen increased the cortical bone parameters (cortical BMC, cortical area and cortical thickness in the diaphysis of femur) in all genotypes. This effect on cortical bone is the first in vivo demonstration of an ERα/β independent effect. One may speculate that estrogen exert its effect on cortical bone via an unknown ER (ERγ) or via the androgen receptor. In conclusion, the effects of estrogen on trabecular bone in ovx mice are mediated via ERα, while the effects on cortical bone are ERα/β independent.

F503

The Trabecular BMD Is Preserved both by an Activation of Estrogen Receptor α and by an Activation of the Androgen Receptor in Male Mice. S. E. Movérare,*[1] M. K. Lindberg,*[1] S. Skrtic,*[1] J. Å. Gustafsson,*[2] C. Ohlsson.[1] [1]Dept. of Internal Medicine/Endocrinology, Sahlgrenska University Hospital, Gothenburg, Sweden, [2]Dept. of Medicial Nuitrition, Karolinska Institute, NOVUM, Huddinge, Sweden.

Androgens may regulate the male skeleton either directly via a stimulation of the androgen receptor (AR) or indirectly via aromatisation of androgens into estrogens and, thereafter, via stimulation of estrogen receptors (ERs). There are two known estrogen receptors, ERα and ERβ. The first aim was to investigate the effect of orchidectomy (orx) in mice lacking one or both of the two estrogen receptors. The second aim was to study the effect of estrogen treatment in these orx mice. Seven months old male mice, lacking ERα (ERKO), ERβ (BERKO) or both receptors (DERKO), where orchidectomized and treated for three weeks with 0.7mg/mouse/day of 17β-estradiol or vehicle. Before orx, the length of femur and serum IGF-1 levels were decreased in ERKO and DERKO but not in BERKO mice. Estrogen treatment of orx mice did not affect the length of femur or serum IGF-1 levels in any genotype. Before orx the trabecular BMD of tibia, measured using peripheral quantitative computerized tomography (pQCT) technique, did not differ between WT, ERKO, BERKO and DERKO mice, demonstrating that neither ERα nor ERβ are required for the maintenance of a normal trabecular BMD in male mice with intact testis. Following orx, there was a similar decrease in trabecular BMD, resulting in equal levels of trabecular BMD in all groups. This reduction was completely reversed after estrogen treatment in WT and BERKO mice, while no effect was found in ERKO and DERKO mice. Thus, our results demonstrate that the trabecular BMD is preserved by an activation of ERα but not ERβ. Furthermore, our data indicate that activation of the AR also results in a preserved trabecular BMD in male mice.

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Osteoclast Activity in Human Osteoclasts Is Decreased by Estradiol and Raloxifene in Vitro. D. Saintier,* M. Burde,* M. C. de Vernejoul, M. E. Cohen Solal. INSERM U349, Paris, France.

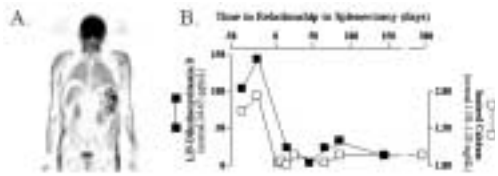
Estrogen defiency is responsible for bone loss in postmenopausal women. The increased bone resorption observed may be reversed by hormone replacement therapy as Estrogen and Raloxifene. The mechanisms of action of both steroids and the target genes regulated by estradiol have been studied in mice both in vivo and in vitro. However, data are rare for humans and might be different from the results obtained in rodents. We therefore studied the effect of estradiol and Raloxifene on bone resorption using a human osteoclast differentiation model in vitro. Peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers. Adherent PBMC were cultured in phenol red-free medium and 10% FCS and in the presence of RANK-L (30 ng/ml) and MCS-F (25 ng/ml). Cells were treated with vehicule (Controls C), 10^{-8}M estradiol (E2) or 10^{-8}M Raloxifene (Rlx) for 18 days. Cultures were performed in duplicate to evaluate the number of TRAP+ and vitronectine receptors+ multinucleated cells and onto dentine bone slices for pit formation. The pit area was significantly decreased in E2-treated cultures (2.4±1.4%) and in Rlx-treated cultures (1.88±1.1%) compared to controls (4.9±2.1%), (all $p<0.05$). However, there were no statistical difference in the number of TRAP+ multinucleated cells treated with E2 (41.2±20.1/mm^2) and Rlx (46.6±16.4) compared to controls (59.3±21.7). At the end of the culture, we observed no expression of estrogen receptors β, whereas estrogen receptors α was expressed in all cultures without any effect of E2 or Rlx. Expression of cathepsine K, TNF-α, c-jun, c-fos and β3 integrin were studied using nested RT-PCR. Cathepsin K expression was not regulated by E2 or Rlx (125±26% and 117.5±37% of controls), as well as TNF-α RNA expression. At day 18, the expression of c-jun mRNA was decreased to 42±15 % and 43±17 % by E2 and Rlx respectively compared to controls ($p<0.05$), in contrast to c-fos expression which remained unchanged (101±24 % and 76±15 % of controls respectively). We observed also that estradiol and Raloxifene decreased the mRNA expression of β3 integrin (60±17 % and 41±24% of controls, $p<0.05$) in parallel with vitronectine receptors + cell number which was decreased in E2 and Rlx-treated cells (38.7±12.2 and 37.9±8.9 /mm^2) compared to controls (47.8±15.4 /mm^2). In conclusion, we found that estradiol and Raloxifene are both effective to directly decrease in vitro bone resorption by human osteoclasts. Both steroids might act more on osteoclast activity than on osteoclast differentiation through β3 integrin, and involve mainly estrogen receptor-α in this human model.

F509

Vitamin D-Mediated Hypercalcemia in Lymphoma: Evidence for Hormone Production by Tumor-Adjacent Macrophages. V. Kantorovich,[1] H. R. Liker,*[2] A. J. Van Herle,*[2] P. Cohan,*[2] D. Zehnder,[3] M. Hewison,[3] J. S. Adams.[1] [1]Burns and Allen Research Institute, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA, [2]UCLA/Center for the Health Sciences, Los Angeles, CA, USA, [3]Medical Sciences, University of Birmingham, Birmingham, United Kingdom.

Hypercalcemia frequently complicates the course of lymphoma. While the most common cause of this phenomenon appears to be PTHrP secretion by the tumor, overproduction of 1,25-dihydroxyvitamin D (1,25(OH)2D) is also frequently observed (Seymour et al. Blood. 1993;82:1383-94). The cellular source of 1,25(OH)2D hormone was assumed to be lymphoma cell itself. Here we provide in vivo and in vitro evidence that lymphoma-adjacent macrophages are the source of the vitamin D hormone. A 75-year old male was diagnosed with symptomatic hypercalcemia resistant to bisphosphonate and mithramycin therapy. Extensive workup showed a high serum concentration of 1,25(OH)2D with a sup-

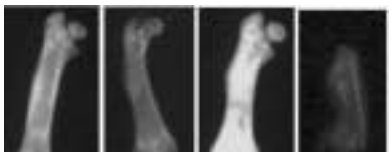
pressed serum PTH level (iPTH <2.0 pg/mL; normal 10-65) and normal PTHrP level (0.2 pmol/L; normal 0-1.3). Whole body computed tomography (CT) and positron emission tomography (PET) revealed evidence of tumor localized to only spleen (panel A, Figure 1). Hypercalcemia was cured with splenectomy and removal of a B-cell lymphoma (panel B, Figure 1). Splenic samples were subjected to immunostaining with antiserum raised against human renal 25-OHD 1alpha-hydroxylase (1-OHase). Lymphoma cells as well as the uninvolved spleen did not stain for the 1-OHase. However, a well-defined sub-population of CD68-positive macrophages bordering the normal spleen-lymphoma interface showed strong expression of 1-OHase. The fact that splenectomy was curative indicated that the spleen harbored cells responsible for either synthesis of 1,25(OH)2D, induction of synthesis, or both. The prominent staining of CD68-positive macrophages bordering stain-negative lymphoma cells, suggested that splenic lymphoma cells produced a locally-active soluble substance which promoted expression of a 1-OHase gene in adjacent macrophages. While the identity of the locally-active paracrine stimulator of the macrophage 1-OHase remains unknown in lymphoma and other granuloma-forming disease, preliminary evidence (Hewison et al., unpublished) suggests that signaling through the toll-like receptor-coupled NFkappaB pathway is a key regulator of enzyme expression.



F510

Vitamin D is a Negative Regulator of the Excessive Bone Formation. H. Tanaka,[1] K. Kinuta,[1] N. Inoue,*[1] M. Shinohara,*[1] S. Kato,[2] Y. Seino.[1] [1]Department of Pediatrics, Okayama University, Okayama, Japan, [2]Tokyo University, Tokyo, Japan.

Vitamin D is an important hormone in bone metabolism, but there is little evidence that vitamin D directly participates in this process. Vitamin D receptor (VDR) null mice have provided new insights in vitamin D metabolism and its role in vivo. However, like other nuclear receptor gene knockout, calcium supplementation experiments aimed at establishing physiological direct functions of VDR in many organs including bone have been inconclusive owing to essential roles of mineral in biological function. Although calcium supplementation showed apparent cure of rickets, we could not exclude the compensatory mechanisms such as hyperparathyroidism in this process. To evaluate the direct function of vitamin D in bone, we analyzed ectopically transplanted VDRKO bone in wild mice. As expected, wild bone in VDRKO showed marked bone resorption because of hyperparathyroidism and high $1,25(OH)_2D$. However, the VDRKO bone in wild mouse (KOW) showed dramatic increase in bone density compared with wild bone in wild mouse (WW). Bone volume was 0.175 mm^3 in KOW and 0.105 mm^3 in WW at 4 weeks after transplantation. Similarly, bone thickness of calvaria was 180 microm, which was 2.7 fold compared to WW. To exclude the possible contamination of the host cells, we next transplanted calvariae wrapped with membrane filter. Even in the absence of the wild type host cells, KO calvarie showed increased bone thickness. To explore the mechanism, we next examined mRNA expression of the genes, which may play key roles in bone metabolism, by quantitative RT-PCR in the transplanted bone. The results demonstrated increased expression of Cbfa1, BMP4 and OPG in KO bone. From these results, we conclude that vitamin D may play an important role in normal bone by protecting it from excessive bone formation through suppressing the expression of Cbfa1, BMP4 and OPG.



F512

Active Vitamin D Inhibits Osteoclastogenesis by Interfering With AP-1/ NF-κB Activity in Osteoclast Precursors. H. Takasu,[1] S. Takeda,*[1] A. Sugita,*[1] T. Kake,*[1] N. Kubota,*[1] E. Ogata,[2] K. Ikeda,[3] Y. Uchiyama.*[1] [1]Fuji Gotemba Res. Lab., Chugai Pharmaceutical Co., Ltd., Shizuoka, Japan, [2]Cancer Inst. Hosp., Japanese Foundation for Cancer Res., Tokyo, Japan, [3]Dept. of Geriatric Res., Natl. Inst. for Longevity Sci., Aichi, Japan.

We have demonstrated that active vitamin D inhibits bone resorption *in vivo* in estrogen deficient rodent models of high turnover osteoporosis (*JBMR 2000*). This contradicts the prevailing notion that $1,25D_3$ induces RANKL in bone marrow stromal cells, thereby promoting differentiation and activation of osteoclasts *in vitro*. In order to solve this discrepancy and to clarify the mechanism by which active vitamin D inhibits osteoclastic bone resorption, we examined the effects of $1,25D_3$ on osteoclastogenesis induced by M-CSF and RANKL in murine marrow cultures. Bone marrow cells from 6-8-week-old male *ddy* mice were cultured with M-CSF for 3 days, and adherent cells consisted mainly of bone marrow macrophage (BMM) were further cultured with M-CSF and sRANKL for additional 3-5 days. The number of TRAP-positive multinucleated cells (more than 3 nuclei) was counted. Addition of $1,25D_3$ inhibited the formation of osteoclasts dose-dependently, with IC50 being 10^{-8}M and 10^{-7}M $1,25D_3$ inhibiting by 70-80%. The expression of VDR in BMM was confirmed, and $1,25D_3$ had no inhibitory effect in bone marrow cells from VDR knockout mice, pointing to a VDR-mediated process. Addition of $1,25D_3$ during the first 3 days had no effect, while its co-presence with sRANKL during the latter half period fully inhibited osteoclastogenesis, and treatment with $1,25D_3$ did not affect RANK level in BMM, suggesting that $1,25D_3$ acts downstream of RANK activation by RANKL. Phosphorylation of IκB at Ser 32 after treatment with sRANKL was not inhibited by $1,25D_3$. A novel vitamin D analog, DD-281, that we have identified on the basis of its greater ability to inhibit AP-1/NF-κB-mediated transcription (25-30x of $1,25D_3$) and weaker activity to induce VDRE-dependent transcription (1/10 of $1,25D_3$) inhibited osteoclast formation 10x more potently than $1,25D_3$ (IC50 being 10^{-9}M), raising the possibility that active vitamin D inhibits osteoclastogenesis by interfering with AP-1/NF-κB function in osteoclast precursors through binding to VDR. In conclusion, we think that the major *in vivo* pharmacological action of active vitamin D is not to induce RANKL ("soil") in marrow stromal cells but to inhibit osteoclastic bone resorption by acting on osteoclast precursors ("seeds") and interfering with RANK signaling, and that the latter action provides an attractive target for developing new VDR-based drugs for osteoporosis.

F513

The Anabolic Effect of Vitamin D Binding Protein-Macrophage Activating Factor (DBP-MAF) and a Novel Small Peptide on Bone. G. B. Schneider,[1] K. J. Grecco,*[1] F. F. Safadi,[2] S. N. Popoff.[2] [1]Division of Basic Medical Sciences, Northeastern Ohio Universities College of Medicine, Rootstown, OH, USA, [2]Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA.

Vitamin D binding protein-macrophage activating factor (DBP-MAF) has previously been shown to stimulate bone resorption and correct the skeletal defects associated with osteopetrosis in two non-allelic mutations in rats. This same protein and a small fragment of the protein have now been shown to demonstrate an anabolic effect on the skeleton of both newborn and young adult, intact rats. The novel peptide fragment was synthetically produced based on the human amino acid sequence at the site of glycosylation in the third domain of the native protein (DBP). The peptide tested is 14 amino acids in length and demonstrates no homologies other than to that region of DBP. Newborn rats were injected i.p. with saline, peptide (0.4 ng/g body wt.) or DBP-MAF (2 ng/g body wt.) every other day from birth to 14 days of age. On day 16 the rats were euthanized and the long bones collected for bone densitometry by pQCT. Serum was collected for evaluation of osteocalcin levels as an indication of bone formation and urine was analyzed for deoxypyridinoline (Dpd) as a measure of bone resorption. After two weeks of treatment with either the whole protein (DBP-MAF) or the small peptide, bone density was significantly increased in the treated animals compared to the saline controls. Serum osteocalcin levels were significantly enhanced and Dpd levels in the urine were significantly decreased in the protein and peptide treated animals. Young adult female rats were given s.c. injections of saline or peptide (0.4 ng/g body wt. or 5 ng/g body wt.) every other day for two weeks; two days after the final injections, the rats were euthanized and the femurs and tibias collected for bone densitometry. Both doses of the peptide resulted in significant increases in bone density as determined by pQCT. Young adult rats were injected locally with a single dose of the peptide (1 μg) or saline into the marrow cavity of the distal femur. One week after the single injection, the bones were collected for radiographic and histological evaluation. The saline controls showed no evidence of bone formation, whereas the peptide treated animals demonstrated bone development at the injection site. These data suggest that DBP-MAF and the synthetic peptide represent therapeutic opportunities for the treatment of a number of bone diseases and skeletal disorders. Systemic administration could be used to treat osteoporosis and a number of other osteopenias and local administration could be effective in fractures, bony defect repairs, spinal surgery and joint replacement.

F515

$1,25(OH)_2D_3$ Synergizes with the PPARγ-Selective Ligand, BRL-49653, to Increase Adipogenesis in Rat Calvaria Cell Cultures. K. Oizumi, Y. Yoshiko, J. E. Aubin. Anatomy and Cell Biology, University of Toronto, Toronto, ON, Canada.

To investigate the effect of $1,25(OH)_2D_3$ on the conversion of osteoprogenitor cells into adipocytes, rat calvaria (RC) cells were treated with $1,25(OH)_2D_3$ and/or BRL-49653, a potent PPARγ-selective ligand. The expression of PPARs and C/EBPs, which are two transcription factor families that regulate adipocyte differentiation, was also assessed. As reported previously, $1,25(OH)_2D_3$ induced adipocyte colonies and adipocyte marker expression, while completely inhibiting bone nodule formation and the expression of most osteoblast markers in RC cultures; an exception was that $1,25(OH)_2D_3$ increased OPN expression at early culture stages. Although the inverse relationship between osteoblast and adipocyte marker expression and osteoblast and adipocyte colony formation suggested the conversion of osteoprogenitor cells into adipocytes, the number of adipocyte colonies in $1,25(OH)_2D_3$-treated dishes was much less than the number of bone colonies/nodules in vehicle-treated dishes. This apparent discrepancy in fate redirection of osteoprogenitors to adipocytes was altered when RC cells were subjected to combined treatment with $1,25(OH)_2D_3$ and BRL-49653, which induced a large number of mature adipocyte colonies, suggesting that $1,25(OH)_2D_3$ has dual roles: inducing adipocyte maturation in some preadipocytes and inducing osteoprogenitor cells to select an adipocyte fate that is then completed in response to the PPARg selective ligand. Although both $1,25(OH)_2D_3$ and BRL-49653 increased PPARγ and C/EBPα expression, BRL-49653 had no effect on osteoblast differentiation. However, our data support the hypothesis that the inhibitory effect of $1,25(OH)_2D_3$ on osteoblast differentiation is based on its induction of C/EBPδ, which is induced eaerlier than PPARγ during initiation of adipogenesis. The present study suggests that committed osteoprogenitor cells in RC cell cultures are redirected in fate choice by $1,25(OH)_2D_3$ but undergo marked conversion into mature adipocytes only after combination treatment with $1,25(OH)_2D_3$ and the PPAR γ selective ligand.

F517

Phosphorylation of the Human Vitamin D Receptor by Protein Kinase A Downregulates $1,25(OH)_2D_3$-dependent Transactivation by Reducing Retinoid X Receptor β Heterodimerization. J. C. Hsieh, H. T. L. Dang,* M.

A. Galligan,* G. K. Whitfield, P. W. Jurutka, P. D. Thompson, C. A. Haussler,* M. R. Haussler. Biochemistry & Molecular Biophysics, College of Medicine, University of Arizona, Tucson, AZ, USA.

Phosphorylation of the human vitamin D receptor (hVDR) includes protein kinase C (PKC) action at serine-51 and casein kinase-II (CK-II) phosphorylation of serine-208, posttranslational modifications that attenuate and potentiate receptor activity, respectively. Preliminary work from our laboratory suggested that protein kinase A (PKA) can also phosphorylate hVDR between amino acids 133 and 201. To elucidate the exact PKA phosphorylation site(s) of hVDR, a series of C-terminally truncated mutants (δ134, δ180, δ190 and δ202) were expressed in transfected COS-7 cells, immunoprecipitated with VDR antibody, and incubated with PKA and [^{32}P]ATP, in vitro. Visualization of these reactions by SDS-PAGE indicated that the major PKA phosphorylation site of hVDR is localized between residues 180-190, a region that contains a cluster of four consecutive serines, ^{182}Ser-Ser-Ser-Ser^{185}, and a single serine at position 187. These serines were individually mutated to alanine using δ190 hVDR, the native receptor, and S51A/S208A (to eliminate PKC and CK-II sites) as templates, and the resulting mutant hVDRs were tested for their ability to serve as PKA substrates, in vitro. The results showed that the S182A mutant hVDR was least able to serve as a PKA substrate. Furthermore, when intact transfected COS-7 cells were treated with [^{32}P]orthophosphate, the S182A mutant displayed the largest reduction in phosphorylation compared to the other alanine-substituted hVDRs. We therefore conclude that serine-182 is a primary PKA phosphorylation site in hVDR, both in vitro and in vivo. As a test of the functional consequence of this phosphorylation event, an aspartate-substituted mutant (S182D) was created to mimic the negative charge of a phosphorylated serine. Utilizing the mammalian two-hybrid assay, it was observed that, while the S182A mutant could associate normally with the retinoid X receptor-β (RXRβ) dimeric partner, S182D was significantly impaired in this interaction. Also, in cotransfection assays with a 1,25(OH)$_2$D$_3$-responsive reporter gene, S182A hVDR exhibited normal transactivation, but the S182D mutant possessed only 50% of wild-type hVDR activity. Taken together, these observations strongly suggest not only that serine-182 can be a target of PKA phosphorylation in hVDR, but that this postranslational event may significantly inhibit hVDR dimerization with RXRβ, thereby attenuating the ability of hVDR to mediate 1,25(OH)$_2$D$_3$-dependent transactivation of target genes.

F519

The Amino Bisphosphonate Ibandronate Prevents Vitamin D Toxicity and Potently Inhibits Vitamin D-Induced Calcification of Arteries, Lungs, and Kidneys. P. A. Price, J. R. Buckley,* M. K. Williamson. Division of Biology, University of California, San Diego, La Jolla, CA, USA.

The present experiments were carried out to determine whether doses of the amino bisphosphonate ibandronate that inhibit bone resorption will inhibit soft tissue calcification and death in rats treated with a toxic dose of vitamin D. These studies were prompted by the recent discovery that ibandronate doses that inhibit bone resorption will potently inhibit artery calcification induced by treatment with the vitamin K antagonist warfarin (Arterioscler. Thromb. Vasc. Biol.(2001) 21(5) in press).

All 16 rats treated with a toxic dose of vitamin D (0.5 million IU vitamin D$_3$/kg at t=0, 1, and 2 days) died by day 6 following the first vitamin D injection (median survival 4.5 days), while the 12 rats treated with vitamin D plus ibandronate (0.25mg/kg/day) were alive and in good health at day 10. Rats treated with the toxic dose of vitamin D alone and examined at day 4 had extensive Alizarin red staining for calcification in the aorta; the carotid, hepatic, mesenteric, renal, and femoral arteries; kidneys; and lungs, while rats treated with vitamin D plus ibandronate showed no evidence of Alizarin red staining for calcification in any of these tissues. Chemical analysis further showed that rats treated with vitamin D alone and examined at day four had 5- to 20-fold higher levels of calcium and phosphate in their abdominal aorta, lungs, and kidneys than was found in control rats ($p < 0.001$), while the levels of calcium and phosphate in tissues from rats treated with vitamin D plus ibandronate were at control values. Serum calcium levels were the same in rats treated with vitamin D alone (13.5 ± 0.9 mg/dl) and in rats treated with vitamin D plus ibandronate (14.0 ± 0.6 mg/dl), which shows that ibandronate does not prevent soft tissue calcification by simply lowering serum calcium levels.

To our knowledge, ibandronate is the first drug that has been shown to prevent soft tissue calcification and death in an animal that has received a toxic dose of vitamin D. The fact that an ibandronate dose that inhibits bone resorption is able to prevent soft tissue calcification induced by toxic doses of vitamin D further supports the hypothesis that soft tissue calcification is linked to bone resorption. The mechanism responsible for this putative linkage remains to be determined.

F520

Identification of a 25-hydroxyvitamin D-1alpha-hydroxylase Splice Variant in Skin, Prostate, and Colon. J. N. Flanagan,*[1] V. Tanpricha,*[1] T. Chen,[1] J. Reichrath,*[2] M. F. Holick.[1] [1]Physiology and Endocrinology, Boston University School of Medicine, Boston, MA, USA, [2]Dermatology, University of Saarland, Homberg, Germany.

The renal 25-hydroxyvitamin D-1α-hydroxylase (1α-OHase), a mitochondrial cytochrome P450, is responsible for producing serum 1α,25(OH)$_2$D$_3$. It has been established that other tissues including skin, prostate, and colon have the ability to convert 25(OH)D$_3$ to 1α,25(OH)$_2$D$_3$ and express the 1α-OHase. The presence of 1α-OHase in non-renal tissues may play an autocrine/paracrine role for locally modulating cell proliferation and differentiation. Since the cloning of the 1a-OHase, its expression has been detected in many other non-renal tissues and when compared to the renal 1α-OHase, the cDNAs sequences were established to be 100% identical. A 1a-OHase specific polyclonal antibody was made and used to characterize the tissue distribution of the enzyme. Western blot analysis of whole cell extracts from skin, kidney, prostate, and colon with 1α-OHase specific antibody revealed the predicted 1α-OHase protein product of 56 kDa in kidney, prostate, and colon but not in skin.However, a larger protein product of 59 kDa was observed in skin, prostate, and colon but not in kidney. A side-by-side comparison of whole kidney extract with whole skin extract demonstrated a size discrepancy of 3 kDa. Thus, no 56 kDa protein product was observed in the skin and there was no 59 kDa protein product observed in the kidney. In order to investigate the possibility of splice variants of the 1a-OHase, we designed sequence specific primers to the 1α-OHase expanding the 8 known introns. Using RT-PCR for the 1α-OHase in skin and kidney, we detected an insertion between exons 2 and 3 in skin but not in kidney. By sequence analysis it was determined that the skin variation contained the 3'-region of intron 2 using an alternative 5'splice signal -AG- in intron 2. This variant was not observed in kidney. This is the first report of an alternative mRNA splice variant and protein product for the 1α-OHase gene in skin, prostate, and colon. The tissue specific alternative spliced variant of 1α-OHase that contains intron 2 may represent a possible regulatory domain that could explain why the renal 1a-OHase is tightly regulated by parathyroid hormone and phosphate whereas the extra-renal 1a-OHase is not. It is also interesting that prostate and contained both splice variants whereas the skin and kidney contained one. The endogenous expression of 1α-OHase gene variants and differential tissue expression of these variants opens up a new paradigm in the understanding of the role of 25-hydroxyvitamin D$_3$ 1α-hydroxylase gene in the regulation of vitamin D physiology.

F522

Renal Cell-Specific Regulation of Type IIa Sodium-Dependent Phosphate Cotransporter Gene Expression by 1,25-Dihydroxyvitamin D3. H. Yamamoto,[1] K. Kobayashi,[1] Y. Tani,[1] Y. Taketani,[1] K. Morita,[1] H. Arai,[1] K. Miyamoto,[1] S. Kato,[2] J. W. Pike,[3] E. Takeda.[1] [1]Clinical Nutrition, School of Medicine, University of Tokushima, Tokushima, Japan, [2]Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan, [3]Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA.

Physiological and pathophysiological alterations in renal Pi reabsorption are related to the altered brush-border membrane expression of the type IIa Na+-dependent phosphate (NaPi) cotransporter. We have shown that the expression of this cotransporter is restricted to renal proximal tubular cells which produce the active vitamin D metabolite 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) and is regulated by 1,25(OH)2D3. The mechanisms through which NaPi cotransporter expression is regulated in renal proximal tubular cells remains unclear, however. To gain a better understanding of the mechanism through which 1,25(OH)2D3 regulates NaPi transporter gene expression, we have characterized the promoter region of the mouse NaPi cotransporter gene with regard to transactivation by the vitamin D receptor (VDR). We first cloned the NaPi-7 gene and isolated the 5' end of the gene containing the promoter. We next examined the activity of this promoter in both renal and nonrenal cell types including porcine LLC-PK1, murine MCT and opposum OK kidney cells as well as COS-7 cells, and contrasted its activity with that of promoters for human osteocalcin and rat 24-hydroxylase (24-OHase) genes. In each case, expression vectors for both VDR and retinoid X receptor were cotransfected together with the promoters to assess basal and 1,25(OH)2D3 response. Basal activity was evident for all of the promoters tested in all the cell types. Interestingly, while 1,25(OH)2D3 was capable of stimulating both the osteocalcin and 24-OHase promoters in all cell types, NaPi-7 promoter activity was enhanced by 1,25(OH)2D3 only in OK cells. A similar cell-selective activation by 1,25(OH)2D3 was also observed with the human NaPi cotransporter (NaPi-3) gene promoter. We next carried out a deletion analysis to define the sequence of the NaPi-7 promoter necessary for vitamin D response. Our results identified a novel region within the 5' end of the gene that acts not as an enhancer element, but rather dictates synthesis of a new transcript containing a novel exon (exon1a). Importantly, northern blot analysis showed that the expression of this exon1a was decreased in VDRKO mice. These findings suggest that an important cell-specific regulatory factor may play an essential role in the regulation of NaPi gene expression by 1,25(OH)2D3 and that this regulation involves a new NaPi-7 promoter.

F524

Histone Acetylation In Vivo at the Osteocalcin Locus is Functionally Coupled to Chromatin Remodeling and Vitamin D Dependent, Bone Tissue-Specific Gene Activation. J. Shen,*[1] J. B. Lian,[1] M. Montecino,*[2] G. S. Stein,[1] J. L. Stein,*[1] A. J. van Wijnen.[1] [1]Cell Biology, UMass Medical School, Worcester, MA, USA, [2]Biologia Molecular, Universidad de Concepcion, Concepcion, Chile.

The accessibility of regulatory elements in chromatin represents a principal rate limiting parameter in gene transcription, and is modulated by enzymatic transcriptional co-factors that alter the topology of chromatin or covalently modify histones (e.g., by acetylation or phosphorylation). The bone-specific activation and 1,25-dihydroxyvitamin D3 (VD3) enhancement of osteocalcin (OC) gene transcription are both functionally linked to modifications in nucleosomal organization. The initiation of tissue-specific basal transcription is accompanied by the induction of two DNase I hypersensitive sites, and this chromatin remodeling event requires binding of the key osteogenic factor RUNX2/CBFA1 to the OC promoter. Here, we define in vivo requirements for bone tissue-specific gene expression that have not been previously identified for Vitamin D responsive or osteoblast related genes. We analyzed the acetylation status of histones H3 and H4 when the gene is active (in osteoblastic ROS17/2.8 cells) or inactive (in fibroblastic ROS24/1 cells) using chromatin-immunoprecipitation (ChIP) assays with antibodies detecting acetylated histone H3 or H4. DNA fragments co-precipitating with the acetylated histones were purified and used in PCR reactions with several sets of gene-specific primers spanning the OC locus. The DNA products were resolved by gel electrophoresis and quantitated by digital imaging. We find that acetylated histone H3 and H4 proteins are associated with nucleosomes in the promoter of the OC gene only when it is transcriptionally active, and the acetylation status is relatively uniform across the OC locus under basal conditions. Acetylation of H4 and H3 at the OC gene is selectively increased following Vitamin D3 enhancement of OC transcription, and the most prominent changes occur in the region between the VD3 enhancer and basal promoter. Thus, acetylation of histones H3 and H4 in specific regions of the OC promoter appears to be functionally linked to the tissue-specific transcriptional activation and Vitamin D enhancement of OC gene expression, as well as chromatin remodeling at the OC locus. These findings provide important mechanistic insights into bone-specific gene activation within a native genomic context in response to developmental regulatory cues and steroid hormones.

F527

Targeted Ablation of the 25-Hydroxyvitamin D 1a-Hydroxylase Enzyme: Evidence for Skeletal, Reproductive and Immune Dysfunction. D. K. Panda,[1] D. Miao,[1] M. L. Tremblay,*[2] J. Sirois,*[2] G. N. Hendy,[1] D. Goltzman.[1] [1]Dept. of Medicine, McGill University, Montreal, PQ, Canada, [2]Dept. of Biochemistry, McGill University, Montreal, PQ, Canada.

The active form of vitamin D, 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D], is synthesized from its precursor 25 hydroxyvitamin D [25(OH)D] via the catalytic action of the 25(OH)D-1α-hydroxylase [1α(OH)ase] enzyme. Many roles in cell growth and differentiation have been attributed to 1,25$(OH)_2$D, including a central role in calcium homeostasis and skeletal metabolism. To investigate the in vivo functions of 1,25$(OH)_2$D and the molecular basis of its actions we developed a mouse model deficient in 1α(OH)ase by targeted ablation of the hormone-binding and heme-binding domains of the1α(OH)ase gene. After weaning, mice developed hypocalcemia, secondary hyperparathyroidism, retarded growth and skeletal abnormalities characteristic of rickets. These abnormalities are similar to those described in humans with the genetic disorder vitamin D dependent ricket type1 (VDDR-1). Altered non-collagenous matrix protein expression and reduced numbers of osteoclasts were also observed in bone. Female mutant mice were infertile and exhibited uterine hypoplasia and absent corpora lutea. Furthermore histologically enlarged lymph nodes in the vicinity of the thyroid gland and reduction in CD4- and CD8- positive peripheral T lymphocytes were observed. Alopecia, reported in vitamin D receptor (VDR)-deficient mice and in humans with VDDR-11, was not seen. The findings establish a critical role for the 1α(OH)ase enzyme in mineral and skeletal homeostasis as well as in female reproduction and also point to an important role in regulating immune function.

SA001

Calcium and Zinc Absorption from Lactose-Containing and Lactose-Free Whey-Based Infant Formulas. S. A. Abrams, I. J. Griffin,* D. C. Powledge,* P. Davila.* Pediatrics, Baylor College of Medicine, Houston, TX, USA.

Although calcium absorption is enhanced by lactose, the quantitative significance of this effect in infant formulas is uncertain as is the possibility of an effect of lactose on zinc absorption. We assessed the absorption of calcium and zinc using a multi-tracer, stable isotope technique in full-term infants fed ad libitum two partially hydrolyzed whey protein-based formulas. The formulas used either 1) lactose or 2) lactose-free (glucose polymers) carbohydrate sources. Calcium content of both formulas was 64 mg/100 kcal. Infants were studied in a blinded cross-over fashion after two weeks adaptation to each formula. Infants were randomized as to which formula was studied first. Isotope absorption studies were done using a 4-tracer method in which 70Zn and 44Ca were provided orally and 67Zn and 46Ca were given intravenously. Subsequently a 24-hour urine was collected to assess calcium absorption and a spot urine obtained at 48-72 hours post-dosing to assess zinc absorption. Eighteen infants completed the calcium absorption study. Data for zinc absorption was available for 14 or these 18. Mean age (78 d), weight (6.3 kg), length (60 cm), and formula intake (1100 ml/d) were similar at each study.

Group	Calcium intake (mg/d)	Percent absorption	Total calcium absorption (mg/d)
Lactose-containing	471 ± 97	66.5 ± 11.9	314 ± 82
Lactose-Free	465 ± 84	56.2 ± 15.3	259 ± 79
P-value of difference (paired t-test)	> 0.5	.002	.006

Zinc intake (5.5 ± 1.1 mg/d)), fractional zinc absorption (32 ± 11%) and total zinc absorption (1.8 ± 0.6 mg/d) were very similar between the two formulas. These data indicate that lactose enhances calcium, but not zinc absorption, from a partially hydrolyzed whey protein-based formula. Fractional calcium absorption from the lactose-containing milk was similar to that reported for human milk. Net calcium absorption from both exceeded that expected from human milk based on the higher calcium intake levels in the formula relative to human milk. We conclude that lactose-free formulas provide adequate absorption of calcium and zinc, but that to obtain levels of absolute calcium absorption comparable to lactose containing formulas, slightly higher concentrations of calcium would be required. This study was funded in part by Nestle, USA.

Disclosures: Nestle USA,2.

SA002

See Friday Plenary number F002.

SA003

Correlates of Forearm Bone Mineral Density in Young Norwegian Women. The Nord-Trøndelag Health Survey. G. A. Hawker,[1] S. Forsmo,*[2] S. M. Cadarette,[1] B. Schei,*[2] S. B. Jaglal,[1] A. Langhammer,*[3] L. Forsén.*[4] [1]University of Toronto, Toronto, Canada, [2]Norwegian University of Science and Technology, Trondheim, Norway, [3]Hunt Research Centre, Norwegian University of Science and Technology, Verdal, Norway, [4]National Institute of Public Health, Oslo, Norway

Peripheral bone densitometry is gaining utility as a less expensive and portable alternative to axial assessments. The main objective of the study was to identify factors associated with forearm bone mineral density (BMD) in young adult women. Population-based data derived from standardized questionnaires among healthy women aged 19-35 years living in Nord-Trøndelag, Norway (N=963) were used. Participants with a history of fracture in the forearm tested, pregnant at the time of the examination, or missing weight data were excluded. Single x-ray absorptiometry was performed at the ultradistal and distal forearm. Multiple linear and logistic regression were used to assess factors associated with BMD (g/cm2), and low BMD (lowest quintile) at the ultradistal and distal sites separately. The mean age and weight of the cohort was 29.7 years (SD=4.7) and 68.6 kg (SD=12.5) respectively. The majority had post-secondary education (54%) and drank milk (91%). The mean BMD values were 0.386 (SD=0.048) and 0.478 (SD=0.042) for the ultradistal and distal sites respectively. In both linear and logistic models, none of vitamin D intake, physical activity, smoking, alcohol consumption, amenorrhea, oral contraceptive use, number of pregnancies, history of breast-feeding or family history of osteoporosis were found to significantly impact BMD. Consistent with prior studies, age and weight were positively associated with BMD at both forearm sites, while increasing age at menarche and lower daily milk consumption were associated with lower BMD levels. Body weight was the most important factor associated with forearm BMD, accounting for at least half of the explained BMD variation in regression models. Given that the forearm is not a weight-bearing site, body weight may be acting as a surrogate for other factors, such as endogenous estrogen exposure. Controlling for age and weight, women who did not drink milk daily were 2 times more likely to have low forearm BMD. This association was strongest at the ultradistal site (odds ratio=2.3; 95% CI=1.6-3.9). Results from this study are consistent with previous reports evaluating peak BMD at the hip and spine, and support the growing body of literature suggesting a benefit of milk consumption on peak BMD.

SA004

See Friday Plenary number F004.

SA005

See Friday Plenary number F005.

SA006

Bone Markers and Bone Mass in Healthy Pubertal Boys and Girls. S. C. C. van Coeverden,*[1] C. J. Netelenbos,[2] C. M. Ridder, de,[1] J. C. Roos,*[3] C. Popp-Snijders,*[4] H. A. Delemarre-van de Waal.*[1] [1]Pediatrics, VU University Medical Centre, Amsterdam, The Netherlands, [2]Internal Medicine, VU University Medical Centre, Amsterdam, The Netherlands, [3]Nuclear Medicine, VU University Medical Centre, Amsterdam, The Netherlands, [4]Clinical Chemistry, VU University Medical Centre, Amsterdam, The Netherlands

The aim of this study was to evaluate associations between the markers of bone formation and bone resorption, the sex steroids and growth factors with bone mineral content (BMC) and bone mineral density (BMD) during puberty in healthy boys and girls. In 155 boys and 151 girls we assessed height, weight, and pubertal stages according to Tanner. BMC and BMD were measured of the lumbar spine, femoral neck and trochanter and the total body using dual energy X-ray absorptiometry. All measurements were repeated after one year. At the first visit fasting blood samples and first-void urine samples were obtained to measure markers of bone formation and bone resorption, estradiol, testosterone, IGF-1, and IGF-BP3. BMC and BMD increased throughout puberty in both sexes. Bone turnover markers significantly increased until maximum values were reached at stage G4 in boys and stage B3 in girls with a significant decline thereafter in girls. Height velocity (HV) had a similar changing pattern. Sex steroids and IGF-1 increased and reached adult values at pubertal stage 4. The correlations between bone markers and BMC were highly significant in boys, while correlations between bone markers and the increase in BMC over one year were significant in both sexes, as was observed for the correlations with HV. Sex steroids and IGF-1 correlated positively with the bone markers in pubertal stages 2+3, but were not significant in pubertal stages 4+5 in boys and girls. Our data suggest that bone markers are good predictors of bone mass in boys and of bone mass increase in both sexes. In early puberty, sex steroids stimulate the pubertal growth spurt which gives rise to an increase in height and to an increase in bone turnover and bone mineral. In late puberty, high levels of estradiol inhibit growth, which leads to a decline in bone turnover. Bone mass still increases under the influence of sex steroids and IGF-1. The data in our study confirm previous reports that markers of bone turnover relate positively to height velocity.

SA007

See Friday Plenary number F007.

SA008

The Influence of Serum Leptin Concentrations on Bone Mass Assessed by Quantitative Ultrasonometry (QUS) in Pre- and Postmenopausal Women. P. Hadji,[1] K. Bock,*[1] M. Gottschalk,*[1] M. Kalder,*[1] G. Emons,*[2] K. D. Schulz.*[1] [1]University of Marburg, Marburg, Germany, [2]University of Göttingen, Göttingen, Germany

This study was aimed to investigate the influence of serum leptin concentration on bone mass assessed by QUS in a large sample of healthy pre- and postmenopausal women. 555 pre- and postmenopausal (n=262 and n=293) women not on estrogens (mean age, 49.5 ± 17.2 years) were recruited at the University of Marburg on the occasion of a routine gynecological visit. Before entry to the study, all women had answered a detailed questionnaire on important risk factors. Speed of sound (SOS), broadband ultrasound attenuation (BUA) and stiffness index (SI) were measured using the Achilles ultrasonometer (GE/Lunar). We systematically investigated the specific influence of age, menopause and BMI on Leptin and QUS variables by allocating women into the following groups: (a) premenopausal women BMI< 25 kg/m² (N=177), (b) premenopausal women BMI>25 kg/m² (N=83), (c) postmenopausal women BMI< 25 kg/m² (N=124) and (d) postmenopausal women BMI> 25 kg/m² (N=168). Additionally we evaluated the influence of serum leptin, age and BMI on QUS variables by performing a multiple linear regression analyses. In the initial analyses premenopausal women showed a significantly lower mean age, weight, BMI, follicle stimulating hormone (FSH) and serum leptin concentration (P<0.001), a higher mean height, serum estradiol and QUS variables compared to postmenopausal women. Irrespective of the menopausal status, women with a BMI>25 kg/m² had significantly higher leptin concentration compared to women with a BMI<25 kg/m² (P<0.001). Only BMI but not Leptin was related to higher QUS variables, whereas increasing age was associated with decreased QUS variables.The multiple linear regression analyses confirmed that only age and BMI but not Leptin, were the only statistically significant independent predictor for QUS variables. Serum leptin concentration is significantly higher in pre- and postmenopausal obese women, compared with normal weight controls. Bone mass assessed by QUS is influenced by age and BMI but not by serum leptin concentration.

SA009

Changes in Bone Structure and Mass with Aging in the Male Mouse. B. Halloran,[1] V. Ferguson,[2] S. Simske,[2] A. Burghardt,*[3] L. Venton,*[4] L. Rudner,*[4] S. Majumdar.[3] [1]Departments of Medicine and Physiology, University of California, Division of Endocrinology, Veterans Affairs Medical Center, San Francisco, CA, USA, [2]Bioserve Space Technologies, University of Colorado, Boulder, CO, USA, [3]Department of Radiology, University of California, San Francisco, CA, USA, [4]Division of Endocrinology, Veterans Affairs Medical Center, San Francisco, CA, USA.

To determine whether the mouse is a suitable model to study age-related bone loss in humans, we examined the changes in bone structure and mass that occur during the normal aging process in the male C57B6 mouse. MicroCT was used to assess the structural and physical properties of the proximal tibia and tibial diaphysis in mice aged 1.5,3,6,12,18 and 24 months. Bone mass reached a peak at 12 months and declined thereafter. Cancellous bone volume declined continuously from a plateau at 6 m of 0.15% to 0.06% at 24 m. Trabecular number decreased and spacing increased beginning at 1.5 m of age. Trabecular thickness increased to 12 m and remained constant thereafter. Connectivity density fell dramatically between 1.5 and 12 m and then remained constant to 24 m. Using beam attenuation as a means of measuring bone density, trabecular mineral density (g hydroxyapatite/ mm3) reached a peak at 12 m and remained constant to 24 m. Cross-sectional area of the tibial diaphysis continued to increase to 18 m. Cortical thickness reached a peak at 6 m and declined continuously to 24 m. Cortical mineral density reached a peak at 3 m and remained constant to 24 m. We conclude that in the male mouse bone mass diminishes with normal aging. Cancellous bone restructures with age resulting in a decrease in total bone volume and a decrease in anisotropy (increased trabecular order). Cortical thickness diminishes but density remains intact. The changes observed in bone in the aging mouse are similar to those observed in human age-related bone loss. These findings support the use of the aging male mouse as an animal model to study age-related bone loss in humans.

SA010

See Friday Plenary number F010.

SA011

Age-associated Changes in Bone Ultrasonometry of the Os Calcis. Expanded Data in Healthy German Women. P. Hadji, M. Meyer-Wittkopf,* M. Gottschalk,* M. Kalder,* K. D. Schulz.* University of Marburg, Marburg, Germany

This study updates age changes for quantitativeultrasonometry (QUS) of the os calcis in a large sample of healthy German women almost four times larger than our preliminary study published earlier. Speed of sound (SOS), broadband ultrasound attenuation (BUA) and stiffness index (SI) of the os calcis were measured in 5148 women (mean age 55.2 ± 10.6 years) using the Achilles ultrasonometer (GE/Lunar). Before entry to the study, all women had answered a detailed questionnaire on important risk factors. The short-term precision in 31 adults was 0.2% for SOS, 1.2% for BUA, and 1.3% for SI. There was an overall decline of 16% for BUA, 4% for SOS and 32% for SI between late adolescence and old age. In premenopausal women, BUA decreased only slightly (-2%), while postmenopausal women showed a significantly increased decline (-12%). In contrast, SOS continuously decreased from the age of 15; there was a decline of 2% from adolescence to the menopause. The SI of premenopausal women decreased only by 9%, but the postmenopausal decline of almost 21% was significantly greater. In accordance to our previous report, the age regression for SI in the larger sample differed from the earlier sample, indicating an increased bone loss with aging after the menopause. The SI values in premenopausal German women were comparable to those for British and American women age 20-50 years. After age 50, the SI of German women was significantly 3-7% higher (Fig.1) in comparison to the British and American reference population.

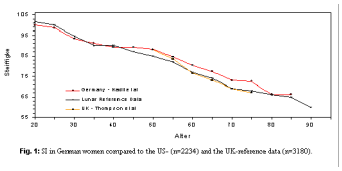


Fig. 1: SI in German women compared to the US- (n=2234) and the UK-reference data (n=3180).

SA012

See Friday Plenary number F012.

SA013

Retinoic Acid Stimulates Transcription of the Type X Collagen Gene Through the BMP Signaling Pathway. School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Vitamin A plays an essential role in endochondral bone formation, acting at least in part by accelerating chondrocyte maturation and hypertrophy. However, the mechanisms by which RA induces expression of the type X collagen gene, the only known gene expressed exclusively in hypertrophic chondrocytes, are at present unknown. Thus we recently initiated experiments to determine whether RA activates type X collagen gene expression at the level of transcription, using a luciferase reporter plasmid driven by various fragments of the type X collagen promoter (Volk et al., J. Bone Min. Res. 13, 1521-1529, 1998). These constructs were transfected into prehypertrophic chondrocytes from embryonic chick sternum, which respond relatively rapidly to physiological concentrations of RA by increased expression of the endogenous type X collagen gene. The 640-bp proximal promoter displayed very low basal activity that was not stimulated by RA. Addition of an upstream 600 bp fragment (nucleotides -2648 to -2008) resulted in significant stimulation by RA, indicating that RA acts at the transcriptional level to stimulate type X collagen production. It is important to note that this same promoter construct is stimulated by bone morphogenetic proteins (BMPs) and by co-transfected Smads (Leboy et al., J. Bone Joint Surg., in press, 2001), suggesting a common pathway for RA and BMP stimulation. We have used 5' end deletion mutagenesis as an initial step toward identification of the sequences required for stimulation by RA and BMPs. We found that a 130-bp region of the type X collagen gene promoter (nucleotides -2648 to -2518) is essential for stimulation by RA and also appears to be important for stimulation by BMPs. This region does not contain recognizable binding sites for retinoic acid receptors (RARs), ligand-activated transcription factors that mediate RA signaling, but contains potential binding sites for Smads, the transcription factors that mediate BMP signaling; we have used electrophoretic mobility shift assays to demonstrate Smad binding to some of these sites. These results suggested that the action of RA on this promoter may be indirect, perhaps through activation of the BMP signaling pathway. We confirmed this suggestion by demonstrating that retrovirus-mediated expression of a dominant-negative BMP receptor IB prevented the increase in type X collagen promoter activity normally induced by RA. These studies provide the first mechanistic insights into the activation of type X collagen gene expression by retinoids.

SA014

See Friday Plenary number F014.

SA015

Interaction Between Systemic Bisphosphonate Therapy and Bone Formation Induced by Local Delivery of rhBMP-2 in Non-Human Primates. M. L. Bouxsein, C. Blake,* C. Luppen,* J. A. Cooper,* J. M. Wozney, H. J. Seeherman. Genetics Institute, Andover, MA, USA.

Recombinant human bone morphogenetic protein-2 (rhBMP-2) is a potent differentiation factor for bone and cartilage precursor cells, and has been shown to induce bone formation in a variety of animal models. We previously reported that, in sheep and non-human primate models, intraosseous placement of rhBMP-2 (soak-loaded on an absorbable collagen sponge (ACS)) led to transient bone resorption followed by new bone formation [Seeherman et al, Trans ORS 1998, 2000]. The goal of this study was to determine whether systemic bisphosphonate therapy would inhibit the transient bone resorption induced by rhBMP-2 and still permit new bone formation. Bilateral core defects (3.5 mm diameter) were surgically created in the distal femurs of 13 adult male cynomolgus monkeys. In each monkey, one defect was treated with an rhBMP-2/ACS implant (360 µg rhBMP-2), and the contralateral defect received a buffer/ACS implant. Monkeys received monthly injections of either bisphosphonate (Ibandronate, 0.3 mg/ml, 0.15 mg/kg, n=8) or saline (VEH, n=5) starting 4 weeks prior to the surgery and continuing throughout the study. Thus, four treatments were evaluated: rhBMP-2/ACS + bisphosphonate (BP), buffer/ACS + BP, rhBMP-2/ ACS + VEH, and buffer/ACS + VEH. In vivo pQCT scans were acquired prior to surgery, immediately post-op, and 2, 4, 8, and 16 wks after surgery to monitor bone resorption and formation by assessing the size and density of the core defect region. At 2 wks, resorption was evident in the rhBMP-2/VEH group, as the area of the defect was approximately twice the size of the defect immediately after surgery. In addition, the defect area was 70% greater in the rhBMP-2/VEH group than in the other groups ($p<0.05$), which were not different from each other. Between 4 and 8 weeks after surgery, bone density in the core defect began to increase. After 16 weeks, the density in the core defect was highest in the rhBMP-2/BP and rhBMP-2/VEH groups (439 ± 137 and 332 ± 27 mg/cm^3, respectively), equaling or exceeding the baseline bone density in all cases, providing evidence of new bone formation and mineralization. In comparison, the density of the defect in the buffer/ BP and buffer/VEH groups was 203 ± 77 and 248 ± 94 mg/cm^3, respectively. In conclusion, these data confirm that delivery of rhBMP-2/ACS in an intraosseous environment induces transient bone resorption followed by bone formation. Systemic bisphosphonate therapy inhibits the bone resorption induced by rhBMP-2, but does not interfere with the subsequent new bone formation and mineralization.

Disclosures: Genetics Institute,3.

SA016

See Friday Plenary number F016.

SA017

Stimulation of Bone Formation by Rho-Associated Kinase Inhibitor. K. Itoh,[1] K. Yoshioka,*[1] T. Nakase,[2] H. Yoshikawa.[2] [1]Biology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan, [2]Orthopedic Surgery, Osaka University Medical School, Suita, Japan.

Bone morphogenetic protein (BMP), a potent inducer of bone formation in vivo, promotes differentiation of mesenchymal cells into osteoblasts. This process is regulated by several signal transduction pathways. The small GTPase Rho and Rho-associated protein kinase (p160ROCK) signal participates in a variety of biological functions including vascular contraction, tumor cell invasion, cellular differentiation and penile erection. However, its involvement in osteoblastic differentiation by BMP remains unknown. Continuous delivery of a specific ROCK inhibitor (Y-27632) using osmotic pumps markedly enhanced

ectopic bone formation induced by recombinant human BMP-2 (rhBMP-2) impregnated into an atelocollagen carrier in mice without affecting systemic bone metabolism. Treatment with Y-27632 also enhanced the osteoblastic differentiation of cultured murine neonatal calvarial, and undifferentiated mesenchymal 10T1/2, MC3T3-E1 and ST2 cells with and without rhBMP-2. These effects were associated with an increase in the gene expression of BMP-4. Transfection of ST2 cells with cDNA encoding a dominant negative mutant of ROCK promoted osteoblastic differentiation with increased expression of the BMP-4 gene. Conversely, the expression of a dominant active mutant of ROCK attenuated osteoblastic differentiation and the ROCK inhibitor reversed this phenotype. These results indicate that ROCK plays a negative role in osteogenesis, and a ROCK inhibitor in combination with the local delivery of rhBMP/collagen composite may be clinically applicable for stimulating bone formation.

SA018

Depot Medroxyprogesterone Acetate Contraception and Bone Mineral Density in Adolescent Women

D. Scholes,[1] A. LaCroix,[1] L. Ichikawa,*[1] W. Barlow,*[1] S. Ott.[2] [1]Center for Health Studies, Group Health Cooperative, Seattle, WA, USA, [2]Dept. of Medicine, University of Washington, Seattle, WA, USA.

Use of depot medroxyprogesterone acetate injectable contraception (DMPA) is associated with decreased bone mineral density (BMD), but bone effects in adolescents have received little study. We conducted a cross-sectional evaluation of the effects of DMPA use on BMD in a recently-recruited cohort of 174 adolescents ages 14-18 years. Participants are enrollees of a Washington state HMO who were selected via computerized databases if they had received DMPA (n=81) or were of similar age (comparison group, n=93). BMD was measured using DEXA at the total hip, P-A spine, and whole body. Data on DMPA and on other factors related to BMD acquisition and retention were collected via questionnaire and exam. Overall, 30% of the cohort is $\leq$ 16 years of age, 30% is non-white. Compared to non-users, DMPA users are significantly more likely to be of African-American ethnicity, to smoke, to have been previously pregnant, and to have lower calcium intake. Unadjusted mean BMD values for DMPA users, although lower at all anatomic sites, did not differ significantly from non-users (mean hip BMD=0.940 g/cm^2 vs. 0.970 g/cm^2, for DMPA users and non-users, respectively (p=0.10); and mean spine BMD=0.970 g/cm^2 vs. 0.992 g/cm^2 (p=0.20)). However, BMD declined with increasing DMPA exposure, with a significant trend at the spine:

		Total hip BMD (g/cm^2)	Spine BMD (g/cm^2)
# DMPA injections	**N**	**Mean**	**Mean**
0	93	0.970	0.992
1	24	0.950	0.994
2-3	26	0.943	0.972
4-7	17	0.927	0.960
8+	14	0.930	0.935
p-value for trend		0.08	0.05

These data suggest that, in this group of young women who have yet to attain peak bone mass, adverse effects on bone may accumulate with increasing duration of DMPA use.

SA019

See Friday Plenary number F019.

SA020

RNA Retrieved from Frozen and Archival Osteoarthritic Tissue Utilizing Laser Capture Microdissection. T. Scharschmidt,*[1] R. Jacquet,*[1] J. Hillyer,*[1] S. Weiner,*[2] P. Flanagan,*[2] W. J. Landis.[1] [1]Biochemistry and Molecular Pathology, Northeastern Ohio Universities College of Medicine, Rootstown, OH, USA, [2]Orthopedic Surgery, Summa Health System, Akron, OH, USA.

This laboratory has initiated skeletal tissue studies utilizing novel laser capture microdissection (LCM) to isolate ("capture") single cells directly from sample sections. Coupled with RT-PCR, the approach permits correlation of molecular data with developmental, temporal and spatial features of a specimen. LCM has been applied to frozen, but not paraffinized archival, skeletal tissue. This investigation demonstrates that LCM can be used to retrieve RNA from paraffinized osteoarthritic (OA) human cartilage. OA tissue was obtained from femoral condyles following surgery of elderly patients. Samples were divided with one segment stored in RNAlater (Ambion, Austin, TX) at -20^{o}C and the remainder fixed in 10% neutral-buffered formalin and processed into paraffin within 4 hrs of surgery. The frozen specimens served as controls for comparing gene expression with that derived from paraffinized material that models archival tissue. Frozen samples were mounted with OTC in a cryostat and sectioned 5 µm thick on glass slides. Slides were fixed in 70% ethanol, stained with eosin, and dehydrated through alcohols. Paraffin-embedded specimens were likewise sectioned on slides, dipped in xylene to deparaffinize them, and stained with eosin. Sections were air dried and observed in a Pixcell LCM system (Arcturus Engineering, Mountain View, CA). Capture of 50-1000 chondrocytes was made with an infrared laser of 15 µm diameter, 70 mW power, and 2.5 ms pulse width. RNA from frozen sections was extracted from cells by microisolation (Stratagene, La Jolla, CA) and from paraffinized sections by a paraffin block RNA isolation kit (Ambion). RNA was DNAse-treated and reverse-transcribed. cDNA was amplified by PCR using AmpliTaq ® DNA polymerase (PE Applied Biosystems, Foster City, CA). Negative controls omitted reverse transcriptase. Ethidium bromide gel analysis revealed 18S rRNA from cells isolated by LCM from both frozen and paraffinized OA tissue. rRNA from frozen and paraffin-embedded specimens was detected from ~300 and ~1000 chondrocytes, respectively. Thus, RNA may be retrieved from cells isolated by LCM from both frozen and paraffin-embedded material. Paraffin processing requires greater cell numbers for measurable RNA compared to frozen samples. However, the fact that RNA can be obtained from paraffinized specimens is significant and shows unequivocally that molecular analysis of archival tissue is possible and can be uniquely correlated with temporal and spatial data from the same material.

SA021

Use of a Hip Structural Analysis Program to Estimate Bone Strength in Children Following Burns. G. L. Klein,[1] T. J. Beck,[2] E. G. Briscoe,*[3] J. I. Rosenblatt,*[4] D. N. Herndon.*[5] [1]Pediatrics, University of Texas Medical Branch & Shriners Burns Hospital, Galveston, TX, USA, [2]Radiology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA, [3]Radiology, University of Texas Medical Branch, Galveston, TX, USA, [4]Biostatistics, University of Texas Medical Branch & Shriners Burns Hospital, Galveston, TX, USA, [5]Surgery, University of Texas Medical Branch & Shriners Burns Hospital, Galveston, TX, USA.

Burn injury results in loss of bone mineral content(BMC) and bone mineral density(BMD) over the first 6 mo post-burn, with low bone mineral accretion thereafter. Although annual extrapolated fracture rate is increased post-burn (J Pediatr 1995; 126:252), bone strength has not been assessed. We used the Hip Structural Analysis Program of Beck et al (J Bone Miner Res 2000; 15: 2297), using dual energy x-ray absorptiometric(DEXA) methods to determine sectional modulus(SeM) of the femoral neck(N) and shaft(S) in relation to subperiosteal width(W), cross-sectional area(CSA) and BMD. SeM is an index of bone bending strength; W is one of the dimensions measured in bone area; CSA, the amount of bone in the cross-section, correlates with BMC.Hip DEXA was performed in 21 children, 6-17 yr old, at 6 wk and 6 mo post-burn(range 5-80% body surface area).11 of 14(79%) had a fall in SeM at N regardless of burn size while at S SeM fell in burns >40% but not smaller(p=0.022, Fisher's exact test).CSA fell in 11/14(79%) at N and 9/14(64%) at S. There was a compensatory rise in W in10/14(71%) at N and in 11/14(79%) at S. Changes in SeM from 6 wk to 6 mo were highly correlated with changes in CSA in N and S, r2= 78%. Changes in BMD only accounted for 16% of changes in SeM at either N or S, possibly due to the compensatory increase in W. These data suggest that there is a decline in bone bending strength at the hip at 6 mo post-burn, consistent with the progressive loss of BMC recently reported in burn patients(Hart et al Ann Surg 2001;233: in press).

SA022

Osteopontin: Defining the Role of Phosphate Groups in Hydroxyapatite Inhibition. K. A. Robertson,*[1] H. A. Goldberg,*[1] T. M. Underhill,*[1] M. D. Grynpas,*[2] G. K. Hunter.*[1] [1]School of Dentistry, University of Western Ontario, London, ON, Canada, [2]Mount Sinai Hospital, Toronto, ON, Canada

The objective this study is to define which phosphorylated residues are important for the crystal inhibiting activity of osteopontin (OPN). OPN is a phosphorylated glycoprotein that is found in high levels in bone and at sites of ectopic calcification. Previous studies in our lab (Hunter *et al.* Biochem. J. 317, 59; 1996) and in others (Jono *et al.* J. Biol. Chem. 275, 20197; 2000) have demonstrated that OPN is a potent inhibitor of hydroxyapatite (HA) formation, and that phosphorylation of the protein is required for this activity. Native OPN was purified as previously described (Goldberg & Sodek, J. Tissue Culture Methods, 16, 211; 1994). Wild-type OPN was expressed in and purified from *E. coli* using the pET system (Novagen). Wild-type and mutant rat OPN were expressed and purified from rat osteosarcoma (ROS) 17/2.8 cells using mammalian expression vector pcDNA3 (Invitrogen). Mutagenesis of rat OPN cDNA was performed in a pGEM-cloning vector (Invitrogen) using the QuikChange 1-day site-directed mutagenesis kit (Stratagene). A constant composition autotitration assay was developed to test OPN inhibiting activity on *de novo* HA crystal formation. Under the conditions of the constant composition assay, HA formation commenced at approximately 10 minutes and was linear thereafter. Addition of native OPN caused a dose dependent increase in nucleation lag time. From plots of 1/lag time vs. log [OPN], it was calculated that lag time became infinite at approximately 6 ug/ml OPN. Prokaryotic recombinant OPN (non-phosphorylated) had no inhibitory effect on nucleation lag time of HA even at concentrations as high as 10 µg/ml. A mammalian OPN expression system has also been developed in our lab. Four sets of mutant constructs were created at identified sites of serine phosphorylation in rat OPN cDNA (Neame & Butler, Connective Tissue Research, 35, 145; 1996). Serine residues were converted to either alanine or glutamic acid to create two N-terminal mutants (10/11; 46/47) and two C-terminal mutants (250; 257; 262; deletion 295-298). These mutants have been expressed for functional analysis. The findings from this study show that phosphorylation of specific serine residues is required for the HA-inhibitory activity of OPN.

SA023

Function of Osteopontin Deduced from Laser Capture Microdissection and RT-PCR. W. J. Landis, R. Jacquet,* J. Hillyer,* J. Zhang.* Biochemistry and Molecular Pathology, Northeastern Ohio Universities College of Medicine, Rootstown, OH, USA.

Osteopontin (OPN), a phosphorylated glycoprotein, appears to play an important role in extracellular matrix development and ultimate mineralization of certain vertebrate connective tissues such as bone and cartilage. With respect to mineral formation, the precise action of OPN is not entirely clear with current evidence supporting alternative possibilites that the protein may be facilitative or inhibitory to mineral deposition. To gain insight into OPN function, normal growth plate cartilage from 1-11 day-old post-natal mice has been studied here by novel laser capture microdissection (LCM) and RT-PCR to measure OPN gene expression by chondrocytes of known age and specific location in this tissue. LCM identifies individual cells within a tissue section and uniquely isolates ("captures") them for a variety of molecular analyses. In this work, mouse tibiae were dissected and stored in RNAlater (Ambion, Austin, TX) at -20^{o}C. They were then mounted with OTC in a cryostat and 5 µm thick sections of the epiphyseal growth plates were obtained on glass slides, fixed in 70% ethanol, stained with eosin, dehydrated through alcohols, and air dried. Sections were examined in a Pixcell LCM system (Arcturus Engineering, Mountain View, CA) where ~200-1200 cells were captured using an infrared laser of 15 µm diameter, 70 mW power, and 2.5 ms pulse width. RNA was extracted from cells by microisolation (Stratagene, La Jolla, CA), DNAse-treated, and reverse transcribed. cDNA was amplified by PCR with AmpliTaq ® DNA polymerase (PE Applied Biosystems, Foster City, CA). Ethidium bromide gels revealed OPN mRNA from groups of chondrocytes isolated from whole cartilage and resting, proliferating, and hypertrophic zones of the mouse growth plates. Brain cells captured by LCM from the same mouse sections were positive controls and negative controls omitted reverse transcriptase. 18S rRNA was used to standardize expressed message from captured cells. RT-PCR of laser-captured whole cartilage showed a progressive qualitative loss of OPN mRNA as animal age increased. Youngest mice expressed equivalent OPN mRNA over all laser-microdissected cartilage zones. OPN expression in 7-11 day-old mice was greatest in resting and lowest in hypertrophic cartilage. Corresponding histology revealed mineral over only hypertrophic cartilage regions of tibiae beginning with 7 day-old mice. OPN expression correlated with mineral appearance in the tissue suggests that OPN functions to inhibit growth plate mineralization and its loss with increasing tissue maturation would be permissive to mineral onset and development.

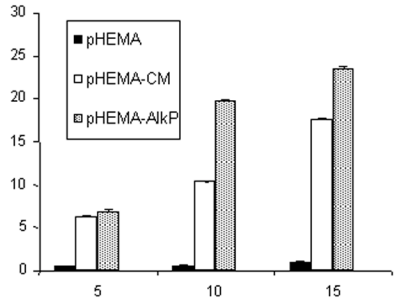
SA024

See Friday Plenary number F024.

SA025

Effect of Charged Groups (Carboxymethyl) or Alkaline Phosphatase on the Calcification of Poly (2-Hydroxyethyl) Methacrylate. R. Filmon,* F. Grizon,* M.F. Basle,* D. Chappard. LHEA, Fac Medicine, Angers, France

Poly(2-hydroxyethyl) methacrylate (pHEMA) has been extensively used as a biomaterial with potentially wide applications. The polymer possess numerous properties: it is highly biocompatible and allows the immobilization of cells or bioactives molecules (e.g., enzymes). When obtained by bulked-polymerization in water-free conditions, pHEMA has an hardness comparable to bone. We have found that immobilization of alkaline phosphatase (AlkP) in the pHEMA can initiate mineralization inside and outside the polymer in a manner that mimics the calcification process of cartilage and woven bone. Because numerous proteins known to initiate bone mineralization possess numerous -COOH species, we have modified the neutral electric surface of pHEMA by carboxy-methylation to study the effect of the negatively charged groups on the calcification process in an in vitro assay. pHEMA was prepared by redox polymerisation with benzoyl peroxide and N-N dimethyl paratoluidine. Calibrated pellets of pHEMA were obtained with a great regularity. Carboxymethylation of the pellets was done by reacting with 1M bromoacetic acid in 2M NaOH solution overnight at room temperature under gentle agitation. Pellets of pHEMA, pHEMA-AlkP and carboxymethylated polymer (pHEMA-CM) were incubated during 5, 10 and 15 days in two types of body fluid. A normal concentration of ions (1X) and a 1.5X concentration were used. Pellets were treated in HCl to completely dissolve the hydroxylapatite crystals and Ca and P were dosed. Carboxymethylation significantly increased the amount of deposited Ca by 1.8 folds in the 1X fluid and 15.8 folds in the 1.5X fluid. Presence of AlkP increased considerably the amount of deposited Ca: 25.9 folds in 1X and 23.3 in 1.5X. ROS 17/2.8 osteoblast-like cells were seeded on the three materials and examined by confocal microscopy after staining with Alexa Fluor 488 phalloidin. Cells grown on pHEMA alone appeared round while cells grown on the crystals deposited on the pHEMA-CM or pHEMA-AlkP were flattened. The presence of AlkP favours the mineralisation process more than the existence of surface negative groups on the polymer. Cells preferentially adhere to the polymer when hydroxylapatite crystals have developed.



SA026

High Concentrations of Urea Alters the Normal Patterns of Chondrogenesis of Limb Mesenchyme In Vitro. C. V. Andrade,*[1] M. A. Mello,[1] M. E. L. Duarte.[2] [1]Universidade Federal Fluminense, Rio de Janeiro, Brazil, [2]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Growth retardation secondary to chronic renal failure has a complex and multifactorial pathogenesis. Basically the extent of growth is determined by endochondral bone formation. Thus, the investigation of diminished linear growth in children with renal failure must include the epiphyseal growth plate. The present study was undertaken to test the possibility that in high concentrations, urea influences the proliferation and maturation of chondrocytes and therefore the determination of bone growth rates. We have used the embryonic limb mesenchyme micromass culture system to investigate the influence of high concentrations of urea on chondrocyte proliferation and maturation *in vitro*. The cells were cultured in DMEM/F12 medium containing 10% FCS and 20-200mg/dl of urea. At regular time points cultures were harvested for posterior analysis. The proliferative potential of the cells were assessed by proliferating cell nuclear antigen (PCNA) immunostaining and by [^{3}H] thymidine uptake. The maturation process was investigated by cell diameter and ECM amount measured microscopically, and by alkaline phosphatase activity. Programmed cell death was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL). In cultures treated with 200mg/dl of urea, PCNA immunoreactivity was detected only in a smalll number of cells and became negative with time in culture. [^{3}H] thymidine uptake was significantly lower ($p<0.01$) in all time points as well as alkaline phosphatase activity. In high concentrations of urea (≥100mg/dl) the cells were smaller ($p<0.001$) and synthetized a lesser amount ($p<0.01$) of extracelullar matrix. Apoptosis was detected as early as day 7 in a high percentage of the cells. Data from the present study have suggested that urea induces a concentration-dependent reduction of cell number accompanied by decreased ECM synthesis. The decrease in cell number results from inhibition of cell proliferation and from increased cell death by apoptosis.

SA027

See Friday Plenary number F027.

SA028

Evidence for the Sequential Cleavage of Aggrecan in IL-1 Stimulated Bovine Articular Cartilage. S. M. Blake,[1] S. M. Hwang,*[2] B. Swift,*[2] A. M. Badger,*[2] T. Newman-Tarr,*[2] B. Bradley,*[2] I. E. James,[2] M. Gowen,[2] S. Kumar,[2] M. W. Lark.[2] [1]Muscoloskeltal Diseases, GSK Pharmaceuticals, King of Prussia, PA, USA, [2]GSK, King of Prussia, USA.

The ability of the large proteoglycan aggrecan to swell and hydrate the collagen fibrils in articular cartilage is central to its function of conferring (or imparting) compressibility and resilience to this tissue. Loss of aggrecan can therefore lead to cartilage degradation and, eventually, loss of joint function as exemplified pathologically by osteoarthritis. Two aggrecanase (1 and 2) enzymes, have recently been cloned. These enzymes are members of the A Disintegrin And Metalloprotease with ThromboSpondin motifs (ADAMTS) family of enzymes and are now referred to as ADAMTS-4 and -5. At least five specific sites, one within the interglobular domain (IGD) and four within the chondroitin sulphate (CS) rich domain of purified bovine aggrecan have been shown to be cleaved by recombinant ADAMTS4. It is still unclear if either ADAMTS 4 or 5, have the ability to generate these fragments in intact native cartilage. In the present study we have used a model system of interleukin-1 (IL-1) mediated matrix degradation of bovine articular cartilage (BAC) to further define the process of aggrecanase mediated aggrecanolysis. Anti-sera generated against two of the proposed CS rich domain cleavage sites as well as the sites within the IGD were used to follow the generation of these neoepitopes remaining within or released from the native articular cartilage by immunocytochemical, Western blot and histochemical techniques. Sections from BAC stimulated with IL-1 for 7 days resulted in an increased staining for the CS rich domain and IGD cleavage sites within the cartilage matrix compared to the unstimulated controls. Serial sections stained with safranin O to detect glycosaminoglycan (GAG) revealed that in the matrix in which GAG content remained high, only CS rich domain cleavage neoepitopes were detectable. Areas of matrix devoid of GAG were strongly immunopositive for the IGD neoepitope. Extending the period of IL-1 stimulation to 18 days resulted in loss of the neoepitopes for the CS rich domain cleavage sites. The intensity of staining for the IGD domain neoepitope was also diminished. Taken together these results suggest that IL-1 induced aggrecanolysis in cartilage occurs sequentially, originating in the CS rich domain of aggrecan prior to cleavage in the IGD domain.

Disclosures: GlaxoSmithKline Pharmaceuticals,3.

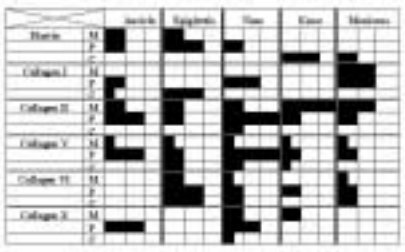
SA029

See Friday Plenary number F029.

SA030

Characterization of Cartilage Sub-types in the Rabbit. J. E. Dennis,[1] A. Naumann,*[2] A. Awadallah,*[3] A. I. Caplan.*[3] [1]Department of Biology, Case Western Reserve University, Cleveland, OH, USA, [2]Department of Otorhinolaryngology, Head and Neck Surgery, Ludwig-Maximilians-University, Munich, Germany, [3]Department of Biology, Case Western Reserve University, Cleveland, OH, USA.

The purpose of this study is to characterize the elastic, hyaline, and fibrous sub-types of cartilage from rabbit by immunochemical localization of extracellular matrix components, biomechanical testing, and glycosaminoglycan (GAG) content. Native cartilage was obtained from several 4-month-old male White New Zealand rabbits. Hyaline (nasal septum, articular), elastic (auricle, epiglottis), and fibrocartilage (meniscus) were removed and either fixed in 10% neutral buffered formalin for immunochemistry or placed in 0.1 M sodium chloride plus protease inhibitors for mechanical testing. For immunohistochemical staining, sections were incubated in 1 mg/ml pronase in PBS and immunostained with antibodies to collagen types I, II, V, VI, X, and elastin. Total GAG content was assayed by colorimetric intensity of saffranin-o, as previously described (1). The intrinsic material properties (aggregate modulus, Poisson's ratio, and permeability) of the different cartilage subtypes were derived from an indentation assay (2)Immunohistochemical staining revealed distinctive patterns of staining for each of the cartilage sub-types. Table I summarizes the intensity of staining within three sub-zones of the matrix: the matrix proper, the pericellular region, and the cellular region. GAG content assays showed that articular and nasal cartilage contain nearly 4 times the amount of GAGs than auricle and epiglottis; meniscus was intermediate in GAG content. The biomechanical testing showed significant differences in material stiffness (aggregate modulus) between cartilage sub-types, with auricle and nose cartilage having similarly high stiffness followed by articular, epiglottis and meniscus. These studies document that distinctive subtypes of cartilage can be identified by immunohistochemistry, and provides a basis for asking whether transplanted chondrocytes change their matrix and mechanical properties to match the transplant environment or retain the properties of the tissue of origin. (1) Carrino, D.A., Arias, J.L., Caplan, A.I. 1991. Biochem. Int. 24, 485-495; (2) Mak, A.F., Lai, W.M., Mow, V.C. 1987. J Biomech 20, 703-714.



SA031

See Friday Plenary number F031.

SA032

A Metabolite of Estrogen, 2-Methoxyestradiol, Suppresses Proliferation and Induces Apoptosis in Rat Growth Plate. J. D. Sibonga, U. Sommer,* R. T. Turner. Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

2-Methoxyestradiol ($2ME_2$), a metabolite of 17ß-estradiol, antagonizes both angiogenesis and induces apoptosis in some tumor cell lines. Suppressive effects on bone longitudinal growth have been previously reported in young rats. We examined the cellular response of growth plate chondrocytes to $2ME_2$ in ovariectomized rats. In a 21-day dose response study (0, 4, 20 and 75 mg/kg), $2ME_2$ was administered orally to recently ovariectomized rats at 10 weeks of age. Five untreated control rats and 5 rats each from higher-dosed $2ME_2$-treated groups (20 and 75 mg/kg/d) were pulsed-labeled with tritiated thymidine 2 hrs before sacrifice. Chondrocyte proliferation was evaluated with radioautography. Apoptosis-induced DNA fragmentation was detected by TUNEL assay. Longitudinal growth rates were determined with fluorochrome labeling. $2ME_2$ reduced the height of the growth plate ($p<0.05$). $2ME_2$ suppressed bone elongation ($p<0.05$), decreased the number of proliferating chondrocytes labeled with tritiated thymidine ($p<0.05$), and increased the percentage of hypertrophic chondrocytes that were apoptotic ($p<0.05$). An additional 2 x 2 experiment with weanling rats treated for one week with ± $2ME_2$ (4 mg/kg/d) or ± ICI 182,780 (1.5 mg/kg) indicated that ICI 182,780 had effects consistent with estrogen antagonism but did not influence the skeletal response to $2ME_2$. Furthermore, the one-week treatment with $2ME_2$ had no effect on the size of hypertrophic chondrocytes in weanling rats suggesting that $2ME_2$ does not influence the production of cartilage matrix. We conclude that $2ME_2$ 1) reduces the number of growth plate chondrocytes by suppressing proliferation and accelerating apoptosis, 2) has no impact on the matrix production by hypertrophic chondrocytes and 3) induces these actions through a non-estrogen receptor pathway.

SA033

See Friday Plenary number F033.

SA034

Identification and Tissue-Specific Expression of Connective Tissue Genes that are Upregulated in Postnatal Chondroinduced Human Dermal Fibroblasts. K. E. Yates,[1] J. Glowacki.[2] [1]Skeletal Biology Research Center, Massachusetts General Hospital, Charlestown, MA, USA, [2]Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA.

Demineralized bone powder (DBP) induces postnatal endochondral bone formation in vivo and is now widely used for skeletal repair and reconstruction. We developed a novel chondroinduction system [Exp Cell Res 227:89, 1996] in which normal human dermal fibroblasts (hDFs) develop a chondrocyte phenotype (collagen type II, aggrecan) after 7 days in culture with DBP [Mat Sci Eng C6:199, 1998]. We used representational difference analysis, a PCR-based method of subtractive hybridization, to identify genes whose expression was altered by cellular interaction with DBP on day 3. Several functional families of genes (extracellular and cytoskeletal elements, protein synthesis/trafficking) were Upregulated early in chondroinduction [Exp Cell Res 265:203, 2001].In this study, we identified additional upregulated genes and surveyed their expression in adult tissues. Differentially expressed cDNAs were used to design and validate PCR primers. We prepared total RNA from human skin, articular chondrocytes isolated from osteoarthritic cartilage, cancellous bone, and bone marrow low-density mononuclear cells. RNA was treated with DNAse I and used in random hexamer-primed cDNA synthesis reactions for RT-PCR. Among the genes that were Upregulated in chondroinduced hDFs were several connective tissue genes that encode collagens (COL11A1, COL3A1, COL6A3), collagen receptors (α-11 integrin, β-1 integrin), and post-translational enzymes (lysyl hydroxylase 2, lysyl oxidase, lysyl oxidase-like protein 2). All of those genes were expressed in articular chondrocytes. In contrast, only a subset was expressed in skin (Table). Thus, several connective tissue genes identified as Upregulated in chondroinduced hDFs were expressed in cartilage but not in skin.

Gene	Skin	Chrondrocytes	Bone	Marrow
α-11 integrin	-	+	-	+
COL11A1	-	+	+	-
Lysyl hydroxlyase 2	-	+	+	+
Lysl oxidase	+	+	+	+
Lysyl oxidase-like protein 2	+	+	+	+
COL3A1	+	+	+	-
COL6A3	+	+	+	+
β-1 integrin	+	+	+	+

These data indicate that specific gene shifts in functional families occurred prior to full expression of cartilage-specific matrix genes and synthesis of extracellular matrix. Taken together, specific changes in architectural, matrix and functional genes characterize early events in chondroinduction. These changes may create an environment supportive of postnatal chondrogenesis. Such signals may be necessary because normal postnatal tissues lack the regulatory signals found in programmed milieux of embryonic tissues.

SA035

See Friday Plenary number F035.

SA036

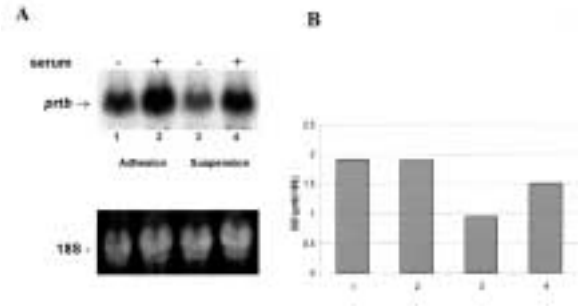
See Friday Plenary number F036

SA037

Expression and Regulation of Proline-rich Transcript of the Brain (prtb) in Osteoblasts. D. W. Sommerfeldt,[1] J. Zhi,*[2] C. T. Rubin,[3] M. Hadjiargyrou.[3] [1]Department of Trauma and Reconstructive Surgery, Hamburg University Hospital, Hamburg, Germany, [2]Center for Biotechnology, State University of New York at Stony Brook, Stony Brook, NY, USA, [3]Department of Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, NY, USA.

To characterize the temporal expression of genes that play a functional role during the process of osteoblast adhesion we used differential display (DD-PCR) on mRNA from attached vs. suspended osteoblasts. A 200-bp fragment showing upregulation after 30 min and 60 min adhesion, was sequenced and showed 97% homology to prtb, a transcript previously only known to be expressed in mouse brain. Northern analysis confirmed a two-fold increase in prtb message during adhesion to tissue culture polystyrene, both in the presence and absence of surface-adsorbed serum proteins. Serum stimulation alone was also able to induce prtb expression, although to a lesser extent (1.5-fold), in cells in forced suspension (see figure below). In a tissue blot experiment strong expression in brain and

bone of adult rats could be detected. Furthermore, prtb expression analysis during osteoblast differentiation revealed high transcription levels independent of proliferation (day 0-7), matrix maturation (day 7-14), and mineralization (day 14-31). Time course analysis of prtb expression during adhesion of sensitized osteoblasts to serum-protein coated surfaces showed detectable mRNA at 5 min post plating and a peak at 10 min. Comparison with the two known serum-inducible immediate early genes c-fos and c-jun showed similar kinetics, with c-jun expression peaking at 15 min and c-fos expression at 20 min.Taken together, these data lead to the hypothesis of prtb functioning as an immediate early, serum-responsive and adhesion-inducible gene and are consistent with a possible involvement in processes such as cell cycle control, proliferation and adhesion.



SA038

See Friday Plenary number F038.

SA039

The Human Extracellular Matrix Signal Molecule hCYR61 Expression is Associated with Conditions of Enhanced Bone Formation and with the Proliferative State of Osteoblasts. N. Schuetze,*[1] J. A. Hoyland,*[2] H. Siggelkow,[3] J. Pfeufer,*[4] C. Hendrich,*[4] J. Eulert,*[4] F. Jakob.*[5] [1]Labor fuer Molekulare Experimentelle Orthopaedie, Orthopaedische Universitaetsklinik, Wuerzburg, Germany, [2]Musculoskeletal Research Group, School of Medicine, Manchester, United Kingdom, [3]Schwerpunkt Endokrinologie, Universitaetsklinik Goettingen, Goettingen, Germany, [4]Orthopaedische Universitaetsklinik, Wuerzburg, Germany, [5]Medizinische Poliklinik, Wuerzburg, Germany

The human cystein-rich protein 61 (hCYR61, CCN1) belongs to a gene family (CCN family) whith plays a role in growth and differentiation, migration, adhesion, wound healing and angiogenesis. Previously, we showed that hCYR61 in human osteoblasts is regulated by 1,25(OH)2-vitamin D3 and growth factors at the mRNA and the protein level. The protein functions as an extracellular signaling molecule. This study was aimed to analyze the hCYR61 expression in human bone samples and in corresponding in vitro systems. Using a 1.5 kB cRNA probe to hCYR61 in in situ hybridization normal bone did not show expression of hCYR61 mRNA. In contrast human bone samples from fracture callus displayed expression of hCYR61 in osteoblasts and mesenchymal cells at sites of new bone formation. Applying immunohistochemistry with a polyclonal anti mouse CYR61 antiserum which crossreacts with the human protein, normal bone and cartilage samples did not show detectable hCYR61 protein. Again samples from fracture callus displayed highly positive staining at chondrocytes, mesenchymal cells and osteoblasts. In addition samples from heterotopic ossifications revealed positive staining on surfaces of mineralized structures. Samples from a human growth plate from a digitus of a 2 year old child indicated high hCYR61 expression in hypertrophic chondrocytes as was controlled by collagen X staining. Using hFOB-cells and primary osteoblasts of different maturation stages a high expression of the hCYR61 protein in proliferating cells was observed whereas differentiated cells revealed much lower signal intensity. In contrast, human chondrocyte cell lines T/AC62, T/C-28a and C-28/I2 displayed a high expression at the mRNA and protein level.hCYR61 expression was observed in situations of enhanced bone turnover/bone formation. Particularly, the expression of hCYR61 in fracture callus points towards a role during fracture repair. hCYR61 could be involved in endochondral ossification as is suggested by the expression at the human growth plate. The expression of hCYR61 in the proliferating stage of osteoblasts could indicate a role in amplifying precursors for bone formation. Thus hCYR61 in human bone combines angiogenic as well as osteogenic activities.

SA040

See Friday Plenary number F040.

SA041

In Situ Analysis of Aggrecanase 1 and 2 Expression in Adult Human Normal and Osteoarthritic Articular Cartilage. J. R. Connor,[1] S. Blake,[2] S. M. Hwang,*[2] M. Gowen,[2] M. W. Lark,[2] S. Kumar.[2] [1]Musculoskeletal Diseases, GlaxoSmithKline, King of Prussia, USA, [2]GlaxoSmithKline, King of Prussia, USA.

The degradation and loss of the proteoglycan, aggrecan, one of the hallmarks of osteoarthritis (OA), results in cartilage degeneration and loss of joint function. The evidence for aggrecanase activity in human cartilage has been demonstrated with the detection of metabolic fragments of aggrecan (1). Recently, two members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) protein family,ADAMTS4(aggrecanase 1) and ADAMTS5 (aggrecanase 2) were cloned and characterized (2,3). This study examined the in situ expression patterns of aggrecanase 1 and 2 mRNA and protein in adult human normal and OA articular cartilage. For in situ hybridization, cryostat sections of normal or OA cartilage were hybridized with 35S-labelled riboprobes to aggrecanase 1 and 2. For immunohistochemical studies, floating sections of cartilage were stained using antibodies generated against peptides derived from the catalytic domains of aggrecanase 1 and 2. By in situ hybridization analysis, aggrecanase 1 mRNA expression was variable between patient samples with similar stages of OA. Additionally, the level of expression varied between the superficial, mid- and deep zone chondrocytes. Aggrecanase 2 mRNA expression could be detected at high levels in some normal and OA cartilage samples. However, some samples which were positive for the expression of aggrecanase 1 had low levels of aggrecanase 2 mRNA expression. Similarly, by immunohistochemistry, aggrecanase 1 protein was detected at high levels in some samples of OA cartilage, while others expressed low levels of the protein. Additionally, the pattern of expression between the superficial, mid- and deep zones varied between samples. Aggrecanase 2 was detected in some OA cartilage, but was lower in most samples when compared to aggrecanase 1. In summary, aggrecanase 1 and 2 mRNA and protein could be detected in adult human normal and OA articular cartilage. Overall, the expression was highly variable between patient samples of cartilage with similar stages of OA. This apparent lack of coordinated aggrecanase expression has been suggested by previous studies (1). An increased understanding of the regulation and control of aggrecanase expression will provide valuable insights for the development of inhibitors of cartilage degradation in OA.Refs 1)Lark MW et al (1997) J Clin Invest 100, 93-106; 2) Tortorella MD et al (1999) Science 284, 1664-1666; 3) Abbaszade I et al (1999) J Biol Chem 274, 23443-23450.

SA042

See Friday Plenary number F042.

SA043

Heparanase mRNA Expression during Fracture Repair. M. Saijo,*[1] R. Kitzazawa,[1] M. Nakajima,*[2] S. Maeda,*[1] S. Kitazawa.[1] [1]Division of Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan, [2]Novartis Pharma K.K., Research, Tsukuba, Japan

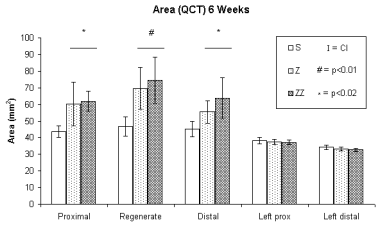
During fracture repair, cartilaginous callus is generated first and then gradually replaced by bony callus. Since angiogenesis is requisite to the recruitment of osteoclasts for the destruction of cartilage, vascular endothelial growth factor (VEGF) is thought to play a role in endochondral ossification. VEGF isoforms ($VEGF_{165}$, $VEGF_{189}$) bind heparan sulfate proteoglycans (HSPG) of the extracellular matrix and are stored in the cartilage and bone matrices. Heparanase is an endoglucuronidase which degrades heparan sulfate and HSPG, and releases heparin-binding growth factors including VEGF. We investigated the mechanism of VEGF recruitment at the boundary of the cartilaginous and bony callus of the fractured bone. After closed fractures were made on the proximal tibia of 12 week-old BALB/c mice, the bones were excised on days 3, 5, 7, 10, 14, 21 and tissue sections were prepared. Blood vessels were assessed by CD34 immunohistochemistry. The localization of VEGF and HSPG was assessed by immunohistochemistry and that of heparanase mRNA by *in situ* hybridization (ISH). The cDNA of mouse heparanase was amplified by RT-PCR, and the digoxigenin-labeled single stranded antisense DNA probe was prepared by unidirectional PCR. Osteoblastic cells of newly formed bone were detected by ISH for osteonectin. Osteoclasts were assessed by TRAP staining. At the initial stage (day 3; hematoma/ inflammatory phase), no signal for heparanase was detected. On day 5 when mesenchymal cells differentiated into chondrocytes and small vessels were formed, a few mature osteoclasts and TRAP-positive precursors expressed heparanase mRNA. On days 7-10 at the endochondral ossification site, VEGF and HSPG were colocalized in hypertrophic chondrocytes. Numerous osteoclasts, resorbing cartilaginous tissue, strongly expressed heparanase mRNA, which was followed by capillary vessel formation and woven bone with osteonectin-positive osteoblasts. On days 14-21 in the woven bone tissue, osteoblasts positive for VEGF and osteoclasts expressing heparanase were observed. These data suggest that VEGF, produced by chondrocytes and stored in HSPG, was released by heparanase from osteoclasts to promote blood vessel formation. Thus osteoclasts may contribute to the promotion of local angiogenesis as well as to the destruction of callus. Mesenchymal cells and osteoclasts, therefore, jointly contribute to local angiogenesis in endochondral ossification during fracture healing.

SA044

Zoledronic Acid Enhances Bone Formation and Mineralization in Distraction Osteogenesis in Immature Rabbits. D. G. Little,*[1] P. R. Williams,*[1] N. C. Smith,*[1] E. J. Smith,[1] J. Briody,*[2] C. T. Cowell,[3] L. E. Bilston.*[4] [1]Orthopaedic Research Unit, Children's Hospital at Westmead, Sydney, Australia, [2]Nuclear Medicine, Children's Hospital at Westmead, Sydney, Australia, [3]Endocrinology, Children's Hospital at Westmead, Sydney, Australia, [4]Mechanical Engineering, University of Sydney, Sydney, Australia

Poor bone formation and stress shielding remain problematic in distraction osteogenesis. We hypothesized that zoledronic acid would address both issues. 42 male 8-week-old NZW rabbits underwent right tibial osteotomy. 14 animals received saline (S), 14 zoledronic acid 0.1mg/kg at surgery (Z), and 14 zoledronic acid 0.1 mg/kg at surgery and two weeks post-op (ZZ). We distracted 10 mm over 2 weeks, followed by 4 weeks consolidation. 30 rabbits had DXA at 2, 4 and 6 weeks for BMC and BMD in 10 mm regions in

regenerate bone and also in the proximal and distal segments. Two equivalent segments were examined in the left (non-operated) leg. QCT was performed at 6 weeks for vBMD and cross sectional area. 4-point bend testing of all tibiae was performed in a standard manner. 12 rabbits underwent histologic analysis at 6 weeks.Bone mineral accretion in the distraction gap of the operated (right) tibia was significantly higher in both Z and ZZ versus S groups between 2 and 4 weeks, and was better maintained at 6 weeks (P<0.01 ANOVA). Stress shielding osteopenia seen in surrounding bone segments in S was abolished in Z/ZZ tibia, with significant increases in BMD and BMC (P<0.01 ANOVA). Cross sectional area increased 49% at 6 weeks in the regenerate in Z, and 59% in ZZ (Fig. 1). We also noted increased cross sectional area in surrounding segments. Z tibiae were 29% stronger in 4-point bending, while ZZ were 89% stronger than S (P<0.01 ANOVA). There was little detectable effect on the non-operated tibiae. Lamellar bone and TRAP positive osteoclasts were present in the regenerate bone at 6 weeks, consistent with active remodelling, despite zoledronic acid administration.



Zoledronic acid administration significantly increased bone area and matrix mineralization in this distraction osteogenesis model. The increases in bone area and retention of mineral despite stress shielding translated into a significant, dose-dependent increase in strength. Further research into the role of zoledronic acid as an adjunctive therapeutic agent in orthopaedic surgery is indicated

Disclosures: Novartis Pharmaceuticals Australia,2.

SA045

The Role of Angiogenesis in the Soft Callus of Long Bone Fracture. J. L. Ford,* D. E. Robinson,* B. E. Scammell. Orthopaedic and Accident Surgery, Nottingham University, Nottingham, United Kingdom

This study was conducted to examine angiogenesis within the cartilage of the soft callus in long bone fractures and to investigate its role in the bone remodelling process.A standardised tibial osteotomy was performed on New Zealand white rabbits, sacrificed at week 1,2,3 and 6 post-osteotomy. Tissue was processed and immunohistochemical techniques were employed to localise vessels using an endothelial cell marker. Basic fibroblastic growth factor (bFGF) was localised as it is known to induce angiogeneisis and initiate proteolysis. Urokinase plasminogen activator (uPA) was immunolocalised to investigate its possible action in the degradation of the cartilage matrix and osteonectin was identified to examine its potential role in the bone remodelling process. A blind arbitrary scoring system was employed to compare the degree of immunoreactivity in different locations within the tissue and at different time points post-osteotomy. Fracture tissue was stained with Sirius red and Alcian blue to examine the bone and cartilage matrix respectively. Electron microscopy was carried out to examine the matrix surrounding the soft callus in more detail.Results revealed that blood vessels formed around and within the soft callus tissue by week 1 post-osteotomy and that vascularisation increased in these areas during the weeks that followed. Osteonectin, bFGF and uPA was localised within the haematoma during the first week and within blood vessels in the following weeks. From week 2 post-osteotomy, uPA was detected in the cartilage of the soft callus and bFGF exhibited a pericellular distribution around the chondrocytes of the soft callus. Histology and TEM revealed that collagen I fibres of the bone matrix formed amongst the chondrocytes at the edge of the soft callus and in areas where cartilage tissue was adjacent to infiltrating vessels. The results indicate that blood vessels newly form in the soft callus as a result of injury and that angiogenesis plays a crucial role in the removal of the cartilage matrix and the formation of new bone in this area. The observed presence of bFGF and uPA in vessels strongly suggests that they are involved in the initiation of angiogenesis and proteolytic activity within the soft callus. Following angiogenesis, bone-producing cells appeared to initiate the production of bone matrix at the edge of the soft callus, infiltrating the cartilage tissue, thus eventually replacing the soft callus with bone matrix. The results presented indicate that angiogenesis within the soft callus is a physiological response to fracture and is likely to play a vital role in the healing process of long bone fractures.

SA046

See Friday Plenary number F046.

SA047

Observation on Interstitial Fluid Flow in the Lacunar-Canalicular Network. S. Qiu, S. Palnitkar,* D. Rao. Bone and Mineral, Henry Ford Hospital, Detroit, MI, USA.

Bone fluid flow plays a fundamental role in the maintenance of osteocyte viability and the regulation of bone modeling and remodeling. To date, there is little empirical data for evaluating the characteristics of bone fluid flow due to lack of appropriate model. In this study, we developed a new methodology to assess interstitial fluid flow in the lacunar-canalicular spaces. Two fluorescent tracers, Evens blue (red) and isothiocyanate (FITC)-dextran (blue), were perfused intravenously in 12 rats and the tibial diaphyses were examined using confocal microscopy. Three different methods (3, 3 and 6 rats in each group) were used to determine the characteristics of fluid flow in the lacunar-canalicular network.1) FITC-dextran was perfused 1 hour before sacrifice and then the bone section was placed in basic fuchsin (red) for 24 hours, which was able to stain the entire lacunar-canalicular network. The lacunae stained with basic fuchsin were all overlapped by those labeled with FITC-dextran. 2) Both tracers were perfused simultaneously 1 hour before sacrifice. The lacunae with different tracers were completely overlapped, but the intensity of their fluorescence was not uniform in the entire lacunar-canalicular network.3) Two tracers were perfused at different times with one hour interval. The rats were sacrificed 1 minute after second perfusion. The rate of fluid flow was calculated by the percentage of overlapped lacunae labeled with different tracers. In this model, the tracer perfused 1 min before sacrifice occupied about 60% of the total lacunae labeled by the first perfusion. Compared with the periosteal side, the majority of lacunae containing second perfused tracer were located in the endosteal side (Fig).The results suggest: 1) one hour is long enough for tracers to penetrate into the entire lacunar-canalicular network, which can be used as control; 2) osteocytes can receive new fluid supply within 2 minutes and 3) the pattern of fluorescent intensities suggests that the interstitial fluid in the lacunar-canalicular network is provided by intermittent bulk volume infusion rather than continues diffusion

SA048

Reciprocal Interaction Between Human Endothelial Cells and Human Bone Marrow Stromal Cells Seems to be Mediated by the Production of Nitric Oxide. B. Guillotin, F. Villars, R. Bareille, L. Bordenave, J. Amedee. U443-INSERM Université Victor Segalen Bordeaux 2, Bordeaux, France

Recent studies revealed that vascular endothelial cells may be pivotal members of complex interactive communication network in bone with the others cell types. Our previous data demonstrated that Human Umbilical Vein Endothelial Cells (HUVEC) and Human Bone marrow Stromal cells (HBMSC) are coupled and that connexin43 may be involved in this heterotypic interaction between these two cell types. This coupling between HUVEC and HBMSC seems to be responsible of the increase of the osteoblastic phenotype (alkaline phosphatase activity, type I collagen synthesis) observed in HBMSC co-cultured with HUVEC. The aim of our study is to explore the signal transduction pathways involved in this cellular interaction. HBMSC were cultured alone in IMDM+10% FCS or with direct contact with HUVEC for 6 days in presence or not with inhibitors of NO synthase (NOS) (5-20µM aminoethylsothiouronium-bromide AET), guanylate cyclase (10µM NS 2028) or Phospholipase C (20µM U73122). These selected concentrations were choosen for their absence of cytotoxicity for HUVEC and HBMSC measured by MTT assays. Cell differentiation was evaluated by the measurement of alkaline phosphatase activity. Using RT-PCR analysis we have investigated the presence of isoforms of NOS (eNOS or iNOS) in both cell types. Among the different targets (phospholipase C, GMPcyclase, NO synthase) reached in our experiments, NO production in these cocultures could play a fonction in the enhancement of the osteoblastic phenotype of HBMSC observed only in direct contact with HUVEC. AET treatment of the coculture at 5,10 or 20µM for 6 days totaly abolished the increase of alkaline phosphatase activity observed in coculture with HUVEC, while the same treatment of HBMSC cultured alone did not modify the enzymatic activity. In conclusion, HUVEC could be considered as a NO donors such as sodium nitroprusside (SNP) tested in our coculture at 100 µM. The next step of our work will consist to establish the relation between the function of connexin43 in this coupling and the production of NO able to travel rapidly between cells through the membrane including perhaps gap jonctions. Others biological mediators involved in this transduction pathways do not be excluded from these investigations.

SA049

See Friday Plenary number F049.

SA050

Isolation and Characterization of Preosteoblastic Cells from Human Long Bone Marrow. L. X. Bi, D. Yngve,* W. L. Buford,* E. Mainous.* The University of Texas Medical Branch, Galveston, TX, USA.

Long bone marrow contains stromal mesenchymal cells that are capable of differentiating into osteoblast cells. Our previous study demonstrated that bone marrow stromal cells comprise several physiologic populations which present a continuum of growth, osteogenic activity. To investigate the osteoblast precursor cells, we have isolated preosteoblasts (marrow sac cells) from the out surface of bone marrow of femoral bone and examined expression of bone morphogenetic protein-2 (BMP-2) and osteocalcin immunocytochemically, and alkaline phosphatase (ALP) histochemically. The human preosteoblast cells were cultured in a-minimum essential medium (a-MEM) and mineralizing medium (a-MEM, Dexamethasone (10^{-8}M), beta-glycerol phosphate (10mM) and ascorbic acid (50ug/ml)) containing 10% FBS for 7 days. Examinations for ALP and BMP-2 (anti-rhBMP-2 monoclonal antibody) and osteocalcin (monoclonal anti-osteocalcin antibody) were performed using commercial kit (Sigma Chemical Co., St. Luis, MO and VECTEASTAIN *Elite* ABC kit Vector Laboratories, Inc. Burlingame, CA). In control medium, the BMP-2 and osteocalcin were detectable in osteoblast precursor cells. The cells were moderately stained for ALP. In mineralizing medium, the expression of ALP was enhanced. The intensity of immunostaining for BMP-2 was significantly increased, for osteocalcin was unchanged, compared to that in a-MEM medium. Taken together, the results indicated that the preosteoblast cells could respond to mineralizing substances to differentiate into osteoblasts by increasing expression of some osteoblastic markers. In conclusion, we have established a new method for isolating and characterizing osteoblast precursor cells which reside proximate to endosteal osteoblasts from long bone marrow. These cells can be induced to differ-

entiate into cells exhibiting the osteoblast phenotype. They could be considered to be an osteogenitor pool from which replacement osteoblasts are normally recruited in vivo. This method provides a new tool to study the regulation of normal bone formation and bone diseases (osteoporosis , osteopenia etc.).

SA051

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SA052

Selective Induction of Opn Expression in Osteoblasts Is Mediated by Different Integrin Ligands. R. S. Carvalho,*[1] P. J. Kostenuik,*[2] A. Bumann,*[3] E. Salih,*[4] L. C. Gerstenfeld.[5] [1]Orthopedic Surgery, Boston University, Boston, MA, USA, [2]Amgen, Thousand Oaks, CA, USA, [3]University of Southern California, Los Angeles, CA, USA, [4]Children's Hospital, Boston, MA, USA, [5]Boston University, Boston, MA, USA.

Bone remodeling is partly regulated by the mechanical environment of skeletal cells. However, some forms of signals derived from mechanical stimulation are only perceived by cells after adhesion has taken place. Therefore, signal transduction and gene expression events activated by the latter maybe similar, if not identical, to those induced by integrin-mediated cell adhesion. Recent studies have shown that osteopontin (OPN) mediates osteoblastic and osteoclastic interaction with the mineralized matrix. Thus, OPN is both associated with remodeling and formation. These studies examined whether osteoblasts discriminate cellular interactions with ECM ligands to mediate opn mRNA expression. Embryonic chicken calvaria osteoblasts were plated using identical cell numbers in plates coated with fibronectin (FN) (1mg/ml), collagen type I (ColI)(1mg/ml), denatured collagen type I (gelatin-G)(1mg/ml), osteopontin (OPN)(1mg/ml), vitronectin (VN)(1mg/ml), laminin (LN)(1mg/ml) or poly-L-lysine (pLp). Subsequently, a determination of the intracellular second signaling systems (protein kinases A and C) that are responsible for mediating the opn gene expression to cellular adhesion were determined. Finally, we evaluated the intracellular distribution of focal adhesion kinase (FAK), p-tyrosine (PT) and vinculin (VN) on surfaces coated with FN or pLp. Results indicated that FN generated the strongest induction of opn expression followed by Col1 and LN. While G was weakly inducing, neither OPN, pLp or VN was capable of inducing opn mRNA expression, suggesting that the latter molecules did not facilitate specific cell adhesion. Induction of PKA and PKC occurred concomitantly only in osteoblasts from FN-coated dishes, while the other ligands promoted PKA solely. Thus, specific effects on kinase activities appear to be dependent on the selective ligand interactions, which are distinct from that of cell adhesion alone. Similarly, osteoblast adhesion did not appear to alter the general intracellular localization of FAC or VN. By contrast, a strong induction was noted for PT with generalized changes of its distribution throughout the cells after adhesion was promoted with a specific integrin binding ligand. Taken together with the opn-mediated gene expression results, these data suggest that induction of intracellular second signal kinase activity are related to the specific nature of the ligand's interactions with the receptor and less with the process of cellular-mediated adhesion alone.

SA053

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SA054

Increased IGF Binding Protein-5 (IGFBP-5) in Extracts of Cancellous Bone Matrix in End-Stage Osteoarthritis. C. A. Sharp,[1] S. J. Brown,*[1] P. Magnusson,[2] M. W. J. Davie,[1] S. Mohan.[2] [1]Charles Salt Centre, RJ&AH Orthopaedic Hospital, Oswestry, United Kingdom, [2]Jerry L Pettis VA Medical Center, Loma Linda, CA, USA.

End-stage osteoarthritis (OA) in the femoral head (FH) is associated with increased matrix turnover and architectural changes to cancellous bone. Of the molecular signals contributing to skeletal changes in OA, we considered IGFs as potential candidates based on their known actions and abundance in bone. IGF actions are modulated by IGF binding proteins (IGFBP). IGFBP-5, the most abundant in bone, fixes IGFs to the bone matrix and is itself a growth factor that acts independently of IGFs. We hypothesized that bone changes in OA FHs are mediated, in part, by increased production of IGFs and/or IGFBP-5. To examine this we have measured IGF-I, IGF-II and IGFBP-5 in extracts of FH cancellous bone powders. FHs were obtained at autopsy from elderly subjects with no bone disease (n=10) and after hip arthroplasty for OA (n=22). Since IGFs in bone are influenced by mechanical loading, bone was taken from 2 FH sites, the Superior (S) and Inferior (I) regions, which experience different loads. IGF system components were measured by RIA after extraction from bone with EDTA in 4M GnHCl and protease inhibitors. Data are expressed as median (range), ng/mg dry bone powder. Comparisons of groups were made using the Mann Whitney test * denotes p<0.05.

	IGF-I		IGF-II		IGFBP-5	
	S	I	S	I	S	I
Normal	0.05 (0.02-0.11)	0.06 (0.03-0.09)	0.21 (0.05-0.41)	0.21 (0.12-0.34)	0.73 (0.14-1.1)	0.98 (0.18-1.9)
OA	0.08 (0.02-0.24)	0.06 (0.02-0.18)	0.22 (0.11-0.32)	0.19 (0.09-0.32)	2.23* (0.7-4.99)	1.59* (0.7-3.19)

Extracts of cancellous bone contain about 3-fold more IGF-II than IGF-I. No sig. differences in levels of any of the components were found between S and I regions in either group. IGFBP-5 was increased in bone from both regions in OA (S p=0.0001; I p=0.009) compared with normals, and remained sig. when results were standardized using extractable protein as a referent. In conclusion, IGFBP-5 levels were significantly higher in extracts of cancellous bone from OA compared with age-selected normals. These findings are consistant with the hypothesis that increased IGFBP-5 production may contribute to changes in the cancellous bone network seen in end-stage OA through increased osteoblast proliferation and/or activity.

SA055

See Friday Plenary number F055.

SA056

Perturbation of Fibronectin Inhibits Osteogenesis in Rat Cranial Suture. A. M. Moursi, P. L. Winnard,* A. V. Winnard.* Pediatric Dentistry, Ohio State University, Columbus, OH, USA.

Craniosynostoses are a group of congenital disorders which involve the premature fusion of the cranial sutures. A therapeutic agent which could prevent re-ossification of the excised sutures by transiently blocking osteogenesis would be an effective adjunct to surgery. Fibronectin (FN), an extracellular matrix molecule, has been shown to play an essential role in calvarial osteoblast osteogenesis. Antagonism of FN with anti-FN antibodies has been shown to selectively and reversibly block osteogenesis in rat calvarial osteoblasts. The purpose of this study was to determine if an anti-FN antibody (anti-FN Ab) could prevent osteogenesis in a rat cranial suture organ culture. The effect of anti-FN Ab was first evaluated in cell culture where fetal rat calvarial osteoblasts were plated on a 1 mm thick resorbable fibrillar collagen gel (Col), 65 mg/ml (NeuColl Inc., Palo Alto, CA), containing anti-FN Ab or IgG at dilutions of 1:40, 1;100 and 1:200. The media was changed every other day, no new anti-FN Ab or IgG was added. Anti-FN Ab released into the media was measured by ELISA. Cell attachment on plastic, Col+anti-FN Ab and Col+IgG was found to be similar at all dilutions, as determined by the alamarBlue dye technique. Proliferation was similar for all conditions at 1:100 and 1:200 dilutions. However, after 3 and 5 days in culture osteoblast proliferation was reduced by 79% and 88%, respectively, on Col+anti-FN Ab (1:40) compared to Col+IgG (1:40). Both Col conditions demonstrated less proliferation than plastic controls. Toluidine-blue staining of fixed cultures confirmed the proliferation data and showed that cells on Col achieved an osteoblastic morphology. The affect of FN perturbation on cranial suture fusion was investigated using a rat calvarial organ culture system in which postnatal day 15 rat calvariae were placed in serum-free, BGJ media. Col+anti-FN Ab (1:40) or Col+IgG (1:40) was placed sub-periosteally on the posterior frontal suture. After 5, 7 and 15 days in culture, histomorphometric analysis showed a decrease of 50%, 72% and 53%, respectively, in the fusion of sutures treated with Col+anti-FN Ab compared to Col+IgG. Together, these results indicate that the Col delivery vehicle can support osteoblast attachment, proliferation and promote normal morphology. They also demonstrate that the Col delivery vehicle can transfer the active antibody to cranial suture sites over time. In addition, the results show that Col+anti-FN Ab can inhibit fusion of rat cranial sutures suggesting potential clinical applications in the treatment of craniosynostosis. Col provided by NeuColl Inc. Supported by NIH grant 5RO3 AR46382-02.

SA057

See Friday Plenary number F057.

SA058

Morphological and Biophysic Study on the Effects of Disuse on Rat Femur Following Sciatic Nerve Neurectomy. H. Yonezu,*[1] S. Takata,[2] N. Yasui.*[2] [1]Orthopedic Surgery, Oe Kyodo Hospital, Oe-gun, Japan, [2]Orthopedic Surgery, The University of Tokushima, Tokushima, Japan

We studied the morphological and biophysic effects of disuse on rat femur following unilateral sciatic nerve neurectomy (USN) using peripheral quantitative computed tomography (pQCT), Fourier transformed infrared spectroscopy (FTIR) and calorimetric analysis in 15 growing Wistar-derived rats, 5 weeks of age. The rats were divided into two groups: operated group (right femur) and non-operated group (left femur). Bone mineral density (BMD) , bone mineral content (BMC), bone area, periosteal circumference, and endosteal circumference were measured by pQCT, and the mineral /matrix ratio was evaluated by FTIR. Furthermore, as for calorimetric experiment, the denatured temperature (Tm), half peak breadth (HPB) and transition temperature (TT) (mj/mg)of type I collagen of the femoral shaft were measured by calorimetry. The operated group showed a significant decrease in cortical BMC, bone area and periosteal circumference compared with the non-operated group ($p<0.05$). The cortical BMD did not vary significantly between the two groups. In the cancellous bone, the operated group showed a significant decrease in BMD and BMC at the metaphysis compared with the non-operated group ($p<0.05$). The mineral/matrix ratio of the cortical bone did no differ significantly between the operated and non-operated groups. The Tm and TT of the operated group were significantly higher than those of the non-operated group ($p<0.05$), whereas the HPB of the operated group was significantly lower than that of the non-operated group. We conclude that in cortical bone, disuse affected by USN inhibits periosteal bone formation but has no significant effect on the mineral/matrix ratio of cortical bone in femurs, and that disuse increases chemical stability of type I collagen.

SA059

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SA060

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SA061

Enhancing Detection Sensitivity of Skeletal Metastases with Green Fluorescent Protein. J. F. Harms,*[1] A. M. Mastro,*[2] C. V. Gay,[2] D. R. Welch.[3] [1]Jake Gittlen Cancer Research Institute, Penn State University College of Medicine, Hershey, PA, USA, [2]Biochemistry & Molecular Biology, Pennsylvania State University, State College, PA, USA, [3]Jake Gittlen Cancer Research Institute, Pennsylvania State University College of Medicine, Hershey, PA, USA.

PURPOSE: Skeletal metastases develop in the majority of breast cancer patients. Our objective was to develop improved models of breast cancer metastasis to bone. METHODS AND RESULTS: We observe metastasis to bone following injection of the human breast carcinoma cell lines, MDA-MB-231 and -435 into the left ventricle of the heart (i.c.) in female athymic mice. Osteolytic skeletal metastases localize predominantly to the trabeculae in femurs, proximal tibia, and vertebrae which are sites for metastases in humans. Development of bone metastasis has not been observed following i.v. (lateral tail vein) or orthotopic (mammary fat pad) injection, underscoring the impact of first-pass clearance in lung capillaries. In order to facilitate examination of tumor cell arrival and initial colonization events in the bone, we engineered 231 and 435 cells that constitutively express enhanced green fluorescent protein (GFP). Fluorescent subpopulations were selected by FACS. Following i.c. injection of 2 x 105 cells, all organs were examined grossly, radiographically and microscopically after 2-6 wk. Except at the latest time points, osseous metastases were not grossly apparent unless tumor replaced the marrow or if a fracture developed. Bones stripped of soft tissues are examined using a epifluorescence dissecting microscope and images digitized. Single fluorescing cells were visible through intact bone, allowing quantification of bone colonization before radiographic evidence of metastasis was apparent. Fluorescent tumors were visible in the bones even when no radiographic evidence of osteolysis was present. H&E sections confirmed osteolysis. GFP-tagging permitted convenient 3D examination of lesions and enables accurate distinction of adjacent, but separate, foci before they coalesced. Use of GFP-tagged cells also appears to be more sensitive than standard histologic examination. The number of femoral metastases per mouse was slightly greater using GFP (2.36 ± 0.65; mean ± SEM) than in H&E analysis (1.25 ± 0.40) in limited sample sets. CONCLUSIONS: In contrast to arduous histological sectioning, GFP-labeling also allows relatively rapid assessment of all skeletal sites. GFP tagging also appears to be more sensitive with regard to detection of boney metastases in these experimental models. Thus, analysis of skeletal metastases at early time points is now possible with increased sensitivity, improved counting accuracy and ease.

SA062

See Friday Plenary number F062.

SA063

Mutational Analysis of Connexin 26 as a Candidate Tumor Suppressor Gene in Parathyroid Carcinoma. J. Costa,* T. M. Shattuck,* A. Arnold. Center for Molecular Medicine, University of Connecticut School of Medicine, Farmington, CT, USA.

One or more tumor suppressor genes critical to the pathogenesis of parathyroid cancers are expected to be present on chromosome 13q, based upon the finding of frequent loss of heterozygosity or deletion on 13q in parathyroid carcinoma. Connexins are encoded by a multigene family, one cluster of which is located on 13q. Connexins make up the functional unit of gap junctional intercellular communication by forming channels that allow the direct passage of small molecules and ions, such as Ca2+, between adjacent cells. Defects in connexin localization and gap junction channel formation have been observed in a variety of neoplastic cell lines and solid tumors. In addition, decreased expression of cyclin D1, an established parathyroid oncogene, has resulted from forced connexin expression in carcinoma cell lines. Furthermore, the rodent homolog of at least one such connexin gene on 13q, connexin 26 (GJB2), is known to be normally expressed in parathyroid tissue. Thus, given its attractiveness as a candidate parathyroid tumor suppressor gene, we rigorously examined the connexin 26 gene for possible inactivating somatic mutations in parathyroid carcinomas. High molecular weight genomic DNA from 6 primary parathyroid carcinomas, 2 recurrences and 2 metastases was analyzed by automated sequencing of the entire coding region of connexin 26. No mutations, insertions, or microdeletions were detected. One base change previously described as a polymorphism, resulting in a valine to isoleucine change at codon 27, was seen in one of the carcinomas and in that patient's corresponding peripheral blood leukocyte DNA. Based on the absence of identifiable inactivating mutations, it is unlikely that connexin 26 commonly functions as a classical tumor suppressor gene in the pathogenesis of parathyroid carcinoma.

SA064

See Friday Plenary number F064.

SA065

PTHrP a Modulator of Breast Cancer Metastasis. R. J. Thomas,* A. M. Tester,* J. A. Sharp,* E. W. Thompson,* T. J. Martin, M. T. Gillespie. St. Vincent's Institute of Medical Research, Melbourne, Australia

The lytic lesions of breast cancer metastasis to bone result from osteoclast activity. PTHrP is commonly expressed by breast cancers and has been shown to enhance RANKL and inhibit OPG production by the osteoblast, which combined are conducive to osteoclast formation. However PTHrP production by breast cancer cells may also affect their tendency to intravasation and extravasation, influencing their metastatic spread. We have examined the expression of MMPs by breast cancers in response to PTHrP, since MMPs are crucial in the degradation of connective tissue for tumor invasion. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are specifically able to degrade type IV collagen, but also degrade elastin, fibronectin and gelatin.Each of the human PTHrP isoforms (139, 141 and 173aa) was overexpressed in MDA-MB-231 cells and stable clones selected. The anchorage-dependent growth rates of the cells did not alter in response to PTHrP production. MDA-MB-231 cells overexpressing each isoform of PTHrP demonstrated elevated levels of MMP-9 mRNA and protein by zymography, however their relative levels differed (139>>141>173). Recombinant PTHrP or PTHrP peptides when added to MDA-MB-231 cells (over a 48hr time course) did not increase MMP-9 mRNA or protein levels, suggesting that MMP-9 was regulated by intracrine actions of PTHrP. We then determined if MMP-9 production by MDA-MB-231 cells could be influenced by bone marrow cells and/or osteoblasts. Coculture of primary osteoblasts or marrow cells with MDA-MB-231 parental or PTHrP overexpressing cells at different cellular ratios did not alter MMP-2 or MMP-9 production, suggesting that marrow cells or osteoblasts do not effect MMP production by MDA-MB-231 cells.We then determined whether PTHrP production by MDA-MB-231 cells modified the ability of cells to migrate through a matrix, or their ability to undergo morphological change in a matrix. In migration assays performed in collagen type IV, no difference was noted in MDA-MB-231 parental cells and PTHrP overexpressing cells. In matrigel outgrowth experiments, MDA-MB-231 cells overexpressing each isoform of PTHrP showed increased outgrowth through the matrigel matrix, with significantly higher branching, which might be indicative of invasive potential, than the MDA-MB-231 parental cells. Notably, PTHrP also increases ductal branching morphogenesis during normal mammary gland development.These data suggest that PTHrP production by breast cancers has distinct roles. Firstly, it promotes outgrowth of cells in matrigel and enhanced MMP-9 production, two phenotypes associated with intravasation and extravasation. Secondly, in bone metastases, PTHrP promotes osteolysis.

SA066

See Friday Plenary number F066.

SA067

Altered Expression of RANK in Human Osteosarcoma Tumors and Cell Lines. P. Bhatia,*[1] A. Deshpande,*[1] D. Ammerman,*[1] M. J. Nellissery,*[1] T. Johnson-Pais,*[2] R. J. Leach,*[2] M. F. Hansen.[1] [1]Molecular Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA.

RANK, a member of the Tumor Necrosis Factor alpha receptor superfamily has been shown to play a major role in mediating communication between osteoblasts and pre-osteoclasts during bone remodeling that results in the differentiation of pre-osteoclasts to mature osteoclasts. RANK is expressed on the pre-osteoclast while its ligand, RANKL, is expressed on osteoblasts. RANK maps to a region of human chromosome 18q21.3 that has been implicated in osteosarcoma tumorigenesis as well as predisposition to familial Paget's disease of bone. Using immunohistochemistry, RT/PCR and northern analysis, we examined RANK expression in primary osteosarcoma tumors and in the established osteosarcoma cell lines SAOS-2, U2-OS, OHS-50, and HT-148. We found that in all cases, RANK was strongly overexpressed. Further, in contrast to the observed pattern in pre-osteoclasts, RANK expression in the osteosarcoma tumors and cell lines was localized to the nucleus. RANK has been shown to activate both the NFkB pathway as well as the Akt1 pathway suggesting that aberrant expression and activation of RANK could lead to inhibition of apoptosis as well as misregulation of cell cycle control resulting in aberrant cellular growth. Thus RANK overexpression may play a critical role in osteosarcoma tumorigenesis.

SA068

See Friday Plenary number F068.

SA069

Increased Bone Remodeling Due to Ovariectomy Dramatically Increases Tumoral Growth in the 5T2 Multiple Myeloma Mouse Model. H. Libouban,*[1] M. Moreau,*[1] M. Basle,*[1] M. Duquenne,[1] R. Bataille,*[2] D. Chappard.[1] [1]LHEA, Fac Medicine, Angers, France, [2]INSERM, Nantes, France

Multiple myeloma (MM) is a B cell malignancy characterized by the presence of osteolytic lesions. In man, MM cells produce local factors such as IL-6, which modulate the bone remodeling and promote bone resorption. IL-6 is also the major growth and survival factor of myeloma cells. Thus, there is a true "vicious circle" in which plasma cells stimulate bone cells, which in return stimulate the neoplastic growth. The graft of 5T2MM mouse plasma cells in the C57BL/KaLwRij mouse is the only model that mimics bone lesions observed in human. The cytokine network is also close to the human one. In this model, 16 weeks are necessary for the development of the disease. We hypothesized that an increased bone remodeling could influence the development of MM.Ovariectomy (OVX) is well known to stimulate bone remodeling and to increase IL-6 levels in the bone marrow. We have ovariectomized mice before injection of the malignant plasma cells (5T2MM). Two groups of mice were injected with 2.10^6 5T2MM cells: control and OVX. 5T2 cells were inoculated intravenously 7 days after OVX (a period necessary to reach a high bone turnover). The development of the disease was monitored from 7 weeks after cells injection by serum electrophoresis to detect the presence of the paraprotein. It was detected 7-8 weeks post injection in the OVX-5T2 group and 9 weeks post injection in the control-5T2 group. At 9 weeks, the concentration of paraprotein was 5 fold higher in the OVX-5T2 group than in the control group. All OVX-5T2MM mice developed a hind-limb paralysis after 9-10 weeks post injection and there were euthanazied due to ethical consideration. In contrast, control 5T2 mice were euthanazied 16 weeks post injection. Osteolytic lesions were quantified by numeric radiography, scanning electron microscopy and X-ray microtomography. At the time of euthanasia, we observed the presence of numerous bone lacunae in the long bones of the OVX-5T2MM mice. The lesions were localized in the metaphysis of the femur and tibia with a preferential localization in the tibial crest. In control animals, the development of bone lesions was followed by X-ray performed under anesthesia, at 10 weeks post injection and at the day of sacrifice. No lesions were observed at 10 weeks post injection; at 16 weeks, numerous lesions were observed in both femur and tibia.In this study, an accelerated bone remodeling (due to OVX) dramatically increased the growth of malignant plasma cells. This is, in turn, associated with an earlier bone destruction confirming the close relationships between bone and MM cells. The usefulness of a bisphosphonate therapy, that would help to limit both mechanisms, is under study.

SA070

See Friday Plenary number F070.

SA071

Expression and Utilization of SDF-1/CXCR4 in Prostate Cancer Metastases. R. S. Taichman,* B. A. Wiedemer,* L. K. McCauley. Periodontics/Prevention/Geriatrics, University of Michigan Dental School, Ann Arbor, MI, USA.

Neoplasms have a striking tendency to metastasize or "home" to bone. Hematopoietic stem cells also "home" to bone during embryonic development where evidence points to the chemokine stromal-derived factor-1 (SDF-1) (expressed by osteoblasts and endothelial cells), and its receptor (CXCR4) as key elements in these processes. We hypothesized that metastatic prostate carcinomas also utilize the SDF-1/CXCR4 pathway to localize to the bone. To test the hypothesis the expression of CXCR4 in several human prostate cancer cell lines by RT-PCR and by Western Blotting was determined. Positive results were obtained with the PC-3 and DU145 cell lines, derived from malignancies which had spread to bone and brain, respectively. Hormone-refractory prostate carcinoma cell lines cloned from a lymph node (LNCaP) and marrow (LNCaP C4-2) also expressed CXCR4. To determine if prostate cancer cells migrate across endothelial cell monolayers in response to SDF-1, confluent bone marrow endothelial cells (Dr. S. Rafii, Cornell University, New York, NY.) were established on microporous membranes. Migrating cells were introduced into the upper chamber, and a gradient of SDF-1 (0 or 200 ng/ml) was established by placing the chemokine in the lower chamber. For LnCaP cells, random or chemokinetic migration accounted were 9,300 ± 3,000 and 7,300 ± 5,000 cells migrating through the endothelial layers/well, respectively. SDF-1 supported migration was significantly greater 35,000 ± 3,000/well ($P<0.05$). In in vitro adhesion assays, pre-treatment of the prostate cancer cells with SDF-1 significantly increased their adhesion to several osteosarcomas in a dose-dependent manor. In addition, invasion of the cell lines through basement membranes was also supported by SDF-1 and inhibited by antibody to CXCR4. Collectively, these results suggest that prostate cancers and perhaps other neoplasms (i.e. breast) may use the SDF-1/CXCR4 pathway during their spread to bone.

SA072

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SA073

Androgen Deprivation Therapy in Patients with Prostate Cancer and Changes in Bone Mineral Density. M. G. Alferos,* S. Yaturu.* Endocrinology, OvertonBrooks VAMC/LSUHSC, Shreveport, LA, USA.

Androgen deprivation therapy in prostate cancer results in low androgen levels. Low androgen levels in men is a risk factor for osteoporosis. We studied the bone density changes in 146 patients who are on androgen deprivation therapy for prostate cancer. All patients received Goserelin injections.In addition to Goserlin, 50 (36%)of the 146 patients received other antiandrogens namely flutamide and casodex. The duration of the anti-androgen therapy ranged from 3 to 122 months. The results of the bone density studies were reviewed and correlated with the duration of the anti-androgen therapy. Osteoporosis was noted in 90 (62%), osteopenia noted in 36(24%) and only 20 patients(14%)had normal bone mineral density studies. Most of the changes were at hip, more so at the ward's triangle of the left hip which constitutes 49%(i.e.72) of the 146 patients.Osteoporosis was noted in either hip in 82 (56%) patients and either hip and or spine in 90 patients which constitutes 62%. The duration of anti-androgen therapy did not correlate with the degree of bone loss. We conclude that patients with prostate cancer on anti-androgen therapy are at high risk for osteoporosis.

SA074

Skeletal Involvement in 14 Advanced Prostate Cancer Patient autopsies, 5 of these Patients Treated by Pamidronate. M. P. Roudier,[1] C. S. Higano,*[2] L. D. True,*[3] S. M. Ott,[4] H. J. Vesselle,*[5] P. H. Lange,*[1] R. L. Vessella.[1] [1]Urology, University of Washington, Seattle, WA, USA, [2]Oncology, University of Washington, Seattle, WA, USA, [3]Pathology, University of Washington, Seattle, WA, USA, [4]Metabolism, Endocrinology and Nutrition, University of Washington, Seattle, USA, [5]Radiology, University of Washington, Seattle, WA, USA.

To better understand the progression of prostate cancer (CaP) into the bone, the anatomical and histological findings of 14 autopsied patients are described.14 advanced CaP patients underwent a necropsy, 12 of them had a least one bone scan taken during the disease and at least 17 bone biopsies taken during the autopsy. 5 patients were treated with pamidronate (BisP) 2 to 13 months before death. Bone biopsies were processed for histology and 6 patients had a second biopsy for histomorphometry analysis. Tumor volume, bone marrow volume, tumor glandular differentiation, PSA and Chromogranin A expressions were evaluated by two pathologists. One pathologist identified bone response to the invading tumor on each slide as either osteodense or normal. The bone histological and bone scan results were correlated to the tumor and clinical data.Bone metastasis ranged from 95% in pelvis to 70% in humeri. Mean number of bone metastasis in BisP-treated patients (BisPp) was 13 and 11.6 by histology and bone scan respectively and 14.57 and 14.28 respectively in non-treated patients. The mean tumor burden was 1097 cm3 with a quasi disappearance of the bone marrow in all cases. Histology detected more metastases than bone scan. The bone scan-histology concordance was nevertheless good (80.32%) and reduced in BisPp (63.38%). Prevalence of abnormality was decreased in the BisPp (0.75) compared to non-treated patients (0.88). The tumor glandular differentiation was low and PSA expression was high with a neuro-endocrine immunophenotype in three patients. Two patients had no histological bone change associated with the carcinoma metastasis; the other 12 were osteoblastic. One of the patients with no bone change was treated with BisP. No clinical or carcinoma features were found to independently explain the other case. Histomorphometric analysis data are underway for 5 BisP-treated patients.In our advanced CaPpatients, the overall skeletal tumor burden in bone is larger compared to earlier studies. This is probably due to a more complete propagation of the tumor that accompanies hormonal treatment of the CaP and prolonged life span. Bone marrow is generally replaced, which accounts for the anemia usually observed in late-stage patients. Bone metastases are generally osteoblastic. The prevalence of abnormality in bone CaP metastasis was decreased in BisPp compared to non-treated patients.

SA075

A Proteome Study of Prostatic Factors Affecting Osteoblastic Activity: Identification and Characterisation of Galectin-1 and Cyclophilin A. H. Andersen,[1] O. N. Jensen,*[2] E. P. Moiseeva,*[3] E. F. Eriksen.[1] [1]University Department of Endocrinology, Aarhus Amtssygehus, Aarhus, Denmark, [2]Protein Research Group, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark, [3]Department of Medicine and Therapeutics, University of Leicester, Leicester, United Kingdom

Prostate cancer cells metastasise to bone causing an osteosclerotic response. We have previously shown that the human prostatic tumour cell line PC3 secretes factors, which stimulate the mitogenic activity of human bone marrow stromal (hBMS) cells. The PC3 derived mitogens have been found to be proteins with a molecular weight of 20 to 30 kDa. The aim of this study was therefore to characterise the protein profile of conditioned medium (CM) from PC3 cells in the molecular weight area 5 to 30 kDa by proteome analysis. A protein profile of the CM from PC3 cells was performed by two dimensional polyacrylamide gel electrophoresis (2D-PAGE). 30 spots in the molecular weight area 5 to 30 kDa were analysed by matrix assisted laser desorption /ionisation time of flight mass spectrometry (MALDI-TOF MS). Two of these spots were identified as galectin-1 and cyclophilin A. Galectin-1 is a lectin that binds to specific carbohydrate structures, which can be

found on a number of glycoproteins (e.g. laminin and fibronectin). Cyclophilin A belongs to the family of immunophilins, which bind to cyclosporin A. We wanted to examine whether galectin-1 (10 and 1000 ng/ml with or without 10 ng/ml of IGF-I) or cyclophilin A (1, 10, and 100 nM with or without 10 ng/ml IGF-I) had any effects on the proliferation (determined by incorporation of [^{3}H]-thymidine) or differentiation (determined by measuring alkaline phosphatase activity) of hBMS cells. Furthermore, we tested whether adhesion of PC3 cells to laminin (10 μg/ml), fibronectin (5 μg/ml), and collagen type I (5 μg/ml) was influenced by lactose (0.1 M), which binds to galectin-1. Neither galectin-1 nor cyclophilin A increased the proliferation of hBMS cells. However, 10 and 1000 ng/ml of galectin-1 increased the differentiation of hBMS cells (Mean (stimulated/control) ± SEM: 1.37±0.08, and 1.31±0.11, $p<0.05$). Cyclophilin A did not have any effect on the differentiation of hBMS cells. Lactose inhibited the adhesion of PC3 cells to laminin (0.72±0.09, $p<0.05$), fibronectin (0.30±0.12, $p<0.01$), and collagen type I (0.86±0.04, $p<0.05$). In conclusion galectin-1 increases adhesion of prostate cancer cells to bone matrix constituents and stimulates osteoblastic differentiation. Thus, it may be involved in the osteoblastic response seen, when prostate cancer cells metastasise to bone. Cyclophilin A showed no such effects.

SA076

See Friday Plenary number F076.

SA077

Why Don't Primary Care Physicians Screen for Osteoporosis? D. H. Solomon,[1] M. Connelly,[2] B. Dawson-Hughes,[3] C. J. Rosen,[4] D. P. Kiel,[5] S. L. Greenspan,[6] E. Leib,[7] A. H. Miguel,*[8] J. S. Finkelstein.[9] [1]Rheumatology and Pharmacoepidemiology, Brigham and Women's Hospital, Boston, MA, USA, [2]Ambulatory Care and Prevention, Harvard Medical School, Boston, MA, USA, [3]USDA Nutrition Center, Tufts University, Boston, MA, USA, [4]Maine Center for Osteoporosis Research, Bangor, ME, USA, [5]Aging, Harvard Medical School, Boston, MA, USA, [6]Medicine, University of Pittsburgh, Pittsburgh, PA, USA, [7]Medicine, University of Vermont, Burlington, MA, USA, [8]Proctor and Gamble, Cincinnati, OH, USA, [9]Endocrinology, Massachusetts General Hospital, Boston, MA, USA.

Data suggest that a large proportion of patients with osteoporosis are never diagnosed and never receive treatment. We examined what characteristics of practitioners are associated with low utilization of bone densitometry. We conducted a cross-sectional survey of primary care physicians (PCPs), including obstetrician-gynecologists (Ob-Gyn), internists (IM), and general practitioners/family practitioners (GP/FP), practicing in any of the six New England states who had a facsimile number listed with the American Medical Association. The questionnaire assessed their demographics, practice characteristics, use of bone densitometry, and attitudes regarding osteoporosis and health maintenance.Fourteen percent (N = 494) of all PCPs responded to the survey and respondents were very similar to non-respondents in terms of age and specialty though respondents were slightly more likely to be female (31% vs 23%). Respondents had a mean age of 51 years and their training included 51% IM, 24% Ob-Gyn, and 25% GP/FP. The mean number of self-reported bone densitometry referrals per month was 10 and 25% of respondents reported referring fewer than four patients per month. In adjusted logistic models, factors significantly associated with referring fewer than four patients per month were: 1) training as an IM (odds ratio, OR, 2.8, 95% confidence interval, CI, 1.5 - 5.0) or a GP/FP (OR 1.9, 95% CI 1.1 - 3.2) versus Ob-Gyn; 2) practicing in a rural (OR 2.1, 95% CI 1.3 - 3.8) or urban (OR 1.7, 95% CI 1.0 - 2.8) versus suburban setting; 3) the belief that calcium and vitamin D are adequate to treat osteoporosis (OR 2.1, 95% CI 1.0 - 4.5); 4) the belief that osteoporosis treatment should not be based on bone density results (OR 4.0, 95% CI 2.3 - 7.0); and 5) reporting confusion about the best anatomic sites for measuring bone density (OR 1.8, 95% CI 1.1 - 2.9).We conclude that several potentially modifiable factors, such as beliefs about adequate treatment and how to use bone densitometry, are associated with low referral rates for bone densitometry. Educational strategies aimed at improving use of bone density testing should consider these factors.

Disclosures: Proctor and Gamble,2.

SA078

See Friday Plenary number F078.

SA079

Effect of Two Different Hip Prostheses on Periprosthetic Acetabular Bone Density. G. Baccalaro,[1] A. Montesano,[1] P. Bertocco,[1] A. Pacico,[1] I. Guarducci,[2] A. Uderzo,[2] S. Ortolani.[3] [1]Unit of Rheumatologic and Metabolic Disorders, Istituto Auxologico Italiano, Verbania, Italy, [2]Divisone di Ortopedia Ospedale S. Biagio, Domodossola (Vb), Italy, [3]Istituto Scientifico Ospedale S. Luca, IRCCS, Milano, Italy

Dual X-Ray Absorptiometry (DXA) can be used in achieving the assessment of bone evaluation and density (BMD) around metal implants after total hip replacement (THR). Acute and persistent periprosthetic bone loss (BL) normally occurs after THR, and it can determine the decrease in local bone reaction and the consequent prosthetic failure. Local BL and reaction are controlled by bone turn-over, by local load applied and by prosthesis carachteristics, cemented (CP) and uncemented (UP). About UP normally used in THR, the cotile could be fixed (UPF) or not (UPU) to the acetabular bone, with differences in elastic and dynamic properties both in prosthesis and bone. The aim of this study was to evaluate the sovracetabular bone density after THR, both in UPF and UPU. Periprosthetic acetabular BMD by DXA was detected in 32 patients (9 M, 23 F), affected by primary osteoarthritis, who underwent THA, with two different prostheses.(see table). Time since surgery ranged from 13 to 46 months. An acetabular fixed region of interest (ROI) was evaluated and analyzed in order to define and assess a more precise weighted variable (RatioROI: BMD-ROI/ BMD total of the controlateral hip). Ratio ROIs of the two groups were compared by a T-Test.

	UPU		UPF	
N	**16 (3 M, 13 F)**		**16 (6 M, 10 F)**	
	Mean	**St. Dev.**	**Mean**	**St. Dev.**
BMD total control. hip	0.82	0.19	0.82	0.14
BMD ROI	1.20	0.36	1.29	0.42
Ratio ROI	1.50	0.34	1.58*	0.42
Age	72.25	5.72	67.00	6.87

*$p< 0.01$ RatioROI of the UPU-Cotex was significantly lower ($p< 0.01$) than UPF-ABG one. We can argue that local periprosthetic bone loss is higher in elastic and dynamic than fixed prosthesis after THR, suggesting that UPF could better control the local periprosthetic bone reaction than UPU. These preliminary data need to be confirmed by a longitudinal study on a larger sample size

SA080

See Friday Plenary number F080.

SA081

Assessing Independent Changes in Bone Mineral Content and Cross-Sectional Area in the Rat Tibia by pQCT. C. S. Tam,[1] P. Cunniff,*[2] C. Gordon,*[3] C. E. Webber,[4] T. V. Sanchez.[2] [1]Osteopharm Limited, Oakville, ON, Canada, [2]Norland Medical Systems, Fort Atkinson, WI, USA, [3]McMasters University, Hamilton, ON, Canada, [4]Hamilton Health Sciences Corp, Hamilton, ON, Canada

Bone mineral density (bone mineral content divided by projectional area) in the rat tibia is often assessed by densitometry as a reflection of bone loss or gain in studies of bone disease. This study details the use of pQCT to independently assess changes of bone mineral content and bone cross-sectional area along the length of the tibia to show how these direct reflectors may better show what is occurring in the total, cortical and trabecular compartments during a treatment study. Tibia from three groups (group A--11 tibia from a test group, group B--11 tibia from a test group, group C--10 tibia from a control group) underwent evaluation of the midshaft and proximal tibia by pQCT. Scans were carried out using standard system settings. Bone mineral content and cross-sectional area were examined independently. Using group C results for content and area as the reference values, the relative content and area in groups A and B were calculated. Examining cross-sectional area, compared to Group C the tibia in Group A showed relative results at the midshaft and proximal tibia of 109% and 110% (Total area), 103% and 93% (Cortical area) and 137% and 88% (Trabecular area). Group B showed relative results at the midshaft and proximal tibia of 113% and 121% (Total area), 107% and 96% (Cortical area) and 126% and 110% (Trabecular area). Examining content, compared to Group C the tibia in Group A showed relative results at the midshaft and proximal tibia of 102% and 95% (Total content), 101% and 97% (Cortical content) and 80% and 42% (Trabecular content). Group B showed relative results at the midshaft and proximal tibia of 104% and 101% (Total content), 104% and 99% (Cortical content) and 78% and 51% (Trabecular content).Results show that while Group A and Group B show significant reductions in trabecular content, the tibia reach reference level total and cortical contents and exhibit cross-sectional areas greater than or equal to reference levels in the total, cortical or trabecular compartments. Independent evaluation of content and cross-sectional area may provide further insight into how the tibia responds to treatment or disease.

SA082

See Friday Plenary number F082.

SA083

A New Approach to Peridental Bone Quality: pQCT Studies of the Human Mandible. E. J. A. Roldan,[1] V. Montangero,*[2] R. Capiglioni,*[2] T. V. Sanchez.[3] [1]Institute of Metabolic Research (IDIM) and Gador, SA, Buenos Aires, Argentina, [2]Institute of Metabolic Research (IDIM), Buenos Aires, Argentina, [3]Norland Medical Systems, Fort Atkinson, WI, USA.

Maxilla and mandible are the only human bones which, besides having muscle and endocrine influence, interact with another hard tissue--teeth. As such, local or systemic bone diseases may be screened and monitored with a differential approach. Low radiation volumetric analysis of maxillar and mandibular bone properties is now feasible by pQCT (Capiglioni, Radiología 1998,7:898), but interpretation of data has not always been manageable in the practice setting. To simplify interpretation we have related an anatomic classification of bone quality (Horner, Dentomaxillofacial Radiol 1998,27:17) to pQCT

measured bone mineral density in 71 cadaveric maxillar and mandibular samples. Methacrilate embedded maxillar and mandibular samples between 0.5 and 0.7 mm wide were fixed and scanned at 10 mm/sec with resolution set at 0.2 mm3 . Volumetric bone mineral density (vBMD) measurements were obtained for all sections (range for CV being 1.2-2.9; n=21x3). With Class I classified as dense bone and Class IV classified as severe osteopenia, bone sections were graded by an independent anatomist as Class I (10 samples), Class II (18 samples), Class III (22 samples) and Class IV (18 samples)--3 samples could not be graded. The pQCT vBMD ranged (in mg/cm3) between 444.5-1003.5 for Class I; 239.4-425.1 for Class II; 106.2-387.4 for Class III and 115.3- 0.0 for Class IV. No Class I sample showed a pQCT vBMD lower than 400mg/cm3, and no Class IV sample showed values above 200mg/cm3. To aid in interpretation each vBMD range could be further defined by a given colour in the software. Hence, a topographic distribution of bone qualities--color defined--within a bone section can be easily interpreted in a given patient.

SA084

See Friday Plenary number F084.

SA085

Mineral Threshold Analysis of the Mandible by pQCT. A New Tool for Selective Diagnosis and Monitoring of Cortical Quality. E. J. A. Roldan,[1] R. Capiglioni,*[2] J. Amaro,*[2] T. V. Sanchez.[3] [1]Institute of Metabolic Research (IDIM) and Gador SA, Buenos Aires, Argentina, [2]Institute of Metabolic Research (IDIM), Buenos Aires, Argentina, [3]Norland Medical Systems, Fort Atkinson, WI, USA.

In order to quantify the cortical mineral composition of the human mandible, the labial, sub-alveolar cortex from 32 healthy adults was analysed using the XCT3000D pQCT system. Volunteers were free of current peri-odontopaties and mean (SD) age of 56 (11) years, body weight of 64 (10) kg and height of 161 (10) cm.. Patients were positioned as previously described (Capiglioni, Radiología 1998,7:898) using a 280mm gantry, 0.5mm3 voxel resolution, 60mm/sec scout-view speed and 20mm/sec measurement speed. A cortical region of interest was manually defined within the axial images. Volumetric bone mineral density (vBMD) using 710 and 900 mg mineral density thresholds, and section areas using 0, 400, 600, 700, 800, 900 and 1,000 mg thresholds were calculated. Mean(SD) and CI95% for cortical vBMD710 was 1008.8 (58.4) and 987-1030 mg/cm3 while cortical vBMD900 was 1128.9 (47.4) and 1111-1146 mg/cm3. CI95% of the area fraction for materials with a density below 400 mg was 26-36%, while between 400-600 mg the area fraction was15-18%, and between 600-700 mg area fraction was 6.7-8.8%. Area fractions for thresholds over 700 mg were minor. There were no significant differences when comparing cortex near dentate or edentate sites. Data suggest that between subjects of different ages and sex, without bone metabolic diseases, the natural variation of cortical material composition is less than 5%. The existence of teeth did not influence densities of cortex material. Hence, cortex and medullar bone behave differently, so that cortical and medullar bone should be evaluated separately by tomographic, not projectional, technology.

SA086

See Friday Plenary number F086.

SA087

The Effect of Bone Density Information and Different Educational Interventions on Osteoporosis Knowledge in Premenopausal Women: Randomised Controlled Trial. G. Jones,[1] L. de Wit,*[1] S. Frendin,*[1] B. Oldenburg.*[2] [1]Menzies Centre for Population Health Research, Hobart, Australia, [2]School of Public Health, Queensland University of Technology, Brisbane, Australia

Preliminary uncontrolled data has suggested that bone density (BMD) feedback may be more effective than provision of information at changing behaviour in women in younger life. However, there is little information available about whether such programs will change knowledge. The aim of this study was to develop a measure of assessment of osteoporosis knowledge and assess the effect of BMD information and different educational interventions on short-term changes in knowledge. We studied 438 women aged 25-44 years who were randomly selected from the electoral role to take part in a three-year study (response rate 57%). Subjects were excluded if they were pregnant or lactating, had a previous BMD or had diseases or were taking medications that may affect BMD. Extensive data were collected at baseline including BMD (Hologic QDR2000) at the spine and hip. Those with a mean T-score at both sites less than zero (N=216) were told that they were at higher risk of fracture in later life while those above (N=222) were told that they were not at a higher risk of fracture. Subjects were also randomly assigned to a four-week small group osteoporosis and self-management course (OPSMC) or a comprehensive pamphlet. Osteoporosis knowledge was assessed by a newly developed 20-item questionnaire with true/false alternatives. This contained items on information contained in both methods of education and, in piloting, was shown to have content validity and exhibit a wide range of scores. All subjects completed the baseline knowledge questionnaire and an identical questionnaire at 6 weeks (without knowledge of the correct answers). In all groups, there was a significant improvement in scores during the 6 weeks (12.5 v 8.9, $p<0.001$). In terms of improvement in knowledge score, the OPSMC was superior to the pamphlet (mean difference 1.41, $p<0.001$) while low BMD was superior to normal BMD (mean difference 0.64, $p=0.049$). These effects were additive. In conclusion, this new instrument for assessing knowledge is sensitive to change in clinical trials and suggests that the best way to improve short-term osteoporosis knowledge is a combination of small group tutorials and BMD information. It remains to be determined whether short-term changes in knowledge will predict behaviour change and alteration in BMD in the longer term.

SA088

See Friday Plenary number F088.

SA089

See Friday Plenary number F089.

SA090

Development of Non-Invasive Diagnostics for Osteoporosis: A Model Based Approach. G. H. Gunaratne,*[1] K. K. Mohanty,*[2] S. J. Wimalawansa.[3] [1]Physics, University of Houston, Houston, TX, USA, [2]Chemical Engineering, University of Houston, Houston, TX, USA, [3]Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA.

Controlling and managing osteoporosis requires the ability to determine the need and optimal time for therapeutic intervention. Non-invasive diagnostic tools are essential for this purpose. Surrogates for bone strength include bone mineral density (BMD), properties of ultrasound transmission through bone, and structural characteristics of a trabecular architecture (TA). Preliminary studies of their applicability requires the use of samples of animal bones (or their digitized images); it is difficult and time consuming to generate suitably uniform samples. A diagnostic tool is useful only if the range of applicability is large; i.e., it can be utilized for many different bones. Consequently, possible forms of diagnostic tools can be deduced via an analysis of a suitable mathematical model of a TA. Only the most promising diagnostic tools need to be subjected to further analysis involving animal bone.We have introduced a mathematical model of a TA which consists of a disordered network of elastic elements. Spatial variations in the structure of TAs motivate a random assignment of spring constants and analogous bond-bending constants. Osteoporosis is modeled by the random removal of elastic elements, and therapeutic regeneration is described by a rule similar to Wolff's law. This model exhibits analogues of several known mechanical features of bone including, (1) an initially linear stress vs. strain relationship that becomes nonlinear following yield, (2) an exponential decay of ultimate stress with a reduction of bone mass, and (3) a dramatic increase of bone strength following therapeutic intervention. Numerical analysis of the model exposes a fundamental difference between healthy and osteoporotic bone. Bonds taking part in stress propagation form a dense subset of the former, and consists of a few coherent pathways in the latter. This observation can be exploited to deduce possible forms of diagnostic tools of osteoporosis. The first example of such a diagnostic is the ratio of responses of the networkto stationary and time-periodic external strain. This ratio is seen to be bi-linearly related to the quantity essential to identify osteoporosis, namely the ultimate stress. The bilinear relationship holds for a wide range of parameters and external strain. Since periodic strain can be applied using ultrasonic techniques, we believe that this measure can be extended to a non-invasive diagnostic of osteoporosis.

SA091

Relationship Between Vertebral Morphometry and Angle of Kyphosis in Osteoporotic Women. D. Diacinti,* S. Minisola, E. D'Erasmo,* E. Tomei,* G. Mazzuoli. Department of Clinical Sciences, Rome, Italy

Hyperkyphosis and loss of height are common in the elderly population, particularly in osteoporotic women. The purpose of this longitudinal study was to evaluate with morphometric radiography (MRX) the contribution of decrease vertebral body heights to the thoracic kyphosis. We enrolled 170 postmenopausal women with an age range of 46-74 years after excluding vertebral fractures on lateral thoracic films centring on level T7 (tube-to-film distance of 115 cm). Lumbar spinal (L2-L4) bone mineral density (LS-BMD) was measured by dual-energy x-ray absorptiometry (DXA) using the Hologic QDR-4500 densitometer (Hologic, USA). On each lateral thoracic radiograph the kyphotic angle was calculated with the method of Fon et al. (1).The films were digitized by means of a scanner and then was performed the vertebral morphometry from T4-T12 using specified software (QR-Verona).The computer automatically calculated the anterior, middle and posterior vertebral bodies heights (Ha, Hm, Hp), the ratios of heights of single vertebrae (Ha/Hp, Hm/Hp, Hp/Hpp), as well as the sum of vertebral body heights (AHs, MHs and PHs). DXA examination and lateral spine radiographs were repeated after two years. After 24 month we observed a decrement of the vertebral heights respect to basal values in all women enrolled. In the group of osteoporotic women the mean vertebral heights loss was -6.5mm; higher in women with a new vertebral deformity (-10.5mm). Non-osteoporotic women had a smaller vertebral height loss (-4.1mm). The highest AHs decrement respect to PHs resulted in a significantly lower AHs/PHs average ratio in all postmenopausal women (0.991 versus 0.984; $p<0.001$). AHs/PHs average ratio correlated significantly with angle of kyphosis($r=-0.49$; $p<0.001$). This longitudinal study demonstrates that hyperkyphosis of osteoporotic women is related to vertebral deformity and the average ratio of the anterior to the posterior height of thoracic spine (AHs/PHs) may be used as index of anterior spine wedging and so of thoracic kyphosis. 1) Fon GT, et al. AJR 1980 ; 134 :979-983

SA092

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SA093

Biomechanical Parameters of Iliac Bone Biopsy Samples in Normal Premenopausal Women. Y. Chung,[1] Y. Won,*[2] K. W. Lee,*[1] H. Kim.*[1] [1]Endocrinology and Metabolism, Ajou University School of Medicine, Suwon, Republic of Korea, [2]Orthopaedic Surgery, Ajou University School of Medicine, Suwon, Republic of Korea

Bone microarchitecture is important in bone biomechanics. Biomechanical properties were investigated in iliac bone biopsy samples of Korean premenopausal women.Transiliac bone biopsies were performed in 5 young healthy premenopausal women using Rochester trephine (inner diameter of 7.5mm). Bone mineral densities (BMDs) were measured by DXA (Lunar Expert-XL, USA). Biomechanical parameters of the biopsy samples were analyzed by MicroCT scan (SkyScan 1072, Belgium).The results are expressed as mean(SD). The mean age of the subjects was 21.4(1.7)years, body mass index 22.1(1.7)kg/m2, and body fat 33.4(2.9)%. BMDs of L2-4 spine were 1.11(0.10)g/cm2, proximal femur 1.03(0.13)g/cm2, and total body 1.19(0.05)g/cm2. Bone volume was 25.1(7.7)%, trabecular thickness 0.041(0.011)mm, trabecular seperation 0.092(0.043)mm, trabecular number 8.45(2.97)/mm, trabecular pattern factor -0.14(1.33)/mm, Euler Number 14.7(59.0), mean intercept length 0.202(0.058)mm, degree of anisotropy 0.548(0.141), and structure model index 1.034(0.258). Bone volume and mean intercept length were correlated with total body BMD (r=0.952, p<0.05, and r=0.899, p<0.05, respectively). Trabecular number and trabecular seperation were correlated with proximal femur BMD (r=0.974, p<0.01, and r=-0.990, p<0.01, respectively). Trabecular thickness and trabecular pattern factor were not correlated with any BMD parameters.Biomechanical parameters were unique represents of bone microarchitecture, and some of the parameters were correlated with BMDs.

SA094

Glycosylation Differences Found Between Human Bone Alkaline Phosphatase Isoforms. P. Magnusson, J. R. Farley. Musculoskeletal Disease Center, Jerry L. Pettis Memorial VA Medical Center, Loma Linda, CA, USA.

High-performance liquid chromatography (HPLC) can separate three human bone alkaline phosphatase (BALP) isoforms in serum: a minor fraction, B/I, and two major isoforms, B1 and B2. The circulating levels of these BALP isoforms can vary independently during the pubertal growth spurt and in metabolic bone diseases. We postulated that structural differences between the BALP isoforms, which allow for HPLC separation, reflect different patterns of glycosylation. The three BALP isoforms were prepared from extracts of human osteosarcoma (SaOS-2) cells and separated by anion-exchange chromatography, using Q-Sepharose beads. Identities of the BALP isoforms were confirmed by HPLC. All three BALP isoforms were similar with respect to: freeze/thaw stability; solubility (100% soluble); heat inactivation; and inhibition by L-phenylalanine, L-homoarginine, and levamisole. The isoforms were also kinetically similar (i.e., no differences in maximal velocity or Michaelis constant at pH 8.8 and pH 10.0). The isoforms differed, however, with respect to sensitivity to precipitation with wheat germ lectin (WGL), p<0.001, but not concanavalin A (Con A). At 3.0 mg/mL, WGL precipitated about 20% of B/I but more than 80% of B1 and B2. At 10 mg/mL, Con A precipitated about 20% of B/I, B1, and B2. Together, these data suggest that B1 and B2 have more (or more reactive) sialic acid residues, compared with B/I, and that the isoforms do not differ with respect to accessible (reactive) mannosyl/glucosyl residues. Gel filtration chromatography appeared to show a difference in the molecular weights (MW) of the BALP isoforms, with B/I at 76 kd; B1 at 178 kd; and B2 at 184 kd. Further studies showed that this difference was also due to differential sialylation. Desialylation of B2 with neuraminidase decreased the apparent MW (to that observed for B/I), suggesting that desialylation results in non-specific interactions between uncharged carbohydrate residues on BALP isoforms and the agarose filtration beads, causing underestimation of the MW. All three BALP isoforms were similarly cross-reactive in the commercial Alkphase-B and Tandem-MP Ostase immunoassays, r = 0.944 and r = 0.985, respectively (p<0.001). Further studies are required to compare their immunoaffinities. In summary, our data indicate that B1 and B2 have more (or more reactive) sialic acid residues compared with B/I. The isoforms do not differ with respect to reactive mannosyl/glucosyl residues, reaction kinetics, or MW. A better knowledge of the molecular structures of these glycoproteins, and the difference between them, should advance our understanding of the function of BALP and of the clinical utility of measuring these isoforms in serum.

SA095

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SA096

Biochemical Markers of Bone Turnover in Response to Dietary Salt. C. Palacios,*[1] K. Wigertz,*[1] B. R. Martin,*[1] M. Peacock,*[2] C. M. Weaver.[1] [1]Foods and Nutrition, Purdue University, W Lafayette, IN, USA, [2]School of Medicine, Indiana University, Indianapolis, IN, USA.

Biochemical markers of bone turnover and hormonal status were measured in 22 black and 14 white female adolescents during a controlled metabolic study. The study comprised 2 sessions of 3-week balances, at 2 levels of dietary sodium. Subjects were randomized to a low (1.3±0.6 g/d) or a high (4.0±1.0 g/d) sodium diet in a crossover design with calcium intake held constant at ~815 g/d. Age was 12±1 y in blacks and 12.9±1.2 y in whites (p<0.05). Subjects were matched by postmenarcheal age (9.5±12.7 months in blacks and 10.1±11.9 months in whites) and weight (55.8±12.7 kg in blacks and 56.3±16.2 kg in whites). Fasting biomarkers of bone turnover and hormonal indices measured and at the end of the study and last 2 weeks average 24-h urinary calcium are shown in the following table:

Biomarker	Dietary Na level	Whites	Blacks	p value
Serum				
25 OH Vitamin D	Low	39.8±7.1	26.6±7.5	0.0001
(ng/ml)	High	44.6±10.6	27.2±9.5	0.0001
1,25 OH Vitamin D	Low	37.7±10.9	44.1±15.1	0.05
(pg/ml)	High	39.3±10.3	46.8±15.4	0.05
Osteocalcin	Low	56.3±38.1	39.2±24.8	NS
(ng/l)	High	60±23.7	46.2±23.7	NS
Bone alkaline	Low	67.5±18.2	66.8±30.5	NS
phosphatase (ng/ml)	High	66.4±29.6	66.4±26.7	NS
Parathyroid hormone	Low	19.6±9.6	22.7±7.9	NS
(pg/ml)	High	22.2±10.7	26.2±10.9	NS
Insulin-like growth	Low	465±112	506±96.6	NS
factor I (ng/ml)	High	499±138	556±73.5	NS
Urine				
Average 24-h urinary	Low	62 ± 35	44 ± 39	NS
calcium (mg/d)	High	101 ± 41	48 ± 36	0.05
N-telopeptide	Low	345±124	320±161	NS
(nmol BCE/mmol Cr)	High	294±90	289±155	NS

* p-value refers to the difference between blacks and whites within dietary sodium level There were no significant differences in any of these parameters due to sodium intake except for urinary calcium. The white girls had significantly higher urinary calcium excretion while consuming the high sodium diet compared to the low sodium diet whereas blacks had no change in urinary calcium excretion due to the sodium intake. Despite the sodium induced urinary calcium output in the white girls, no changes in the bone turnover variables were observed. Whites had significantly higher 25-OH vitamin D but lower 1,25-OH vitamin D compared to blacks. Fasting biochemical markers of bone turnover did not reflect changes in calcium metabolism during a 3-week period in adolescents.

SA097

Sandwich CLIAs for the Variants of the Aminoterminal Propeptide of Type I Procollagen. E. Marjoniemi,*[1] L. Hakalahti,*[2] M. Immonen,*[2] S. Niemi,*[2] A. Novamo,*[2] J. Risteli.[1] [1]University of Oulu, Oulu, Finland, [2]Orion Diagnostica, Oulu, Finland

At least two variants of type I collagen are found in vivo. The classical form contains two different gene products, two α1-chain and one α2-chain of type I collagen, whereas the type I α1- homotrimer collagen contains three similar α1-chains. During the biosynthesis of these collagen variants also two different aminoterminal propeptides (abbreviated hetPINP and hotPINP) are formed. The presently available immunoassays for the intact PINP can not distinguish them. The ability to detect the synthesis of type I α1-homotrimer collagen would be interesting, since the Sp1 polymorphism of the COL1A1 gene has been suggest to lead to its synthesis e.g. in advanced age. The α1-homotrimer collagen is less mineralized and the stabilizing cross-links are also formed in a different way. The decreased tensile strength of the α1-homotrimer collagen fibers could explain the increased fracture risk.The hotPINP was purified from human ascitic and pleural fluids. It could be separated from the hetPINP by DEAE-chromatography in low pH (5.0), since the hotPINP contains 50% more negatively charged phosphate groups. Since there may be variation in the extent of phosphorylation, also other methods such as size exclusion and reverse phase chromatographies would be needed to totally remove the other variant from the standard preparations. After separation of these two forms, their consentrations can be estimated by the intact PINP radioimmunoassay, which measures both PINP variants equally. We selected a sandwich chemiluminescence immunoassay (CLIA) technology to distinguish if there is two or three similar α1-chains in the propeptide. At first thirteen differents monoclonals antibodies agaist the intact PINP were tested both in capture and reporter positions thus giving 169 different antibody combinations. The antibodies were biotinylated or labeled with an acridiniumester. The platform used was a 96-well plate coated streptavidin. A flash-type chemiluminescence was analyzed by a Victor[2] multilabel counter (PerkinElmer Wallac Inc). Thirty four antibody combinations gave an immunoassay which detected both the heterotrimer and homotrimer PINP and several assays measured them equally. A CLIA for the intact PINP gave a correlation of 0.953 (n = 100) with the intact PINP radioimmunoassay. Seven pairs were able to react preferentially with the hotPINP. Since our hetPINP preparations still seem to contain some hotPINP, the exact determination of the cross-reaction needs further work. Also the clinical significance of the presence of hotPINP in serum will be then evaluated.

Disclosures: Risteli J,7.

SA098

See Friday Plenary number F098.

SA099

Quantitative Ultrasound and Its Association to Hormone Replacement Therapy - Results of the SEMOF-Study. F. C. Hartl,[1] J. Cornuz,[2] A. Tyndall,*[1] M. A. Krieg,[2] C. Ruffieux,*[3] P. Burckhardt.[2] [1]Rheumatology, University, Basel, Switzerland, [2]Internal Medicine, University, Lausanne, Switzerland, [3]Statistics, University, Lausanne, Switzerland.

Cross-sectional data of the SEMOF-study (7550 postmenopausal women, mean age 75.3 years ± 3) were used to analyse the association of hormone replacement therapy (HRT) and quantitative ultrasound measurements (QUS). SEMOF is a prospective, population based cohort study in Switzerland to asses the predictive value of QUS for hip fractures. QUS measurements were performed by trained personnel in 10 centres with 3 different ultrasound devices (Lunar Achilles, Hologic Sahara and IGEA DBM Sonic). Additionally a questionnaire regarding risk factors for osteoporosis was completed. 6225 subjects were eligible for further analysis after exclusion of individuals with missing information about HRT intake (n=139), unknown duration of HRT use (n=228), unidentified drug (n=401) and treatment with non-recognised drugs (n=557). Only subjects not indicating a diagnosis of osteoporosis in the questionnaire were further evaluated (n=5076). Of those, subjects with current use of HRT since at least 5 years or longer were regarded as current users (n=398 or 7.8%). Women never being exposed to such a treatment were defined as never users (n=4182 or 82.4%). All QUS measurements were significantly higher in current users compared to never users, this difference persisted after adjustment for confounding variables. It was observed that current users were significantly younger (73.7 vs. 75.5 years), had less weight (63.7 vs. 66.2 kg), were taller (160.2 cm vs. 158.5) and presented a lower BMI (24.8 vs. 26.4 kg/m²). Additional variables being associated with HRT were identified using a logistic regression analysis. Therefore current HRT users had a better formation, reported a higher rate of surgical menopause and were living more often with their husbands than never users. Bone status measured by QUS in elderly women seems to be preserved in current HRT users (HRT 5 years or longer), even so significant differences for health and socio-economic profiles were observed between never and current users in this large population based sample.

Table: Adjusted QUS variables of 4545 subjects in never and current users:

QUS device	Never users n=4156	Current >5 years. n=389	p-value current (>5 years) vs. never users
Achilles Stiffness	69.6 ± 12.3	79.0 ± 13.6	<0.0001
Achilles SOS	1511.5 ± 26.1	1530.3 ± 29.3	<0.0001
Achilles BUA	99.7 ± 9.5	105.9 ± 10.2	<0.0001
HologicQUI	74.8 ± 16.4	86.6 ± 18.5	<0.0001
Hologic SOS	1517.3 ± 26.5	1535.6 ± 30.6	<0.0001
Hologic BUA	57.9 ± 15.0	68.4 ± 16.0	<0.0001
DBM SOS* *Missing values in 47 subjects	1844.9 ± 98.7	1905.0 ± 100.9	<0.0001

SA100

Differential Effects of Multi-site SOS and DXA on Cortical Bone with HRT. K. M. Knapp,[1] G. M. Blake,[1] T. D. Spector,[2] I. Fogelman.[1] [1]Osteoporosis Unit, Guy's Hospital, London, United Kingdom, [2]Twin Research and Genetic Epidemiology Unit, St Thomas' Hospital, London, United Kingdom.

Hormone Replacement Therapy (HRT) is commonly used for the prevention and treatment of postmenopausal osteoporosis. The aim of this study was to investigate the effects of HRT use on speed of sound (SOS) measurements in cortical bone. A group of premenopausal women (n=278, mean age 38 ± 9 years), postmenopausal women (n=191, mean age 59 ± 7 years) and women receiving HRT (n=126, mean age 56 ± 7 years, mean HRT duration 7 ± 4 years) were recruited from Guy's and St Thomas' Hospitals. All women were normal healthy subjects as judged by the absence of any risk factors for osteoporosis. All patients had SOS measurements of their non-dominant third proximal phalanx, 1/3 radius, midshaft tibia and fifth metatarsal using the Sunlight Omnisense (Rehovot, Israel) and DXA measurements of the lumbar spine and proximal femur using Hologic QDR densitometers (Bedford, MA). T-scores were calculated using the premenopausal controls aged 20-40. Multivariate regression analysis was used to examine the effect of age, HRT usage, and their interaction on T-score values.

Site	Mean T-score Controls[a]	T-score difference[b]	p-value	Age coefficient[c]	p-value
RAD	-1.00	0.55	<0.001	-0.06	<0.001
TIB	-0.80	0.51	<0.001	-0.04	0.001
PLX	-1.09	0.29	0.053	-0.10	<0.001
MET	-0.65	0.26	0.055	-0.04	0.007
LS	-0.70	0.42	<0.001	-0.06	<0.001
THIP	-0.37	0.08	0.519	-0.05	<0.001

[a] = mean T-score at mean age for whole study group (58 years), [b] = mean increment in T-score due to HRT usage, [c] = mean decrease in T-scores per year. A positive effect of HRT usage on SOS T-scores was found at all four sites, although the effect at the phalanx and metatarsal was smaller and just failed to reach significance. The DXA T-scores for the lumbar spine are significantly different between the two groups, although the differences for the proximal femur are considerably smaller and insignificant. These results show that HRT has a positive effect on SOS measurements in cortical bone, which is greater than the effect found in the proximal femur using DXA. This suggests that SOS measurements may be measuring different aspects of bone than density (BMD) and that HRT may affect bone structure as well as BMD.

SA101

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SA102

Changes in QUS and BMD Measurements with Antiresorptive Therapy: A Two-Year Longitudinal Study. M. L. Frost, G. M. Blake, I. Fogelman. Osteoporosis Screening & Research Unit, Guy's Hospital, London, United Kingdom.

The aim of this two-year longitudinal study was to assess whether calcaneal quantitative ultrasound (QUS) measurement variables respond to antiresorptive therapy and whether these measurements display adequate long-term precision to be useful for monitoring purposes. The study population consisted of 195 postmenopausal women who were placed into one of three groups: (i) 39 women treated with antiresorptive therapy who commenced treatment at baseline; (ii) 25 women treated with antiresorptive therapy who had been on treatment for at least two years at baseline; (iii) 131 women who did not taken antiresorptive therapy during the two year study period. Subjects had baseline and 12 and 24 months follow-up BMD measurements at the lumbar spine (LS), femoral neck (FN) and total hip (HIP) and calcaneal QUS measurements of broadband ultrasound attenuation (BUA) and speed of sound (SOS) on the Hologic Sahara. For women in Group 1, all BMD and QUS variables increased significantly from baseline after two years of treatment. For women in Group 2, only HIP BMD and BUA increased significantly after two years and the changes were less than those observed in women in Group 1. The overall treatment effect for each measurement variable, defined as the difference in the mean absolute changes between Group 1 and Group 3 after two years, was 0.08, 0.03 and 0.04 g/cm^2 for LS, FN and HIP BMD, and for BUA and SOS was 5.8 dB/MHz and 13.1 m/s respectively. When the overall treatment effect was expressed in T-score units, the effect was greatest for LS BMD (0.65 T-score units) and lowest for FN BMD (0.31 T-score units). QUS measurement variables yielded intermediate values of 0.43 to 0.52 T-score units. The average least significant change (LSC = precision error * 2.8) was 0.38 T-score units for BMD measurements, while the LSC for QUS measurements was three-times greater at approximately 1.20 T-score units. Ninety-four percent of the women in Group 1 showed changes in LS BMD that exceeded the LSC after two years, while the percentage was lower for the other measurement variables ranging from approximately 6% for FN BMD and SOS to 50 % for HIP BMD. A lower percentage of women in Group 2 and 3 displayed changes that exceeded the LSC for both BMD and QUS measurement variables. In conclusion, calcaneal QUS measurement variables were found to show a highly significant response to antiresorptive therapy. However, the precision of QUS measurements was not good enough to allow QUS to be used for monitoring response to treatment. Future improvements in the precision of calcaneal QUS measurements are required to increase the utility of QUS for monitoring purposes.

SA103

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SA104

Normative Data for Ultrasound measurement of the Calcaneus within a Male Caucasian Population. H. J. Hinkley, I. P. Drysdale, D. Bird,* N. J. Walters.* British College of Naturopathy and Osteopathy, LONDON, United Kingdom.

After routine scanning of male subjects it was observed that a higher than expected proportion were diagnosed as being at risk of Osteoporotic fracture. This was seen particularly in younger age groups and suggests that the McCue normative data line may be too high.The purpose of this study was therefore to re-determine normative data for Caucasian men aged 29 to 80 years, using the McCue Cubaclinical II device.A questionnaire was used to determine handedness and eligibility for the study. Exclusions were use of Corticosteroids or Thyroxine for more than 6 months or a previous history of fracture. There were 276 men within the age range 20 to 80 years, recruited from various sources.Broadband ultrasound attenuation (BUA) was determined for the left and right calcaneus, each three times in succession. Both heels were re-positioned between each measurement to compensate for anatomical variation.There was a significant difference between non-dominant and dominant measures (P<0.05). For the purpose of normative data, the mean of the non-dominant BUA was used. Linear regression gave an R^2 of 0.089 (P<0.0001). The lin-

ear regression line resulting from this study was found to be lower than the MCCue line, with BUA values of 96 db/MHz as opposed to 113 db/MHz at age 20 and 77 db/MHz instead of 85 db/MHz at age 80.The same procedure was applied to 360 female Caucasians previously assessed (linear R^2 value of 0.143, P<0.0001). A significant difference was found between non-dominant BUA measurements for male and female, across all ages, with male being higher.

SA105

Maternal History of Osteoporosis Is a Major Determinant of Peak Bone Mass in Southern Chinese Premenopausal Women. A. Y. Ho,[*1] H. L. Lau,[*1] S. S. Yeung,[*1] J. L. Chan,[*1] A. W. Kung.[*1] [1]University of Hong Kong, Queen Mary Hospital, Hong Kong, Hong Kong Special Administrative Region of China.

Genetics influence is known to be a major determinant of peak bone mass and subsequent risk of osteoporosis in later life. To determine the role of genetic and environmental impact on bone mass, we measured bone mineral density (BMD) of the anterior-posterior lumbar spine and the hip in 464 premenopausal southern Chinese women by dual energy X-ray absorptiometry (DEXA, Hologic QDR 2000+). 265 of them had a maternal history of osteoporosis (Group A, maternal T-score hip/spine less than or equal to -2.5 or maternal history of hip/vertebral fracture) and 199 women were born to mother with T-score greater than -2.5 (Group B). Women in group A had significantly lower BMD at both the lumbar spine and the hip (L1-L4 BMD 0.958 ± 0.117 g/cm^2 vs 0.987 ± 0.116 g/cm^2, p = 0.01; Total hip BMD 0.816 ± 0.143 g/cm^2 vs 0.841 ± 0.103 g/cm^2, p = 0.04). The two groups were similar in their body weight, serum calcium, phosphate, total alkaline phosphatase, exercise levels (standing time and walking time per day) and daily calcium intake, but women in Group A were significantly shorter (p = 0.02) and older (p = 0.001). After adjusting for age and height using analysis of covariance, women in Group A still had significantly lower BMD at the lumbar spine (3.0 %, p < 0.05) and the hip (3.1%, p = 0.05) than those in Group B. Multiple regression analysis showed that body weight, maternal history of osteoporosis and serum total alkaline phosphatase were significant predictors of BMD at both lumbar spine and total hip. We concluded that genetic influence in terms of maternal history of osteoporosis is a major determinant of peakbone mass in southern Chinese premenopausal women.

SA106

Two Inbred Rat Strains Which Differ Substantially in Hip Fragility. Q. Sun,[*1] F. M. Hinant,[*2] C. H. Turner.[1] [1]Department of Orthopaedic Surgery, Indiana University, Indianapolis, IN, USA, [2]School of Medicine, Indiana University, Indianapolis, IN, USA.

One approach for identifying the genetic influences on skeletal phenotypes involves the creation and genetic mapping of a population of F2 offspring derived from a cross of two inbred strains of rodents. The two inbred strains should be chosen based upon a large difference in the phenotype of interest, e.g. bone fragility. We found previously that considerable variation exists in fragility phenotypes among inbred strains of rats, and the phenotypic variation was site specific. The aim of this study was to identify useful inbred rat strains for studies of genetic influences on hip fragility. Two inbred rat strains, Copenhagen 2331 (COP) and DA, were found to differ significantly in femoral neck size and strength as assessed by pQCT, biomechanics and geometry measurements. At six months of age, COP rats had 16 % greater proximal femur bone mineral content (BMC), 31% higher ultimate force (Fu), 47% higher ultimate displacement (du), 73% higher work to failure (U), and a significantly wider femoral head and neck, as compared to DA rats (Table 1). These data indicate that significant phenotypic variation at the femoral neck site exists between these two inbred strains, and COP rats appear to have genes that specifically enhance the femoral neck structure and strength. Therefore the cross of these two inbred strains, COP with DA, may provide a compelling model for the genetic studies of hip fragility.

SA107

See Friday Plenary number F107.

SA108

Studies Using Congenic Mice Reveal That Chromosome 1 QTL Locus in Cast/EiJ Mouse Contributes to 30% Variation in BMD Difference Between CAST and C57BL Mice. W. Gu,[1] B. Edderkaoui,[1] W. Beamer,[2] X. Li,[1] H. C. M. Sheng,[1] J. Wergedal,[1] K. Shultz,[*2] K. H. W. Lau,[1] L. R. Donahue,[*2] C. Rosen,[*3] S. Mohan,[1] D. J. Baylink.[1] [1]MDC, Pettis VAMC, Loma Linda, CA, USA, [2]The Jackson Lab, Bar Harbor, ME, USA, [3]St. Joseph's Hospital, Bangor, ME, USA.

Peak BMD is one of the strongest determinants of subsequent osteoporotic fracture risk. More than 70% of variance in peak BMD is determined by genetic factors. In previous studies using F2 female mice generated from CAST/EiJ (CAST) and C57BL/6J (B6) mouse strains, which exhibit high and low peak BMD, respectively, we identified four statistically significant quantitative trait loci (QTL) that contributed to BMD variation between these two strains. In this study, we focused on the genetic effect of a QTL locus located on chromosome 1 because: 1) this QTL locus exhibited the highest LOD score of 8.0 among the 4 QTLs; and 2) this QTL is located on the syntenic region of human chromosome 1Q21-23, a region implicated in peak BMD regulation in humans. To determine whether chromosome 1 QTL contains biologically active gene(s) that contribute to a significant peak BMD difference between CAST and B6 strains, we generated congenic strains by transferring CAST chromosome 1 QTL region (donor) into the B6 strain (recipient). We found that the volumetric BMD at the mid diaphysis of the femur by PQCT was significantly higher in the congenic mice compared to age-matched B6 mice at 16 weeks of age (685±34 vs 638±41 mg/cm^3; P<0.05). In order to determine if this QTL exerts sex-specific effects, we evaluated BMD difference in both male and female mice. BMD was significantly increased in both male and female congenic mice compared to corresponding sex and age-matched B6 mice, thus suggesting that the effect of this QTL was not dependent on sex-specific hormones. To determine if the chromosome 1 QTL acted in a dominant or additive manner, we evaluated BMD difference between CAST/CAST homozygous and CAST/B6 heterozygous mice for this QTL. We found that, while the homozygous CAST genotype of the QTL locus contributed to 30% of the variation in BMD between CAST and B6 mice, the heterozygous genotype contributed to approximately 15% of the BMD variation, thus suggesting that chromosome 1 QTL acts in an additive manner. Conclusions: 1) The QTL region on mouse chromosome 1 contains a gene(s) that contributes to 30% of the variation in femur BMD between B6 and CAST strains. 2) Allele in CAST chromosome 1 QTL acts in a sex-independent and additive manner. 3) Congenic approach can be used to evaluate the relative contribution of a QTL to a phenotypic variation between the two inbred strains used for QTL mapping.

SA109

See Friday Plenary number F109.

SA110

Areal and Volumetric Bone Mineral Density (BMD) and Bone Volume in Men with Primary Osteoporosis and Their First-Degree Male Relatives: Further Evidence for a Familial Effect. P. R. Ebeling,[1] B. Erbas,[*2] S. Ristevski,[*1] C. Poon,[*1] S. Yeung.[*1] [1]Diabetes and Endocrinology, The Royal Melbourne Hospital, Parkville, Australia, [2]General Practice and Public Health, The University of Melbourne, Parkville, Australia.

Osteoporosis in men is set to become an important public health problem. By 2010, 30% of all hip fractures will occur in men. While areal BMD is lower in daughters of women with postmenopausal osteoporosis, there are few data examining familial effects on areal and volumetric BMD, or bone volume, in relatives of men with primary osteoporosis. We assessed areal and volumetric BMD, and bone volume in men with primary osteoporosis and their first-degree male relatives (FDMR) to determine if there was a familial effect on the development of primary osteoporosis in men.Sixty-five normal, healthy men (Gp1); 113 men with primary osteoporosis (Gp2); and 66 of their FDMR were examined. FDMR were divided into 2 groups according to whether they had a t-score -2 (Gp 4). Participants were recruited by advertising through local newspapers; via a staff member, family, friends; and sporting clubs. BMDs at the spine (LS) and femoral neck (FN), and total body (BMC) were measured by DXA and vertebral and FN volumes were calculated by standard formulae. Values in all groups were corrected for age.Osteoporosis (t< -2) occurred in 41% of FDMR. BMD at all sites was lower in OP men and FDMR than in normal men. Age was negatively related to areal and volumetric BMDs at the FN and also to FN volume, while fat mass was positively related to age. All values were lower in OP men than in normal men, except fat mass and FN volumetric BMD. While LS and FN areal BMDs were both lower in FDMR only LS, but not FN volumetric BMD, was lower in FDMR.

Value	Gp1 vs Gp2	Gp1 vs Gp3	Gp1 vs Gp4
LS aBMD b	-0.25 (0.02)**	-0.21 (0.03)**	-0.04 (0.03) NS
FN aBMD b	-0.24 (0.02)**	-0.21 (0.03)**	-0.02 (0.03) NS
TBBMC b	-0.28 (0.10)**	-0.15 (0.13) NS	-0.14 (0.13) NS
% Fat	1.56 (1.12) NS	2.54 (1.47) NS	4.61 (1.44)**
Vert vol b	-0.06 (0.03)*	-0.04 (0.04) NS	0.010 (0.03) NS
LS vBMD b	-0.24 (0.03)**	-0.20 (0.04)**	-0.03 (0.03) NS
FN vol b	-0.17 (0.07)*	-0.12 (0.10) NS	-0.03 (0.09) NS
FN vBMD b	-0.12 (0.07) NS	-0.11 (0.10) NS	-0.02 (0.09) NS

Indicates Log to the base transformation of data; *, p < 0.05; **, p < 0.001.The high prevalence of low BMD in FDMR provides further evidence for a familial effect in primary osteoporosis in men. The familial effect may have a greater impact on spinal volumetric BMD, while age-related effects may predominate at the proximal femur

SA111

Extensive Screening of Candidate Genes for Ossification of the Posterior Longitudinal Ligament of the Spine. K. Furushima,[*1] T. Tanaka,[*1] S. Maeda,[2] K. Ikari,[1] T. Nobukuni,[*3] K. Shimo-onoda,[*2] T. Nakajima,[*3] S. Komiya,[*2] S. Harata,[*1] I. Inoue.[*3] [1]Dept. of Orthopaedic Surgery, Hirosaki Univ., Aomori, Japan, [2]Dept. of Orthopaedic Surgery, Kagoshima Univ., Kagoshima, Japan, [3]Division of Genetic Diagnosis ,The Institute of Medical Science,The University of Tokyo, Tokyo, Japan.

Ossification of the posterior longitudinal ligament of the spine (OPLL) showing ectopic bone formation in the posterior ligament is a common disorder among Asian populations, where an estimated prevalence of 2-4 % of the general population. Ectopic ossification

compresses the spinal cord and leads to various degrees of neurological symptoms from discomfort to severe myelopathy. OPLL is a hyperostotic disease with a pathology almost the opposite of osteoporosis, a typical bone loss disease. Despite the late onset, OPLL has a strong genetic background, as shown in the classical epidemiological study and by the estimated relative risk to siblings of ~10. Because genetic determinants appear to play a crucial role in the etiology of OPLL, we began a large scale screening for genetic loci linked with OPLL. In the present study, we performed an extensive non-parametric linkage study with 126 affected sib-pairs using markers for various candidate genes by two distinct analyses, SIBPAL and GENEHUNTER. 88 candidate genes were selected based on two distinct investigations: 1) genes identified by cDNA microarray analysis of systematic gene expression profiles during osteoblastic differentiation of human mesenchymal stem cells, and 2) genes known to be involved in bone metabolism. The newer technique using cDNA microarray-based technology provides a more extensive range of biological information on the interplay of genes. Of the 24 genes regulated during osteoblastic differentiation, only one, the alpha B crystalline gene, showed evidence of linkage (P=0.016, NPL=1.83). Of 64 genes known to be associated with bone metabolism, 6 showed weak evidence of linkage by SIBPAL analysis (P<0.05): BMP4, PRG1, TGFb3, OPN, PTHR1, and IGF1. Among these genes, BMP4 (NPL=2.23) showed evidence of linkage by GENEHUNTER. Because the functional role of BMP-4 in bone metabolism is well established, further investigation of molecular screening for BMP-4 is needed to detect the exact causality of OPLL.

SA112

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SA113

Genetic Determinants of Osteoporosis Susceptibility in a Female Ashkenazi Jewish Population. V. Kantorovich,[1] D. H. Cohn,*[2] H. Yang,*[2] L. S. C. Cheng,*[2] S. Chen,*[1] J. I. Rotter,*[2] J. S. Adams.[1] [1]Burns and Allen Research Institute, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA, [2]Human Genetics, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA.

Although multiple environmental factors influence the development of osteoporosis, the major determinant for the disease is oligogenetic control of the achievement of peak bone mass. To identify loci that determine susceptibility to osteoporosis, we initiated the study of a single ethnic group, Ashkenazi Jews, where the incidence of osteoporosis is known to be high. Here we present bone phenotype data from 162 subjects in 56 different families in our Ashkenazi Jewish cohort and preliminary results from candidate gene linkage analysis. We also studied 69 control patients who included both non-Jewish and non-Ashkenazi subjects from families with osteoporosis/osteopenia, as well as Ashkenazi Jewish subjects without a family history of osteoporosis. Compared to the control population, Ashkenazi Jewish probands had significantly lower age- and sex-matched values of mean (±SE) bone mineral density at both lumbar spine (lumbar Z score 0.060±1.380 vs. -0.510±1.050; p=0.006) and femoral neck (hip Z score -0.400±0.900 vs. -0.900±0.620; p=0.0003). Variant Component Analysis, based on 94 sib-pairs and with adjustment for age, was used to estimate the genetic component of bone density. While heritability was found to be insignificant for lumbar bone mass, it showed high significance for both T and Z scores at the femoral neck, 0.34 (p=0.0001) and 0.38 (p=0.0017), respectively. We further carried out linkage analysis for candidate genes, starting with the genes that encode type I collagen. Genotypes were determined at microsatellite loci either tightly linked or within the COL1A1 and COL1A2 genes, respectively. Using SIBPAL, both qualitative and quantitative analysis of linkage to osteoporosis was calculated. No significant support for linkage was found with these markers. However, the proportion of allele sharing at COL1A2 among affected pairs was 0.62 (p=0.051) suggesting that the shared allele may contribute to disease susceptibility. We conclude that: 1] the Ashkenazi Jewish population shows significantly lower bone mass at the femoral neck and lumbar spine compared to our mixed-ethnicity control population; 2] there is significant inheritance of decreased bone density at the femoral neck; 3] with the initial number of families studied, there was no linkage between bone mineral density and the markers tested for the type I collagen; and 4] there was increased allele-sharing at COL1A2 among affected pairs, providing evidence that this locus may contribute to osteoporosis.

SA114

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SA115

Geometry as a Heritable Determinant of Bone Strength. D. H. Goddard,[1] C. Goddard,*[2] J. Hecht,*[3] E. Kim,*[4] E. R. Myers,[4] R. D. Blank,[4] R. S. Bockman.[4] [1]New York Methodist Hospital & Arthritis & Osteoporosis Center, Brooklyn, NY, USA, [2]Arthritis & Osteoporosis Center, Brooklyn, NY, USA, [3]HAFTR High School, Lawrence, NY, USA, [4]Hospital for Special Surgery, New York, NY, USA.

Fracture susceptibility is determined by a combination of genetic and environmental factors, which act in concert to establish whole bone strength. Whole bone strength depends on the mineral and organic content as well as geometry and the integrity of the structural components of the bone. Recent studies have focused on bone geometry as a potential predictor of fracture risk in specific family groups with high fracture susceptibility. Hypothesizing that individuals with similar volumetric bone mineral densities (vBMD) might differ markedly with regard to bone geometry, we used peripheral QCT scans of the forearm to measure vBMD, bone diameters, and to calculate the maximum cross-sectional moment of inertia (I) in a 3-generation family. Typical scans of the radius using a Stratec XCT-2000, peripheral QCT are shown for the 68 year old father, the 67 year old mother and the 45 year old daughter in the figure below.

family	vBMD (mg/cc)	1 mm4
father	1140	1379
mother	1225	628
daughter	1215	356

While mother and daughter have similar vBMDs, they display considerable differences in the cortical diameters. This results in large differences in the calculated I. It is not possible to determine the contribution of aging to the mother-daughter difference in bone size from these data alone. A striking sexually dimorphic difference was observed in the I values for the father and mother. All members of this family have low BMD values, however, it is the striking difference in bone size that highlights the potential importance of geometry as a determinant of bone strength and in evaluating fracture susceptibility.

Disclosures: Merck & Co,2,8; Eli Lilly Co,2; Proctor & Gamble,8.

SA116

Genetic Regulation of the Bone Regenerative Capacity: Studies of 12 Inbred Strains of Mice. X. Li,* W. Gu,* G. Masinde,* C. Rundle,* S. Mohan,* D. Baylink.* Jerry L. Pettis VA Medical Center, Loma Linda, CA, USA.

Bone is one of the few tissues that has innate ability to regenerate upon damage. Based on the recent findings that ear-tissue regeneration in mice is genetically controlled, we hypothesized that bone regenerative capacity is different among the inbred strains of mice and that the variation in bone regeneration is determined by genetic factors. To evaluate this hypothesis, we have developed a "drill-hole" model in the tail vertebra of mice, a model which allows us to reproducibly introduce an injury with a defined boundary and quantify the rate of bone healing (CV≈6%) using the combination of high resolution Faxitron X-ray images and a ChemiImager^{TM} 4000 Low Light Imaging System. Using this model, we have found that bone regenerative capacity differs significantly among the inbred strains of mice (ANOVA, p<0.001). Of the 12 inbred strains tested, the order of bone regenerative capacity was Sencar/PtJ>129J>C3H/HeJ>DBA/1J>NZB/BinJ>LP/J>LG/J>KK/HiJ>RIIIS/J>C57BL/6J >FVB/NJ>CBA/J. Sencar/PtJ has been identified as a suitable model for the study of hard-tissue regeneration. It healed 3 times as fast as slow healer, CBA/J (70.7±14 relative density unit for Sencar/PtJ vs 23.6±5.5 for CBA/J. p<0.001). Heritability estimate revealed that 72% of variation in the healing of tail vertebra is genetically controlled. To determine if bone and ear tissue regeneration is under the control of common genetic mechanisms, we evaluated the correlation between the two phenotypes. We found no significant correlation in healing capacity between soft-tissue and bone-tissue among the 12 inbred strains (r=-0.08, p=0.67), suggesting that different set of genes may regulate the two phenotypes (the order of ear-healing capacity: LG/J>CBA/J>Sencar/PtJ>RIIIS/J>DBA/1J >LP/J> C3H/HeJ>KK/HiJ >C57BL/6J>FVB/J>NZB/BinJ>129J). We next determined if the ability to heal bone is correlated with the BMD phenotype. It was found that tail vertebral regeneration correlated positively (r=0.49, p<0.01) with total body BMD by DEXA, suggesting that BMD and bone regeneration may be regulated by some common genetic mechanisms. Conclusions: 1) Inbred strains of mice exhibit variation in the ability to heal drill hole in the tail vertebra. 2) 72% variation in bone healing capacity is genetically regulated. 3) Some common genetic mechanisms may regulate BMD and bone regeneration. 4) Different genetic mechanisms may regulate bone- and soft-tissue regeneration.

SA117

See Friday Plenary number F117.

SA118

Genetic Heterogeneity in Familial Renal Magnesium Wasting. V. Kantorovich,[1] R. K. Rude,[2] J. E. Gaines,*[2] X. Guo,*[3] M. R. Pandian,*[4] D. H. Cohn,*[3] J. S. Adams.[1] [1]Endocrinology, Diabetes and Metabolism, UCLA/Cedars-Sinai Medical Center, Burns and Allen Research Institute, Los Angeles, CA, USA, [2]Endocrinology, Diabetes and Metabolism, USC/LA County Hospital, Los Angeles, CA, USA, [3]Human Genetics, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA, [4]Corning Nichols Institute, San Juan Capistrano, CA, USA.

Although Mg depletion is usually an acquired disorder, there are several rare inherited forms of isolated Mg deficiency. Two familial forms of primary hypomagnesemia due to renal Mg wasting have been described. One form (OMIM #248250) is inherited in an autosomal recessive fashion and results from mutations in the paracellin 1 gene (PCLN1) on chromosome 3. Another is an autosomal dominant form (OMIM #154020) originally described in two unrelated Dutch families. The related gene was localized to chromosome 11q23 and identified as the Na+, K(+)-ATPase gamma subunit gene FXYD2. Its product is expressed in the epithelial cells of the renal distal convoluted tubule and participates in Mg reabsorption. Here we describe an American family with a phenotype similar to OMIM #154020 but without linkage to 11q23. The index case, a 36 year old female, presented with paraesthesias, muscle weakness and seizures. Her evaluation showed hypomagnesemia and hypermagnesuria, which persisted despite aggressive Mg replacement. Prospective assessment revealed 6 additional subjects with frankly low serum Mg from a

total of 22 family members. Compared to unaffected relatives and normal controls, affected family members harbored significant reductions in serum (1.1±0.2 vs. 1.9±0.1, p<0.001 [normal 1.7-22 mg/dL]) and lymphocyte (1.0±0.2 vs. 1.5±0.4, p=0.01 [normal 1.2±0.2 µg/mg protein]) magnesium concentrations. The mean fractional clearance of Mg during a normomagnesemic clamp was significantly elevated in 7 affected subjects (proband and 6 family members) compared to unaffected family members (p<0.001) and controls (p<0.001). Bone mineral density at the femoral neck, but not at the lumbar spine, was significantly reduced (p=0.03) in affected family members. Genotypes were determined using reported markers: D11S4092, D11S4127, D11S4195, D11S1356, and D11S4104. Regardless of the level of penetrance specified, multi-point LOD scores for 5 different loci from the 11q23 region were less than 2.97 in all 22 individuals. We conclude that this hypermagnesuric hypomagnesemic pedigree differs from previously reported families in that 1] it shows no linkage to the 11q23 locus and 2] hypomagnesemia in this family is associated with low bone mass at the femoral neck.

SA119

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SA120

The Relationship Between Serum Insulin-like Growth Factor-I(IGF-I),IGF-I Gene Polymorphism and Bone Mineral Density in Postmenopausal Korean Women. J. Kim,*[1] K. Roh,*[1] V. Yoo,[2] S. Moon,*[1] J. Lee.*[1] [1]Department of Obstetrics & Gynecology, Seoul National University Hospital, Seoul, Republic of Korea, [2]Cheongvak Primebeyond Hospital, Seoul, Republic of Korea.

The objectives of this study were to test the hypothesis that cytosine adenine (CA) polymorphism in insulin-like growth factor-I (IGF-I) gene is associated with serum levels of IGF-I and biochemical markers of bone turnover, and to investigate the relationship between this IGF-I gene polymorphism and bone mineral density (BMD) in postmenopausal Korean women.. IGF-I CA polymorphism was analyzed in 300 postmenopausal Korean women by GeneScan and direct DNA sequencing, and IGF-I was measured by radioimmmunoassay after Bio-gel P-10 preparation of serum samples. Serum bone alkaline phosphatase (BAP) and CrossLaps were measured by ELISA and BMD at the lumbar spine and proximal femur by dual energy X-ray absorptiometry. Ten IGF-I alleles were observed with product sizes ranging between 176-196 base pair (bp), and five most common allele distributions were as follows: 188 bp 26.2%, 194bp 19.8%, 186bp 18.2 %, 190bp 15.5%, and 192bp 14.3%. Guanine and adenine deletion were found following 3' end of CA repeats. After adjusting for age, years since menopause, and body mass index, serum IGF-I and BMD at most skeletal sites measured in women homozygous for 194bp allele among five major alleles were significantly higher than that in the 194bp heterozygotes or women who did not possess 194bp allele. A significantly decreased prevalence of 194/194 genotype in osteopenic and osteoporotic women was observed, compared with normal women. No correlation between IGF-I genotypes and adjusted biochemical markers of bone turnover was found. Compared with normal women, serum BAP levels were higher in osteoporotic women while no significant differences in serum crosslaps levels were observed. IGF-I gene CA polymorphism does not affect serum levels of biochemical markers of bone turnover and that this polymorphism is one of genetic factors which may affect circulating IGF-I levels and BMD at the lumbar spine and proximal femur.

SA121

Hyperthyroidism, Loss of Bone Mass, and their Relation to Vitamin D Receptor (VDR) Gene Bsm1 and Interleukin-1 Receptor Antagonist (IL-1RN) Gene VNTR Polymorphisms in Postmenopausal Women. E. Bajnok,* I. Takacs, A. Tabak,* G. Speer,* Z. Nagy, C. Horvath, P. Lakatos. 1.st Department of Medicine, Semmelweis University, Budapest, Hungary.

The genetic mechanisms on thyroid hormone-stimulated bone loss have not yet been established. Recently, an association has been found between the number of VNTR polymorphism of the IL-1RN gene and postmenopausal bone loss. Moreover, "allele A2" has been implicated in the development of Graves' disease (GD). The relationship between Bsm1 polymorphism of VDR gene and bone mineral density (BMD) has been reported in numerous population, however the results are controversial. Bsm1 genotypes seem to be associated with GD, and with BMD in hyperthyroidism (HT), too. In this study, we determined the previously described IL-1RN VNTR and VDR-Bsm1 polymorphisms to test their possible functional contribution to the pathogenesis of hyperthyroidism in 208 women. We genotyped 61 patients with GD (18-69 years) and 84 with toxic adenoma (TA) (27-78 years) and compared the genotype frequencies to that of 63 healthy controls (45-83 years). From this cohort, were investigated an association between these polymorphisms and BMD in 162 postmenopausal women. This group consisted of 62 healthy (mean age 56,2 ± 6,6 years) and 100 hyperthyroid subjects (mean age 53,7 ± 8,3) 40 patients with GD (mean age 50,1 ± 7,9 years) and 60 with TA (mean age 55,5 ± 8,1 years). BMD was measured by DXA at the lumbar spine and femoral neck. We found significant difference in the distribution of VDR genotypes, but not in case of IL-1RN. The bb genotype was significantly more frequent in TA than in the control and G group (p=0,015). No genetic polymorphism was associated with BMI and age adjusted BMD at any measured site neither in HT nor in control group. Our data do not support the hypothesis that IL-1RN gene VNTR or VDR Bsm1 polymorphism have an impact on BMD in hyperthyroid postmenopausal women. However, VDR gene might affect the development of toxic adenoma.

SA122

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SA123

Lack of Association Between CaSR Gene A986S Polymorphism and Bone Density in Hungarian Postmenopausal Women. I. Takacs, G. Speer,* E. Bajnok,* Z. Nagy, A. Tabak,* C. Horvath, L. Kiss,* P. Lakatos. 1.st Department of Medicine, Semmelweis University, Budapest, Hungary.

Calcium-sensing receptor (CaSR) is an attractive candidate gene for osteoporosis susceptibility. CaSR A986S genotype has been shown to have an effect on serum calcium. Recently an association has been reported between the CaSR gene A986S polymorphism and the bone mineral density in healthy Caucasian girls. In this study, we examined whether CaSR gene A986S polymorphism is associated with decreased bone mass in 231 Hungarian postmenopausal women (age range:40-70). From this cohort, 109 osteoporotic (OP) patients (mean age:57.6±0.6) were compared with 122 (mean age:58.5±0.5) healthy control (C) women. Bone mineral density (BMD) was measured at the lumbar spine (L2-4) and femoral neck using DEXA method. Allele-specific PCR was used to amplify A986S polymorphisms of the CaSR gene. No significant effect of CaSR genotype on BMD was observed either in the whole population or in the subgroups. Our data do not support the idea that CaSR gene A986S polymorphism has an impact on bone mass in postmenopausal women.

SA124

Two Recently Described SNPs in a COL1A1 Regulatory Region Lie Within Nuclear Factor Binding Sites. N. Garcia-Giralt,*[1] A. Enjuanes,*[2] X. Nogués,*[3] L. Mellibovsky,*[3] A. Díez-Pérez,[3] D. Grinberg,*[4] S. Balcells.*[4] [1]Genetics, Universitat de Barcelona, Barcelona, Spain, [2]IMIM, Barcelona, Spain, [3]Hospital del Mar, Barcelona, Spain, [4]Dept. of Genetics, Universitat de Barcelona, Barcelona, Spain.

Polymorphisms in the regulatory region of the genes encoding type I collagen chains might be important for BMD variability. We have recently described two SNPs in the COL1A1 promoter and showed that they were associated to BMD in Spanish postmenopausal women. The aim of the present study was to analyze the binding of the regions containing the SNPs to primary osteoblast nuclear factors as a functional assay to contribute to the understanding of the molecular basis of this association.Electrophoretic mobility shift assays (EMSA) were used to detect binding of nuclear proteins to 25-30-mer oligonucleotides containing the polymorphic site in a central position. Both single and double-stranded DNA radiolabelled probes were used and competition assays with cold oligonucleotides containing known DNA binding site were performed. Nuclear extracts were prepared from 70% and 100% confluent primary osteoblast cells and from MG-63 osteosarcoma cell line. Southwestern studies were also carried out as a first characterization of the proteins bound to the probes. EMSA showed specific binding for both polymorphismic sites. For one of the SNPs, named PCOL1, only the forward ssDNA probe binds to the nuclear proteins. Competition assays allowed us to detect different binding capacities for the two alleles, the most frequent one having the highest. A Southwestern analysis performed to characterize the proteins involved in the binding, detected an 80 kD protein. A weaker band corresponding to a 48 kD protein was also seen.Nuclear factor binding to the other polymorphic site, PCOL2, was shown by EMSA both with ss-DNA (reverse) or ds-DNA probes, but was clearly stronger when the ss-DNA probe was used. The forward strand displayed a different higher molecular weight shift. Again, clear differences between the two alleles were observed. Southwestern studies for PCOL2 detected a protein in the 60-70 kD range and a smaller one of around 40 kD.In conclusion, we showed that the two SNPs lie within nuclear factor binding sites which preferentially bind proteins as ss-DNA. We also demonstrated differential binding capacities for the two alleles of each polymorphism. Finally, a preliminary characterization, in terms of molecular weight, was performed for the proteins binding to these COL1A1 regulatory regions.

SA125

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SA126

Short Term Fibroblast Growth Factor-2 Treatment Increases Osteoblast Differentiation and Bone Nodule Formation, in Bone Marrow Cultures From Young and Adult Mice. M. M. Hurley, T. Sobue, X. Zhang. Endocrinology & Metabolism, University of Connecticut Health Center, Farmington, CT, USA.

Fibroblast growth factor-2 (FGF-2) has been shown stimulate bone formation in vitro and in vivo in rats. However, there are few studies in mice and only limited data on the mechanism (s) by which FGF-2 induces new bone formation. We therefore assessed whether short term treatment of bone marrow stromal cells from 2 month and 6 month old black swiss/129 mice with FGF-2 would increase alkaline phosphatase positive (ALP), mineralized colony formation as well as the expression of genes important in osteoblast maturation and bone formation. Bone marrow cells were plated at $3x10^6$ cell/well in complete media, αMEM with 10% heat inactivated fetal calf serum, 10 nM dexamethasone, 50 µg/ml ascorbic acid, and 8 mM beta-glycerophosphate in the absence or presence of varying concentrations of FGF-2 (0.01, 0.1, 1.0 nM). After 3 days, media were changed to control media without further additon of FGF-2. Cultures were stained for ALP and von-Kossa

for mineralization at 14 and 21 days. Colony area was measured using NIH IMAGE-1-61. Short term treatment with FGF-2 (0.01, 0.1, 1.0 nM) for 3 days increased the number and area of ALP colonies and colony at 14 and 21 days in marrow stromal cultures from 2 month as well as 6 month old mice. At 14 days, the maximum increase in colony number (14.7±1.4 vs 7.0±1.5) and colony area (0.26±0.03 vs 0.09±0.02) was observed in marrow stromal cultures from 2 month old mice treated with 0.1 nM FGF-2. There was also a significant increase in colony area (0.58±0.07 vs 0.24±0.05) in marrow stromal cultures from 6 month old mice that were treated with 1 nM FGF-2. Northern analysis of cultures from 2 month old mice treated with FGF-2 for the first 3 days of culture showed that FGF-2 (0.1 nM) increased type 1 collagen (COL1A1) mRNA by 152 and 134%; osteocalcin (OC) by 227 and 177%; core binding factor alpha (Cbfa1) by 123 and 121%; and insulin-like growth factor 1 (IGF-1) by 153 and 134% at 14 and 21 days respectively. Similar results were obtained in marrow cultures from 2 and 6 month old mice treated with 1 nM FGF-2 for the first 3 days of a 14 or 21 day culture period. In conclusion, short term exposure of bone marrow stromal cultures derived from young and older mice to FGF-2 significantly increased ALP colony number, size and mineralization. The increase in colony number probably represents enhanced recruitment of osteoblast progenitors by FGF-2. The increase in colony size is probably due to expansion of clones in response to FGF-2. Since these cultures showed increased expression of COL1A1, OC, Cbfa1 and IGF-1 mRNAs, we conclude that short term FGF-2 treatment can also enhance osteoblast maturation in vitro.

SA127

Basic Fibroblast Growth Factor (bFGF) Directly Stimulates Bone Pit Resorption by Isolated Osteoclasts (OC) and Promotes OC Recruitment, Development and Resorption in Association with Neoangiogenesis In Vivo. P. Collin-Osdoby, L. Rothe, S. Bekker,* F. Anderson, P. Osdoby. Biology, Washington University, St. Louis, MO, USA.

Increased local bone resorption coincides with regional angiogenesis in bone development, fracture healing, and pathological disorders such as inflammatory-related periodontal disease, rheumatoid arthritis, or tumor-associated osteolysis. Angiogenic stimulation facilitates delivery of hematopoietic precursor cells into tissues and causes the adhesion, activation, transmigration, and differentiation of cells via signals expressed on the surface of vascular endothelial cells (VEC). Thus, angiogenesis may enable greater numbers of pre-OC to emigrate from the circulation into bone and develop into resorptive OC. Recently, we showed that VEC can directly promote OC development in vitro from circulating monocytic or bone marrow precursors, and that inflammatory cytokines upregulate RANKL on human VEC thereby stimulating co-culture human OC formation and bone pit resorption. Here, we employed a chick chorioallantoic membrane (CAM) model of angiogenesis and OC development and function to investigate whether a potent angiogenic stimulator, bFGF, could promote OC recruitment, differentiation and resorption in vivo. Focal implantation of bFGF (0.5 ng) in an agarose plug adjacent to a bone chip on the CAM elicited an angiogenic response within 9 days. This was accompanied by significant increases (1.5 to 2.5-fold) in the mean number of OC formed, number of pits excavated, and total area of bone resorbed by OC in vivo. Increased resorption was a function of both increased OC numbers as well as OC activation since the area resorbed/OC was also increased (>1.5-fold), primarily due to OC initiating more resorption sites (pits/OC) rather than producing larger lacunae (area/pit). In vitro, bFGF also directly targeted mature isolated avian OC to stimulate their bone pit resorption. In contrast, bFGF did not directly influence in vitro pre-OC differentiation in avian bone marrow cell cultures. Thus, angiogenesis is an important component of bFGF stimulated OC development in vivo, and may involve bFGF-induced RANKL in VEC. Although bFGF has recently generated much interest as a potential bone anabolic agent, such effects may be transient and newly formed bone subsequently resorbed. Moreover, inflammatory elevated bFGF levels are directly linked to the extent of OC formation, joint destruction, and disease severity seen in rheumatoid arthritic patients. Our results therefore suggest that bFGF may contribute to inflammatory bone loss through direct and indirect mechanisms that stimulate angiogenesis and promote localized OC recruitment, formation and bone resorption.

SA128

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SA129

Elevated mRNA Gene Expression of Fibroblast Growth Factor 23 (FGF23) in the Thymus of X-Linked Hypophosphatemic (*Hyp*) Mice. R. A. Meyer, M. H. Meyer. Department of Orthopaedic Surgery, Carolinas Medical Center, Charlotte, NC, USA.

Mutations of fibroblast growth factor 23 (FGF23) cause bone disease in autosomal dominant hypophosphatemic rickets (ADHR; Nature Genetics 26:345, 2000) as do mutations of *Phex* in X-linked hypophosphatemia. The similarities in physical symptoms and biochemical abnormalities in these two diseases suggest a common mechanism. To test whether FGF23 activity is altered in animals with mutation of the *Phex* gene, expression of FGF23 mRNA was measured in several organs of normal and X-linked hypophosphatemic (*Hyp*) mice. FGF23 has been reported to be expressed in the thalamus and thymus of the mouse (Biochem.Biophys.Res.Commun. 277:494, 2000). These organs were examined along with kidney and liver. In this study, primers were designed from the mouse cDNA sequence for FGF23 (GenBank Accession: AF263536). mRNA expression was measured by reverse transcription - polymerase chain reaction (RT-PCR) from mRNA prepared from individual mice at 4 weeks of age, normal and *Hyp*, male and female, on a C57BL/6J background. The amplimers were separated by electrophoresis and blotted to nylon membranes. The blots were probed with a ^{32}P-labeled internal oligonucleotide specific for FGF23. The ^{32}P was quantified with a phosphor imager in units of photo stimulated luminescence (PSL). No detectable amplimers were found for FGF23 in the diencephalon (epithalamus, thalamus and hypothalamus) or in the kidney. Amplimers of the predicted size were found in the liver and thymus. The mRNA expression in the liver was too variable to use for conclusions. However, the thymus had higher expression of FGF23 in the *Hyp* mice (2067 ± 217 (7) PSL, mean ± SEM (n)) than in the genetically normal mice (1275 ± 206 (7), P = 0.02). These data indicate that inactivation of *Phex* leads to up-regulation of FGF23 expression. There may be an interrelationship between these two genes in the regulation of phosphate homeostasis.

SA130

Recombinant FGF-23 Interacts in vitro with FGF Receptors 2 and 4. K. B. Jonsson,[1] M. Pragnell,*[2] T. Larsson,*[1] K. White,[3] M. Econs,[3] S. Schiavi.*[2] [1]Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [2]Applied Genomics, Genzyme, Framingham, MA, USA, [3]University of Indiana School of Medicine, Indianapolis, IN, USA.

Mutations in the gene encoding fibroblast growth factor 23 (FGF-23) are the most plausible cause of autosomal dominant hypophosphatemic rickets (ADHR) and high concentrations of its mRNA were recently identified in tumors that are associated with oncogenic osteomalacia (OOM). It remains uncertain, however, how ADHR-specific mutations or overexpression of FGF-23 in OOM tumors leads to phosphaturia or which receptor(s) mediates these effects. To address these issues, we expressed cDNAs encoding FGF-23 tagged at the C-terminus with a V5-epitope (FGF-23[V5]) and FGF-23 with one of the ADHR mutations (FGF-23[R176Q-V5]) in E. coli, Sf9, and COS-7 cells. Immunoblot analysis of supernatants from COS-7 and Sf9 cells expressing FGF-23[V5] revealed two distinct protein bands of =35 kD and =15 kD. In the presence of the R176Q mutation, the intensity of the lower molecular weight form of FGF-23 was dramatically reduced. Furthermore, purified recombinant, full-length FGF-23[R176Q-V5] was not cleaved when incubated with untransfected COS-7 cells. To investigate whether FGF-23 interacts with any of the known FGF receptors, we assessed binding of FGF-23[V5] to the recombinant extracellular domains of the FGF receptors 1-4 fused to the IgG-Fc fragment. Conditioned media from COS-7 cells expressing FGF-23[V5] was incubated with each of these FGF receptor-Fc chimeras and the resulting complexes were precipitated with immobilized protein A; FGF-1 and anti-FGF-1 antibodies served as positive controls. FGF4R-Fc showed the highest interaction with FGF-23[V5]. A weaker interaction was detected with FGFR2-Fc whereas binding to FGFR1-Fc and FGFR3-Fc was not detected. These results suggest that FGFR4, and possibly FGFR2 are receptors for FGF- however, an additional yet unknown receptor for FGF-23 cannot be excluded.

SA131

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SA132

Different Signaling Pathways Are Involved in Runx2 Expression and RUNX2 Activation by FGF/FGFR Signaling. H. Kim,*[1] M. Park,*[1] J. Kim,*[1] S. Bae,*[2] H. Kim,*[3] H. Ryoo.[1] [1]Biochemistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [2]Biochemistry, School of Medicine, Chungbuk National University, Chungju, Republic of Korea, [3]Pediatric Dentistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea.

Fibroblast growth factor-2 (FGF-2) or FGF-4 strongly induced the expression of Runx2, a master transcription factor of osteoblast differentiation. However, underlying molecular signaling mechanisms involved in the FGFs-induced Runx2 expression haven't been clearly understood. We identified FGF-signaling not only induced Runx2 expression but also activated transcriptional function of the RUNX2 protein. Consistent with the result by FGFs, overexpression of constitutive active FGFR2 mutants (human Crouzon syndrome) also increased Runx2 expression and RUNX2 transactivational activity. FGF-signaling cascades were tested to determine their role in Runx2 expression and RUNX2 transcriptional function by using pathway specific inhibitors. PD98059, an Erk-specific inhibitor, and SB203580, a p38-specific inhibitor, didn't influence on FGF2-induced Runx2 expression but suppressed FGF2-induced RUNX2 transcriptional activity 5 folds and 2 folds, respectively. In contrast, blocking of JNK pathway didn't change Runx2 expression as well as its transcriptional activity. Calphostin C, a protein kinase C-specific inhibitor, strongly blocked FGF2-induced Runx2 expression and consequently almost completely suppressed RUNX2 transactivation function determined with 6XOSE2-Luc stable cells. Moreover, PKC pathway blocker also suppressed the transactivation function of exogenously expressed RUNX2 in Runx2-/- cells. These results indicate FGF/FGFR-induced Runx2 expression is resulted from the activation of PKC pathway, and the enhancement of RUNX2 transcriptional activity by FGF siganling is resulted from the activation of PKC pathway or Erk (for minor part by p38) MAP kinase. To analyze functional relevance of Runx2 in the pathogenesis of FGFR mutation-related craniosynostosis, we introduced mouse calvarial organ culture system. FGF2 beads placed directly on the osteogenic fronts of the sagittal suture of E15 mouse calvaria strongly accelerated the suture closure and Runx2 expression after 24 hours compared to control group. As expected in previous results, FGF2-induced acceleration of suture closure was blocked by PKC or ERK inhibitors. These results suggest expression of Runx2 and activation of RUNX2 by FGF/FGFR signaling employ different signaling machineries and provide the direct relevance of Runx2 in the pathogenesis of FGFR mutation related premature suture closure.

SA133

PDGF-CC A Novel Gene That Exhibits BoneAnabolic Activity. G. V. Krishnan,[1] S. Na,[2] L. Hammond,[2] A. Glasebrook,[2] W. Yeh,[2] L. Myers,[2] A. Kharitonenkov,[2] E. W. Su,[2] H. Song,[2] A. G. Geiser,[1] Y. L. Ma,[1] Q. Zeng,[1] E. C. Black,[1] C. A. Frolik.[1] [1]Gene Regulation, Bone & Inflammation, Eli Lilly and Company, Indianapolis, IN, USA, [2]Bio RT&P, Eli Lilly and Company, Indianapolis, IN, USA.

PDGF-CC is a novel cDNA encoding an N-terminal CUB-like domain with a C-terminal PDGF/VEGF motif. It is a member of the cysteine-knot family of proteins that includes NGF, TGF-β and VEGF. The 3.4 kilobase transcript of PDGF-CC is expressed in most tissues and it encodes a 354 amino acid protein with an approximate MW of 38 kDa. Full-length PDGF-CC shares approximately 26% identity with PDGF-B. PDGF-CC protein was purified to near homogeneity after over-expression in HEK293T cells. We have employed a rat neonate metatarsal organ culture model that measures increases in endochondral ossification in response to bone anabolic agents. PDGF-CC was shown to increase bone anabolic activity (17% ± 4) compared to PTH 1-38 (20% ± 3) in an ex-vivo organ culture model. PDGF-CC has been shown to target the PDGF-α receptor in vitro and co-treatment with Tyrphostin 1295 an inhibitor of PDGF-R tyrosine kinase, blocks its bone anabolic activity. PDGF-CC induces its bone anabolic activity in the organ culture model via a MAPK dependent step. Co-treatment with the MEK/ MEKK inhibitor U0126 results in complete loss of PDGF-induced bone anabolic activity. PDGF-CC does not directly induce osteocalcin promoter activity or expression and co-treatment of antisense Cbfa1/ Osf-2 oligonucleotides along with PDGF-CC results in minimal change in bone anabolic activity. In contrast, co-treatment with antisense c-fos oligonucleotides results in partial reversal of bone anabolic activity. In this report we have identified a novel target for bone anabolic activity that mediates its activity via the PDGF-α receptor.

SA134

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SA135

Enhancement of Bone Induction by in-vivo Reduction of Proinflammatory Cytokines in the Rat. G. Voggenreiter,[1] M. Majetschak,*[1] M. Bardenheuer,[1] U. Obertacke,[1] F. U. Schade.*[2] [1]Department of Trauma Surgery, University Hospital Mannheim, Mannheim, Germany, [2]Clinical Research Group Schock & Multi Organ Failure, University Hospital Essen, Essen, Germany.

The influence of the reduction of proinflammatory cytokines on bone induction by demineralized bone matrix (DBM) was investigated. Cytokine synthesis was reduced by repeated injections of lipopolysaccharide (LPS). This constitutes a state of endotoxin tolerance with a consecutive reduction of the synthesis of proinflammatory cytokines. Materials and Methods: Prior implanting DBM (day 0) endotoxin-tolerance (ET) was induced by i.p. injection of LPS in concentrations of 0.1 mg/kg BW on day -7 and of 0.5 mg/kgBW on days -6, -5, -4 and -3. On day 0 50mg of DBM were implanted into the abdominal wall of adult Lewis rats in six groups (7 rats each): isogeneic (iDBM) and xenogeneic (xDBM) DBM (controls); iDBM and xDBM with short-term ET (SET) and iDBM and xDBM with long-term ET (LET). For LET injections of LPS (0.5mg/kgBW) were continued after surgery every other day. In-vitro cytokine synthesis was evaluated by TNF-alpha ELISA on days -7, 0, 7 and 28. Animals were sacrificed after 4 weeks and the bone remodeling of the proximal tibial metaphysis as well as the induced new bone were evaluated by quantitative histomorphometry (guidelines of the ASBMR). Comparisons between groups were done using unifactorial analysis of variance and with the Scheffè test. Linear regression analysis was used for correlation of TNF-synthesis and histomorphological parameters.Results: Implantation of iDBM and xDBM alone had no influence on TNF-synthesis. Induction of ET yielded in a significant reduction of TNF-synthesis on day 0 ($p < 0.05$). Furthermore in the LET groups the TNF-synthesis was reduced significantly at day 7 but no longer at day 28. However TNF-synthesis was reduced in 7 out of 14 rats at day 28. In all groups histomorphometry revealed no differences in the parameters obtained from the tibial metaphysis, but SET and LET showed a twofold increase of the induced bone volume. However it has to be marked that LET was only effective in 7/14 rats on day 28. The subgroup with effective LET demonstrated a threefold increase in bone volume compared to control animals. Based on the values of the isogeneic control group and the isogeneic SET and LET animals a significant linear relationship between the induced bone volume and TNF- synthesis was evident ($r=-0.68$, $P < 0.001$).Conclusion: Induction of endotoxin tolerance increases bone induction in isogeneic DBM. By means of TNF-alpha synthesis a linear relationship between the reduction of cytokine synthesis and bone formation has been detected.

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SA137

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SA138

Expression and Localization of VEGF and its Receptors in Osteoblasts and Bone Tissues. G. Hirata, K. Urabe, C. Li,* T. Shuto, A. Matsuo,* Y. Iwamoto.* Department of Orthopaedic Surgery, Kyushu University, Fukuoka, Japan.

Angiogenesis is an important process involved in normal bone growth and repair. Vascular endothelial growth factor (VEGF), an potent angiogenic factor, is reported to be involved in osteoclastogenesis. M-CSF and RANKL are essintial for osteoclastgenesis. VEGF substitutes for M-CSF in RANKL induced steoclast formation. Some osteogenic cytokines, BMP-4, BMP-7, TGF-β1 andFGF-2, and hormones, PTH and 1,25-$(OH)_2$Vit.D_3, induce the production of VEGF in osteoblasts. VEGF may be important in bone and mineral metabolism. However, the role of VEGF in osteoblastic bone formation is not well understood. We hypothesized that VEGF is expressed in osteoblasts and is involved in osteoblasic bone formation. The aim of the present study is to clarify the presence of VEGF in osteoblasts using various type of octeoblastic cells and bone tissues. Initially, we examined the expression of VEGF and its receptors in rat calvarial osteoblasts, human osteoblastic cell lines, rat and human bone tissues by RT-PCR. Fetal rat calvaria (FRC) cells were prepared as we previously reported (K. Urabe, J. Orthop. Res. 17:920, 1999). Rat calvaria for immunohitochemical study was obtained from 3 week old SD rats. Human trabecular bone was obtained from patients that underwent knee arthroplasty. Isoforms of rat VEGF, VEGF-122, 144 and 164, Flt-1/VEGFR1 and KDR/Flk-1/VEGFR2 were detected in FRC cells, and isoforms of human VEGF, VEGF-122, 165, 189 and 206, and Flt-1 were detected in SaOS2 and MG-63, human osteoblastic cell lines using RT-PCR. In order to localize VEGF and its receptor in bone tissues, immunohistochemical study was performed. Osteoblasts were positively stained with antibodies against VEGF and Flt-1 in rat calvaria and human trabecular bone. Because VEGF and its receptors are co-localized in osteoblasts and osteoblastic cells, VEGF may act on osteoblasts in an autocrine/paracrine fashion. It is possible that VEGF induces migration of osteoblasts to bone surface or regulates osteoblastic differentiation as is suggested previously. VEGF isoforms and its receptors may be important in regulation of bone metabolism.

SA139

Effect of TP508, A Synthetic Thrombin Peptide, on Growth Factor Expression During Femoral Fracture Healing. H. Wang, J. Convery,* J. T. Ryaby. OrthoLogic, Tempe, AZ, USA.

TP508 is a synthetic 23 amino acid peptide, representing a receptor-binding domain of human thrombin. When clots dissolve, thrombin fragments are released and act on thrombin receptors to initiate healing. The aim of this study was to determine the effects of a single percutaneous injection of TP508 on the expression of Cbfa1, Collagen type II and VEGF during femoral fracture healing in rats. Close fractures at the midshaft of the femurs were created in Sprague-Dawley rats. TP508 was injected percutaneously into the fracture site. The expressions of three genes in fracture calluses were analyzed in TP508 treated groups and PBS control groups on days 4, 7, 9, 11, 14, 17, and 21 after fracture by using Relative Quantitative RT-PCR. At 1μg/100ml, Cbfa1 expression increased 3-fold at 4 and 21 days. Collagen type II was expressed at 4 days in TP508 treated calluses but not in the controls, and was approximately 5-fold greater than controls at 14 and 17 days. The expression level of VEGF was increased at almost all time points. At 10μg/100ml, Cbfa1 although reduced at 4 days but increased at all other time points. Collagen type II expression was increased 2-3 fold at 14 and 17 days; this was less than the stimulation observed with 1mg/100μl. VEGF expression was increased more than 2 fold at 17 days. TP508 has the potential to promote fracture healing since it increases the expression of Cbfa1, VEGF, and collagen type II.

Disclosures: OrthoLogic,1,3.

SA140

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SA141

IGF-I Modulates Bone Resorption by Decreasing Osteoprotegerin/ RANKL Ratios in vitro and in vivo. J. Rubin,[1] L. Zhu,*[1] X. Fan,*[1] T. Murphy,*[1] C. Ackert,[2] W. G. Beamer,[2] C. J. Rosen.*[3] [1]Emory University and VAMC, Decatur, GA, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA, [3]Maine Center for Osteoporosis Research and Education, St. Joseph Hospital, Bangor, ME, USA.

Insulin-like growth factor-I (IGF-I) is considered a coupling factor for bone remodeling as it has a potent role in stimulating bone formation and collagen synthesis. By contrast, the effects of IGF-I on bone resorption are not well defined. We previously reported that growth hormone (rhGH) treatment in elders is associated with increased serum IGF-I, enhanced N-telopeptide (NTx) excretion, and bone loss at several skeletal sites. We thus hypothesized that IGF-I might induce resorption via changes in osteoprotegerin (OPG) and RANKL expression. Here we examined OPG and RANKL expression in response to IGF-I in mouse ST-2 stromal cells, and measured serum OPG levels in elderly women treated with placebo, low or high doses of rhGH. In ST2 cells we found a dose dependent decrease in OPG expression by RT-PCR such that IGF-I concentrations of ≥20 ng/ml were associated with a significant reduction in OPG in ST2 cells. Northern analysis confirmed that

OPG expression was maximally suppressed (37.0 ± 1.8%; p<0.002) by 100 ng/ml IGF-I at 24, 48 and 72 hours (p<0.01). Northerns also showed that IGF-I coordinately upregulated RANKL mRNA by 24 h to levels comparable to those achieved with 10 nM vitD in ST2 cells. To test the in vivo effects of IGF-I on OPG, we examined serum in 9 subjects from an earlier randomized trial of rhGH; 3 subjects were treated with placebo (P), 3 with 0.0025 mg/kg/day rhGH (LD-Rx), and 3 with 0.005 mg/kg/day rhGH (high dose-HD) for 1 year. At 3 and 12 mos in P, there were no changes in serum IGF-I, NTx or OPG, although serum IGF-I correlated inversely with OPG at all time points(r=-0.82, p=0.006). For the LD-Rx group, IGF-I increased significantly and there was an inverse relationship between change in NTx and change in OPG (r=-0.71,p=0.05). In the HD-Rx group, IGF-I levels increased two fold and were related to NTx (r=0.72,p=0.02); OPG levels declined nearly 25% (p<0.05) by 3 months with HD-Rx and correlated inversely with NTx (r=-0.60, p<0.09) at all time points. In summary, we report that IGF-I suppresses OPG and upregulates RANKL mRNAs in stromal cells in vitro and, in vivo, IGF-I is associated with decreased OPG and increased bone turnover. These data suggest that IGF-I may be important in coordinating OPG/RANKL expression both within the skeleton and in the circulation. This effect of IGF-I may have clinical significance with respect to rhGH as an anabolic agent in osteoporosis, and provide a possible mechanism for the observation that rhGH enhances bone resorption as well as formation.

SA142

See Friday Plenary number F142.

SA143

Differential Promoter Use and Tissue Specific Expression of IGF-I in Inbred Strains of Mice: Implications for Understanding the Role of Circulating and Skeletal IGF-I in Modulating Bone Formation. M. L. Adamo,[1] C. Ackert,[2] L. Donahue,*[2] W. G. Beamer,*[2] D. Powell,*[3] C. J. Rosen.[4] [1]The University of Texas, San Antonio, TX, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA, [3]Lexicon, The Woodlands, TX, USA, [4]St. Joseph Hospital, Bangor, ME, USA.

We previously reported that a high BMD mouse strain C3H/HeJ(C3H) has 30% greater serum IGF-I concentrations than a low BMD strain C57B/6(B6). Although bone formation is greater in C3H mice than B6 mice, the relationship of serum IGF-I to skeletal production and function is not clear. Thus, we examined the expression of IGF-I in several tissues, and in association with ongoing genetic studies, identified QTLs for the circulating IGF-I phenotype. We performed RNase protection assays(RPA), to assess IGF-I mRNA transcripts synthesized from both the major (P1) and minor (P2) promoters. In the liver of both strains, promoter 1(P1) transcripts exceeded promoter 2(P2) by >2:1 a pattern not different from other species. But, hepatic P2 expression was 2 fold greater in C3H than B6 (p<0.01). By contrast, in calvarial bone cells, there was a nearly five fold increase in P1 transcripts for C3H compared to B6(p<0.005). P2 transcripts were also increased in C3H compared to B6, but there were low levels in both strains (<10% of total IGF-I mRNA). Heart, kidney, brain and intestine showed no differences in either P1 or P2 transcripts by strain. In calvarial bone cells, P1 nuclear RNA was increased threefold in C3H vs B6 and whole bone mRNA from the femur demonstrated that C3H had 40% greater exon 1 mRNA transcripts than B6(p<0.05). In conditioned media from C3H calvarial OBs, there was twice the amount of secreted IGF-I /cell than B6 (2.4ng/10^6 cells vs 1.2 ng/10^6 cells;p<0.05). Finally, preliminary experiments indicate markedly increased phosphoAKT levels in OBs from C3H vs B6, suggesting increased autocrine IGF-I activation of the PI3 kinase/Akt pathway. In summary, we report both tissue and promoter specific expression of IGF-I for 2 inbred strains that differ in bone acquisition. Overall, C3H has enhanced IGF-I P1 and P2 expression compared to B6, a pattern that mirrors strain differences in serum IGF-I. In conclusion, we believe interstrain differences in serum IGF-I are due to altered hepatic expression of the peptide. The influence of strain on IGF-I expression in bone reflects differences in serum levels, but promoter use(P1vs P2)remains tissue specific . Quantitative trait analysis of serum IGF-I regulatory determinants should identify the transcriptional regulators of IGF-I that are growth hormone independent, but promoter and tissue specific.

SA144

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SA145

IGF-I Effects on Bone Accretion During Prepubertal Growth Phase Are Mediated Predominantly Via Mechanisms Independent of Growth Hormone (GH). S. Mohan,[1] L. R. Donahue,[2] C. Richman,[1] R. Guo,*[1] J. Wergedal,[1] D. Baylink.[1] [1]Pettis VAMC, Loma Linda, CA, USA, [2]The Jackson Lab, Bar Harbor, ME, USA.

Our data in mice show that prepubertal and pubertal growth periods are critical in the regulation of peak BMD. IGF-I knockout mice exhibited severe reduction in peak BMD, thus demonstrating that IGF-I is an important determinant of peak BMD. Based on the findings that IGF-I expression in bone is regulated by other osteoregulatory agents (estradiol, PTH), in addition to GH, we propose that IGF-I effects on peak BMD are mediated via a GH-independent mechanism, in addition to GH-dependent mechanism. Skeletal changes were evaluated at day 23 (prepubertal), 31 (pubertal), and 56 (postpubertal) in the whole femur by DEXA and in the mid-diaphysis by pQCT in mice lacking GH (*lit/lit*) and compared to mice lacking functional IGF-I. Bone strength was measured by 3-point bending. Values are % of corresponding age-matched control mice (mean; 8-20) and significant at p<0.01.

	Day 23 GH	IGF-I	Day 31 GH	IGF-I	Day 56 GH	IGF-I
Femur length (mm)	90	74	80	59	75	60
BMD (mg/cm^2)	93	61	76	54	68	44
BMD (mg/cm^3)	93	71	89	69	85	68
PC (mm)	92	62	86	58	75	57
Breaking strength (N)	73	38	53	26	45	20

The much larger deficit in length, BMD, periosteal circumference (PC), and breaking strength in mice lacking IGF-I compared to GH at day 23 suggests that skeletal changes during prepubertal growth is dependent on IGF-I expression largely independent of GH. To further evaluate the relative contribution of the GH-independent pathway during puberty, we determined the rate of gain in various parameters between day 23-31. The rate of gain in length was 35% and 32%, respectively, in mice lacking IGF-I or GH compared to control mice, thus suggesting that IGF-I effects on length are mainly mediated via GH. In contrast, PC increased by 50% in mice lacking GH compared to control mice during puberty while no increase in PC occurred in mice lacking IGF-I, thus suggesting that the increase in PC during puberty is IGF-I dependent and mediated equally via both GH-independent and GH-dependent mechanisms. Similarly, IGF-I effects on rate of gain in BMD and bone strength during puberty are mediated in part by GH-independent mechanism. Conclusions: 1) Mice deficient in IGF-I exhibit greater impairment in bone accretion than mice deficient in GH. 2) IGF-I effects on bone accretion during prepuberty are mediated predominantly via mechanisms independent of GH, while during puberty they are mediated via both GH-independent and GH-dependent mechanisms.

SA146

Skeletal Unloading Induces Resistance to Insulin-like Growth Factor I on Bone Formation. T. Sakata,[1] B. P. Halloran,[1] H. Z. ElAlieh,*[1] S. J. Munson,*[1] L. Rudner,*[1] L. Venton,*[1] C. J. Rosen,[2] D. D. Bikle.[1] [1]Medicine, University of California, Veterans Affairs Medical Center, San Francisco, CA, USA, [2]Medicine, Maine Center for Osteoporosis Research, Bangor, ME, USA.

Skeletal unloading results in an inhibition of bone formation associated with a decrease in osteoblast number, impaired mineralization of bone, and altered proliferation and differentiation of osteoprogenitor cells. Although such changes are likely to be mediated by multiple factors, resistance to the growth promoting action of insulin-like growth factor I (IGF-I) has been hypothesized to play an important role. To determine whether skeletal unloading induces resistance to IGF-I on bone formation, we examined the response of unloaded (hindlimb elevation) and normally loaded tibia and femur to IGF-I treatment. To eliminate the variable of endogenous growth hormone production and secretion during exogenous IGF-I treatment, we used growth hormone-deficient dwarf rats (dw/dw). Beginning at 3 months of age these rats were given IGF-I (2.5 mg/kg/day) or vehicle via osmotic minipumps during 7 days of unloading or normal loading. This increased the serum level of IGF-I from 233±19 and 225±22 ng/ml to 390 ±79 and 393±32 ng/ml in the normally loaded and unloaded rats, respectively.In the histomorphometric study: IGF-I treatment markedly increased the bone formation rate (BFR/BS) at the tibiofibular junction of normally loaded rats (196% of the control). Unloading decreased the BFR/BS at the tibiofibular junction in the vehicle treated rats, and blocked the ability of IGF-I to increase the BFR/BS (62% and 68% of the control, respectively). On the other hand, IGF-I treatment increased the BFR/BS at the mid point of the humerus (normally loaded in this model) in both hindlimb-elevated and normally loaded rats (293% and 262% of the control, respectively). In the bone marrow osteoprogenitor (BMOp) cell study: IGF-I treatment significantly increased the osteogenic colony number on day 7 and 12, the total ALP activity on day 12 and 21, and the total mineralization on day 21 in BMOp cells of normally loaded rats. Unloading reduced the osteogenic colony number, the total ALP activity, and the total mineralization in the vehicle treated rats, and blocked the ability of IGF-I to increase these parameters. Furthermore, IGF-I treatment (10 ng/ml) in vitro significantly increased the cell proliferation (bromodeoxyuridine incorporation) of the BMOp cells isolated from normally loaded bone, but not from unloaded bone. These results indicate that skeletal unloading induces resistance to IGF-I on bone formation.

SA147

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SA148

Growth/differential Factor-5 Enhances Cellular Proliferation and Up-Regulates Its Receptor Transcript in Human Cementum-Derived Cell. M. Yamamoto,[1] T. Nakamura,[1] W. J. Grzeski,[2] J. Pohl,*[3] Y. Izumi.*[1] [1]Department of Periodontology, Kagoshima University Dental School, Kagoshima, Japan, [2]Dental Research Center, Department of Periodontics, School of Dentistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [3]BIOPHARM GmbH, Heidelberg, Germany.

Growth/differential factor-5 (GDF-5), also designated cartilage-derived morphogenetic protein-1, is a member of TGF beta super family. Gdf-5 is expressed during the mesenchymal condensation and later localized in joint formation in axial skeletal development of limbs. Mutations in gdf-5 gene are known to be responsible for brachypodism in mice and chondrodysplastic diseases in human. In addition to biological roles in embryonic develop-

ments, recombinant human GDF-5 protein shows inductive activities such as an ectopic induction of tendon/ligament-like tissue in rat, ectopic cartilage and bone formation in rodents, and enhanced healing of long bone defect in primate. Previous in vitro study indicated that GDF-5 transduced these effects through the BMP receptor IB. Tooth is connected to alveolar bone in jaws, with attachment apparatus composed of alveolar bone proper, periodontal ligament, and cementum. This apparatus is derived from dental follicle, in which gdf-5, gdf-6 and gdf-7 are reported to be expressed strongly in root formation area. The purpose of this study was to examine the biological effects of recombinant human GDF-5 protein (rhGDF-5) on human periodontal ligament cells (PDL) and human cementum-derived cells (HCDC), both of which were isolated from teeth extracted on clinical demands. First, proliferation assay was performed in order to determine the effect of rhGDF-5 on the cells cultured in 96-well plate in the presence of various concentrations of the protein. The rate of cellular proliferation was significantly increased in both PDL and in HCDC at 200-1000 ng/ml of rhGDF-5 concentrations on day 1, and also in HCDC in same range of concentration on day 3. Second, the expressions level of osteogenesis markers such as parathyroid hormone receptor (PTHR), osteocalcin (OCN), and bone morphogenetic protein receptor (BMPR) IA, IB, II, were compared by semi-quantified RT-PCR. The total RNA was isolated from cells cultured with 200 ng/ml or 1000 ng/ml of rhGDF-5 for 3 days. Interestingly, the levels of IB receptor were increased only in HCDC at both protein concentrations tested. On the other hand, the expression levels of BMPR IA, II, PTHR, and OCN were not altered or slightly decreased in both of cells. These results indicate that rhGDF-5 protein affects on cellular functions of PDL and HCDC in an in vitro culture system. This suggests that GDF-5 may be possibly involved in maintenance and healing of periodontium.

SA149

See Friday Plenary number F149.

SA150

Expression of GDF-7 mRNA During Endochondral Ossification and Tooth Development. Y. Miyoshi,* M. Yokozeki, K. Terai,* K. Hiura, K. Moriyama. The Univ. of Tokushima, Tokushima, Japan.

Bone morphogenetic proteins (BMPs) are secretory signal molecules which have a variety of regulatory functions during morphogenesis and cell differentiation. Growth and differentiation factor-7 (GDF-7), which is known as a member of BMP family, induces formation of tendon and ligament when is implanted at ectopic sites in vivo. Since tendon and ligament are attached to bones, these tissues might be closely related to early skeletogenesis. GDF-7 may also be a potent regulatory molecule in the tooth development since it was reported that the expression of GDF-7 mRNA was detected in the dental follicle. Therefore, the objective of this study is to analyze the expression of GDF-7 mRNA during endochondral ossification and tooth development in mouse embryo and to elucidate the roles of GDF-7 in skeletogenesis and odontogenesis. C57BL/6N mouse embryo at day 14.5 and 16.5 were used in this study. Reverse transcription polymerase chain reaction (RT-PCR) was performed using specific primers for 3'-untranslated region of mouse GDF-7 mRNA. In situ hybridization was performed using digoxigenin-labeled single stranded cRNA probes for GDF-7 and BMP-2, BMP-4 and osteopontin. RT-PCR analysis confirmed the expression of GDF-7 mRNA in developing mouse embryo at day 14.5 and 16.5. In situ hybridization analysis of mouse embryo at the day 14.5 and 16.5 showed that GDF-7 mRNA expression was observed in the periosteal cells around ossified ribs and chondrocyte of proliferating zone in the first rib cartilage where endochondoral ossification took place. Expression patterns of BMP-2 and BMP-4 mRNA were analogous with that of GDF7. The expression of osteopontin mRNA was not observed in periosteal cells expressing GDF-7, BMP-2, and BMP-4, but in osteoblasts within the ossified rib. In the developing tooth germ, GDF-7 mRNA was expressed in the dental papilla at the cap stage and the bell stage. GDF-7 mRNA expression was also observed in the preodontoblasts at differentiation stage. These results suggested that GDF-7 might be regulatory factor in skeletogenesis and odontogenesis during mouse development.

SA151

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SA152

Serum Activin-A Levels in Metabolic Bone Diseases. R. Nuti,[1] G. Martini,[1] G. Florio,*[2] R. Valenti,*[1] S. Salvadori,*[1] A. Picchi,*[1] F. Petraglia.*[2] [1]Metabolic Disease Unit, University of Siena, Siena, Italy, [2]Obstetrics and Gynecology, University of Siena, Siena, Italy.

Activin is a dimeric protein that inhibits follicle-stimulating hormone secretion. It structurally belongs to the transforming growth factor beta family and shows some biological properties of growth factors. Increasing evidence suggests the involvement of Activin-A in the regulation of bone metabolism.Aim of the study was to evaluate the relationships between serum Activin-A levels and bone turnover, measured by serum markers of osteoblast and osteclast activity.We studied 58 subjects: 15 healthy postmenopausal women, 25 women with involutional osteoporosis characterized by one or more vertebral crushes, 10 patients with poliostotic Paget's disease of bone, 5 patients with multiple myeloma, and 3 patients with secondary osteoblastic bone lesions. All patients had normal renal function.Bone metabolism was evaluated by: serum and urinary calcium, serum phosphate (standard methods), serum bone alkaline phosphatase (BAP: Metrabiosystem), serum crosslaps (CTx: Osteometer), serum iPTH 1-84 (DRG-Int). Activin-A was measured using a specific two-site enzyme immunoassay (Oxford Bio-Innovation LTD).No differences among groups were appreciated as regards serum calcium, serum phosphate, urinary calcium excretion, serum iPTH. A statistically significant increase of CTx and BAP was found in Pagetic patients with respect to healthy postmenopausal women (10823±8158 pmol/l vs 4228±2528 pmol/l, $p<0.05$; 122.8±119 U/l vs 32.4±27 U/l, $p<0.05$ respectively). In postmenopausal women serum level of Activin-A was 0.628±0.2 ng/ml; increased but not statistically significant values, were detected in Paget's disease (0.700±0.15 ng/ml), in osteoporotic women (0.716±0.26) and in multiple myeloma (0.804±0.13). A dramatic increase of Activin-A (1.986±1.06) was found in three patients with solid cancer and osteoblastic metastasis.No relationships were found between Activin-A and bone metabolism parameters, while a positive correlation with age was confirmed ($r=0.42$; $p<0.05$).These preliminary data indicate that serum Activin-A levels may not actually considered a valuable marker of bone turnover.

SA153

Cell Swelling Activation of Membrane Currents, Ca2+ Transients and Regulatory Volume Decrease in Bovine Articular Chondrocytes (BAC). C. E. Yellowley,[1] J. C. Hancox,*[2] H. J. Donahue.[1] [1]Musculoskeletal Research Laboratory, Penn State College of Medicine, Hershey, PA, USA, [2]Department of Physiology, Bristol University, United Kingdom.

During mechanical loading, the osmotic environment of the chondrocyte is perturbed by changes in local proteoglycan concentration and, therefore, the ionic concentration and osmolarity. It is possible that cells may sense mechanical changes in their environment via changes in osmolarity. In this study we examined the effect of hypo-osmotic stress on early signaling events in chondrocytes by making measurements of intracellular calcium [Ca2+]i and of macroscopic ionic currents. In addition, we looked at the ability of BAC to regulate cell volume during a hypo-osmotic challenge. Cells were perfused with Tyrode's solution containing in mM; 140NaCl; 2MgCl2; 5K2ATP; 10 HEPES and 5 Glucose titrated to pH 7.4. For cell swelling external NaCl content was reduced to 100, 80 and 60mM, reducing osmolarity from 295mOsm (normal) to 220, 185 and 145 respectively. [Ca2+]i was quantified using the fluorescent dye fura-2 AM. Cell membrane ionic currents were recorded using perforated patch clamp. Relative changes in cell volume were measured by monitoring the fluorescence of calcein loaded cells. BAC continuously exposed to hypotonic solution (-150mOsm) swelled in size to a peak response at 4 min, with a mean decrease in dye fluorescence of 12.6±1.1% (±SEM), and recovered back to near normal within 30 min. Volume recovery was inhibited by Gd3+ (50mM), cell fluorescence decreased by 23.4±4.0% and showed no sign of recovery after 30min. When exposed to a decrease in osmolarity of -75mOsm, 28.7± 3.6% of cells responded with an increase in [Ca2+]i, 43.9±10.7% of cells responded to -110mOsm and 73.5± 6.7% to -150mOsm. The proportion of cells responding to -150mOsm (73.5± 6.7%) was not affected by nifedipine (20mM), (67.5±11.2%), but was reduced by Gd3+ (50mM) and thapsigargin (50nM) to 3.8± 2.6% and 8.1± 3.0% respectively. Inward and outward membrane currents increased over 4-fold when exposed to -150mOsm Tyrode's. These results indicate that BAC possess mechanisms by which they can regulate cell volume and that this mechanism may require Ca2+. The cell Ca2+i response to swelling was dependent on the magnitude of the hypotonic shock and was blocked by Gd3+ and thapsigargin suggesting roles for stretch activated channels and intracellular Ca2+ stores. Activation of ion channels and Ca2+i have been shown to play significant roles in volume regulation in other cell types, however, they may also play a role in transducing mechanical signals into cellular metabolic responses. These effects may be involved in a mechanism by which mechanical loads are transmitted to chondrocytes.

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SA155

PTH potentiates Volume-sensitive Calcium Influx Pathways through the Activation of Adenyl cyclase in Mechanically Stretched Human Osteocytes. A. Miyauchi,[1] K. Naruse,*[2] M. Itoman,*[2] M. Goto,*[3] K. Notoya,[3] K. Okabe,[4] Y. Takagi,[1] K. Jinnai,*[1] Y. Yoshimoto,[1] T. Sugimoto,[5] K. Chihara,[5] T. Fujita,[6] Y. Mikuni-Takagaki.[7] [1]Medicine, National Hyogo-Chuo Hospital, Sanda, Japan, [2]Orthopaedic Surgery, Kitasato Univ., Sagamihara, Japan, [3]Takeda Chemical Industries Ltd., Osaka, Japan, [4]Oral Physiology, Fukuoka Dental College, Fukuoka, Japan, [5]Third Div., Med., Kobe Univ., Kobe, Japan, [6]Ca Res. Inst., Kishiwada, Japan, [7]Oral Biochemistry, Kanagawa Dental College, Yokosuka, Japan.

In mechanically loaded bone, osteocytes transduce signals that lead to bone formation. The mechanisms of mechanosensing and transduction, however, are not well elucidated especially in human cells. Previously, we have found that mechanosensing in rat osteocytes depends on a volume-sensitive calcium influx pathways that is regulated by activation of stretch activated cation channels (SA-Cat) (J. Biol. Chem. 275: 3335, 2000). These Ca^{2+} influx pathways are upregulated by PTH through the activation of adenyl cyclase. In this study, we found that the mechanosensing machinery operates similarly in human osteocytes. For this purpose, primary cultures of human long bone osteocytes were prepared from bone chips obtained during total hip replacement surgery. Cytosolic calcium concentrations, $[Ca^{2+}]i$, were measured using single cell video-image analysis with fura-2. Rapid and progressive increases (70±4nM above basal (n=11)) in $[Ca^{2+}]i$ were induced by stretch loading of human osteocytes in hypoosmotic solution (50% of normal osmolarity). Influx of extracellular Ca^{2+} is primarily responsible for the hypotonicity stimulated increase in $[Ca^{2+}]i$ as the response was abolished by Ca^{2+} free media containing EGTA. Gadolinium chloride (Gd^{3+}), a selective SA-Cat blocker, eliminated the $[Ca^{2+}]i$ increase caused by hypotonic solution. PTH(1-34) specifically potentiated the hypotonicity-induced $[Ca^{2+}]i$

increase in a dose dependent manner. The adenyl cyclase pathway appears to be involved in this potentiation, because dibutyryl cAMP had comparable stimulatory effects on the hypotonicity-induced $[Ca^{2+}]i$ increase. The $[Ca^{2+}]i$ increases induced by both PTH and hypotonicity were observed primarily in the cell processes of osteocytes. In cyclically stretched osteocytes on flexible-bottomed plates, PTH(1-34) or dibutyryl cAMP acted synergistically in elevating osteocalcin mRNA levels. Furthermore, Gd^{3+} inhibited the stretch-induced elevation of osteocalcin mRNA. The volume-sensitive calcium influx pathways of osteocytes, demonstrated here for the first time in human cells, represent a mechanism by which PTH potentiates mechanical responsiveness, an important aspect of bone formation.

SA156

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SA157

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SA158

Differential Expression of the AP-1 Protein Complex in Mechanically Strained Human Osteoblasts. M. Wozniak, K. A. Hruska. Internal Medicine, Washington University, St.Louis, MO, USA.

We have recently demonstrated the role of the $\alpha_v\beta_3$ integrin plaques in osteoblast differentiation and deposition of mineralizing extracellular matrix in response to mechanical strain (JBMR 2000). Our current goal is to further investigate the molecular signals, particularly the transcription factors, leading to osteoblast proliferation, differentiation and mineralization of matrix. The AP-1 (activator protein-1) transcription factor consists of either Jun/Jun homodimers or Fos/Jun heterodimeric complexes, and is formed by Fos (c-Fos, Fra-1, Fra-2, Fos B) and Jun proteins (c-Jun, JunB, JunD). Our hypothesis is that members of the AP-1 protein complex are differentially expressed during osteoblast proliferation, differentiation and mineralization of the extracellular matrix, and that mechanical strain affects their expression and cellular localization. Primary human osteoblasts were developed from bone marrow stromal cells and analyzed at distinct stages of differentiation (osteoprogenitor, preosteoblast and osteoblast) under conditions of chronic, repetitive mechanical strain. The magnitude of the non-uniform strain at the site of analysis was 70,000 με, applied in the form of a square wave at the frequency of 0.05 Hz for 48 hours. The results demonstrate that nuclear expression of Fra-1 is higher in preosteoblasts than in osteoprogenitors or in mineralizing osteoblasts, thus correlating well with previous studies of fra-1 mRNA levels, and that mechanical strain affects osteoprogenitor Fra-1 expression pattern. The Fra-1 protein was uniformly distributed throughout the nucleus of non-strained progenitors, but was confined to the endoplasmic reticulum following strain. In contrast to Fra-1, c-Fos expression was higher in osteoprogenitors than in preosteoblasts and osteoblasts. Accordingly, c-fos mRNA levels were previously shown to be high during the proliferative period of the osteoblast development. There was a shift in c-Fos localization pattern that occurred concurrently with the progression of osteoblastic differentiation - in progenitors c-Fos was present in the endoplasmic reticulum, later it was found in nuclear vacuolar structures and uniformly distributed throughout the nucleus in preosteoblasts and osteoblasts, respectively. We thus conclude that in ostoprogenitors c-Fos is synthesized in the endoplasmic reticulum, then transported into the nucleus during early preosteoblast stage, where it later (during the osteoblast stage) induces gene transcription (observed as uniform nuclear expression pattern). In summary, the proteins of the AP-1 transcription complex show osteoblast development stage-dependent expression and localization pattern.

SA159

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SA160

Signaling Pathways for the Fluid Shear Stress Induction of Cyclooxygenase-2 in Murine Osteoblats. S. Wadhwa,[1] S. Godwin,*[1] L. G. Raisz,[2] C. C. Pilbeam.[2] [1]Oral Biology, University of Connecticut Health Center, Farmington, CT, USA, [2]Medicine, University of Connecticut Health Center, Farmington, CT, USA.

Anabolic effects of mechanical loading of bone can be abrogated by inhibition of prostaglandin (PG) production. Fluid shear stress (FSS), hypothesized to transduce mechanical loading into cellular signals, generates PGs in osteoblasts by inducing new gene transcription of cyclooxgenase-2 (COX-2). To examine signaling pathways involved in this induction, we used immortalized murine osteoblastic MC3T3-E1 cells stably transfected with -371/+70 bp of the murine COX-2 5' flanking DNA fused to a luciferase reporter (Pluc) and primary osteoblastic cells derived from calvaria of mice transgenic for Pluc. Cells were plated on collagen-coated glass slides, grown to confluence, and subjected to 10 dynes/cm^2 of steady laminar FSS in a parallel plate flow chamber. On Northern blot analysis, COX-2 mRNA was induced by FSS within 30 minutes and peaked at 4 h. An inhibitor of new protein synthesis, puromycin (10 μg/ml), did not affect the small FSS induction of COX-2 mRNA at 1 h but inhibited the large peak induction at 4 h by 90%. COX-2 promoter activity, measured as luciferase activity normalized to total protein, correlated with COX-2 mRNA expression. Inhibitors of the protein kinase A (PKA) signaling pathway, H-89 (30 μM) and PKI (1 μM), used at doses determined to be most specific for the PKA pathway, reduced FSS stimulated COX-2 mRNA expression and luciferase activity at 4-4.5 h by 60% and 66%, respectively. In contrast, a specific inhibitor of the protein kinase C (PKC) pathway, GF109203X (1.25 μg/ml), and down regulation of the PKC pathway by 24 h of pretreatment with phorbol myristate acetate (1 μM) had no effect on the FSS induction of COX-2 mRNA and luciferase activity at 4-4.5 h. On Western analysis, FSS induced phosphorylation of ERK1/2 within 5 min. A specific inhibitor of the ERK1/2 pathway, PD98059 (40 μM), reduced FSS stimulation of COX-2 mRNA and luciferase activity at 4-4.5h by 65% and 91%, respectively. We conclude that maximal induction of COX-2 by FSS requires new protein synthesis, involves the PKA but not the PKC pathway, and depends on activation of ERK1/2.

SA161

See Friday Plenary number F161.

SA162

Surface Topography Modulates Osteoblast Response to Shear Force. F. Del Toro,[1] C. H. Lohmann,*[2] S. R. Bannister,*[3] Y. Liu,*[1] V. L. Sylvia,[1] D. L. Cochran,*[4] Z. Schwartz,[5] D. D. Dean,[1] B. D. Boyan.[1] [1]Orthopaedics, University of Texas Health Science Center, San Antonio, TX, USA, [2]Orthopaedics, Georg-August University, Goettingen, Germany, [3]Periodontics, Wilford Hall Medical Center, Lackland AFB, TX, USA, [4]Periodontics, University of Texas Health Science Center, San Antonio, TX, USA, [5]Periodontics, Hebrew University, Jerusalem, Israel.

Previous studies have shown that osteoblasts are sensitive to surface roughness and morphology. When cultured on titanium (Ti) surfaces, MG63 osteoblast-like cells exhibit decreased proliferation and increased differentiation with increasing roughness. To examine how the topography of the surface modulates osteoblast response to shear force, MG63 cells were cultured in a continuous flow device on glass disks (R_a < 0.2 μm) or Ti disks with three different R_a values and topographies (smooth [PT]: R_a = 0.60 μm; sand-blasted and acid etched [SLA]: R_a = 3.97 μm; and titanium plasma sprayed [TPS]: R_a = 5.21 μm). Flow rates were varied, resulting in shear forces of 0, 1, 5, 14, and 30 dynes/cm^2. Confluent cultures were exposed to fluid flow for one hour. After an additional 23 hours in culture under static conditions, cell number, alkaline phosphatase specific activity, and levels of osteocalcin, TGF-β1, and PGE_2 in the conditioned media were determined. Cell number on smooth surfaces (glass and PT) was unaffected by shear force. In contrast, there was a dose-dependent reversal of the decrease in cell number seen on rough SLA and TPS surfaces. On smooth surfaces alkaline phosphatase specific activity was unaffected by shear force, but shear force caused a biphasic reduction in the roughness-dependent increase on SLA and TPS that was maximal at 14 dynes/cm^2. There was a similar effect seen with TGF-b1 levels. Osteocalcin was unaffected on smooth surfaces; shear force caused a dose-dependent reduction in the roughness-stimulated increase seen on SLA and TPS. PGE_2 production was increased by shear force on all surfaces. There was a 2-fold increase on glass and PT in response to 14 dynes/cm^2, but on SLA and TPS, 14 dynes/cm^2 shear force caused a 9-10-fold increase. These results show that surface roughness and topography modulate osteoblastic response to shear force. The effect of shear force is greater on rougher surfaces. The shear force-mediated decrease in osteoblast differentiation seen in cultures on rough surfaces may be due to increased production of PGE_2.

Disclosures: ITI Foundation, Waldenburg, Switzerland,2.

SA163

See Friday Plenary number F163.

SA164

Combined Estrogen and Exercise Completely Prevented Marrow Cancellous Bone Loss and the Elevated Intracortical Bone Remodeling Following Ovariectomy in the Femoral Neck. W. Yao, J. Chen,* C. Y. Li,* A. Mo,* R. B. Setterberg,* W. S. S. Jee. Radiobiology Division, University of utah, Salt Lake City, UT, USA.

The current study was designed to investigate the effects of estrogen (E_2) and exercise on the femoral neck (FN) of the ovariectomized (OVX) rats. Exercise was conducted by employing a raised cage (RC) model so as to make the rats rise to bipedal stance for feeding. Six-month-old female Sprague-Dawley rats were bilateral ovariectomy at day 0. They were housed in normal height or RC and injected twice a week with 10μg/kg of E_2 or vehicle for 8 weeks. We found that 1) Marrow cancellous bone (B.Ar) lost by 39% and intracortical porosity area (Po.Ar) increased by 108% while total bone area (TB.Ar) did not change significantly due to the periosteal expansion following OVX. 2) E_2 alone partially prevented the decrease of B.Ar and the increases of Po.Ar by inhibiting endosteal bone erosion (Es-E.Pm)and decreasing the periosteal bone formation (Ps-BFR). 3) RC alone also partially prevented the decrease of B.Ar by preventing the increased Es-E.Pm maintained the elevated Ps-BFR. 4) E_2 plus RC completely preserved the B.Ar and prevented the intracortical remodeling by having additive effect on reducing Es-E.Pm. RC helped to partially prevent decreased of Ps-BFR after E_2 administration (Table), as a result, TB.Ar increased. In conclusion, apart from inducing marrow cancellous bone loss, OVX also increase intracortical remodeling in the FN. Both E_2 and RC partially prevented these changes. Combination treatment completely prevented OVX-induced bone loss by having additive effect on reducing endosteal bone erosion and RC partially counteracted the

decrease of bone formation by E_2 treatment.

Parameters	Ct.Ar	Po.Ar	TB.Ar	B.Ar	Ps-BFR	Es-BFR	Es-E.Pm
Sham	72.5	1.4*	82.5	11.0*	19.7*	2.3	3.1*
OVX	75.7	2.8	80.3	6.7	60.2	3.9	5.6
E	70.1	1.5*	77.6	8.6	16.3*	3.4	2.9*
RC	72.0	1.8	80.6	9.8*	50.2	2.4	3.0*
E+RC	80.2	1.4*	90.9*	11.7*	32.1*	1.9*	1.2*
Two-way ANOVA							
E	0.666	0.001	0.440	0.122	0.000	0.188	0.000
RC	0.008	0.571	0.000	0.058	0.011	0.018	0.000
E × RC	0.054	0.040	0.001	0.109	0.958	0.273	0.609

*,P < 0.05

SA165

See Friday Plenary number F165.

SA166

Age, Sex, and Grip Strength Determine Architectural Bone Parameters Assessed by Peripheral Quantitative Computed Tomography (pQCT) at the Human Radius. P. Schneider,[1] Y. Hasegawa,[2] C. Reiners.[2] [1]Clinic for Nuclear Medicine, University of Würzburg, Würzburg, Germany, [2]University of Würzburg, Würzburg, Germany.

The purpose of this study was to estimate the relation of some noninvasively derived mechanical characteristics of radial bone including architectural parameters for bone strength to grip strength and muscle cross-section. 63 males between 21 and 78 years of age and 101 females between 18 and 80 years of age were measured at the nondominant forearm using peripheral quantitative computed tomography (pQCT). We assessed volumetric bone mineral density (BMD) and content (BMC) by pQCT at the distal and at the midshaft radius. Bone area (Area), cortical thickness (C-th), and a newly proposed index for bone strength, the stress strain index (SSI) were also calculated. The dynamometrically measured maximum grip strength was taken as a mechanical loading parameter and muscle cross-section as a substitute for it.Sex, grip strength, BMC and BMD (distal radius) were identified in a multiple regression analysis to predict 81% of bone strength as expressed by SSI, after adjusting for all other independent variables. Further analysis showed grip strength closest related to age, sex, BMD and SSI of the distal radius. The cross-sectional area of muscle was not significantly determining the grip strength within the analysis model. The correlation between grip and SSI was 0.6.

Table

	Beta	St. Error of Beta	Partial correlation	p-level
BMC (distal radius)	0.693	0.068	0.488	<0.0001
BMD (distal radius)	0.281	0.052	0.378	<0.0001
grip strength	0.199	0.055	0.262	0.0003
Sex	0.134	0.058	0.177	0.02

The table shows the results of a forward stepwise regression (6 steps) with SSI of the distal radius as dependent variable. Sex was entered as 0=female, 1=male; with multiple R=0.910; p<0.0001; and adjusted R-square = 0.817.In conclusion, our results suggested that architectural parameters at the distal radius were better related to grip strength than to cross-sectional muscle area in both males and females. Only 19% capacity remained in our model to explain factors not investigated, such as bone genetics. A strong association between muscle and bone cross-section as found by others (JBMR 14, 1999, S495) could not be confirmed.

SA167

See Friday Plenary number F167.

SA168

Bone Formation Decrease and Bone Resorption Increase with Decreased Activity Level. M. K. Karlsson,[1] C. Karlsson,*[1] S. Ljunghall,*[2] K. J. Obrant.*[1] [1]Department of Orthopedic Surgery, Malmö, Sweden, [2]Department of Medicine, Uppsala, Sweden.

The aim of this study was to (i) evaluate the relationship between bone formation, bone resorption and regional bone mineral density in elite male soccer players and controls and (ii) follow bone turnover with decreased and increased activity level. Bone formation was assessed by serum osteocalcin (OC) and carboxy-terminal propeptide of type I collagen (PICP). Bone resorption was assessed by carboxyterminal cross-linked telopeptide of type I collagen (ICTP). Bone mineral density (BMD; g/cm2) total body, lumbar spine, hip was measured by Dual X-ray Absorptiometry (DEXA) and BMD calcaneus by Quantitative Ultraosund (QUS; speed of sound (SOS, m/s), broadband attenuation (BUA, db/MHz) and stiffness index). Included were 12 male professional soccer players (mean age 23 years, range 17-34) exercising 12 hours/week, (range 8-15) for a mean of 5 years (range 1-15). The athletes were followed from full activity through four weeks of rest (weekly) between two seasons with recreational activity of mean 3 hours/week (range 1-7) into the new season. Twenty-seven age- and gender matched volunteers served as controls for the biochemical measurements and 24 individuals for the BMD measurements. Data is presented as mean ± SEM.Male soccer players had 22 ± 12 % higher OC ($p<0.05$), 13 ± 9 % higher PICP (NS) and 34 ± 17 % higher ICTP ($p<0.05$) compared to controls at the end of the season. Soccer players had also higher BMD than controls; 7 ± 1 % total body BMD, 15 ± 3 % lumbar spine BMD, 14 ± 2 % femoral neck BMD, 5 ± 1 % SOS, 13 ± 2 % BUA and 30 ± 3 % stiffness index (all $p< 0.001$, respectively). Bone formation (PICP) decreased by 21 ± 7 % ($p <0.05$) and bone resorption (ICTP) increased by 8 ± 10 % ($p = 0.07$) after two weeks rest and did not change during the rest of the resting period. Bone formation (PICP) increased by 26 ± 5 % ($p < 0.01$) and bone resorption (ICTP) decreased by 8 ± 2 % ($p<0.10$) after 10 days recommencing of seasonal activity level at the end of the study period. After this period, no differences was found in PICP- and ICTP values compared to baseline.In summary, exercising male soccer players have higher bone turnover conferring higher BMD in weight loaded skeletal regions compared to controls. Decreased activity level decreases bone formation and increases bone resorption within weeks. We conclude that decreased activity level with cessation of active career may confer a higher rate of loss of BMD in former athletes compared to controls with possibly no residual higher BMD to be found in old ages when fragility fractures occur.

SA169

See Friday Plenary number F169.

SA170

Physical Activity Increases Bone Size in Prepubertal Boys and Bone Mass in Prepubertal Girls: A Combined Cross-sectional and 3-Year Longitudinal Study. M. Sundberg,*[1] I. Sernbo,*[1] P. Gardsell,[1] E. Ornstein,*[2] B. Sandstedt,*[2] O. Johnell,[1] M. K. Karlsson.[1] [1]Department of Orthopedic Surgery, Malmö, Sweden, [2]Department of Orthopedic Surgery, Hassleholm, Sweden.

The purpose of this study, following 44 boys and 42 girls from age 13 to 16 years, was to evaluate the skeletal effect of physical activity from age 9 to 13 and from age 13 to 16 years. The children were divided into two groups to achieve comparative numbers of children in each group, one high and one low activity group. Accrual of bone mass and growth in bone size were evaluated annually by dual-energy X-ray absorptiometry (DXA) from age 13 to 16 years. The outcome of discrepancies in physical activity from age 9 to 13 yeas were cross-sectionally evaluated at baseline (age 13) and the outcome of discrepancies in physical activity from age 13 to 16 years were longitudinally evaluated. Girls with a high activity level from age 9 to 13 years, had at age 13 years higher femoral neck (FN) bone mineral content (FN BMC; g) ($p = 0.07$), higher FN areal bone mineral density (FN aBMD; g/cm2) and higher FN volumetric BMD (FN vBMD; g/cm3) (both $p < 0.05$, respectively) compared with girls with a low activity level. FN width (cm) and head aBMD (an unloaded region) showed no differences when comparing the two groups. Three years of further high and low activity level (from age 13 to16) did not confer any increased differences when the two groups were compared, and the changes in bone mass and bone size during this period (adjusted for changes in height, weight and Tanner stage) showed no discrepancies when the high and the low activity group were compared.Boys with a high activity level from age 9 to 13 years had at age 13 higher FN BMC, FN aBMD and FN width (all $p < 0.05$, respectively) compared to boys with a low activity level. FN vBMD and head aBMD showed no differences in the comparison between the two groups. Three years of further high and low activity level (from age 13 to 16 years) did not confer any increased discrepancies when the two groups were compared, and the changes in bone mass and bone size during this period (adjusted for changes in height, weight and Tanner stage) showed no discrepancies when the high with the low activity group were compared.We summarize that exercise may confer gender specific skeletal benefits before age 13 years, and that three years of continued high or low activity level do not confer any increased discrepancies in bone size or bone mass in neither girls nor boys. In conclusion, the present study suggests that efforts by the community in order to enhance the skeletal bone status in the population by means of increased physical activity, should probably be initiated before the age of 13 years.

SA171

Phospholipids as Survival Factors in Osteoblastic Cells - A Role for Phosphatidylinositiol 3-Kinase. A. Grey, Q. Chen,* K. Callon, C. Xu,* B. Hill,* I. R. Reid, J. Cornish. Medicine, University of Auckland, Auckland, New Zealand.

The naturally occurring phospholipid growth factors lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) have been shown to influence fundamental cellular processes in a number of cell types in vitro. We and others have shown that both LPA and S1P are mitogenic to osteoblastic cells. In the current study, we examined the effect of these compounds on osteoblast survival in vitro. Using TUNEL and DNA fragmentation assays, we found that both LPA and S1P dose-dependently inhibit the apoptosis induced by serum withdrawal in cultures of primary rat osteoblasts or SaOS2 cells. This effect was observed

at concentrations between 0.01mM and 10mM, which are similar to those reported in the systemic circulation. LPA reduced osteoblast apoptosis by 74% at a concentration of 10mM, while 10mM S1P induced a 65% reduction. We further examined the signaling pathways involved in these anti-apoptotic effects using a DNA fragmentation assay. In primary rat osteoblasts, the ability of both LPA and S1P to prevent apoptosis was completely blocked by pertussis toxin (PTx, 10ng/ml). LY294002, a specific inhibitor of phosphatidylinositol 3-kinase (PI 3-K), dose-dependently inhibited the survival-promoting actions of each phospholipid. Neither PD-98059 nor U-0126, specific inhibitors of MEK, the kinase responsible for activating p42/p44 MAP kinases, abrogated the anti-apoptotic effect of either phospholipid. In Swiss 3T3 fibroblastic cells, PTx blocked the anti-apoptotic actions of LPA and S1P, but LY294002 had no effect on phospholipid-induced cell survival. We conclude that (a) the phospholipids LPA and S1P are potent survival factors in osteoblastic cells, (b) Gi proteins and PI 3-K, but not p42/44 MAP kinases, are involved in transducing the survival signal activated by LPA and S1P in osteoblasts and (c) the signaling pathways which mediate the pro-survival effects of LPA and S1P are cell-type specific.

SA172

See Friday Plenary number F172.

SA173

The Role of IGF-I Receptor in Cell Survival of Trabecular Osteoblasts Derived from the Hypophysectomized Rat. J. F. Evans,* J. K. Yeh, J. F. Aloia. Medicine, Winthrop-University Hospital, Mineola, NY, USA.

It has been previously established that trabecular bone cells isolated from hypophysectomized (HX) animals 6-weeks after surgery have a higher rate of proliferation than their intact control counterparts when placed in culture. We have hypothesized that this increase is in part due to an up-regulation of the IGF-I receptor. Since active IGF-I receptor imparts an anti-apoptotic effect on the cell we compared the survival potential of HX vs control trabecular cells in response to the apoptosis inducing levels of inorganic phosphate and serum-free medium conditions. After a 72-hour incubation with an additional 9mmol/L inorganic phosphate as sodium phosphate in the medium, control cultures decreased in viability by 47% while the HX cultures experienced only a 12% decrease. When 200ng/ml of IGF-I was added to these cultures neither the control nor the HX cells experienced a decrease in viability after 72 hours, demonstrating that an increase in IGF-I receptor activity provides a protective effect. To further test cell survival we placed control and HX trabecular cells in serum-free medium with and without antibody to IGF-I receptor. After 48 hours of culture in serum-free medium 65% of cells survived in the HX cultures while only 50% remained in the control ($P< 0.01$), as assessed by the MTT viability assay. The addition of IGF-I receptor antibody decreased cell survival by 30% in the HX but only by 10% in the control, demonstrating that IGF-I receptor plays a role in their increased survival. In order to confirm whether or not the greater number of surviving cells in the HX cultures is due to a decrease in apoptosis, we examined the bcl-2/bax ratio in both groups using quantitative multiplex PCR. The bcl-2/bax ratio was 2X greater in the HX cultures than in the controls (3.03 vs. 1.54) indicating that the increased cell survival is through an anti-apoptotic effect. In conclusion the increased proliferation observed in trabecular bone cultures of the HX rat compared to those of the intact control is likely due to an increase in IGF-I receptor which imparts a resistance to apoptosis and therefore increases cell number. These cells provide a good model that can be used to further the study of anti-apoptotic effects induced through the IGF-I receptor.

SA174

Study of Cell Proliferation and Apoptosis During Fracture Healing. G. Li, G. White,* C. Connolly,* D. Marsh.* Department of Trauma and Orthopaedic Surgery, The Queen's University of Belfast, Belfast, United Kingdom.

Fracture repair is a complex physiological process during which bone shows the remarkable ability to mount a repair process, restoring its mechanical integrity and anatomical configuration by original osseous tissue. Programmed cell death, or apoptosis, is a naturally occurring cell suicide pathway with a homeostatic function in the maintenance of continuously renewing tissues. The present study investigated the relation between cell proliferation and apoptosis during fracture healing in a mouse femoral model.Left femoral osteotomies were performed in 20 male CFLP mice (35-45g), immobilised with uniplanar external fixators. 4 animals were sacrificed on days 2, 4, 8, 16 and 24 post-fracture and fracture callus collected for paraffin embedding. Localisation of cell proliferation was examined using immunohistochemistry with proliferating cell nuclear antigen (PCNA) monoclonal antibody. Apoptotic cells were visualised with the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labelling (TUNEL) method. Random images of each time specific specimen were captured via a digital camera and the positive labelling indices of PCNA and TUNEL labelling were calculated. Cell proliferation and apoptosis were found co-existing during the entire period of fracture healing studied. Cell proliferation was predominant in the early phases of fracture healing (days 2-8). PCNA positive labelling index peaked at day 8 and PCNA-positive cells were not limited to the fracture gap mesenchymal tissues but extended in the periosteum along most of the fractured femur. TUNEL positive labelling was minimal in the early stages (days 2-8). In later stages of fracture healing (days 16-24), PCNA expression declined as intramembranous and endochondral ossification spread within the fracture site and apoptosis was the dominant cell activity with the TUNEL positive labelling index peaked at day 16 and then declined sharply at day 24. The current study indicated that apoptosis was a normal concomitant during fracture repair, confirming programmed cell death in chondrocytes and bone cells, and that cell proliferation and apoptosis were tempero-spatially dependent. These findings support the view that apoptosis is a natural process, genetically programmed and active during fracture repair. The demonstration of a mixture of proliferative and apoptotic cell populations in the regenerating tissues of fracture callus, suggests that apoptosis and cell proliferation may be regulated by local factors during fracture healing.

SA175

See Friday Plenary number F175.

SA176

Autocrine Glutamate Signaling Regulates Osteoblast Apoptosis. P. G. Genever, T. M. Skerry. Biology, University of York, York, United Kingdom.

Osteoblasts express functional receptors for the neurotransmitter glutamate that are similar to those located at synaptic sites in the central nervous system. We have shown that osteoblasts also accumulate glutamate using specific transporters and release glutamate constitutively under steady-state conditions to regulate extracellular glutamate concentrations. This continuous glutamate recycling in osteoblasts is in contrast to glutamatergic activity in neurons where prolonged exposure to glutamate has cytotoxic effects. We therefore investigated the functional role of constitutive glutamate signalling on cell survival in cultures of primary human osteoblasts. Using a fluorimetric assay we determined that osteoblasts release approximately 1-30μM glutamate into their incubation medium, though concentrations at the cell surface are likely to be much greater. Addition of riluzole, a pharmacological inhibitor of neuronal glutamate release, significantly inhibited glutamate release in osteoblasts. However exposure to riluzole at neuroprotective concentrations (25μM), induced biochemical and morphological characteristics of apoptosis in osteoblasts (membrane blebbing, DNA fragmentation, chromatin condensation, TUNEL). By western blot analysis of osteoblast lysates we demonstrated that riluzole (25μM, 24h) markedly increased the Bax/Bcl-2 ratio (+400%), a characteristic of cells beginning to apoptose. Riluzole treatment increased intracellular glutamate concentrations in these cells by approximately 50% compared to controls, however dimethylglutamate (5mM), a membrane permeable glutamate precursor, caused similar elevations in intracellular glutamate (+45%) but did not have proapoptotic effects. These findings suggested that riluzole-induced apoptosis was caused through decreased glutamate release and not merely increased intracellular glutamate accumulation. To test this hypothesis we cultured differentiated osteoblasts in glutamate/serum-free medium in the presence of varying concentrations of exogenous glutamate (0-500μM) for 24h. MTT assays revealed that glutamate significantly increased osteoblast viability, with 50μM glutamate causing an approximate 15% increase in viable cell numbers compared to untreated controls ($P<0.001$). Glutamate induced a marked dose-dependent decrease in the Bax/Bcl-2 ratio in these cells (-75%, 50μM glutamate) and rescued riluzole-induced increases in the Bax/Bcl-2 ratio, without affecting expression levels of PCNA, a protein involved in DNA replication and repair. These data suggest strongly that glutamate acts as an osteoblast survival factor and will impact significantly on our understanding of remodelling events and pathological bone disorders.

SA177

Retroviral Delivery of Genes to Transit Amplifying Osteoblast Progenitors: Inhibition of Apoptosis by Overexpression of Bcl-2. I. Gubrij, C. A. O'Brien, A. A. Ali, S. C. Manolagas, R. L. Jilka. Div. Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. of Arkansas for Med. Sci., Little Rock, AR, USA.

Murine marrow-derived colony forming unit-osteoblasts (CFU-OBs) are transit amplifying progenitors capable of both replication and differentiation into osteoblasts. Modulation of CFU-OB behavior influences bone homeostasis as shown by evidence that suppression of CFU-OB replication mediates the anti-remodeling effect of estrogen,and that decreased CFU-OB replication may contribute to age-related bone loss. To facilitate study of the regulation of CFU-OB using a genetic approach, we developed a strategy to transduce them with a replication defective murine retrovirus expressing genes of interest. A construct containing an internal ribosomal entry site was made to allow expression of two proteins from a single mRNA. For preliminary experiments, we made a construct expressing enhanced green fluorescence protein (EGFP) and neomycin phosphotransferase (neo). The former permitted nondestructive monitoring of transduction efficiency, and the latter allowed selection of transduced cells with G418. Marrow cells were obtained from mice expressing β-galactosidase under the control of an osteocalcin promoter so that osteoblast formation from CFU-OB could be detected by X-gal staining. The cultures were infected 4 days after plating, when CFU-OB are known to be dividing and thus susceptible to retroviral infection. Then, cells were subcultured and maintained for 20 days in medium containing 1 mM ascorbate-2-phosphate to induce osteoblast differentiation. We found that 40-60% of X-gal-positive colonies were composed exclusively of cells expressing EGFP, indicating efficient transduction of CFU-OB. After selection in G418, all such colonies expressed EGFP. To begin analysis of the role of apoptosis in CFU-OB behavior and fate, marrow cells were infected with a retrovirus expressing neo and human Bcl-2, which inhibits apoptosis. As a negative control, cells were infected with the EGFP/neo retrovirus. After G418 selection, all the cells in fibroblastic colonies that developed in cultures transduced with the Bcl-2/neo retrovirus, but not the EGFP/neo retrovirus, exhibited immunostaining for human Bcl-2. More important, only Bcl-2-transduced cells were resistant to apoptosis stimulated by etoposide, as determined by immunostaining for active caspase-3. These findings show that human Bcl-2 was expressed at levels sufficient for blockade of the death program in CFU-OB and their progeny; and they set the stage for investigation of the role of apoptosis in osteoblast differentiation and bone formation.

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Bcl-2 Overexpression Prevents Glucocorticoid-induced Apoptosis in Osteoblasts from the H2K-BCL-2 Transgenic Mouse. G. Gronowicz,*[1] M. B. McCarthy,*[1] L. Aguila.*[2] [1]Orthopaedics, UCONN Health Center, Farmington, CT, USA, [2]Center for Immunotherapy, UCONN Health Center, Farmington, CT, USA.

The anti-apoptotic protein Bcl-2 has been shown to be inhibited by glucocorticoids, which increases apoptosis in primary osteoblasts. The transgenic mice (H2K-BCL-2) overexpresses human bcl-2 (hbcl-2) in all cells of the hematolymphoid system including spleen, thymus and lymph nodes (Domen,et al., 1998 Blood 91:2272). H2K is a major histocompatibility (MHC) class I promoter. We found that human bcl-2 is also expressed in mouse osteoblasts from this transgenic mouse, therefore the goal of this study was to use osteoblasts from the transgenic mouse to determine if overexpression of bcl-2 would prevent glucocorticoid-induced apoptosis. Primary osteoblasts were isolated by hyaluronidase/collagenase digestion (third digestion, fraction 3) of 1 month-old mouse calvaria from transgenic and control littermates. The transgenic osteoblasts expressed hbcl-2 as determined by Western blot with an antibody specific to the human isoform (Santa Cruz Biotechnology, Inc.) while osteoblasts from the nontransgenic littermate did not demonstrate hbcl-2. Treatment with 1 to 100 nM corticosterone caused a dose-dependent decrease in mouse bcl-2 levels in osteoblasts from the nontransgenic mice. Apoptosis assessed by acridine orange/ethidium bromide staining and TUNEL increased in a dose-dependent manner to a maximal 4-fold increase compared to control, untreated osteoblasts. In contrast, the osteoblasts from the H2K-BCL2 mouse demonstrated no changes either in hbcl-2 or mouse bcl-2 levels and did not undergo apoptosis in response to 1 to 100 nM corticosterone. Therefore, transgenic overexpression of bcl-2 driven by the H2-K promoter prevents glucocorticoid-induced apoptosis in calvarial osteoblasts.

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Raloxifene Protects Osteoblasts from Apoptosis Induced by Sodium Nitroprusside and Low Serum Concentration. S. Olivier,*[1] C. Ribbens,*[1] B. Relic,*[1] P. Durez,*[2] M. P. Merville,*[3] M. Malaise,*[1] N. Franchimont.[1] [1]Rheumatology, CHU Sart-Tilman, Liège, Belgium, [2]Eli Lilly, Brussels, Belgium, [3]Chimie Médicale, CHU Sart-Tilman, Liège, Belgium.

Raloxifene is a selective estrogen receptor modulator (SERM) currently used for the treatment of postmenopausal osteoporosis. Raloxifene has agonist estrogen-like effects on bone remodeling and increases bone mass in osteoporotic women. Clinical studies revealed that raloxifene was even more potent in reducing vertebral fractures in osteoporotic women, suggesting additional positive properties on bone quality. However, the exact mechanisms of raloxifene effects on bone cells are not fully understood. We tested the hypothesis that raloxifene decreases osteoblast apoptosis induced by the nitric oxide (NO) donor sodium nitroprusside (SNP). It is believed that high concentrations of NO in response to proinflammatory cytokines inhibit proliferation and induce apoptosis of osteoblasts. For this purpose, we studied the effects of SNP at 1 to 4 mM in the presence or absence of raloxifene or estradiol on the survival of mouse osteoblastic cells MC3T3-E1. MC3T3-E1 apoptosis was evaluated by quantitative colorimetric cell viability assay, by DNA fragmentation and FACS analysis using Annexin-V labelling. The assays were conducted in 1% charcoal treated fetal calf serum (FCS) to reduce steroid interactions. We demonstrated significant levels of estrogen receptor-beta in MC3T3-E1 by Western blotting while levels of estrogen receptor-alpha were barely detectable.In 1% charcoal treated FCS, low levels of apotosis were observed in the absence of SNP, indicating that serum and/or steroids are required to prevent apoptosis in osteoblasts. Treatment with SNP at 2 mM for 24 h reduces cell viability to 30-40% of the control in both untreated and estradiol (10^{-6} M) treated cultures. In contrast, in the presence of raloxifene (10^{-6} M), 90% of the cell viability was sustained after treatment with SNP at 2 mM. Similar results were obtained whether the cells were pretreated with raloxifene for 48 h before SNP treatment or treated simultaneously with SNP. In addition, raloxifene positive effect on cell viability was dose-dependent, a significant effect being observed at a dose of 10^{-10} M. Interestingly, when using 10% non charcoal treated FCS, a dose of 4 mM SNP was required to induce a 50% cell mortality and raloxifene protective effect was abolished. In contrast, estradiol had a small positive effect on cell viability in 4 mM SNP treated cultures in the presence of 10% serum. These data suggest that raloxifene decreases SNP-induced apoptosis in conditions of low serum and/or steroid concentrations, possibly through mechanisms distinct from estrogen.

Disclosures: Eli Lilly and Company,2.

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Withdrawn

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Cell Cycle Regulation During Osteoblast Differentiation, and the Role of c-Fos in Post-Confluence Growth Control. A. Sunters,* D. P. Thomas, D. Harmey, A. Tumber,* K. Beedles,* W. A. Yeudall,* A. E. Grigoriadis. Craniofacial Dev, KCL, London, United Kingdom.

The c-Fos proto-oncogene has been shown to play an important role in osteoblast differentiation, as exemplified by the formation of osteosarcomas in c-Fos overexpressing transgenic mice. We have demonstrated previously that alterations in the expression of key cell cycle regulators occur upon the expression of a c-Fos transgene *in vivo*. Furthermore, induction of exogenous c-Fos expression in primary mouse osteoblasts and in AT9.2 cells, a clone of MC3T3-E1 that expresses c-Fos under the control of a tetracycline (Tc) regulatable promoter, results in elevated cyclin A expression and accelerated S-phase entry. However, how cell cycle control is regulated during osteoblast differentiation and how c-Fos controls both these processes is unclear. Primary mouse osteoblasts were induced to differentiate by the inclusion of ascorbate and beta-glycerophosphate in the culture medium, which resulted in the expression of alkaline phosphatase and the formation of mineralised bone nodules after 15 days. We measured the expression of cell cycle control genes in differentiating compared to non-differentiating cultures (without ascorbate and beta-glycerophosphate) in order to determine what changes in cell cycle regulation were differentiation specific, and which were effects of long term culture. Early changes that were differentiation specific were upregulation of cyclin D3, prolonged expression of cyclin A, CDK1, CDK2, and p27, and a decrease in p57 expression. At later time points, we observed a dramatic increase in p16 expression in differentiating cultures. Expression of p16 is associated with senescence, and we also observed an increase in staining for senescence-associated-beta-galactosidase activity in differentiating cultures, suggesting a link between osteoblast differentiation, cell cycle regulation and senescence.Overexpression of c-Fos in primary mouse osteoblasts or in Tc-regulatable osteoblast-like clones caused a reduction in specific markers of osteoblast differentiation. Furthermore, whilst overexpression of c-Fos in AT9.2 cells did not result in an increase in prolliferation in exponentially growing cells, overexpression in post confluent cultures resulted in increased cell number and a higher number of cells in S-phase as determined by flow cytometry. The overexpression of c-Fos in postconfluent cells also resulted in prolonged expression of cyclin E.Taken together, these data show that osteoblastic differentiation is characterised by specific changes in cell cycle control, which are not observed in non-differentiating cultures, and that c-Fos can regulate post confluent growth in osteoblasts.

SA182

Effects of Estrogen on Human Osteoblast Gene Expression. D. C. Ireland,* S. Bord, S. R. Beavan,* J. E. Compston. University of Cambridge, Cambridge, United Kingdom.

Histomorphometric data in women given long-term, high-dose estradiol therapy show evidence of increased osteoblast activity whereas conventional HRT does not appear to have such an effect. We have investigated the effects of high (10^{-7}M) and physiological (10^{-10}M) concentrations of 17β-estradiol on the expression of genes for estrogen receptors (ERs), osteoprotegerin (OPG), RANKL, alkaline phosphatase (ALP), osteocalcin (OC) and type I collagen (COLIA1) in cultured low-passage, non-transformed human osteoblasts. Human osteoblasts were grown for two days in flasks coated with type I collagen in medium supplemented with vitamin C (100μM), hydrocortisone (200nM), β-glycerophosphate (7.5mM) and human male serum (10% v/v)and then treated with medium containing 10^{-7}M, 10^{-10}M or no added 17β-estradiol for 48 hours. RNA isolated from harvested cells was used for real-time, fluorescence-based quantitative RT-PCR. Following normalisation to GAPDH, mRNA levels relative to cultures with no added estradiol were calculated. Results shown in the table below demonstrate that estradiol increases levels of mRNAs for ER beta, OPG, RANKL, ALP and COLIA1 in human osteoblasts although the functional significance of the changes remains to be determined.

Relative ratios of mRNAs in estradiol-treated human osteoblasts

	no estradiol mean (std dev)	10-10M estradiol mean (std dev)	10-7M estradiol mean (std dev)
ER alpha	1.0 (0.3)	1.1 (0.2)	1.2 (0.1)
ER beta	1.0 (0.2)	1.4* (0.1)	1.5* (0.2)
OPG	1.0 (0.2)	1.3 (0.1)	2.6* (0.4)
RANKL	1.0 (0.3)	1.8 (0.8)	28.0* (3.0)
ALP	1.0 (0.2)	1.3 (0.4)	2.1* (0.1)
OC	1.0 (0.3)	1.1 (0.2)	1.2 (0.1)
COLIA1	1.0 (0.2)	1.4* (0.2)	2.4* (0.1)

Statistically significant changes are indicated with an asterisk ($p<0.05$)

SA183

Reduced Estrogen Influences Bone Remodeling by Delaying the Differentiation of Bone Marrow-Derived Osteoclast-Inductive Precursors into Osteoblastic Cells. J. Owens, M. Bouxsein, V. Rosen. Genetics Institute, Cambridge, USA.

Post-menopausal women who develop low bone density are at an increased risk of fragility fractures. In normal individuals, rhBMP-2 enhances fracture repair. However, it is not known whether rhBMP-2 would have the same positive effect on bone healing in estrogen deficient individuals. We addressed this question by comparing the response of bone marrow cells to rhBMP-2 stimulation before and after ovariectomy (ovx). We acquired bone marrow aspirates from ten skeletally mature female baboons before ovx and 6 and 12 months post-ovx. Compared to pre-ovx, the post-ovx samples had a 3 fold increase in the number of osteoblastic colonies which formed in our bone marrow cell cultures ($p<0.01$). RhBMP-2 increased alk phos activity in both pre- and post-ovx cells. However, the increase in alk phos activity in response to rhBMP-2 was 10 times lower post-ovx ($p<0.01$).The addition of estrogen to post-ovx cells for 14 days in vitro failed to restore the response to rhBMP-2 to pre-ovx levels. In contrast, increasing the exposure time to rhBMP-2, (from 14 to 21 days), restored alk phos activity to pre-ovx levels. These results suggest that without estrogen, the response to rhBMP-2 is delayed. Northern analysis of the type I BMP receptor, ALK3, showed decreased levels in post-ovx cells. The loss of

BMP receptors was not restored by the addition of estrogen in vitro.To test our hypothesis that ovx leads to a delay in the differentiation of bone marrow stromal cells, we investigated changes in RANKL and OPG expression using Northern analyses. We found that OPG mRNA levels increase and RANK-L mRNA levels decrease during osteoblast differentiation. The RANK-L:OPG ratio of mRNA expression was greater in our post-ovx samples compared to pre-ovx samples, indicating the presence of a greater proportion of osteoclast-inductive stromal cells and therefore, delayed osteoblast differentiation and increased osteoclast formation. In conclusion, these data suggest that the reduced response of post-ovx bone marrow to rhBMP-2 stimulation is due in part to a decrease in BMP receptor expression levels and in part to a delayed differentiation of osteoclast-inductive stromal cells into osteoblastic cells, resulting in increased osteoclastogenesis.

SA184

Participation of Signal Transducer and Activator of Transcription Proteins in Estrogen-induced Signaling. A. Maran, A. M. Kennedy,* R. T. Turner. Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

Signal transducers and activators of transcription (STATs) transduce signals initiated by many growth factors and cytokines. Estrogen is important for the normal growth and remodeling of bone. While the physiological role of estrogen has been well defined, the signal transduction pathways utilized by estrogens in bone cells are largely unknown. To better understand the molecular mechanism of action of estrogens, we have studied the effects of 17-β estradiol on Janus Kinase (JAK)/STAT regulation in human fetal osteoblast (hFOB) cells in culture. 17-β estradiol stimulated various members of Stat family. The induction of Stat 1, -2, -3, -5a, -5b and -6 were noticed in estrogen treated human osteoblasts. Estrogen treatment resulted in the activation of Stat1 protein, which accompanied an increased binding to interferon-stimulated response element in the DNA. This provides first direct evidence that Stat 1 is a transducer for the estrogen signaling in bone cells. The estrogen receptor antagonist ICI 182, 780 blocked the regulation of Stat1 by estrogen indicating that this pathway is estrogen receptor dependent. Induction of Stat gene expression and activity by estrogens in osteoblastic cells suggests that components of JAK/STAT signal transduction pathway may mediate distinct estrogen mediated functional processes in skeletal system.

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The Phytoestrogen Genistein Stimulates the Production of Osteoprotegerin by Human Trabecular Osteoblasts. V. Viereck,[1] C. Gruendker,*[1] S. Blaschke,*[1] H. Siggelkow,[1] G. Emons,*[1] L. C. Hofbauer.[2] [1]University of Goettingen, Goettingen, Germany, [2]University of Marburg, Marburg, Germany.

The anti-resorptive effects of estrogen on bone metabolism are thought to be mediated through modulation of paracrine factors produced by osteoblastic lineage cells that act on osteoclastic lineage cells. Receptor activator of nuclear factor-kappaB ligand (RANKL) is the essential factor for osteoclast formation and activation, and enhances bone resorption. By contrast, osteoprotegerin (OPG) which is produced by osteoblastic lineage cells acts as a decoy receptor that neutralizes RANKL, and prevents bone loss. Recently, 17beta-estradiol was found to enhance OPG mRNA levels and protein secretion in a human osteoblastic cell line through activation of the estrogen receptor (ER)-alpha. In this study, we assessed the effects of the phytoestrogen genistein on OPG mRNA steady state levels (by semiquantitative RT-PCR and Northern analysis) and protein production (by ELISA) in primary human trabecular osteoblasts (hOB) obtained from healthy donors. Genistein increased OPG mRNA levels and protein secretion by hOB cells by up to two- to six-fold in a dose- ($P < 0.0001$) and time-dependent ($P < 0.0001$) fashion with a maximum effect at 10-7 M. Co-treatment with the pure ER antagonist ICI 182,780 completely abrogated the stimulatory effects of genistein on OPG protein secretion, indicating that the effects observed were specific and directly mediated through the ER. Pre-treatment with genistein partially prevented the inhibitory effects of the glucocorticoid dexamethasone on OPG mRNA and protein production. The stimulation of OPG mRNA levels by genistein was not affected by the protein synthesis inhibitor, cycloheximide, and was shown to be due to enhancement of OPG gene transcription. In conclusion, these data suggest that the phytoestrogen genistein is capable of up-regulating the production of OPG by human osteoblasts. Thus, dietary sources of phytoestrogens may help to prevent bone resorption and bone loss by enhanced osteoblastic production of OPG.

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Differential Effects of Genistein (GEN) and Daidzein (DIZ) on MC3T3-E1 Osteoblastic Cell Proliferation and Differentiation. X. W. Chen,*[1] S. C. Garner,[2] J. J. B. Anderson.[1] [1]Dept. of Nutrition, University Of North Carolina, Chapel Hill, NC, USA, [2]Dept. of Surgery, Duke Medical Center, Durham, NC, USA.

The mechanisms by which estrogen-like molecules, including isoflavones, improve bone retention in ovariectomized rodent models remain unclear. In this *in vitro* study, the effects of genistein (GEN) and daidzein (DIZ), two major isoflavones, were compared to 17β-estradiol (E_2) in MC3T3-E1 osteoblastic cells. Cell proliferation was evaluated by BrdU staining and by MTT assay. At high concentrations (~10^{-3} M), GEN or E_2, but not DIZ, strongly inhibited cell proliferation through stimulation of apoptosis ($P<0.05$). At nutritionally relevant concentrations (10^{-9} - 10^{-6} M), only slight stimulatory effects of GEN or E_2 were found; however, DIZ increased cell viability about 47%. In cell differentiation studies, MC3T3-E1 cells were cultured up to 16 days, and GEN or DIZ were added with fresh medium on either day 8 or day 12 for 8-day or 4-day treatments, respectively. Cells and culture medium were harvested for isolation of total RNA, and measurements of alkaline phosphatase (ALP), and interleukin-6 (IL-6). Although no significant effects of GEN, DIZ, or E_2 on ALP activities (adjusted for DNA) were detected during 4-day treatment periods, DIZ, but not GEN or E_2, significantly increased ALP activities 100%-120% during 8-day treatment periods ($P<0.05$). IL-6 synthesis (adjusted for DNA) was inhibited about 50%-70% by either GEN, DIZ, or E_2 during both 4-day and 8-day treatments, and the stronger inhibitory effects were found in the cells treated with DIZ or E_2. In addition, semi-quantitative RT-PCR was performed with gene-specific primers, and the predicted product sizes of 360 bp, 460 bp and 631 bp for osteoprotegerin (OPG)/osteoclastogenesis inhibitory factor (OCIF), receptor activator of NF-kB ligand (RANKL)/osteoclast differentiation factor (ODF), and GAPDH, respectively, were obtained. The ratios of OPG/OCIF mRNA to RANKL/ODF mRNA increased 282%, 254%, 218% in the MC3T3-E1 cells treated with GEN, DIZ, or E_2 at 10^{-8}M, respectively, in comparison to vehicle (control). In summary, because of the critical functions of IL-6, OPG/OCIF, and RANKL/ODF in osteoclast differentiation, the modulation of these three osteoblastic products by genistein and daidzein may be one important mechanism through which isoflavone molecules reduce the risk of osteoporosis. The differential effects of genistein and daidzein, compared to 17β-estradiol, suggest that two different pathways (estrogen receptor-dependent vs. estrogen-independent pathways) of regulation of osteoblastic functions may be involved.

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Estrogen and Raloxifene Modulate the Expression of Osteoprotegerin (OPG) and RANK-Ligand (RANKL) in Human Osteoblast-like Cells at Supra-physiological/Pharmacological Concentrations. G. Hampson,[1] J. Cheung,[1] Y. T. Mak,[1] S. Papaioannou,*[2] B. Evans,*[3] I. Fogelman,[4] A. E. Grigoriadis.[2] [1]Chemical Pathology, St Thomas Hospital, London, United Kingdom, [2]Orthodontics and Paediatric Dentistry, Kings College, London, United Kingdom, [3]Child Health, University Hospital of Wales, Cardiff, United Kingdom, [4]Nuclear Medicine, Guy's Hospital, London, United Kingdom.

Oestrogen (E2) inhibits osteoclastic activity and resorption, at least in part, by regulating the production of IL-6, IL-1 and TNF by osteoblasts. It is now thought that the final step in the osteoclast regulatory pathway may be determined by the relative ratio of RANK-ligand (RANKL) to osteoprotegerin (OPG). The aim of the current study was to assess the regulation of IL-1, OPG and RANKL by E2 and the selective estrogen receptor modulator, Raloxifene (RAL). We studied an immortalised human bone marrow stromal cell line with osteoblastic characteristics (HCC1) and primary human osteoblastic cells (HOB). We tested physiological (10-8M) and supra-physiological (10-7, 10 -6 M) concentrations of E2. IL-1 alpha and beta and OPG were measured by ELISA. The expression of RANKL and OPG protein was also determined by immunolocalisation. Gene expression of OPG and RANKL in the HCC1 cells was measured using the SYBR Green PCR assay. IL-1 alpha and beta were significantly decreased following treatment with E2 and RAL at 10-7 and 10-6 M (E2 :73%, 66%. RAL : 69%, 73% of control, p =0.01). There was a significant reduction in OPG in the HCC1 and HOB cells at E2 and RAL concentrations of 10-6 M only (HOB, E2 : 63%, RAL : 55%. HCC1 , E2 : 54%, RAL : 39%, p=0.001). The same trend was observed when OPG expression was determined by immunolocalisation. The reduction in OPG was less than that found following treatment with Dexamethasone (DEX) at 10 -9, -8 and -7 M (HCC1 , DEX 10-9M : 15%, 10-8M : 16% , 10-7M : 25% of control p=0.001). There was also a decrease in RANKL protein expression in the HCC1 and HOB cells at E2 and RAL concentrations of 10-6M (HOB % RANKL positive cells Control : 12.3%, E2 : 3.8%, RAL : 2.8%. HCC1 , Control :12.9%, E2 : 4.6%, RAL : 7.1%). RANKL mRNA expression was significantly reduced following treatment with RAL at all concentrations tested (RAL 10-8 M : 52%, 10-7M : 62%, 10-6M : 53% of control p =0.001). However the ratio of RANKL/OPG mRNA did not differ significantly from control following either E2 or RAL treatment. E2 and RAL modulate the production of OPG and RANKL in human osteoblastic cells at supra-physiological/ pharmacological concentrations (10-6M). Although both E2 and RAL lead to a decrease in OPG and RANKL at this concentration, the ratio of these two factors remains unchanged.

SA189

The Orphan Receptor, Estrogen Related Receptor alpha (ERRα), May Regulate Bone Formation by Its Direct Effect on Bone Sialoprotein (BSP) Expression. V. Kung,* E. Bonnelye, J. E. Aubin. Dept of Anatomy & Cell Biology, University of Toronto, Toronto, ON, Canada.

The orphan estrogen related receptor alpha, ERRα, is known to be expressed by osteoblastic cells and, in promoter-reporter assays, to transactivate at least one osteoblast associated gene, osteopontin (OPN). This, together with its relationship to the estrogen receptors, ERα and ERβ, makes it an interesting and important potential regulator of bone formation and maintenance of bone mass. We recently described the expression of ERRα *in vivo*in sections of fetal (21 d. fetal rat calvaria (RC)) & adult (femur) bones, and in RC cell populations *in vitro*. To dissect further possible involvement of ERRα in bone development, we next blocked ERRα in differentiating RC cell cultures using phosphorothioate-modified antisense oligonucleotides. Concomitant with a dose-dependent total inhibition of bone nodule formation, we observed by RT-PCR and confirmed by immunocytochemistry a marked decrease in bone sialoprotein (BSP) expression in antisense-, but not in sense-, treated RC cultures. Interestingly, we found no evidence for changes in OPN expression levels in the same antisense-treated samples. BSP and ERRα were found to be co-expressed in very early osteoprogenitors *in vitro*(by PCR) and in mature osteoblasts *in vivo*(by immunocytochemistry). Given these observations, we next asked whether the effect of ERRα on BSP expression was direct or indirect. A sequence screen of the rat BSP promoter revealed a potential ERRα core binding site (-32 AAGAAGGGTT**TATAGGT-CA**GCAAGA -8). Moreover, by EMSA, we found evidence for potential interaction of

ERRα on this core binding site. Our data indicate that ERRα interacts with the BSP promoter, and strengthens the possibility that BSP is a potential target gene of ERRα, suggesting one pathway by which ERRα exerts its effect on osteoblast development.

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Molecular Mechanisms of Osteoblastic Transformation in HumanOsteosarcomas. D. J. Papachristou,1 A. Bourli,*2 N. Arnogiannaki,*3 M. Assimakopoulou,*1 J. Varakis,*1 A. G. Papavassiliou.4 1Anatomy, University of Patras, School of Medicine, Patras, Greece, 2St. Olga Anticancer Hospital, Athens, Greece, 3St. Sabbas Anticancer Hospital, Athens, Greece, 4Biochemistry, University of Patras, School of Medicine, Patras, Greece.

Transcription factor AP-1 (a homo-/heterodimer comprised of c-Jun-and c-Fos-related proteins) binds to specific control elements in thepromoters of osteoblastic genes and modulates their expression. Thecomposition of AP-1 complexes, the phosphorylation status of c-Jun, andthe presence of auxiliary proteins dictate AP-1 activity in variouscontexts. AP-1-mediated gene expression contributes towards a diversityof important end-points, including proliferation, differentiation, andneoplastic transformation. Alpha-NAC (a-NAC) is an AP-1 co-activatorrestricted to osteoblasts, which is considered to be implicated in theregulation of gene expression during osteoblastic differentiation.Specifically, a-NAC augments the transcriptional potential of thehomodimeric c-Jun complex, but has no effect on the c-Jun/c-Fosheterodimer. Because AP-1 has a fundamental role in osteoblast geneprograming, it is plausible that alterations in theexpression/activation profile of AP-1 components/auxiliary factors mightbe involved in the biology of osteogenic tumors. We performedimmunohistochemical studies in paraffin sections of human osteosarcomas(anaplastic, intramedullary, periosteal, paraosteal) to investigate theexpression profile of a-NAC, in conjunction with theexpression/activation state of c-Jun and the kinases that are known tostimulate its transactivation potential (c-Jun N-terminal kinases[JNKs1/2]). In a high percentage of interspersed tumor cells of allosteosarcomas examined, there was a strong a-NAC immunoreactivity aswell as a co-localized enhancement in the expression of JNK1, JNK2, andtheir cognate substrate c-Jun. These inductive effects paralleled thepresence of the activated forms of c-Jun and JNKs and appeared tocorrelate with the histopathologic grading of the tumors. In accordancewith the minimal expression of the aforementioned proteins innon-proliferating osteoblasts in vitro, non-tumorous parts of thespecimens were devoid of the above immunoreactivities. Our resultsindicate the involvement of the JNK/c-Jun signaling cassette inosteoblastic transformation and imply that activation of c-Jun via theJNK pathway is a primary event in a cascade of changes related toincreased proliferative activity of human osteosarcoma cells. Our dataalso support the hypothesis that a high level of specific AP-1 complexes(i.e. c-Jun/c-Jun dimers) may play decisive role in the process of osteoblastictransformation.

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The Decrease in Adipocyte Differentiation Observed In Vivo and In Vitro in Osteosclerotic ΔFosB Transgenic Mice Occurs at Early Adipocyte Commitment. M. Kveiborg, G. Sabatakos, M. Wu,* R. Baron. Yale University School of Medicine, New Haven, CT, USA.

Several members of the AP-1 family of transcription factors are known to play important roles in the regulation of bone cell differentiation. We have previously reported that overexpression of δFosB isoforms, naturally occurring splice variants of the *fosB* transcript, in transgenic mice resulted in a dramatic increase in bone density due to increased bone formation, shown to be cell-autonomous to the osteoblast lineage (Sabatakos et al. Nature Med 6: 985-990, 2000). In contrast, abdominal fat concentrations were decreased in δFosB transgenic mice, and marrow smears exhibited a reduced adipocyte number. In vitro, both bone marrow stromal cells and calvarial cultures from δFosB mice showed a significant decrease in adipocyte number and maturation, as opposed to increased expression of osteoblastic marker genes. The observation of decreased adipocyte differentiation in the presence of increased osteoblastogenesis in vivo and in vitro raised the possibility that the two events are linked. Therefore, the present study was aimed at determining whether the inhibitory effects of δFosB isoforms on adipocyte differentiation are the result of a direct action on adipocyte precursors or indirectly, via the effects of δFosB isoforms on osteoblasts. δFosB as well as δFosB mutants encoding only the full-length δFosB or the truncated δ2δFosB isoform were overexpressed in both the preadipocytic cell line 3T3-L1 and the mesenchymal cell line ST2, which has the potential to differentiate into both osteoblast- and adipocyte-like cells. Stably or transiently transfected cell lines were induced to differentiate into adipocytes by addition of adipogenic agents. Subsequent morphological and biochemical analysis demonstrated an inhibitory effect of δFosB on adipocyte differentiation, as revealed by reduced Oil Red O staining of lipid droplets and decreased induction of the adipogenic transcription factors PPARγ and C/EBPα. The repression of adipogenesis appeared to be mediated via the truncated δ2δFosB isoform and was more profound in the ST2 cell-line, representing an earlier stage of adipocyte differentiation, than in the already committed 3T3-L1 cell-line. Thus, these results suggest that δ2δFosB may exert cell autonomous inhibitory effects on adipocyte differentiation both at the level of early stem cell commitment, possibly via a decrease in the pool of adipocyte precursors as a consequence of increased osteoblast differentiation, and also on more committed adipocyte precursors.

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SA195

Cbfa1- and Smad-binding Elements Mediate TGF-β Stimulation of Osteoprotegerin (OPG) Gene Expression. K. Thirunavukkarasu,*[1] R. R. Miles,*[2] D. L. Halladay,*[2] X. Yang,*[2] R. J. S. Galvin,[2] S. Chandrasekhar,[2] T. J. Martin,[3] J. E. Onyia.*[2] [1]Elanco Animal Health, Eli Lilly and Company, Greenfield, IN, USA, [2]Gene Regulation, Bone and Inflammation Research, Eli Lilly and Company, Indianapolis, IN, USA, [3]St. Vincent's Institute for Medical Research, Fitzroy, Victoria, Australia.

Transforming growth factor-β (TGF-β) regulates osteoclastogenesis and osteoclast survival, in part through the induction of osteoprotegerin (OPG), a protein known to inhibit osteoclast formation and function. To explore the molecular basis of TGF-β regulation of OPG expression, we evaluated the effects of TGF-β on osteoclast formation, OPG protein secretion, mRNA expression and gene transcription. The marked inhibitory effect of TGF-β on osteoclast differentiation was confirmed in a co-culture model utilizing murine stromal/osteoblastic BALC cells and bone marrow hematopoietic precursors. This inhibition in osteoclast differentiation was preceded by a decrease in RANKL mRNA expression (5-fold) and a reciprocal increase in OPG mRNA (6.1-fold) and protein (7.1-fold) expression in BALC cells. At the promoter/transcriptional level, TGF-β treatment resulted in a 3- to 10-fold increase in reporter gene activity directed by a 5.9-kb fragment of the human OPG promoter in transfection assays performed in UMR106 cells. The effect of TGF-β was mimicked by TGF-β2 and -β3, but not BMP-4, suggesting a TGF-β signal-specific effect. Deletion analysis revealed that a 183-bp region (-372 to -190) in the promoter was required for TGF-β responsiveness, and this region was sufficient to confer TGF-β inducibility to a heterologous (osteocalcin) minimal promoter. Substitution mutations that disrupted the Cbfa1- and/or Smad- binding elements present in the 183-bp region resulted in a decrease in baseline expression and in the responsiveness to TGF-β and Cbfa1. Collectively, these studies indicate the involvement and possible interaction of Cbfa1 and Smad proteins in mediating the effects of TGF-β on OPG transcription.

Disclosures: Eli Lilly and Company,3.

SA196

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SA197

YKL-40 Gene Expression Is Inhibited by Cyclosporin A in Human Osteoblast-Like Cell Line MG63

E. Musacchio,* C. Valvason,* A. Pozzuoli,* G. Priante,* L. Punzi,* F. S. Netto,* L. Sartori. Department of Medical and Surgical Sciences, University of Padova, Padova, Italy.

YKL-40, also known as HC gp-39, is a human glycoprotein related in aminoacid sequence to the chitinase family, but shows no chitinase activity. It is a major secretory protein of human cartilage mainly produced, in vitro and in vivo, by chondrocytes and synoviocytes. It is also actively secreted in vitro by the osteoblast-like cell line MG63. YKL-40 mRNA is undetectable in normal human cartilage, but it is prominent in patients with rheumatoid arthritis (RA) and appears to play a role in cartilage remodelling perhaps as a glycosidic bond hydrolase with polysaccaride specificity. It has been identified as a target of the immune response in RA, while its circulating levels have been suggested to be used as a marker of inflammatory stage in several rheumatic disorders. The very effective immunosuppressant cyclosporin A (CyA) is widely used in the treatment of rheumatic diseases, yet nothing is known about its effect on YKL-40 release in patients. We have previously reported that CyA has several direct effects on osteoblast activity both at the phenotyipical and molecular level. The aim of the present study was to investigate the effect of CyA on YKL-40 gene expression in the human osteoblast-like cell line MG63 treated with CyA 0.1, 1.0, 2.5 and 5.0 ug/ml for either 48 or 96 hours. After treatments, total RNA was extracted, and gene expression was determined using the comparative semi-quantitative RT-PCR approach, glyceraldehyde-3P-dehydrogenase being the housekeeping gene. Our results indicate that CyA induces a marked down-regulation of YKL-40 gene expression in this cell line. The response was maximum after 96 hrs of treatment being 60% less than controls when the highest CyA dose (5 ug/ml) was used and showed a dose-dependent decreasing pattern, with a 25% down-regulation achieved already with CyA 0.1 ug/ml. The same trend was observed after 48 hrs with a decrease ranging from 20% (CyA 0.1 ug/ml) to almost 50% (CyA 5 ug/ml).To our knowledge, these results represent the first report of YKL-40 expression modulation in vitro by a pharmacological agent and may contribute to a better understanding of the role of this protein in rheumatic diseases.

SA198

See Friday Plenary number F198.

SA199

Synergistic Induction of Transcriptional Activity of the cAMP Response Element Binding Protein by Parathyroid Hormone and Epidermal Growth Factor in Osteoblastic Cells. J. T. Swarthout,[*1] D. R. Tyson,[*2] S. C. Jefcoat,Jr.,[*1] N. C. Partridge.[1] [1]UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, United States, [2]University of California, Irvine, CA, United States.

We have previously shown that transactivation of the cAMP response element binding protein (CREB) by parathyroid hormone (PTH) requires both serine 129 (S129) and serine 133 (S133) in UMR 106-01 cells. Furthermore, while PKA is responsible for phosphorylation at S133, glycogen synthase kinase-3 (GSK-3) activity is also required and may be responsible for phosphorylation of CREB at S129. The AP-1 member c-fos has been implicated in the mitogenesis and/or differentiation of osteoblasts in the skeletal system, and CREB is a major activator of c-fos transcription. It is known that both epidermal growth factor (EGF) and PTH activate c-fos transcription in addition to phosphorylating CREB. The ability of EGF to stimulate CREB and the requirement for both S129 and S133 in the transactivation of CREB by EGF in osteoblastic cells is unknown. Here we show, using the GAL4-CREB reporter system, that EGF can transactivate CREB in UMR 106-01 cells in addition to PTH's effects. Furthermore, using mutational analysis we demonstrate that S129 and S133 are required for EGF-induced transcriptional activity of CREB. Furthermore, EGF activates both p38 and ERKs in UMR 106-01 cells and pre-treatment with either the p38 inhibitor (SB203580) or the MEK inhibitor (PD98059), respectively, prevented activation of p38 and ERKs by EGF. These agents also inhibit phosphorylation of CREB at S133 and transactivation of CREB by EGF, but not by PTH. In addition, treatment of UMR 106-01 cells with both PTH and EGF results in a greater phosphorylation of CREB at S133 than that seen with either PTH or EGF alone, and the synergistic transactivation of CREB. The latter effect is significantly reduced following pre-treatment with a PKA inhibitor (H-89) or by co-treatment with SB203580 and PD98059. Therefore, transactivation of CREB by EGF occurs following activation of p38 and ERKs, and requires both S129 and S133. Furthermore, co-treatment with both PTH and EGF results in the synergistic transactivation of CREB. This work emphasizes the different pathways that these two agents signal through and their possible synergistic effects in vivo on common endpoints.

SA200

See Friday Plenary number F200.

SA201

Glucocorticoid Treatment Inhibits Gene Expression of Heparin Binding Growth Associated Molecule (HB-GAM) in Mouse Primary Osteoblasts. M. Lorentzon,[*1] C. Ohlsson,[*1] A. Bäckman,[*1] R. Lorentzon,[1] U. H. Lerner.[2] [1]Department of Surgical and Perioperative Sciences, Sports Medicine Unit, Umeå University, Sweden, [2]Department of Oral Cell Biology, Umeå University, Sweden.

Glucocorticoids are known to have deleterious effects on the skeleton. Suggested pathogenetic mechanisms include stimulation of bone resorption due to increased osteoclastogenesis and inhibition of osteoblastic bone formation. The molecular mechanisms, however, are poorly understood. Since the key cell in bone metabolism is the osteoblasts we have studied the effect of glucocorticoids on gene expression in mouse calvarial osteoblasts using cDNA expression arrays (Clontech, Paulo Alto, CA, USA) containing a total of 2352 cDNAs. Primary mouse osteoblasts were isolated from calvarial bones from 2 to 3 days old CSA mice, cultured in a-MEM containing 10% fetal calf serum, with or without dexamethasone (10-7 M). Cells were harvested, after 20 hrs, 40 hrs, and 16 days, and RNA was subsequently isolated. 32P -labelled first-strand cDNA probes were synthesized using Superscript II (Life Technologies, USA), hybridized to Atlas Array Mouse 1.2 and Mouse 1.2II (Clontech), and scanned on a Storm 860 PhophoImager (General Dynamics). Image analysis was performed using Atlas Array v1.5 software (Clontech). Heparin binding growth associated molecule (HB-GAM) gene expression was lower at 20 hrs (ratio 0.24), 40 hrs (ratio 0.26-0.36), and at 16 days (ratio 0.16) in cell cultures treated with dexamethasone (10-7 M), compared to controls. A reverse transcription (RT)- polymerase chain reacion (PCR) was chosen to, semi-quantitatively, verify the observed downregulation of HB-GAM gene expression at 40hrs. RNA from dexamethasone treated osteoblasts showed less intense RT-PCR fragments (ethidium bromide stained) for HB-GAM for all tested number of PCR cycles (28-36), while no differences could be detected for GAPDH (used as an internal control) RT-PCR fragments. Mineralization of bone nodules in 16-day-old cultures was significantly inhibited by dexamethasone demonstrating the inhibitory effect of glucocorticoids on in vitro bone formation. HB-GAM is an extracellular matrix-associated protein, believed to play an important role in osteoblast recruitment and attachment, processes necessary for bone formation. Most interestingly, transgenic mice overexpressing HB-GAM develop increased bone thickness [Imai, S. et al, J Cell Biol, 43: 1113-1128, 1998].The downregulation of HB-GAM gene expression following dexamethasone treatment suggests a role for HB-GAM in glucocorticoid-induced bone loss.

SA202

See Friday Plenary number F202.

SA203

Thrombin Peptide TP508 Regulates BMP-2 and -7 Expression By Human Osteoblasts. L. X. Bi,* Y. Ji,* R. S. Crowther,* E. Mainous,* W. L. Buford.* The University of Texas Medical Branch, Galveston, TX, USA.

Previous studies have shown that osteoblastic cells express a non-proteolytically activated receptor (NPAR) that can be activated by a synthetic thrombin peptide (TP508). To investigate potential effects of TP508 on human osteoblasts, we examined expression of BMP-2 and -7, alkaline phosphatase activity (ALP) after treatment of cells with TP508. Human osteoblasts, obtained from Clonetics, Inc, San Diego, CA, were cultured in mineralizing-growth medium and treated with TP508 [10^{-6}M] for 3, 5, 7 days, respectively. The level of ALP and cell proliferation were assayed using a commercial kit (ALP and MTT, Sigma Chemical Co., St. Luis, MO). ALP activity was expressed on the basis of cell numbers. Expressions of BMP-2 (anti-rhBMP-2 monoclonal antibody, Genetics Institute, Cambridge, MA) and –7 (polyclonal antibody, Santa Cruz Biotechnology, Inc. CA) were examined using western blot analysis. The relative densities of different bands were quantified by Scion Image densitometry and normalized to a-tubulin. ALP activities were elevated (22-80%, $P<0.05$) within the first 5 days of culture by TP-508, compared to control group. BMP-7 expression was significantly increased at day 3 (68%, $P<0.05$) and day 5 (132%, $P<0.01$) after TP-508 treatment compared to control. There was increase in the level of BMP-2 protein (83%, $P<0.05$) within the first 3 days of culture, above the baseline. We conclude that thrombin peptide, TP-508, increases the expression of osteoblastic markers which play an important role in fracture healing in early stage. It may trigger the cascade of bone repair in vitro after fracture.

SA204

See Friday Plenary number F204.

SA205

Microarray Analysis of Early Gene Expression Profile in Primary Human Osteoblast Cells Treated with Connective Tissue Growth Factor-Like Protein. C. R. Hanning,[*1] S. C. Tice,[*1] S. Ghosh,[*2] B. J. Votta,[1] M. W. Lark,[1] M. Gowen,[1] S. Kumar.[1] [1]Musculoskeletal Diseases, GlaxoSmithKline, King of Prussia, PA, USA, [2]Transcriptome Analysis Group, GSK, King of Prussia, PA, USA.

Connective Tissue Growth Factor-Like protein (CTGF-L) belongs to the CCN family of growth factors and has been shown to bind IGFs, promote adhesion of osteoblasts, and inhibit osteocalcin production. CTGF-L is expressed at high levels in primary osteoblasts and bone. We have examined the expression of CTGF-L during in vitro mineralization of primary osteoblasts. The expression of CTGF-L mRNA correlated with the expression of type I collagen and mineralization. CTGF-L mRNA was induced at day 14 and remained elevated up to day 28 when the mineralization was at maximum. We engineered a stable TF274 cell line expressing CTGF-L protein and examined their mineralization potential. TF274 cells expressing CTGF-L mineralized faster by day 9 compared to the wild type. These data suggest that CTGF-L protein is involved in mineralization of osteoblast cells. In order to understand the role of CTGF-L in osteoblast differentiation, we have utilized microarray technique to evaluate the effect of recombinant hCTGF-L on the expression of ~900 genes. Primary osteoblasts were treated with CTGF-L for 24 and 72 hours, total RNA was isolated, and microarray analysis was performed using 32P labeled cDNA probes. Two different blots were used; the first was a commercially available human Atlas Array with 588 genes. The second was a custom blot containing 277 genes known to play a role in bone and cartilage metabolism. Data was analyzed using Clontech's Atlas Image and other statistical and clustering software. Using criteria of a minimum 1.5-fold change in expression, we identified 54 (~9%) and 30 (~11%) genes on Atlas and custom blots, respectively, that were up or down regulated. Some of the genes with significant differential expression were BRCA2, MUC18 glycoprotein, glutaredoxin, APC, prothymosin alpha, cyclin D1, transferrin receptor, colon carcinoma kinase-4, integrin linked kinase, Prostaglandin G/H synthetase precursor, Cyr61 protein, Apolipoprotein E precursor, Hc-GP39 precursor, Interleukin 6, EGR-1, and Tumor necrosis factor receptor 1. The expression patterns of genes represented on the custom blot were further analyzed by a non-supervised clustering technique known as self-organizing maps (SOM). Eleven distinct patterns of expression were identified by SOM analysis with a dominant cluster (63 genes) representing genes with reduced expression at 24 and 72 hours post CTGF-L treatment. These results illustrate the utility of microarray technology to identify genes involved in the osteoblast differentiation process.

SA206

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SA208

Osteoblastic Expression of the Rat Osteocalcin-EGFP Transgene in Mice and ex vivo. T. A. Owen,[1] S. L. Smock,[*1] C. Banerjee,[*2] K. S. Harrington,[*2] B. Lu,[*1] T. A. Castleberry,[1] L. C. Pan,[1] S. C. Marks,[2] A. J. van Wijnen,[2] J. L.

Stein,*[2] J. B. Lian,[2] G. S. Stein.[2] [1]Cardiovascular and Metabolic Diseases, Pfizer Global Research & Development, Groton, CT, USA, [2]Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA.

Mice expressing a reporter gene that reflects the growth, differentiation and metabolic activity of osteoblasts would facilitate studies on bone anabolism. To provide a sensitive in situ marker of bone formation and examine osteoblast responses to physiologic mediators of bone formation in longitudinal studies, we fused the DNA sequence encoding enhanced green fluorescent protein (EGFP) to the 1775 bp proximal portion of the rat osteocalcin (OC) gene promoter to construct the OC-EGFP transgene. Transgenic mice were produced on the FVB background and weanlings were phenotyped by observation of EGFP expression in tail snips by fluorescence microscopy. Expression of EGFP was then analyzed in cryosections of calvaria, tibia, femur, lumbar spine, kidney and liver. We found that at 28 days of age, expression of EGFP as observed by fluorescence microscopy, was strong in cells lining both the periosteal and endosteal surfaces of the long bones and calvarium, as well as lining the trabeculae, especially in the lumbar spine. These cells were distinguished as osteoblasts based on morphology and on localization within the bone. Weaker staining was evident in osteocytes. No EGFP was observed in either kidney or liver of the transgenics and no EGFP was observed in any tissue from non-transgenic littermates. Bone marrow cultures initiated from 9 week old mice, as well as calvarial cell cultures initiated from newborn pups, formed bone-like nodules after approximately 14 days and expression of EGFP was observed in the cells on the nodules and in cells immediately surrounding the nodules. Temporally, the expression of EGFP correlated with the appearance of the nodules and, on the RNA level, EGFP was correlated with the onset of osteocalcin expression. Taken together, these data show that we have marked active osteoblasts in transgenic mice with EGFP, a marker that can be easily followed both in tissue sections and during longitudinal studies of differentiation in cell culture.

SA209

Smad3 Promotes ALP Activity and Mineralization of Osteoblastic MC3T3-E1 Cells: Involvement of Type I Collagen, Osteocalcin and p38 MAP Kinase. H. Sowa,* H. Kaji, T. Yamaguchi, T. Sugimoto, K. Chihara.* The third division, Department of Medicine, Kobe University School of Medicine, Kobe, Japan.

TGF-β, abundantly stored in bone matrix, appears to regulate bone metabolism in various ways, including skeletal development and bone remodeling. Although the diverse effects of TGF-β on osteoblasts have been reported, the local administration of TGF-β stimulates bone formation in vivo. On the other hand, the Smad family proteins are critical components of the TGF-β signaling pathways but the role of Smad3 in the expression of osteoblastic phenotype remains unknown. The present study was performed to clarify the role of Smad3 in the proliferation, expression of bone matrix protein and mineralization by employing stably Smad3-transfected mouse osteoblastic MC3T3-E1 cells. Smad3 stable transfection significantly inhibited [^{3}H]-thymidine incorporation and MTT-dye assay, compared to empty vector-transfection(control). Moreover, Smad3 significantly inhibited the level of osteocalcin mRNA, compared to control in Northern-blotting. Smad3 increased the level of type I procollagen, osteopontin, and matrix Gla protein mRNA, compared to control. TGF-β mimicked the effects of Smad3 on osteoblast prolifearation and the expression of bone matrix proteins in wild-type MC3T3-E1 cells. On the other hand, Smad3 greatly enhanced ALP activity (biochemical ALP asssay and ALP staining) and mineralization (Von Kossa and Alizarin Red-S staining) of MC3T3-E1 cells, compared to control, although TGF-β inhibited ALP activity and mineralization of wild-type MC3T3-E1 cells. Smad3δC, MH2 region-deleted Smad3 construct, stable transfection did not affect these phenotypes of MC3T3-E1 cells. Type I collagen synthesis inhibitor, L-azetidine-2-carboxylic acid, as well as osteocalcin and a p38 MAP kinase specific inhibitor, SB203580, significantly antagonized Smad3-stimulated ALP activity and mineralization in MC3T3-E1 cells. In summary, Smad3 inhibited proliferation and altered the level of bone matrix proteins in the manner similar to TGF-β. On the other hand, Smad3 enhanced ALP activity and mineralization through the synthesis of type I collagen, the reduction of osteocalcin, as well as p38 MAP kinase. We propose that Smad3 plays an important role in osteoblastic bone mineralization, and Smad3 may provide a beneficial clue for the investigation of transcriptional mechanism of bone formation and the development of bone-forming drugs.

SA210

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SA211

Continuous Inhibition of MAPK Signaling Promotes Osteogenesis in Mesenchymal Cells. C. Higuchi,*[1] N. Hashimoto,*[1] A. Myoi,[1] K. Itoh,[2] H. Yoshikawa.[1] [1]Orthopaedic Surgery, Osaka University Medical School, Suita, Japan, [2]Biology, Osaka Medical Center for Cancer and Cardiovascular diseases, Osaka, Japan.

We screened the small molecule compounds which induce bone formation by themselves or promote bone morphogenetic proteins (BMPs)-induced osteogenesis. Final goal of our study will be clinical application of them. We found that a specific inhibitor for MAPK/ ERK kinase 1 (MEK1), PD98059 promoted the osteoblastic differentiation in C2C12 myoblastic cells treated with recombinant human BMP-2 (rhBMP-2). It also promoted the differentiation of MC3T3-E1 osteoblastic cells in the absence or presence of rhBMP-2. Alkaline phosphatase (ALP) activity was synergistically increased by the treatment with 25 to 100 microM PD98059 in C2C12 cells stimulated by 300 ng/ml rhBMP-2 in a dose-dependent fashion and it was also increased in MC3T3-E1 cells in the absence or presence of 50 ng/ml rhBMP-2. PD98059 reduced osteocalcin production of C2C12 cells stimulated with 300 ng/ml rhBMP-2, but increased osteocalcin secretion from MC3T3-E1 cells into the culture media. Twenty-five microM PD98059 also promoted mineralization of extracellular matrix in both C2C12 cells stimulated by 300 ng/ml rhBMP-2 and MC3T3-E1 cells. We also confirmed these biological phenomenon by introducing mutant MEK1 cDNA into C2C12 cells. C2C12 cells stably expressing constitutively-active MEK1 showed little ALP activity and osteocalcin production. In contrast, dominant-negative MEK1 transfectants represented higher ALP activity and lower osteocalcin production in the presence of rhBMP-2 than a mock transfectant did. These results were consistent with those of parent C2C12 cells treated with MEK1 inhibitor.Our results indicate that BMP-2-induced meneralization of mesenchymal cells and osteoblastic cells could be regulated by a fine tuning of MAPK signaling pathway and that MEK1 inhibitors would be useful in vivo for promotion of bone formation, for instance, fracture healing.

SA212

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SA213

Potential Role of PPAR-γ in the Inhibition of Human Osteoblastic Cell Proliferation by Polyunsaturated Fatty Acids. A. C. Maurin,* P. M. Chavassieux, P. J. Meunier. INSERM Unit 403, Faculty R.T.H. Laennec, Lyon, France.

We have previously shown that the inhibitory effect induced by adipocytes on the proliferation of primary human osteoblastic cells derived from bone explants (hOB cells) may be mediated by polyunsaturated fatty acids (PUFAs) released by mature adipocytes. PUFAs have been reported to activate peroxisome proliferator-activated receptors (PPARs), specially PPAR-γ which has been implicated in cellular proliferation and differentiation. The purpose of the present study was to determine the role of PPAR-γ in the PUFA-induced inhibition of hOB cell proliferation. A PPAR-γ specific ligand 15-deoxy-prostaglandin J2 (15d-PGJ2) at a dose of 10 μM significantly decreased the hOB cell proliferation (- 50 %, $p < 0.01$). The inhibitory effects of 30 μM of docosahexaenoic and arachidonic acids were confirmed (- 27 and - 42 %, respectively). These effects were not related to cell apoptosis as shown by Hoechst dye staining. Immunofluorescence and western blot analysis showed the presence of PPAR-γ protein in the hOB cell nucleus. Besides the effect on cell proliferation, long-term (3 weeks) exposure of hOB cell cultures to PUFAs or 15d-PGJ2 induced morphological changes with the development of lipidic vacuoles in the presence of horse serum. Moreover, western blot experiments indicated an increase in PPAR-γ protein expression with PUFAs or 15d-PGJ2 treatment, markedly enhanced in the presence of horse serum, in hOB cells as in the 3T3-L1 preadipocytic cell line. In conclusion, as fatty acids, PPAR-γ specific ligands inhibited the proliferation of hOB cells, activated the expression of PPAR-γ in these cells, and also induced an adipocyte-like phenotype. These *in vitro* observations showed that fatty acids and their metabolites favor the commitment of bone-derived cells to the adipocyte pathway. These results raise the hypothesis that adipocyte-released compounds may initiate a switch between the osteoblast and adipocyte lineages. This mechanism may contribute to the age-related bone loss.

SA214

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SA215

Cortisol Enhances the Expression of Notch 1 in Osteoblasts and Stromal Cells. R. M. R. Pereira,*[1] A. M. Delany,[2] E. Canalis.[2] [1]Saint Francis Hospital and Medical Center, Hartford, CT, USA, [2]Saint Francis Hospital and Medical Center and The University of Connecticut School of Medicine, Hartford, CT, USA.

Notch 1, 2, 3 and 4 are a family of closely related transmembrane receptors that mediate signaling mechanisms controlling cell fate decisions. Notch 1 is expressed by skeletal cells, but information about regulation and function of Notch receptors in osteoblasts is limited. Overexpression of Notch 1 in osteoblasts appears to decrease cell differentiation and functional markers of the osteoblast, such as type I collagen and Cbfa-1. These effects are similar to the reported inhibitory actions of cortisol on osteoblastic differentiation and function. Consequently, we postulated that cortisol might regulate Notch expression in cells of the osteoblastic lineage. Murine osteoblastic MC3T3 cells and primary bone marrow stromal cells were grown to confluence and then cultured in medium enriched with ascorbic acid and 5 mM beta glycerolphosphate in the presence or absence of cortisol at 1 μM for periods of 24 h to 4 weeks following confluence. Both MC3T3 and stromal cells expressed Notch 1, 2, 3 and 4 transcripts. Cortisol increased the expression of Notch 1 mRNA by 2 to 4 fold in MC3T3 and stromal cells, as determined by Northern blot analysis and densitometry. The effect was detected 24 h after exposure to cortisol, and it was sustained for 4 weeks, both in MC3T3 and stromal cells. In MC3T3 cells, the stimulatory effect on Notch 1 mRNA was observed with a cortisol dose as low as 10 nM. Cortisol also caused a modest increase in Notch 2 gene expression, but did not regulate Notch 3 or 4 mRNA levels. Supporting the inhibitory role of Notch 1 and cortisol on osteoblastic cell differentiation, bone morphogenetic protein 2, an inducer of this process, had an opposite effect to that of cortisol and suppressed Notch 1 expression. In conclusion, cortisol up-regulates Notch 1 and 2 expression in cells of the osteoblastic lineage, an effect that could mediate the inhibitory actions of cortisol on osteoblastic cell differentiation.

SA216

See Friday Plenary number F216.

SA217

Coactivation of FAK After Hormone- and Growth Factor-Treatment in Osteoblastic Cells. A. Schroeder,* G. Delling,* E. A. Kaiser. Dept. Bone Pathology / Center for Biomechanics, University Hospital Hamburg-Eppendorf, Hamburg, Germany.

Aims: Physiological processes of osteoblastic cells, e.g. proliferation, differentiation or migration, are influenced by a wide spectrum of extracellular stimuli such as matrix proteins, growth factors and hormones. Their cognate receptors, i.e. integrins, receptor protein kinases or G-protein coupled receptors are linked to a variety of signal tranduction pathways that influence gene expression to allow the cells to react to changes in their extracellular milieu. Little is known about the signal transduction of PDGF and PTH in osteoblastic cells via the non-receptor protein-tyrosine kinase FAK. We observed an increase in FAK phosphorylation following stimulation of metaphyseal and diaphyseal derived marrow stromal cells with PDGF or PTH. Therefore we examined the pathways leading from the receptors to FAK using specific inhibitors to selected signal transduction molecules as, e.g. PI3K, PKC and PKA. Methods: Metaphyseal and diaphyseal marrow cells were isolated from 4 week old male Spraque Dawley rats; only cells of the first passage were used for the experiments. Cells were preincubated with the selected inhibitors for 30 min, then incubation with PTH or PDGF followed for 30, 60 or 90 min. Cells were lysed and lysates were subjected to western blot analysis with antibodies against FAK protein and FAK-autophosphorylation site. Results: PTH-stimulated diaphyseal marrow cells show a slight increase in FAK-autophosphorylation. This effect can be reduced by preincubation of the cells with H-89, a specific inhibitor of PKA. Preincubation with the PKC-inhibitor Calphostin C does not abolish the phosphorylation signal. PDGF-stimulation of metaphyseal marrow cells also leads to a FAK-autophosphorylation increase. This effect could be reversed by preincubation with LY294002, a specific inhibitor of phosphatidyl-inositol-3-kinase (PI3K). In this experiment preincubation with Calphostin C was again unable to decrease the activation of FAK after PDGF-incubation. Discussion: These results lead to the suggestion, that the signals following PTH stimulation in diaphyseal marrow cells are transduced by a PKA-dependent pathway. PDGF stimulation of metaphyseal marrow cells seems to activate a PI3K involving pathway.

SA218

Ionic Changes in Parathyroid-Stimulated Osteoblasts: Evidence for G-protein Activation. A. Chatoo,*[1] F. Arrebola,*[2] F. McDonald,[2] A. Warley.*[2] [1]GKT Schools of Medicine and Dentistry, LONDON, United Kingdom, [2]GKT School, London, United Kingdom.

Osteoblasts have well characterised receptors for parathyroid hormone (PTH); this ligand acts by heterotrimeric G protein activation. Its precise actions on osteoblasts are unclear although previous experiments suggest that PTH causes an increase in cell volume in osteoblasts. The current series of experiments were designed to further clarify the ionic component of the signalling pathway. Transformed human osteoblasts, derived from an osteogenic sarcoma cell line (Saos-2), were cultured in alpha modification Dulbecco's Modified Eagle's medium (DMEM), with 10% heat inactivated foetal calf serum, 100 U/ml penicillin, 10 µg/ml streptomycin, 2.5 µg/ml amphotericin B and 4 mM L-glutamine at 37°C in a humidified atmosphere with 5% carbon dioxide. Cells (2 x 10^4/ml)were seeded on to Pioloform covered gold electron microscopy grids contained in a 4-well tissue culture plate. Six hours prior to stimulation the culture medium was removed and replaced with DMEM containing 250 ng/ml cholera toxin (CTX). After incubation the cells were exposed to 2.5 µM PTH for 0, 5, 15, 30 and 60 seconds. They were then washed in ice-cold distilled water for 10 seconds; excess water was removed by blotting, and the cells cryofixed by immersion in liquid nitrogen (-210°C). The cells were freeze dried and coated with carbon. Areas of cytoplasm and nucleus (1x 2.5µm) were analysed for 60 seconds live time using a Zeiss electron microscope with a Link AN 10 000 microanalysis system. Cells (n=30) cultured in PTH showed a generalised decrease in the concentration of Cl^- and an increase in K^+/Na^+ ratio within the cytoplasm after 30 secs, which recovered towards control values by 60 secs (Table 1). In cells cultured with PTH + CTX concentrations of Cl- remained low, whilst K^+/Na^+ ratio was high after 30 secs and remained at this level (Table 1). Concentrations of Mg^{2+} were inconsistent but generally reduced and remained at a lower level.Table 1. Concentrations (mmol/kg dry weight; mean ± SEM; PTH; PTH + CTX) of Na^+ and K^+ in Saos-2 cells after stimulation with PTH or PTH + CTX

Time (s)/Ion	Mg^{2+}	Cl^-	K^+/Na^+
0	33.3 ± 1.3; 41.2 ± 2.6	93.8 ± 6.3; 84.7 ± 3.2	19.5 ± 0.7; 13.3 ± 0.4
30	12.6 ± 1.2; 24.6 ± 1.9	81.2 ± 4.3; 67.9 ± 5.2	21.9 ± 1.1; 24.1 ± 1.6
60	20.3 ± 1.6; 24.5 ± 1.6	99.4 ± 4.9; 68.7 ± 4.4	12.8 ± 0.6; 21.7 ± 1.1

Ionic changes of Cl- and K^+/Na^+ ratios induced by PTH appear to be linked to G-protein activation and can be modified by CTX.

SA219

See Friday Plenary number F219.

SA220

Growth Factor Stimulation of Human Osteoprogenitor Proliferation Requires Activation of p70 S6 Kinase and Akt. L. R. Chaudhary, K. A. Hruska. Renal Division, Washington University School of Medicine, St. Louis, MO, USA.

The growth factors PDGF-BB and FGF-2 regulate the recruitment of osteoprogenitor cells, their proliferation and differentiation into mature osteoblasts, and play important roles in bone remodeling. We have shown PDGF to be a more potent stimulator of normal human osteoblastic (HOB) cell proliferation than FGF-2. However, the molecular mechanisms by which these growth factors differentially regulate osteoblast growth, bone formation and their signaling pathways have not been defined. We have demonstrated that while both growth factors activate ERK1/2, PDGF-BB but not FGF-2 stimulates PI 3-kinase and the cell survival signal, protein kinase B (PKB/Akt). These results demonstrate differential signal transduction by the two growth factors. Here, we analyze the importance of PI 3-kinase in the activation of p70 S6 kinase (p70S6K) responsible for stimulation of phosphorylation of S6 ribosomal protein, protein synthesis and cell proliferation. Quiescent HOB cells were treated with PDGF-BB (30 ng/ml) or FGF-2 (30 ng/ml). Cell lysates were subjected to SDS-PAGE, and activation of p70S6K was assayed by immunoblotting with two different anti-phospho-p70S6K antibodies that specifically recognize phosphorylation at Thr389 or Thr421/Ser424. Our results demonstrated that PDGF-BB treatment of HOB cells increased phosphorylation of p70S6K at both Thr389 and Thr421/Ser424 in a time-dependent manner, while FGF-2 failed to stimulate phosphorylation of p70S6K. The phosphorylation of Thr389 was mediated via PI 3-K and the mammalian target of rapamycin (mTOR) and was sensitive to the PI 3-K inhibitor, wortmannin or the mTOR inhibitor, rapamycin. The activation of p70S6K requires activation of both ERK1/2 and PI 3-K/PDK1/2. FGF-2 activated ERK1/2 but did not activate PI 3-K and p70S6K in HOB cells. Our results demonstrate for the first time differential activation of p70S6K by PDGF-BB and FGF-2. These results are of critical importance in understanding the mechanisms of PDGF and FGF-2 action in translational control of protein synthesis. PDGF-BB but not FGF-2 may be involved in the up-regulation of osteoblast protein synthesis by stimulating p70S6K. Taken together, our data demonstrate that the activation of Akt and p70S6K by PDGF-BB but not by FGF-2 contributes to increased proliferation of cells in the osteoblast lineage by PDGF-BB.

SA221

T Cell Induction of MMP-13 in Human Osteoblasts is Dually Regulated by p38 and ERK1/2 MAP Kinases. L. Rifas, S. Arackal.* Division of Bone & Mineral Diseases, Washington University School of Medicine & Barnes-Jewish Hospital, St. Louis, MO, USA.

T cells play a major role in the connective tissue degradation that occurs in chronic inflammatory diseases such as rheumatoid arthritis. We hypothesized that MMP-13 is induced in hOB by T cells and is regulated by MAP kinases. We have demonstrated that T cells induce the expression of MMP-13 in normal human osteoblasts (hOB). Using neutralizing antibodies and ELISA assays we have excluded TNF-alpha and IL-6 as mediators of MMP-13 production. To explore the mechanism of MMP-13 regulation by MAP kinases, hOB were treated with either the ERK 1/2 specific inhibitor PD98059 or the p38 specific inhibitor, SB203580, over a dose range of 0.1-40 µM. PD98059 induced, while SB203580 inhibited, MMP-13 in a dose dependent fashion demonstrating that p38 stimulates and ERK 1/2 inhibits MMP-13 expression. To further detail the MAP kinase signaling pathways regulating MMP-13 induction in hOB by activated T cell conditioned medium (ATCM), we examined the phosphorylation state of ERK 1/2 and p38 in the presence or in the absence of ATCM over a 16-72 hr period. Specific antibodies recognizing the phosphorylated forms of ERK 1/2, p38 and JNK were used in Western blots of whole cell extracts from untreated and ATCM treated hOB. ATCM slightly activated p38 and ERK 1/2 at 24 hours, an effect that lasted up to 72 hours. Interestingly, treatment with PD98059 did not inhibit phospho-ERK demonstrating that ERK is activated in resting, unstimulated osteoblasts and may play a role in maintaining the differentiated state. Although SB203580 dramatically inhibited MMP-13 production, it surprisingly increased phosphorylation of p38 while not affecting its protein level over the time course examined. Phosphorylated ERK 1/2 was also increased in the presence of SB203580 as well, but not ERK 1/2 protein. JNK protein was unchanged throughout the time course while phospho-JNK was not detected which indicates that this pathway does not play a role in MMP-13 regulation in hOB. Our data demonstrate that SB203580 inhibits the activity, but not the activation of p38 in human osteoblasts. Furthermore, SB203580 induces the activation of ERK 1/2. This process occurs through the activation of Ras and Raf-1, the upstream regulators of ERK1/2 activation. Thus, a crossover between the p38 and ERK1/2 pathways results in an increase in ERK1/2 and an inhibition of p38 activity, suggesting that this is the mechanism of inhibition of MMP-13 by SB203580. Our data show that MMP-13 is regulated by both the ERK pathway and the p38 pathway in response to T cell cytokines. Selective inhibition of p38 activity may offer a target for phamacological inhibition of bone loss in rheumatoid arthritis.

SA222

Effects of Risedronate, Alendronate and Etidronate on Viability and Activity of Rat Stromal Cells in vitro. K. Still,*[1] R. J. Phipps,[2] A. Scutt.*[1] [1]Children's Hospital, Sheffield, United Kingdom, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

The effects of bisphosphonates (BP) on osteoclasts (OC) have been studied extensively, and current evidence shows that BP suppress bone resorption by directly inhibiting OC activity. This BP-mediated inhibition of OC activity is achieved by (i) inhibition of OC differentiation from haematopoietic precursors, (ii) inhibition of activity of mature OC, (iii) stimulation of release of an osteoblast-derived OC inhibitory factor and/or (iv) by induction of OC apoptosis. In contrast, effects of BPs on osteoblasts have received less attention. It has recently been suggested that BPs may possess some mild anabolic activity that contributes to their efficacy in treating osteoporosis. This is based on the finding that long-term treatment with BPs increases wall thickness, which is considered to indicate a localised increase in osteoblastic activity. Consistent with this is the finding that BPs can prevent induction of apoptosis in murine osteoblasts and osteocytes, and can stimulate fibroblastic-colony formation by murine and human bone marrow. The purpose of this study was to investigate the effects of risedronate, alendronate and etidronate in two models of bone formation in rat stromal cells *in vitro*; high-density bone marrow cultures, and calcifying fibroblastic colony forming unit (CFU-f) cultures. Biphasic effects on bone formation were observed. In CFU-f cultures, high concentrations (10^{-5} to 10^{-4}M) of alendronate and risedronate inhibited colony formation and cell activity whereas etidronate had relatively little effect. At lower concentrations (10^{-9} to 10^{-7}M) all three drugs increased colony numbers suggesting a mild anabolic effect. These results were not entirely paralleled in the high-density cultures where all three drugs inhibited colony numbers and cell activity. Etidronate, however, was considerably less potent than the other two drugs. The inhibitory effects of alendronate and risedronate could be partially reversed by addition of geranylgeraniolpyrophosphate suggesting a similar mechanism to BP-induced apoptosis in OC. In contrast, the anabolic effects of alendronate and risedronate were augmented by co-incubation with geranylgeraniolpyrophosphate. Indeed, geranylgeraniolpyrophosphate, geranylgeraniol and farnesol all stimulated colony formation. These data suggest that high doses of BP can induce cell death in bone marrow-derived osteoprogenitor cells, by a mechanism similar to that seen in OC. However, at lower doses a mild anabolic activity is present which is not obviously mediated via inhibition of the cholesterol biosynthetic pathway.

SA223

See Friday Plenary number F223.

SA224

Investigation into the Plasticity of Adult Human Mesenchymal Stem Cells. S. Ioannidou,*[1] K. Seehra,*[1] P. Wood,*[2] W. Staley,*[1] R. J. Byers,*[1] J. A. Hoyland,[1] A. J. Freemont.*[1] [1]Musculoskeletal Research Group, University of Manchester, Manchester, United Kingdom, [2]Department of Immunology, University of Manchester, Manchester, United Kingdom.

Osteoblasts and adipocytes are derived from the same stem cell (osteoprogenitor) in adult marrow and plasticity exists between the two lineages. However, the stage of adipocytic differentiation at which plasticity is lost and commitment is established is not known. Adult human mesenchymal stem cells (HMSCs) were investigated for the expression of STRO-1, a marker of osteoprogenitors, by fluorescence activated cell sorting. They were cultured in adipogenic medium (AM), osteogenic medium (OM) or mineralising medium (MM). After 2 weeks, morphology and histochemical staining for oil red O, alkaline phosphatase and alizarin red S was noted. Moreover, HMSCs cultured in AM for 8 and 16 days were switched to OM and MM and their ability to undergo osteoblastic differentiation and form mineralised calcium was determined by noting morphology and histochemical staining in these cells 8 and 16 days after medium switch. HMSCS were shown to be 82 per cent positive for STRO-1. These cells underwent adipocytic differentiation, osteoblastic differentiation and formed mineralised calcium upon appropriate stimulation. Moreover, HMSCs cultured in AM for 8 and 16 days could be stimulated to undergo osteoblastic differentiation and form mineralised calcium by medium switch. In conclusion, HMSCs are osteoprogenitors in their majority. They can undergo both osteoblast and adipocyte differentiation and there is plasticity of differentiation between early and late adipocytic precursors and osteoblasts as evidenced by morphology and histochemical staining. Future work will be centered on confirming these findings by specific PCR for known markers of osteoblast and adipocyte differentiation.

SA225

See Friday Plenary number F225.

SA226

Human Dental Pulp Stem Cells (DPSCs): Characterization and Developmental Potential. S. Gronthos,*[1] P. Gehron Robey,[1] A. Boyde,*[2] S. Shi.*[1] [1]Craniofacial & Skeletal Diseases Branch, NIDCR, National Institutes of Health, Bethesda, MD, USA, [2]Department of Anatomy & Developmental Biology, University College, london, United Kingdom.

We have previously identified a clonogenic cell population, termed dental pulp stem cells (DPSCs), derived from adult human third molars. Ex vivo expanded DPSCs have the unique ability to regenerate a dentin-pulp complex following xenogeneic transplantation using hydroxyapatite/ tricalcium phosphate (HA/TCP) as a carrier vehicle. Transplants were composed of mineralized tubular structures lined with odontoblasts, and fibrous tissue containing blood vessels, analogous to normal human teeth. To further characterize the stem cell-like properties of DPSCs, we harvested cells from three-month old DPSCs transplants and expanded these populations in vitro. Human cells were isolated from these cultures based on their reactivity to a human specific anti-integrin b1 antibody using fluorescence activated cell sorting. These selected cells were then re-transplanted along with HA/TCP into immunocompromised mice. A mineralized dentin-like structure was formed following re-transplantation by the human donor cells. Moreover, regenerated dentin was also found to contain dentin sialophosphoprotein (DSPP) by immunohistochemical staining. Analysis of twenty individual colonies from primary cultured DPSCs demonstrated that only 10% to 15% of the clones showed a high proliferative potential in vitro. We next determined the ability of individually ex vivo expanded clones to form a dentin-pulp structure following xenogeneic transplantation. Approximately 20% of the transplanted clones showed the potential to develop a mineralized dentin-pulp structure in vivo. In conclusion, our data indicate that human DPSCs possess stem cell-like characteristics that include clonogenic potential, the ability to self-renew, and the capacity to differentiate into functiuonal odontoblasts. In analogy to bone marrow stromal stem cells, DPSCs represent another kind of mineralized tissue forming stem cell population, and may provide a potential resource for the regeneration of viable dental structures in vivo for clinical applications.

SA227

Cystatin C Inhibits Osteoclast Formation and Activation via Different Mechanisms. M. Brage,*[1] A. Grubb,*[2] M. Abrahamsson,*[2] M. Ransjö,[1] U. H. Lerner.[1] [1]Dept Oral Cell Biology, Umeå university, Umeå, Sweden, [2]Dept Clinical Chemistry, University of Lund, Lund, Sweden.

Cysteine proteases, particularly cathepsin K, have previously been shown to be key enzymes in the degradation of extracellular matrix in osteoclastic resorption lacunae. In line with this, we have reported that cystatin C, an inhibitor of papain-like cysteine proteases, inhibits bone resorption in mouse calvariae primarily by inhibiting the degradation of bone matrix proteins. This effect is mimicked by the amino-terminal segment Arg8-Leu9-Val10-Gly11 (RLVG) [Johansson et al. Bone 26:451, 2000]. We have previously reported that not only osteoclast activity but also osteoclast formation is dependent on cysteine protease activity. Thus, cystatin C, Z-RLVG-CHN2, E-64 and leupeptin inhibit osteoclastogenesis in mouse bone marrow cultures stimulated by PTH and D3. In the present study, we have studied the effect of cysteine protease inhibition on the expression of different phenotypic markers of osteoclast and osteoblast/stromal cell differentiation and in addition investigated the effect of cystatin C on bone resorption in vivo as assessed by decreased serum calcium. PTH did not stimulate osteoclast formation at 2 days but at and after 4 days of culture the number of osteoclasts increased with maximal number seen after 7 days. Addition of cystatin C at day 5 was sufficient to obtain inhibition of osteoclastogenesis. Using a semi-quantitative RT-PCR it was found that cystatin C did not inhibit the stimulatory effect of PTH on TRAP and cathepsin K mRNA expression at 2 or 9 days. After 9 days, cystatin C reduced PTH stimulated mRNA for calcitonin receptor and carbonic anhydrase II, without affecting that of RANK. Cystatin C did not affect osteocalcin, a1-procollagen and alkaline phosphatase mRNA. However, down regulation of OPG by PTH was abolished by cystatin C. Cystatin C and D were equipotent as inhibitors of bone resorption in mouse calvariae, whereas cystatin D was clearly less potent than cystatin C as inhibitor of osteoclast formation. Injection of cystatin C i.p. at 15 mg/100 g into rats fed a calcium deficient diet resulted in a transient inhibition of ionized blood calcium. These data show that cysteine proteinase inhibitors decrease bone resorption in mouse calvariae, inihibit osteoclast formation in mouse bone marrow cultures and decrease blood calcium in rats. The inhibition of osteoclast formation and activity seems to be due to different mechanisms (different sensitivities to cystatin C and D) and the inhibitory effect on osteoclast differentiation pathway may be mediated via effects on preosteoclast differentiation, rather than on osteoblast/stromal cell differentiation

SA228

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SA229

Localisation of Collagen Fragments (CTx, ICTP, NTx, NTP and Dpd) in Resorption Sites: A 3D Confocal Study. S. A. Nesbitt,[1] S. P. Robins,[2] J. Risteli,[3] P. Qvist,[4] S. Apone,[5] D. R. Eyre,[6] M. A. Horton.[1] [1]Bone and Mineral Centre, University College London, London, United Kingdom, [2]Rowett Research Institute, Aberdeen, United Kingdom, [3]University of Oulu, Oulu, Finland, [4]Osteometer BioTech, Herlev, Denmark, [5]Ostex Int., Seattle, USA, [6]University of Washington, Seattle, USA.

During bone resorption osteoclasts liberate degraded bone matrix collagens and transport them through the cell before their release in the extracellular space. Herein, antibodies to the C telopeptides (CTx, ICTP), the N telopeptides (NTx, NTP) and the deoxypyridinoline collagen crosslinks (Dpd) have been used to assess the localisation of degraded collagens within resorption sites. The analysis was performed using an in vitro resorption model with fluorescence immunostaining and confocal microscopy with post hoc 3D image reconstruction. Human osteoclasts were cultured on biotinylated dentine slices for 24h. After fixation and permeabilisation, the cells were triple-stained for F-actin, biotinylated matrix and the degraded collagens. 3D images of osteoclasts and adjacent stromal cells were reconstructed from a series of 64 immunofluorescence xy optical sections (0.35 microns thick) taken by confocal microscopy to provide internal views of epitope distribu-

tion in the cells. Image analysis of the resorbing osteoclasts showed CTx and NTP in the resorption pit, across the ruffled border and forming discrete intracellular aggregates in the basolateral cell body. Minimal amounts of NTx were seen in osteoclasts compared to CTx, NTP and Dpd. Most NTx was localised along the ruffled border where Dpd and ICTP were absent. Furthermore, exposed pits were positive for ICTP, but negative for Dpd and NTx, and these sites were infiltrated with stromal cells. In summary, these data suggest that degraded collagen fragments NTx, NTP and CTx are liberated at the ruffled border and, in part, traffic through the osteoclast. Further intracellular degradation generates Dpd epitopes and, subsequently, ICTP is exposed following stromal cell modification of the resorption sites. Our data demonstrate that collagen fragments are generated both extracellularly and during transcytosis through osteoclasts; these findings will have implications for clinical tests of osteoclast activity that are based upon measurement of the levels of collagen fragments.

SA230

Development of Quantitative ELISAs to Pro- and Mature Cathepsin K: Their Utility in Mechanistic Studies of Osteoclastic Bone Resorption. C. J. Gress,*[1] I. E. James,*[1] N. Dada,*[2] M. Gowen,[1] M. W. Lark.[1] [1]Musculoskeletal Diseases, GlaxoSmithKline, King of Prussia, PA, USA, [2]diaDexus, Santa Clara, CA, USA.

Cathepsin K is the principal cysteine protease involved in osteoclast-mediated bone resorption(1). It has been proposed that small molecule inhibitors of this enzyme would be efficacious in the treatment of metabolic bone diseases such as osteoporosis. Indeed, small molecule, non peptide cathepsin K inhibitors have been described that inhibit osteoclast-mediated bone resorption both in vitro and in vivo. In an effort to understand the mechanisms of cathepsin K inhibition, it has been previously demonstrated, using Western blot analysis, that resorbing human osteoclasts treated with inhibitors of human cathepsin K cause a dose dependent accumulation of intracellular levels of mature enzyme(2). In this study we confirm and extend these observations using quantitative ELISAs specific for either pro- or mature cathespin K. In addition, we show that other classes of bone resorption inhibitors such as small molecule inhibitors of vATPase do not affect the intracellular levels of mature or pro-cathepsin K. Furthermore, in an in vitro human osteoclast resorption assay we show that normal osteoclastic resorption is resumed upon removal of the cathepsin K inhibitors. Importantly, preliminary studies suggest that this resumption of osteoclastic function does not appear to be accompanied by a resorption rebound effect from the accumulated mature cathepsin K. These data have important implications with regard to using cathepsin K inhibitors in the treatment of osteoporosis. Also, the development of the quantitative ELISAs may provide an important tool in the diagnosis and treatment of metabolic bone diseases such as osteoporosis.1) James et al., J Biol. Chem. 276: 11507-11511, 2001, 2) Rieman et al., J Bone Min. Res. 14: S357, 1999

SA231

Dissociation of Angiogenesis and Osteoclastic Resorption During Endochondral Bone Formation in Neonatal Mice. M. M. L. Deckers,*[1] G. van der Pluijm,[1] E. R. van Beek,*[1] A. Wetterwald,*[2] L. van der Wee-Pals,*[1] M. Cecchini,[2] S. E. Papapoulos,[1] C. W. G. Löwik.[1] [1]Endocrinology, LUMC, Leiden, The Netherlands, [2]Gene Therapy Laboratory, Department of Clinical Research & Urology Clinic, University of Bern, Inselspital, Bern, Switzerland.

Invasion of the mineralized cartilage by endothelial cells and osteoclasts is a key event in endochondral bone formation and is at least partly mediated by actvity of matrix metalloproteinases (MMP). Through the action of osteoclasts, the mineralized matrix in the center of embryonic bones is resorbed and a primitive bone marrow cavity is formed. During this process, endothelial cells invade the bone directing osteoblast progenitors to the cartilaginous scaffolds of the growth plate to form bone. To selectively examine the putative role of osteoclastic resorption and angiogenesis in this process we used an in vivo model of suppressed bone resorption and/or suppressed angiogenesis.Angiogenesis and osteoclastic resorption was examined in tails and long bones of newborne mice treated with bisphosphonates (BPs), osteopetrotic mice (c-fos knockout or op/op mice) and newborne mice treated with selective MMP inhibitors (MMPIs). The tail vertebrae of newborne mice, represent all stages of endochondral bone formation of which the most proximal vertebrae represent fully differentiated mineralized cartilage as opposed to immature non mineralized cartilage at the most distal vertebrae. During the next 7 days of postnatal development, all vertebrae in the tail reach their mature stage represented by the formation of a primitive marrow cavity.In control mice PECAM-1 positive sinusoids were detected in the vicinity of TRAcP positive osteoclasts. Treatment with BPs completely abolished bone resorption and eliminated TRAcP positive osteoclasts while angiogenesis remained unaffected. Similarly, bone sections of osteopetrotic mice revealed that capillaries invaded the mineralized cartilage. In contrast, in mice treated with a broad spectrum MMPI (marimastat) both processes were inhibited whereas a new selective MMPI solely blocked angiogenesis without affecting osteoclastic resorption.In conclusion we show that 1) endothelial cells invade the mineralized cartilage in the absence of osteoclasts. 2) BPs have no effect on angiogenesis. 3) a selective MMPI that has no effect on osteoclastic resorption strongly inhibits angiogenesis, suggesting that different MMPs are involved in osteoclastic resorption and angiogenesis. Together, these data strongly indicate that, during endochondral bone formation, invasion of cartilage by capillaries can occur independently of osteoclastic resorption and vice versa.

SA232

Expression and Role of Mannose Receptor/Terminal High Mannose on Osteoclast Precursors During Osteoclast Formation Via Cellular Fusion Events. S. Morishima,*[1] I. Morita,[2] T. Tokushima,[2] S. Enomoto,*[1] S. Murota.*[2] [1]Oral Surgery, Department of Oral Restitution,Division of Oral Health Sciences, Tokyo Medical and Dental University, Tokyo, Japan, [2]Celluler Physiological Chemistry, Tokyo Medical and Dental University, Tokyo, Japan.

It is well known that osteoclast is formed from hematopoietic precursors via cell-cell fusion. We have already presented that mannose residues wereexpressed on outer membranes of monocytes and that they were involved in the osteoclast formation via cellular membrane fusion events. In the present paper, we will demonstrate the mechanisms for expression of terminal high mannose (TM) and also show that mannose receptor, a counterpart of TM, is expressed on osteoclast precursor cells. Osteoclasts were formed by 3 different systems: 1) mouse bone marrow cells were incubated with 10-8M of PGE2 for 7 days, 2) mouse spleen cells wereco-cultured with stromal cells (TMS-14) in the presence of 10-8M of 1α,25(OH)2VitaminD3 (VitD3) for 7 days, and 3) RAW264.5 cells were cultured with 50 ng/ml of sRANKL in a spot culture for 4 days. The expression of TM was quantitatively determined by fluorescence intensity of cell membraneassociated FITC-conjugated pradimicine, which is recognized to bind TM, andthat of mannose receptor was immunostained with antibody against alveolar macrophage mannose receptor. During osteoclast differentiation the expression of TM increased graduallyand reached its maximum at the stage of fusion in the 3 different systems. The mannose receptor was not detected in bone marrow cells and spleen cellsin the absence of PGE2 or Vit D3, and gradually increased during osteoclastdifferentiation. In contrast, the expression of mannose receptor in RAW264.7 cells.was already detected without sRANKL and it was not changed during osteoclast differentiation. The expression of TM on outer membrane ofosteoclast precursor cells was abrogated by the addition of castanospermine,an inhibitor of glucosidase I, and was enhanced by the addition of either deoxymannojirimycin, an inhibitor of α-mannosidase I or swainsonine, aninhibitor ofα-mannosidase II. In parallel with TM expression, the number of osteoclast was inhibited by castanospermine, and stimulated bydeoxymannojirimycin or swainsonine. These results indicated that mannose receptor was expressed on the osteoclast precursor cells as a counterpart of TM, and the degradation of N-linked oligosacchadide regulates osteoclast differentiation via cellularfusion events. These data also suggest that the inhibitors of gluocidase I may be postulated as novel drugs against osteoporosis.

SA233

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SA234

A Series of Echistatin Analogs Containing A Benzophenone Which Flanks the RGD Triad: Novel Tools in Photoaffinity Crosslinking Studies of the Osteoclast $\alpha_v\beta_3$ Integrin. D. Yahalom,* M. Rosenblatt, J. A. Alexander, M. Chorev.* Division of Bone and Mineral Metabolism, Charles A. Dana and Thorndike Laboratories, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA.

The benzopheneone (BP)-based photoaffinity scanning (PAS) methodology is employed by us to map the bimolecular interface between $\alpha_v\beta_3$integrin, the dominant integrin in human osteoclasts, and its RGD-containing ligands. We have previously identified the contact sites within the sequences β_3[99-118] {Bitan et al. Biochemistry (1999) 38, 3414-3420} and β_3[167-171] {Bitan et al. Biochemistry (2000) 39, 11014-11023} of the $\alpha_v\beta_3$ receptor for two small peptides, containing BP moieties at either the carboxyl or the amino terminal side of the RGD triad, respectively. More recently, we have employed an echistatin analog modified by insertion of a BP moiety on Lys^{45}, which is located within the C-terminus and removed from the $R^{24}GD^{26}$ triad {JBMR (1999) 14, S481}. We have identified β_3[209-220] as a putative auxiliary binding site for the C-terminus in echistatin. In order to identify the contact sites in $\alpha_v\beta_3$ for the residues flanking the RGD triad in echistatin and compare them with the analogous sites in small RGD-containing peptides, we have developed echistatin analogs containing *p*-benzoylphenylalanyl (Bpa), a BP-containing amino acid residue, in positions 21, 23, and 28. The [Bpa^{21},Leu^{28}], [Bpa^{23},Leu^{28}]and [Bpa^{28}]-Echistatin analogs bind with high affinity to the purified $\alpha_v\beta_3$ integrin. The radioiodinated analogs crosslink effectively to the recombinant human $\alpha_v\beta_3$ receptor overexpressed in HEK-293 cells. This photocrosslinking is blocked in the presence of excess echistatin. Unlike echistatin, the three new analogs are very poor ligands for the closely related $\alpha_{IIb}\beta_3$ receptor, suggesting high specificity toward $\alpha_v\beta_3$ integrin. We will present the results of the receptor binding assays and discuss them in the context of ligand structure-function relations. We will also report our photoaffinity crosslinking studies, and discuss their implications for the emerging experimentally-based model of the ligand-$\alpha_v\beta_3$ integrin bimolecular interface.

SA235

CpG Containing Oligodeoxynucleotide Modulates Osteoclastogenesis. W. Zou,*[1] H. Schwartz,*[1] G. Hartmann,*[2] S. Endres,*[2] Z. Bar-Shavit.[1] [1]Hebrew University faculty of Medicine, Jerusalem, Israel, [2]University of Munich, Munich, Germany.

The ability of the macrophage to distinguish bacterial from mammalian DNA is mimicked by single-stranded oligonucleotides containing unmethylated CG dinucleotides ("CpG" motifs) in specific sequence context (CpG ODN). CpG ODN interaction with the macrophage changes the transcription of a number of genes by activating nuclear translocation of nuclear factor κB (NF-κB). Activation NF-κB is also a key event in the differentiation of the osteoclast from its precursor cell, a member of the macrophage family. Therefore we examined the ability of CpG ODN to modulate osteoclast differentiation. We observed TRAP positive cells when CpG ODN (but not control GC ODN) was added to bone marrow macrophage (BMM) cultures in the presence of M-CSF. However. these cells did not fuse significantly, did not express calcitonin receptors and the intensity of the

TRAP stain was weak as compared to RANK ligand (RANKL)-treated cells. Furthermore, CpG ODN inhibited in a dose dependent manner the osteoclastogenic activity of RANKL. At 50 nM CpG ODN we observed 99.5% inhibition. On the other hand, a two fold enhancement of osteoclateogenesis was recorded if the CpG ODN was added to RANKL-containing cultures for the last 30 hours of a five day experiment. CpG ODN stimulated osteoclastogenesis in RANKL-pretreated BMMs, even in the absence of RANKL. CpG ODN treatment of BMMs increased the mRNA and protein levels of IL-1 and TNF-α. While these two cytokines show very little osteoclastogenic activity in BMMs, they are very effective in promoting osteoclast differentiation in RANKL-treated cells. The osteoclastogenic activities of IL-1 and TNF-a are inhibited by IL-1 receptor antagonist (IL-1ra) and antibodies to TNF-α, respectively. Induction of osteoclast differentiation in RANKL-pretreated BMMs by CpG ODN was not affected by IL-1ra, but was blocked by antibodies to TNF-a. In summary, CpG ODN is capable of modulating osteoclast differentiation. It exerts an inhibitory effect on the precursor, but markedly enhances osteoclastogenesis of RANKL-treated BMMs, namely cells which started to differentiate into osteoclasts. The enhancement of differentiation is mediated by TNF-a, while the inhibitory phase is may be due to reduction of M-CSF receptors caused by CpG ODN. Currently, potential target indications of CpG oligonucleotides comprise infections (current clinical trials as vaccine adjuvant) and malignancy (effective adjuvant for dendritic cell-based tumor therapy in animal tumor models). The present study suggests that CpG ODNs may be beneficial in treating osteoporosis.

SA236

See Friday Plenary number F236.

SA237

Osteoclasts Generated from Cultures of Peripheral Blood Stem Cells from Human Osteopetrotic Patients after Stimulation with Interferon Gamma Resorb Bone. **P**. R. Madyastha,* W. L. Ries, L. L. Key. Pediatrics (Endocrinology), Medical University of South Carolina, Charleston, SC, USA.

Osteopetrosis is a metabolic disorder caused by the impairment of osteoclastic bone resorption. The chief manifestation is the accumulation of bone. Superoxide production, which appears to be necessary for normal osteoclastic bone resorption, is defective in osteoclasts (OCL) and white blood cells of osteopetrotic patients. We earlier have shown that in OCL generated from PBMC of osteopetrotic patients, IFNγ-stimulation increased the superoxide generation to levels present in control OCL. In the present study, we show that OCL generated from cultures of PBMC of four osteopetrotic patients were dysfunctional and failed to form resorption pits *in vitro* on bovine bone slices, representing the pathological features of osteopetrosis *in vivo*. In comparison, osteoclasts generated *in vitro* from non-osteopetrotic control subjects formed numerous resorption pits. When stimulated with IFNγ, the ability of osteopetrotic OCL to form resorption pits was improved (number of pits/experiment in triplicate bone slices: patient, unstimulated, 0; IFNγ -stimulated, 68; control, 121. The average total area resorbed was less (control, 549943 μm^2; osteopetrotic patient, 289449 μm^2), but the average resorption area per pit was similar (control, 4545±960 μm^2; osteopetrotic patient, 257±971 μm^2). These findings were complimentary to our earlier *in vivo* histomorphological data that demonstrated a decrease in trabecular bone volume on the bone biopsy specimens pre- and post-treatment with IFNγ. NBT staining, a marker of superoxide generation was significantly increased in IFNγ-stimulated OCL from osteopetrotic patients, compared to unstimulated OCL ($p<0.0001$). Our current data provide evidence that IFNγ-stimulated the generation of superoxide in osteopetrotic OCL, improving bone resorptive activity. In contrast to observations made in OCL harvested from non-osteopetrotic subjects where IFNγ exposure has suppressed OCL formation and bone resorption, the data from the current study suggest that IFNγ increases osteoclastic numbers in cultures of osteoclastic precursor cells harvested from osteopetrotic patients. This unique response may explain improved bone resorption seen in osteopetrotic patients treated with IFNγ.

SA238

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SA239

See Friday Plenary number F239.

SA240

TNFα Stimulates Osteoclast Differentiation Through Direct Effects on Osteoclast Precursors. J. L. Nalepka,* E. M. Greenfield. Orthopaedics, Case Western Reserve University, Cleveland, OH, USA.

Most bone resorptive agents stimulate osteoclast differentiation indirectly by increasing the RANKL:OPG ratio produced by stromal cells. In contrast, TNFα, in the presence of M-CSF, directly stimulates differentiation of osteoclast precursors in the absence of stromal cells. This study examined whether the primary effect of TNFα is through this direct pathway in the more physiological situation where both stromal cells and other cytokines are present. Osteoclast differentiation was assessed in co-cultures of precursors obtained from murine spleen and CIMC-4 cells, a conditionally immortalized murine calvaria cell line that supports osteoclast differentiation in response to 1,25-D3, IL-1, IL-6, IL-11, and TNFα. Addition of OPG (200 ng/ml) completely blocked formation of both TRAP+ multi- and mono-nuclear cells induced by 1,25-D3 and all of the cytokines, with the exception of TNFα. OPG also had no effect even in the presence of a wide range of TNFα concentrations and when a 10-fold higher concentration of OPG was studied. To confirm that TNFα primarily acts directly on the osteoclast precursors, spleen cells isolated from mice lacking both TNF-receptor-1 and TNF receptor-2 were studied. TNFα did not induce differentiation of osteoclast precursors that lack TNF receptors, even when they were co-cultured with CIMC-4 cells with normal TNFα responsiveness. Specificity of this effect was demonstrated since the TNF receptor-deficient precursors differentiate normally in response to 1,25-D3. Low concentrations of the cytokines synergistically stimulate osteoclast differentiation when added together and this effect was blocked by OPG. However, use of the TNF receptor-deficient osteoclast precursors shows that TNFα primarily acts directly on the osteoclast precursors even in the presence of the other cytokines. Thus, the cytokine mixture induced significantly less differentiation of TNF receptor-deficient osteoclast precursors than of control precursors. Moreover, the extent of differentiation of TNF receptor-deficient osteoclast precursors that is induced by the cytokine mixture is indistinguishable from that induced by the cytokine mixture lacking TNFα. Taken together, these results show that TNFα stimulates differentiation primarily by acting directly on the osteoclast precursors even in the presence of other cytokines and TNF responsive mesenchymal support cells. This mechanism is distinct from other resorptive agents that act through the RANKL pathway but does not imply that osteoclast differentiation induced by TNFα is completely independent of RANKL. Instead, it is likely that activation of RANK by RANKL is permissive for direct stimulation of osteoclast precursors by TNFα.

SA241

See Friday Plenary number F241.

SA242

Flt3 Ligand Can Substitute for M-CSF in Support of Osteoclast Differentiation and Function. J. M. Lean,* K. Fuller, T. J. Chambers. Department of Cellular Pathology, St. George's Hospital Medical School, London, United Kingdom.

Although bone resorption and osteoclast numbers are reduced in osteopetrotic (op/op) mice, osteoclasts are nevertheless present and functional, despite absence of macrophage colony-stimulating factor (M-CSF). In fact, unresorbed bone is removed and a well-developed marrow cavity forms within a few weeks of birth. This suggests that alternative factors can compensate for the crucial actions of M-CSF in osteoclast-induction. We found that when non-adherent bone marrow cells were incubated in RANKL with flt3 ligand (FL) without exogenous M-CSF, tartrate-resistant acid phosphatase (TRAP)-positive cells were formed, and bone resorption occurred. Without FL, only macrophage-like, TRAP-negative cells were present. GM-CSF, stem cell factor, IL-3 and vascular endothelial growth factor could not similarly replace the need for M-CSF in these cultures. TRAP-positive cell induction by FL was not due to synergy with M-CSF produced by the bone marrow cells themselves, since FL also enabled their formation from the hemopoietic cells of op/op mice, which lack any M-CSF. FL appeared to substitute for M-CSF by supporting the differentiation of adherent cells that express mRNA for RANK and responsiveness to RANKL. FL did not compensate for M-CSF in precursor expansion, so that functional osteoclasts were formed, but in small numbers. To determine whether FL can account for the compensation for M-CSF-deficiency that occurs in-vivo, we blockaded FL signaling in op/op mice by injection of the soluble receptor for FL. We found that the soluble receptor induced a substantial decrease in osteoclast number, strongly suggesting that FL is responsible for the partial compensation for M-CSF deficiency that occurs in these mice. The actions that we have observed of FL on osteoclast progenitors are consistent with a role for FL in determining the natural history of osteopetrosis in the op/op mouse, in which the number of osteoclasts formed is adequate to cope with bone resorption except in early development, when the resorptive burden is greatest.

SA243

TNF Induces Osteoclast Formation Independent of NF-kB p50 and p52 Expression and Promotes the Formation of RANK-expressing Osteoclast Precursors. L. Xing,[1] J. Hofecker,*[1] Z. Tai,*[1] P. Li,*[1] T. Bushnell,*[1] E. Schwarz,[1] F. Young,*[1] M. Tondravi,[2] B. Boyce.[1] [1]Univ. Rochester, Rochester, NY, USA, [2]American Red cross, Rockville, MD, USA.

TNF is implicated in postmenopausal and inflammation-mediated bone loss. It stimulates RANKL production by stromal cells and like RANKL, activates NF-kB. NF-κB is required in osteoclast (ocl) precursors for ocl formation, it up-regulates TNF expression and thus mediates an up-regulatory osteoclastogenic cycle. Although TNF promotes ocl formation indirectly, it may also act directly on ocl precursors through or independent of NF-κB. To study this latter possibility, we treated wild-type (wt) and various NF-kB knockout (KO) mice, including p50KO, p52KO, and p50;p52 double KO (dKO) mice with TNF and examined its effects on ocl formation *in vivo* and *in vitro*. TNF injections (1μg 3/d x 3d): 1) increased ocl numbers (per mm^2 bone area) in calvarial sections: wt (55+/-12), p50KO (82+/-32) and p52KO (79+/-21) mice (vs 16+/-10 in PBS-treated wt mice) and eroded surface: wt (25+/-2%), p50KO (36+/-12) and p52KO (33+/-7.4%) mice (vs 7.6+/-4.7% in PBS controls). Importantly, ocls (which are absent in calvariae of untreated dKO mice) formed in dKO mice treated with TNF (7+/-1.5), but not with IL-1; 2) increased ocl formation from *ex vivo* cultures of splenocytes from TNF-treated mice: wt (626+/-32 ocl#/well); p50KO (730+/-155), p52KO (683+/-192) (vs 101+/-54 in PBS controls); 3) increased RANK+ve splenocytes from TNF-treated wt mice (8.1 vs 1.3% in PBS controls) and their proliferation response to RANKL/M-CSF (5 +/-0.1 fold increase vs 1.7+/-0.2 in an MTT assay), CFU-GM colony number (199+/-15 vs 39+/-5) from splenocytes and ocls formed from these colonies (460+/-23 vs 202+/-7) and resorption pit area per dentine slice (0.41+/-0.01 vs 0.17+/-0.03 mm^2). In addition, TNF promoted a 5-fold increase in CFU-

GM colony formation from wt, NF-kB p50KO and p52KO splenocytes, while spleen cells from untreated dKO mice formed 3-fold more CFU-GM colonies and 3-fold more RANK expressing cells than wt mice. Finally, splenocytes from TNF transgenic mice formed more ocls earlier in culture (5 days) than wt controls (441+/-13 vs 50+/-7), an effect that was not prevented by maximal doses of the TNF receptor blocker, hTNFR:Fc. Our findings indicate that 1) although the major osteoclastogenic effects of TNF are mediated by NF-kB, TNF, unlike IL-1, can promote ocl formation by an NF-kB independent pathway; 2) TNF primes RANK-positive ocl progenitor formation in vivo; 3) NF-kB expression is required for ocl formation in RANK-expressing cells beyond the CFU-GM stage. Thus, prevention of postmenopausal and inflammation-mediated bone loss is likely to require inhibition of both TNF and NF-kB signaling.

SA244

See Friday Plenary number F244.

SA245

Impaired Osteoclastogenesis, but Normal Bone Resorption, in Mice Deficient in Tumor Necrosis Factor (TNF). W. Hofstetter,[1] R. Balga,*[1] J. Portenier,*[1] C. Mueller.*[2] [1]Department Clinical Research, University of Berne, Berne, Switzerland, [2]Department for Pathology, University of Berne, Berne, Switzerland.

Growth factors of the TNF family are essential for physiological bone metabolism. In the present study, osteoclastogenesis and bone resorption were investigated in mice deficient in TNFα and lymphotoxin (TNFβ) and in TNFα,β null-mice carrying a transgene encoding a transmembrane form of TNF (tmTNF). Mice of 3 months of age were ovariectomized (OVX). Bone density (BD) and bone mineral content (BMC) were measured by peripheral quantitative computer tomography (pQCT) in weekly intervals. After three weeks, bones were embedded in methylmetacrylate (MMA) for histological analysis and co-cultures of bone marrow cells (1.5 x 10^5 cells/well) together with primary murine osteoblasts from wt donors (10^4 cells/well) were prepared and cultured for 6 days. By pQCT, a significant decrease in total BMD and BMC was detected in the distal femur and the proximal tibia of TNF null mutants and of tmTNF transgenic mice within 3 weeks after OVX. Separation of total BMD and BMC into the cortical and trabecular compartments revealed a similar decrease in the cortical bone. Virtually no effect was detected in trabecular bone, which may be due to limited sensitivity and/or resolution. The data was confirmed by histological analysis of MMA embedded tibiae. In the metaphysis, total bone was reduced, and a significant reduction of trabecular bone was evident. TRAP staining of sequential sections revealed no obvious changes in the number and distribution of osteoclasts in the bones derived from the genetically different mouse strains. In co-cultures of total bone marrow with osteoblasts, the formation of osteoclasts was reduced by ca. 40% in marrow cells from TNF null-mice with respect to wt. A further decrease to ca. 20% as compared to wt was observed when the marrow cells were derived from tmTNF transgenic mice. The data demonstrates that in mice deficient in TNF or carrying a transgene for tmTNF, deficiency in estrogen induces an increase in bone resorption, which is not different to the increase in bone resorption observed in normal animals after OVX. While *in vivo* bone resorption is not affected by the mutations, the formation of osteoclasts in vitro is impaired by the deficiency in TNF, and is further reduced when only tmTNF is present. From the present data, it can be concluded that TNF is essential for the efficient formation of osteoclasts *in vitro*. In the regulation of physiological bone resorption, however, TNF is redundant and the increase in bone loss after ovariectomy can be mediated by other factors than TNF.

SA246

See Friday Plenary number F246.

SA247

The Immunosuppressant Rapamycin, alone or with Transforming Growth Factor-β, Enhances Osteoclast Differentiation of RAW264.7 Monocyte-Macrophage Cells in the Presence of RANK-Ligand. C. Shui,* B. L. Riggs, S. Khosla. Endocrinology, Mayo Clinic, Rochester, MN, USA.

Immunosuppressant therapy has been shown to cause high-turnover osteoporosis both clinically and experimentally. Since this may result from direct or indirect stimulation of osteoclast development, we investigated whether cyclosporin A (CsA), rapamycin, or FK506 affect osteoclastic differentiation of RAW264.7 cells induced by RANK-ligand (RANK-L). Furthermore, since the rapamycin receptor protein binds TGF-β receptors, and TGF-β enhances osteoclastogenesis induced by RANK-L, we also examine potential synergistic effects of rapamycin and TGF-β_1. Cell proliferation was assessed using ^{3}H-thymidine incorporation and osteoclast differentiation was determined by tartrate-resistant acid phosphatase (TRAP) activity, TRAP-positive multinucleated cell (TRAP$^+$MNC) formation, and quantitative RT-PCR analysis of TRAP and calcitonin receptor (CTR) mRNAs. We found that rapamycin, in the presence of 50 ng/ml RANK-L and 5 ng/ml macrophage colony-stimulating factor (M-CSF), inhibited cell proliferation and stimulated TRAP activity in a dose-dependent manner (0.1-1000 ng/ml). At the optimal concentration of 10 ng/ml, it increased the number of TRAP$^+$MNC more than 20-fold and enhanced the expression of TRAP and CTR mRNAs 2.1- and 10-fold, respectively. CsA, at 125-2000 ng/ml, similarly inhibited proliferation, but at high doses (1000-2000 ng/ml) it decreased TRAP activity, TRAP$^+$MNC formation, and the expression of TRAP and CTR mRNAs. FK506 showed no detectable effect on cell proliferation or TRAP activity at concentrations up to 2000 ng/ml; however, similar to CsA, the addition of 1000 ng/ml FK506 inhibited TRAP$^+$MNC formation and the expression of TRAP and CTR mRNAs. The combination of rapamycin (10 ng/ml) and TGF-β_1(1 ng/ml) increased TRAP$^+$MNC 3.1- and 6.9-fold as compared to rapamycin or TGF-β_1 alone, respectively, and enhanced the CTR mRNA expression induced by TGF-β_1 by 1.9-fold. By contrast, both CsA and FK506 significantly antagonized the effect of TGF-β_1 or rapamycin on the expression of the osteoclast phenotype. These data thus indicate that rapamycin, alone or in synergy with TGF-β directly enhances osteoclastogenesis and may affect bone metabolism in vivo after long-term use. In contrast, CsA and FK506 seem to have an inhibitory effect on osteoclast development.

SA248

See Friday Plenary number F248.

SA249

Isolation of a Human Homolog of Osteoclast Inhibitory Lectin That Inhibits Osteoclastogenesis. Y. S. Hu,*[1] D. Myers,[2] H. Zhou,[2] J. M. W. Quinn,[1] V. Kartsogiannis,[2] J. Elliott,*[1] C. Gange,*[1] W. J. McKinstry,*[1] M. T. Gillespie,[1] K. W. Ng.[2] [1]St Vincent's Institute of Medical Research, Fitzroy, Australia, [2]Department of Medicine, The University of Melbourne, St Vincent's Hospital, Fitzroy, Australia.

Murine and rat Osteoclast Inhibitory Lectins (mOCIL, rOCIL) are type II membrane C-type lectins expressed by osteoblasts and other extraskeletal tissues. The extracellular domain of mOCIL, expressed as a recombinant protein, inhibited osteoclastogenesis by adherent murine spleen cells treated with M-CSF and soluble RANKL (JBC, 2001).We have isolated the human homolog of OCIL (hOCIL) from a human fetal cDNA library using [32P] dCTP-labeled rOCIL as a probe under low stringency hybridisation conditions. hOCIL cDNA predicts a 191 amino-acid type II membrane protein, with the 112 amino-acid C-type lectin region in the extracellular domain having 54% homology to the C-type lectin sequences of rOCIL and mOCIL. The hOCIL gene, located in chromosome 12p, is 46.5 kb in length and comprised 6 exons, with evidence of 5' alternative splicing. Unlike mOCIL, hOCIL mRNA was not regulated in human osteoblastic cells (SaOS2 or MG-63) treated with calciotropic agents, PGE2, dexamethasone or calcitriol.The extracellular domain of hOCIL was expressed as a soluble recombinant protein in E. coli, and its biological effects were compared with recombinant mOCIL on human and murine osteoclastogenesis. Human osteoclastogenesis was performed with CD14+ cells separated from peripheral blood mononuclear cells. CD14+ cells were cultured on bone for 21d in the presence of hM-CSF (25 ng/ml), sRANKL (40 ng/ml) and three concentrations (2, 8 and 32 ng/ml) of hOCIL. 32 ng/ml hOCIL resulted in 90% inhibition of multinucleate osteoclast formation and 75% reduction in pit formation. In comparison, 32 ng/ml mOCIL also resulted in 90% inhibition of MNC formation and 80% reduction in pit formation.The effect of hOCIL on murine osteoclast formation was determined with spleen cells cultured for 7d in the presence of M-CSF and sRANKL. 50 and 500 ng/ml mOCIL caused near-complete inhibition of murine osteoclastogenesis, while hOCIL at these concentrations only inhibited osteoclast formation by 70%. This implies a degree of species specificity for OCIL action, with hOCIL having a higher affinity for human cells than murine cells: this is similar to growth factors such as M-CSF.In summary, hOCIL is highly conserved with mOCIL in its primary amino acid sequence, genomic structure, and activity to inhibit osteoclastogenesis. In contrast to mOCIL, hOCIL mRNA was not regulated by calciotropic factors. While there was cross-species efficacy in inhibiting osteoclast formation, hOCIL was more effective in blocking human osteoclastogenesis.

Disclosures: Pfizer Limited,2.

SA250

See Friday Plenary number F250.

SA251

RAW264 Cell Clones are Transcriptional Osteoclast Precursors. B. L. Vincent,* A. J. O'Donoghue,* M. J. Glimcher, K. P. McHugh. Orthopaedic Research, Children's Hospital, Boston, MA, USA.

In this study we isolated and characterized osteoclast (Oc) precursors from RAW264 cells for studies of Oc transcriptional regulation. We cloned RAW264 cells which, with RANKL treatment, form functional phenotypic Ocs at high frequency. We also employed gene expression profiling of 1^o Ocs, their precursors, and Oc-genic RAW264 cell clones to characterize transcription in these cells. RAW264 cells have been used for >20 years, are well characterized as phagocytic macrophages, and have recently been shown capable of forming Oc-like cells when cultured with RANKL. While working with the RAW264 model we observed that not all cells give rise to Oc-like cells, demonstrating clonal variation. Thus we isolated Oc precursor clones for use in further studies of transcription. RAW264 cells (ATCC: TIB-71) were cloned by limiting dilution and random clones were grown +/- recombinant RANKL. After 5 days of culture, cells were fixed and stained for TRAP activity. All clones showed some degree of TRAP staining after RANKL treatment, while untreated cells did not. Approximately 1/4 of clones gave rise to TRAP positive multinuclear giant cells demonstrating various degrees of spreading. RNA was purified from parallel cultures and used as template for RT-PCR reactions for Oc markers (RANK, TRAP, cathepsin-K, calcitonin receptor (CTR), and β_3 integrin). All clones were found positive for RANK and, with RANKL treatment, for TRAP. All clones tested were capable of induced cathepsin-K expression, yet some clones constitutively express this Oc protease. CTR and β_3 were expressed in few clones with RANKL treatment. While all β_3 positive clones express CTR, not all CTR positive clones express β_3, making β_3 the most limited Oc marker. Clones were also assayed for their ability to form pits on dentine. We

found that pit formation correlated with cell spreading and β_3 expression. We also employed expression profiling using commercially available dense cDNA arrays to measure the steady-state expression levels of 1,200 known genes. As expected, the constitutive expression profiles of 1^o cells and RAW264 clones largely overlap. The genes coordinately repressed or induced by RAW264 clones and 1^o cells during Oc formation represent ~10% of the total detected, however the magnitude is not equal in all cases. Additionally, expression of some genes was specific to 1^o cells or RAW264 clones, perhaps reflecting the M-CSF dependent nature of 1^o cells or the transformed nature of RAW cells. We conclude that Oc-genic RAW264 clones represent osteoclast precursors that closely mirror the morphological, functional, and gene expression profile of 1^o Ocs, and are an appropriate model with which to study Oc transcriptional machinery.

SA252

See Friday Plenary number F252.

SA253

Osteoblasts Prepared from LPS-Tolerant C3H/HeJ Mice Normally Support Osteoclast Formation in Response to LPS. K. Suda,*[1] M. Takami,[2] J. Woo,[1] N. Udagawa,[2] T. Suzawa,[2] K. Itoh,[2] N. Takahashi,[2] K. Nagai.*[1] [1]Bioengineering, Tokyo Institute of Technology, Yokohama, Japan, [2]School of Dentistry, Showa University, Tokyo, Japan.

Lipopolysaccharide (LPS), a major constituent of Gram-negative bacteria, is a potent stimulator of bone loss in inflammatory diseases such as periodontitis and some types of arthritides. We previously showed that LPS enhanced RANKL expression in osteoblasts and stimulated the survival and fusion of mononuclear osteoclasts. Recently, it was shown that C3H/HeJ mice, characterized by hyporesponsiveness to LPS, have a mutation within the Toll-like receptor (TLR) 4 gene. However, we have shown here that osteoblasts obtained form C3H/HeJ mice can support osteoclast formation in response to LPS similarly to those from C3H/HeN wild type mice.(1) When co-cultures of bone marrow cells and osteoblasts, both of which were prepared from C3H/HeJ mice, were treated with LPS, TRAP-positive osteoclasts were formed within 7 days. The number of osteoclasts induced by LPS in the co-culture of C3H/HeJ mice was similar to that in the co-culture of C3H/HeN normal mice. (2) OPG completely blocked LPS-induced osteoclast formation in the both co-cultures. (3) LPS stimulated RANKL mRNA expression in C3H/HeN wild type osteoblasts with two peaks; the first peak was observed at 3 hr, and the second peak at 48 hr. In osteoblasts prepared from C3H/HeJ mice, LPS stimulated RANKL mRNA expression with one peak at 48 h. LPS failed to induce RANKL mRNA expression at 3 hr in the mutant osteoblasts. (4) PD98059, a specific inhibitor of Erk1/2, inhibited RANKL mRNA expression induced by LPS at 3 h in normal osteoblasts, but had no effects on the LPS-induced RANKL mRNA expression at 48 h. In contrast, NS-398, a selective inhibitor of COX-2, inhibited LPS-induced RANKL mRNA expression at 48 h but not at 3 h in normal osteoblasts. (5) NS398 but not PD98059 completely blocked LPS-induced osteoclast formation in the co-culture. (6) LPS similarly stimulated COX-2 mRNA expression in both C3H/HeN and C3H/HeJ osteoblasts. These results suggest that LPS stimulates RANKL mRNA expression and induces osteoclast formation independent of TLR4-mediated signals. It is also suggested that RANKL mRNA expression induced by LPS in the late phase that depends on the prostaglandin E_2 production, plays important roles in LPS-induced osteoclast formation.

SA254

See Friday Plenary number F254.

SA255

Induction of Osteoclastogenesis in Rat Bone Marrow Cultures by RANKL. W. Grasser,*[1] A. P. Baumann,*[2] V. Paralkar.*[2] [1]Pfizer Research Global Division, Groton, CT, USA, [2]Pfizer Global Research Division, Groton, CT, USA.

Osteoclasts differentiate from hematopoietic stem cell precursors of the monocyte/macrophage lineage. The terminally differentiated osteoclast is characterized as being large, multinucleated with phenotypic markers such as, tartrate-resistant acid phosphatase (TRAP), integrin ?vb3, calcitonin receptors and the ability to resorb calcified bone matrix. RANKL (receptor activator of NF-kB ligand)also known as TNF-related activation-induced cytokines (TRANCE), osteoprotegrin ligand (OPGL) or osteoclast differentiation factor (ODF) has been shown to be important in the formation differentiation and survival of osteoclasts from bone marrow cells. However, a majority of the in vitro studies carried out to study RANKl mechanism have been carried out using stem cells derived from either wild type or knockout mice. The rat has been used accepted as a preclinical model of choice osteoporosis. However, very little information has been generated on the role and action of RANKL in rat stem cell cultures. We have previously shown that in vitro cultures of adherent and non-adherent marrow cells from ovariectomized rats can be induced to form osteoclasts in the presence of platelet-derived growth factor (PDGF) and Vitamin D3. In this study we show that whole bone marrow from ovariectomized rats can be induced to differentiate in to osteoclasts in the presence of 25 ng/ml RANKL, 25 ng/ml M-CSF and 10-8 M Vitamin D3 after 6 days of culture. These cells express many of the phenotypic markers associated with osteoclasts. The addition of RANKL induces multinucleated osteoclats formation in a dose dependent fashion with optimal response at 25 mg/ml. We also show that estrogen can inhibit the formation of osteoclasts generated as a result of RANKL treatment. Our data shows for the first time that RANKL can be used to induce rat bone marrow differentiation into osteoclasts. Although rat has been the pre-clinical animal of choice in osteoporosis research majority of in vitro studies on osteoclasts have been carried out using murine cells. The relative ease of our culture conditions and the short time frame (six days) as a result of treatment with RANKL makes it possible to study rat osteoclasts formation and function in vitro.

SA256

See Friday Plenary number F256.

SA257

Direct Negative Regulation of Bone Remodeling by Thyrotropin (TSH). Evidence from a TSH Receptor Knockout Mouse Displaying Increased Osteoblast and Osteoclast Formation, Osteosclerosis and Disordered Mineralization. E. Abe,[1] R. Marians,*[1] X. B. Wu,*[1] L. Sun,[1] H. C. Blair,[2] T. F. Davies,*[1] M. Zaidi.[1] [1]Endocrinology, Diabetes, and Bone Diseases; The Mount Sinai Bone Program; and the Bronx VA GRECC, Mount Sinai Medical Center, New York, NY, USA, [2]Department of Pathology, University of Pittsburgh, Pittsburgh, PA, USA.

Effects of thyrotropin (TSH) other than those on the thyroid gland itself are unknown. We report, for the first time, a direct, thyroxine-independent, effect of TSH on bone remodeling exerted *via* the TSH receptor. Thus, TSH receptor (TSH-R) null mice showed significant enhancements in osteoblast and osteoclast formation. Histostaining revealed foci of enhanced bone remodeling characterized by atypical osteoblasts and osteocytes, large osteocyte lacunae, focal osteosclerosis, and an increased diaphysial cortical thickness. Plain radiographs showed disordered mineral deposition in the femoral diaphysis and articular cartilage. To determine the skeletal localization of the TSH-R, we inserted Green Fluorescent Protein (GFP) at TSH-R gene deletion site. Multiple optical sections examined by dual photon confocal imaging revealed fluorescent periosteal mesenchymal cells in the inner table of the skull. Examination of bone marrow cultures showed intense green fluorescence in colony forming unit-fibroblast (CFU-F) colonies (at 10 days), as well as surprisingly, in osteoclast progenitors (between 4 and 6 days). This was consistent with TSH-R expression detected by RT-PCR in bone tissue and bone marrow cell cultures of wild type and not of TSH-R null mice. To understand the functional significance of TSH-R expression in bone, we examined osteoblast and osteoclast formation in bone marrow cell cultures. There was a significant, ~2-fold, enhancement of alkaline phosphatase-positive CFU-Fs, mineralizing CFU-OBs and TRAP-positive osteoclasts in both null and heterozygotic mice compared with wild type littermates. Finally, TSH-R null mice with or without thyroid supplementation showed essentially a similar phenotype. The main difference was that the femurs of those not given thyroid hormone were smaller, reminiscent of a hypothyroid state. It is notable that even the heterozygotes with normal circulating T3 and T4 displayed enhanced bone remodeling linking the observed phenotype to the TSH-R, rather than to circulating T3 or T4. We thus provide compelling evidence for the *negative, direct and simultaneous* regulation of osteoblastic bone formation and osteoclastic bone resorption by the TSH-R.

SA258

See Friday Plenary number F258.

SA259

Inhibitory Effects of Minodronate (YM529) on Tumor-Induced Osteolysis in Mice with Bone Metastases. K. Shibasaki,*[1] M. Ito,[2] N. Amizuka,[2] S. Tanaka,*[1] H. Yuyama,*[1] U. Matsukawa,*[1] H. Asano,*[1] K. Miyata,*[1] H. Ozawa.[3] [1]Yamanouchi Pharmaceutical Co., Ltd., Tsukuba, Japan, [2]Niigata University, Niigata, Japan, [3]Matsumoto Dental University, Matsumoto, Japan.

The effects of minodronate (YM529), a potent third-generation bisphosphonate, on tumor-induced osteolysis caused by bone metastases in nude mice were investigated. Metastases were induced by intracardiac injection of the human breast cancer cell line MDA231 into BALB/c nu/nu mice. Minodronate was intravenously or orally given to mice after radiologically defined osteolytic metastases were observed. Histological examination confirmed that numerous osteoclastic (TRAPase-positive) cells appeared on the bone surface near the tumor. Either intravenous or oral administration of minodronate dose-dependently reduced both the number of osteoclasts and the ratio of osteoclast surface to bone surface at each tumor site. These effects were statistically significant at doses of 0.1 mg/kg i.v., 0.3 and 3 mg/kg p.o. In contrast, a dose of 10 mg/kg i.v. of pamidronate, the treatment currently available, was necessary to cause a significant decrease in the number of osteoclasts and the ratio of osteoclast surface to bone surface. The effects of minodronate on the total metastatic tumor area and ash weight of femurs were also evaluated. Minodronate at a dose of 0.1 mg/kg i.v. significantly reduced the total metastatic tumor area and slightly reversed the decrease in ash weight of normally seen in femurs with metastases. In conclusion, minodronate inhibits osteolysis in nude mice caused by bone metastases of MDA231 cells, regardless of its administration route. Consequently, minodronate holds promise as an intravenously and orally available treatment for bone lesions arising from metastases of breast tumors to bone, and may be a more effective treatment than the widely used treatment pamidronate.

SA260

See Friday Plenary number F260.

SA261

Fc-Osteoprotegerin (Fc-OPG) Fusion Protein Reduces Osteoclast Numbers and Prevents Bone Destruction in Rats with Collagen-Induced Arthritis. E. Romas,[1] N. A. Sims,[1] D. Hards,*[1] M. Lindsay,*[2] P. F. J. Ryan,*[2] J. M. W. Quinn,[1] C. Dunstan,[3] T. J. Martin,[1] M. T. Gillespie.[1] [1]St. Vincent's Institute of Medical Research, Melbourne, Australia, [2]Medicine, Monash University, Melbourne, Australia, [3]AMGEN Corporation, Thousand Oaks, CA, USA.

DA rats in which collagen-induced arthritis (CIA) is induced by a single intradermal injection of type-II collagen (C-II) are a suitable model for analysis of experimental arthritis. These rats exhibit marked inflammation (paw swelling and massive leukocyte infiltration of the synovium) and skeletal damage (reduced bone mineral density and extensive bone and cartilage destruction) affecting many joints. Osteoclasts are present at sites of focal bone destruction in CIA and the infiltrating synovial cells express abundant RANKL mRNA.In this study, DA rats with CIA were treated with an Fc-OPG fusion protein (Fc-OPG) to assess whether blockade of RANKL would reduce osteoclast numbers, prevent focal bone erosion and joint destruction. Fc-OPG was administered by SC injection for up to 7 days at a dose of 3mg/kg daily beginning at the onset of paw swelling (day 14 after C-II inoculation). Osteoclasts were detected in joint tissue sections by TRAP staining. The mRNA for endogenous RANKL and OPG was detected by in-situ hybridization. Synovitis, bone erosion and osteoclast numbers were scored in a blinded fashion using a tiered, semi-quantitative grading scale. Compared to control human IgG1, Fc-OPG administration dramatically reduced numbers of osteoclasts in the juxta-articular bone. After 5 days of Fc-OPG treatment, osteoclasts were completely absent at sites of synovial/bone attachment, bone erosions were absent and joint structure was preserved. In contrast, the control animals exhibited extensive osteoclasts lying in erosive pits at the synovial-bone-cartilage junctions. The inflammatory synovitis, degree of leukocyte infiltration and levels of RANKL or OPG mRNA expression assessed by in situ hybridization within the synovial tissue were not affected by administration of Fc-OPG. Paw swelling was not significantly different between Fc-OPG and control rats with CIA. The low level of osteoclast-mediated joint destruction in the presence of continued inflammation in Fc-OPG treated CIA rats support the concept that osteoclasts principally mediate bone destruction in arthritis and indicate that Fc-OPG is able to protect bone integrity in CIA by regulating osteoclastogenesis.

SA262

See Friday Plenary number F262.

SA263

Interleukin-4 Directly, and Indirectly Though TSA-1 Production by T-lymphocytes, Inhibits Osteoclast Formation. D. Mirosavljevic,* J. M. W. Quinn, N. J. Horwood,* B. J. Classon,* J. Elliott,* T. J. Martin, M. T. Gillespie. St. Vincent's Institute of Medical Research, Melbourne, Australia.

IL-4 is expressed by cells present in the bone microenvironment, in rheumatoid arthritis joints and is secreted by activated TH2 cells. IL-4 is a proliferation factor for both B and T cells, regulates macrophage differentiation and inhibits osteoclast formation. We examined the mechanism of IL-4 action on hematopoietic cells and on T and B cells to modulate osteoclast formation.To exclude potential effects of IL-4 on osteoblast/stromal cells, cultures of adult murine spleen cells from normal C57BL/6J mice were treated with M-CSF and RANKL for 7 days. IL-4 potently and dose-dependently inhibited osteoclast formation in these cultures as well as in M-CSF and RANKL treated adherent splenic cultures or spleen cultures established from Rag1 mice: both cultures are depleted of lymphocytes. The inhibitory actions of IL-4 occurred during the first and last three days of these cultures. This implied a direct effect of IL-4 on hemopoietic cells, which may result from PPARg action (PNAS 98, 2443, 2001). In contrast, in RANKL-stimulated cultures of the M-CSF independent RAW264.7 cells (ATCC) treated with IL-4, TRAP+ MNC formation increased 30% and nuclei number were also increased suggesting IL-4 may enhance cell fusion. All TRAP+ MNCs expressed CTR, confirming their osteoclast identity. Thus IL-4 has differential actions on osteoclast formation dependent upon the source of hemopoietic precursor. When T (Thy1.2+, CD4+ or CD8+), but not B, lymphocytes were added to RANKL-stimulated RAW264.7 cultures, IL-4 abolished osteoclast formation. The T cell-derived inhibitor elicited by IL-4 was concluded to be membrane associated since cultures where T cells were physically separated from RAW264.7 cells, IL-4 did not inhibit osteoclast formation, and conditioned media of T cells treated with IL-4 was also unable to inhibit osteoclast formation of RANKL-stimulated RAW264.7 cells. This contrasts with many of the known T lymphocyte-derived osteoclastogenesis inhibitors that are secreted. Using neutralising antibodies to potential candidate molecules, the membrane-associated factor was identified as TSA-1, a known osteoclast inhibitor. TSA-1 is a member of the Ly6 family and is bound to the cell surface through a GPI-anchor. Neutralizing antibodies to TSA-1 rescued osteoclast formation in RAW264.7 cultures containing T lymphocytes in response to IL-4.Thus, IL-4 has dual roles in that it can inhibit or enhance osteoclast formation depending on the cell system employed and mediates its inhibitory actions directly or through T-lymphocyte production of TSA-1.

SA264

Pyrrolopyrimidine Class Src Inhibitors Inhibit Osteoclast and Bone Metastatic Tumor Cell Functions. I. Recchia,*[1] N. Rucci,*[1] S. Migliaccio,*[1] A. R. MacKay,*[1] C. Festuccia,*[1] M. Bologna,*[1] D. Fabbro,*[2] M. Susa,[3] A. Teti.[1] [1]Experimental Medicine, University of L'Aquila, L'Aquila, Italy, [2]Oncology, Novartis Pharma, Basel, Switzerland, [3]Arthritis and Bone Metabolism, Novartis Pharma, Basel, Switzerland.

The discovery that c-Src is critical for osteoclast (OC) mediated bone resorption has had a profound impact on the field of bone biology. In this study we report the effects of novel Src inhibitors (SrcIs) of the pyrrolopyrimidine class, which reduce bone loss in ovariectomized animals, on OC and bone metastatic tumor cell behaviour. SrcIs CGP77675 and CGP76030 exhibited a concentration-dependent inhibition of OC maturation in a mouse bone marrow OC formation assay as determined histochemically and biochemically by TRAP activity. SrcIs also reduced the capacity of mature differentiated OCs to reabsorb bone in a bovine bone slice pit formation assay. SrcIs impaired OC adherence and spreading on a serum protein substrate, with a greater effect on mature vs immature cells. These two inhibitors also reduced dose-dependently proliferation of the human bone metastasis-derived prostate cancer cell line PC3 as assessed by the 3H-thymidine incorporation test. Crystal violet staining revealed that the two SrcIs reduced the capacity of PC3 cells to adhere to and spread upon a serum protein matrix in a concentration- and time-dependent manner. However, the SrcIs were modestly active at promoting PC3 cell detachment. When PC3 cells were previously attached to substrate prior to treatment with the inhibitors, crystal violet staining showed only 15% reduction of cell adherence at the highest concentration (20 uM). However, cells rounded up, confirming a role of Src on cell spreading. To evaluate the effect of SrcIs on cell invasion, we plated PC3 cells in transwells on porous membranes coated with recostituted Matrigel or gelatin substrates. Cells were then subjected to chemotaxis by inoculating in the lower compartment of the transwells culture medium previously conditioned by NIH3T3 cells. Results showed a potent concentration-dependent inhibition of PC3 cell ability to invade these substrates. In conclusion, these data highlight a role for Src activity in the regulation of OC and PC3 behaviour and, therefore, suggest a novel therapeutic avenue for Src inhibitors in osteopenic and cancer-induced bone diseases.

SA265

Tamoxifen Inhibits Acid Transport Activity and Binds Specifically to a 76kDa Protein in Isolated Osteoclast Ruffled Membranes. M. A. McKenna,*[1] J. P. Williams,[2] L. Zhang,*[1] J. M. McDonald.[1] [1]Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Department of Internal Medicine, University of Kentucky, Lexington, KY, USA.

Tamoxifen, a potent calmodulin antagonist, directly inhibits osteoclastic bone resorption by a mechanism independent of estrogen (*J Biol Chem* 1996; 271:12488-12495). However, the molecular events responsible for this inhibition are unknown. We have previously demonstrated that osteoclasts cultured on bone have increased intracellular calmodulin levels (*J Cell Physiol* 1994; 160:17-28). Furthermore, the inhibitory effects of tamoxifen on osteoclast activity are mimicked by the calmodulin antagonist, trifluoperazine. Chemical crosslinking was used to identify tamoxifen target proteins in osteoclast lysates and ruffled membrane preparations. Avian osteoclasts and ruffled membrane preparations were from egg-laying hens maintained on a calcium-deficient diet and mouse osteoclasts were differentiated from bone marrow cell preparations. Tamoxifen and trifluoperazine inhibit avian and mouse osteoclast bone resorption activity by 40-50% with IC50's of ~1.0µM, as measured by ^{3}H- proline release from labelled bone. Similarly, both agents inhibit acid transport in preparations of isolated ruffled membrane vesicles with IC50's of 0.5-2.0µM. These concentrations are similar to those that are reported to inhibit calmodulin-dependent activity in other systems. Chemical crosslinking was accomplished using tamoxifen aziridine, a modified, active tamoxifen molecule that acts as native tamoxifen, but binds covalently to tamoxifen target proteins. In intact avian osteoclasts and lysates from untreated avian and mouse osteoclasts, tamoxifen aziridine binds specifically to two proteins with molecular weights of 76 and 52 kDa. Using isolated ruffled membrane preparations from avian osteoclasts, the 76 kDa protein is the major, specific tamoxifen binding protein. The Kd for tamoxifen aziridine binding to the 76 kDa protein in isolated vesicles is ~1µM, similar to the concentrations at which tamoxifen half maximally inhibits acid transport and bone resorption. In conclusion, the direct inhibitory effects of tamoxifen on osteoclastic bone resorption and acid transport activity in isolated membrane vesicles are non-genomic and using chemical crosslinking we have identified a 76 kDa candidate protein as a potential molecular target through which tamoxifen exerts its inhibitory activity.

SA266

See Friday Plenary number F266.

SA267

Fas-Mediated Apoptosis in Mouse Osteoclasts. X. Wu,* M. McKenna,* X. Feng, J. McDonald. University of Alabama at Birmingham, Birmingham, AL, USA.

The balance between bone formation and degradation is critical for maintaining bone mass. This balance is dependent upon both the number and activity of osteoblasts and osteoclasts. The cell number is determined not only by the rate of cell formation but also by the rate of apoptosis. Research in the past several years has provided greater insights into

the molecular mechanisms of osteoclast formation. However, the molecular mechanisms underlying osteoclast apoptosis remain largely unknown. Given that a) the Fas/Fas ligand (FasL) system is one of the most important systems regulating apoptosis in the development of the immune system and b) increasing evidence supports crosstalk between the immune system and bone homeostasis, we have initiated characterization of the Fas/FasL system in osteoclasts.To investigate whether Fas is expressed in osteoclasts, we generated mouse osteoclasts in vitro by treating osteoclast precursors (bone marrow macrophages) isolated from C57bl/6 mice with soluble mouse RANKL and M-CSF. RT-PCR using total RNA prepared from these osteoclasts demonstrated the presence of Fas transcripts in these cells. Furthermore, both Western analysis and immunohistochemistry showed that Fas proteins are also expressed in mature mouse osteoclasts. To determine whether Fas plays a role in regulating osteoclast apoptosis, mature mouse osteoclasts were treated with Fas activating antibody and apoptotic activity was determined by monitoring chromatin condensation by fluorescent nuclear staining. Fas activating antibody induced osteoclast apoptosis in time- and dose-dependent manners. Apoptosis of multinucleated osteoclasts was maximal at 0.5ug/ml of activating antibody with 40% of the cells undergoing apoptosis. Maximal apoptosis was seen at 18 hours after treatment with Fas antibody with no further increase at 24 hours. Interestingly, IFN-r up-regulated Fas expression dose-dependently in osteoclasts and induced osteoclast apoptosis by itself but did not sensitize the cells to Fas stimulation. IFN-r (10ng/ml) for 24 hours increased basal apoptosis 3 fold. In addition, FasL was also expressed in mouse osteoclasts as determined by both RT-PCR and immunohistochemistry, and its protein expression level could also be up-regulated by IFN-r.The observation of FasL expression and functional expression of Fas on mouse osteoclasts implies that the Fas/FasL system may play an important role in regulating bone remodeling. Furthermore, the regulation of apoptosis and Fas/FasL expression by IFN-r further supports a functional link between the immune system and bone remodeling.

SA268

See Friday Plenary number F268.

SA269

Leupaxin Is a Cytoskeletal Protein in the Sealing Zone of the Osteoclast

A. Gupta,[1] B. S. Lee,[2] M. A. Chellaiah,[1] M. A. Khadeer,*[1] K. A. Hruska.[2 1]OCBS, University of Maryland, Baltimore, Baltimore, MD, USA, [2]Department of Medicine, Washington University, St. Louis, St. Louis, MO, USA.

Leupaxin (Leu), a novel cytoplasmic protein, was first identified in human (hu) macrophages, and was found to share homology with the focal adhesion protein paxillin. Leu was shown to associate with Pyk2, a member of the FAK family, and to be a substrate for a tyrosine kinase in lymphoid cells. Like other members of the paxillin superfamily, Leu possesses LIM and LD domains that have been implicated in targeting proteins such as focal adhesion kinase (FAK) to focal adhesions. Cross-species conservation of the LD motifs and sequence differences therein, are critical determinants of unique distribution and specificity of functions of these "adaptor" molecules. We have now cloned Leu from an osteoclast (OC) cDNA library (Leu-OC) as a 1.9 kb clone, comprising a 5' untranslated region of 72 bases, followed by 1161 bases of coding region, and 540 bases of 3' untranslated region. The overall amino acid identity between the OC and the hu-macrophage form of Leu was found to be 90%. In our preliminary studies, we have reported that Leu-OC is associated with Pyk2, similar to its association in macrophages. Although Leu-OC contains 10 tyrosine residues, sequence analysis of the Leu-OC amino acid sequence identified only tyrosines #9 and #10 as being potential phosphotyrosine sites. Treatment of osteoclasts (OCs) with TNF-alpha, a potent activator of osteoclastogenesis, and which is known to cause cytoskeletal rearrangements in macrophages, stimulated tyrosine phosphorylation of Leu-OC. We found Leu-OC to co-immunoprecipitate with the protein tyrosine phosphatase PTP-1B in murine OCs, although we have not determined whether Leu is indeed a substrate for PTP-1B. In hu-macrophages, both Leu and PTP-1B colocalized at the cell periphery. Furthermore, Leu-OC was also found to associate with FAK, the paxillin kinase linker p95PKL, and the ARF-GTPase PAK in murine OCs. In addition, Leu-OC was found to coimmunoprecipitate with the recently characterized focal adhesion protein called actopaxin, which has the ability to bind actin through its calponin-homology domains. Finally, we found that Leu-OC is distributed to actin-rich areas in the cell periphery of hu-OC, particularly those associated with the specialized adhesion structures known as podosomes. The localization of Leu to the OC podosomal complex may be explained by its putative association with actopaxin. These results indicate that Leu, which is a cytoskeletal protein preferentially expressed in hematopoietic cells, is likely to play a crucial role in adhesion and motility of the osteoclast.

SA270

See Friday Plenary number F270.

SA271

Bone Mass in Women With Their First Acute Coronary Ischemic Episode. C. Valero,* J. A. Riancho,* L. Perera,* J. L. Olmos,* J. A. Amado,* J. González Macías.* Internal Medicine, Hospital Universitario Marques de Valdecilla, Santander, Spain.

Objective: To know if women with their first acute coronary ischemic episode have a low bone massPatients and methods: 28 women with first myocardical infarction or unstable angina and 47 normal women matched by age(range 40-75 y.) were studied. BMD at the spine and hip (Hologic QDR 4500) and QUS at the calcaneous (Sahara) were measured .BMI, smoking habit (considered as yes or no) and time since menopause were also noted. Results: No differences in age or time since menopause were found between both groups. However ,both BMI and smoking habits were higher among patients.BMD was similar in both groups (table 1).

*** T Student ** Chi2**

	Controls	Patients	P
Age (years)	59.7 ± 8.9	61.8 ± 10.0	0.34
BMI(Kg/m2)	25.4 ± 4.1	29.9 ± 5.3	<0.05*
Smoking habits	8/47	7/28	<0.05**
Years since menopause	10.8 ± 6.8	14.6 ± 10.1	0.21
Z-score spine	-0.08 ± 1.13	-0.01 ± 1.31	0.81
Z-score hip	0.35 ± 1.01	0.60 ± 0.97	0.30

After adjusting for BMI and smoking habit,the lack of difference persisted. Conclusion: We have found no differences in bone mass between women with their first acute coronary syndrome and normal women matched by age. However, they differed in BMI and smoking habits, and therefore new studies may be needed to further clarify the relationship between bone mass and atherosclerosis in wome

SA272

Recreational Physical Activities Are Associated With Increased Bone Mineral Density in Elderly Chinese in Beijing: Beijing Osteoarthritis Study. L. Xu,*[1] Y. Zhang,[2] D. Felson,[2] M. Qin,*[3] W. Yu,*[3] L. Lui,*[3] S. Cummings,*[4] M. Nevitt.*[4 1]Dept of Obstetrics and Gynecology, Peking Union Medical College Hospital, Beijing, China, [2]Rheumatology, Boston University School of Medicine, Boston, MA, USA, [3]Peking Union Medical College Hospital, Beijing, China, [4]University of California at San Francisco, San Francisco, CA, USA.

While several studies have shown that low levels of physical activity may contribute to decreased bone mineral density (BMD) in women, the epidemiologic evidence has not been consistent. To date, few studies have evaluated the effects of occupational and recreational physical activities simultaneously on BMD among elderly, especially elderly men. We examined the effects of these two types of activities on BMD among Chinese men and women in Beijing, China, a population with high levels of physical activity. Residents (age >60 years) were recruited door-to-door in randomly selected neighborhoods. Of those contacted, 82% (n=2,048) completed an interview and had BMD measured at hip and spine using a Lunar DPX-L or Lunar Prodigy. A cross-calibration study (40 subjects) was done to estimate Prodigy equivalent BMD from DPX measurements. For each subject, data was collected on past occupational physical activities performed daily, including walking >2 miles, standing >2 hours, digging >2 hours, kneeling or squatting >30 minutes, climbing up or down >10 flights, or lifting >10 kg objects. We also inquired into the nature of and time spent on current recreational physical activities. For each gender, we examined each activity in relation to total hip and spine (L1-L4) BMD adjusting for age, height, BMI, education, smoking, health status and densitometer using a linear regression model. There was little relation of past occupational activities on BMD at the hip and spine. In contrast, time spent on recreational activities was associated with an increased BMD in men and a threshold effect of recreational activities (> 10 min/day) on BMD in women.

Adjusted Mean of BMD

	Men (n=827)			Women (n=1221)		
Time spent on recreational physical activity (min/day)	N	Hip	Spine	N	Hip	Spine
None	484	0.920	1.079	823	0.803	0.890
10-30	76	0.931	1.088	61	0.825	0.922
31-60	135	0.939	1.095	85	0.824	0.917
>60	132	0.941	1.113	152	0.822	0.909
P for trend		<0.033	<0.049		<0.005	<0.012

The specific recreational activities related to an increased BMD were running, Taichi and stretching in men (differences in mean BMD ranging 2.2%-7.6% in hip, 2.5%-3.1% in spine), and Taichi and stretching in women (differences ranging 2.0%-2.5% in hip, 2.0%-3.8% in spine). In conclusion, time spent on current recreational physical activities instead of past occupational physical activities was associated with increased BMD among elderly men and women in Beijing

SA273

Study of Bone Health Among Middle-Aged Chinese Canadian Immigrants. A. M. Cheung,[1] G. Chan,*[2] E. Fuller-Thompson,*[1] J. Law,*[1] C. Chan,*[1] D. Lui-Yee,*[1] N. Forde,*[1] N. Diaz-Granados,*[1] R. Chaudhry,*[1] A.

Shik,*[1] L. Thompson.*[1] [1]University of Toronto, Osteoporosis and Women's Health, University Health Network, Toronto, ON, Canada, [2]Yee Hong Community Centre, Toronto, ON, Canada.

The purpose of this community-based cross-sectional study is to examine the risk factors for osteoporosis and osteoporotic fractures and the prevalence of osteoporosis among middle-aged Chinese Canadian immigrants. We recruited community-dwelling individuals aged 45 and over through newspaper, radio and television announcements, as well as posters in community centres in the Greater Toronto Area. Interviews were done in person at a community centre or hospital, and data were collected on demographics, risk factors for osteoporosis and osteoporotic fractures, calcium intake, physical activity, medication use, comorbid conditions, and fall and fracture history. Data was collected on height and weight and heel bone density measured using the Sahara ultrasound device (Hologic, Inc).A total of 491 individuals (334 women and 157 men) aged 44 to 94 (mean age = 60) were included in our study. Approximately 3/4 immigrated from Hong Kong, and the rest mainly from China and Taiwan. On average, the participants have been in Canada for 11.5 years (range = 2 months to 46 years). Less than 2% are current smokers and 10% are ex-smokers. Common reported comorbid conditions include hypertension (29%), hypercholesterolemia (18%), diabetes (9%), gastrointestinal diseases (8%), lung disease including asthma and COPD (7%), coronary artery disease (7%), liver disease (3%), and stroke (3%). Among the 334 women, 290 had children and 57% of these breastfed. Mean age at menarche was 13.6 years and over 70% of the women were postmenopausal (mean age at menopause = 48 years). Approximately a quarter of the participants have had at least one accidental fall and one fifth report a family history of osteoporosis. For women, average height and weight were 61 inches and 121 lbs, and average heel BMD was 0.509 g/cm2 with a T-score of -0.63. For men, average height and weight were 66 inches and 144 lbs, and average heel BMD was 0.511 g/cm2 with a T-score of -0.62. Overall, 4% of women and 2% of men had T-score at or below -2.5 and 39% of women and 41% of men had T-scores between -1 and -2.5.This is the first community-based study of bone health among Chinese immigrants in Canada. In this sample of Chinese Canadian immigrants, the prevalence of osteoporosis among men is similar to what has been described for the Canadian population in general, however, the prevalence of osteoporosis among Chinese Canadian women is less than would be expected. Further study is needed to compare the characteristics of this population to that of the general Chinese immigrant population in Canada.

Disclosures: Eli Lilly,2; Novartis,2; Procter and Gamble,2.

SA274

See Friday Plenary number F274.

SA275

Effects of Pregnancy, Risk Factors and Calcium Intake on Bone Mass in a Sample of Venezuelan Women. G. A. Galue, L. Mora,* C. Padron,* M. Camacho.* Centro de Ultrasonido Oseo, Maracay, Venezuela.

There have been conflictive reports on the effect of pregnancy and calcium intake on bone mass. This study aimed to elucidate the effects of pregnancy on bone mass ascertained by quantitative ultrasonometry (QUS) and on a basic biochemical profile in a group of healthy Venezuelan women. A group of 30 pregnant Venezuelan women (mean age of 26.23+/-7.26 SD years) in their second trimester underwent ultrasonometry at the heel (Achilles Express, Lunar Corp). Laboratory tests to determine serum calcium, serum phosphorus and total alkaline phosphate were performed at the beginning of the second trimester of pregnancy and during the third. A questionnaire on estimated calcium intake and risk factors for low bone mass was done. The ultrasonometry variables-speed of sound, broadband ultrasound attenuation, and Stiffness Index-were measured during the second and third trimester. Our results showed that the incidence of risk factors for low bone mass during the second and third trimester of pregnancy were: coffee intake of more than 3 cups/day (33 %), unspecified upper tract digestive disorders (33%), body weight under 45 kilograms (20%), family history of osteoporosis (20%), past use of glucocorticoids (20%). 43.3% of the women reported inadequate calcium intake. 46.6% had past history of oral contraceptive use and 60 % did not perform regular physical activity. There was a significant decrease in the total serum calcium (9.19 mg/dl vs. 8.39 mg/dl p<0,0001), increase of total alkaline phosphate (36 IU/l vs. 40,86 IU/l, p<0,0512) and decrease of the Stiffness Index (90,49 vs. 86,51, p<0,0379). Our study shows that in a sample of Venezuelan pregnant women there is a decrease in bone mass during the second and third trimester of pregnancy which can be safely assessed by quantitative ultrasonometry at the heel and that during this period there is a high bone turnover as can be inferred through a basic biochemical profile. Low calcium intake, sedentary lifestyle, high coffee intake and digestive disorders are frequent in this group of women.

SA276

The Hip Bone Mass of U.S. Adults Living in the First Half of the 20th Century. W. E. Duncan,[1] L. W. Poulsen,*[2] D. L. Stott,*[1] A. S. Chang.*[3] [1]Department of Medicine, Walter Reed Army Medical Center, Washington, DC, USA, [2]Department of Forensic Medicine, University of Aarhus, Aarhus, Denmark, [3]Department of Clinical Investigation, Walter Reed Army Medical Center, Washington, DC, USA.

Osteoporosis is a significant health problem for modern populations. The study of the bone mass of past populations may give insights into the influence of current lifestyles on bone health. Therefore, the bone mass of 293 femora (from 49 Black men, 96 White men, 51 Black women, and 97 White women) from a well-characterized and preserved group of American skeletons (the Terry collection, Smithsonian National Museum of Natural History) was measured by DEXA (Hologic QDR-2000). The bone mass was then compared to the hip bone mass of a recent (1988-1991) American population sample (NHANES III). The age of the Terry collection subjects was 52.6 ± 15.7 (mean ± 1SD) years (range: 20-89), the date of birth was 1885 (range: 1843 to 1934) and the date of death, 1938 (range: 1921 to 1965). The bone mass (gms/cm2) of the total hip was higher in the Black men (1.072 ± 0.159) and lower in White women (0.703 ± 0.149) than in Black women (0.887 ± 0.160) or White men (0.946 ± 0.127) (p<0.01). This trend was observed at the femoral neck, trochanter, intertrochanter, and Ward's triangle. Loss of bone mass with age was observed in all four gender/ethnic groups. Compared to the NHANES III reference population, the total hip bone mass of the Terry collection femora was 1.05 ± 0.10 SD lower for White women (p<0.0001), 0.42 ± 0.11 SD lower for Black women (p<0.001), 0.19 ± 0.08 SD lower for White men (p=0.03) and unchanged (+0.04 ± 0.12) for Black men. These differences were constant from the third through the ninth decade of life. Similar differences were observed at the other regions of the hip and could not be explained by differences among the study subjects in the date of death, body weight, cause of death or the size of their femurs. From 1938 to 1990, American women have significantly increased the bone mass of their femurs. This improvement was greatest for White women. While the explanation for these findings are likely to be multifactorial, the use over the last 60 years of hormone replacement therapy and possibly oral contraceptives is most likely the major reason for the observed improvement in bone health of American women.

SA277

Relationships between Anthropometric and Bone Mineral Density Variables Differ between Men and Women in the Early Adult Years. J. M. Beiseigel,* S. M. Nickols-Richardson. Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.

Osteoporosis prevention for both males and females is important; however, strategies for osteoporosis prevention among men and women may differ depending on relevant anthropometric and body composition variables. The purpose of this study was to investigate relationships between anthropometric measures, body composition variables, and bone mineral density (BMD) among men (n = 29; age = 22.7 ± 0.6 years; mean ± SEM) and women (n = 60; age = 20.3 ± 0.2 years) in their early adult years. Body height and weight were measured by an investigator. BMD of the total body (TB), lumbar spine (LS), nondominant total proximal femur [TPF, including Ward's triangle (WT), femoral neck (FN), and trochanter (Troch)] and nondominant forearm [including ultra-distal (UD), mid, and proximal regions] were measured by dual energy X-ray absorptiometry (DXA). Fat mass (FM), fat-free soft tissue mass (FFST), and percent body fat (%BF) were calculated from TB DXA scans. Correlation analyses showed that for men, height and FFST were positively associated with TB BMD ($r = 0.38$; $p < 0.05$ and $r = 0.31$; $p < 0.05$, respectively). In men, %BF was negatively associated with TB ($r = -0.37$; $p < 0.05$) and Troch BMD ($r = -0.42$; $p < 0.05$). Among women, body weight was positively associated with TB ($r = 0.28$; $p < 0.05$), LS ($r = 0.36$; $p < 0.01$), TPF ($r = 0.48$; $p < 0.001$), FN ($r = 0.34$; $p < 0.001$), Troch ($r = 0.40$; $p < 0.01$), and UD BMD ($r = 0.29$; $p < 0.05$). In women, FFST was positively associated with TB ($r = 0.38$; $p < 0.001$), LS ($r = 0.35$; $p < 0.01$), TPF ($r = 0.52$; $p < 0.001$), WT ($r = 0.26$; $p < 0.05$), and FN BMD ($r = 0.34$; $p < 0.001$). FM was positively associated with Troch ($r = 0.28$; $p < 0.05$) and UD BMD ($r = 0.31$; $p < 0.05$) in women, and %BF was positively associated with UD BMD ($r = 0.31$; $p < 0.05$) in women. Among these young adults, FFST was positively associated with measures of BMD for both men and women. In men, however, height was positively associated with TB BMD while for women, body weight, FM, and %BF were positively related to measures of BMD. These results suggest that for both men and women, maintaining or increasing FFST, such as skeletal muscle, appears beneficial for bone and osteoporosis prevention. For men, a lean body composition (i.e., lower %BF) may be beneficial, while among females, overall body weight, either from FM or FFST may be important to BMD and osteoporosis prevention.

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SA278

Association of Weight with Bone Mineral Density in Older African Americans. J. Robbins,*[1] C. Hirsch,*[1] J. Cauley,[2] T. Harris.[3] [1]Internal Medicine, UC Davis, Sacramento, CA, USA, [2]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA, [3]Geriatric Epidemiology, National Institute on Aging, Bethesda, MD, USA.

Purpose: To assess correlates of bone mineral density (BMD) in African American (AA) men and women over the age of 64.**Methods:** Two sites of the Cardiovascular Health Study (CHS) (Pittsburgh PA and Sacramento CA) evaluated BMD by DEXA in 304 AAs. CHS is an observational study of older Americans. In 1995-6 DEXAs were obtained as well as medication usage, and multiple cardiovascular risk factors, demographic, physiologic variables, including estrogen use, diabetes, depression, alcohol use, self reported health. Correlation coefficients were calculated for multiple independent variables compared to BMD in the PA lumbar spine and total hip. Those that showed even marginal significance (p<0.1) were tested in sex stratified liberally configured linear regression models. (Backwards stepwise, p<0.1 to enter, 0.2 to remove.)**Results:** The study included 187 women and 119 men with an mean age of 75, range 67 to 96. The means and standard deviations for the BMDs were Spine: 1.11 (±0.26) gm/cm2, Total Hip: 0.92(±0.18)gm/cm2 . Gender was significantly associated with BMD (t-test, p<0.001). R2 for variables which stayed in the model are:

	TOTAL HIP	LUMBAR SPINE	TOTAL HIP	LUMBAR SPINE
	WOMEN		MEN	
WEIGHT	22%***	16%	27%***	

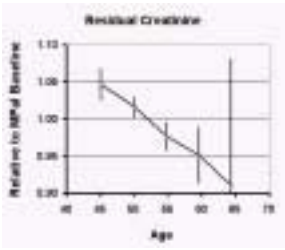
AGE	2%*		9%
IADL	1%		
ESTROGENS	1%		
FAMILY INCOME		2%	

Women only *p<0.05, **p<0.01, ***p<0.0001The mg/cm2 change in BMD per lb of weight ranged from: 2.4 to 3.1 **Conclusions:** As expected gender is associated with BMD. In this elderly population age is less important than might be predicted. In older AAs most of the identified variance in BMD is associated with weight. Fully 27% of BMD of the hip in AA men and 22% in women could be explained by weight. Thin older AA men and women are at significantly increased risk for low BMD and likely more risk for fracture

SA279

Mid-Life Muscle Mass Decrease. K. M. Davies,[1] R. P. Heaney,[2] R. Ryan,*[1] K. Rafferty.*[1] [1]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [2]John A. Creighton University Professor, Creighton University, Omaha, NE, USA.

A cohort of healthy, active, nulliparous white women (The Omaha Nuns) has been studied for 34 years to understand calcium metabolism, bone health, and menopause. For the first 25 years, participants had 8-day inpatient balance studies every five years with detailed analysis of diet on study. This period largely predated the scanner era, when body composition measurements have become routine. We have investigated urine creatinine as a surrogate of muscle mass in a subcohort of 131 women who averaged 4 visits each. Using a regression of urine creatinine on meat protein eaten (Urine creatinine = 0.909 + 0.00739 * Meat_Protein), we determined Residual Creatinine as Urine creatinine - 0.00739*Meat_Protein, which we took to be a better surrogate, and expressed all values in terms of each woman's peri-menopausal baseline value. Aggregating by age groups (40, 45, 50, etc) or by time with respect to menopause or including only those with natural menopause, we found virtually indistinguishable linear declines from age 45 to age 65 with no menopausal effect. The rate is -0.69 %/y (95%CL: -0.79,-0.59) The figure illustrates the linearity of the aggregate data. Conclusion: Muscle mass in these women decreased at the rate of -0.7 %/y.



SA280

More Evidence for the Beneficial Effects of Moderate Alcohol Intake and Adverse Effects of Caffeine Consumption on Bone Mass in Postmenopausal Women. J. Z. Ilich, R. A. Brownbill. University of Connecticut, Storrs, CT, USA.

It is well established that excessive alcohol and caffeine consumption, as well as cigarette smoking have detrimental effects on bone. Some studies however, report that moderate alcohol intake may be beneficial for bone, and moderate coffee consumption with, otherwise adequate calcium (Ca), may not adversely affect bone. The purpose of our study was to determine the relationship between alcohol, caffeine, cigarette smoking and bone mineral density (BMD) of different skeletal sites in healthy, Caucasian, women, n=136, age 68.6±7.1 y (mean±SD), not taking any medication known to affect bone. Alcohol and caffeine consumption was assessed using questionnaires designed to determine frequency, amount, and source of each. Additionally, 3-day dietary records were collected for more complete assessment and Ca intake. History of smoking was recorded and the years of smoke exposure (SE) calculated. BMD of various skeletal sites was measured by a Lunar DPX-MD densitometer (Lunar Corp., Madison, WI). Mean Ca intake was 834±365 mg/d (range 250-1857). Mean alcohol and caffeine consumption was 5.2±9.9 g/d and 210±188 mg/d, respectively. 46% women consumed wine as a main alcohol source, while 11% and 5%, consumed liquor and beer, respectively. 36% women did not drink any alcohol. 63% women consumed coffee as a main caffeine source, while 13% and 2%, consumed tea and soda, respectively. 20% women did not consume any caffeine. There were no current smokers and 54% of women never smoked. The average SE was 11.5±20.7 y*package, (range 0-102). Multiple regression models were constructed in which alcohol, caffeine and SE were regressed on BMD of different skeletal sites. Each model was corrected for age, weight and dietary Ca. The results showed statistically significant positive effect of alcohol and Ca intake and negative effect of caffeine intake on all regions of spine and total body BMD, with R2(adjusted) ranging from 19% to 35%. This effect was less significant in the models with forearm and non-existent in the models with hip BMD. SE did not show statistically significant effect on any of the skeletal sites examined. Our data indicate the beneficial effect of moderate alcohol, mostly consumed as wine, on BMD of spine and total body. The negative effect of caffeine could have been exacerbated by the relatively low Ca intake in our population (as Ca showed strong positive effect on BMD of all measured skeletal sites). The lack of association between SE and BMD could be due to the fact that majority of women have never smoked and those who have, were not heavy smokers. More investigation is necessary to clarify the above relationships.

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SA282

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SA283

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SA284

Secondary Causes of Hip Fracture in Community-Dwelling Patients. L. A. Fitzpatrick,[1] J. Loftus,*[2] M. Faurot,*[1] M. Bolander.[2] [1]Department of Medicine, Mayo Clinic, Rochester, MN, USA, [2]Department of Orthopedics, Mayo Clinic, Rochester, MN, USA.

Mortality due to hip fractures can exceed 20% in one year and less than one-third of patients regain their independence. To determine the potential for prevention of hip fractures in a community population, we assessed laboratory values for bone loss in all patients sequentially admitted to our medical center for hip fracture. Patients with trauma as a cause were excluded and a total of 33 patients were tracked through the daily surgical list. Baseline demographics were collected by chart review and bedside interview. All patients had BMD measurements, basic laboratory parameters and a follow-up appointment with an endocrinologist, with a speciality interest in bone metabolism, at 6 to 8 weeks post fracture. Average age was 79±8 years with 30% male and 70% female distribution. Average serum calcium was 9.7±0.43 mg/dl, phosphorus 3.49±0.47 mg/dl, 25(OH)D 17.2±6.3 ng/ml, urinary Ca 114±96 mg/24h, PTH 3.5±3.0 pmol/ml, NTX 509±482, BMD LS 0.88±0.23 (T-score -1.9±1.6), BMD FN 0.49±0.15 (T-score -1.91±1.3). 55% of patients had had a prior fracture (12% ankle, 18% hip, 3% pelvic, 27% Colles'). In spite of this, only 9% of patients were aware that they had a diagnosis of osteoporosis. Only 3% were chair- or bedridden and could not ambulate without assistance. Regarding recognized secondary causes of bone loss, 3 patients had been on long-term glucocorticoids and 1 was hypogonadal (prostate cancer). Laboratory evaluation revealed that 4 patients were hypercalcemic and 4 had secondary HPT with elevated PTH levels. 13/33 had U_{CA} <100 mg/24h. One patient had an elevated TSH level. 22 of 32 (69%) patients had 25(OH)D levels ≤20 ng/ml and 5/32 (16%) had levels ≤12 ng/ml. In addition, 7/30 (23%) had $1,25(OH)_2D$ levels ≤15 pg/ml. By T-score criteria, (<-2.5), 11/30 at LS, 11/22 at FN and 27/30 (90%) at any site (LS, FN, Ward's, total hip Troch, Inter), who met criteria for osteoporosis. In community-dwelling subjects with hip fx, there is a high incidence of abnormal laboratory tests (77%) that are correctable with appropriate treatment. Patients with hip fx commonly have secondary causes of bone loss and require careful metabolic evaluation. Targeting patients with prior fx may provide an early intervention for reversal of secondary causes and prevent future hip fxs.

SA285

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SA286

Predictive Demographics in 200 Consecutive Community-Dwelling Residents With Hip Fracture. L. A. Fitzpatrick,[1] J. Loftus,*[2] M. Faurot,*[1] M. Bolander.[2] [1]Department of Medicine, Mayo Clinic, Rochester, MN, USA, [2]Department of Orthopedics, Mayo Clinic, Rochester, MN, USA.

Hip fracture results in significant mortality and morbidity in elderly patients. In order to understand the risk factors for hip fracture in a community population, we evaluated all consecutive community hip fracture (fx) patients admitted to our medical center. Patients with trauma as a cause were excluded and 200 patients were tracked through the surgical register and cases confirmed by operative reports. Baseline demographics were collected by chart review and bedside interview. The average age was 73±12 and 67% of the patients were female. Average BMI was 26.09±7.62. Remarkably, 41% of the patients had a prior fracture. Previous Colles' fracture (25%) was predominant followed by contralateral hip fracture (13%), ankle fracture (9%), pelvic fracture (2%), and vertebral fracture (2%). In spite of the large number of prior fxs, only 16% of subjects had a prior diagnosis of osteoporosis. Baseline demographics revealed that 27% of the patients had dementia/chronic brain syndrome; 10% had a history of transient ischemic attacks and only 2% complained of vertigo. Only 1% of 200 patients had problems such as Parkinsonism or ataxia. Gait instability, focal cortical degeneration and functional gait problems were each less than 1%. Of the 122 patients with interview information regarding ambulatory status, only 2% were chair- or bedridden prior to fracture; 24% were community ambulators, 35% were home ambulators, and 23% of patients were fully active and brisk walkers.In summary, a large number (41%) of patients with hip fx had prior fx, especially Colles' fx (25%). Few patients were bed- or chair-bound prior to fx and vertigo was rare at the time of fx. Organic brain syndrome/dementia was associated with fracture in one-quarter of the cases. These data suggest that selective interventions for patients with dementia may also reduce hip fxs and early intervention in patients with a prior fracture may be important to prevent future

hip fractures.

SA287

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SA288

Accuracy of Physical Examination for Detection of Thoracic Vertebral Fractures. K. Siminoski,[1] R. Warshawski,*[2] H. Jen,*[2] K. Lee.*[3] [1]Endocrine Centre of Edmonton and Medical Imaging Consultants, Edmonton, AB, Canada, [2]Medical Imaging Consultants, Edmonton, Canada, [3]Endocrine Centre of Edmonton, Edmonton, Canada.

Vertebral fracture is the most common type of broken bone in osteoporosis patients. Little information is available about the accuracy of physical examination in detecting such fractures. We have assessed two simple physical examination maneuvers for their accuracies in determining the presence of thoracic vertebral fractures. The maneuvers were measurement of (1) kyphosis angle (KA) from T4 to T12, quantified with a handheld digital inclinometer (in degrees), and (2) wall-occiput distance (WOD), quantified with a tape measure, as the distance from the wall to the occipital prominence with the patient standing, heels to the wall, looking straight ahead (in cm). A total of 216 women referred for assessment of osteoporosis were studied. The average age was 53 years (range: 18 to 92). Vertebral fracture was defined as a decrease in vertebral height of 20% or more on lateral radiographs (from T4 to T12 for the purposes of this study). One or more thoracic fractures were present in 29% of subjects. Among those with fractures, the average number of fractures was 1.9. KA and WOD were correlated ($r = 0.32$; $p<0.0001$). Both KA and WOD increased with age in those without fractures ($r = 0.29$ and 0.34; $p < 0.001$ for both) The areas under the receiver operating characteristic curves were 0.71 (95% CI: 0.61-0.80) for KA and 0.74 (0.66-0.83) for WOD. Accuracy results are shown in the first table. Likelihood ratios are in the second table. Upper cut-off points were present that produced very high LRs, but there were no lower cut-offs that produced very low LRs.The following applications can be made from this data. KA > 43^o or WOD > 7.0 cm rules in a fracture with a high degree of accuracy. KA < 20^o or WOD = 0 reduces the chance of fracture, but does not reliably rule it out These results show that physical examination of the thoracic spine using simple methods can produce clinically useful accuracy in detecting thoracic vertebral fractures. KA and WOD should be incorporated into the routine physical examination of osteoporosis patients.

KA (o)	Sens	Spec	PPV	NPV
>43	22	99	85	78
>30	63	81	55	86
>20	84	28	30	83
WOD (cm)	Sens	Spec	PPV	NPV
>7	21	99	92	76
>3	38	93	69	79
>0	60	87	65	85

Sens = sensitivity. Spec = specificity. PPV = positive predictive value. NPV = negative predictive value.

SA289

See Friday Plenary number F289.

SA290

Predictors for Atraumatic Vertebral Fracture in Japanese Women as Assessed by Dual Energy X-ray Absorptiometry. T. Ogawa,*[1] S. Takata,[1] H. Yonezu,*[2] N. Yasui.*[1] [1]Orthopedic Surgery, The University of Tokushima, Tokushima, Japan, [2]Orthopedic Surgery, Oe Kyodo Hospital, Oe, Japan.

We clarified the characteristics of bone mineral density (BMD) and soft tissue composition in Japanese women with atraumatic vertebral fractures (AVF). Sixty-four women, 55 to 75 years of age, were divided into two groups: women with AVF (fracture group, n=30) and women without AVF (non-fracture group, n=34). The BMD of the 2nd to 4th lumbar vertebrae (L2-4BMD), the BMD of the head, arms, legs, ribs, thoracic vertebrae, lumbar vertebrae and pelvis as well as the lean mass and the fat mass of the head, arms, legs, and trunk were measured by dual energy X-ray absorptiometry. L2-4BMD, total body BMD and BMDs of the lumbar spine, thoracic spine and pelvis of the fracture group were significantly lower than those of the non-fracture group ($P <0.001$). The total as well as regional lean and fat mass did not differ significantly between the two groups. However, total lean and fat mass of the fracture group tended to be lower than that of the non-fracture group. We conclude that the BMD of weight-bearing bones, except for the bones of the legs of the fracture group, is significantly lower than that of the weight-bearing bones in the nonfracture group.

SA291

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SA292

Cost of Hip Fractures and Estimate of Treatment Cost Effectiveness in Wisconsin Nursing Home Residents. M. E. Elliott,[1] L. D. Willsey,*[1] P. B. Beutel,*[2] D. R. Zimmerman,*[2] A. Gudmundsson,[3] P. D. Meek.*[1] [1]School of Pharmacy, University of Wisconsin, Madison, WI, USA, [2]Center for Health Systems Research and Analysis, University of Wisconsin, Madison, WI, USA, [3]Hrafnista Nursing Homes, Reykjavik, Iceland.

Residents in long term care facilities (LTCF's) have a 3-11 fold higher fracture risk than community-dwellers, but osteoporosis in LTCF's is rarely assessed. Economic constraints likely contribute to this lack of attention. It is unknown whether management of osteoporosis in LTCF's is cost-effective. Goals of this project were to estimate the cost of hip fractures occurring in Wisconsin LTCF's and the number needed to treat to prevent one hip fracture.To estimate hip fractures for 1999, we used fracture data from 95 not-for-profit LTCF's that constitute 30% of Wisconsin LTCF beds. Costs were obtained by combining hospital costs and LTCF charges above baseline after fracture. Fractured LTCF residents were assumed to incur hospital costs similar to Wisconsin community-dwellers. For incremental LTCF costs after fracture, mean daily cost for all non-Medicare residents ($109) was subtracted from the mean Medicare charge of $232 for the first 20 days post discharge to LTCF. To estimate number needed to treat (NNT), we assumed that there was a 20% annual death rate, that transfer independence and below-median bone density increased fracture risk 3-fold, and that alendronate reduced risk 50%.Based on a 2.8% annual hip fracture rate, an estimated 1120 hip fractures occurred in the 40,000 Wisconsin LTCF residents in 1999. Costs of $12,849 per fracture were based on hospital costs of $8477, in-hospital MD fees of $1912 and incremental LTCF costs of $2460. Total costs estimated for hip fractures for Wisconsin LTCF's in 1999 were $14,390,000. NNT for one year to prevent one hip fracture was 26. The model assumed that alendronate treatment of 100 high-risk residents (see above) reduced hip fractures from 8.4 to 4.2 per year. NNT to prevent any clinical fracture would be substantially lower than the estimate of 26.To our knowledge this report is the first to estimate combined cost of hospitalization, MD costs, and LTCF costs of hip fracture, as well as NNT in LTCF's. Direct medical costs for hip fracture were substantially less than that for hip fractures in community-dwellers (~$25,000). This is largely because estimates for community-dwellers include LTCF costs after fracture. Despite the lower figure of $12,849 per hip fracture occurring in LTCF's, the overall cost is considerable, because of the very high fracture risk in LTCF's.Given the high risk and high cost for hip fractures in LTCF's, and a relatively low number needed to treat, further research on assessment and treatment of osteoporosis in LTCF's is warranted.

Disclosures: Merck,2,5; Aventis,2; Novartis,2.

SA293

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SA294

The Expected Cost of New Fractures in the Year Following a Vertebral Fracture. R. Lindsay,[1] R. T. Burge,*[2] D. M. Strauss.*[2] [1]Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Vertebral fractures are believed to be important predictors for future vertebral and other fractures, leading to at least a 4 to 5-fold increase in the risk of subsequent fractures. However, healthcare payers and providers generally view vertebral fractures as being relatively unimportant from a cost perspective. The purpose of this study was to estimate the expected costs of all new fractures occurring within one year of an incident vertebral fracture among osteoporosis patients. A probabilistic decision tree model was developed to identify the expected cost of all subsequent fractures emanating from an incident vertebral fracture event. A total of 381 subjects in the placebo arm (Ca + Vit D) in the risedronate VERT and HIP studies with incident vertebral fractures were identified and followed up for 1 year. Kaplan-Meier time-to-event models were used to estimate the cumulative incidence of new vertebral, hip, wrist, and other fractures occurring within one year of the incident vertebral fracture. Expected costs for each fracture type were obtained from published literature and national hospital discharge database analyses. The 1-year probability of any new fracture was 26.1% (17.3% vertebral; 3.6% hip; 3.5% other (humerus/leg); 1.6% wrist). The unit costs for each fracture type used in the analysis were $36,864 (hip), $1,880 (vertebral), $1,740 (forearm/wrist), and $3,950 (other). For patients with a subsequent fracture the weighted cost was $7,004 during the course of the year of follow-up. Overall the calculated cost of new fractures within one year of an incident vertebral fracture was estimated at $1,828 per patient (excluding the costs of the index vertebral fracture); a weighted average of fracture patients ($7,004; 26.1%) and non-fracture patients ($0; 73.9%). All patients were assumed to not receive any osteoporosis therapies in the model. All patients with incident vertebral fractures are at a 4-5-fold higher risk of a subsequent vertebral fracture within the following 12 months. This analysis predicts that the expected cost of subsequent fractures over the next 12 months following an incident vertebral fracture is $1,828 averaged over all vertebral fracture patients. These results should alert health care payers and providers to both the clinical need for, and economic benefits in, proactively treating vertebral fracture patients to prevent additional, costly fractures associated with osteoporosis.

SA295

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SA296

Bone Loss Following Fracture of the Tibial Shaft. S. C. Findlay,* R. Eastell, B. M. Ingle. Division of Clinical Sciences, University of Sheffield, Sheffield, United Kingdom.

Delayed union and non-union are common complications after fracture of the tibial shaft. Healing of the fracture could be monitored non-invasively using techniques currently used in the study of osteoporosis. The aims of our study were 1) to evaluate the decrement in bone measurements made close to the fracture using dual x-ray absorptiometry (DXA), quantitative ultrasound (QUS) and peripheral quantitative computed tomography (pQCT); 2) to calculate the short term precision for BMD, QUS and pQCT and 3) to calculate the ratio of decrement to precision (response ratio) to determine the optimal test for monitoring changes after tibial fracture. We recruited 28 subjects (14 women, 14 men, ages 17 to 78 years, mean 41) following a fracture of the tibial shaft. BMD of the hip, ultra-distal tibia and fibula (UD) and heel were measured by DXA (Hologic 4500/A). pQCT of the distal tibia and fibula and proximal tibia and fibula (10% site) were measured using the Norland Stratec XCT 2000. Speed of sound (SOS) and broadband ultrasound attenuation (BUA) of the heel were measured using the Lunar Achilles + (LA+) and DMS UBIS 5000 (UB). SOS was also measured at the mid-tibia using the Sunlight Omnisense (SL). Two measurements were made 1 week apart to calculate the short-term coefficient of variation (CV) for the fractured limb. Paired t-tests were used to test for significant differences between the contralateral and ipsilateral limbs (the contralateral side acted as a control for measurements). Finally, we calculated a response ratio (RR) using the following equation: RR = % difference/% CV. The results are shown in the table (*P<0.05, **P<0.01, ***P<0.0001).

Measurement	Mean,diff	%, diff	CV, %	RR
UD tib/fib BMD, g/cm ^{2}	-0.13	-19.1***	6.8	2.8
Heel BMD, g/cm ^{2}	-0.05	-6.5***	4.7	1.4
Proximal trabecular, mg/cm^3	-36	-27.6**	3.0	9.0
Proximal cortical, mg/cm^3	-38.5	-7.7**	3.0	3.0
LA+ SOS, m/s	-14	-0.9***	0.6	1.5
UB SOS, m/s	-15	-0.9***	0.8	1.3
SL SOS, m/s	-109	-2.8*	1.8	1.6
LA+ BUA, dB/MHz	-2	-2.0**	2.8	0.7
UB BUA, dB/MHz	-2	-3.3**	4.8	0.7

The biggest decrement in BMD occurred at sites closest to the fracture when measured by DXA and pQCT. The response ratios were high for pQCT of trabecular bone and low for heel BUA. We conclude that pQCT of the trabecular regions of either the proximal or distal tibia and fibula should prove the most sensitive measurement for monitoring changes in bone adjacent to a tibial shaft fracture.

SA297

Does Cumulative Estrogen Exposure Explain the Association Between Osteoporosis and Coronary Heart Disease? B. I. Gulanski,[1] J. M. Robbins,*[2] A. L. Arnold,*[1] V. Vaccarino.*[3] [1]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA, [2]Population Studies Center, University of Pennsylvania, Philadelphia, PA, USA, [3]Department of Medicine, Division of Cardiology, Emory University School of Medicine, Atlanta, GA, USA.

The purpose of this study was to investigate the contribution of cumulative estrogen exposure, as measured by reproductive variables, to the association between osteoporosis and coronary heart disease (CHD) in women. We analyzed data from 2770 postmenopausal non-Hispanic White, African-American and Mexican-American women aged 40 or older who participated in the Third National Health and Nutrition Examination Survey and had a total-hip bone mineral density (BMD) scan performed. Logistic regression models were used to examine the association between BMD and CHD after adjusting for demographic factors, CHD risk factors, and reproductive variables. Mean total-hip BMD was lowest among non-Hispanic Whites and highest among African Americans. Age, hypertension, and HDL cholesterol were associated with lower BMD, while education, physical activity, BMI, early age at menarche, hormone use, ovariectomy and hysterectomy were associated with higher BMD. History of CHD showed a significant association with lower BMD in non-Hispanic Whites and a borderline association in Mexican Americans, but no association in African Americans. In multivariate analysis, reproductive variables did not explain the association between BMD and CHD in non-Hispanic Whites and Mexican Americans. When all the study factors were controlled for, non-Hispanic White women in the lowest BMD tertile, compared to the highest tertile, had 2.66 higher odds of a previous myocardial infarction, 1.98 higher odds of angina pectoris, and 2.03 higher odds of either myocardial infarction or angina pectoris. In conclusion, cumulative estrogen exposure may not explain the association between BMD and CHD. Further investigation of alternative mechanisms is warranted.

SA298

Model-Fitting and Its Validation of Bone Mineral Density Using an Exponential-Type Nonlinear Mixed-Effect Model in the Lumbar Spine of Postmenopausal Women After Natural Menopause Based on a Longitudinal Study. H. Watanabe,*[1] M. Fukunaga,[2] M. Shiraki,[3] Y. Ohashi.*[1] [1]Department of Biostatistics, University of Tokyo, Tokyo, Japan, [2]Department of Nuclear Medicine, Kawasaki Medical School, Okayama, Japan, [3]Research Institute and Practice for Involutional Diseases, Nagano, Japan.

An exponential-type nonlinear mixed-effect model was fitted to estimate and predict bone mineral density (BMD) at the lumbar spine - in 833 postmenopausal women. Years since menopause (YSM) and age at menopause (AAM) were used as explanatory variables. The model was assumed as $(BMD)_{ij}=a+a_i+(b+b_i)\exp(c(YSM)_{ij})+d((AAM)_i-AAM_{(mean)})+e_{ij}$ (i: subject; j: time point; a, b, c and d are the population means; a_i and b_i are the random effects among subjects; e_{ij} are random error). Because BMD decreases rapidly after menopause and changes slowly thereafter, an exponential model was assumed. The fitted model was $(BMD)_{ij}=0.838+a_i+(0.312+b_i)\exp(-0.0922(YSM)_{ij})-0.00175((AAM)_i-48.7)+e_{ij}$, $\sigma_a^2=(0.162)^2$, $\sigma_b^2=(0.112)^2$, $\sigma_e^2=(0.0290)^2$. The maximum likelihood model was done with a specific algorithm which took account of highly unbalanced data. These formulae were also used to derive a prediction formula for each individual using a Bayesian approach. This prediction formula was validated using other data, and found to be useful for BMD prediction except extremely abrupt change. This prediction formula makes it possible to predict the future BMD and to estimate the risk of osteoporosis accurately in individual subjects.

SA299

Menopausal Symptoms and Bone Loss in Chinese Perimenopausal Women. S. C. Ho,[1] S. S. G. Chan,[1] V. Yip,*[2] A. Sham,*[1] C. Chan,*[1] J. Woo.*[1] [1]Department of Community & Family Medicine, The Chinese University of Hong Kong, New Territories, Hong Kong Special Administrative Region of China, [2]Department of Nursing & Health Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong Special Administrative Region of China.

Perimenopause is a period of transition from premenopausal to postmenopausal years. This transitional period is usually accompanied by an increase in reported symptoms. This period is also characterized by rapid bone loss. This study aims to investigate if increased number of reported symptoms at baseline is related to increased bone loss over 24month period. 438 women aged 45 to 55, not on hormonal replacement therapy, were recruited through random telephone dialing and family medicine clinic into a cohort study on bone changes. Standardized questionnaire, including a 22-item symptom check list, was administered at baseline. Five symptom clusters, namely psychological, musculoskeletal/gastrointestinal, non-specific somatic, respiratory, and vasomotor have been identified by the principal component analysis method. We had also obtained baseline measurements of bone mineral density (BMD) at the spine (L2-L4) and the hip with the use of dual energy x-ray densitometre (Hologic 2000). (Standardized questionnaires and measurements for dietary and lifestyle variables were also obtained at baseline.) Repeated measurements of BMD were obtained after 24 month followup.Analyses were carried out in 265 women with measurements at baseline and followup. The mean age of this study population was 49.9 y (sd = 2.65), and mean followup time was 2.56 y (sd= 0.09). Univariate analysis revealed that women with less than 5 reported symptoms had significantly higher percentage loss of BMD at the spine, femoral neck, trochanter, intertrochanter, wards and total hip, compared with women with fewer reported symptoms. The differences remained significant for intertrochanter and the wards after adjustment for age and menopausal status. Women with at least one reported symptom in the psychological symptom group had higher bone loss at the hip sites, while women with reported vasomotor symptoms had significantly higher bone loss at the wards.Our longitudinal data revealed that reported symptoms during menopause and possibility the accompanying stress level and or endocrine changes may be detrimental to bone health during menopausal transition. Such association is worth further investigation.

SA300

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SA301

Endogenous Estrogen Levels and Its Relationship With Bone Mineral Density in Postmenopausal Women. A. Bagur,[1] M. Belotti,*[1] M. B. Oliveri,[1] A. López Cabanillas,*[1] D. Yankelevich,*[2] F. Sayegh.*[2] [1]División Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Sección Climaterio, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina.

Postmenopausal women whose serum estradiol levels are between 5-25 pg/ml would have higher bone mineral density (BMD) and a lower prevalence of vertebral deformities than women with lower serum estradiol levels and these salutary effects would be moderated by reduced bone turnover. The aim of this study is to evaluate the effect of endogenous estrogens on BMD in healthy postmenopausal women. Upon completion, this cross sectional study will have included 100 healthy postmenopausal women from 55 to 75 years of age. The BMD of L2-L4, Total Femur (TF) and Total Body (TB) are measured by DXA (Lunar Prodigy). The following bone markers are measured in serum: calcium, bone alkaline phosphatase (BAP) and crosslaps (CTX), and in urine calcium. Estradiol and estrona

are measured with sensitive assays that detect lower limit at 5 pg/ml. To date, 48 women have been included in the study. The general characteristics of the population are as follows (Mean±DS): age 65.0±5.8 years, weight 63.2±9.7 kg, height 155±0.05 cm, BMI 26.2±4.2, onset of menopause 48.8±6.1 years, L2-L4: 0.99±0.02 g/cm2 (Z score -0.2), TF 0.85±0.10 g/cm2 (Z score -0.02), TB 1.01±0.02 g/cm2 (Z score -0.2), BAP 74.7±18.7 UI/l (Normal values: 31-95 UI/l) and CTX 522.9±267.8 ng/l (Postmenopausal Normal values: 80-590 ng/ml) , estradiol 8.5±5.7 pg/ml (Postmenopausal Normal values: up to 20 pg/ml), estrona 23.5±16.0 pg/ml (Normal values: 14-100 pg/ml).The 48 women were stratified into 4 groups according to their serum estradiol levels: 10 pg/ml and into 2 groups based on their serum estrona: 15 pg/ml. The results show no differences in BMD areas according to estradiol and estrona levels in these postmenopausal women (range L2-L4 from 1.02 to 0.98 g/cm2, TF from 0.84 to 0.89 g/cm2 and TB from 1.04 to 0.98 g/cm2).Bone alkaline phosphatase was significantly higher in group >10 pg/ml (p< 0.02). Estradiol and estrona did not correlate with BMD and bone markers. Estradiol only correlated significantly with estrona (p<0.02). These preliminary results obtained with 48 post menopausal women show: 1- No association between the low levels of estradiol and estrona determined using ultrasensitive assays and differences in BMD of L2-L4, Total Femur and Total Body. 2- The higher BAP levels observed in the group with the highest estradiol levels (>10 pg/ml) might indicate a positive influence of estrogens on bone mass.

SA302

Low Bone Mineral Density Is Associated With Low Serum Insulin-like Growth Factor-1 in Perimenopausal but Not in Premenopausal Women. D. H. Schussheim,[1] M. R. Rubin,*[1] C. A. M. Kulak,*[2] E. S. Kurland,[1] C. J. Rosen,[3] S. J. Silverberg,[1] J. P. Bilezikian,[1] E. Shane.[1] [1]Medicine, Columbia University, New York, NY, USA, [2]Medicine, Federal University of Parana, Curitiba, Brazil, [3]Maine Center for Osteo. Research, Bangor, ME, USA.

Although osteoporosis (OP) predominantly affects older postmenopausal women, unexplained low bone mineral density (BMD) also occurs in men and younger women. We have previously observed reduced IGF-1 levels in men, premenopausal (PreM) and perimenopausal (PeriM) women with unexplained OP, suggesting a possible pathogenic link between reduced IGF-1 and idiopathic low BMD. However, it is unclear whether this association in women is linked to changing estrogen status prior to menopause. We therefore compared 18 (15 PreM and 3 PeriM) women with a Z score <-2.0 at the lumbar spine (LS) and/or femoral neck (FN) and compared them with 32 (28 PreM and 4 PeriM) healthy volunteers. The PreM and PeriM patients and controls did not differ in age (PreM, 35+2 years vs. 38+1 years; PeriM, 50+2 years vs. 50+2 years, respectively). BMD (Z score) was significantly lower in patients than controls at the LS (-2.063+0.17 vs 0.726+0.13) and FN (-2.022+0.14 vs 0.743+0.16, both P<0.0001). A family history of OP was reported by 67% of patients vs. 37% of controls (P<0.05). Fragility fractures were reported by 44% of patients vs. 19% of controls (P=0.05). All patients had normal serum calcium, electrolytes, renal function, PTH and urinary calcium. Although serum osteocalcin and urinary N-telopeptide (NTX), were normal, NTX was higher in patients (37+3.8 vs. 26.8+2.2 ng/ml, P<0.05). Serum estradiol E2 levels, measured during the follicular phase of the cycle (days 1-5), were lower in PreM patients than in controls (31+4 vs. 50+7 pmol/L; P<0.05) but not in PeriM patients. Serum IGF-1 (168.9+15 vs. 158.7+8.3 ng/ml) and IGFBP3 did not differ between PreM patients and controls. However, in PeriM subjects, serum IGF-1 was lower in patients than controls (105.0+17.1 vs. 166.5+14.67 ng/ml; P<0.05), as were IGFBP3 levels (2815+191 vs. 4368+464 ng/ml; P<0.05). Serum IGF-1 values correlated inversely with age (r=-0.55, P<.0001) and directly with LS and FN BMD (r=0.72 and r=0.89, respectively, both P<0.05) in PeriM women but not in PreM women. Serum E2 did not correlate with IGF-1, LS or FN BMD. In summary, lower IGF-1 levels are associated with low BMD in PeriM but not PreM women. This observation, together with the lack of correlation between IGF-1 and E2, suggests that as E2 levels decrease during the perimenopause, IGF-1 may play a more important role in preserving BMD.

SA303

Are Bone Density and Mammographic Parenchymal Density Related? G. A. Greendale,[1] S. Carter,[2] B. Reboussin.*[2] [1]Department of Medicine/Division of Geriatrics, University of California, Los Aangeles, Los Angeles, CA, USA, [2]Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, USA.

Women with breast cancer have higher bone mineral density (BMD) than those who do not have this disease; higher endogenous estrogens and/or greater estrogen responsiveness are postulated explanations. Higher mammographic density is an independent risk factor for breast cancer. We therefore conducted a study to explore whether mammographic density and BMD were positively associated. Between 1989-91, PEPI enrolled 875 postmenopausal women, aged 45-64 years, who were between 1-10 years postmenopause and not taking HRT within 2 months of screening. All women for whom we were able to retrieve baseline mammograms, and who did not have breast implants were eligible for this study, which included 594 participants (68% of original PEPI sample). BMD was measured using Hologic 1000-QDR densitometers. A computer-assisted method was used to assess mammographic density, the percent of the breast made up of dense tissue. Age and BMI averaged 56 years and 26.2 kg/m^2, respectively. 171 women (29%) were recent HRT-users (stopped for PEPI). Mammographic density ranged from 0-90%. Linear regressions were run with BMD as the outcome and mammographic density, age, and BMI as predictors (Model 1). Then baseline estrone was added (Model 2). The table below shows the β-coefficients (p values) for each of the predictor variables at each bone site among the 415 women who were not recent HRT-users.

Variable	Spine BMD (1)	Spine BMD (2)	Hip BMD (1)	Hip BMD (2)
Mammog. density	0.076 (0.07)	0.064 (.13)	0.059 (.06)	0.054 (.08)
Age	-0.006 (0.00)	-0.005 (.00)	-0.005 (.00)	-0.004 (.00)
BMI	0.012 (0.00)	0.010 (.00)	0.013 (.00)	0.013 (.00)
Estrone	N/A	0.001 (.00)	N/A	0.005 (.10)

Results were similar when estradiol was added to Model 1 (data not shown). In recent HRT-users, after adjustment for the same covariates, BMD and mammographic density were not significantly associated. We conclude that bone and breast densities are positively related in postmenopausal women who have not recently stopped HRT. Adjusting for baseline estrone or estradiol did not substantively alter this association, suggesting that the "common pathway" is more complex than higher circulating endogenous estrogens.

SA304

Effect of Menopause on Bone in Postmenopausal Women with High Bone Volume. S. Qiu,[1] S. Palnitkar,*[1] D. Rao,[1] A. M. Parfitt.[2] [1]Bone and Mineral, Henry Ford Hospital, Detroit, MI, USA, [2]Division of Endocrinology, University of Arkansas for Medical Sciences, Little Rock, MI, USA.

It has been claimed that some postmenopausal women suffer from increased bone fragility without obvious bone loss. There is little information, however, on bone histologic changes in such women. The current study was aimed at this problem.From our archived iliac biopsy sections, we included 94 healthy white women in whom the osteocyte density had been measured. Postmenopausal women with lower values of cancellous bone volume were excluded until the mean value for BV/TV was close to that in premenopausal women. Results of cancellous bone histomorphometry, in 38 premenopausal and 37 postmenopausal women, were compared. As well as standard measurements we added interstitial bone fraction [Ib.F = (Tb.Th-W.Th*2)/Tb.Th]. Additionally, 10 unbroken areas were randomly selected in each section stained with Goldner's trichrome for determination of osteocyte number per bone area (Ot.N/B.Ar). The results are shown in the following table.

	Pre	Post		Pre	Post
BV/TV	24.88(7.46)	24.81(4.58)	MS/BS	5.79(3.09)	8.50(5.55)
Tb.Th	140.56(23.57)	151.02(36.84)	MAR	0.58(0.19)	0.55(0.10)
Tb.N	1.76(0.37)	1.69(0.31)	Aj.Ar	0.31(0.19)	0.24(0.14)
Ib.F	46.32(9.59)	52.30(9.11)*	BFR/BS	12.71(6.94)	17.41(11.71)
W.Th	36.86(3.67)	34.86(5.51)	BFR/BV	14.68(8.90	18.57(12.81)
OS/BS	13.60(7.82)	21.95(12.17)***	Ac.f	0.35(0.19)	0.49(0.31)
ES/BS	6.63(3.17)	7.62(3.49)	Ot.N/BAr	416(57)	344(38)***

Pre: Premenopausal; Post: Postmenopausal. *p < 0.05; **p < 0.01; ***p < 0.001.

The increase in bone remodeling in the postmenopausal women without apparent bone loss was less clear than that shown in the complete data set. However, there was a significant decrease in osteocyte density and a significant increase in interstitial bone fraction in such postmenopausal women. Loss of osteocytes may impair detection of microdamage in bone matrix, and may also lead to focal hypermineralization. We have previously demonstrated that osteocyte loss is most prevalent in interstitial bone. Loss of osteocytes and increased interstitial bone fraction may contribute to enhanced bone fragility in some postmenopausal women without apparent bone loss. It needs to be emphasized that loss of osteocytes is more likely due to aging as we have previously reported

SA305

See Friday Plenary number F305.

SA306

Testing and Validation of Simple Clinical Risk Indices to Identify Postmenopausal Women with Osteoporosis. M. C. Hochberg,[1] D. E. Thompson,[2] P. D. Ross.[2] [1]University of Maryland, Baltimore, MD, USA, [2]Merck Research Labs, Rahway, NJ, USA.

Several risk assessment tools have been developed for identifying postmenopausal women with osteoporosis, as defined by low bone mineral density (BMD), with the goal of targeting BMD measurements and reducing the number of tests and related health care costs. These tools include the Osteoporosis Self-assessment Tool for Asians (OSTA), SOFSURF, ORAI, and the Simple Calculated Osteoporosis Risk Evaluation (SCORE). The objective of the current study was to evaluate the performance of these indices among Caucasian women ages 45-93 who were screened for eligibility for the Fracture Intervention Trial (FIT). After completing a detailed questionnaire, BMD was measured. There were 17,572 women with age, weight, and BMD data; 3637 (21%) of these women had osteoporosis, as defined by a femoral neck BMD T score ≤ -2.5. Among the selected Caucasian women screened for FIT, at least 3 of the risk tools yielded reasonably good (>45%) specificity when sensitivity was set to approximately 90% for diagnosing osteoporosis (Table); similar results were obtained using T ≤-2.0 (data not shown). Likelihood ratios (LR) are provided for calculating the post-test odds (equal to LR*pre-test odds). Three risk categories have been previously reported using the OSTA index. A high risk subgroup was identified (OSTA score < -3, 1309 [7%] of women) that had a high prevalence of osteoporosis (59%). The prevalence of osteoporosis was 26% in a medium risk group (OSTA = -3 to 0, 9474 [54%] of women), and only 6% in a low risk group (OSTA > 0,

6789 [39%] of women). These results are very similar to those reported in the original Asian sample (Osteoporos Int 2000;11[Suppl 5]:S9), except that the risk category cutoffs were shifted by one unit among Caucasians compared to Asians. While the women who were screened for FIT may not be representative of the general population as they were selected on certain characteristics, the performance of these risk tools was similar to that in the original development populations. We conclude that these free and simple risk assessment tools could help clinicians target BMD testing among postmenopausal women.

Index	Cutoff	Sensitivity	Specificity	LR (+)	LR (-)
OSTA	<1 vs ≥1	89	46	1.65	0.24
ORAI	>10 vs ≤10	92	26	1.24	0.31
SOFSURF	>1 vs ≤1	82	55	1.82	0.33
SCORE	>10 vs ≤10	88	47	1.66	0.26

Disclosures: Merck & Co., Inc,2,8; Aventis,2,5; Eli Lilly,5; Proctor & Gamble,5.

SA307

See Friday Plenary number F307.

SA308

Effect of Smoking and Alcohol on Quantitative Ultrasound Parameters in the Elderly. R. J. Barr,*[1] A. Stewart,*[1] B. McDonagh,*[2] D. J. Torgerson,*[3] D. M. Reid.*[1] [1]University of Aberdeen, Aberdeen, United Kingdom, [2]Strakan Ltd, Galashiels, United Kingdom, [3]University of York, York, United Kingdom.

Background: Quantitative ultrasound assessment (QUS) of bone predicts hip fractures in elderly women. Few studies have investigated the relationship between lifestyle factors, such as smoking and alcohol consumption in elderly women and QUS parameters. Methods: This study investigated the association between smoking and alcohol consumption with the calcaneal QUS parameters broadband ultrasound attenuation (BUA) and speed of sound (SOS) in 712 women aged 70-98 years. Information about smoking habit and alcohol consumption were collected by questionnaire. BUA (dB/MHz) and SOS (m/s) were determined in the left heel using a McCue CUBA clinical system. Weight and height were also measured. Results: BUA was significantly associated with weight, height and age ($r=0.36$ $p<0.001$, $r=0.19$ $p<0.001$ and $r=-0.18$ $p<0.001$). SOS was significantly associated with age ($r=-0.112$, $p=0.003$) but not with weight and height. The BUA (mean±SD) of smokers was significantly lower than never and ex smokers (51.3±14.1, 56.7±16.2 and 56.3± 15.4; p=0.009 and p=0.019 unadjusted). However after adjustment for age, weight and height the BUA of smokers was significantly lower than the never smokers but not the ex smokers (p=0.003 and p=0.082). The SOS (mean±SD) of smokers, ex smokers and never smokers was 1581.6±48.7, 1577.7±48.6 and 1578.7±48.1m/s respectively, with none of the differences significant. Women who consumed alcohol 3-4 times a week had a significantly higher BUA (mean±SD) than women who consumed alcohol every day or less than 3-4 times a week (62.5±16.4, 53.4±16.5 and 55.9±15.6 dB/MHz; p=0.007 and p=0.01 unadjusted). After adjustment for age, weight and height women who consumed alcohol 3-4 times a week still had a significantly higher BUA than those who consumed alcohol every day (p=0.046) but not than those women who consumed alcohol less than 3-4 times a week (p=0.237). The SOS (mean±SD) of women who consume alcohol every day, 3-4 times a week and less than 3-4 times a week was 1585.4±54.3, 1573.7±42.4 and 1580.5±48.6m/s respectively. No significant difference was observed between the groups.Discussion: BUA, but not SOS, was linked to both smoking and alcohol consumption in elderly women. Interestingly the data on alcohol reflect BMD findings in a group of peri-menopausal women from Aberdeen1. Further work is needed to establish whether moderate alcohol consumption is related to a reduced incidence of hip fracture in elderly women.References: 1MacDonald HM et al., 2000. Nutritional influences on peri- and early menopausal bone loss: effects of alcohol and calcium intake. Osteoporos. Int. 11,(Suppl 1) S19.

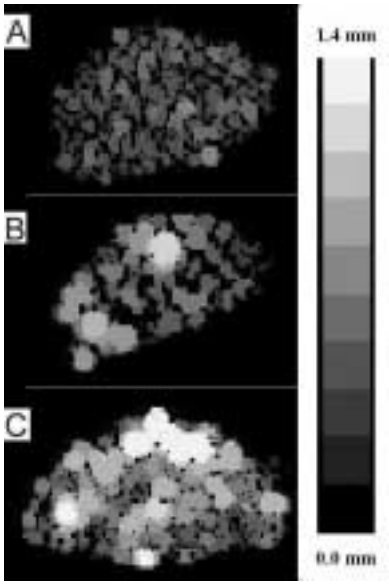
SA309

See Friday Plenary number F309.

SA310

Magnetic Resonance Image Analysis of Trabecular Bone Structure with Distance Transformation Maps. K. J. Hall,* A. Laib,* D. Newitt,* S. Majumdar. Radiology, University of California, San Francisco, CA, USA.

MR imaging of trabecular bone shows promise for predicting fracture risk, yet it has limited spatial resolution and signal-to-noise ratio. Laib et al (ASBMR 2000) presented a new image analysis technique in which characteristic distances in bone and bone marrow spaces are directly determined by Euclidean distance transformation. In this work, this technique is used to produce three-dimensional distance transformation maps of trabecular bone parameters Tb.Th (thickness), Tb.Sp (separation), and Tb.N (number). Two patient groups were analyzed: a mixed group of normal and osteopenic patients, and a group of patients with vertebral fractures. Qualitative analysis of these distance maps highlights differences among the normal, osteopenic, and fractured patients. The distance maps in the figure show the distance between the surfaces of neighboring trabeculae (Tb.Sp) of an axial slice from the distal radius. Lighter gray-levels represent areas of larger trabecular separation. The size of a sphere also yields information about the homogeneity of the local trabecular structure. Images whose distance maps have similarly-sized spheres have a more homogeneous structure than those whose distance maps are made up of a variety of sphere sizes. Normal subjects (A) typically have distance maps with even-sized spheres and a fairly homogeneous distribution of brightness, while osteopenic subjects (B) show more inhomogeneity in their distance maps. Subjects with vertebral fractures (C) have even more inhomogeneous distance maps that typically show greater separation between the trabeculae with increasing distance from the distal endplate. Valuable internal structural information can be extracted from the homogeneity of the distance maps, making their analysis a promising tool for studying trabecular bone structure. Further investigations will examine the distance maps for patterns in the radius and for assessing response to therapy and changes with age.



SA311

See Friday Plenary number F311.

SA312

Magnesium Deficiency: Effect on Bone & Mineral Metabolism in the Mouse. R. K. Rude,[1] H. E. Gruber,*[2] L. Y. Wei,*[1] A. Frausto,*[1] B. G. Mills.*[1] [1]University of Southern California and Orthopaedic Hospital, Los Angeles, CA, USA, [2]Carolinas Medical Center, Charlotte, NC, USA.

Insufficient dietary magnesium (Mg) intake has been associated with low bone mass and/or increased bone loss in humans and in animals. The purpose of this study was to explore the hypothesis that inflammatory cytokines may contribute to alterations in mineral metabolism and to the Mg deficiency-induced bone loss in the mouse. BALB/c female mice were fed either a normal (.06%) or low (.002%) Mg diet for up to 6 weeks. Serum Mg, Ca and PTH, and bone mineral (Mg,Ca,PO4) content were determined. Histomorphometry was used to quantitate the effect on bone cells and immunohistochemical staining was used to localize the effect of substance P, TNFa, and IL1. Hypomagnesemia developed and skeletal Mg content fell significantly in Mg deficient mice within 3 weeks. Serum Ca in the Mg deficient mice was higher than in the control mice although serum PTH was not significantly different. Osteoprotegerin (OPG) inhibited osteoclastic bone resorption but did not prevent hypercalcemia in Mg deficient animals. However concomitant 50% reduction of dietary Ca prevented hypercalcemia suggesting that compensatory intestinal absorption accounted for the hypercalcemia. The growth plate width decreased by 33% in Mg deficient animals and the chondrocyte columns decreased in number and length. There was an increase of 135% in osteoclast number. The result was a decrease in trabecular bone volume in the metaphysis of the tibia. Osteoblast number was significantly reduced. Immunohistochemistry of cells in the medullary spaces revealed that substance P increased 227% and 200% in the megakaryocytes and lymphocytes respectively after 1 day of Mg depletion. IL-1 increased by 143% in osteoclasts by day 3, while TNFa increased in osteoclasts by 123% and by 500% in megakaryocytes at day 12. This study demonstrates a profound effect of Mg depletion on bone characterized by impaired bone growth, decreased osteoblast number, increased osteoclast number and loss of trabecular bone. Increased inflammatory cytokines in bone of Mg deficient animals may provide an explanation of increased osteoclastic bone resorption accompanying Mg depletion.

Disclosures: Blaine Pharmaceuticals,5.

SA313

See Friday Plenary number F313.

SA314

Short-Term, High Dose Vitamin A Does Not Affect the Skeleton. T. N. Kawahara,*[1] D. C. Krueger,[1] J. A. Engelke,*[2] J. M. Harke,[1] N. C. Binkley.[1] [1]Institute on Aging, University of Wisconsin, Madison, WI, USA, [2]Biochemistry, University of Wisconsin, Madison, WI, USA.

Excess vitamin A produces skeletal toxicity. While studies are limited, currently available data in humans and animals indicates that excess vitamin A stimulates bone resorption and inhibits bone formation. Although the animal studies have utilized extremely high doses, it has been suggested that unappreciated human subclinical vitamin A toxicity may be associated with high skeletal turnover and bone loss. If this is the case, vitamin A supplementation could be a heretofore unappreciated osteoporosis risk factor. To further evaluate this possibility, a prospective, randomized, single-blind pilot study was conducted in 80 healthy men age 18-50 utilizing the highest vitamin A supplement dose available over the counter. One half of study participants received 25,000 IU of retinol daily with their evening meal, the others received placebo. Screening laboratory parameters including complete blood count and serum chemistry panel were required to be normal for enrollment. Volunteers with a medical history of malabsorption, renal or hepatic disease were excluded from study participation. Fasting serum specimens were collected between 0730-1030 at baseline and following two, four and six weeks of supplementation. Serum bone specific alkaline phosphatase (BSAP) and n-telopeptides of type I collagen (NTx) were measured at all timepoints. At this time, 29 men (15 control/14 vitamin A supplemented, mean age 29 ± 1.4 years) have completed the study.Supplementation was well tolerated. Compliance was excellent (90%) with no difference between groups. At baseline, BSAP and NTx were higher ($p < .05$) in the placebo group. No differences in BSAP or NTx were demonstrated between the supplemented and placebo groups over the course of the study (Table 1).

Table 1: Markers of bone turnover

	Baseline	2 weeks	4 weeks	6 weeks
BSAP (U/L) Vitamin A	24.0 (1.6)	24.1 (1.5)	24.3 (1.6)	25.1 (2.3)
BSAP (U/L) Placebo	31.9 (3.0)	30.7 (2.9)	30.6 (2.7)	32.0 (2.4)
NTx (nM BCE) Vitamin A	17.1 (1.4)	16.6 (1.3)	15.4 (1.4)	16.5 (1.3)
NTx (nM BCE) Placebo	23.4 (1.6)	22.7 (1.8)	21.8 (2.2)	25.0 (1.8)

Data as mean (SEM)

In conclusion, short-term vitamin A supplementation, in a dose above the recently published tolerable upper intake level, but available over the counter, does not alter serum markers of skeletal turnover. Whether long term supplementation might have adverse skeletal effects remains to be determined

SA315

Potassium Citrate and Bone Metabolism in Rats. M. Horcajada-Molteni,*[1] B. Chanteranne,*[1] M. Davicco,*[1] V. Coxam,[1] S. Miller,[2] J. Barlet,*[1] C. Rémésy.*[1] [1]INRA, Theix, France, [2]Radiobiology Division, University of Utah, Salt Lake City, UT, USA.

The role of nutritional influences on bone metabolism remains undefined because most studies have focused attention on calcium intake. Consumption of nutrients found in abundance in fruits and vegetables is positively associated with bone health. Indeed, fruits and vegetables contribute to an alkaline environment due to their organic salts content (mainly potassium citrate). This study was thus undertaken to probe the hypothesis that potassium citrate, and then a high K/Na ratio in diet (as in fruits and vegetables) may influences ovariectomy-induced bone loss in rats. The experiment was carried out on forty 3-month old Wistar rats. Twenty of them were ovariectomized (OVX), while the remainings were sham-operated (SH). Among the OVX, ten were fed for 3 months after surgery (day 0) with the same diet (0.4% calcium ; 0.8% phosphorus ; K/Na = 2) as ten SH (10TSH, 10 TOVX). The twenty other rats (10 KSH, 10 KOVX) received the same diet in which K/Na = 14 ; K being given as citrate ($K_3C_6H_5O_7$, H_2O). The OVX rats were pair-fed to the SH. Urine of each animal was collected over 24h-periods from day 83 until day 89. At necropsy, on day 90, blood and femurs were collected. At the end of the experiment, the decrease in uterine weight was not different in TOVX and KOVX. On day 89, urinary pH was significantly increased by citrate potassium intake (KSH : 6.64 ± 0.06 ; $p<0.01$ vs TSH ; KOVX : 6.77 ± 0.06 ; $p<0.01$ vs TOVX). The lowest calciurias were observed in KSH and KOVX groups. Ovariectomy induced a significant decrease in femoral failure load (N) which was not prevented by potassium citrate consumption. In the opposite, total (T-BMD) (TSH : 0.2264 ± 0.0055 ; TOVX : 0.2142 ± 0.0025 ; $p<0.05$) and distal metaphyseal (M-BMD) (TSH : 0.2307 ± 0.0027 ; TOVX : 0.2163 ± 0.0032 ; $p<0.05$) femoral density ($g.cm^{-2}$) were significantly decreased by ovariectomy, this decreased being prevented by potassium citrate intake [(T-BMD : KSH : 0.2358 ± 0.0023 ; KOVX : 0.2281 ± 0.0025) ; (M-BMD : KSH : 0.2491 ± 0.041 ; KOVX : 0.2305 ± 0.0012)]. Moreover, on day 90, urinary deoxypyridinolin excretion (nM DPD.mmol creatinin-1) was higher in TOVX (164.8 ± 13) and TSH (121.9 ± 8.8) than in KSH (93.6 ± 10.3). In conclusion, this study allows the concept that organic salts with alcalinizing effects of the diet play a positive role in bone metabolism.

Disclosures: Aprifel, France,2.

SA316

Do the Patients with Chronic Atrophic Gastritis Have Secondary Hyperparathyroidism? **C.** Lee,[1] Y. Jeon,[1] I. Park,[2] W. Choi.[3] [1]Department of Internal Medicine, Hanyang University Kuri Hospital, Kuri, Republic of Korea, [2]Department of Clinical Pathology, Hanyang University Kuri Hospital, Kuri, Republic of Korea, [3]Department of Internal Medicine, Hanyang University Seoul Hospital, Seoul, Republic of Korea.

It is well known that Hydrochloric acid is important factor for the absorption of calcium and the bone loss is often seen in human after total gastrectomy. Chronic atrophic gastritis causes a decrement of acid secretion of gastric mucosa. The aim of our study was to document the change of calcium metabolism in the patients with decreased gastric acid secretion. The study sample consisted of the gastritis group which had 19 post-menopausal females (age: 58.5 ±6.1 yr) followed in our hospital for chronic atrophic gastritis, and the control group which had 22 healthy females (age: 59.9 ±7.5 yr) with normal gastrofiberscopic findings. Serum levels of calcium, phosphorus, total alkaline phosphatase and intact PTH(IRMA, Nicol inc), were monitored in all the patients in both group before medication. There were no significant differences of calcium (9.1 ±0.9 vs 9.0 ±1.4 mg/dL, $p > 0.05$), phosphorus (3.5 ±1.1 vs 3.6 ±0.9 mg/dL, $p > 0.05$), alkaline phosphatase (60.5 ±34.6 vs 59.2 ±20.4 U/L, $p > 0.05$) between the gastritis group and control group. Serum intact PTH showed increased average value of the gastritis group comparing the control group (21.3 ±6.7 vs 17.0 ±3.7 pg/ml, $p = 0.09$). In conclusion, in patients who have decreased capacity of gastric hydrochloric acid secretion, there may be a possibility of abnormal calcium and bone mineral metabolism.

SA317

Vitamin K Depletion and Repletion in Postmenopausal Women Does Not Alter Biomarker of Bone Metabolism. L. Martini, S. Booth, E. Saltzman, R. J. Wood.* Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts Univeristy, Boston, MA, USA.

Vitamin K has been implicated in bone metabolism. Low dietary vitamin K intake and status has been associated in epidemiological studies with low bone mineral density and increased risk of hip fractures. The only known biochemical role of vitamin K is as a cofactor in an enzymatic reaction that post-translationally carboxylates specific glutamine residues in a small number of vitamin K-dependent proteins. Despite a potential role of vitamin K in osteoporosis, little is known about the effects of vitamin K status on the underlying components of bone metabolism. The aim of the present study was to assess the effects of vitamin K depletion and repletion on biomarkers of bone formation (serum osteocalcin) and bone resorption (serum and urinary type 1 collagen cross-link N-telopeptide (NTX)). Fifteen postmenopausal women participated in a 3-month in-house study that consisted of five dietary phases during which they were fed a low vitamin K depletion diet fortified with varying levels of vitamin K: 2-week “baseline” (65 mcg vitamin K/d) 4-week “depletion” (10 mcg vitamin K/d), 2-week “repletion –1” (65 mcg vitamin K/d), 2-week “repletion-2” (200 mcg vitamin K/d) and 2-week “repletion – 3” (500 mcg vitamin K/d). In addition, to test a possible interaction of vitamin K status with vitamin D, one-half of the subjects were randomly assigned to receive calcitriol (1,25 dihydroxyvitamin D3) supplementation (1 mcg/d) during the last 7d of baseline, depletion and repletion-3 dietary phases. Vitamin K depletion and repletion status was monitored by measurements of serum PIVKA-II, a measure of undercarboxylated prothrombin that increases in vitamin K deficiency states. There was a significant increase ($p<0.05$) in PIVKA-II during the dietary vitamin K depletion phase (1.5 ± 1 (baseline, mean±SD); 2.5 ±1.8 (depletion); 2.2 ± 1.7 (repletion-1); 1.7 ± 1.0 (repletion-2) and 1.0 ± 0.7 (repletion-3) ng/ml) confirming a functional vitamin K depletion state. However, there was no changes in bone metabolism during the study. Serum osteocalcin, a biomarker of bone formation, was similar in all dietary periods: 7.0 ± 2.0, 7.7± 2.7, 7.2 ±, 7.3 ± 1.5, 7.3 ± 2.0. Likewise, serum and urinary NTX, measures of bone resorption activity, were not affected by dietary vitamin K depletion: serum NTX (18 ± 3, 19 ± 4, 19 ± 4, 19 ± 5, 20 ± 5, 18 ± 3 nM BCE); urinary NTX (48 ± 14; 48 ± 15; 47 ± 13; 53 ± 15 and 44 ± 10 nM BCE/nM Cr). These findings suggest that short-term consumption of a low vitamin K diet can cause a functionally significant change in vitamin K status (increased PIVKA-II), but does not alter biomarkers of bone formation or resorption in elderly women.

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Caloric Restriction Decreases Calcium Absorption in Mature Obese and Lean Rats. M. Cifuentes,* A. B. Morano,* H. Chowdhury,* S. A. Shapses.* Rutgers University, New Brunswick, NJ, USA.

Weight loss has been associated with loss of bone mass, however the mechanisms involved remain unclear. We hypothesize that weight loss reduces estrogen activity and alters intestinal Ca absorption thereby contributing to the negative effect on bone. Lean subjects who lose weight may be at greater risk of bone loss than heavier individuals. We investigated the effects of body weight (wt) and caloric restriction on Ca metabolism in mature female rats. Rats (n=56) were fed either a high fat (47%) or low fat (16%) diet to generate obese and lean animals, respectively. At 6 months of age, lean and obese groups were each divided into controls (CTL; fed ad-libitum) and calorie restricted (CR; at 40% restriction, matched for vitamins and minerals) to total 4 groups. Markers of bone turnover were measured before and after 10 weeks of CR or CTL diets. Traditional Ca balance method estimated net Ca absorption, whereas true Ca absorption, endogenous fecal Ca and intestinal Ca secretion also included the use of Ca45 radioisotopes (1). Uterine wt was measured, as a bioassay for estrogenic activity. At baseline, there were no differences between obese and lean rats in Ca balance, true absorption, endogenous fecal Ca or intestinal Ca secretion. Net Ca absorption, however, was slightly higher in obese compared with lean rats (33.6 6.5% and 29.1 7.0%, $p<0.05$). Lean rats presented higher serum osteocalcin levels and lower 24-h pyridinium crosslink excretion than obese rats ($p<0.001$). Caloric

restriction reduced body wt by -24.2 7.6% compared to CTL (+7.2 7.3%; p< 0.001) and decreased true intestinal Ca absorption (-23.7 16.3%) compared to CTL animals (-9.6 23.8%), regardless of body size (p 0.77 , p < 0.001), net absorption was not able to detect the effect of CR. Intestinal Ca secretion and endogenous fecal Ca also decreased due to CR (-29.3 28.5% and -15.4 29.5%, respectively; p<0.05). Uterine wts were lower in CR (249.9 0.1mg) than CTL rats (516.8 0.1mg, p< 0.0001), and these differences were greater in lean than obese rats (p<0.01). These data show that CR decreases true Ca absorption and may contribute to bone loss due to weight reduction. This may be due to a relative estrogen deficiency. The greater loss of estrogen activity, but not Ca absorption, in lean than obese animals suggests that other factors may be regulating Ca absorption during CR. In addition, lean individuals who lose weight may be more susceptible to bone loss than obese due to a greater loss of estrogen. 1. O'Loughlin PD & Morris HA. J Nutr 124:726-31, 1994.

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Cancellous Bone Loss in Adult Rats Following Reduction in Chow Calcium and Vitamin D Contents to NRC-Recommended Levels. M. R. Allen, A. C. Currado,* S. A. Bloomfield. Health & Kinesiology, Texas A&M University, College Station, TX, USA.

In two separate studies, we have found evidence that alterations of rodent chow calcium and Vitamin D content, even if meeting the NRC-recommended levels, can lead to significant decreases in cancellous bone mineral density (cBMD) in skeletally mature male Sprague-Dawley rats. Animals for both experiments were fed a standard chow diet (Harlan Teklad Global Diet #2018) by the vendor until animals were shipped (5-months-old). After 1 wk acclimation, rats were then acclimated to their new diet for an additional 14 d prior to the start of each study. In Study 1, male rats were fed a Vitamin D-deficient diet (D-; n=4) (Purina Mills Test Diet) while in Study 2, male rats were fed AIN93-M chow (AIN; n=9) (Dyet) designed to provide adequate nutritional intake for adult maintenance (Reeves et al., J. Nutr., 1993). Both diets met the minimal daily requirement for calcium as defined by the NRC; however, these calcium contents were ~50% lower than the diet the animals were raised on. All animals were housed singly and allowed to eat ad lib. On day 0 of each study, baseline data were collected using in-vivo peripheral quantitative computed tomography (pQCT; Stratec Research-M) at the tibia proximal metaphysis (3 slices centered at 5.5mm from tibial plateau) and mid-diaphyseal shaft (1 slice at 50% total bone length). After 28d of normal cage activity, pQCT scans were repeated. Study 1: Over 28d, D- animals gained body mass (28 ± 7g) and consumed 24 ± 1.2 g of food/day over the final week of the study. In-vivo pQCT showed a significant (p < 0.05) loss (-21%) of cBMD over 28d (pre: 229 ± 12; post: 179 ± 7 mg/cm3) at the proximal tibia. There were also significant decreases in total BMD (-7%), while total area (+6%) and marrow area (+11%) both increased at the proximal tibia. There were no significant changes at the mid-diaphysis in D- animals over 28d. Study 2: AIN-fed animals gained body mass over 28d (32 ± 6g) and consumed 21 ± 1.1 g of food/day during the final week of the study. In-vivo pQCT showed a significant decline (-18%) in cBMD at the proximal tibia over 28d (pre: 198 ± 6.0; post: 162 + 5.7 mg/cm3). However, there were no significant changes in proximal tibia total BMD or area, nor in any variables at the tibial mid-diaphysis. Given similar losses in proximal tibia cBMD on both the D- and AIN diets, it appears that reduced calcium intake and not reduced Vitamin D-3 intake was the key factor affecting cBMD. Reducing exogenous D-3 intake did appear to impact on bone geometry at the proximal tibia. Possible confounders in these studies might be 1) reduced physical activity associated with single housing of rats and 2) alterations in the Ca:P molar ratio. Further studies are necessary to better control for these factors.

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Cortical Bone Parameters for the Characterization of Asthmatic Patients with Glucocorticoid-Induced Vertebral Fractures. H. Tsugeno,*[1] B. Goto,*[2] T. Fujita,*[2] T. Sugishita,[3] T. Yokoi,*[1] S. Takata,*[1] M. Okamoto,*[1] K. Nishida,*[1] Y. Hosaki,*[1] K. Ashida,*[1] F. Mitsunobu,*[1] Y. Tanizaki,*[1] [1]Misasa Medical Branch, Okayama University Medical School, Tottori, Japan, [2]Calcium Research Institute, Kishiwada, Japan, [3]Third Division, **Dept of Medicine, Kobe University School of Medicine, Kobe, Japan.**

Despite a deepening understanding of the trabecular bone dynamics, little is known about glucocorticoids (GC)-induced cortical bone loss and fractures. Using peripheral quantitative computed tomography (pQCT), we have recently demonstrated oral GC-induced fall in cortical bone volume and density of adult asthmatic patients. For further study of the relationship between cortical bone loss and fractures, we examined the pQCT parameters and the presence of vertebral fractures in 83 postmenopausal (> 5 years after menopause) asthmatic patients on high-dose oral GC (> 10g of cumulative oral predonisolone) (oral GC group) and 194 age-matched controls. Cortical and trabecular bone was measured using pQCT (Stratec XCT960). Relative cortical volume was obtained by dividing the cortical area by the total bone area. Strength Strain Index (SSI) was calculated in the radius based on the density distribution around the axis. Spinal fracture was assessed on lateral radiographs. Fifty-four patients (65.1%) in oral GC group and 45 (23.2%) in the control group had at least 1 vertebral fracture. High-dose oral GC (> 10g of cumulative oral predonisolone) increased the risk of fracture (odds ratio, 8.64; 95% C.I., 4.11-18.1). In each group, diagnostic and predictive ability of vertebral fractures was determined by areas under the receiver operating characteristic (ROC) curves of the parameters. All parameters were significant predictor (p < 0.0001) in the control patients. Cortical bone mineral density (BMD) (p = 0.003), relative cortical volume (p < 0.0001) and SSI (p = 0.001) were significant predictors, but trabecular BMD was not (p = 0.242) in the oral GC group. Thresholds of all parameters for vertebral fractures were also calculated by Chi-square test, 90 percentile, 95% confidence interval and ROC analysis, and compared between the two groups. Although a rise of fracture threshold in GC group was suggested, variations depending on the method of calculation precluded a definite conclusion.We confirmed an outstanding risk of fracture on high-dose oral GC administration. Cortical bone parameters obtained by pQCT were good predictors of glucocorticoid-induced vertebral fractures.

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Osteodensitometric, Biochemical and Histomorphometric Parameters of Bone after Longterm Corticosteroid Therapy. T. Eidner,* G. Lehmann,* G. E. Hein.* Dpt. of Internal Medicine IV, Friedrich-Schiller-University, Jena, Germany.

We investigated the influence of longterm corticosteroid therapy on osteodensitometric, biochemical and histomorphometric parameters of bone in postmenopausal women. Bone mineral density (BMD; DXA, QDR Hologic 4500A) at lumbar spine (LS) and femoral neck (FN), biochemical markers of bone turnover (serum osteocalcin - OC, urinary excretion of pyridinolin - Pyd and desoxypyridinolin - DPyd) and bone histomorphometric parameters from iliac crest biopsies were determined in 42 postmenopausal women. 25 patients (group A, Æ 62,6 y.) had primary osteoporosis. 17 patients (group B, Æ 63,0 y.) had longterm corticosteroid treatment (at least 7,5 mg prednisolon per day for more than one year) due to inflammatory rheumatic diseases (13), COPD (2) or chronic inflammatory liver diseases (2).

DXA, Bioch.	BMD LS	BMD FN	OC	Pyd	DPyd
	g/cm²	g/cm²	ng/ml	nmol/mmolKrea	
group A	0,73±0,12	0,65±0,09	8,1±2,4	54,7±29,7	20,8±8,2
group B	0,72±0,18	0,58±0,08	5,8±2,9	79,5±41,6	21,3±9,0
p (u-test)	n.s.	0,03	0,02	(0,06)	n.s.
ref. range	1,04±0,22	0,88±0,20	8,5±3,5	40±10	10±3,5

Histomorphometry	BV	OS	Ob.S	ES	Oc.S	N.Oc	BFR
	% BS	% BS	% BS	% BS	% BS	/mm²	µm³/µm²*d
group A	14,8±4,8	9,8±8,1	3,8±2,8	14,3±6,0	4,4±4,2	0,50±0,49	0,081±0,084
group B	13,4±4,1	11,9±9,9	3,4±3,5	15,5±12,7	2,3±2,2	0,25±0,22	0,058±0,029
p (u-test)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

(BV bone vol., BS bone surf., OS osteoid surf., Ob.S osteoblast covered surf., ES erosion surf., Oc.S osteoclast covered surf., N.Oc number of osteoclasts, BFR bone formation rate, n.s. not significant)

In patients with longterm corticosteroid therapy (group B) we found a significantly lower BMD at FN (despite a comparable BMD at LS), a significantly lower OC-level as sign of reduced bone formation, an elevated Pyd-level as sign of increased bone and additional cartilage resorption (predominantly inflammatory rheumatic diseases) as well as a comparably elevated DPyd-level pointing towards increased bone resorption in both groups. Bone histomorphometry did not show any significant differences between both groups, but there was a tendency towards a lower bone volume, a lower bone formation rate and a lower osteoclast surface under corticosteroids. We conclude, that increased bone resorption can be found in postmenopausal women with osteoporosis relatively independent of longterm corticosteroid therapy, but there seems to be an additional inhibitory effect of corticosteroids on bone formation.

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Prednisolone Administration Ameliorates the Increased Osteoclastogenesis But Aggravates the Loss of Osteocytes and Bone Strength That Follow Orchidectomy. R. S. Weinstein, C. C. Powers,* R. D. Landes,* A. M. Parfitt, S. C. Manolagas. Div. of Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Glucocorticoid excess causes decreased bone turnover and a profound depression in the numbers of both osteoblasts and osteoclasts due to inhibition of both osteoblastogenesis and osteoclastogenesis as well as to increased apoptosis of osteoblasts. Sex steroid deficiency also causes osteoblast and osteocyte apoptosis but, in contrast to glucocorticoids, results in upregulation of osteoblastogenesis, osteoclastogenesis and bone turnover. The two disorders could either aggravate or ameliorate each other. We used established murine models of glucocorticoid-induced osteoporosis and of androgen deficiency to examine the interactions of these conditions. Prednisolone or placebo was administered to 5-mo-old Swiss Webster mice for 28 days after sham operation or orchidectomy. Serial bone densitometry determinations revealed that bone loss was more rapid with prednisolone than after orchidectomy but, after 28 days, the amount of bone loss was similar in the orchidectomy and orchidectomy plus prednisolone groups. Vertebral compression strength was, however, less when both conditions were present. In the orchidectomized animals receiving prednisolone, the expected increases in CFU-F and in osteoclastogenesis were attenuated, as

determined in ex-vivo bone marrow cell cultures. Likewise, the orchidectomized animals receiving prednisolone failed to show the expected increase in cancellous osteoblasts and bone turnover. In addition, the prevalence of osteoblast apoptosis was less after orchidectomy plus prednisolone than after orchidectomy alone due to the decline in bone turnover. In contrast, *osteocyte* apoptosis was greatest when both conditions were present. These data demonstrate that glucocorticoid excess suppresses osteoclastogenesis in the marrow and the number of osteoclasts in cancellous bone, even in the face of the potent counter regulatory signals generated by orchidectomy. Moreover, they strongly suggest that changes in osteoblast and osteocyte apoptosis in glucocorticoid excess have graver consequences for bone homeostasis than the suppression of the rate of bone turnover. This latter contention is consistent with the evidence that whereas both bisphosphonates and glucocorticoids suppress turnover in sex steroid deficiency, the former increases while the latter, as shown here, further decreases bone strength–perhaps due to the combined effect of glucocorticoid excess and loss of androgens on osteocyte apoptosis.

SA324

Pre-receptor Activation of Glucocorticoids in Osteoblasts Increases With Age and Steroid Therapy. M. S. Cooper, E. R. Rabbitt,* P. Goddard,* P. M. Stewart,* M. Hewison. Medicine, University of Birmingham, Birmingham, United Kingdom.

The risk of glucocorticoid-induced osteoporosis increases substantially with age but is subject to considerable individual variation. We have proposed that this may be due to pre-receptor regulation of glucocorticoid responsiveness by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD1): human osteoblastic cells (hOB) express 11β-HSD1 and synthesis of active cortisol by these cells is sensitively stimulated by inflammatory cytokines. In data presented here we have used a large number of primary hOB cultures to assess the effects of age on 11β-HSD expression and activity at different bone sites. In addition we show that prednisone is a target for 11β-HSD1 and that 'pre-receptor' activation of glucocorticoids via this enzyme is, in turn, induced by therapeutic steroids. HOB cells were generated from collagenase-digested trabecular bone chips obtained from orthopaedic specimens. Basal 11β-HSD1 activity (synthesis of cortisol from cortisone) increased significantly with age: e.g. 1.2 pmols/hr/mg protein age 11; 6.5 pmols/hr/mg protein age 63 ($r=0.59$, $p<0.05$). 11β-HSD1 expression and activity was also increased following treatment with various naturally occurring and synthetic steroids (eg. dexamethasone 2.1-fold ± 0.3; cortisol 1.7-fold ± 0.1 both $p<0.01$; $n=12$). Similar increases in expression of mRNA for 11β-HSD1 were demonstrated using real-time PCR (3.6-fold with dexamethasone; 2.5-fold with cortisol, both $p<0.01$). hOB as well as cells transfected with 11β-HSD1 also demonstrated the ability to convert prednisone to prednisolone, emphasizing that hOB cells have the ability to 'concentrate' active steroid through a pre-receptor mechanism. The significance of this *in vivo* was assessed by measuring circulating levels of steroids in normal males before and after treatment with oral prednisolone (5mg). 0900 cortisone levels were 110 ± 5 nmol/L and prednisone peaked at 78 ± 23 nmol/L 120 min. after administration of prednisolone. Thus therapeutic use of steroids has the potential to increase substrate availability for 11β-HSD1 in bone. These studies confirm that glucocorticoid availability in bone is dependent on the expression of 11β-HSD1. We propose that pre-receptor activation of glucocorticoids plays an important role in age-related decrease in bone formation and increased risk of steroid-induced osteoporosis.

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Skeletal Effects of Ovariectomy in New Zealand White Rabbits. R. J. Colman,[1] M. Butz,*[2] N. C. Binkley.[2] [1]Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI, USA, [2]Institute on Aging, University of Wisconsin, Madison, WI, USA.

Animal model utilization has played a major role in understanding osteoporosis pathogenesis and development of effective treatments. Ideally, animal models for skeletal research would possess bone remodeling processes similar to adult humans, i.e., Haversian remodeling. This is not the case with current small animal models. However, laboratory rabbits possess Haversian remodeling and many other attributes of an outstanding model including ready availability at modest expense and ease of handling. Despite these advantages, it is not established that another important animal model criterion, bone loss following estrogen depletion, occurs in rabbits. To this end, we conducted a study evaluating the effects of ovariectomy (oxv) on bone mass in eleven, 5-month old, New Zealand white rabbits (Oryctolagus cuniculus). Animals were individually housed and fed standard laboratory chow ad libitum. Baseline study was followed by randomization to receive bilateral ovx (n=6) or sham (n=5) surgery. Following surgery, animals were assessed, utilizing acepromazine and ketamine, at 0.5, 1, 1.5, 3 and 6 months. Assessments included blood sampling and bone density measurement by dual-energy x-ray absorptiometry (DXA). DXA consisted of lumbar spine (L1-L7) scans utilizing a Lunar DPX-L densitometer (GE/Lunar, Madison WI) with pediatric software, and distal femur/proximal tibia scans utilizing a Lunar PIXImus densitometer. Longitudinal data was analyzed by repeated measures analysis of variance. Body weight increased in both groups over time ($p<0.0001$). In the ovx group, lumbar spine BMD initially decreased and did not fully recover, leading to an ovx-sham difference of ~9% over time ($p<0.05$). Lumbar spine BMD increased 9% in the sham group during this study. Modeling of distal femur data showed a strong correlation with body weight and a difference between sham and ovx in the reaction to surgery ($p<0.05$). Although these animals were hypercalcemic (range: 11.8-13.1 mg/dl) and hypercalciuric relative to humans, both serum calcium and phosphorus were stable through 30 days post surgery, indicating tight homeostatic regulation. In summary, our findings, while preliminary, indicate that five month old rabbits have not reached peak skeletal mass. However, the development of relative osteopenia demonstrates promise for a rabbit model of estrogen-depletion bone loss. With its relative ease of handling and cost-effectiveness, we believe rabbits may possess many of the characteristics of an ideal animal model for osteoporosis research and, due to their Haversian remodeling, may be particularly useful for assessment of skeletal anabolic agents.

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Evidence that Postmenopausal Women with Vertebral Fractures Due to Type I Osteoporosis Have Enhanced Responsiveness of Bone to Estrogen Deficiency. B. L. Riggs,[1] L. J. Melton, III,[1] E. J. Atkinson,*[1] W. M. O'Fallon,*[1] C. Dunstan,*[2] S. Khosla.[1] [1]Mayo Clinic and Mayo Foundation, Rochester, MN, USA, [2]Amgen Corporation, Thousand Oaks, CA, USA.

We have hypothesized that osteoporosis (OP) can be divided into type I and type II fracture syndromes (Am J Med 75:899, 1983). Type I osteoporosis occurs within 20 years after menopause and may be due to estrogen (E) deficiency plus some additional factor predisposing to excessive bone loss. One such factor might be a greater degree of sex steroid deficiency, which could not be excluded previously because assays were not sufficiently sensitive to measure low postmenopausal sex steroid levels accurately. Thus, we studied 40 women with typical high turnover type I postmenopausal osteoporosis and vertebral fractures and 40 normal postmenopausal women (controls) using new ultrasensitive assays with detection limits of 1 and 5 pg/ml for estradiol (E_2) and estrone (E_1), respectively, and 5 ng/dl for testosterone (T). Bone turnover was assessed by measuring serum osteocalcin (OC) and bone alkaline phosphatase (BAP) and 24-h excretion of urinary pyridinoline (PYD) and deoxypyridinoline (DPD).

Variable (mean ± SEM)	Controls	Type I OP	P (rank sum)
Age, yrs	64.8 ± 0.9	64.6 ± 0.8	NS
Yrs after menopause	16.7 ± 1.1	17.3 ± 1.4	NS
E_2, pg/ml	6.3 ± 0.8	6.1 ± 1.2	NS
E_1, pg/ml	15.9 ± 0.9	16.8 ± 2.2	NS
T, ng/dl	24.8 ± 1.6	27.6 ± 1.8	NS
OC, ng/ml	9.0 ± 0.3	10.2 ± 0.	<0.02
BAP, U/L	20.3 ± 1.0	30.4 ± 1.9	<0.001
PYD, nmol/mmol Cr	42.7 ± 2.1	55.5 ± 2.5	<0.001
DPD, nmol/mmol Cr	13.0 ± 0.6	18.8 ± 0.9	<0.001

Although levels of serum sex steroids were identical in both groups, bone turnover was increased by up to 50% in the women with type I osteoporosis. Moreover, compared with controls, the osteoporotic women had 13% lower ($P<0.05$) serum PTH and 53% higher ($P<0.01$) serum osteoprotegerin levels, which were likely compensatory responses to the increased bone turnover. Because many studies have shown that E replacement will normalize the elevated bone turnover in these patients, those postmenopausal women who develop type I osteoporosis may have a genetically-determined, increased responsiveness of bone to E deficiency that is evident in the presence of low E levels but is overcome by high E levels. This hypothesis is consistent with recent reports that postmenopausal women with polymorphisms of the E receptor genes have low bone density and fractures

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Changes in Activation Frequency are More Influential Than Changes in Focal Bone Balance in Predictive Models of BMD Response to Estrogen Depletion and Replacement. C. J. Hernandez,* G. S. Beaupré, R. Marcus, D. R. Carter. VA Palo Alto HCS, Palo Alto, CA, Stanford University, Stanford, CA, USA.

The decreases in bone mass associated with estrogen depletion and replacement are the result of changes in bone remodeling. Bone remodeling is performed by the coupled activity of groups of cells in basic multicellular units (BMUs). Although changes in BMU activity such as increased activation frequency and decreased focal bone balance have been associated with estrogen loss there are conflicting reports regarding how these changes are associated with estrogen depletion and how they may be reversed through estrogen replacement. In this study we use a computational model of BMU activity to determine the changes in BMU activity that are most consistent with clinical bone mineral density (BMD) measurements made during estrogen depletion and replacement. In this analysis bone remodeling in the lumbar spine is simulated. Changes in BMU activity are applied in response to changes in serum estradiol concentrations. Changes in estradiol levels are considered to cause changes in: 1) activation frequency; 2) focal bone balance or 3) both. The magnitude of changes in BMU activity are determined by parametrically fitting simulation results to the changes in BMD observed in patients experiencing menopause or starting hormone replacement therapy (Recker et al. JBMR 2000 15(10):1965-73). All three simulation methods were able to predict the changes in BMD caused by estrogen depletion at menopause. Only simulations that considered both changes in focal bone balance and activation frequency gave predictions that were consistent with the BMD changes observed after hormone replacement therapy. Simulations that were consistent with both estrogen depletion and hormone replacement had only small changes in focal bone balance, suggesting that modification of activation frequency is the primary change in BMU activity that occurs in response to estrogen depletion and replacement. Since BMD changes resulting from modification of activation frequency are reversible, our finding that activation frequency is most likely the primary change in BMU activity implies that most of the bone mass lost due to estrogen depletion could be recovered with the appropriate estrogen

replacement therapy (although concurrent bone loss from factors like disuse or nutritional deficiencies would not be returned). Knowing the changes in BMU activity caused by estrogen depletion and replacement may help identify ways to modify BMU activity in postmenopausal women so that the greatest increases in bone mass can be achieved.

SA329

Number of Years Since Menopause: Spontaneous Bone Loss Is Dependent but Response to HRT Is Independent. N. H. Bjarnason,* P. Alexandersen, C. Christiansen. Clinical Dept., Center for Clinical and Basic Research, Ballerup, Denmark.

We wished to study the influence of number of years since menopause (YSM) on spontaneous bone loss and response to HRT in postmenopausal women under consideration for HRT. For this purpose we used data from 274 women within 20 years after menopause participating in 2 placebo-controlled mono-center trials of sequential, continuous and interrupted HRT in traditional and low doses without calcium addition. Both cross-sectionally at baseline in the total cohort (n=274) and longitudinally in the placebo group(n=71), bone loss in untreated women was greatest closest to the menopause and declined thereafter (r=0.34, p<0.01 for lumbar spine and r=0.25, p<0.05 for the femoral neck), such that the loss was eliminated in the femoral neck and bone mass increased in the spine in women more than 10 years after menopause.

Associations between YSM categories and BMD in untreated women

Cross-sectional (n=274 untreated women at baseline; mean(std))

	YSM<=2	YSM>2 and <=4	YSM>4 and <=7	YSM>7 and <=10	YSM>10
BMDspine(g/cm2)	0.99(0.13)	0.94(0.13)	0.92(0.13)	0.91(0.13)	0.84(0.11)
BMDneck(g/cm2)	0.76(0.10)	0.73(0.11)	0.70(0.09)	0.68(0.08)	0.68(0.11)

Longitudinal (n=71 placebo-treated women; mean(sem))

	YSM<=2	YSM>2 and <=4	YSM>4 and <=7	YSM>7 and <=10	YSM>10
aBMDspine(%/yr)	-1.54(0.38)	-0.33(0.22)	-0.18(0.53)	-0.04(0.47)	0.39(0.60)
aBMDneck(%/yr)	-1.50(0.26)	-0.63(0.25)	-0.44(0.51)	-0.24(0.92)	-0.01(0.91)

In contrast, bone turnover (S-OC and S-CTX) in untreated women was consistently elevated all through menopause, both cross-sectionally and longitudinally. The association between bone mass and YSM was counteracted both by 1 and 2 mg estradiol sequentially or continuously combined with gestodene, by piperazine estrone sulphate in combination with interrupted norethisterone and by a continuous combination of 2 mg estradiol and 1 mg norethisterone acetate. The response to the various HRT regimens was also independent of baseline bone mass.In conclusion, whereas bone loss was significantly related to number of years since menopause in untreated women, all HRT regimens arrested bone loss regardless of number of years since menopause. In addition, this effect seemed to be independent of type of estrogen, type of progestin and regimen chosen. Interestingly, the increase in spinal bone mass in women more than 10 years after menopause can not - as it has previously been suggested - be contributed to calcium supplementation, since calcium was not administered in our study. The constant difference between bone resorption and formation markers may be an essential component to explain the exponential pattern of bone loss in untreated women, but further experiments are needed to characterise this function which involves several compartments and eliminations

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Bone Response to Estrogen Replacement in BRKO (Estrogen Receptor-β) Null Mice. M. A. Gentile, J. G. Seedor, G. A. Rodan, D. B. Kimmel. Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA, USA.

α and β isoforms of the estrogen receptor (ER) exist, but their role in bone has not been fully defined. This study examined the skeletal effects of 17-β-estradiol (E2) in ovariectomized (OVX) ERβ null (BRKO) mice, obtained by F2 crosses of ERβ(+/-) females with ERβ(-/-) males.BRKO and wild type (WT) mice were OVX or Sham-OVX at age 26 weeks. OVX groups were given 0, 0.02, 0.04, or 0.2mg/kg 3X/wk E2 in sesame oil for eight weeks. Uterine weight (UWt, mg) and bone mineral density of distal 20% of femur (DF-BMD, mg/cm2, pDXA Norland) were measured after necropsy. The five groups of each genotype (see Table, all N=~12) were compared by Kruskal-Wallis ANOVA with Student Neuman-Keuls post-hoc test.

		Wild Type		BRKO	
Surg	**E2**	**UWt**	**DF-BMD**	**UWt**	**DF-BMD**
Sham	0	115±36#	62±7*	99±41#	65±4#
OVX	0	22±6	57±4	21±4	59±6
OVX	0.02	72±16#	60±7*	99±29#	65±7#
OVX	0.04	86±23#	64±3#	89±40#	67±6#
OVX	0.2	123±46#	66±4#	101±29#	67±7#

Mean±SD; #greater than OVX/same genotype (P<.01); *(P<.08)

There were no significant differences in UWt or DF-BMD between BRKO/Sh and WT/Sh mice. OVX reduced UWt (P<.001) and DF-BMD (P<.04) in both genotypes. All doses of E2 protected equally against loss of UWt and DF-BMD in WT and BRKO mice. We conclude that in the absence of ERb: 1) OVX induces bone loss; and 2) OVX-induced bone loss is prevented by E2. The similar protective action of E2 against loss of uterine weight and bone in OVX BRKO and WT, suggests that ERb is not required for these effects in mice

SA332

Effects of Ovariectomy on Bone Histomorphometric Parameters in Old Cynomolgus Monkeys. J. Legrand,[1] A. Bécret,[2] C. Fisch,[2] P. Persil,*[2] M. Attia,*[2] R. Forster,[2] J. Claude.*[3] [1]Beaufour-Ipsen, Paris, France, [2]CIT, EVREUX, France, [3]Faculty of Pharmacy, Paris, France.

Mature female cynomolgus monkeys from Mauritius, approximately weighing 4 kg, were randomly allocated to two groups. Seven animals were ovariectomized (OVX) and thirteen were sham-operated (SHAM). Bone mineral density (BMD) of lumbar vertebrae was measured by DXA before and 15 months after surgery. At this latter time, biopsies of the iliac crest and rib were obtained after dual calcein labeling (13-day interval). The bone samples were cut, undecalcified, stained with Goldner stain or examined under fluorescence to quantify the bone static and dynamic endpoints of bone formation and resorption histomorphometric parameters, using a validated image analysis software (Biocom). Fifteen months after surgery, the BMD of SHAM and OVX animals were respectively identical (99 ± 1 %) and decreased (93 ± 1 %) when compared to the BMD measured before surgery. Trabeculae could only be found in 4/13 rib biopsies of SHAM animals and in 5/7 rib biopsies of OVX monkeys. Trabecular tissue was lacking in only two iliac crest biopsies of the 20 animals (1 SHAM and 1 OVX). Histomorphometric parameters measured on trabecular bone of iliac crest and on cortex of both iliac crest and rib are summarized in the table (mean ± SEM,*: parameter quantified at iliac crest).

Parameter	SHAM	OVX	Parameter
BV/TV (%)*	18.93 ± 2.74	12.52 ± 1.72	MS/BS (%)*
Tb. Th. (μm)*	119 ± 8	98 ± 2	MAR (μm/day)*
Tb. N. (mm^{-1})*	1.53 ± 0.17	1.27 ± 0.16	BFR ($\mu m^2/\mu m$/day)*
Tb. Sp. (μm)*	634 ± 95	742 ± 88	
ObS/BS (%)*	5.86 ± 0.76	5.62 ± 2.00	Ct. Por. (%)*
OS/BS (%)*	16.70 ± 2.17	27.70 ± 4.57	Ct. Por. (rib) (%)
ES/BS (%)*	4.86 ± 0.66	6.62 ± 1.24	

Histomorphometric parameters in SHAM animals were similar to previously reported values in iliac crest of mature (10-15 years) cynomolgus monkeys. Most parameters of bone resorption and bone formation were higher in OVX monkeys than in SHAM animals. This reflects that the ovariectomy-induced bone turnover is still ongoing 15 months after surgery, a fact that should be considered when using the old OVX cynomolgus monkey as a model of established human osteoporosis.

SA333

The Relation of Renal Calcium Excretion and Bone Mineral Metabolism in Korean Postmenopausal Women. K. Oh,*[1] S. Lee,[1] D. Lee,*[1] E. Oh,*[2] W. Lee,*[2] M. Kang.*[2] [1]Miz Medi Hospital, Seoul, Republic of Korea, [2]St. Mary's Hospital, The Catholic University Medical College, Seoul, Republic of Korea.

Although all postmenopausal women are estrogen-deficient, women with postmenopausal osteoporosis may have some another defects, that explain for their higher rates of bone resorption and greater bone loss, compared to postmenopausal women without osteoporosis. In this cross-sectional study, we tested the hypothesis that one of the defects is an impairment of renal calcium conservation. In 177 postmenopausal women and 88 premenopausal women, we measured 24-hour urinary calcium levels, serum FSH, Estradiol (E2), intact PTH levels and bone tunover markers (serum osteocalcin and urine deoxypyridinoline). Bone mineral density using DEXA was also measured.Twenty-seven percent (47/177) of Korean postmenopausal women had urinary calcium excretion exceeding 4 mg/kg per day. The postmenopausal women had higher (p<0.05) values for mean urinary calcium to creatinine ratio (U cal/cr ratio) of 0.26±0.12 mg/mg of creatinine vs. 0.22±0.13 mg/mg of creatinine and higher (p<0.001) mean serum calcium level of 9.2±0.3 mg/dL vs. 9.0±0.3 mg/dL than the premenopausal women. Significant positive correlations were observed between U cal/cr ratio and both turnover markers (for serum osteocalcin, r=0.72, p<0.001; for urine deoxypyridinoline, r=0.213, p<0.01). However, there was no correlation with serum FSH level, E2 level, or intact PTH level. Although U cal/cr ratio was not significantly different according to bone mineral density status, there was increasing tendency of U cal/cr ratio in postmenopausal women with osteoporosis compared to women with normal bone mineral density (0.26±0.14 vs 0.22±0.13 mg/mg of creatinine). We suggest that some of Korean postmenopausal women have a defect in renal calcium conservation and bone turnover could be higher in postmenopausal women with a defect than those without this defect. Further studies are needed to identify the specific cause of the renal leak in cal-

cium in postmenopausal osteoporotic women.

SA334

See Friday Plenary number F334.

SA335

Ovariectomy Dramatically Reduces Bone Innervation. B. Burt-Pichat,*[1] M. Lafage-Proust,[2] F. Duboeuf,*[1] C. Itzstein,*[1] P. D. Delmas,[1] C. Chenu.[1] [1]INSERM Unit 403, Lyon, France, [2]LBTO, INSERM-E9901, Saint-Etienne, France.

We recently demonstrated that sciatectomy induces a bone loss in rat tibia associated with a reduction of nerve profiles immunostained for several nerve markers, suggesting a link between bone innervation and remodeling. In order to rule out the effect of limb immobilization in these experiments, we studied the innervation in rat tibiae after ovariectomy and compared the results with the neurectomy model. A total of 27 female Wistar rats, aged 12 weeks, were randomly divided into 2 groups. 13 rats were sham-operated (SHAM) and 14 were ovariectomized (OVX). The rats were sacrified 14 days after surgery and infused with 1% glutaraldehyde in PBS by intracardiac injection. Bone mineral density (BMD) was measured by dual energy x-ray absorptiometry (DEXA) (Hologic 1000 plus) on the left tibiae, which were then processed for bone histomorphometry analysis. Right tibiae were fixed in 1% glutaraldehyde and processed for immunocytochemistry of nerve markers. BMD was significantly decreased in OVX tibiae compared to SHAM after 14 days ($P<0.005$, Mann-Whitney's Test). Bone histomorphometry analysis of tibiae demonstrated a significant reduction of trabecular bone volume (BV/TV) in both primary ($P<0.005$) and secondary ($P<0.0005$) spongiosa of OVX rats compared to SHAM. Immunocytochemistry of tibiae was performed using antibodies directed against the specific neuronal markers, neurofilament 200 (NF200) and synaptophysine (SY). We observed an important reduction of nerve profiles immunolabelled for both nerve markers in OVX tibiae. Quantitative image analysis (Osteolab, Biocom) of immunostaining for NF200 showed a 70% and 60% decrease of labelled profiles areas, respectively in primary ($P<0.0001$) and secondary spongiosa ($P<0.005$) of OVX tibiae compared to SHAM. A two-fold decrease of the percent area of immunolabelling for SY was also shown in primary spongiosa ($P<0.01$) and secondary spongiosa ($P<0.05$) of OVX Tibiae. While the decrease of innervation after sciatectomy was more marked in the modeling zone, ovariectomy lead to a strong reduction of nerve profiles in both modeling and remodeling zones. Our results indicate that ovariectomy of rat tibiae induces a bone loss associated with a marked reduction of nerve profiles density, similar to that observed after neurectomy. The decrease of innervation associated with bone loss in both models suggests that neural regulation may play a role in bone loss during immobilization and in osteoporosis.

SA336

Anabolic Effect of Vitamin K2 on Endocortical Bone in Orchidectomized Rats. J. Iwamoto,[1] T. Takeda,*[1] J. K. Yeh,[2] S. Ichimura,*[3] Y. Toyama.*[4] [1]Sports Medicine, Keio University School of Medicine, Tokyo, Japan, [2]Metabolism Laboratory, Winthrop-University Hospital, Mineola, NY, USA, [3]Orthopaedic Surgery, National Defense Medical College, Saitama, Japan, [4]Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan.

The aim of the present study was to examine the effect of vitamin K2 on cortical bone loss in orchidectomized rats. Fifty male rats, 6 weeks of age, were randomized by stratified weight method into 5 groups with 10 rats in each group: baseline controls (BLC), age-matched controls (AMC), vitamin K2 administration (K), orchidectomy (ORX), and ORX+K. Vitamin K2 (menatetrenone) was administered subcutaneously at dose of 30 mg/kg on 2 days a week in the K and ORX+K groups. The experimental period was 8 weeks, and cortical bone histomorphometry was performed on double fluorescent labeled 40 um-thick sections of the tibial shaft. Total tissue area (Tt Ar) and cortical area (Ct Ar) were significantly higher in the AMC, K, ORX, and ORX+K groups that those in the BLC group (all $P<0.0001$). Tt Ar and Ct Ar were significantly lower in the ORX and ORX+K groups than that in the AMC group (all $P<0.05$), but they did not differ significantly between the AMC and K groups and between the ORX and ORX+K groups. Percent Ct Ar did not differ significantly between the AMC and K groups. Percent Ct Ar was significantly lower and percent marrow area (Ma Ar) was significantly higher in the ORX group than those in the AMC group (Ct Ar 83.1 % vs 84.9 %, $P<0.05$; Ma Ar 16.9 % vs 15.1 %, $P<0.05$). Percent Ct Ar was significantly higher and percent Ma Ar was significantly lower in the ORX+K group (84.9 % and 15.1 %, respectively) than those in the ORX group (both $P<0.05$), and they did not differ significantly from those in the AMC group. Endocortical eroded surface (ES/BS) and mineralizing surface (MS/BS), and periosteal MS/BS and mineral apposition rate (MAR) did not differ among any groups. However, endocortical MAR was significantly higher in the ORX group than that in the AMC group (1.20 um/day vs 1.02 um/day, $P<0.01$). Endocortical MAR was significantly lower in the ORX+K group (1.02 um/day) than that in the ORX group ($P<0.001$), and it did not differ significantly from that in the AMC group. These findings suggested that ORX increased MAR and subsequent bone turnover on the endocortical surface compared with age-matched controls, resulting in endocortical bone loss, and that vitamin K2 could prevent ORX-induced endocortical bone loss by normalizing MAR and subsequent bone turnover to the control level. Vitamin K2 could not prevent the ORX-induced reduction in the age-related gain in Tt Ar and Ct Ar. The present study provides evidence indicating the anabolic effect of vitamin K2 on endocortical bone in orchidectomized rats.

SA337

Sex Hormone and Adrenal Androgen Concentrations in Men with Idiopathic and Steroid-induced Osteoporosis. N. Bhargava,[1] J. Cheung,[1] S. Vaja,*[1] P. Seed,[2] I. Fogelman,[3] G. Hampson.*[1] [1]Chemical Pathology, St Thomas' Hospital, London, United Kingdom, [2]Obstetrics and Gynaecology, St Thomas' Hospital, London, United Kingdom, [3]Nuclear Medicine, Guy's Hospital, London, United Kingdom.

Changes in gonadal and/or adrenal steroids have been implicated in the pathogenesis of male osteoporosis and corticosteroid-induced osteoporosis. This study was undertaken to assess and compare adrenal androgen, testosterone and oestrogen status in a group of men with idiopathic and steroid-induced osteoporosis. We investigated 77 men (Group A, Idiopathic osteoporosis, n = 38 age (mean (SD) 57.7 (12.1) years, Group B, Steroid-induced osteoporosis, n = 39 age 55.3(13.1) years). The current steroid dosage was (mean (SD)) 14.3 (11.3) mg/day and the duration of oral steroid intake was 9.6 (8.1) years for the patients in Group B. There was no significant difference in bone mineral density (BMD) expressed as 'T' score at the spine, femoral neck and total hip between the 2 groups (Group A : spine -0.85 (0.83), femoral neck -1.56 (0.09), hip -2.54 (1.09), Group B : spine -0.89 (0.14), femoral neck -1.54 (0.81), hip -2.2 (.22)). However the prevalence of fragility fractures was higher in Group A (53% of patients in Group A v/s 23% in Group B, $p=0.0019$). Serum androstenedione, dehydroepiandrosterone sulphate (DHEAS), testosterone and and oestradiol were determined on all patients. The results were analysed by multiple regression with robust standard errors. Androstenedione and DHEAS were significantly reduced in Group B patients (Androstenedione:Group A 4.99(1.8), Group B 2.1 (1.6) $p = 0.0001$, DHEAS Group A 3.3 (2.4),Group B 1.4 (2.2) $p = 0.001$)). Serum DHEAS was undetectable in 23 (59%) patients in Group B compared with only 2 (5%) patients in Group A. We observed no significant difference in total and free testosterone and oestradiol between the 2 patient groups.Oestradiol/Sex hormone binding globulin (SHBG) ratio was significantly lower in Group A (Group A 1.24 (0.7), Group B 2.03 (1.3) $p = 0.007$). In the whole population studied, there was a significant positive association between serum oestradiol and BMD at the spine ($t = 2.622$, $p = 0.01$). The relation between serum oestradiol and spine BMD was more marked in Group B patients ($t = 2.974$, $p = 0.004$). In conclusion, this study confirms the association between serum oestradiol and BMD in men. Adrenal androgens production is significantly impaired in patients on steroids in contrast to sex steroids. Suppression of adrenal androgen synthesis may contribute, at least in part, to the reduction in bone formation observed in steroid-induced osteoporosis.

SA338

See Friday Plenary number F338.

SA339

Densitometric Diagnosis of Osteoporosis in Men: Effect of Measurement Site and Normative Database. N. L. Vallarta-Ast,[1] D. C. Krueger,[2] N. C. Binkley.[2] [1]Department of Radiology, Wm S Middleton VAMC, Madison, WI, USA, [2]Institute on Aging, University of Wisconsin, Madison, WI, USA.

Controversy exists regarding which sites to measure, and the appropriate database to use, for the densitometric diagnosis of osteoporosis in men. While bone mineral density (BMD) measurement of the proximal femur (F) and lumbar spine (LS) is routine, LS osteoarthritis often elevates measured BMD in older men. Additionally, use of a male normative database is standard practice, however, recent data suggest a female database may be more appropriate. As such, the purpose of this cross-sectional study was to evaluate the effect of sites measured and normative database utilized on osteoporosis diagnosis in men. From 1997 to 2000, 647 male veterans were referred for initial BMD measurement; 52 were excluded based on inability to image a site. In the remaining 595 men (mean age 65 ± 12 years), DXA measurements were obtained at the LS, F and ultradistal radius (UD) using a Lunar Expert-XL densitometer. The manufacturers male normative database was used for initial analysis and World Health Organization (WHO) diagnostic criteria were applied. Osteoporosis (T-score < -2.5) was present at one or more sites in 282/595. The most sensitive single site was F identifying 75% of osteoporotic men compared to 56% and 37% at the UD and LS respectively. Combination of F plus UD was more sensitive (p 70, F plus UD detected 98% of men with osteoporosis. Of the 595 subjects, 129 had radiographically documented fractures (94 vertebral, 13 hip, and 28 other). In this group, scans were analyzed using male and female normative data. WHO diagnostic criteria and National Osteoporosis Foundation (NOF) treatment guidelines were applied. More men with prior fracture were diagnosed as osteoporotic ($p < .0001$), and NOF treatment indications were met more frequently ($p < .0001$), when a male normative database was utilized. Specifically, use of female normative data would lead to 29.5% of these men with prior fracture not meeting criteria for pharmacologic therapy. Furthermore, the percentage classified as having normal bone BMD was higher using female reference data (16%) than when male (10%) was used. In conclusion, BMD measurement at only the spine and hip leads to underdiagnosis of osteoporosis in men. This situation will be exacerbated by utilization of a female normative database. Many men with prior fracture will be categorized as not meeting a pharmaceutical intervention threshold or as having normal bone mass. Clinicians need to be aware of this impact on osteoporosis treatment recommendations if use of female reference data becomes standard densitometric practice in men.

SA340

See Friday Plenary number F340.

SA341

The Effect of Chronic Estrogenic Treatment on the Bone Mineral Metabolism of the Man. M. Sosa,[1] E. Arbelo,*[2] M. Domínguez,*[3] D. Hernández,[4] A. Arbelo,*[3] J. Gómez.*[4] [1]Bone Metabolic Unit, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [2]University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [3]Hospital University Dr. Negrín, Las Palmas de Gran Canaria, Spain, [4]Hospital University Insular, Las Palmas de Gran Canaria, Spain.

Background: There is a clear relationship between oestrogen deficiency and the decrease in bone mass among women, but the aetiology of osteoporosis in men in somewhat different. It has been described an excess of glucocorticoids, hypogonadism, and a variety of other systemic conditions, medications, and lifestyle factors, but often there is no obvious cause. It has also been linked with testosterone deficiency, and recently with serum estradiol levels. Taking into account that there is no ethically approved method to treat osteoporosis in men with estrogens -due to the feminisation caused-, we have searched for a population of men that self-administered estrogens, because they view themselves as women. They are transvestites. Not having found any reference to similar studies in Medline, we have studied the effects of a long-term estrogens intake in a middle-age male population on the bone mineralMethods: We studied 53 men (age 30-59) and divided them into two groups:Group 1: 27 transvestites that had been taking estrogens for a minimum period of 36 months. Group 2: 26 healthy men without any intake of estrogens as controls.To perform this study, we included men that received estrogens for a minimum time of 36 months. Those with chronic hepatitis, HIV and other diseases that affect the bone such as chronic renal failure, hepatic insufficiency, hyperparathyrodism, hypothyroidism, were excluded, the same as those transvestites that had undergone surgery in order to change their gender (transsexuals). Results: We found no statistically significant differences in biochemical bone remodeling markers and hormones between both groups with the exception of serum estradiol levels who were higher in transvestites and testosterone who were lower in this group compared to controls. Bone mineral density and bone mineral content values were similar in both groups (Table 1). Conclusion: Chronic treatment with estrogens dos not produce significant changes in bone mineral metabolism in men, either in biochemical bone remodelling markers or in bone mass.

	Transvestites	Controls	P values
BMD (g/cm^3)			
L2L4	1.068 ± 0.175	1.043 ± 0.145	0.59
Femoral neck	0.882 ± 0.154	0.826 ± 0.124	0.16
Trochanter	0.772 ± 0.136	0.758 ± 0.090	0.66
Intertrochanter	1.154 ± 0.212	1.160 ± 0.136	0.90
Mean (total)	0.997 ± 0.164	0.989 ± 0.118	0.84
Ward	0.707 ± 0.150	0.635 ± 0.145	0.86

SA342

Decrease in Serum Leptin by Troglitazone Is Associated With Preventing Bone Loss in Type 2 Diabetic Patients. S. Watanabe,*[1] Y. Takeuchi,[1] S. Fukumoto,[2] T. Nakano,*[3] T. Fujita.*[1] [1]Department of Medicine, University of Tokyo School of Medicine, Tokyo, Japan, [2]Department of Laboratory Medicine, University of Tokyo School of Medicine, Tokyo, Japan, [3]Tokyo Metropolitan Tama Geriatric Hospital, Tokyo, Japan.

Thiazolidinedione (TZD) class of antidiabetic drugs decreases the expression of leptin in adipocytes. Troglitazone, a member of TZD, often suppresses serum leptin level in type 2 diabetic patients. Less leptin can be associated with high bone mass based on analyses of mice deficient in leptin actions. Therefore, treatment with TZD may influence bone metabolism in diabetic subjects. The present study was undertaken to clarify effects of one year-treatment with troglitazone on bone mineral density (BMD) and bone metabolism in non-obese type 2 diabetic patients. Twelve male (mean 71.3 year-old) and 15 postmenopausal female (mean 72.6 year-old) with type 2 diabetes (HbA1c 8.4 +/- 0.5%) were enrolled in 1998-1999 to take 400 mg/day troglitazone for one year. Serum leptin decreased in 70% of subjects after one month-treatment. HbA1c significantly decreased after 3 months, while body mass index and %body fat did not change until 12 months in patients whose serum leptin level either increased or decreased. Although lumber BMD in each patient was equivocally changed during the treatment period, %change of BMD was significantly higher in the leptin-decrease group than in leptin-increase group at 6 and 12 months. Both of urinary excretion of type I collagen N-telopeptide (NTx) and serum bone alkaline phosphatase (ALP) decreased at one month but increased thereafter in either group. %Change of BMD was negatively correlated with %change of serum leptin, whereas it was not associated with that of other parameters including NTx, bone ALP, body mass index and HbA1c. There was no significant correlation between basal level of serum leptin and BMD. These observations suggest that the decrease in serum leptin with no change in body fat mass by troglitazone is involved in preventing bone loss in type 2 diabetic patients. Hence, TZD may have advantage for diabetic patients who have a risk for osteoporosis, and leptin may negatively affect bone metabolism in humans.

SA343

Role of Insulin-like Growth Factor-1 and Transforming Growth Factor b1 in Experimental Osteoporosis in Rat. X. Liang.* Shenzhen Institute of Geriatrics, Shenzhen, China.

Previous studies suggested that insulin-like growth factor-1 (IGF-1) and transformaing growth factor b1 (TGFb1) may involve in the development of osteoporosis. To document the their roles in the pathogenesis of osteoporosis, the levels of IGF-1 and TGFb1 were followed in the rats with experimental osteoporosis induced by the twin ovaries removal. The bone mineral density was measured by HOLOGIC dual energy X ray bone densitometer. IGF-1 and TGFb1 were measured by enzyme-linked immunosorbent assay. Osteoporosis was documented by the low bone mineral density in the experimental group (n=30 each group, P<0.01, compared to the control group with sham operation). Serum IGF-1 levels were lower in the experimental group (P<0.05, compared to the control group) 8 to 16 weeks after the operation. Experssion of TGFb1 by chondrocytes and osteoblasts was different in the experimental group compared to the control group. (P<0.05). These data suggests that both IGF-1 and TGFb1 may be important in the pathogenesis of the postmenopausal osteoporosis.

SA344

See Friday Plenary number F344.

SA345

Trauma due to Fracture Reduces Biomechanical Properties of Non-Fractured Bone in the Aged Rat Model. S. Juma,[1] M. P. Akhter,[2] D. Chakkalakal,*[3] J. R. Novak,*[4] D. A. Khalil,[1] E. A. Lucas,[1] M. El-Osta,*[1] B. J. Stoecker,[1] E. D. Fritz,*[4] B. H. Arjmandi.[1] [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [3]Biomedical Engineering Center, Creighton University, Omaha, NE, USA, [4]VA Medical Center, Creighton University, Omaha, NE, USA.

Fracture healing involves various local and systemic factors. The purpose of this study was to investigate whether the process of fracture healing in one bone affects the bone density and biomechanical properties of other bones in sham and ovariectomized (ovx) aged rats. Fifty-six female 12-month old Sprague-Dawley rats were divided into four groups; two sham and two ovx groups. Animals in all groups were placed on a semi-purified diet for a period of 120 days to allow significant bone loss to occur in the ovx animals as confirmed by assessing whole body bone mineral density (BMD) using DXA. Thereafter, osteotomy was performed to create an experimental fracture model in both fibulae in animals from one sham and one ovx group. In this model a surgically created 4-mm-long defect was fitted with an 8-mm-long tubular specimen of demineralized bone matrix over the cut ends of fibula. Animals were killed 100 days after osteotomy and bone specimens were collected for BMD and biomechanical assessments. As expected, ovx significantly decreased 4th lumbar vertebra and femoral BMD and bone mineral content (BMC) but fracture had no significant effects on BMD and BMC of these bones in either the sham or ovx group. However, fibular fracture in sham animals significantly decreased structural properties of femur such as yield (25%) and ultimate load (27%) without affecting BMD and BMC. Thus, trauma in one bone and the repair response compromised the mechanical properties of distant bones without decreasing BMD or BMC. These findings suggest a disruption of bone remodeling characterized by dystrophic mineralization rather than ossification of newly formed matrix. In ovx rats, the higher rate of bone turnover may be responsible for overcoming this deficiency. These mechanisms are currently being investigated. Supported by USDA NRI grant No. 99-35200-7606

SA346

Serum Leptin Levels and Bone Mineral Density in Postmenopausal Women. Z. Nagy, G. Speer, I. Takács, É. Bajnok,* P. Lakatos. 1st Department of Medicine, Semmelweis University, Budapest, Hungary.

Leptin is secreted by adipocytes and regulates the bone formation via the receptor located in hypothalamus. Beside, leptin controlls the body weight and gonadal functions, which are also in connection with bone mineral density. Animal studies have shown that leptin inhibits bone formating function of osteoblasts and the leptin resistant animals have obesity, hypoganadism and high bone mass. In humans, the connection between serum leptin levels and bone mineral density (BMD) is controversial.In our work, we examined whether serum leptin levels, vitamin D receptor (VDR) gene BsmI, and estrogen receptor (ER) gene XbaI/PvuII polymorphisms are associated with BMD in 179 Hungarian postmenopausal women. From this cohort, 98 osteoporotic (mean age: 56.5±7.1 yr.) patients were compared with 81 healthy control women (mean age: 54.3±5.7 yr.). No one had been treated with hormone replacement therapy or other medication which could affect bone metabolism. Serum leptin levels were measured by RIA technique, while PCR was used to identify VDR and ER gene polymorphisms. BMD was measured at the lumbar spine (L2-L4) and at the femoral neck by dual X-ray absorptiometry (DEXA) and radius midshaft (SPA). Significant correlation was found between the BMD and the body mass index (BMI) ($p<0.0001$), also between the serum leptin levels and the BMI ($p<0.0001$). The VDR gene BsmI polymorphism shows a significant correlation with the femoral neck BMD ($p<0.05$). We found a negative correlation between serum leptin levels and BMD measured at radius ($p<0.03$) and femur ($p<0.05$) in the hole population. Subjects with BB genotype exhibited significantly higher serum leptin levels ($p<0.05$). No significant correlation was found between BMD and ER gene XbaI/PvuII polymorphisms and leptin levels. Our data raises the possibility that leptin plays a role in the regulation of bone metabolism

in humans.

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See Friday Plenary number F347.

SA348

Effects of Intravenous Zoledronic Acid on Bone Remodeling in Postmenopausal Osteoporosis: a Histomorphometric Analysis of Transiliac Biopsies after One Year. M. E. Arlot,[1] J. P. Roux,*[1] N. R. Portero,*[1] Z. Horowitz,[2] P. Richardson,[2] U. Trechsel,[2] P. J. Meunier.[1] [1]INSERM U403, Lyon, France, [2]Zoledronic Acid Study Group, Novartis, Basle, Switzerland.

The third generation bisphosphonate zoledronic acid (CGP 42446) is a very potent anti-resorptive agent. To assess its effects on human bone after menopause, intravenous bolus zoledronic acid were given in osteoporotic and osteopenic postmenopauseal women with DXA T score ≤ -2 and no more than one osteoporotic vertebral fracture in a Phase II study. Patients received placebo or zoledronic acid given every 3 months (0.25, 0.5, 1 mg), or every 6 months (2mg) or only once (4 mg) for one year. In addition, all patients received 1000 mg calcium daily. At the end of one year treatment, 7.5 mm transiliac biopsies were obtained from 43 patients aged 64 ±7 yrs (48 to 78 yrs), after double labeling with tetracycline. 27 biopsies were measurable. The sections were undecalcified and stained with Goldner's trichrome or remained unstained for tetracycline measurements.When compared to placebo, treated patients, whatever the dose, demonstrated a significant decrease in mineralizing surfaces, bone formation rate, adjusted apposition rate, activation frequency (- 71 to - 84 %; $p<0.05$) , and a borderline decrease in eroded surface (-39%; $p<0.06$) and volume (-48%; $p<0.07$). No change was noted for cortical thickness and porosity, cancellous bone volume, trabecular thickness, separation and number, wall width of trabecular packets, number of node/tissue volume and osteoid maturation time. No dose effect has been detected. No osteomalacia was diagnosed neither qualitatively nor quantitatively (osteoid thickness and volume, mineral apposition rate). Zoledronic acid did not induce any qualitative bone abnormalities.In conclusion, one year treatment with zoledronic acid markedly decreased the rate of bone remodeling without inducing osteomalacia and maintained bone quality without loss of the lamellar organization of bone.

Disclosures: Novartis,2.

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SA350

Identical Acute Reduction in Bone Resorption are Obtained with Alendronate Administered on a Daily or Weekly Schedule in Patients with Baseline High Bone Resorption. J. R. Talbot,[1] M. Moran,*[2] G. Bollero,*[2] D. Mata,*[3] O. Messina.[3] [1]School of Medicine, UAP University, LSM, Argentina, [2]Endocrinology, British Hospital, Buenos Aires, Argentina, [3]CIRO, Rheumatology & Osteology Research Institute, Buenos Aires, Argentina.

We were concern to start a weekly dose of Alendronate (70mg/once/week) instead 10 mg/daily in patients who have extremely high bone resorption at their baseline determination. Therefore, we decided to compare the antiresortive acute effect of 70 mg of Alendronate administered during 4 weeks in a daily schedule of 10mg/day vs. a weekly schedule of 70mg/once/week. Fourteen patients with high bone resorption (i.e. deoxypyridinoline/ creatinina > 18 nmol/mmol) were randomized to receive Alendronate 10 mg/day (n=7) or Alendronate 70 mg/once/week (n=7). Second urinary morning void were collected to determine deoxypyridinoline and creatinina concentration (D-Pyr/Cr) at baseline and at the end of week 1, 2, 3 and 4. The baseline urinary concentration of D-Pyr/Cr was 22 ± 4 nmol/mmol decreasing »60% after Alendronate therapy in both groups ($p<0.001$), but in a similar pattern: -30% vs. -26 % at the first week; -51% vs. -49% at the second week; -51% vs. -65% at the third week and -60% vs. -65% at the fourth week (p=NS, between groups. Our results suggest that Alendronate 70mg-once-weekly is same effective as 10 mg daily to decrease acutely bone resorption on patient with baseline high bone resorption.

SA351

See Friday Plenary number F351.

SA352

Once a Week Alendronate Therapy: A Convenient and Effective Way of Prevention and Treatment of Osteoporosis: A Four-Year Clinical Study. S. J. Wimalawansa. Department of Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ, USA.

Oral bisphosphonate therapy such as alendronate and residronate are highly effective in prevention and treatment of osteoporosis. The main aim of this study was to see whether the administration of alendronate once a week (i.e, infrequent dosing) has the same efficacy on bone mineral density (BMD), as the daily therapy. The secondary aims were to assess the adverse reaction profile and whether this regimen improves the compliance, in comparison with the daily administration of alendronate. A total of 180 patients with BMD <2.0 SD in the lumbar spine was included in this study. The first group of patients (n=89) received alendronate once a week (6 x 10 mg tablets) for two years. At the end of two years, they were given the choice of continuing the same dose of alendronate (60 mg once a week), or taking 40 mg of alendronate (one tablet) once a week for further two-years (a total follow up period of four years). This group was compared with a group of patients who received the original recommended dose of alendronate 10 mg taken once a day (n=91) for a four-year duration. BMD was measured by DXA in the lumbar spine and in the total hip at the beginning and then annually, and these data were compared between these three groups. Biochemical markers of bone turnover; serum osteocalcin and N-telopeptides were also measured. BMD increased significantly, and changes of both BMD and biochemical markers were remarkably similar in three groups over the four years duration of the study. Patients on both regimens had equal and significant suppression of biochemical markers; serum osteocalcin and urinary N-telopeptides. Patient compliance was 88% in the once a week regimen and 72% in the once daily regimen. Drop out rates due to adverse effects of alendronate were 8% for the once a week group and 14% for the once daily treatment group, respectively. All alendronate regimens that were tested significantly increased the bone mass in both lumbar spine and in hip, decreased markers of bone turnover, and generally well tolerated over the four-year duration. However, the compliance and the adverse effect profiles were better with the once a week regimen. This study gives an insight into a highly effective, safe and economical way of administration of alendronate to improve BMD in patients with established osteoporosis.

Disclosures: Proctor & Gamble Pharmaceuticals,5.

SA353

See Friday Plenary number F353.

SA354

Bisphosphonate Incadronate Preserves Cancellous Bone Mass and Strength in Corticosteroid-treated Rats. N. Miyakoshi, T. Kudo,* Y. Tamura,* T. Tsuchida,* Y. Kasukawa, E. Itoi,* K. Sato.* Orthopedic Surgery, Akita University School of Medicine, Akita, Japan.

Corticosteroids have been used for treating a variety of diseases such as systemic lupus erythematosus, asthma, and rheumatoid arthritis. However, long-term corticosteroid administration results in significant bone loss. Although incadronate disodium (YM175) has been proved to be an effective drug in estrogen-deficiency osteopenia, little is known about its preventive effects on corticosteroid-induced osteopenia. The purpose of this study, therefore, was to evaluate the preventive effects of incadronate disodium on cancellous bone loss in rats treated with corticosteroid. The protocol was designed for the simultaneous or pre-treatment of incadronate disodium.Seven-month-old female Wistar rats were divided into six groups: the vehicle saline treated group, PSL group (prednisolone 2.5 mg/kg s.c., 6 times a week for 8 weeks), YM group (YM175 1 ug/kg s.c., 6 times a week for 8 weeks), YM+PSL group (YM175 1 ug/kg + prednisolone, 6 times a week for 8 weeks), pre-YM group (YM175 10 ug/kg, 3 times a week for 2 weeks prior to 8 weeks of vehicle injection), and pre-YM+PSL group (YM175 10 ug/kg, 3 times a week for 2 weeks prior to 8 weeks of prednisolone administration). At necropsy, their femurs, tibiae, and urine were collected. Histomorphometric studies of the proximal tibia revealed that PSL group exhibited a marked decrease in cancellous bone volume relative to vehicle group, as expected ($p<0.001$). In contrast, bone volumes in both YM+PSL and pre-YM+PSL groups showed significantly higher values compared to that in the PSL group ($p<0.01$). Bone formation and mineral apposition rates in the PSL, YM, YM+PSL, pre-YM, and pre-YM+PSL groups were significantly lower than those in the vehicle group ($p<0.05$). Eroded and osteoclast surfaces in the YM+PSL and pre-YM+PSL groups were significantly lower than those in the PSL group ($p<0.01$). Values in these histomorphometric parameters in YM+PSL or pre-YM+PSL groups were comparable with those in YM or pre-YM groups. Bone mineral density of the distal femur measured by DXA and metaphyseal strength of the femur were significantly lower in the PSL group than in other five groups. We also found that YM+PSL group and pre-YM+PSL group showed significantly lower urinary deoxypyridinoline compared to PSL group. We conclude that both simultaneous and pre-administration of incadronate disodium preserve cancellous bone mass and strength in corticosteroid-treated rats.

SA355

See Friday Plenary number F355.

SA356

Alendronate as a Potential Countermeasure to Microgravity Induced Bone Loss. A. D. LeBlanc,[1] L. Shackelford,*[2] T. Driscoll,*[1] H. Evans,*[1] N. Rianon,*[1] S. Smith.*[2] [1]Baylor College of Medicine, Houston, TX, USA, [2]Johnson Space Center, Houston, TX, USA.

It is well documented that unloading the skeleton in microgravity or bed rest simulation results in rapid bone loss. Restoring the appropriate stimulus to the skeleton should, presumably, ameliorate bone loss in space. Exercise therefore is the logical countermeasure to restore physiological balance. Although preliminary results are encouraging, the appropriate type, amount and frequency of in-flight exercise is still unclear. Whatever the actual exercise prescription that is ultimately implemented, it will likely be time intensive and may not always be performed properly or effectively by in-flight crew members. All human data to date, indicate that space flight and bed rest dramatically increase bone resorption. Since the primary effect of bisphosphonates is to decrease bone resorption, their

use as a prophylactic agent during space flight is attractive. Earlier studies have indicated that during bed rest, bisphosphonates may reduce loss of urinary Ca and possibly bone. We report preliminary results of 10 mg/day of alendronate treatment in 8 subjects bed rested for 4 months compared to 8 control untreated bed rest subjects. Bone mineral density (BMD), bone resorption and formation markers were measured at the beginning and end of bed rest and at several intermediate time points. Subjects were maintained on a controlled metabolic diet and total urine and feces were collected throughout the study to determine Ca excretion and balance. Our preliminary results show that compared to baseline, formation markers were unchanged in controls while reduced 5-18% in the treated group. Resorption marker n-telopeptide, was increased 45% in the control group while decreased 24% in the treated group. Urinary Ca was elevated 53 mg/day in controls while decreased 22 mg/day in the treated group. No significant decreases, except for the calcaneus were observed in any bone regions in the treated group, while controls showed decreased bone density in the trochanter, intertrochanter, calcaneus, pelvis and legs. These data suggest that alendronate treatment may be an effective alternative countermeasure to exercise or more likely as an adjunct to in-flight exercise. The optimum dosage, bisphosphonate type, and dose schedule as well as the combined effects of exercise and alendronate have not been evaluated and need further study.

SA357

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SA358

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SA359

Daily (5 mg) vs. Weekly (30 mg) Dosage of the Bisphosphonate (BP) Risedronate (R): Effects on Markers of Bone Remodeling in Early Postmenopausal Women, With Consideration of Circadian Rhythm Effects. A. Shields,*[1] E. Laschansky,*[2] K. Beach,*[2] S. Downing,*[2] M. Burns,[3] C. H. Chesnut III.[2] [1]Radiology, Osteoporosis Research Group, Univ of WA Medical Center, Seattle, WA, USA, [2]Radiology, Univ of WA Medical Center, Seattle, WA, USA, [3]Procter & Gamble, Mason, OH, USA.

The BP R decreases bone resorption, increases bone density, and decreases vertebral and non-vertebral fractures. Weekly oral administration of R may be more acceptable than daily administration in terms of convenience and compliance; whether a higher weekly R dosage (30 mg) is biologically equivalent to a lower daily R dosage (5 mg) in terms of an effect on markers of bone resorption (and by extrapolation, an effect on BMD/fracture) remains unconfirmed. There is also concern regarding the magnitude of an observed circadian diurnal variability on bone markers, which may influence the timing of sample collection for distinguishing therapeutic effects on bone markers of differing dosing schedules. A six month randomized active-control parallel study group in 30 early postmenopausal women is currently underway, 1) to assess the biological equivalence, or lack thereof, of 6 months of daily or weekly oral R administration in terms of effect on BR markers, 2) to determine the 24 hour magnitude of circadian rhythm of such BR markers , and 3) to define the effect of circadian rhythm on such bioequivalence studies.Preliminary data (n=17) notes a 24 hr circadian rhythm of 30% in serum n-telopeptide (S-NTx), and 39% for urine NTx/creatinine (U-NTx). Percent changes in S-NTx / U-NTx for 5 mg daily and 30 mg weekly after the first 4 weeks of the 6 mo follow-up are:

R(n)	S-NTx (± SE)	U-NTx (± SE)
5 mg daily	(9) –30% ± 7.5	(7)–40.4% ± 8.6
30 mg weekly	(9) –24% ± 6.3	(9)–39% ± 8.5

The data available to date indicate the ability of this study design to compare 5 mg daily to 30 mg weekly R, and potentially to define the effects of circadian rhythm on such dosage comparability. A portion of this work was conducted through the Clinical Research Center Facility at the University of Washington and supported by the National Institutes of Health, Grant M01-RR-0037

SA360

Effect of Once Weekly Risedronate on Bone Density in Postmenopausal Osteoporosis. M. F. Delaney, J. Shaw,* S. Hurwitz,* M. S. LeBoff. Endocrine-Hypertension, Brigham and Women's Hospital, Boston, MA, USA.

Risedronate is FDA-approved as a daily treatment for postmenopausal osteoporosis. Risedronate 5mg daily increases bone density (BMD) at the spine (LS) by 5.4% and femoral trochanter (TR) by 3.3% (Harris et al, JAMA). Due to its long skeletal half-life, Risedronate may have a therapeutic effect if given as a once weekly dose. Risedronate 30mg once weekly decreases bone turnover (NTX) by 44% (Delaney et al, ASBMR 2000). 58% of patients had a previous history of GI symptoms and when treated with Risedronate once weekly, only 6% experienced GI symptoms (Delaney et al, Endo Soc 2001). In this retrospective study using Risedronate 30mg once weekly, we evaluate BMD changes in 60 postmenopausal women being treated for low BMD in the Osteoporosis Clinic at Brigham and Women's Hospital between 2/98 and 3/2001.Patients ages ranged from 46-85 years. After a baseline BMD (Hologic; QDR), follow up BMD was performed at 4-16 months of treatment (median 12months). Many patients (87%) had previous therapy with antiresorptive agents, Fosamax (F), Estrogen (E), Evista (Ev) and Calcitonin (C); 60% had previously taken Fosamax. During the study, 17% continued an additional baseline antiresorptive treatment (E, Ev, or C). Patients with secondary causes of bone loss were excluded (e.g. hyperparathyroidism, glucocorticoids, malignancy).

Percentage Change in BMD

BMD site	No. of patients	% change BMD	p value
FN	42	+1.1%	0.08
TR	40	+2%	0.009
LS	29	+1%	0.24
Total	42	+1.9%	0.002

We found an increase of 2% at the TR, 1.9% at the total hip and 1.1% at the FN, but no significant increase at the LS. In a small number of patients (n=14) never previously treated with a bisphosphonate, we found an increase of 2.7% at FN, 4.3% at TR, 2.3% at LS and 2.9% at Total hip. We conclude that once weekly Risedronate 30mg increased BMD at the total hip and trochanter ($p<0.01$). Risedronate 35mg may be the optimum weekly dose, however larger randomized controlled studies are necessary

Disclosures: Proctor and Gamble Pharmaceuticals,2,5.

SA361

Glucocorticoid Induces Osteopenia in Young and Adult Mice and Alendronate Partially Prevents this Bone Loss. C. Liu,[1] V. Shen,[1] M. Heggem,*[1] R. Leininger,*[1] H. Chen,*[1] P. Shao,*[1] M. Bailey,*[1] R. Velasco,*[1] G. Nickols,[2] M. Thiede,[2] S. Bain.[1] [1]Skeletech, Inc., Bothell, WA, USA, [2]Pharmacia, Inc., St. Louis, MO, USA.

Glucocorticoid (GC) excess is associated with osteopenia in humans. Although various animal models have been developed to study the mechanisms associated with GC-induced bone loss, most remain unsatisfactory or not fully characterized. Recently, a murine model has been developed in adult mice (JCI 102:274-282, 1998). As GC therapy in adolescents is also associated with osteopenia, the present study was undertaken to evaluate and compare the effects of GC and alendronate (ALN) on bone in young adult and adult mice. Swiss Webster mice, 3 and 7 mo old, were divided into 4 groups (n=7-10/group) and treated as follows for 4 wk: 1) vehicle-treated control, 2) low dose (LD) GC (0.7 mg prednisolone/kg/d), 3) high dose (HD) GC (3 mg prednisolone/kg/d), and 4) HDGC+alendronate (ALN). GC was given by slow release pellets, while ALN by oral gavage. GC caused dose-dependent decreases in lumbar vertebral total bone mineral density (BMD) (-5.5% and -11.8% in 3 mo old mice; -0.83% and -12.0% in 7 mo old mice) and comaprable changes in total bone mineral content (BMC) (-5.8% and -12.5% in 3 mo old mice; 1.8% and -12.9% in 7 mo old mice) (all significant at HD) as evaluated by DXA scans. Similar dose-dependent decreases in total BMD (-7.0% and -16.0% in 3 mo old mice; -11.9% and -20.9% in 7 mo old mice) and total BMC (-9.7% and -16.4% in 3 mo mice; -8.4% and -17.6% in 7 mo old mice) were observed in the distal femur as evaluated by pQCT. The above GC-induced bone changes were further confirmed by static histomorphometry at these bone sites. Co-administration of ALN partially prevented these bone changes. Our results suggest that the mouse model is an effective system for evaluating the skeletal effects of GC in both young and adult mice and has the potential for assessing the therapeutic efficacy of agents that may prevent GC-induced bone loss.

SA362

Comparison of the Efficacy of Alendronate With of Calcitriol in the Treatment of Postmenopausal Osteoporosis-3 Years' Data. Y. Kirazli, F. Erkisi, B. Durmaz. Physical Medicine, Ege University, Izmir, Turkey.

80 postmenopausal women with a mean age of 58.2 ± 4.2 were included in this study which was designed to compare the efficacy of alendronate with of calcitriol , which are widely used agents in the treatment of osteoporosis. The patients were randomly divided into two groups. Alendronate 10 mg+ elementary calcium 500 mg a day was given to the patients in the first group for three years while the patients in the second group took calcitriol 0.5 mg +elementary calcium 500 mg a day for the same period of time. The primary endpoint was bone mineral density(BMD) of the lumbar spine and the hip. The patients were evaluated with DEXA (Hologic) at baseline, at the end of the 1st, 2nd and the 3rd year.BMD values for the first group was 0.75 g/cm2 for the lumbar spine, 0.63 g/cm2 for the femoral neck, 0.53 g/cm2 for the trochanter region and 0.43 g/cm2 for the Ward's triangle. Baseline values were similar in the second group. All of the patients had gone through biochemical tests at baseline, and every three months through the treatment period. BMD values and T scores for the first group are presented in the Table. For the first group, there was statistically significant improvement in all areas except the Ward's triangle whereas the increase in BMD was significant in all areas at the end of the three yearsLumbar 2-4 Baseline 1st year 2nd year 3rd yearBMD 0.75 0.79* 0.78 0.81** T score -2.82 -2.51* -2.44 -2.37**Neck BMD 0.63 0.66* 0.65 0.65 T score -2.68 -2.28* -2.33 -2.04**Trochanter BMD 0.53 0.56* 0.57 0.58** T score -2.24 -1.79* -1.72 -1.51**Ward's triangle BMD 0.43 0.43 0.43 0.49** T score -3.28 -3.10 -2.19 -2.35***The difference between baseline and 1st year ($p< 0.05$)** The difference between baseline and 3rd year ($p<0.05$)There was statistically significant improvement in regards of BMD values also in the second group but alendronate was superior to calcitriol .It is concluded that both pharmacological agents are efficacious in the treatment of postmenopausal osteoporosis but alendronate is superior to calcitriol in regards of increasing BMD.

SA363

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SA364

Combination Therapy with Ibandronate and Calcitriol Is Effective in Patients Following Liver Transplantation. A. Fahrleitner,[1] G. Prenner,*[2] G. Leb,*[1] K. Tscheliessnigg,*[2] C. Piswanger-Sölkner,*[1] H. Dobnig.[1] [1]Div. of Endocrinology, Graz, Austria, [2]Div. of Transplantation, Karl Franzens University, Graz, Austria.

Bone loss and fractures are common complications in patients following liver transplantation (LTX). Aim of this study was to evaluate the effectiveness of a combination therapy with ibandronate (1 or 2 mg iv./3mos) and calcitriol (0.5- 1.5µg/d) in 15 liver transplant recipients, as well as serum osteoprotegerin (OPG) at baseline and under therapy. The median age of the patients was 57 (37 – 71) years, the mean time since TX 21 (13 – 83) mos and none of the patients was on any osteoprotective therapy. DXA measurement of the hip and spinal X- rays were performed at baseline and after 12 months of therapy.

	Baseline	6 months	12 months
Current prednisolon dose (mg/d)	3.7 +/- 1.1	2.3 +/- 0.7	3.7 +/- 1
S-Creatinine (0.6-1.3 mg/dl)	1.5 +/- 0.1	1.6 +/- 0.	1.7 +/- 0.1
iPTH (10 - 65 pg/ml)	71 +/-14	59 +/- 10	51 +/- 8
S-Cross Laps (1.0 - 2.4 nmol/l)	8.4 +/- 1.7	5.6 +/- 1.3 *	3.4 +/- 0.5 **
S-Osteocalcin (1 - 35 ng/ml)	86 +/- 20	54 +/- 14 *	35 +/- 4*
S-Osteoprotegerin (pg/ml)	85 +/- 7	56 +/- 4*	63 +/- 4 **
Z-Score femoral neck (SD)	-1.32 +/- 0.35	NA	-0.76 +/- 0.31*
Bone mass femoral neck (g/cm²)	0.657 +/- 0.03	NA	0.710 +/- 0.03*

*p<0.05 vs baseline; ** p< 0.05 vs 6 months and baseline;At baseline 80 % of the patients fulfilled WHO criteria of having osteoporosis, 53% had at least 1 vertebral fracture (mean 2.1 + 0.5). Laboratory analysis further revealed a high prevalence of renal impairment (60%), vitamin D deficiency (60%), 2° HPT (40%) as well as elevated cross laps- (73%) and osteocalcin- (67%) values indicating high bone turnover. After one year of therapy a highly significant bone gain (p = 0.0007) could be shown at the femoral neck. The median increase of absolute bone mass was 9.2 percent (p = 0.001). The cumulative number of fractures didn´t change significantly. A significant correlation between OPG and cross laps (r=0.63;p=0.01) and osteocalcin (r=0.69;p=0.004) could only be seen at baseline, and the OPG decrease during the first six months correlated with the decrease of the bone markers, probably simply reflecting a normalisation of bone turnover. At later time points no correlation could be shown. Whereas the bone markers showed a further decrease during the second half of the year, OPG levels increased. As this increase correlated with the bone gain at the femoral neck (r=0.63;p=0.01), we postulate that the OPG increase under osteoprotective therapy (after normalisation of bone turnover) may be an independent predictor for treatment response. In summary we found calcitriol and ibandronate to be a successful well tolerated treatment option in patients following liver transplantation.

SA365

PTH and Bisphosphonates in the Treatment of Osteoporosis: The PTH and Alendronate (PaTH) Trial. D. M. Black,[1] C. Rosen,[2] S. Greenspan,[3] K. Ensrud,[4] J. Bilezikian,[5] J. McGowan.[6] [1]UC San Francisco, San Francisco, CA, USA, [2]U. Maine, Bangor, ME, USA, [3]U. Pittsburgh, Pittsburgh, PA, USA, [4]U. Minn., Minneapolis, MN, USA, [5]Columbia U, NY, NY, USA, [6]NIAMS, Bethesda, MD, USA.

Recent studies have shown that PTH use over 1-2 years can increase BMD and may decrease fracture risk. Antiresorptive agents, used in combination or following PTH therapy, could increase or sustain this effect but little is known about how best to combine PTH with antiresorptive agents. To examine this question, we are studying different combinations of PTH (RhPTH, 1-84, 100 microgm/day) with alendronate (ALN, 10 mg/day) in an NIAMS-funded randomized trial of 240 women. The study is being conducted at 4 clinical centers in the U.S. The main goal is to compare 1 year of PTH given concurrently with alendronate to 1 year of PTH followed by alendronate to 1 year of PTH with no bisphosphonate. Women must be between 55-85 years of age and have low BMD together with a risk factor for subsequent fracture. The women will be randomized into one of 4 treatment groups and followed for 2 years:

Treatment Group	Year 1 PTH	ALN	Year 2
1	yes	placebo	ALN
2	yes	yes	ALN
3	placebo	yes	ALN
4	yes	placebo	ALN placebo

Primary outcomes include change in BMD at the hip and spine by DXA and biochemical markers. In addition, we will use QCT to assess the impact of PTH and combination therapy on trabecular and cortical bone at the spine and the hip. Other endpoint measurements include calcaneal ultrasound, radiographic absortiometry and adverse experiences. As of April 15, the study had randomized 110 participants and we anticipate completing enrollment by September, 2001.

SA366

Effects of Continuous Combined HRT and Clodronate on Bone Mineral Density in Osteoporotic Postmenopausal Women. M. Tuppurainen,*[1] L. Sandini,*[2] E. Alhava,*[2] J. Jurvelin.*[3] [1]Dept. of Obstetrics and Gynecology, Kuopio University Hospital, Kuopio, Finland, [2]Surgery, Kuopio University Hospital, Kuopio, Finland, [3]Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland.

Recent studies suggest that adding bisphosphonates to HRT may improve the antiresorptive effects of HRT. In the Kuopio Osteoporosis Study a sample of 3200 women was selected for bone density measurement (BMD) by DXA in 1995-97. If the participants were osteoporotic according to WHO criteria at the lumbar spine and/or femoral neck, they were offered participation to an intervention trial. In absence of contraindications for HRT or bisphosphonates, they were randomized to estradiol hemihydrate (E2) 2 mg + noretisterone acetate (NETA) 1 mg (Kliogest®, Novo Nordisk, Denmark) + Boneplac. Boneplac consists of either 800 mg/day clodronate (Bonefos®, Leiras Ltd, Finland) or placebo. In case of contraindications or refusal of HRT, the woman was offered clodronate 800 mg/day. One and 3-year BMD follow-up measurements have been done. Full compliance and BMD values were available for 136 participants out of 168: Kliogest® + Bonefos® (KB, n=46), Kliogest® (K, n=44), Bonefos® (B, n=46). MANOVA was used to analyse BMD changes in time. After one year of treatment, the KB and K groups showed similar increases in lumbar spine BMD of 5.0±5.1 % and 5.3±3.5% respectively, while the B group showed an increase of 2.2±4.8%. After 3 years, the KB and K groups showed increases of 3.5±6.2% and 4.1±4.8%, while group B showed a decrease of -2.8±5.4% (p<0.01). After one year of treatment, the KB and K groups showed increases in femoral neck BMD of 3.1±4.5% and 3.1±3.6% respectively, while the B group showed a decrease of -0.1±5.6%. After 3 years, the KB and K groups showed increases of 2.9±6.0% and 2.9±5.9%, while group B showed a decrease of -3.1±5.8% (p<0.001). In a MANOVA analysis, after correction for age and BMI, there was a significant time effect on the femoral neck BMD (p<0.01). There was also a significant treatment group effect (p<0.001), suggesting that the effect of treatment was different in time. Post-hoc comparisons showed that the B group was constantly different from the others. There was no time effect on the lumbar BMD (p=0.34), but there was a significant treatment group effect (p<0.001), suggesting that the type of treatment was determinant. However, BMI has also a significant effect on the BMD changes in all groups (p=0.038). In conclusion, E2 2mg in combination with NETA 1 mg increases spinal and femoral BMD in patients with postmenopausal osteoporosis. However, the addition of clodronate 800mg does not increase further the BMD values. In contrast, 800 mg/day clodronate had no effect on BMD.

SA367

Anabolic Effect of Combined Estrogen and PTH (1-34) Therapy in OVX Mice Assessed by Micro-Computed Tomography. D. von Stechow,*[1] S. Fish,*[2] M. Chorev,[2] M. Rosenblatt,[2] R. Müller,[3] J. Alexander.[2] [1]Orthopedic Biomechanics Laboratory, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA, [2]Division of Bone and Mineral Metabolism, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA, [3]Institute for Biomedical Engineering, ETH and University, Zürich, Switzerland.

We have developed an ovariectomized (OVX) mouse model of osteoporosis to study the effects of anabolic and anti-resorptive agents. The aim of this study was to investigate the effect of a combined 17 beta-estradiol (E2) and parathyroid hormone [PTH(1-34)] therapy on bone in mice over a period of 9 weeks. 3-month-old Swiss-Webster mice underwent either OVX or Sham operation. 5 weeks post-OVX the mice were administered either E2,or E2 and PTH, or vehicle for 4 weeks. Animals were sacrificed after week 1 (Sham and OVX), week 5(Sham and OVX) and week 9 (Sham or E2 or , E2 & PTH or vehicle-treated OVX). Femoral bones were analysed by micro-computed tomography. The bone tissue was segmented using a global thresholding procedure. Morphometric indices, such as bone volume density (BV/TV), bone surface density (BS/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and cortical thickness (Ct.Th) as well as connectivity (Conn.D) were measured in three dimensions using direct 3D morphometry. OVX vehicle treated control mice showed a constant loss of metaphyseal trabecular bone over time (week 1 to week 9). This loss was associated with marked decreases in trabecular number and connectivity density. While E2 only treated mice showed a 7-fold increase of these parameters over vehicle-treated mice, the combination of E2 and PTH resulted in a 14-fold increase. Cortical endosteal bone formation under E2 treatment showed a 22 % increase while the combination of E2 and PTH showed a 45 % upregulation, resulting in significantly increased cortical thickness. Both E2 and the combination of E2 and PTH increased the numbers of trabecular and cortical bone indices above the level of the sham operated controls. Three-dimensional µCT of mouse trabecular and cortical bone offers quantitative structural endpoints that are highly correlated with static and dynamic histomorphometric parameters. We conclude that a combined E2 and PTH therapy has a highly beneficial effect on cancellous and cortical bone properties in OVX mice.

SA368

A Double-blind Placebo-controlled Prospective Study Comparing Active Absorbable Algal Calcium (AAACa) with CaCO3 on Spinal Fracture and Spondylotic Deformity. T. Fujita,[1] Y. Fujii,[1] A. Miyauchi,[2] Y. Takagi.[2] [1]Katsuragi Hospital, Calcium Research Institute, Osaka, Japan, [2]Medicine, National Hyogo-Chuo Hospital, Sanda, Japan.

The data obtained in the double-blind placebo-controlled study comparing the effect of Active Absorbable Algal Calcium (AAACa)on osteoporosis with that of CaCO3 reported in Calcif Tissue Int (1996) 58:226-230 by Fujita et al were reevaluated as to the fracture incidence assessed by X-ray morphometry and intraindividual variation of lumbar spine density and vertebral body area measured by DXA to assess the vertebral deformity due to compression fracture and spondylosis deformans quantitatively. In 57 women with a mean age of 80 admitted to a geriatric hospital randomly divided into 3 groups, 900mg/day Ca supplement was given as AAACa in Group A(18 subjects), the same amount of Ca as CaCO3 in Group B (19 subjects) and indistinguishable placebo containing no Ca in Gruop C (20 subjects) for 27 months, in addition to the hospital diet containing 600mg/day Ca. New fractures occurred in 0/1000 subjects-years in Group A, 357/1000 subjects-years in Group B and 500/1000 subjects-years in Group C. In addition to the significant increase of lumbar BMD in Group A over Group C, but not in Group B after the 6th month reported previously, intraindivudual variation of L1-L4 BMD was significantly lower in Group A than in Group C in the 18th month (p=0.0264,Fisher's PLSD) and than Groups B (p=0.0432 and 0.0454) and C and (p=0.0445 and 0.0390) in the 24th and 27th month respectively, suggesting an improvement of vertebral deformity mainly due to spondylosis deformans in response to AAACa, but not CaCO3 or placebo. Decrease of projected area of lumbar vertebral body to less than 80% of the previous value occurred in 4 of 18 subjects in Group A (22.2%),7 of 19 in Group B (36.4%) and 8 of 20 in Group C (40%). Decrease of vertebral body area by more than 20% occurred in 8 of 184 vertebral bodies in Group A (4.3%), 15 of 200 in Group B (7.5%) and 17 of 212 in Group C (8.0%). In addition to increasing spinal BMD, AAACa decreased the occurrence of vertebral body deformity as demonstrated by decreases of the intraindividual variation of both BMD and projected area of vertebral bodies significantly better than CaCO3 and placebo.

SA369

Effects of Repeated Ingestion of Calcium-Fortified Spring Water on Serum Ionized Calcium and Type I Collagen Cross-Linked N-telopeptide (NTx). J. Guillemant,*[1] C. Accarie,*[2] V. de la Guéronnière,*[3] S. Guillemant.[4] [1]Faculté de Médecine Pitié-Salpêtrière, Paris, France, [2]EPHE Pharmacologie et Nutrition Hydrominérale, Paris, France, [3]Pôle Expertise Eau, Bourg-la-Reine, France, [4]Faculté de Médecine Pitié-Salpêtrière and EPHE Pharmacologie et Nutrition Hydrominérale, Paris, France.

In a previous study (Am J Clin Nutr 2000) we showed that a single oral intake of calcium-rich mineral water could significantly increase the serum concentration of ionized calcium (iCa) and suppress serum concentrations of CTx for 4 hours demonstrating that calcium-rich mineral water could be considered as an efficient source of dietary calcium. Nevertheless, the availability of natural calcium-rich mineral water is limited and the possibility to fortify natural spring water with calcium salt is to be considered. Ten young adult males (21-26 y) ingested at three times (at 08.00, 11.00 and 14.00) 660 ml of either calcium-fortified (as bicarbonate, chloride and sulfate) spring water (300 mg/L of calcium) or calcium-poor (<10 mg/L) mineral water. Blood was collected at 08.00, 11.00, 14.00 and 17.00 referred as to P0, P3h, P6h and P9h, immediately before every intake of water for measurement of iCa and NTx. Oral intake of calcium-fortified water resulted in a progressive increase in serum iCa (from 1.252 to 1.319 mmol/L) while serum NTx decreased (by 19.3% at P3h, 24.7% at P6h and 23.1% at P9h) gradually. Since ingestion of calcium-poor mineral water induced a modest (-10.6%) but significant (P<0.01) decrease in NTx we compared the two sets of assays with repeated-measures two-factor ANOVA with interaction. Ingestion of calcium-fortified water resulted in a significant increase in serum iCa (time, P<0.0001; treatment,P<0.0001; time-by-treatment, P<0.0001) and a significant decrease in serum NTx (time, P<0.0001; treatment, P=0.006; time-by-treatment, P=0.09) demonstrating that usually drinking calcium-fortified water could efficiently lower the serum NTx concentrations throughout the day.

Disclosures:,Société anonyme des eaux minérales d'Evian, 2.

SA370

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SA371

Vitamin K Status Does Not Affect Bone Loss in Ovariectomized Rats. N. C. Binkley,[1] J. A. Engelke,*[2] J. W. Suttie.[2] [1]Institute on Aging, University of Wisconsin, Madison, WI, USA, [2]Department of Biochemistry, University of Wisconsin, Madison, WI, USA.

Many reports suggest that vitamin K is important in bone metabolism. Notably, high dose vitamin K2 (menatetrenone, MK4) has been reported to reduce both ovariectomy (ovx) induced bone loss in Fischer 344 rats and osteoporotic fracture in postmenopausal women. However, it is unclear whether these reductions reflect a vitamin K physiologic effect or a direct pharmacologic result of MK4. To further evaluate this, we conducted three studies in which 6-month old nulliparous Sprague-Dawley rats were randomized to ovx or sham groups. All studies utilized a vitamin K deficient diet (Harlan-Teklad #93308, Madison, WI) containing 1% calcium, .55% phosphorous and 2.2 IU vitamin D/gram to which vitamin K1 (phylloquinone, [K1]) or MK4 was added as follows: study 1, 72 rats received diets containing low, control or high amounts of K1 (0.2, 1.37 and 500 mg per kg of chow respectively); study 2, 36 rats received diets containing 1.37 mg K1 or 500 mg MK4 per kg of chow; study 3, 56 rats received diets containing 1.3 mg K1, 882 mg K1 or 882 mg MK4 per kg of chow. Distal femur bone mineral density (DFBMD) in an eight mm region of interest was measured in vivo at baseline and one month post-operatively in all studies utilizing a GE Lunar (Madison, WI) DPX-L densitometer (studies 1 and 2) or a GE Lunar PIXImus densitometer (study 3). In study 1, no differences in ovx-induced DFBMD reduction versus baseline were observed in the control, low and high K1 groups (9.8%, 9.7% and 11.4% respectively). In study 2, no difference in ovx-induced bone loss was observing between the ovx control K1 (5.6%) and ovx high MK4 group (5.9%). Unlike studies 1 and 2, study 3 utilized paired-feeding to minimize the ovx-induced weight increase and also permitted direct comparison of K1 and MK4 effects on bone mass. Again, no effect of either K1 or MK4 on ovx-induced bone loss was observed. Specifically, DFBMD declined 10.5%, 9.2% and 11.2% following ovx in the control, high K1 and high MK4 groups respectively. In conclusion, in these studies of adult ovx rats, neither high dose K1 nor MK4 blocked the development of estrogen deficiency osteopenia. We conclude that for the relationship of vitamin K and ovx-induced bone loss, Sprague-Dawley and Fisher rats appear to give different results. It is possible that currently unappreciated strain and species differences in vitamin K metabolism explain why these data do not reproduce published work or reflect the reduced fracture risk observed in postmenopausal women.

Disclosures: Eli Lilly,2; Aventis,2; Merck,8.

SA372

Dose-Effect Relations of Loop- and Thiazide-Diuretics on Calcitropic Hormones and Biochemical Bone Markers in Postmenopausal Osteopenic Women: A Randomized Double-Blinded Latin Square Multiple Crossover Study. L. Rejnmark,[1] P. Vestergaard,[1] L. Heickendorff,*[1] F. Andreasen,*[2] L. Mosekilde.[1] [1]Aarhus Bone and Mineral Research Group, Aarhus University Hospital, Aarhus, Denmark, [2]Dept of Clinical Pharmacology, Aarhus University Hospital, Aarhus, Denmark.

Thiazide diuretics (TD) reduce whereas loop diuretics (LD) increase urinary calcium. We studied the effects of different doses of TD and LD on electrolytes, calcitropic hormones and biochemical bone markers. In a five period crossover study, comparing four active doses with placebo, 40 postmenopausal women with osteopenia were treated with different doses of the LD bumetanide (n=20, 0.5 to 2.0 mg per day) or the TD bendroflumethiazide (n=20, 2.5 to 10 mg per day). Each treatment period lasted one week. Urinary calcium decreased dose-dependently in response to bendroflumethiazide; 5 mg/day decreased urinary calcium significantly compared with placebo and 2.5 mg/day. Doses above 5 mg/day did not further decrease urinary calcium. Total plasma calcium levels increased, whereas ionized calcium at ambient pH-values decreased due to increased pH-values in response to bendroflumethiazide. Plasma PTH levels did not change, whereas a slight dose-dependent increase occurred in plasma 1,25(OH)2D levels. As a marker of bone formation, plasma osteocalcin levels increased. Conversely, bumetanide dose-dependently increased renal calcium losses with concomitant increased plasma PTH and 1,25(OH)2D levels. Bumetanide did not affect urinary NTx levels, whereas plasma osteocalcin levels increased and bone-specific alkaline phosphatase levels decreased dose-dependently. It makes a difference on calcium homeostasis whether a LD or a TD is chosen as diuretic therapy. An optimal hypocalciuric effect can be obtained by administrating bendroflumethiazide 5 mg/day. The effects of LD are potential harmful to bone. Further studies are needed to evaluate whether long term treatment with LD causes osteoporosis. Until than, we suggest that TD rather than LD are preferred, if possible, as diuretic therapy in order not to cause any deleterious effects on bone metabolism.

SA373

The Orthosis Spinomed Improves Posture, Lung Function, Trunk Muscle Strength, and Quality of Life in Postmenopausal Women with Spinal Osteoporosis: Results of a Prospective, Randomized, and Controlled, Cross-over Study. M. Pfeifer, B. Begerow,* H. W. Minne. Institute of Clinical Osteology "Gustav Pommer", Bad Pyrmont, Germany.

Thoracolumbar braces are widely used in the care of patients with vertebral fractures due to osteoporosis. Their usefulness, however, has never been tested under standardized conditions. To our knowledge, this is the first randomized, controlled study to determine the efficacy of an orthosis in the treatment of spinal osteoporosis. In this cross-over study, patients had been randomized into two groups: group A (n = 31) started with an intervention period of six months, while group B (n = 30) served as controls. After six months the groups changed. Measurements include trunk muscle strength, body sway, angle of kyphosis, pain, and limitations of everyday life and were performed at base-line and every three months thereafter. At baseline, we did not observe any differences between both groups concerning age (p = 0.77), height (p = 0.90), weight (p = 0.84), loss of height (p = 0.25), and number of vertebral fractures (p = 0.87).

Table 1 presents initial values and changes at 6 months in 61 study subjects:

	Initial value	Change	P-Value
Angle of kyphosis (°)			
Group A	-2.2 ± 4.1	-2.2 ± 4.1	
Group B	69.8 ± 9.9	1.4 ± 4.3	0.007
Back extensor strength (N)			

Group A	264 ± 131	122 ± 107	
Group B	262 ± 119	18 ± 55	<0.001
Abdominal flexor strength (N)			
Group A	165 ± 71	66 ± 61	
Group B	155 ± 64	25 ± 41	0.005
Body Sway (mm)			
Group A	84 ± 70	-14 ± 45	
Group B	78 ± 37	10 ± 43	0.045
Vital capacity (%)			
Group A	83 ± 21	0.5 ± 13	
Group B	93 ± 16	-8.2 ± 13	0.020
Pain (Score)			
Group A	4.0 ± 1.1	-0.9 ± 1.0	
Group B	3.9 ± 1.0	0.1 ± 0.9	<0.001
Limitations of daily living (Score)			
Group A	4.8 ± 1.9	-1.3 ± 1.4	
Group B	4.1 ± 1.7	0.2 ± 0.8	0.007

Spinomed improves posture, trunk muscle strength, lung function, body sway, and quality of life in postmenopausal women with osteoporosis. We believe that the efficacy of thoracolumbar braces needs to be investigated in prospective, randomized and controlled clinical trials prior to their introduction in patients' care.

SA374

See Friday Plenary number F374.

SA375

Insulin-like Growth-Factor and IGFBP-3 Levels in Patients with Osteoporosis Before and After Physical Exercise. H. Franck,[1] F. Blum.*[2] [1]Internal Medicine, Center of Rheumatology, Oberammergau, Germany, [2]University of Tübingen, Tübingen, Germany.

IGF-1 and IGFBP-3 are known to influence bone turnover. The aim of our study was to examine whether patients with osteoporosis have different levels of IGF-1 or IGFBP-3 than patients with osteoarthritis.130 patients with osteoporosis (WHO-definition) (mean age 51,6 ± 15 years) and 90 patients with lumbar spine syndrome (mean age 50,8 ± 16 years) were included in the study. IGF-1 and IGFBP-3 were determined as described by Blum et al. Patients performed a standarized physical exercise program. All patients with osteoporosis and osteoarthritis were included in a randomized open controlled trial into two arms one group I: classic physiotherapy, group II: weight bearing exercise. Patients with osteoporosis presented with a significant increase of IGF-1 (121,8 ± 37,2 µg/l ® 128,9 ± 35 µg/l) after physical therapy. In contrast, patients with osteoarthritis didn't show a significant increase after physical therapy. There was no significant change in IGFBP-3 either.Looking at different therapy strategies in patients with osteoporosis and patients with osteoarthritis group I didn't show any significant increase in IGF-1 (102 ± 26 µg/l ® 101,7 ± 38 µg/l) in patients with osteoporosis and osteoarthritis (144 ± 48 µg/l ® 145 ± 47 µg/l). The same was evident for IGFBP-3 in patients with osteoporosis (2,7 ± 0,46 µg/l ® 2,87 ± 0,61 µg/l) and osteoarthritis (2,89 ± 0,7 ® 3,12 ± 0,61 µg/l). In contrast, in group II IGF-1 increase significantly from 103,8 ± 25 µg/l to 120,0 ± 27,6 µg/l (p > 0,01) in patients with osteoporosis with no significant change in patients with degenerative disease (135,6 ± 30® 135,2 ± 33 µg/l). Correspondingly there was a significant increase in IGFBP-3 (2,91 ± 0,18 ® 2,97 ± 0,18 µg/l, p > 0,0001).Weight bearing exercise leads to a significant increase of IGF-1 and IGFBP-3 in patients with osteoporosis.

SA376

Effects of Chronic Symptomatic Instability of the ACL on Bone Mineral Density, Soft Tissue Mass and Muscle Strength of the Leg. M. Kobayashi,*[1] S. Takata,[1] S. Kashiwaguchi,*[1] Y. Takeda,*[1] T. Matsuura,*[1] H. Yonezu,*[2] N. Yasui.*[1] [1]Orthopedic Surgery, The University of Tokushima, Tokushima, Japan, [2]Orthopedic Surgery, Oe Kyodo Hospital, Oe, Japan.

We studied the effects of chronic symptomatic instability of the ACL on bone mineral density, soft tissue mass and muscle strength of the leg in 33 patients (22 men and 11 women) aged 15 to 39 years with unilateral ACL injury. Bone mineral density, lean mass and fat mass were measured by dual energy X-ray absorptiometry, and the isometric and isokinetic muscle strength was assessed by an isokinetic machine. There was not significant difference in bone mineral density between injured and intact legs. Lean mass of the injured leg was significantly smaller than that of the intact leg (p =0.0001), whereas fat mass and %fat of the injured leg were significantly greater than those of the intact leg (p =0.0301, p =0.0001). The isometric and isokinetic muscle strength of hamstrings and quadriceps muscles of the injured leg was significantly smaller than that of the intact leg (p <0.05). We conclude that the unilateral ACL injury does not affect the bone mineral density of the injured leg, and that it affects muscle strength and muscle mass, predominantly that of quadriceps muscles, on the injured side, compared with hamstrings muscles.

SA377

Androgen Deficiency-induced Bone Loss Can Be Prevented by the Combined Intervention of Exercise and Genistein Administration in Mice. J. Wu,[1] X. Wang,*[1] H. Chiba,*[1] C. Miyaura,[2] Y. Ishimi.[1] [1]Food Science, The National Institute of Health and Nutrition, Tokyo, Japan, [2]Biochemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Both estrogen and androgen deficiency caused by ovariectomy (OVX) and orchidectomy (ORX) result in a marked bone loss because of increased bone resorption. Estrogen administration completely restored it in both OVX and ORX animals. Recently, attention has been focused on nonsteroidal estrogen-like plant compounds called phytoestrogens that selectively act on bone without exhibiting substantial estrogenic action in reproductive tissues. We reported that genistein, one of the phytoestrogens, prevented bone loss caused by estrogen deficiency without substantial effects on the uterus in osteoporotic animal models. We also found that the combined intervention of moderate intensity of exercise and a sub-maximal dose of genistein exhibited cooperative effects on the prevention of bone loss in OVX mice. In this study, we investigated the effects of running exercise and genistein administration on bone mass in ORX mice to examine whether the both treatments exhibit cooperative effect on bone mass similar to that in OVX mice. Male ddY mice aged 7 weeks were either sham-operated or ORX and divided into six groups: (1) Sham; (2) ORX; (3) ORX, treated with genistein (0.4mg/day) subcutaneously (G); (4) ORX, exercised on a treadmill daily for 30min/day at 12m/min on a 10°uphill slope (Ex); (5) ORX, given genistein and exercised (ExG); and (6) ORX, treated with 17β-estradiol (0.03µg/day) in the same manner as genistein (E2). Four weeks after the intervention, weight of seminal vesicles was measured and bone mass was estimated by dual energy x-ray absorptiometry (DXA). The weight of seminal vesicles was strikingly decreased in ORX mice. Treatment with neither genistein nor estrogen recovered it at all. Bone mineral density (BMD) of the whole femur was significantly reduced by ORX, and restored by the combined intervention of exercise and genistein. Histomorphometric analysis showed that bone volume (BV/TV) and trabecular number (Tb.N) in the distal femoral cancellous bone were significantly lower in ORX group than those in Sham group, and these were completely restored in ExG group, as in the E2 group. These results indicate that the combined intervention of moderate exercise and genistein administration shows a cooperative effect in preventing bone loss in ORX mice similar to the case in OVX mice.

SA378

BMD Follow-up of Patients on Alendronate, Etidronate, Estrogens or Food Supplements; Significant Differences in Mean Response Between the Groups. A. Hoiseth.* Sentrum Rtg., Oslo, Norway.

Change in lumbar and femoral BMD among women being on no-medication, any systemic estrogen, or the bisphosphanate alendronate or etidronate, has been determined as part of an ordinary clinical follow up. The groups were sub-divided according to compliance and further according to the use of food supplements (calcium and/or vitamin D). The purpose of this on-going follow up of ordinary patient treatment is to reveal any systematic differences between the groups stratified according to the above criteria. A total of 1230 women, mean age 62.4 years (SD 10.8) had repeat BMD measurements made after at least 1 year. Mean age in the group on etidronate (n=123) was 70 years (SD 7.4), on alendronate (n=135) 66 years (SD 13), on an estrogen (n=474) 60 years (SD 8.1), on no medication (n=418) 61.5 years (SD 12.0) and on a combination of estrogen and a bisphosphanate (n=80) 64.8 years (SD 10.5). At baseline BMD (g/cm2, Hologic calibration) in the lumbar spine in the same groups were respectively 0.73, 0,74, 0,83, 8.87 and 0.78; in the total femur, respectively 0.68, 0.69, 0.76. 0.79 and 0.69. Mean (years) and SD of interval between measurements were in the same respective groups 2.2 (0.9), 2.1 (1.1), 3.2 (1.5), 2.9 (1.3) and 2.6 (1.1). In each medication group a substantial number of the women reported non-compliance (50% in the estrogen group). These had significant poorer response than the compliars. These non-compliars were removed from further analyses. In the etidronate and estrogen groups there was a slight tendency for a poorer response among those who did not report taking calcium. The groups not on an anti-resorptive medication had a statistically significant mean reduction in both lumbar and total femoral BMD (respectively 0.9% and 0.7% reduction). There were no differences depending on use or not use of calcium and/or vitamin D supplements in this group. The compliars on anti-resorptive medication had significant increase in lumbar and femoral BMD: Alendronate (5.9% and 2.9%); estrogen (4.0% and 2.3%); etidronate (3.1% and 1.4%); estrogen and a bisphosphanate in combination (5.0% and 4.0%). Several pertinent questions concerning anti-resorptive medical intervention are not addressed by randomized blinded clinical trials. Not least, few have compared different alternative medications or considered aspects related to monitoring the patients by means of repeat BMD measurements. This study indicates substantial differences in the effect by different medications, alendronate showing a superior effect to the other alternatives. Monitoring the individual patients by means of BMD measurements may be useful in patients on other medications; a poor response indicated by such measurements may call for a change in medication.

SA379

Fractures in Women Over 50yr: Implications for the Secondary Prevention of Osteoporotic Fractures of the Application of the NOF and RCPLondon Treatment Guidelines. A. R. McLellan,[1] M. Fraser.*[2] [1]Medicine & Therapeutics, Western Infirmary, Glasgow, United Kingdom, [2]Western Infirmary, Glasgow, United Kingdom.

Audit of demographic and bone mineral density (BMD) data from 1061 women over 50yr, who had sustained a fracture(fx) and who were referred consecutively to our Direct Access DEXA Service(for Primary Care) and to our Osteoporosis-Orthopaedic Liaison Service, is reported; this highlights the needs of the target population for the secondary prevention of osteoporotic fx and the implications of the application of current treatment guidelines for reducing fracture risk are reported.1061 women had sustained 1705 fx including fx of humerus(n=189), ankle(n=181), vertebra(n=177), hand/foot(n=171), hip(n=134) and other sites (n=147). 62.5% had a history of only one fx, while 25.4%, 8.7%, 2.4% and 1% had sustained respectively 2, 3, 4 or at least 5 fx in total (including the fx that led to referral). BMD was measured by Hologic QDR1000plus DEXA scanner: Z-scores (mean(SD)) in patients with 1 fx were -0.34(1.38) while those in patients who had sustained 2, 3, 4 and at least 5fx respectively were -0.54(1.22), -0.73(1.21), -0.84(1.03) & -1.31 (1.27), ANOVA p<0.0001. Similar trends were seen for absolute BMD, T-score and Z-scores at hip and spine. The prevalence of T-scores of '<-2.5', '<-2', '<-1.5' & '<-1.0' at Total Hip (and at Total Hip or Lumbar Spine L1-L4) among the 1061 women was also assessed and were as follows: 26.6(52.4)%, 41.9(67.2)%, 59.8(78.8)% & 74.7(87.9)%. Application of the Royal College of Physicians of London treatment guideline(http://www.rcplondon.ac.uk/pubs/wp_osteo_update) suggests that treatment should be offered to 87.9% of patients (based on hip+/-spine DEXA). Application of the NOF guideline(http://www.nof.org/physguide) suggests treatment should be offered to 59.8% of patients (based on hip DEXA) or to 70% (based on hip BMD if <70yr and treatment for all those >70yr).Osteoporotic fx are a recurring problem- 37.5% of our patients referred because of fx, had previously had a fx and those with a history of multiple fx had lower BMD, emphasising both the opportunity and need to offer treatment for the secondary prevention of fx. The proportion of our patients who would be offered treatment with the aim of reducing subsequent fx risk differs substantially depending on which current treatment guideline is employed.

SA380

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SA381

Impact of Osteoporosis Health Beliefs on Preventive Behaviors in the Postmenopausal Post-fracture Patient. M. T. Cuddihy,[1] S. E. Gabriel,*[2] C. S. Crowson,*[2] J. A. Sloan,*[2] L. J. Melton.[2] [1]General Internal Medicine (Area), Mayo Clinic, Rochester, MN, USA, [2]Health Sciences Research, Mayo Clinic, Rochester, MN, USA.

To investigate the influence of osteoporosis health beliefs on preventive behaviors after fracture, the cohort of 343 postmenopausal women who sustained a minimal trauma distal forearm fracture in Olmsted County between 1993-97 and was previously identified using the resources of the Rochester Epidemiology Project, was sent written survey in 1999.Preventive behaviors included: exercise, calcium intake and osteoporosis medication use. The Osteoporosis Health Belief Scale (OHBS) provided 7 scores (health motivation, susceptibility and seriousness of osteoporosis, exercise barriers and benefits, calcium barriers and benefits). Fracture history, prior diagnosis of osteoporosis, bone densitometry after fracture, osteoporosis medication use, and Charlson co-morbidity index were determined by medical record review. Chi-square and rank sum tests were used for univariate comparisons, and multivariate procedures to identify predictors of preventive behaviors.Among 285 survivors, 168 (59%) responded (mean age 66 years). Only 20 (12%) exercised vigorously, while 98 (60%) exercised moderately and 46 (28%) little or none. Daily calcium intake was >1200 mg in 114 (68%) and <1200 mg in 52 (31%). Prior diagnosis of osteoporosis was associated (p1200 mg and ongoing use of medication for osteoporosis. Clinical factors correlated (p<.05) with higher OHBS susceptibility scores included education < high school, osteoporosis diagnosis, osteoporotic fractures, and bone densitometry; whereas health motivation correlated only with bone densitometry. Higher scores in health motivation, and perceived susceptibility to develop osteoporosis, correlated (p1200 mg. Higher exercise benefit score was correlated (p1200 mg, and a higher exercise barrier score with less frequent exercise (p<.001). Independent predictors of exercise were exercise benefit score (Odds Ratio [OR] 1.9, 95% confidence interval [CI] 1.1-3.1) and exercise barriers (OR 0.6, CI 0.4-0.9); and of calcium intake >1200 mg a higher susceptibility score (OR 1.5, CI 1.1-2.0).Some osteoporosis health beliefs are able to predict exercise behavior and calcium use, but not osteoporosis medication use. Patient perceived susceptibility to osteoporosis was associated with: prior osteoporotic fracture, past osteoporosis diagnosis, adequate use of calcium and osteoporosis medication use. Further work to clarify determinants of preventive behaviors, including medication use, will help focus our educational efforts.

Disclosures: Eli Lilly and Company,2.

SA382

See Friday Plenary number F382.

SA383

Information Needs of Family Physicians for the Management of Osteoporosis. S. B. Jaglal,[1] G. Hawker,[2] J. Carroll,[3] W. McIsaac,[3] L. Jaakkimainen,[3] S. Cadarette,[3] D. Davis.[3] [1]Rehabilitation Science, University of Toronto, Toronto, ON, Canada, [2]Medicine, University of Toronto, Toronto, ON, Canada, [3]University of Toronto, Toronto, ON, Canada.

In Ontario, Canada which has a population of 10 million inhabitants, family physicians are largely responsible for managing osteoporosis. More than 80% of all BMD tests are ordered by family physicians. The objective of this study was to determine the information needs of family physicians for managing osteoporosis and their preferences for receiving this information. A needs assessment was conducted. Purposive sampling through community physicians was used to identify participants in communities that vary in population demographics and utilization rates of BMD testing. Focus groups were conducted between October and December 2000 with male and female physicians who represented a range of ages and practice types. The focus groups were audio-taped. Analyses were performed through independent identification of themes by the investigators. Saturation of themes was reached after 4 focus groups. A total of 32 family physicians (12 men, 20 women) participated from two urban and two rural areas. Average years in practice was 14.4, ranging from 2 to 50 years. Overall these physicians saw osteoporosis as an important issue in their practices with demand for testing often coming from their patients. However, major clinical themes that emerged were uncertainty about screening, BMD testing and treatment. Screening questions included: who to screen, when to start and how to handle the elderly. These physicians were uncertain about the workup for osteoporosis, how often a BMD test should be repeated and how soon you should expect to see a change with treatment. They would like to see better interpretation in the reporting of BMD test results from specialists. Information on what drugs to combine, what is best for preventing fractures, whether drugs work equally well at all sites, long-term safety and the efficacy of non-pharmacologic interventions were identified as needs. Another theme identified was that family physicians find it difficult to remain current. They want guidelines or information aids about osteoporosis with significant input from family physicians, developed by a credible source, which also reflects the reality of busy family practice. Patient education was identified as a key factor in information dissemination because educated patients prompt the physician to think about osteoporosis. In summary, these focus groups clearly indicated that the format of the current information available on the management of osteoporosis does meet the needs of family physicians or their patients.

SA384

See Friday Plenary number F384.

SA385

Effect of Raloxifene on Osteoporosis in Rat. X. Liang.* Shenzhen Institute of Gerontology, Shenzhen, China.

Effect of Raloxifene on osteoporosis in rat. Xiaoping Liang. Shenzhen Institute of Gerontology, Shenzhen, Guangdong, People's Republic of China.In this study, the effect of Reloxifene on osteoporosis was investigated in an experimental osteoporosis model in rat. Experimental osteoporosis model was established by removing both ovaries. The rats were treated with Raloxifene at a dose of 1.2mg/kg/day (n=20) or saline (n=20) for three months starting from three months after operation. Bone mineral density was measured over time by dual energy X-ray bone densitometer. Serum insulin-like growth factor-1 (IGF-1) measured by enzyme-linked immunosorbent assay and expression of transforming growth factor beta-1 in the bone detected by immunohistochemistry were also followed. Raloxifene significantly increased the bone mineral density in the lumbar spine (Figure, P<0.01, compared to the saline control) starting 4 weeks after the treatment. Increased levels of serum IGF-1 were detected in the rats treated with Raloxifene between 4 to 8 weeks after treatment. Expression of TGF-beta by chondrocytes and asteoblasts were different in the treatment group compared to the saline control group. These data suggest that Raloxifene is effective in treating osteoporosis presumably through increasing the level of serum IGF-1 and the expression of TGF-beta in the bone. Raloxifene may be useful for the treatment of osteoporosis in human.

SA386

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SA387

Near Complete Prevention of Musculoskeletal Losses in Hind-limb Suspended Rat Model, using the Combination Therapy with Alendronate and Testosterone

S. M. Wimalawansa,*[1] S. J. Wimalawansa.[2] [1]Department of Medicine, Robert Wood Johnson Medical School, New Brunswicck, NJ, USA, [2]Robert Wood Johnson Medical School, New Brunswick, NJ, USA.

Disuse or the lack of gravity (as encountered during space flights) is known to cause rapid loss of musculoskeletal tissues. Hindlimb-elevated rat model has been validated as a ground-based model to simulate microgravity (i.e., it simulate the musculoskeletal changes observed in rats in space flight). Using this animal model, previously we demonstrated a significant decrease of serum testosterone levels ($p<0.001$) in male rats (International Congress of Endocrinology, 1996; Endocrine, 10:253-60, 1999). Although testosterone replacement and bisphosphonate therapies by themselves have been shown to significantly decrease these losses, neither drug prevented it completely (Journal of Applied Physiology, 86: 1841-6, 1999). These experiments led us to hypothesize that it may be possible to alleviate musculoskeletal losses to near completeness by administering appropriate doses of the combination of these two agents. Rats were hindlimb suspended (n=10 per group) and treated with either vehicle (placebo control), alendronate (30 microgram/kg, twice a week, s.c.), or depot testosterone 6 mg/kg, i.m.). Additional 10 rats (non-suspended) were used as ground controls. In all rats, a loose ligature was placed around the inguinal canal to prevent testes from moving into the abdominal cavity. Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DXA) and the muscle mass by magnetic resonance imaging (MRI). A significant losses of BMD (12% decrease, $p<0.01$), and muscle mass (a decrease of 25%, $p<0.01$) were seen in these 26 week old male Wistar rats after 12 days of hindlimb suspension (n=10 each group). Rats treated with alendronate had a 75% lesser bone loss, but there was no protection against the muscle losses. Testosterone treated rats, bone loss was decreased by 65%, and the muscle losses by 70%. However, the group of rats treated with testosterone and alendronate together had 95% protection against the bone loss and 70% protection against the muscle losses. These data suggest in male rats that the combination therapy with androgen, testosterone, and bisphosphonate, alendronate (at their optimum combination) may completely alleviate the musculoskeletal lossse associates with weightlessness conditions.

Disclosures: Proctor & Gamble Pharmaceuticles,5.

SA388

See Friday Plenary number F388.

SA389

Cenestin® (Synthetic Conjugated Estrogens, A) Maintains Trabecular Bone Mass, Structure And Bone Strength; Suppresses Bone Turnover; And Maintains Estrogenic Uterine Effects In Ovariectomized Rats. N. E. Lane, MD,[1] J. Kumer,*[1] M. Khan,*[1] R. Klein, MD,*[2] R. E. Stevens, Ph.D.,[2] K. V. Phelps.[2] [1]Department of Medicine, University of California at San Francisco, San Francisco, CA, USA, [2]Department of Clinical & Medical Affairs, Duramed Pharmaceuticals, Inc., Cincinnati, OH, USA.

Estrogen products, when given in adequate doses, reduce the risk of osteoporosis through their anti-resorptive effect on bone. This study evaluated the effect of Cenestin, a synthetic conjugated estrogens product synthesized entirely from plant source, on the maintenance of trabecular bone micro-architecture, bone strength, suppression of bone turnover, and uterine effect in the ovariectomized (ovx) rat model. Two doses of Cenestin were chosen in an attempt to approximate the equivalent human oral doses of 0.3- and 0.625mg. Fifty, 6-month retired female breeder Sprague Dawley rats were randomly assigned to one of four groups; 1) sham-operated, 2) ovx + vehicle, 3) ovx + day 1 post-ovx Cenestin, 8.12 mg/kg, 4) ovx + day 1 post-ovx Cenestin, 16.24 mg/kg, for 8 weeks. Trabecular structure of the right proximal tibia of each rat was imaged non-invasively by microCT. A strength failure test determined the mechanical properties of the tibial plateau. Bone markers, Deoxypyridinoline crosslink (DPD) and Osteocalcin (OC), were analyzed by ELISA. Uteri were removed at sacrifice, were thin sectioned, and H & E stained. The sections were scored by 2 readers for uterine myometrial hypertrophy and endometrial cell height (score ranges 0-4). Measurements of trabecular bone mass, structure, and connectivity density in the Cenestin-treated groups were not statistically different ($p>0.05$) from the sham rats, but were all significantly higher ($p<0.05$) than the ovx treated rats. Cenestin-treated rats Structure Model Index (SMI), which measures trabecular morphometry, maintained a more equal mix of plate- and rod-like structure similar to the sham, while the ovx group had predominantly rod-like trabeculae. Fracture load from the Cenestin-treated group (16.24mg/kg/d) was 31% ($p<0.01$) higher than the sham and 61% ($p<0.05$) higher than the ovx animals. Cenestin-treated groups showed significantly lower biochemical markers of bone turnover (OC and DPD) than the ovx group. Uterine histology showed that Cenestin-treated groups had uterine cell hypertrophy and cell lining changes that were similar to the estrogenic uterine effects as the sham group, and significantly different than the ovx group ($p<0.05$). Cenestin, given at the time of ovariectomy, prevented the increase in bone turnover from estrogen deficiency, maintained trabecular bone volume, structure and strength, and exhibited estrogenic effect on rat uteri.

SA390

See Friday Plenary number F390.

SA391

Effects of Raloxifene on the Severity of New Vertebral Fractures in Postmenopausal Women with Osteoporosis: Results from the MORE Trial. E. Siris,[1] G. Crans,*[2] S. Sarkar,*[2] M. Wong,[2] K. D. Harper.[2] [1]Columbia University, New York, NY, USA, [2]Lilly Research Laboratories, Indianapolis, IN, USA.

The 4-year MORE (Multiple Outcomes of Raloxifene Evaluation) trial studied the effects of placebo (PL), or raloxifene (RLX) at 60 or 120 mg/day in 7705 postmenopausal women (≤ 80 years old) with osteoporosis, with or without prevalent vertebral fractures (VF). New VF were identified in radiographs taken at 2, 3, and 4 years [JAMA 282(1999): 637]. Semiquantitative criteria defined VF severity as normal (no VF) or mild, moderate or severe VF, based upon the percent reduction in vertebral height [JBMR 8 (1993): 1137]. The table shows relative risks (RR) and 95% confidence intervals (CI) for new moderate or severe VF at 4 years in women with or without prevalent VF. RLX decreased the risks of at least one new moderate or severe VF in women without prevalent VF by 40 to 51% and in women with prevalent VF by 37% to 47%, with no significant differences between the RLX doses in either group of women. Both RLX doses decreased the risk of multiple new moderate or severe VF by 88% in women without prevalent VF. The 3-year risk reductions for moderate or severe new VF were similar to the 4-year reductions in each RLX group.

Relative Risk (95% CI)

No Prevalent VF	At least one new VF	RLX60	0.49 (0.27, 0.78)
		RLX120	0.60 (0.35, 0.98)
	Multiple (≥2) new VF	RLX60	0.12 (0.00, 0.35)
		RLX120	0.12 (0.00, 0.39)
With Prevalent VF	At least one new VF	RLX60	0.63 (0.50, 0.79)
		RLX120	0.53 (0.40, 0.68)
	Multiple (≥2) new VF	RLX60	0.58 (0.32, 0.91)
		RLX120	0.47 (0.26, 0.80)

RLX60 and RLX120 decrease the risk of moderate or severe new VF at 3 and 4 years, in women with and without prevalent VF.

Disclosures: Eli Lilly and Company,2,3,5,8.

SA392

Dose-Dependent PTH Effects in Young, Intact, Female Rats After 9 Months of Treatment. M. Sato, A. Schmidt,* L. Y. Ma, S. Smith,* E. Rowley,* H. Cole,* M. Westmore. Lilly Research Labs, Indianapolis, IN, USA.

The long-term, dose-dependent, skeletal effects of human recombinant parathyroid hormone PTH (1-34) in ovary-intact Fischer 344 rats were evaluated for up to 9 months of treatment. Young females (1.5 month-old) were subcutaneously administered 0, 0.03, 0.3, 5, 30, or 75 ug/kg/day PTH and evaluated longitudinally by DXA for whole body composition and computed tomography (QCT) for skeletal effects in the proximal tibia metaphysis. No consistently significant differences in body weight or whole body lean mass were observed between groups; however, 30 and 75 ug/kg PTH elevated whole body bone content for the duration of the study after 1 month of treatment. Volumetric bone mineral density (BMD) for the proximal tibia became elevated for 5, 30 and 75 ug/kg after 3-4 months of treatment. After 9 months, dose-dependent increases in BMD were observed for vertebra and femora, with BMD = 1.2 g/cc (75 ug/kg PTH) for the whole femur diaphysis in cross-section which was due to a dose dependent 86% increase in BMC. Biomechanical analyses confirmed substantial, dose dependent increases in the strength and stiffness of the vertebra, femoral midshaft and proximal femur. Interestingly, the optimal dose for vertebral toughness was 30 ug/kg, indicating that duration is an under appreciated aspect of PTH efficacy. Serum osteocalcin levels for all groups were considerably lower than for baselines, consistent with an age dependent reduction in osteoblastic activity. PTH dose-dependently increased osteocalcin levels compared to vehicle controls, confirming PTH stimulation of osteoblastic activity. PTH at 30 ug/kg and above substantially increased whole body BMC, and BMD in the proximal tibia, femoral midshaft and vertebra to well beyond normally attained peak levels. Midshaft analyses showed that the rat cortical bone is as responsive to PTH treatment as cancellous bone with long-term administration, although with slower kinetics. Rat cortical bone and its responsiveness to PTH represent a significant physiological difference between rodents and primates, based on the cortical bone data of PTH effects in cynomolgus macaques and humans. The data taken together showed that treatment duration is an under appreciated aspect of PTH skeletal efficacy.

Disclosures: Eli Lilly and Co.,3.

SA393

See Friday Plenary number F393.

SA394

Dramatic Skeletal Effects of PTH in Male and Osteopenic Ovariectomized Rats after 1 Year of Subcutaneous Administration. Y. L. Ma,[1] M. Westmore,[1] C. Turner,[2] J. Hock,[2] J. Vahle,*[1] M. Sato.[1] [1]Lilly Research Laboratories, Indianapolis, IN, USA, [2]Indiana University Medical Center, Indianapolis, IN, USA.

Skeletal effects of PTH were evaluated in intact male and osteopenic, ovariectomized F344 rats, after 1 year of treatment with 0, 8, or 40 ug/kg/day sc PTH. Males and females were about 6 months of age at baseline, but the females were ovariectomized (Ovx) for 5 weeks prior to initiation of PTH treatment. To ascertain PTH effects on longitudinal growth, femoral length was measured and found not to differ between male groups; but PTH slightly increased femoral length of Ovx rats. No significant differences in femoral

length were observed between Sham and Ovx controls. Histomorphometric analysis of the proximal femur showed a dose-dependent 84-91% increase in trabecular bone volume (BV/TV), 122-189% increase in cortical thickness, 170-189% increase in trabecular number, and 325-664% increase in connectivity for males and Ovx females, respectively. The substantial increase in trabecular and endocortical apposition coincided with a -74% and -61% loss of marrow space for males and females, respectively. Failure analysis of the femoral neck showed 140% and 180% increase in strength for males and Ovx females. QCT analyses of the femoral midshaft showed dose-dependent 200% and 70% increases in BMC, 67% and 55% increases in BMD, and 22% and 10% increases in cross-sectional area, which resulted in 104% and 47% increases in moments of inertia for males and Ovx females, respectively. 3-point-bending analyses of the midshaft showed substantial, dose-dependent increases in strength and stiffness, but a reduction in ultimate strain was observed, which may reflect the significantly altered geometry. Histologic evaluation of sections from the proximal tibia and kidneys showed no treatment related anomalies after 1 year of PTH treatment, indicating that the bone structural changes occurred without histologic lesions or abnormalities. Therefore, 1 year (nearly half-a-lifetime) of PTH treatment substantially increased bone mass and strength to beyond normally attained levels. The data show that treatment duration is an under appreciated aspect of PTH skeletal efficacy.

SA395

The Response of the Mouse Skeleton to Estrogen-Deficiency and Parathyroid Hormone (PTH) Treatment Differs According to Skeletal Sites. A. Iida-Klein,[1] S. S. Lu,[1] M. Ducayen-Knowles,*[1] K. Yokoyama,*[1] J. Nieves,[2] D. W. Dempster,[1] R. Lindsay.[2] [1]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Clinical Research Center, Helen Hayes Hospital, West Haverstraw, NY, USA.

We have previously demonstrated that the anabolic action of parathyroid hormone (PTH) is skeletal-site specific in intact mice (ASBMR, 2000). However, the effects of PTH in mice with estrogen-deficiency are not defined. To determine whether ovariectomy (ovx)-induced bone loss is also skeletal-site specific in mice, and whether the PTH response in ovx mice is similar to that in intact mice, we initiated PTH treatment (hPTH 1-34, 40mcg/day, 5 days per week) in 16 week-old, female, sham-operated and ovx C57BL/J6 mice at 4 weeks of post operation for an additional 7 weeks (11 weeks total ovx). BMD was longitudinally measured in vivo by DEXA. At 4 weeks post-ovx, BMD was significantly reduced at all the skeletal sites examined. The magnitude of bone loss at 4 weeks was 9.8%, 5.3%, 5.1% and 3.8%, (all $p<0.05$) in the lumbar spine, total body bone, femur, and tibia, respectively. Lumbar spine BMD further decreased with time in ovx-control animals and was 17.8% below that of sham-control at 11 weeks after ovx. From 4 to 11 weeks post-ovx, age-related slight increases in BMD were evident at other skeletal sites in both sham and ovx animals. PTH partially reversed the ovx-induced bone loss in the spine in a time-dependent manner, but at the dose used failed to completely reverse bone loss after 7 weeks of treatment. However, PTH did reverse the ovx-induced bone loss completely in the tibia, the femur, and total body by 1, 3 and 7 weeks of treatment, respectively. The lumbar spine was the most sensitive site to estrogen deprivation, while the tibia was most sensitive to the anabolic action of PTH. In summary, ovx-mice are a good model of estrogen-deficiency bone loss and PTH alone reverses ovx-induced bone loss at most skeletal sites in mice. However, there is clear heterogeneity among skeletal-site response to both estrogen and PTH.

SA396

See Friday Plenary number F396.

SA397

Effects of Parathyroid Hormone on Bone Formation in a Rat Model for Chronic Alcohol Abuse. M. Zhang,* R. T. Turner, G. L. Evans,* J. D. Sibonga.* Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

Alcohol is a risk factor for osteoporosis and it is not clear whether the detrimental effects of alcohol on bone are reversible. Parathyroid hormone (PTH) is a potent stimulator of bone matrix synthesis and is being investigated as a therapeutic agent to reverse bone loss. The present investigation was designed to determine the effects of PTH on bone formation in a rat model for chronic alcohol abuse. Alcohol was administered in the diet of female rats (35% caloric intake) for two weeks. Human (I-34) PTH (80 µg/kg/d) was administered sc during the second week of the study. Alcohol resulted in a transient reduction in steady-state mRNA levels for the bone matrix proteins type 1 collagen, osteocalcin, and osteonectin compared to rats fed an alcohol-free (control) diet. As expected, alcohol decreased and PTH increased histological indices of bone formation and two-way ANOVA demonstrated that alcohol antagonized PTH-induced bone formation. In spite of antagonism, bone formation and mRNA levels for bone matrix proteins in alcohol-fed rats treated with PTH greatly exceeded the values in rats fed the control diet. The results of this study contribute to a growing body of evidence that alcohol-induced bone loss is primarily due to reduced bone formation. We conclude that alcohol does not prevent the stimulatory effects of PTH on bone formation. This is evidence that the effects of alcohol on the skeleton are reversible. Additionally, the positive effects on bone formation in rats consuming high concentrations of alcohol suggest that PTH may be useful as an intervention to treat alcohol-induced osteoporosis.

SA398

Effects of Basic Fibroblast Growth Factor on Bone Gene Expression in Aged Ovariectomized Rats. R. A. Power, T. J. Wronski. Physiological Sciences, University of Florida, Gainesville, FL, USA.

Histomorphometric studies indicate that basic fibroblast growth factor (bFGF) has a strong anabolic effect on bone. Treatment with bFGF in ovariectomized (OVX) rats results in markedly increased osteoblast surface compared to vehicle-treated OVX rats. Osteoblast, mesenchymal stem cell, and osteoprogenitor cell culture studies have yielded varied results regarding mRNA levels for bone matrix proteins in the presence of bFGF. The purpose of this study was to examine the effects of bFGF therapy on gene expression for osteocalcin (OC), type I collagen (COL I), and several growth factors and cytokines (IGF-I, TGF-β, IFN-γ), *in vivo*, in OVX Sprague Dawley rats. Animals were ovariectomized at 3 months of age, and maintained untreated for one year after surgery. At 15 months of age, catheters were inserted in the jugular veins of all rats for IV injections. One group of OVX rats was treated daily with bFGF (Chiron Corp., CA) for 14 days, at a dose of 200 µg/kg, then euthanized. Another OVX group was injected daily with vehicle. At necropsy, lumbar vertebrae were collected for RNA analyses. Total RNA was isolated from vertebral bodies using a guanidine thiocyanate-phenol-chloroform extraction method. Following northern blot analysis for mRNAs encoding bone matrix proteins, and RNase protection assay for mRNAs encoding growth factors and cytokines, mRNA levels were quantified by densitometric scanning of phosphor images. mRNA levels for OC and COL I, relative to 18S rRNA, were significantly higher in bFGF-treated OVX rats than in vehicle-treated OVX rats by a factor of greater than 10 ($P < 0.05$). IGF-I mRNA levels, relative to rL32 housekeeping gene, were also significantly higher, by nearly a factor of 3, in the bFGF-treated OVX rats ($P < 0.01$). Treatment of OVX rats with bFGF did not appear to affect message levels for TGF-β and IFN-γ. These *in vivo* results suggest that bFGF treatment upregulates gene expression for IGF-I, which may mediate, at least in part, the increased gene expression for bone matrix proteins and the bone anabolic effects of bFGF in aged OVX rats.

SA399

Statin Lipid-Lowering Drugs and Bone Density. D. H. Solomon,[1] J. S. Finkelstein,[2] P. S. Wang,*[3] J. Avorn.*[3] [1]Pharmacoepidemiology and Rheumatology, Brigham and Women's Hospital, Boston, MA, USA, [2]Endocrinology, Massachusetts General Hospital, Boston, MA, USA, [3]Pharmacoepidemiology, Brigham and Women's Hospital, Boston, MA, USA.

HMG Co-A reductase inhibitors (statins) have been associated with a reduced rate of fractures in some observational studies but not others. We are defining the relationship between statin use and bone mineral density (BMD) among 400 patients referred for DXA scans by their local physicians. After scanning, participants responded to a telephone survey assessing a range of known osteoporosis risk factors, use of anti-resorptive agents, and statin exposure. Among the first 245 patients enrolled in the study, 69 (28%) patients reported current or recent use (last 3 months) of statins. Mean age of respondents was 60 ± 11 years and 90% were female. Statin users and non-users were similar with respect to: age, gender, menopausal status (statin users 95% postmenopausal vs non-users 94%, P = 0.81); prior fracture history (statin users 42% vs non-users 33%, P = 0.16); use of an anti-resorptive agent (statin users 45% vs non-statin users 50%, P = 0.56); current or recent oral glucocorticoid use (statin users 3% vs non-statin users 8%, P = 0.15); ≥ 1 alcoholic drink per day (statin users 16% vs non-statin users 20%, P = 0.48); and current cigarette smoking (statin users 6% vs non-statin users 6%, P = 0.95). BMI was significantly higher in statin users vs non-users (27.2 ± 5.7 vs 25.0 ± 4.7, P = 0.005). Total BMD of the lumbar spine and proximal femur did not differ between statin users and non-users in crude analyses (see Table) or after adjustments for the above variables.

	Statin Use	No Statin Use	P
Total femoral BMD, mean (g/cm^2)	0.897 ± 0.144	0.867 ± 0.146	0.35
Total lumbar BMD, mean (g/cm^2)	0.946 ± 0.165	0.938 ± 0.135	0.82

These preliminary data fail to demonstrate a significant difference in lumbar spine or total hip BMD between statin users and non-users, though spine BMD was slightly higher in users. Our data suggest that if statin use is associated with a reduced risk of fractures, then factors other than BMD may be responsible for this effect. However, we cannot yet exclude the possibility that differences in BMD may be demonstrated with a larger sample size.

SA400

See Friday Plenary number F400.

SA401

Long-Term Strontium Ranelate Administration in Monkeys: Effects on Mineral Crystals and on the Degree of Mineralization of Bone. G. Y. Boivin,[1] D. Farlay,*[1] G. Panczer,*[2] I. Dupin-Roger,*[3] C. Simi,*[1] A. Buffet,*[1] I. Tupinon-Mathieu,*[3] P. J. Meunier.[1] [1]Laboratoire d'Histodynamique Osseuse, Faculté de Médecine R. Laennec, Lyon, France, [2]LPCML, CNRS UMR 5620, Université C. Bernard, Villeurbanne, France, [3]Institut de Recherches Internationales Servier, Courbevoie, France.

The analysis of the interactions of Strontium Ranelate (Protos®) with bone mineral is of interest as this agent has a dual effect on bone remodeling by increasing bone formation and decreasing bone resorption leading to prevention of bone loss and increase of bone

mass and strength in normal and ovariectomized rats. Strontium (Sr) has already been shown to be heterogeneously distributed in the bone of normal monkeys receiving Strontium Ranelate (SR) for 13 weeks with higher concentrations in new than in old bone (Boivin et al. 1996, JBMR 11:1302-11). Similar findings have been reported in post-menopausal osteoporotic women treated for 2 years with SR (Boivin et al. 2000, JBMR 15 suppl.1:S305). The interactions of Sr with bone mineral were investigated in monkeys after a long-term SR treatment and at the end of a recovery period. Iliac bones were obtained from 30 monkeys: 7 untreated controls, 12 sacrificed at the end of a 52-week SR treatment (200, 500, 1250 mg/kg/day PO) and 11 sacrificed 10 weeks after the end of a 52-week SR treatment (same 3 doses of SR). Sr uptake and distribution in bone mineral were quantified by X-ray microanalysis, changes at the crystal level were evaluated by X-ray diffraction, and the degree of mineralization of bone (DMB) was measured by quantitative microradiography. In monkeys sacrificed at the end of a 52-week SR treatment, Sr was uptaken in a dose-dependent manner into compact and cancellous bone, with higher contents (1.6 times) in new bone than in old bone. Sr content greatly decreased (1.7-2 times) in animals sacrificed 10 weeks after the end of treatment but this affects almost exclusively new bone and not old one. After SR treatment, there were no significant changes in crystal characteristics. Easily exchangeable in bone mineral, Sr was slightly linked to crystals by ionic substitution (generally less than 1 calcium ion substituted by 1 Sr ion in each unit cell). DMB was not significantly different in the various groups of monkeys. In conclusion, at the end of a long-term SR treatment and after a period of withdrawal, Sr was uptaken in a dose-dependent manner into new bone without alteration of DMB and with no major modification of bone mineral at the crystal level. These findings confirm the safety of Strontium Ranelate usage at the mineral level and of its potential interest as anti-osteoporotic treatment which simultaneously increases bone formation and decreases bone resorption.

Disclosures: Institut de Recherches Internationales Servier,2.

SA402

Effect of Melatonin on Bone Metabolism in Ovariectomized Rats. Dependence with Estradiol Serum Levels. M. G. Ladizesky,*[1] R. A. Cutrera,*[2] V. Boggio,*[2] J. Somoza,*[1] M. Centrella,*[2] C. A. Mautalen,[1] D. Cardinali.*[2] [1]División Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

The objective of this work was to assess the effect of pharmacological dose of melatonin on bone metabolism in ovariectomized (OVX) rats. Animals received melatonin in the drinking water (25 ug /ml water) or drinking water alone. Baseline and secuential determinations of urinary deoxypyridinoline (UD PYR) (a marker of bone resorption), calcium excretion, as well as circulating levels of calcium, phosphorous and bone alkaline phosphatase (BAP) activity (a marker of bone formation), were measured in adult rats for up to 60 days after OVX. At this time, the animals were killed, and bone mineral density (BMD), bone mineral content (BMC) and bone area (BA) of total body, were measured. UD PYR increased significantly after OVX by 51% (30 days after surgery) and by 47% (60 days after surgery) ($p<0.01$, anova dunnet´s t test). Melatonin treated OVX rats, showed a significantly decrease in UDPYR, 30 days after surgery, compared with values detected at the same time, in OVX rats drinking water, indicating a diminution in bone resorption. ($p<0.001$, anova dunnet ´s t test). Urinary calcium concentration was similar in the 4 experimental groups studied, as was the circulating calcium concentration at every time interval examined. Fifteen days after surgery, a significant increase in serum BAP levels occurred in OVX rats receiving melatonin as compared with their controls, indicating an increase of bone formation. Sixty days after surgery , BMD, BMC and BA decreased significantly in OVX rats, an effect not modified by melatonin. Serum estradiol decreased significantly by 30 days after OVX to attain values close to the limit of detection of the assay by 60 days after OVX. In summary, post-OVX disruption of bone remodeling (a highly regulated process in the mammalian skeleton) could be prevented in rats by administering a pharmacological amount of melatononin in a way apparently related to the levels of circulating estradiol. Further studies are needed to define the precise roles that melatonin plays in bone development and in formation and degradation of bone and their dependence of circulating estrogen concentration

SA403

Adenovirus-mediated VEGF-A Gene Transfer Induces Bone Formation In Vivo - A Preliminary Report. J. Huuskonen,[1] M. O. Hiltunen,*[2] M. Ruuskanen,*[3] A. Mähönen,*[2] A. Herranen,*[2] A. Mahonen,*[4] H. Kroger,[3] S. Ylä-Herttuala.*[2] [1]Department of Surgery, University of Kuopio, Kuopio, Finland, [2]A.I. Virtanen Institute, University of Kuopio, Kuopio, Finland, [3]Department of Surgery, Kuopio University Hospital, Kuopio, Finland, [4]Department of Biochemistry, University of Kuopio, Kuopio, Finland.

We tested the hypothesis that vascular endothelial growth factor (VEGF-A) transfer is an appropriate way to enhance recruitment of osteoblasts in vivo. We used adenovirus vectors (1.4x1010 particles) for targeted gene therapy in six sixteen weeks-old female New Zeland White rabbits. Adenovirus vectors (2 ml per injection) containing VEGF-A and lacZ genes were injected locally into three right distal femurs. Saline was injected into all six contralateral distal femurs. One week after the injections the femurs were collected for analyzes. X-Gal staining was used to quantify transfected bone marrow cells. Trabecular bone hard tissue histomorphometry of the distal femurs was performed to study the effect of gene transfer on trabecular bone turnover.Up to twenty percent of the bone marrow cells were transfected. Compared to contralateral saline injected side, VEGF-A injected trabecular bone had 7 % lower bone volume, 157 % higher osteoblast number, 155 % higher osteoblast surface, 61% higher osteoid volume and 32 % higher total erosion surface. When compared to unilateral bacterial lacZ received trabecular bone, VEGF-A bone had 17 % lower trabecular bone volume, 150 % higher osteoblast number, 137 % higher osteoblast surface, 171 % higher osteoid volume and 66 % lower total erosion surface.We have demonstrated that injection of adenovirus vector is capable of transfecting bone marrow cells in vivo with high efficiency. Our preliminary results suggest that adenovirus-mediated VEGF-A gene transfer induces bone formation via increasing osteoblast activity.

SA404

See Friday Plenary number F404.

SA405

Long-Term Treatment with Strontium Ranelate (S12911) Increases Vertebral Bone Mass Without Deleterious Effect in Mice. P. Delannoy,*[1] D. Bazot,*[2] B. Robin,*[3] Y. Tsouderos,[3] P. J. Marie.[1] [1]Inserm U349 affiliated CNRS, Lariboisiere Hospital, Paris, France, [2]Biologie Servier, Gidy, France, [3]Institut de Recherches Internationales Servier, Courbevoie, France.

It has been shown previously that strontium salts modulate bone metabolism in rats and mice. In this study, the effect of a long term treatment with strontium ranelate (S12911 - PROTOS®), has been investigated on bone metabolism in mouse caudal vertebrae. Strontium ranelate (200, 600 or 1800 mg/kg/d, i.e. 0.78, 2.34 or 7.03 mmol Sr^{2+}/kg/d) or vehicle, was given orally in the diet to normal 6-week old $B_6C_3F_1$ male and female mice over 104 weeks. Histomorphometric analysis of indices of bone formation and resorption were determined in the endosteal caudal vertebrae of 20 male and 20 female animals per group. A 104-week treatment with strontium ranelate dose-dependently increased the trabecular bone volume (BV/TV) by 25% and 59% in females treated with 600 and 1800 mg/kg/d, respectively ($p<0.05$ *vs* controls in both cases). This increase of BV/TV was linked to a similar increase in the mineralized bone volume (Md.V/TV): +27% and +62% in the same animals ($p<0.05$ *vs* controls in both cases). An increase of BV/TV was also observed in males treated with 200 and 1800 mg/kg/d (+17% and +38%, respectively, $p<0.05$ *vs* controls in both cases). In parallel, a dose-dependent increase of the osteoblastic surface (Ob.S/BS) was observed in treated males (+131% for the highest dose group, $p<0.05$ *vs* controls). In addition to this stimulatory effect on bone formation, strontium ranelate decreased histomorphometric indices of bone resorption in males and females. Osteoclast surface (Oc.S/BS) was decreased in a dose-related manner in females and was significantly different from the control group at the highest dose (-52%). Furthermore, the number of osteoclasts (N.Oc/T.Ar) was dose-dependently diminished by 30% to 47% in female mice ($p<0.05$ *vs* controls for each dose group). Osteoid thickness remained unchanged in all treated groups as compared with control animals, suggesting that strontium ranelate, even at the highest dose tested (1800 mg/kg/d), had no deleterious effect on bone mineralization. These findings show that, in mice, strontium ranelate simultaneously increases indices of bone formation and decreases indices of bone resorption. This results in increased vertebral bone mass in mice without deleterious effects on bone mineralization.

Disclosures: IRI Servier,2.

SA406

Growth Hormone (GH) Decreases Bone Mineral Mass and Bone Strength in Adult Female Rats Fed a Low Protein Diet. P. Ammann,[1] L. Rodriguez,[1] M. L. Aubert,[2] J. P. Bonjour,[1] R. Rizzoli.[1] [1]University Hospital, Div of Bone Diseases, Geneva, Switzerland, [2]University Hospital, Div of Pediatric Endocrinology and Diabetology, Geneva, Switzerland.

Isocaloric protein undernutrition is associated with accelerated bone loss and decreased bone strength. This seems to be related to a decrease in GH and/or IGF-I production and/or action. Whether GH administration can reverse the protein undernutrition-induced alterations in bone turnover, bone mineral mass and bone strength when the diet is low in protein, a dietary situation frequent in elderly, is not known. Six-month old female rats were fed isocaloric diets containing 2.5% (Low Protein, LP) or 15% (Normal Protein, NP) casein for 2 weeks. Then, GH (0.5 or 2.5 mg/day x kg BW) or its solvent were given subcutaneously to rats on either diet twice daily for 4 weeks. Proximal and midshaft tibia bone mineral density (BMD) and ultimate strength, together with serum osteocalcin and IGF-I were measured. In rat fed the NP diet, 4 weeks of GH did not significantly influence proximal tibia BMD (254.4±5.0 and 263.0±4.5 for 0.5 and 2.5 mg GH vs 253.1±4.9 mg/cm2 in controls, n=8 per group, x±SEM) nor bone strength (209.9±5.4 and 191.1±14.9 vs 208.0±11.9 N). In contrast, GH dose-dependently decreased BMD (248.8±6.4 and 234.0±4.7 vs 262.7±3.5, $p<0.05$ ANOVA) and bone strength (199.7±12.2 and 139.9±13.9 vs 225.7±9.4, $p<0.05$) in rats fed the LP diet. On both diets, GH caused a dose-dependent increase in IGF-I in NP and LP diets (585±17, 709±20 for 0.5 and 2.5 mg GH vs 476±22 ng/ml in controls, $p<0.05$, and 450±30, 556±29 vs 304±19, $p<0.05$, respectively). GH increased serum osteocalcin in rats on both NP (17.5±1.1, 25.7±2.5 vs 16.2±1.6 ng/ml, $p<0.05$) and LP (13.5±0.7, 18.7±1.6 vs 10.9±0.9, $p<0.05$) diets. Thus, whereas GH treatment increased IGF-I and osteocalcin levels on both NP and LP diets, it was associated with a negative bone balance and with a lower bone strength when administered to animals on a low protein diet. These results emphasize the major importance of dietary protein intake in the bone response to GH administration.

SA407

Simvastatin Did Not Prevent Bone Loss In Ovariectomized Rats. W. Yao,[1] C. Y. Li,*[1] R. W. Farmer,*[2] J. L. Chen,*[1] A. Mo,*[1] R. Cooper,*[2] P. Chmielewski,*[2] R. B. Setterberg,*[1] W. S. S. Jee,[1] M. W. Lundy.*[2] [1]Radiobiology Division, University of Utah, Salt Lake City, UT, USA, [2]Bone Biology, Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Statins decrease the hepatic biosynthesis of cholesterol and thereby reduce serum cho-

lesterol concentration. Some recent reports showed that they could also stimulate bone formation *in vivo* and *in vitro*. To investigate whether statins have beneficial effects on bone metabolism, we designed a study in which Sprague-Dawley rats were ovariectomized (OVX) at 6 months of age, allowed to lose bone for 60 days and followed with oral administration of Simvastatin at dose levels of 0.3, 1, 3 and 10 mg/kg/d for 60 days. Study endpoints included bone histomorphometry on the proximal tibial metaphysis (PTM) and the tibial diaphysis (TX) and mCT on the 5th lumbar vertebra (LV). As expected compared to sham, 60 days of OVX decreased cancellous bone volume in the PTM (41%) and LV (15%); with a continued bone loss 120 days post-OVX (PTM, 80%; LV 18%). The OVX-induced bone loss was accompanied by increased bone formation and resorption. Simvastatin did not cause significant changes in bone volume, bone formation rate or bone erosion surface when compared to 120d-OVX animals (Table).

Groups	**Sham**	**OVX**		120d	**Simvastatin**	
Parameters	60d	0.3mg	1mg	3mg	10mg	
PTM	BV/TV	10.6±3.3*#	7.6±1.7	3.1±1.9	4.0±1.4	3.7±1.5	4.6±1.8
BFR/BS	23.7±4.5*	35.7±1.7	31.5±3.5	27.9±2.0	30.6±2.9	31.4±1.5	28.7±5.9
ES	3.4±1.4*	12.9±3.4	12.2±3.9	10.3±3.0	10.5±1.0	10.76±0.76	10.7±2.3
TX	Ps-BFR	15.4±19.0#	74.9±28.1	30.5±23.2	30.1±4.3	37.0±18.1	13.5±10.4
Ec-BFR	6.6±12.7*#	19.4±6.9	28.1±11.8	20.2±6.9	34.4±7.5	27.9±8.5	24.7±8.9
Ec-ES	5.5±2.7*#	14.1±4.3*	9.4±6.4	10.4±1.8	7.5±2.9	9.1±3.2	11.3±8.1
LV	BV/TV	41.0±1.9*#	34.8±2.8	33.5±2.1	33.2±1.8	31.5±2.4	33.0±2.1
CortTh	201.5±17.1*#	172.1±17.2	177.6±6.3	175.6±2.8	167.6±12.7	174.1±11.7	174.5±8.7

Mean ± SD; *p < 0.01 vs 120d-OVX; # p < 0.01 vs 60d-OVX; BV/TV, bone volume (%); BFR, bone formation rate ($\mu m^3/mm^2/d$); ES, eroded surface (%); Ps, periosteal surface; Ec, endocortical surface; CortTh, cortical thickness. In conclusion, this study demonstrated that Simvastatin did not prevent cancellous bone loss and did not stimulate cancellous and cortical bone formation following ovariectomy in the PTM, TX and LV.

SA408

Statins and Bone Mineral Density. S. Yaturu,*[1] M. G. Alferos,*[1] C. Deprisco,*[2] J. Tynes,*[3] S. Wade.*[4] [1]Endocrinology, OvertonBrooks VAMC/ LSUHSC, Shreveport, LA, USA, [2]OvertonBrooks VAMC/LSUHSC, Shreveport, LA, USA, [3]Medicine, LSUHSC, Shreveport, LA, USA, [4]Pharmacy, OvertonBrooks VAMC, Shreveport, LA, USA.

Treatment of osteoporosis remains a major challenge despite an increase in the choice of therapeutic agents. Experimental evidence has shown that the statins, the cholesterol lowering agents, increase the bone formation. We reviewed the bone density studies of patients who were on statins for at least one year (12-15months). Additionally, twenty-seven of them were on other antiresorptive agents like estrogens or bisphosphonates or calcitonin. We compared the changes in the bone density of those on statins alone with the changes in the bone density (BMD) of those on statins and other antiresorptive agents.

Change in BMD±Standard error

Site	Statins alone	Statins+antresorptive	*p*
Spine:Total	0.009±0.02	-0.005±)0.002	0.22
RightHip:Total	0.004±0.01	-0.004±0.004	0.16
Left Hip:total	-0.01±0.01	3.7±0.004	0.17

We did not observe any significant differnce between the changes in BMD whether they were on statins alone or other antiresorptive agents in addition to statins.We conclude that addition of antiresorptive agents to statins may not improve the BMD further

SA409

Short-Term Treatment With Atorvastatin Does not Change Bone Turnover in Patients With Hypercholesterinemia: A Randomised, Controlled Study. P. Salbach,*[1] J. Kreuzer,*[2] M. J. H. Seibel,[3] [1]Dept. of Cardiology, University of Heidelberg, Heidelberg, Germany, [2]Cardiology, University of Heidelberg, Heidelberg, Germany, [3]Dept. of Endocrinology, University of Heidelberg, Heidelberg, Germany.

Osteoporotic fracture risk has been reported to be significantly lower in patients treated with statins than in subjects treated with non-statin lipid lowering drugs or non-users. In one study, the statin effect was already observed after short-term treatment. So far, however, no data are available regarding the acute and short-term effect of statins on bone turnover.In a prospective study, 54 patients with hypercholesterinemia (mean age: 65.5 ± 9.7 yrs) were randomised to either 40 mg Atorvastatin (AS) b.i.d. or a low fat diet. Blood and urine samples were collected 0, 3 and 30 days into the study. Serum bone specific alkaline phosphatase (bALP) and osteocalcin (OC; ng/mL), and urinary carboxyterminal telo-peptide (CTX-I, pmol) were determined in 23 male (mean age 62.8 ± 8.8) and 11 female (mean age 64.5 ± 12.1) patients treated with AS, and in 13 male (mean age 65.9 ± 8.4) and 7 female (mean age 67.4 ± 9.5) patients receiving diet. All values were expressed as mean ± SD.In the AS treated group, bALP decreased significantly (p< 0.01) from day 0 to day 3 (19.2 ± 8.3 to 16.8 ± 7.2 U/L), and returned to baseline levels at day 30 (19.4 ± 8.8 U/L). These changes were more pronounced in males than in females. In the diet group, bALP decreased non-significantly and remained at that level over 30 days. Serum OC levels remained unchanged in both groups. Urinary CTX excretion decreased significantly (p< 0.05) from day 0 to day 3 (2914 ± 1454 to 2585 ± 1287 pmol) and remained at that level until day 30. Again, changes were more pronounced in males than in females. In contrast, no significant changes were observed in the group receiving diet.We conclude that except for minor (though statistically significant) changes 3 days after initiating AS treatment, no systematic changes in bone turnover were observed in this study. Therefore, neither AS nor a fat modified diet seem to affect bone metabolism on a short term basis. This does not exclude alterations in bone turnover over longer periods of time.

SA410

Statin Increases Cortical Bone in Young Male Rats by Single, Local Administration but Fails to Restore Bone in Ovariectomized (OVX) Rats by Daily Systemic Administration. D. T. Crawford, H. Qi, K. L. Chidsey-Frink, H. A. Simmons, D. D. Thompson, H. Z. Ke. Pfizer Global Research and Development, Groton Labs., Groton, CT, USA.

HMG-CoA reductase inhibitors (statins) are widely used as cholesterol-lowering agents. It was recently reported that statins may protect against skeletal fractures. We attempted to characterize the local and systemic skeletal effects of one of the statins, lovastatin, in young male rats and in an established osteopenic, OVX rat model. In the first study, a single injection of either vehicle (n=8) or lovastatin at 3 mg/kg (n=8) was given locally through the cortex into the bone marrow cavity of the proximal tibial metaphysis of 6-week-old male rats. Seven days post-injection, the rats were necropsied and the injection site was analyzed by pQCT and bone histomorphometry. Compared with vehicle controls, lovastatin significantly increased cortical bone area (+17%), cortical thickness (+13%) and axial area moment of inertia (+29%). In addition, a non-significant increase in total content (+12%) and area (+11%), trabecular content (+30%) and periosteal circumference (+5%) was found in lovastatin-treated rats compared with vehicle-treated rats. Histomorphometric results showed that periosteal and endocortical bone formation increased in lovastatin-treated rats compared with vehicle-treated rats. In the second study, S-D female rats were sham-operated or OVX at 3.5 months of age. The OVX rats were untreated for 6 weeks to allow development of osteopenia. Thereafter, OVX rats were treated with either vehicle (n=10) or lovastatin at 10 mg/kg (n=10) by daily s.c. injection for 4 weeks. Compared with sham controls, OVX significantly decreased total content and density, trabecular density, cortical content and cortical thickness in the distal femoral metaphysis as determined by pQCT analysis. The above parameters in lovastatin-treated OVX rats did not differ significantly from vehicle-treated OVX controls, indicating no skeletal effect of lovastatin on bone in this OVX rat model. Similarly, lovastatin had no effect on trabecular bone volume, trabecular number or bone turnover parameters in proximal tibial metaphyseal cancellous bone in OVX rats. In conclusion, we found that lovastatin increases cortical bone in young male rats by single, local administration to the bone marrow cavity but fails to restore bone in OVX rats by systemic, daily administration. The mechanism for the different response to local and systemic administration of lovastatin is not clear.

Disclosures: Pfizer Inc.,3.

SA411

Decreased Bone Turnover in Postmenopausal Women Treated with Statins: A Cross-Sectional Study. L. Rejnmark,[1] N. H. Buus,*[1] P. Vestergaard,[1] F. Andreasen,*[2] M. L. Larsen,*[3] L. Mosekilde.[1] [1]Dept of Endocrinology and Metabolism, Aarhus University Hospital, Aarhus, Denmark, [2]Dept of Clinical Pharmacology, Aarhus University Hospital, Aarhus, Denmark, [3]Dept of Medicine and Cardiology, Aarhus University Hospital, Aarhus, Denmark.

Statins have been suggested as potential agents in the management of osteoporosis. In a cross-sectional design, we compared 140 postmenopausal women who had been treated with a statin for more than two years (median 4 years) with 140 age- and gender-matched population based controls.We studied the effects of treatment with statins on calcium homeostasis, bone turnover, bone mineral density, and body composition. Plasma PTH levels were 16% higher in the statin treated subjects than in the controls (p<0.01). Conversely, plasma levels of biochemical bone markers were lower in the statin treated subjects than in the controls: osteocalcin (-9%, p=0.03), bone-specific alkaline phosphatase (-14%, p<0.01), and CTx (-11%, p<0.01). No correlation could be demonstrated between changes in biochemical quantities and dose or duration of statin use. Moreover, body composition and BMD at the lumbar spine, hip, forearm, and whole body did not differ between the two groups. Our data suggest that statins reduce bone turnover and exert an antiresorptive effect on bone metabolism. Prospective studies are needed in order to resolve whether this effect is clinical relevant in the management of osteoporosis.

SA412

Fluvastatin and Cerivastatin Are Not Anabolic for Bone After Local or Systemic Administration of Non-Toxic Doses in Mice and Rats. J. A. Gasser. Arthritis & Bone Metabolism, Novartis Pharma AG, Basel, Switzerland.

The purpose of the experiments was to extend the knowledge on the proposed bone anabolic properties of HMG Co-A reductase inhibitors (statins) following their local administration over calvaria in mice (fluvastatin) or daily oral administration in rats (cerivastatin).Male Swiss mice were injected locally twice daily over the central calvaria with fluvastatin at concentrations ranging between 0.001 and 100mM or with 100nM hPTH(1-34) for 5 consecutive days, and sacrificed on day 14 for histomorphometric analysis of fluorochrome labels.Seven month old skeletally mature intact or ovariectomized vir-

gin Wistar rats were administered daily by gavage with 0.01 or 0.1mg/kg of cerivastatin for 4 weeks and changes in cancellous and cortical bone parameters monitored in the proximal tibia metaphysis by pQCT.Local administration of fluvastatin at concentrations between 1 and 100mM led to acute local capillary damage and visible bleeding forcing the termination of the highest dose. Treatment with lower doses did induce formation of woven bone but only at doses where histological hemorrhage was detected (1 and 10mM). The increase in the mineral apposition rate was statistically significant at 10mM ($p<0.01$, Dunnett-test). Our results suggest that the measured bone activity may represent a damage repair response rather than a direct anabolic property of the compound.In estrogen competent rats, daily oral administration of cerivastatin did not induce any change in cancellous or cortical bone mass, density or structural cortical parameters as measured by pQCT which would be indicative of a bone anabolic response. Similarly, the significant decrease in cancellous bone mineral density (-15.3%, $p<0.01$) and cortical thickness (-14.5%, $p<0.01$) or any of the other bone parameters in OVX-rats was not reduced by daily administration of cerivastatin. Total serum cholesterol was reduced significantly in OVX-rats at 0.01 (-14.6%, $p<0.01$) and 0.1mg/kg (-19.8%, $p<0.01$).Taken together, these studies in mice and rats are not supportive for the proposed anabolic activity of statins in bone and suggest, that disruption of capillary integrity and local bleeding may explain some of the previously reported bone responses in the mouse calvaria model.

Disclosures: Novartis,3.

SA413

Effect of Statins on Bone Mass and Turnover in Ovariectomized Rats. P. Masarachia, G. Wesolowski, J. G. Seedor, C. Weiss,* B. L. Pennypacker,* M. A. Gentile, G. A. Rodan, D. B. Kimmel. Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA, USA.

Statins, cholesterol controlling drugs, have been reported to reduce fractures in humans and prevent cancellous bone loss in ovariectomized (OVX) rats, via stimulation of bone formation. The object of this study was to compare skeletal effects of simvastatin (SIM) and atorvastatin (ATOR) to those of alendronate (ALN) and PTH in adult OVX rats.Eight months old virgin female rats were OVXd or Sham-OVXd. OVX rats were treated for 35 days with: vehicle; 10mg/kg/d po SIM; 10mg/kg/d po ATOR; 0.01mg/kg SC 3X/week ALN; or 0.08mg/kg SC 3X/week PTH(1-34). We measured: urinary deoxypyridinoline/creatinine (uDPD); static and dynamic histomorphometry of proximal tibial metaphysis using dual fluorochrome labeling; including cancellous bone volume (BV/TV, %), osteoclast surface (OcS/BS, %) and mineralizing surface (MS/BS, %); and distal femoral metaphyseal bone mineral density (DF-BMD, mg/cm2) by DXA (Hologic 4500A). Groups (N=13) were compared by ANOVA with Fisher PLSD post-hoc test. Results show that:

Variable	Sham	Vehicle	SIM	ATOR	ALN	PTH
DF-BMD	180±19*	160±14	169±11	163±18	180±12*	185±12*
BV/TV	24±6*	14±6	20±5&	18±7#	24±5*	27±7*
uDPD	20±5*	48±12	55±15	47±14	22±8*	52±23
OcS/BS	3.6±1.3&	5.2±2.3	5.1±1.6	4.9±1.0	3.6±1.3*	6.0±1.6
MS/BS	20±9*	37±7	30±6&	31±4#	15±4*	46±15&

Mean±SD; #diff from OVX (P<.11); &diff from OVX (P<.05); *diff from OVX (P<.001)

1) ALN or PTH fully prevented OVX-induced bone loss; SIM was partially effective [BV/TV]; 2)Only ALN lowered uDPD and osteoclast surface; 3)ALN suppressed and PTH augmented, the OVX-induced rise in formation; and 4)SIM and ATOR partially reduced that rise in formation. We conclude that SIM but not ATOR partially prevented OVX-induced bone loss. Although no suppression of uDPD or osteoclast surface occurred, SIM's modest bone loss prevention effect here seems likely due to inhibition of bone resorption, since both statins tended to reduce fluorochrome labeled surface (MS/BS) below OVX+VEH.

SA414

See Friday Plenary number F414.

SA415

Effects of Short-term Cerivastatin on Bone Turnover. F. Cosman, J. Nieves, M. Zion,* J. Cruz,* S. Gordon,* R. Lindsay. Clinical Research Center, Helen Hayes Hospital, West Haverstraw, NY, USA.

Since statin drugs share a common mechanism of action with bisphosphonates in the cholesterol synthesis pathway was hypothesized that statins could also affect the skeleton. In vitro and in vivo animal data suggested that statins could stimulate bone formation and inhibit bone resorption but observational studies on statins and fracture risk have been mixed and the few clinical trial data are not definitive. To help settle the controversy, we performed a short-term randomized blinded clinical trial to determine if cerivastatin (Becol, Bayer Corp) could increase biochemical variables of bone formation and/or reduce biochemical indices of bone resorption. Fourteen postmenopausal, healthy women, mean age 58, on no medications known to influence bone metabolism, were randomly assigned to receive cerivastatin 0.4 mg/d or an identically appearing placebo for 12 weeks. Biochemical indices of bone formation (propeptide of Type I procollagen, PICP and osteocalcin, OC) as well as bone resorption (urinary N-telopeptide, NTX and C-telopeptide, CTX) were obtained in the AM in the fasting state at baseline, and 1, 2, 4, 6, 8 and 12 weeks after treatment assignment. Compliance with medication was >90%. There were no demographic differences (years from menopause, height, weight, baseline BMD) between groups and mean baseline biochemical indices were the same in both groups. During the 3-month follow up, there were no significant differences in either variable of bone formation in the cerivastatin group compared to baseline or compared to the placebo group. However, biochemical indicators of bone resorption were modestly reduced within 6 weeks by <20% in the cerivastatin group (NTX, P = 0.08; CTX, P = 0.19). These results suggest that in vivo cerivastatin does not substantially stimulate bone formation, and therefore, is unlikely to produce a significant anabolic effect on bone mineral density. However, cerivastatin might have modest anti-resorptive potential.

SA416

Effect of Simvastatin on Three-Dimensional Trabecular Architecture of Ovariectomized Rats. Y. Jiang,[1] J. Zhao,[1] G. Gutierrez,[2] G. R. Mundy,[2] I. R. Garrett,[2] H. K. Genant.[1] [1]Osteoporosis and Arthritis Research Group, University of California, San Francisco, CA, USA, [2]OsteoScreen, San Antonio, TX, USA.

The statins, orally bioavailable drugs that decrease hepatic cholesterol biosynthesis, are commonly prescribed and administered to patients to reduce serum cholesterol concentrations and lower the risk of heart attack, and have shown to be bone activation agents by enhancing the expression of BMP-2 gene in bone cells. This study evaluated effect of the statin simvastatin, administered systemically on trabecular structure and connectivity of ovariectomized (OVX) rats after oral administration, using µCT, a non-destructive advanced imaging technique. Unlike traditional histomorphometry based on 2D sections parallel plate model, µCT directly measures three-dimensional (3D) trabecular architecture without stereological model assumption, which may improve our ability to understand the pathophysiology of osteoporosis and to estimate bone biomechanical properties as the mechanical competence of trabecular bone is a function of its apparent density and 3D distribution. Thirty 3-month old virgin female SD rats were equally divided, based on body weight, into 3 groups: 1) Control: sham-OVX rats receiving vehicle (sham rats), 2) OVX: OVX rats receiving vehicle (OVX rats), and 3) OVX treated: OVX rats receiving simvastatin at a dose of 10 mg/kg/day by oral gavage for 35 days. Treatment with the statin started one day post-OVX. Animals were pair fed. After 5 weeks the animals were sacrificed by anesthetic overdose, and the left femur was dissected and scanned using a µCT scanner with isotropic resolution of 11 µm. 3D trabecular structure in the secondary spiongiosa in the distal femur was directly measured. There was a significant change in 3D trabecular bone volume fraction (BV/TV, -51%), trabecular number (Tb.N, -49%), trabecular thickness (Tb.Th, -34%), trabecular separation (Tb.Sp, +68%), structure model index (SMI, +30%), degree of anisotropy (DA, +2.5%), and connectivity density (CD, -58%) in OVX rats (group 2), compared with those in sham rats (group 1). Compared with OVX treated with vehicle (group 2), simvastatin treatment (group 3) caused a significant change in 3D BV/TV (+81%), Tb.N (+32%), Tb.Th (+52%), Tb.Sp (-22%), SMI (-22%), DA (-3.4%), and CD (+62%). 3D trabecular thickness was preserved at the sham level after simvastatin treatment. Thus, OVX induces deterioration of 3D trabecular structure in rats. The trabeculae become more rod-like (indicated by structure model index) and more isotropic after OVX. Simvastatin treatment appears to prevent OVX-induced bone loss.

SA417

Effect of Disodium Pamidronate on the Interface of External Fixation Half Pins and Fracture Healing. K. Yang,* J. Jahng, J. Park,* E. S. Kang.* Orthopaedic Surgery, Yonsei University College of Medicine, Seoul, Republic of Korea.

The purpose of this study was to evaluate the antiresorptive effect of the bisphosphonate around the metal implant, which was inserted in the bone and its influence on fracture healing and stress-shielding effect. Twelve dogs were divided into 2 groups; Six dogs were treated with disodium pamidronate (0.5mg/kg, im injection) every two weeks for 6 weeks (Group 1) and the other six dogs were treated with placebo (Group 2). External fixators with 6 pins were applied on both tibiae. Perpendicular osteotomy was performed at mid shaft of the right tibia and gap of one millimeter was made in all 12 dogs. Right side was for evaluation of fracture healing and pin bone interface in unstable situation. Left side was for evaluation of stress-shield effect. Torque strength (extraction torque of the pin) could not be measured in 4 cases of 72 pins in Group1 and in 12 cases of 72 pins in Group 2 ($p<0.01$). It was due to pin loosening and infection at pin bone interface. The means of torque strength in measureable pins were 0.97 ± 0.50Nm in Group 1 and 0.94 ± 0.51Nm in Group 2. It was statistically insignificant. Results of maximal breakage strength of the right tibia, which was measured by 3 point bending test, showed no difference between Group 1 (227 ± 129Nm) and Group 2 (256 ± 45Nm). That means that pamidroanate had no deteriorative effect on fracture healing. Three point bending test in left tibia showed 202 ± 25Nm in group 1 and 68 ± 16Nm in group 2. Stress-shielded left tibia showed significantly higher ultimate strength in Group 1 ($p<0.01$). Disodium parmidronate, an aminobiphosphonate, could decrease the incidence of severe pin loosening and prevent bone loss in stress-shielded tibia. It did not deteriorate fracture healing process.

SA418

A Herbal Extract and its Major Component Induce Growth Hormone Release and Prevent Bone Loss in Ovariectomized(OVX) Rats. C. Kim,[1] H. K. Ha,[1] J. H. Lee,*[1] J. S. Kim,*[1] K. Y. Song.*[2] [1]Korea Institute of Oriental Medicine, Seoul, Republic of Korea, [2]College of Medicine, Chung-Ang University, Seoul, Republic of Korea.

Estrogen deficiency after menopause induces bone loss and results in osteoporosis. To

find any estrogen replacement from herbs, effects of herbal extract (F4) and BB, a major compound from the F4 extract, were investigated on growth hormone (GH) release in a pituitary cell culture and on osteoporosis in vivo and in vitro models. Proliferations of osteoblast (MG-63, Saos-2) were tested with MTT and alkaline phosphatase (ALP) assays. Adult OVX SD rats (10 weeks old) were divided into eight groups; sham, control, 17-beta-estradiol at 1 micro-g/kg/day (E2), OVX and various concentrations of F4 and BB (F41, F42, F410, BB1, and BB10). Animals were given an i.p. injection everyday for 9 weeks. Trabecular bone areas (TBA) of tibia and lumbar spine were measured by bone histomorphometry. Plasma levels of ALP, calcium, inorganic phosphate, cholesterol and HDL-cholesterol were analyzed. In results, extract F4 and BB were induced GH release upto 157% and 166% of control corresponding to 407 nM and 446 nM of GH-releasing factor (GRF) using rat pituitary cells. F4 increased cell proliferations of MG-63 and Saos-2 and BB stimulated ALP activity of Saos-2. And F4 inhibited osteoclast activity a little less than genistein, a phytoestrogen. Plasma levels of HDL-cholestrol decreased and those of LDL-cholesterol inceased during the study in both F4 and BB treated groups. Plasma concentrtions of ALP decreased following ages. TBA of tibia in control group was reduced 58.1% compared to sham ($P<0.01$). The TBAs of tibia in F41, BB1, and BB10 groups were increased upto 143% of control ($P<0.01$) which was similar to that in E2 group. But TBAs of tibia in F42 and F410 groups were not increased. Interestingly, TBA of lumbar in BB1 and BB10 groups were increased significantly ($P<0.01$). In conclusions, herbal extract F4 and BB, a major compound of F4, prevent OVX-induced cancellous bone loss for 9 weeks in OVX rats.

SA419

See Friday Plenary number F419.

SA420

Melatonin Could Prevent Cancellous Bone Loss in Ovariectomized Rats. Y. Hattori,* T. Kaku, H. Koyama, O. Nakade.* Health Sciences University of Hokkaido, Ishikari-Tobetsu, Japan.

We have recently demonstrated that daily I.P. injection of melatonin (aMT) could increase cancellous bone mass in young growing mice in vivo, mostly through suppressing bone resorption. The present study was to determine whether aMT could prevent bone loss in ovariectomized (OVX) rats. Female rats were sham-operated or OVX at 6 months of age and treated for 4 weeks with vehicle or aMT. Sham-operated control rats (CON + VEH) and one group of OVX rats (OVX + VEH) were injected I.P. with vehicle between 3 and 5 p.m. The remaining OVX rats were injected I.P. with either 10 (OVX + 10 aMT) or 50 (OVX + 50 aMT) mg aMT / kg BW / day at the same time with vehicle-injected groups. The proximal tibial metaphyses were processed undecalcified for quantitative bone histomorphometry osteopenic with a cancellous bone volume at only 50 % of vehicle-treated control level. This bone loss was derived from increased indices of bone turnover such as osteoclast surface, osteoid surface and bone formation rate. Compared to vehicle-treated OVX group, aMT treatment with low dose (10 mg/kg /day) increased cancellous bone volume by 80 % and decreased osteoclast surface, osteoid surface and bone formation rate. However, these effects by aMT treatment were minimized at high dose (50 mg/kg/day) and increased osteoid surface without a significant effect on mineral apposition rate was apparent, suggesting that aMT treatment could exert to increase bone formation at high dose. These results indicate that melatonin treatment could prevent bone loss in OVX-rats through decreased bone resorption activity, although melatonin would induce diverse effects on bone depending on its dose.

SA421

Combined Assessment of Both Bone Formation and Bone Resorption for Improved Prediction of Long-Term Skeletal Effect of Anti-Resorptive Therapy. P. Qvist,[1] E. G. Henriksen,[1] N. Bjarnason,*[2] P. Ravn,*[2] C. H. Christiansen.[2] [1]Osteometer Biotech, Herlev, Denmark, [2]CCBR, Ballerup, Denmark.

Markers of bone turnover have been applied for monitoring of anti-resorptive therapy, partly because they, in contrast to image technologies, respond rapidly to medical intervention. Several studies have demonstrated that early change in markers following anti-resorptive therapy predict treatment efficacy as assessed by measurements of bone mineral density over several years. Recently statistical models have been developed to improve both sensitivity and specificity of the biochemical prediction of the long-term BMD response to treatment (1-3). However, none of the models included both a bone resorption marker and a bone formation marker and we therefore investigated if such a combined model could improve prediction of the long-term skeletal effect of anti-resorptive therapy.We performed the data analysis on prospective studies of hormone replacement therapy (HRT) (2) and alendronate (4) in prevention of postmenopausal osteoporosis. In brief, the calculations included data from individuals receiving either 2 mg estradiol (combined sequentially with gestodene) or placebo (n=56), or for the bisphosphonate study either 1, 5, 10 or 20 mg of alendronate or placebo (n=67).The data analysis demonstrated that Serum CrossLaps and N-MID Osteocalcin ELISA were independent predictors of change in bone mineral density of the spine in postmenopausal women receiving bisphosphonate or HRT. In a logistic regression analysis combining the change of Serum CrossLaps and N-MID osteocalcin during 6 months of therapy the sensitivity and specificity was in the range 85-95% for early prediction of bone gain (alphaBMD>0) and bone loss (alphaBMD<0), respectively, which was better than each of the markers alone.(1) Garnero et al., Bone (1999) 24:603-609. (2) Bjarnason and Christiansen, Bone (2000) 26:561-569. (3) Delmas et al., Bone (2000) 26:561-569. (4) Ravn et al., Bone (1999) 24:237-244.

Disclosures: Osteometer Biotech,1.

SA422

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SA423

Osteoprotegerin (OPG) Abrogates Chronic Alcohol Ingestion-induced Bone Loss in Mice. J. Zhang,[1] J. Dai,*[2] D. Lin,[2] P. Habib,*[2] P. Smith,*[2] J. Murtha,*[2] Z. Fu,*[3] E. T. Keller.[4] [1]Department of Pathology, University of Michigan, Ann Arbor, MI, USA, [2]ULAM, University of Michigan, Ann Arbor, MI, USA, [3]Department of Immunology, University of Michigan, Ann Arbor, MI, USA, [4]Pathology, ULAM, Immunology, University of Michigan, Ann Arbor, MI, USA.

Alcoholism is a widespread problem accompanied by many pathophysiological manifestations. For example, a large proportion of chronic alcoholics have osteopenia. There is evidence that alcohol (ethanol) induces bone loss through both inhibition of osteoblast activity and induction of osteoclast activity. Osteoclastogenesis occurs through the binding of receptor activator of NFkB (RANK) ligand, which is present on osteoblasts, to RANK, which is found on the osteoclast precursors. Osteoprotegerin (OPG), a protein produced by osteoblasts and bone marrow stromal cells, binds RANK ligand, effectively blocks RANKL from binding to RANK, and thus inhibits osteoclastogenesis. We have previously documented that alcohol induces osteoclast activity and bone resorption in mice. In addition, we showed the ethanol-induced osteoclast activity was associated with increased RANKL mRNA expression in bone marrow cells. Thus, we hypothesized that inhibition of RANKL should diminish ethanol-induced bone loss. Accordingly, the purpose of this study was to determine if the RANKL inhibitor, OPG, could inhibit alcohol-mediated bone loss in a murine model. To investigate the role of osteoprotegerin (OPG) on alcohol (ethanol)-mediated osteoporosis, we measured a variety of bone remodeling parameters in mice that were either on a control diet, an ethanol (5%) diet, or an ethanol (5%) diet plus OPG administration. OPG diminished the ethanol-induced decrease of bone mineral density as determined by dual-energy densitometry. OPG also abrogated (1) ethanol-induced decrease of cancellous bone volume and trabecular width and the increase of osteoclast surface as determined by histomorphometry of the femur, (2) increased urinary deoxypyridinolines as determined by ELISA, and (3) increased colony forming unit-granulocyte monocyte (CFU-GM) formation and osteoclastogenesis as determined by ex vivo bone marrow cell cultures. Additionally, OPG diminished ethanol-induced decrease of several osteoblastic parameters including osteoblast formation and osteoblast culture calcium retention. These findings were supported by histomorphometric indices in the distal femur. Taken together, these data demonstrate that OPG diminishes ethanol induced bone loss. Furthermore, they suggest that OPG achieves this through it ability to abrogate ethanol-induced promotion of osteoclastogenesis and promote osteoblast proliferation.

SA424

Anti-Resorptive and Survival Actions of Selective Integrin Peptidomimetics on Authentic Human Osteoclasts. P. Collin-Osdoby,[1] F. Anderson,[1] L. Rothe,[1] X. Yu,[1] Y. Huang,*[1] W. Maloney,*[2] W. Westlin,*[3] G. A. Nickols,[3] P. Osdoby.[1] [1]Biology, Washington University, St. Louis, MO, USA, [2]Orthopedics, Washington University, St. Louis, MO, USA, [3]Pharmacia, St. Louis, MO, USA.

Mature osteoclasts (OC) undergo functional cycles of migration and resorption, processes dependent upon specific cell surface integrins. The integrin $\alpha v\beta 3$ becomes highly expressed in OC development, mediates OC motility and attachment to RGD-containing extracellular matrix proteins, and is crucial for OC bone resorption and survival in vivo and in vitro. Conversely, RGD ligands and $\alpha v\beta 3$ antagonists inhibit OC function and prevent bone loss in ovariectomized animals. However, the precise mechanisms of action and specificity of integrin antagonists for human OC (HOC) have not been well characterized. Here, we investigated two integrin peptidomimetics designated S787 and S783, with S787 ($IC50 = 0.7$ nM) being a more potent inhibitor than S783 ($IC50 = 14.7$ nM) for human $\alpha v\beta 3$ binding, for their effects on HOC function and survival. Authentic HOC were isolated from femoral heads obtained during hip replacement surgery, cultured (10 days) on ivory with or without S783 or S787 (0.01 to 10 uM), and the ivory analyzed for TRAP stained HOC and resorption pits. Both peptidomimetics dose-dependently inhibited HOC pit resorption via decreasing the mean number of HOC remaining attached to the ivory, the number of pits formed per HOC, the area resorbed per HOC, and the size of individual pits. Inhibition was more pronounced with S787 than S783 and was maximal (up to 95%) at 1 uM of either S787 or S783. Attachment assays using in vitro RANKL-induced monocyte-generated OC (HMN-OC) demonstrated that the number of HMN-OC binding to ivory within 1 h was reduced by 55% or 65% in the presence of 1 uM S787 or S783, respectively, compared to the number of HMN-OC that attached in the absence of these agents. Whether decreased HOC numbers also reflected increased apoptosis was evaluated using HOC or HMN-OC treated with S787 or S783 (0.01 to 10 uM, 20 h) and then stained with annexin V-FITC. Both peptidomimetics elicited HOC and HMN-OC apoptosis (but not necrosis), S787 was more potent than S783 for inducing apoptosis, and maximal HOC apoptosis (2 to 4-fold over control levels) occurred with 0.1 uM S787 or 10 uM S783. An RPA multiprobe kit was used to quantify potential changes in bcl family gene expression during peptidomimetic-induced apoptosis of HMN-OC (at 6 and 24 h) and revealed decreases in anti-apoptotic bclx (6 h) and bfl1 (6, 24 h). Thus, integrin peptidomimetics potently and dose-dependently inhibit HOC bone pit resorption, induce HOC apoptosis via a bcl-related pathway, and may exert differential effects as a function of their $\alpha v\beta 3$ potency.

SA425

See Friday Plenary number F425.

SA426

Celecoxib Does Not Prevent Osteopenia or Impair the Action of Anti-resorptive Therapies in Ovariectomized Rats. S. L. Settle,*[1] R. K. Rader,*[1] S. Hornby,[2] G. A. Nickols,[1] M. A. Thiede.[1] [1]Discovery Pharmacology, Pharmacia Corporation, St. Louis, MO, USA, [2]SkeleTech Inc, Bothell, WA, USA.

Prostaglandins play a role in bone cell function, and several studies have suggested that administration of COX nonselective NSAIDs can influence skeletal metabolism. New selective inhibitors of COX-2 now make it possible to test the role of COX-2 in high turnover bone loss associated with ovarian hormone deficiency. The study aims were to determine the effect of selective COX-2 inhibition on ovariectomy (OVX)-induced bone loss and COX-2 selective inhibition on anti-resorptive therapy in this model. Sprague-Dawley rats (6 mo) underwent either OVX or sham-operation (S). In study 1, treatment (TX) was begun on post-op day 14. In study 2, TXs were initiated on the day of surgery. Groups of rats (n=8) were administered vehicle (V) (0.5% methylcellulose, 0.025% Tween-20), celecoxib (C, 10 mg/kg/d), 17a-ethynylestradiol (EE, 30 ug/kg, PO) or combinations thereof by gavage. Alendronate (A, 28 ug/kg) was administered sc 2 twice weekly. Calcein (10 mg/kg, BID) was injected on days 49 and 56. BMD of the left proximal tibia was determined by pQCT on days 0, 14, 28, 42 and 56. TBD of the lumbar spine was measured by DEXA on day 56. Trabecular bone strength of the distal femur was measured by an indentation method.

	Study 1 Tx	Study 2 Prevention	Study 1 Tx	Study 2 Prevention
Group	Day 56 Tibial BMD (mg/ccm)	Day 56 Tibial BMD (mg/ccm)	Day 56 Maximum Load (N) (n= 6-8)	Day 56 Maximum Load (N) (n= 6-8)
1. Sham +V	386 +/- 23	346 +/- 36	5.5 +/- 3.2	6.7 +/- 6.1
2. Ovx +V	233 +/- 28	191 +/- 18	1.5 +/-1.2	1.5 +/-1.2
3. Ovx +EE	323 +/- 34	306 +/- 18	6.3 +/- 7.3	5.9+/-6.4
4. Ovx + A +V	342 +/- 28	424 +/- 23	5.6+/- 6.1	10.5+/-9.1
5. Sham + C	419 +/- 21	357 +/- 17	6.7 +/-5.3	6.2 +/-3.4
6. Ovx +C	201 +/- 23	168 +/- 15	3.2 +/-2.3	1.4 +/-1.5
7. Ovx + EE +C	305 +/- 25	315 +/- 20	4.0+/-3.6	4.5 +/-2.9
8. Ovx + A + C	325 +/- 12	423 +/- 19	3.4 +/-2.5	9.0 +/-6.7

Changes in BMD and trabecular bone strength following OVX and anti-resorptive TX were as expected for the model. Concomitant TX with C had no measurable effect on the response of the skeleton to OVX or the response to Tx. Static and dynamic histomorphometry was performed on proximal tibiae with similar findings. Together these data suggest that anti-inflammatory doses of celecoxib do not prevent bone loss associated with OVX, but that its use to treat pain and inflammation during the post-menopausal period is not likely to impact the benefits of current anti-resorptive therapies either.

Disclosures: Pharmacia Corporation,3.

SA427

Current Opinion of Experts About the Prevention and the Treatment of Postmenopausal Osteoporosis. S. Rozenberg,[1] A. Peretz,*[2] H. Ham.[1] [1]Interdisciplinary Group on Osteoporosis, St Pierre Hospital, Brussels, Belgium, [2]CHU Brugman, Brussels, Belgium.

The National Institutes of Health (NIH) convened a consensus conference about the diagnosis, prevention and therapy of osteoporosis. A consensus has the merit to reflect the opinion of a panel of experts, but this view is not necessarily shared by others. We conducted an opinion survey on the therapy of osteoporosis. One physician not familiar with this subject reviewed the titles of all papers published during the last 5 years in major journals. Fourty papers were found to be closely related to the therapy of osteoporosis and a questionnaire was then sent to the first authors of these papers. They were assured that their participation would remain anonymous, and that the study would not be commercially exploited. They were asked what effect they thought that some medications (HRT, Biphosphonates, SERMS, Calcium and vitamin D) have in modifying the future risk of either vertebral- or hip fracture. After 1 months, we had obtained 20 returns (1 blank and 19 filled returns). The results show that the surveyed experts are quite critic towards the proposed treatments. If we consider that only a risk modification superior to 20% is clinically relevant, only 12 (63%) and 10 (53%) experts considered that HRT has a favourable effect on prevention and 14 (74%) and 11 (58%) on treatment of vertebral or hip fracture. Others considered that there were not enough data to conclude. For the biphosphonates, 10 (53%) experts considered that these drugs have a favourable effect on vertebra or hip prevention and 18 (95%) and 15 (79%) on vertebral or hip fracture treatment. About half of the expert think that the efficacy of the biphosphonates on prevention of osteoporosis has not been proven, while it has as a treatment of established osteoporosis. Only respectively 10 (53%) and 7 (37%) experts considered that SERMS has a favourable effect on prevention of vertebral or hip fracture, and 16 (84%) on treatment of vertebral and 6 (32%) on treatment of hip fracture. Concerning the calcium plus vitamin D, 4 (21%) and 6 (32%) experts considered this regimen has a favourable effect on prevention and 9 (48%) and 10 (53%) on treatment of vertebral or hip fracture. These data suggest that some experts are much more critical in their views than the views which appeared in the consensus conference.

SA428

A New Active Vitamin D Analog, ED-71, Increases Bone Mass with Preferential Effects on Bone in Osteoporotic Patients. T. Matsumoto,[1] T. Miki,[2] T. Sugimoto,[3] R. Teshima,*[4] Y. Kato,*[5] S. Okamoto,[6] H. Tsurukami,[7] Y. Tanigawara,*[8] T. Nakamura,[7] M. Shiraki,[9] Y. Hayashi,*[10] M. Fukunaga.*[11] [1]University of Tokushima, Tokushima, Japan, [2]Osaka City University, Osaka, Japan, [3]Kobe University, Kobe, Japan, [4]Tottori University, Yonago, Japan, [5]Shimane Medical University, Izumo, Japan, [6]Sanyo Osteoporosis Research Foundation, Oita, Japan, [7]University of Occupational and Environmental Health, Kitakyushu, Japan, [8]Keio University Hospital, Tokyo, Japan, [9]Research Institute and Practice for Involutional Diseases, Nagano, Japan, [10]Tokyo Metropolitan Tama Geriatric Hospital, Tokyo, Japan, [11]Kawasaki Medical School, Kurashiki, Japan.

ED-71 [1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3] is a potent analog of active vitamin D, 1α,25$(OH)_2D_3$, bearing a hydroxypropoxy substituent at the 2β-position. In ovariectomized rats, ED-71 prevented the reduction in bone mass and strength without causing hypercalcemia by inhibiting bone resorption and enhancing bone formation. These results suggested that ED-71 preferentially acts on bone to increase bone mass with less effect on intestinal calcium absorption. We therefore conducted a randomized controlled clinical trial in 108 osteoporotic subjects (101 females and 7 males) 49 to 81 years of age (mean 65.0 years). The patients were randomly assigned to either 0.25, 0.5, 0.75 or 1.0 μg/day of ED-71 administered orally. They were treated for 6 months, and bone mineral density (BMD) and bone markers were evaluated. ED-71 treatment increased the BMD at L_{2-4} in a dose-dependent manner (0.34±0.73, 0.50±0.91, 3.00±0.65 and 2.66±0.71% in the 0.25, 0.5, 0.75 and 1.0 μg groups, respectively, mean±SE). The percentages of patients that showed an increase in the L_{2-4}BMD over 3% after 6 months also increased dose-dependently (21.7, 26.1, 54.2 and 45.5% in the 0.25, 0.5, 0.75 and 1.0 μg groups, respectively). Although 24 patients (23.3%) showed serum 25(OH)D levels below 20 ng/mL, the effect of ED-71 on the L_{2-4}BMD was not affected by the serum 25(OH)D level. ED-71 also exhibited a dose-dependent suppression of urinary deoxypyridinoline and Crosslaps excretion as well as serum bone-type alkaline phosphatase, whereas serum osteocalcin was not suppressed, suggesting a maintenance of bone formation with a suppression of bone resorption. ED-71 was well tolerated without causing hypercalcemia, and no patient exhibited sustained postprandial hypercalciuria over 0.4 mg/dL GF. These results demonstrate that ED-71 can effectively increase bone mass without causing hypercalcemia possibly via its preferential effects on bone, and suggest that ED-71 is a new modality that may be a candidate for the treatment of osteoporosis.

SA429

Alfacalcidol in the Prevention of Bone Loss After Heart Transplantation. H. U. Stempfle,[1] C. Werner,*[1] S. Florian,*[1] R. Frost,*[1] W. A. Rambeck,*[2] R. Gärtner.*[3] [1]Cardiology, Medizinische Klinik Innenstadt, Munich, Germany, [2]Tierärztliche Fakultät, Munich, Germany, [3]Endocrinology, Medizinische Klinik Innenstadt, Munich, Germany.

Background: Immunosuppressive therapy induced osteoporosis is a well known complication after heart transplantation (HTx). The aim of this prospective, placebo-controlled study was to assess the effect of alfacalcidol (1 μg) in the prevention of bone loss after HTx. Methods: Study patients (n=56) received a triple-drug immunosuppression including FK506, azathioprine or mycophenolate mofetil and glucocorticoids. Patients were treated with elemental calcium (500 mg/d) and sex hormone replacement in hypogonadismus. 38 patients (mean age: 48±9 yrs.; 5±1 months post HTx) received alfacalcidol and were compared to 18 cardiac transplants with placebo (age: 54±12 yrs.; 5±4 months post HTx) at baseline and 12 months. 16 and 9 patients completed already a 24 months treatment intervall. Bone mineral density (BMD) was measured at the lumbar spine (LS) and at the femoral neck (FN) with DEXA (Lunar Expert, g/T-score %). Fractures were assessed by X-rays of chest, thoracic and lumbar spine. Biochemical markers included gonadal hormones, gonadotropins, urinary and serum para-meters of calcium metabolism, intact PTH, 25OHVitD3 and renal function. Results and Conclusions: Calcium supplemention and sex hormone replacement in hypogonadism proved a sufficient therapy to increase BMD and to prevent fractures after HTx. The additional dose of 1 μg Alfacalcidol demonstrated a significant extra benefit regarding BMD (see table).

SA430

See Friday Plenary number F430.

SA431

Alfacalcidol Restores Vertebral Bone Mass in Ovariectomized Rats. M. Li, Y. Li,* D. R. Healy,* H. A. Simmons, D. D. Thompson. Pfizer Global Research and Developemt, Groton, CT, USA.

Vitamin D has been demonstrated to reduce vertebral and hip fractures in elderly patients. However, the role that vitamin D play in reducing fractures in the elderly is unclear. Some in vitro and in vivo pre-clinical studies have suggested that the vitamin D may effectively stimulate osteoblastic activity and exert an anabolic effect on bone. The current study was designed to further explore the ability of active vitamin D to restore bone in a skeletal site with established osteopenia in ovariectomized (OVX) rats. Female Sprague-Dawley rats at five months of age and 8 weeks after sham ovariectomy (OVX) or

OVX were randomly divided into 7 groups with 10 per group. At the beginning of the treatments, one group of sham-operated rats and one group of OVX rats were sacrificed to serve as baseline controls. Another group of sham operated rats and one group of OVX rats was treated with vehicle for 4 weeks. The OVX rats in the remaining groups were treated with 0.05, 0.1 or 0.2 mg/kg/d body weight of alfacalcidol [1a(OH)D3] by daily oral gavage, 5 days/week for 4 weeks. As expected, estrogen depletion caused high bone turnover and cancellous bone loss in lumbar vertebra of OVX rats. Alfacalcidol treatment at 0.1 or 0.2 but not 0.05 mg/kg/d increased serum calcium and phosphorus in OVX rats as compared with vehicle treatment. In addition, serum parathyroid hormone was suppressed, whereas serum osteocalcin was increased by alfacalcidol at all dose-levels. Furthermore, histomorphometric data of lumbar vertebra revealed that cancellous bone volume in OVX rats treated with alfacalcidol at 0.1 or 0.2 mg/kg/d was increased to the level of sham-controls treated with vehicle. This increment in cancellous bone mass was accompanied by increases in trabecular number and thickness and a decrease in trabecular separation. Moreover, osteoclast surface was decreased, whereas bone formation variables such as mineralizing surface and bone formation rate were increased in alfacalcidol-treated OVX rats compared with those of vehicle-treated OVX rats. Finally, a linear regression analysis showed that alfacalcidol treatment dose-dependently altered most of the variables measured in the current study. In conclusion, alfacalcidol completely restores cancellous bone by stimulating bone formation and suppressing bone resorption in lumbar vertebra of OVX rats. Increased serum calcium and decreased serum parathyroid hormone accompany such effects of alfacacidol on bone. These results support the use of active vitamin D analogs for the treatment of osteoporosis.

Disclosures: Pfizer Inc,3.

SA432

An Unusual Pattern of Bone Formation by Alfacalcidol Treatment in Aged Male Rats. M. Li, D. R. Healy,* Y. Li,* H. A. Simmons, D. D. Thompson. Pfizer Global Research and Development, Groton, CT, USA.

Vitamin D is an essential component for maintenance of health. Further, it has been demonstrated to prevent vertebral and hip fracture in elderly patients. The role that vitamin D play in preventing fractures in the elderly is unclear. Calcitriol [1a25(OH)2D3] and alfacalcidol [1a(OH)D3] have been reported to increase bone mass and bone strength in relative young rats. However, these effects of vitamin D have not been assessed in aged animals. Therefore, we sought to investigate the effects using aged male rats to understand the relationship between changes in bone mass and bone strength. Eighteen-month-old male Sprague-Dawley rats were treated with 0, 0.1, or 0.2 mg/kg/d body weight of alfacalcidol by daily oral gavage, 5 days/week for 12 weeks. At the end of the study, the second lumbar vertebrae were processed for standard bone histomorphometric analysis and the fourth lumbar vertebrae were subjected to a compression test. The results of current study confirmed the previous finding of increasing bone mass and improving bone strength by alfacalcidol in aged male rats. However, an atypical pattern of bone formation on trabeculae was observed in the rats treated with alfacalcidol. The atypical bone formation was characterized by small, focal packets of newly formed bone on trabecular bone surfaces. This gave the appearance of the formation of "bone buds" emanating from trabecular surfaces. The "bone buds" appeared to be randomly scattered along the surfaces. Also they were fully mineralized and demonstrated significant fluorochrome label indicating recent mineralized and their lamellae did not run parallel to those of the trabecular plate to which they are attached. No evidence of osteomalacia was observed in these animals. In summary, alfacalcidol treatment increased cancellous bone and improved bone strength of lumbar vertebrae in aged male rats. However, a unique pattern of bone formation was observed in this study, a finding not previously recorded. Further study will be necessary to assess if these effects are restricted to the aged.

Disclosures: Pfizer Inc,3.

SA433

Lack of Significant Toxicity of a Daily Supplementation of 1 g Elemental Calcium and 880 IU Vitamin D in Postmenopausal Women. J. P. Devogelaer,* G. Depresseux.* Rheumatology Unit, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium.

Calcium and vitamin D deficiencies are relatively common in elderly population, leading to secondary hyperparathyroidism potentially detrimental for bone.Vitamin D and calcium supplementations are more and more frequently recommended. However, blind systematic supplementation could potentially be at risk of toxicity. We have, therefore, administered physiologic doses of elemental calcium (1 g) and vitamin D (880 IU) daily for 2 months to 30 ambulatory postmenopausal women with osteopenia [T-score = - 1.5 (1.2)], aged 67.7 (6.4 SD) whose 25OHD level was lower or equal than 30 ng/ml. Following therapy, 25OHD level increased from 14.7 (7.0) to 29.2 (7.0) ng/ml, 50 % of patients reaching levels higher than 30 ng/ml. Serum calcium (total and corrected) and creatinine clearance did not change significantly. iPTH decreased significantly from 34.1 (14.8) to 25.5 (8.7) pg/ml ($p < 0.01$). 24-H urinary calcium excretion (both total and body weight-corrected) increased from 173 (83) to 223 (93) mg and from 2.7 (1.2) to 3.5 (1.6 mg/kg), respectively ($p < 0.01$). 24-H urinary N-telopeptide (NTx)/creatinine and NTx/whole body BMC decreased non significantly. 13.3 % of patients increased their total serum calcium and 24-H urinary calcium/body weight over 10.4 mg/dl (up to 10.9 mg/dl) and 5 mg/kg (up to 6.6 mg/kg), respectively, and 20 % developed 24-H urinary calcium excretion superior to 300 mg (up to 444 mg).In conclusion, calcium and vitamin D supplementations in elderly patients are able to induce a significant decrease in serum iPTH and a non-significant decrease in urinary NTX excretion, potentially favorable for bone mechanical resistance. However, 24-H urinary calcium excretion should ideally be checked after 2 months in order to detect frank hypercalciuria which can occur in up to 20 % of patients. This could be of some clinical significance in a minority of patients.

SA434

A Vitamin D Analog, ED-71, Is a More Potent Anti-Osteoporosis Drug than Alfacalcidol in an Estrogen-deficient Rat Model of Osteoporosis. S. Takeda,[1] Y. Uchiyama,[1] Y. Higuchi,[1] T. Masaki,[1] A. Shiraishi,[1] K. Sato,[1] N. Kubodera,[1] K. Ikeda,[2] E. Ogata.[3] [1]Chugai Pharmaceutical Co., Ltd., Shizuoka, Japan, [2]Dept. of Geriatric Res., Natl. Inst. for Longevity Sci., Aichi, Japan, [3]Cancer Inst. Hosp., Japanese Foundation for Cancer Res., Tokyo, Japan.

Although active vitamin D is used in certain countries for the treatment of osteoporosis, the risk of causing hypercalcemia/hypercalciuria results in a narrow therapeutic window, and has precluded worldwide approval. The results of our previous animal studies suggested that the therapeutic effect of active vitamin D on bone loss after estrogen deficiency can be dissociated at least partly from its effect of enhancing intestinal calcium absorption and suppressing parathyroid hormone (PTH) secretion. In the present study, we compared the effects of ED-71, a propoxy derivative of 1α,25-dihydroxyvitamin D_3, with alfacalcidol on bone mineral density (BMD) and the bone remodeling process as a function of their effects on calcium metabolism and PTH secretion, in a rat ovariectomy (OVX) model of osteoporosis. Eight-month-old female Wistar rats were subjected to OVX and treated p.o. with ED-71 (0.05-0.2 micro g/kg BW) or alfacalcidol (0.1-0.4 micro g/kg BW) for 12 weeks. ED-71 increased BMD in the lumbar vertebra to a greater extent than alfacalcidol, while enhancing calcium absorption (reflected by urinary calcium excretion) and decreasing serum PTH levels to the same degree as alfacalcidol. ED-71 lowered the biochemical and histological parameters of bone resorption, such as urinary deoxypyridinoline and osteoclast surface (Oc.S/BS), more potently than alfacalcidol, while maintaining bone formation markers such as serum osteocalcin and bone formation rate (BFR/BS). These results suggest that active vitamin D exerts an anti-osteoporotic effect by inhibiting osteoclastic bone resorption while maintaining osteoblastic function, and that these anti-catabolic/anabolic effects of active vitamin D take place independently of its effects on calcium absorption and PTH secretion. The demonstration that ED-71 is more potent in these properties than alfacalcidol makes it an attractive candidate as an anti-osteoporotic drug.

SA435

Measurement of Tartrate-Resistant Acid Phosphatase (TRAP) and the Brain Isoenzyme of Creatine Kinase (CK-BB) Accurately Diagnoses Type II Autosomal Dominant Osteopetrosis but Does Not Identify Gene Carriers. S. G. Waguespack,[1] S. L. Hui,[1] K. A. Buckwalter,*[2] M. J. Econs.[1] [1]Medicine, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Radiology, Indiana University School of Medicine, Indianapolis, IN, USA.

Autosomal dominant osteopetrosis, Type II (ADO2) is a metabolic bone disorder that results from ineffective osteoclast-mediated bone resorption. The diagnosis is typically made on radiographs, which demonstrate the pathognomonic findings of osteosclerosis and endobone formation. Individuals with ADO2 also have elevated serum levels of TRAP and CK-BB. In the current study, we tested the utility of these enzymes in making or refuting the diagnosis in asymptomatic patients at risk for ADO2. Furthermore, because ADO2 has incomplete penetrance, we examined whether TRAP and CK-BB were helpful in identifying gene carriers. We studied eight families and measured serum levels of TRAP and CK-BB in 44 affected patients and 12 obligate carriers (individuals with normal radiographs, but who have an affected descendant as well as another relative with the disorder). We then compared their values to age-matched controls without osteopetrosis (Table). The reference ranges for TRAP are 4.3-21.1 U/l in children <18 years of age and 3.5-9.1 U/l in adults. CK-BB should be undetectable in adults whereas in children, small quantities of CK-BB activity may normally be observed.

	Affected <18 yrs	Controls <18 yrs	Affected >18 yrs	Controls >18 yrs	Obligate Carriers
N	**14**	**23**	**30**	**89**	**12**
Age (yrs±SD)	9.0±4.1	7.1±3.3	45.3±17.2	44.3±16.0	41.3±9.8
M / F	9 / 5	13 / 10	13 / 17	44 / 45	4 / 8
TRAP (U/L±SD)	81.4±17.3*	21.2±4.8	49.5±18.4*	9.62±2.9	11.3±5.4#
CK-BB (U/L±SD)	190.6±96.0*	1.2±2.4	59.6±43.7*	0.0±0.0	0.8±2.6#

*$p < 0.0001$ vs controls; #$p > 0.3$ vs controls

Our results demonstrate that affected patients have significantly elevated levels of both TRAP and CK-BB. In contrast, gene carriers have values that are no different from controls. In our study population, TRAP is 100% sensitive and 94% specific when a cutoff of 1.5X the age-appropriate upper limit of normal is used. In children, using a CK-BB value of 15 U/L gives a sensitivity and specificity of 100%. In the adult group, a cutoff of 0 U/L gives a diagnostic sensitivity and specificity of 93% and 100%, respectively. From this large study of ADO2 patients and carriers, we conclude that: 1) TRAP and CK-BB are significantly and consistently elevated in patients with radiographically-proven ADO2, 2) gene carriers cannot be adequately identified by measurement of these analytes, and 3) TRAP and CK-BB are highly sensitive and specific diagnostic tests that can efficiently and effectively screen high risk individuals who have not had previous radiographic assessment

SA436

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SA437

Intravenous Pamidronate Improved Bone Mineral Density and Diminished Bone Turnover in Patients with Fibrous Dysplasia. M. S. Parisi,*[1] M. B. Oliveri,[1] C. Gómez Acotto,[1] C. Tau,[2] F. Solís,*[1] C. A. Mautalen.[1] [1]División Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Metabolismo Cálcico y Óseo, Endocrinología, Hospital de Pediatría J. P. Garraham, Buenos Aires, Argentina.

We report the effect of one year treatment with intravenous Pamidronate (APD) on bone mineral density (BMD) and bone turnover in six patients with Fibrous Dysplasia (FD), 5 women and 1 man. Mean age was 37.3 years (r:21-79). A complete clinical, biochemical and radiographic follow-up was performed. BMD of Total Body (TB) was assessed by DEXA at baseline and one year after the onset of treatment. In patients with unilateral location of the disease, areas of FD (aFD) (3 located in the arms, 5 in legs and 1 in pelvis) were delimited by ROI analysis and compared with their contralateral non-affected side (C). The difference in BMD was calculated as a percentage where the healthy side was taken as 100%. The same analysis was performed in 6 sex and age matched controls. Bone alkaline phosphatase (BAP) and urinary cross-laps (CTX) were measured at baseline and at 3 months intervals. A 60mg/day dose of iv APD was administered on 3 days every 6 months.At baseline, TB BMD values were in the normal range, except for one postmenopausal woman who presented diminished BMD. Mean difference in BMD (ROI analysis) between aFD and C was -8.4% (r:-36.2 to +4.6). The difference in BMD between right and left sides in control subjects was +0.5±4.3(X±SD). BAP and CTX levels were above the normal range in 5 and 3 patients respectively.After one year, we observed 2.6% increments in mean TB BMD ($p<0.05$), while mean BMD of aFD and C increased 6.9% and 1.9% respectively. The mean difference between aFD BMD and C BMD diminished to –4.8% (r:-31.5 to +19.5). BAP levels were found to decrease an average 30% in the five patients presenting abnormal baseline values, but were still above the normal range. CTX levels decreased in the three patients presenting abnormal baseline values. Only one patient with markedly high baseline values still showed high levels of CTX after one year of treatment, although a 40 % reduction was observed. CTX and BAP levels remained unchanged in the patients with normal baseline values.Whereas TB BMD was mostly within normal ranges, BMD of FD areas was lower than their corresponding healthy contralateral side. Treatment with iv APD produced an increase in Total Body and areas of FD BMD and diminished markers of bone turnover. Our results lead to the conclusion that specific densitometric evaluation of areas of FD by means of ROI analysis, together with determinations of specific markers of bone turnover allow to assess the effectiveness of one year treatment with iv APD in most patients with FD.

SA438

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SA439

Identification of EDR3, a Candidate Gene for the Cornelia - de Lange Syndrome (CDLS). A. M. Deshpande,*[1] M. J. Nellissery,*[1] X. Reveles,*[2] S. L. Naylor,*[2] L. G. Jackson,*[3] R. J. Leach,[2] M. F. Hansen.[1] [1]Center for Molecular Medicine, Univ. Connecticut Health Center, Farmington, CT, USA, [2]Department of Cellular and Structural Biology, Univ. Texas Health Science Center, San Antonio, TX, USA, [3]Department of Medical Genetics, Thomas Jefferson University, Philadelphia, PA, USA.

CDLS is a syndrome characterized by pre- and post-natal growth delay, mental retardation, microbrachycephaly, synorphrys, hirsutism, delayed skeletal maturation especially upper limb abnormalities and a characteristic facies. Ireland et al. first described a [3:17] balanced translocation in a classical case of CDLS. This and other studies suggest localization of the gene(s) involved, to the region 3q26 - q27. We have identified a candidate for CDLS, a novel member of the polycomb group of proteins, which we have named EDR3. Southern and FISH analyses reveal that EDR3 is triplicated in cell lines derived from CLDS patients even in the absence of gross cytogenetic abnormalities. Northern and RT-PCR analyses indicate that EDR3 is expressed in mesenchymal stem cells (MSC), but is expressed at very low levels in normal human osteoblasts and adult bone. The gene has an ORF of 2748 bp and a 5'- and 3'- UTR. The mouse homologue of EDR3 is approximately 92% homologous to the human EDR3 protein. The EDR3 protein is similar to other members of the polycomb protein family including human EDR2 (48%) and mouse mPh2 proteins (52%). Members of the PcG family regulate gene expression through protein-protein and protein-DNA interactions. These proteins play an important role in axial patterning of organisms through regulation of expression of homeotic genes. PcG proteins antagonize the function of trithorax group of proteins and mediate long term lineage-specific silencing of genes. Mutations in polycomb proteins [e.g.: Ezh2, BmiI] cause shifts in the boundaries of homeotic gene expression, which result in anterior or posterior transformations and skeletal dysmorphogenesis. Based on the observed localization of the EDR3 gene and its potential role in regulation of skeletal dysmorphogenesis, we propose that overexpression of EDR3 caused by triplication gives rise to the skeletal phenotype associated with CDLS.

SA440

See Friday Plenary number F440.

SA441

Bone Marrow Triglyceride Accumulation and Hormonal Changes During Chronic Alcohol Consumption. F. H. Wezeman,*[1] Z. Gong.*[2] [1]Orthopaedic Surgery, Loyola University Medical School, Maywood, IL, USA, [2]Loyola University Medical School, Maywood, IL, USA.

The relationship between chronic alcohol consumption and bone marrow fat content was studied in a rat model. Clinical observations during orthopaedic surgical procedures frequently note an increase in bone marrow fat content in chronic alcoholics. Chronic alcohol consumption may influence the metabolism of adipocytes, the most abundant stromal cell phenotype in bone marrow, and promote increased bone marrow triglyceride. Male and female rats 35 days old were fed the Lieber-DeCarli liquid diet containing 36% of the calories as ethanol and were compared to pair-fed rats (weight-matched to ethanol-fed rats) given an isocaloric liquid diet in which maltose-dextrin substituted for the calories supplied by ethanol. Other control rats were fed chow ad libitum. The rats were maintained on these diets for 64 days, after which the femurs were recovered. Individual femur marrow triglyceride content was quantified after lipid extraction and alkaline hydrolysis. Serum concentrations of insulin, insulin-like growth factor-1, testosterone, estradiol, progesterone, and leptin were determined by radioimmunoassay at the time of sacrifice. End-weights of male and female ethanol-fed rats were significantly lower than both control groups. Femur marrow triglyceride levels were significantly increased in ethanol-fed male and female rats compared to both control groups. Femur marrow cavity diameters were significantly increased and cortical thickness was significantly decreased by alcohol in both males and females. Serum insulin levels were significantly decreased by alcohol only in female rats compared to the ad libitum but not the pair-fed control group, and insulin-like growth factor-1 levels were significantly reduced in male and female rats given the ethanol diet compared to both controls. Male testosterone and female estradiol levels remained unchanged. Male estradiol levels were significantly elevated by ethanol compared to both controls, and female progesterone levels were significantly reduced by ethanol compared to pair-fed rats. Whereas female leptin levels were unchanged by ethanol, male leptin levels were significantly increased by ethanol compared to pair-fed rats. Hormonal and growth factor changes during chronic alcohol consumption accompany triglyceride accumulation in bone marrow, and may parallel the effects of alcohol on mesenchymal stem cells and the balance between osteogenic and adipogenic lineages and their cellular progenies.

SA442

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SA443

Bone Mineral Impairment and Body Composition Evaluated by DXA (Dual-Energy X-Ray Absorptiometry) in Black HIV-Positive Patients. S. Daens,*[1] M. Guillaume,[2] P. Bergmann,[3] M. Fuss,*[2] R. Karmali,*[2] A. Peretz.*[1] [1]Rheumatology, CHU Brugmann, Brussels, Belgium, [2]Internal Medicine, CHU Brugmann, Brussels, Belgium, [3]Clinical Chemistry, CHU Brugmann, Brussels, Belgium.

Background. The aim of the study was to determine the influence of HIV infection and its treatment on bone mineral density and body composition in African black patients.Methods. 24 HIV-positive (HIV+) black patients (9 men and 15 women) and 16 HIV-negative black control subjects (C) were consecutively enrolled. All patients were already treated with an anti-retroviral therapy with two third of them receiving in addition a protease inhibitor (PI). Nutritional status and hormonal functions were evaluated at the initiation of the study and one year later. Body composition, lumbar (BMDL) and whole body BMD (BMDW) were assessed using DXA (Hologic, QDR 1000).Results. No differences were found between HIV+ and C in anthropometric parameters or nutritional status. HIV+ had significantly higher TSH level than C ($p<0.001$) indicating perhaps a lower thyroid activity. BMDW and BMDL were similar in female HIV+ and C. However in HIV+ men the BMDL, the bone mineral content (BMC) and the lean mass (LM) were respectively 9%, 16% and 11% lower than in C ($p<0.05$). After a year of follow up, DXA measurements and endocrine functions did not change. In the patients who were also treated with PI, fat distribution remained unchanged a year later.Conclusions. Male black HIV+ had a significantly lower LM, BMC and BMDL when compared to C. In addition, treatment with PI did not change fat distribution.

SA444

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SA445

Diffuse Crippling and Disfiguring Bone and Skin Lesions as a Presentation of Systemic Mastocytosis. A. Halabe,[1] R. Shor,[1] I. Tolatov,[2] E. Rachmilewitz.[2] [1]Department of Internal Medicine and Metabolic Bone Diseases, The Edith Wolfson Medical Centre, Holon, Israel, [2]Hematology, The Edith Wolfson Medical Centre, Holon, Israel.

Mastocytosis is a disease characterized by an abnormal increase in mast cells associated usually with skin lesions (urticaria pigmentosa) and systemic bone involvement. We report a 77-years-old Russian male who was referred to our clinic because of intractable pain in his vertebral column and lower extremities. His past medical history included severe chronic dermatitis diagnosed as vasculitis, following a skin biopsy in Russia. The patient was treated with high dose of corticosteroids without any improvement in his skin lesions. On physical examination the most evident finding was a severe disability affecting mainly the thoracic and lumbar vertebral regions, causing inability to flex the spine and maintain an upright position (the patient was forced to use a metal corset). A maculo-papular skin eruption was also observed over his trunk. Laboratory tests including CBC, chemistry analysis and serum protein electrophoresis were within normal range. An Osseous CT and MRI revealed severe osteoporosis with diffuse large lytic lesions in the iliac bones and in the spinal area at the level of L4-L5. CT guided FNA from one of the bone lesions and bone marrow biopsy, were not diagnostic. An open biopsy from the iliac area demonstrated focal paratrabecular mast cell infiltration compatible with the diagnosis of systemic mastocytosis. A repeat biopsy from one of the skin lesions supported the diagnosis of mastocytosis-urticaria pigmentosa. The patient was treated with monthly I.V. Pamidronate 90 mg, alpha interferon 3 X 10-6 U/weekly and H2 receptors blockers.Three months later, his bone pain subsided considerably and the skin eruption was very much improved. A repeat MRI showed improvement in the extent of the lytic lesions of the iliac region. This is an interesting case of a rare disease, which responded dramatically to treatment. The most striking feature was an improvement in the quality of life of one who suffered for two decades from a crippling and disfiguring disease.In conclusion, mastocytosis is a rare disease with protean manifestations; very seldom considered in the differential diagnosis of osteoporosis and possibly even overlooked.

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SA448

Specificity of DSL Salmon Calcitonin ELISA. N. Khaja,*[1] S. K. Durham,*[1] J. Mistry,*[1] M. Nicar,[1] A. Consalvo,*[2] A. Sturmer,*[2] N. Mehta.*[2] [1]DSL, Inc, Webster, TX, USA, [2]Unigene Laboratories, Fairfield, NJ, USA.

Calcitonin plays a major role in calcium homeostasis by inhibiting osteoclast-mediated bone resorption. Salmon calcitonin (sCT), a 32-amino acid polypeptide, is used clinically in the treatment of bone disorders such as Paget's disease, hypercalcemia and osteoporosis in humans. Unigene has developed a dual recombinant process for the cost-effective, large-scale production of bioactive sCT. The technology involves production of a glycine-extended sCT precursor in *E. coli*, followed by *in vitro* amidation. An enzyme-linked immunosorbent assay (ELISA) was recently developed by DSL to measure sCT in human serum. This ELISA was used to study the specificity of the assay towards synthetic and recombinant full-length sCT as well as various peptide fragments of sCT including amino acid 1-11, 12-18, 19-24, 25-32 NH_2, 1-15, 16-32 NH_2, 1-24 and 12-32 NH_2. The synthetic and recombinant intact antigens showed equal immunoreactivity in the ELISA throughout the dynamic range (10-500 pg/mL) of the assay. Peptide fragments 1-11, 12-18, 19-24, 25-32 NH_2, 1-15, 16-32 NH_2 (at concentrations 0.06-500 ng/mL) displayed insignificant cross-reactivity (<2%) whereas, fragment 12-32 NH_2 showed minor (3.3-16.9%) cross-reactivity at 0.06-1.95 ng/mL. The only peptide fragment that cross-reacted in the assay was sCT 1-24, and the extent of cross-reactivity was greater than 100% in the linear range of the assay. Human calcitonin (up to 500 ng/mL), katacalcin (up to 1 ng/mL), CGRP (up to 1 ng/mL), ACTH (up to 2 ng/mL) and PTH (up to 0.2 ng/mL) did not display any cross-reactivity in the assay. We conclude that the DSL Active™ Salmon Calcitonin ELISA is species-specific; the pair of antibodies used in this sandwich ELISA do not recognize peptide fragments of sCT (except for sCT 1-24). The availability of this highly specific ELISA provides a useful tool for monitoring treatment and dosage of salmon calcitonin in human serum or plasma.

Disclosures: Diagnostic Systems Laboratories,3; Unigene Laboratories,3.

SA449

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SA453

Predictive Value of Bone Markers in the Long-Term Response to Bisphosphonates in Paget's Disease. N. Guanabens, L. Alvarez, P. Peris, S. Vidal-Sicart,* I. Ros,* A. Monegal,* J. Bedini,* R. Deulofeu,* F. Pons,* A. M. Ballesta,* J. Muñoz.* Metabolic Bone Diseases Unit, Hospital Clínic, University of Barcelona, Barcelona, Spain.

The purpose of this study has been to evaluate if short-term changes in bone marker levels predict long-term Paget's disease activity after bisphosphonate treatment.This prospective study was carried out in 15 patients with Paget's disease of bone treated with tiludronate (400 mg/day for 3 months). The activity of the disease was determined by quantitative bone scans (scintigraphic activity index -SAI), at baseline and after 9 and 21 months. Biochemical markers of bone turnover, including serum levels of total alkaline phosphatase (TAP), bone alkaline phosphatase (BAP) and procollagen type I N-terminal propeptide (P1NP) and urine levels of hydroxyproline (HYP) and N-terminal telopeptide of type I collagen (NTx) were measured at the same times.According to the SAI changes between 9 and 21 months, the patients were divided into two groups: group I: no changes or reduction (9 patients), and group II: increased SAI (6 patients). Short-term changes (0-9 months) in the markers of bone formation were similar in both groups (I vs II. TAP -48 ± 7 % vs -68 ± 6%; BAP: - 63 ± 9% vs -81 ± 3%; PINP: -67 ± 6 % vs -75 ± 4%). By contrast, long-term changes (9-21 months) in the markers of bone formation were clearly associated with the SAI, since the patients with high scintigraphic activity had significant increases of TAP (69 ± 20% vs 9 ± 4%, $p<0.01$), BAP (120 ± 18% vs 33 ± 12%, $p<0.01$) and PINP (105 ± 10% vs 23 ± 12%, $p<0.05$). No significant changes in the markers of bone resorption were observed between both groups.In conclusion, the short-term changes in bone markers did not predict the long-term response to tiludronate. Changes in bone formation markers, measured between 9 and 21 months, provide the best biochemical approach for assessing long-term response to treatment.

SA454

Paget's Disease of Bone with Normal Serum Alkaline Phosphatase Activity - Effects of Bisphosphonates on Clinical Symptoms and Bone Markers. F. F. A. Bandeira,[1] G. J. C. Caldas,*[2] L. H. M. Griz,*[2] C. H. C. Bandeira.*[2] [1]Dept. of Endocrinology/UPE/CpqAM, Centro de Osteoporose, Recife, Brazil, [2]Dept of Endocrinology, Centro de Osteoporose, Recife, Brazil.

In most series the majority of patients with Paget's disease of bone are asymptomatic and are usually accidentally discovered during a clinical,radiological or biochemical evaluation for an unrelated condition. For many years serum alkaline phosphatase(AP) activity has been used as the most common index of activity of the pagetic lesions. In a serie of 77 consecutive patients seen at our Institution we identified 6 patients with normal serum AP activity but high levels of deoxypiridinoline(DPD) or N-telopeptide(NTX) excretion. The patients were 4 males and 2 females with mean+/-SD age 74.5+/-6.7 and 74.3+/-9.5 years respectively, all of them with poliostotic disease and 2 of them asymptomatic.Mean serum AP was 86.8+/-15.8 U/L(normal up to 110).One patient had urinary DPD 82% above the upper limit of normal, and 5 patients had NTX 123.2+/-72.3% above the upper limit of normal, with absolute levels of 125+/-66.7 nmol/mmol creatinine(range 107.2 to 188).In 4 patients with symptomatic disease treatment were offered, oral alendronate(ALN)in 2 patients and intravenous pamidronate(PAM)in 2 patients. There was great improvement in pain after treatment and the changes in serum AP were -48+/-9.7%.One patient had 70% decrease in DPD excretion with ALN, and the other 3 had 63+/-18.6% decrease in NTX (-50% with ALN,-82% with PAM,-87% with PAM respectively).In conclusion in 8 % of a our patients with Paget's disease of bone basal serum AP activity were unable to express the disease activity in comparison with markers of bone resorption.In at least the symptomatic patients, treatment with bisphosphonates induced a significant decreases in serum AP,and more pronounced decreases in urinary DPD and NTX along with clinical improvement.

SA455

Symtomatic and Scintigraphic Improvement Following Pamidronate Treatment of Paget's Disease with Normal Serum Alkaline Phosphatase. G. Ang,* A. Moses. Medicine, SUNY-Upstate Medical University, Syracuse, NY, USA.

Pamidronate is well known to be effective in the treatment of patients with mild to moderate Paget's disease of the bone. It relieves pain and induces prolonged remissions. We report 3 cases of patients with symptomatic Paget's disease who presented with normal serum alkaline phosphatase (SAP). All had typical x-ray findings, abnormal bone scans and had pain in the involved area. They were treated with IV pamidronate 60 mg once weekly for 2-3 consecutive weeks. SAP and urinary markers of bone resorption were monitored every 3 months post-treatment. Bone scans were repeated after symptomatic improvement was achieved. Vitamin D deficiency was ruled out with appropriate laboratory tests. The first case is a 79 year-old man who had Paget's disease of the left pelvis with associated pain and bone scan revealed intense uptake in the involved area. Pretreatment SAP was 88 (normal 21-129 U/L) and urine deoxypyridinoline (d-pyr) was 15 (normal 2-7 nmol/mmol creatinine). He was treated with 180 mg of pamidronate. Post treatment SAP was 56 and d-pyr was 3.7. He remained symptom free for 6 years. At that time, SAP was 63 and bone scan did not show any increased uptake in the left pelvis. The second case is a 78-year old woman who had Paget's disease of the left iliac bone, vertebrae L2 and L4 who initially presented with pain the left hip and lower back. Her pre-treatment SAP was 124 and NTX was 124 (normal 15-110 nmol BCE/mmol creatinine). She became asymptomatic soon after treatment and at 6 months the SAP decreased to 84. Bone scan showed marked improvement six months after treatment. She was asymptomatic for 17 months, at which time the SAP rose to 122 and NTX was 177. She was retreated and 3 months later, the pain disappeared, the SAP was 76 and NTX was 61. The third case is a 67 year-old man with Paget's disease of the right proximal femur and complained of pain in the right hip. The pagetic femur was bowed. He received 180 mg of pamidronate. Pre-treatment SAP was 120 and NTX was 68. Three months post-treatment SAP was 97 and NTX was 36. He was asymptomatic after treatment. Bone scan repeated after one year showed marked decrease in radiotracer uptake. Conclusion: There are little data on the treatment of Paget's disease with normal SAP, which occurs in a small percentage of symptomatic patients. We observed that pamidronate is effective treatment for these individuals. It afforded symptomatic relief and decreased the levels of SAP and urinary markers of bone resorption. A normal SAP does not rule out active Paget's disease and in such patients, urinary markers of bone resorption and bone scans should be used to assess and monitor the activity of the disease.

SA456

See Friday Plenary number F456.

SA457

New England Registry for Paget's Disease of Bone. M. Seton,[1] R. Sebaldt,[2] C. Cooper.[3] [1]Arthritis, Massachusetts General Hospital, Boston, MA, USA, [2]McMaster University, Hamilton, ON, Canada, [3]MRC Environmental Epidemiology Unit, Southampton, United Kingdom.

Paget's disease of bone (PDB) is a focal disorder of bone re-modeling, with clinical complications resulting from the location, extent of affected skeleton, and activity of the bony lesions. Some scientists postulate that PDB is the late expression of a viral infection of osteoclasts; others contest the theory of antecedent viral infection and explain the familial and geographic clustering that is reported in PDB by genetic predisposition. They point to genetic studies in some families with PDB where a candidate gene on the long arm of chromosome 18 has been identified. The New England Registry for Paget's Disease of Bone was established in 1999, designed to be the platform for a national / international database of patients with Paget's disease. The goal of the Registry is to provide clinical investigators with data to assess the prevalence, epidemiology and treatment outcomes; and to provide scientists with tissue for correlative science. Enrollment began February 2001, with completion of the database. Enrollment is voluntary, initiated by a patient questionnaire exploring the distribution and determinants of disease frequency. This information is linked to a clinical database and banked tissue in selected patients. Data from the first 100 patients entered into the registry show a familial incidence of Paget's disease of 13%; 5 patients were second generation PDB, with maternal history positive in all cases. In one, both parents had PDB. These mothers came from Scotland, Italy and Ireland, with no correlate to birth date or year of immigration; the proband reported measles infection in each case, but no consistent pet ownership. 8 patients had siblings with PDB; one was his twin sister, 4 reported more than one sibling affected. Overall, PDB affected the 1st in birth order 27%, 2nd 24%, the 3rd 19%, the 4th 11% and the 5th 12%, with 7 affected in later birth order. The most common ancestral country was Italy (Sicily) 42%, USA 36%, England (Scotland) 13% and Canada 16% with the Middle Eastern countries, Germany, central Europe (Poland, Czech) and Russia next in frequencies. Two patients emigrated from the Cape Verde Islands. 61% of patients were male. A summary of skeletal distribution of PDB: 42% hip, 41% skull, 31-34% lower back and pelvis, 26% tibia. Further correlative studies will help to define this focal disorder of bone. www.massgeneral.org/pagetregistry

Disclosures: Alliance for Better Bone Health,2.

SA458

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SA459

Treatment of Paget's Disease of Bone with Alendronate 60mg. G. M. Tsoukas,[1] A. Arzoumanian.[2] [1]Endocrinology, McGill University, Montreal, PQ, Canada, [2]The Montreal General Hospital, Montreal, PQ, Canada.

One of the aims of treatment of Paget's disease should be the normalization of the activity of the disease with the shortest possible exposure to the drug. Alendronate has demonstrated effectiveness in the oral treatment of of Paget's disease of bone. With a 40mg daily dose for 6 months, 63% of treated patients normalized their serum alkaline phosphatase,(Siris,et al. J Clin Endocrinol Metab 1996). The aim of our study was to examine the effects of a higher treatment dose of alendronate (60mg per day) over a shorter period (3 months). There were 28 patients with Paget's disease, 18 male and 10 female, with a mean age of 69 years, who were treated with an oral alendronate at a dose of 60mg per day for 3 months. Ten patients had never been treated before and 18 had previously received anti-osteolytic drugs. The mean period without treatment prior to alendronate 60mg was 14 months. Baseline alkaline phosphatase levels fell from 266.6 to 82.2 (mean difference 183.8, P=0.000). Osteocalcin levels fell from a baseline of 25.62 to 16.53 (mean difference 9.1, P=0.017). PTH levels rose from a baseline of 5.1 to 8.7 (mean difference 3.6, P=0.0002). All patients normalized their alkaline phosphatase levels. Follow-up was carried out on all 28 patients two years later. All but three were in remission, for a rate of 89.2%. No side effects noted in any of the patients treated. In summary, a three month course of treatment with alendronate 60mg proved to be very effective and well tolerated for the treatment of Paget's disease.

SA460

A Novel Tandem Duplication Identified in the TNFRSF11A Gene in Two Unrelated Patients with Familial Expansile Osteolysis. F. R. Singer,[1] T. L. Johnson-Pais,*[2] H. G. Bone,[3] M. J. Nellissery,*[4] C. T. McMurray,*[5] M. F. Hansen,[4] R. J. Leach.*[6] [1]Skeletal Biology, John Wayne Cancer Institute, Santa Monica, CA, USA, [2]Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [3]Michigan Bone and Mineral Clinic, Detroit, MI, USA, [4]Center for Molecular Medicine, University of Connecticut, Farmington, CT, USA, [5]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic and Foundation, Rochester, MN, USA, [6]Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA.

Familial expansile osteolysis (FEO) is an autosomal dominant disorder that is characterized by dramatic bone remodeling. It is extremely rare, with only three families reported in the literature. Last year, Hughes et al. (2000) identified identical mutations in the first exon of the TNFRSF11A gene in all published families with FEO. The TNFRSF11A gene codes for RANK, which has been demonstrated to be important in bone remodeling and the differentiation of pre-osteoclasts to osteoclasts. The mutation appears to arise as a tandem duplication of 18 bases in the sequence coding for the signal peptide of RANK. The resulting mutation causes an increase in RANK-mediated NF-6B signaling, consistent with a gain-of-function mutation. We have identified two additional FEO patients. One has no family history of FEO, but presented with classic symptoms including bilateral hearing loss at an early age, deterioration of teeth by the late 20's, and severe pain and swelling in distal tibia before the age of 20. The second patient had an extensive expansile tibial lesion, which was first noted in adolescence, and less prominent lesions in one humerus and a phalanx. Her hearing deteriorated after age 10, as did her teeth in her 20's. Her father also had skeletal involvement, hearing loss, and loss of dentition. Mutational analysis of the TNFRSF11A gene in these patients demonstrated an 18 base pair tandem duplication in the first exon, one base proximal to the tandem duplications reported by Hughes et al. (2000). This novel mutation results in the addition of the same 6 amino acids to the RANK signal peptide that has been observed in all other FEO patients. Further analysis of this sequence in this region demonstrated that it has the ability to form stable secondary structure. Formation of this secondary structure may facilitate the generation of these tandem duplications. Although the mutations we described are not identical on the DNA level to those previously described, the resulting proteins are identical. Thus, it appears that the mutations in the TNFRSF11A that give rise to FEO are highly specific.

SA461

Compound Heterozygous Mutations of the AIRE-1 Gene Causing Autoimmune Polyendocrinopathy Type 1. M. R. Bowl, J. J. O. Turner,* M. A. Nesbit,* B. Harding,* R. V. Thakker. Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom.

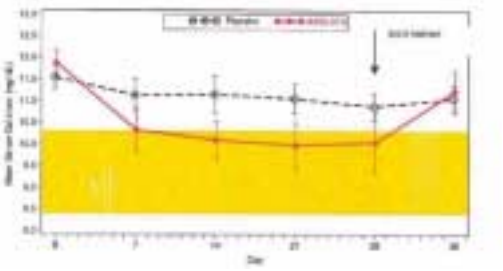
Autoimmune polyendocrinopathy type 1 (APS1) is an autosomal recessive disorder characterised by hypoparathyroidism, adrenocortical failure, mucocutaneous candidiasis, pernicious anaemia, alopecia (totalis or areata) and vitiligo. The gene causing APS1, which is located on chromosome 21q22.3, is referred to as the autoimmune regulator (AIRE-1) gene and consists of 14 exons that encompass a 1635bp coding region. The AIRE-1 protein, which consists of 545 amino acids, contains two plant homeodomain (PHD) zinc-finger motifs, a proline rich region and four LXXLL motifs, and these features suggest that it may have a role as a transcription factor. Over 20 different AIRE-1 mutations have been reported and two of these account for more than 85% of the mutations observed in APS1 patients. These two mutations are a nonsense mutation, Arg257Stop, that occurs in >80% of Finnish and >30% of USA patients, and a 13bp deletion (1094-1106del) that occurs in

>70% of British and >55% of USA patients. We have therefore investigated a 7 year old boy with APS1 for AIRE-1 mutations. The patient, whose parents are non-consanguineous, presented at 3.5 years of age with carpo-pedal spasms and investigations revealed hypocalcaemia (serum calcium = 1.58mmol/L) with an undetectable circulating PTH concentration. In addition to the hypoparathyroidism, he also developed moniliasis and alopecia totalis. Adrenocortical and thyroid function are normal. Treatment with alfacalcidol has restored normocalcaemia. Leukocyte DNA was utilised together with 14 pairs of primers to amplify, by use of PCR, the 14 exons and their respective exon-intron boundaries. The DNA sequences of the PCR products were then determined. This revealed the presence of two mutations which were the Arg257Stop mutation that was inherited from his mother, and the 13bp deletion (1094-1106del) that was inherited from his father. The nonsense mutation was confirmed by Taq I restriction enzyme analysis, and the deletion by agarose gel electrophoresis of the PCR products. The nonsense mutation predicts a truncated AIRE-1 protein of 256 amino acids, and the 13bp deletion predicts a frameshift with 50 missense amino acids followed by a premature termination signal at codon 372. Both of these mutations will result in the loss of the zinc-finger motifs, the proline rich region and two of the four LXXLL domains, and hence a loss of AIRE-1 activity. Thus, the patient is a compound heterozygote for inactivating AIRE-1 mutations, whilst the parents, who are phenotypically normal, are heterozygous carriers. These findings are consistent with an autosomal recessive inheritance for APS1.

SA462

An Evaluation of the Calcimimetic AMG 073 in Patients with Hypercalcemia and Primary Hyperparathyroidism (PHPT). D. M. Shoback,[1] A. F. Firek,[2] R. B. Mims,[3] T. A. Binder,*[4] T. Graves,*[4] S. A. Turner,*[4] M. Peacock.[5] [1]SF Veterans Affairs Med Ctr, UCSF, San Francisco, CA, USA, [2]Jerry L. Pettis VAMC & Loma Linda Univ Med Ctr, Loma Linda, CA, USA, [3]Diabetes and Endocrinology Associates for Research, Santa Rosa, CA, USA, [4]Amgen Inc., Thousand Oaks, CA, USA, [5]Indiana Univ Sch Med, Indianapolis, IN, USA.

Calcimimetics reduce PTH secretion and, subsequently serum Ca, by increasing the sensitivity of the parathyroid calcium-sensing receptor to extracellular Ca. In a prospective, double-blind, 5 week trial, 10 patients (6 AMG 073, 4 placebo) with PHPT and Ca levels ≥ 11.0 mg/dL were randomized to receive twice daily oral doses of 65 mg AMG 073 or placebo for 4 weeks of treatment and a 1 week follow up period (no drug administered). The effects of AMG 073 and placebo on serum Ca are shown below. The normal range for serum Ca is shaded.All but one (83%) of the AMG 073-treated patients experienced a reduction in serum Ca to the normal range (≤ 10.3 mg/dL) as compared with only 1 of 4 (25%) of placebo-treated patients. Serum Ca returned to predose levels 1 week after cessation of AMG 073 treatment. Baseline mean (SD) PTH was 189.9 (± 89.6) pg/mL in the AMG 073 group and 92.7 (± 25.3) pg/mL in the placebo group. Maximum reductions from baseline in mean PTH were observed in the AMG 073 group at 2 to 4 hours post-dose (approx. 39%). On day 28, mean (SD) PTH was reduced by 14.5 (± 7.62)% at 12 hours post-dose in the AMG 073 group as compared with an increase of 10.6 (± 7.57)% in the placebo group.AMG 073 was generally well-tolerated at this dose. In this 5-week study of patients with moderate to severe PHPT, 65 mg bid AMG 073 was efficacious in reducing serum Ca with concurrent reductions in PTH.



Disclosures: Amgen Inc.,3,5.

SA463

Genetic Analysis of the MEN1 Gene and HPRT2 Locus in 2 Kindreds with Familial Isolated Hyperparathyroidism. F. Cetani,[1] E. Pardi,*[1] E. Vignali,[1] A. Giovannetti,*[1] F. Golia,*[1] L. Cianferotti,[1] A. Picone,*[1] E. Ambrogini,*[1] P. Viacava,*[2] A. Pinchera,*[1] C. Marcocci.[1] [1]Endocrinology, University of Pisa, Pisa, Italy, [2]Oncology, University of Pisa, Pisa, Italy.

Familial hyperparathyroidism may occur as part of hereditary syndromes, including MEN1 and MEN2A), hyperparatyroidism-jaw tumor (HPT-JT) syndrome, and familial isolated hyperparathyroidism (FIHPT). It is unclear whether FIHPT represents a distinct genetic entity or is a variant of MEN1 or HPT-JT. We describe 2 unrelated Italian kindreds with FIHPT, in whom the clinical, histopathological and genetic analysis of the MEN 1 gene and HPRT2 locus at 1q21-32 suggest that both represent a variant of MEN1 or HPT-JT syndromes. Genomic DNA was isolated from peripheral blood leukocytes from all family members and from parathyroid tissues of subjects submitted to parathyroidectomy. Ionized calcium and PTH were measured in all family members. The coding sequence of the MEN1 gene were PCR-amplified and sequenced. Parathyroid glands were obtained from 5 subjects submitted to parathyroidectomy (III-2 and IV-4 of family 1; II-3, III-2 and III-4 of family 2). LOH at 11q13 and 1q21-32 was analyzed using polymorphic DNA markers PYGM and D11S449, and D1S212, D1S222, D1S428, D1S412, D1S413, respectively. Forward primers were conjugated with 5' fluorescent dye. PCR products were subjected to ABI PRISM 310 sequencer. Five members of family 1 and 2 of family 2 had biochemical evidence of primary hyperparathyroidism. A heterozygous deletion of 1 bp (1785delA) was found in exon 10 in affected members of family 1. The mutation was confirmed by subcloning in pCR4-TOPO vector. No MEN1 gene mutation was found in any member of family 2. LOH at 11q13 was observed in the tumors of family 1 members (PYGM in III-2; PYGM and D11S449 in IV-4) but not in tumors of family 2 members. Studies at 1q21-32 showed LOH at D1S428 and D1S212 markers in the tumors of the 2 affected members of family 2, whereas a retained heterozygosity was found in the remaining family 2 member (II-3) and in any tumor of family 1 members. The histological diagnosis in the 2 affected members of family 1 was chief cell hyperplasia with diffuse-nodular pattern of growth. Cut surface showed no cystic structures. Typical parathyroid adenoma was diagnosed in 1 member of family 2 and atypical adenoma in the remaining 2. All tumors showed cystic structures. We describe two unrelated kindreds clinically diagnosed as FIPHPT, which, on the basis of histological and genetic studies, can be classified as variants of MEN1 syndrome and HPT-JT. Therefore an extensive analysis of these genes should be performed in FIHPT families to identify the subset which are linked to MEN 1 gene or HPRT2 locus.

SA464

See Friday Plenary number F464.

SA465

Increased RANKL Expression and Osteoclast Numbers, but No Change in Osteoblast Number or Apoptosis in a Murine Model of Sustained PTH Elevation: Mechanism of Decreased Bone Density and Strength. C. A. O'Brien, R. S. Weinstein, C. Powers,* S. C. Manolagas. Div. of Endo/Metab, Center for Osteoporosis & Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Whereas intermittent administration of PTH results in net bone gain possibly via inhibition of osteoblast apoptosis, sustained elevation of PTH results in net bone loss. However, the cellular and molecular mechanisms by which continuously elevated PTH leads to bone loss remain unclear. In vitro studies reported elsewhere in this meeting show that PTH directly stimulates RANKL gene expression. To determine whether sustained PTH elevation stimulates RANKL in vivo and to quantitate the cellular changes associated with this condition, we induced secondary hyperparathyroidism in mice via a calcium-deficient diet. Five-month-old C57BL/6 mice were stratified into two groups (n = 4) with equal spinal bone mineral density and one group was placed on normal mouse chow (0.97% calcium, 0.85% phosphorus, Harlan/Teklad) and the second group on a calcium-deficient chow (<0.01% calcium, 0.4% phosphorus diet, ICN) for 7 days. Body weight was similarly maintained in the two groups but spinal density (BMD by DEXA) decreased by 8.4% and compression strength of L5 by 36% in the calcium-deficient group. As expected, calcium-deficiency raised serum intact PTH levels by 3-fold (18.2±4.7 vs 53.7±7.7 pg/ml, P <0.003) and there was an inverse relationship between PTH and BMD (r = -0.85, P <0.01) and between PTH and compression strength (r = -0.78, P <0.05) reflecting the role of the increased PTH in loss of skeletal assets. In contrast to the decrease in osteoblast and osteocyte apoptosis and anabolic effects seen with intermittent PTH administration, longitudinal thin sections of L1-4 vertebra showed no change in the prevalence of osteoblast or osteocyte apoptosis. However, the group fed the calcium-deficient diet had decreased cancellous bone area and trabecular width as well as increased cancellous osteoclasts, mineralizing perimeter and bone formation rate without a significant increase in cancellous osteoblasts. Consistent with the increased number of osteoclasts, Northern blot analysis of tibial RNA revealed increased RANKL mRNA expression. Based on these results we propose that dietary calcium deficiency and the resultant continuous elevation of PTH cause bone loss by increasing osteoclast number and bone turnover, via RANKL, without altering osteoblast production or lifespan, thereby resulting in an inadequate number of osteoblasts to reconstitute the increased number of resorbed cavities.

SA466

See Friday Plenary number F466.

SA467

Dynamics of Bone Resorption Changes in Pediatric Patients with Osteogenesis Imperfecta Treated with Cyclical Pamidronate. J. Marowska,* E. Jelonek,* M. Olszaniecka,* M. Kobylinska,* M. Lebiedowski,* R. Lorenc. The Children's Memorial Health Institute, Warsaw, Polandn

Osteogenesis imperfecta (OI) is an incurable heterogenous group of genetic disorders of collagen type I, characterized by osteopenia and recurrent fractures. Recently, administration of aminobisphosphonates to children with OI has been reported to improve bone mass and reduce fracture incidence with no significant adverse effects.The purpose of our study was to investigate rapid and long-term changes of bone resorption in children with OI treated for 12 months with pamidronate given according to two regimens, with special emphasis on the safety of treatment, considered as the absence of excessive and/or permanent inhibition of bone resorption.Seven patients with OI type I, III and IV, aged 7-16 yrs, with low L2-L4 BMD by DEXA (z score: -5.41±2.08) and high bone resorption as measured by urinary pyridinoline (ZS: 3.23±2.40) and deoxypyridinoline excretion (ZS: 2.53±2.88), entered the study. Pamidronate was administered iv in cycles of 3 consecutive days with 4 month intervals at a dose of 1 mg/kg/d (6 patients with OI type III and IV) or once a month (1 patient with OI type I) at a dose of 30 mg/m2 body surface. Urinary Pyr

and DPyr was measured by HPLC and L2-L4 BMD by DEXA (Lunar DPX-L) before and after 1 year of treatment.Three days of pamidronate infusion caused a profound, but transient, suppression of bone resorption, with maximal response observed around day 7 (% change from baseline: -56±9% and -70±6% for Pyr and DPyr, respectively; p<0.0001), corresponding with the decrease of Pyr ZS to -0.32±1.77 and DPyr ZS to -1.30±1.30, followed by the gradual gain (within 30 days) of new plateau, maintained till the end of the cycle at the level 20-30% lower as compared to baseline. The pattern of bone resorption changes was similar during all cycles. Pamidronate treatment in one-month cycle resulted in less profound maximal suppression of bone resorption (-36±11% for Pyr and -45±12% for DPyr), which was returning to baseline values within 30 days, up to the end of the 3rd cycle. Beginning from the 4th cycle, however, urinary excretion of crosslinks at day 30 of each cycle stabilized at the level 20-30% lower than baseline.One year pamidronate treatment resulted in relief of bone pain, improvement of mobility and decreased fracture incidence (from 2.3 to 1.3 per year), whereas L2-L4 BMD increase was observed only in a patient under 1 month cycle of bisphosphonate administration (ZS improvement from -5.21 to -3.50).It is suggested that pamidronate treatment of pediatric patients with OI does not result in excessive and prolonged suppression of bone resorption, which could be deleterious to bone health.

SA468

See Friday Plenary number F468.

SA469

Use of Representational Difference Analysis to Identify Candidate RFLPs Linked to Modifiers of the Murine *Cola2$^{oim/oim}$* Osteogenesis Imperfecta Phenotype. A. N. Patani,*[1] A. Pincavage,*[2] J. Bankston,*[2] N. P. Camacho,[2] R. D. Blank.[1] [1]Endocrinology/Medicine, University of Wisconsin, Madison, WI, USA, [2]Research Division, Hospital for Special Surgery, New York, NY, USA.

The *Cola2$^{oim/oim}$* mouse harbors a nonsense mutation in the gene encoding the a2 chain of type I collagen and consequently produces type I collagen composed of α1 homotrimers rather than $(\alpha1)_2$(a2) heterotrimers. This mutation is maintained on an outbred B6C3 background, but we have noted that inbreeding results in attenuation of the bone fragility phenotype that is characteristic of *oim/oim* mice. Notably, there is considerable phenotypic heterogeneity among these animals. The outbred animals averaged 3.2 ± 1.6 long bone fractures each under normal cage activity while the partially inbred animals averaged 1.0 ± 1.1 long bone fractures. In an attempt to identify loci whose segregation modifies the *oim/oim* phenotype, we used pools of genomic DNA prepared from outbred and partially inbred *oim/oim* animals to perform representational difference analysis. This PCR-assisted subtraction method results in preferential amplification of restriction fragments present exclusively in one of the two DNA sources. We amplified representative populations of BamH1 restriction fragments from the outbred and inbred DNA pools by PCR to generate "amplicons." We iteratively subtracted outbred from inbred amplicons and amplified the difference products. Following 3 rounds of subtraction and amplification, difference products were cloned into a plasmid vector and analyzed. Unique, polymorphic clones obtained by this method are putative markers for genes that modify the *oim/oim* phenotype. Representational difference analysis provides a complementary approach to linkage mapping in localizing genes of interest.

SA470

Early Treatment Improves Prepubertal Growth in X-linked Hypophosphatemic Rickets. O. Makitie,* E. Sochett.* Division of Endocrinology, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

X-linked hypophosphatemic rickets (XLH) is a disorder of renal phosphate wasting characterized by hypophosphatemia, defective bone mineralisation, rickets and impaired growth. Treatment with oral phosphate (Pi) and calcitriol is usually started in infancy and results in improved blood biochemistry, resolution of the skeletal changes and improved height gain. However, even with optimal medical treatment, many patients do not achieve normal stature; one contributing factor may be the height loss occurring prior to diagnosis and onset of treatment. To assess whether the time of inititation of treatment impacts on pre-pubertal growth, we retrospectively examined the growth data in 17 well controlled patients with XLH followed regularly at our clinic. Individual growth curves were drawn using growth data obtained in clinic until puberty. Heights at 6-monthly intervals (until 9.5 yrs) were interpolated and transformed into SD scores (SDS). Height SDS for males and females were pooled and medians calculated for the two groups separately at each age. Treatment was started in 8 patients (3 girls) in the first year of life (median age 0.35 years, range 0.15 - 0.58 years, Group 1) and in 9 patients (8 girls) after one year of age (median 2.0 years, range 1.3 - 8.0 years, Group 2). All in Group 1 and 4 patients in Group 2 had a positive family history of XLH. At the start of treatment the median height SDS for Group 1 was -0.76 SDS (range -1.4 - +0.14 SDS) and for Group 2, -1.7 SDS (range -2.8 - -1.0 SDS) (P=0.0028). The median height SDS for Group 1 remained > -1.0 SDS (range for median -0.96 - -0.50 SDS) and for Group 2, <-1.3 SDS (range for median -1.9 - -1.3 SDS) throughout childhood. The height SDS at commencement of treatment correlated significantly with the height SDS later in childhood (at 9.5 years P<0.02 for each group). There was no significant difference in the rate of treatment-related complications i.e. secondary and tertiary hyperparathyroidism and nephrocalcinosis between the two groups. We conclude that patients commencing oral Pi/calcitriol treatment before a year of age experience improved pre-pubertal growth overall when compared with those starting after a year of age. Our data also suggest that height lost prior to treatment may not be completely recovered and may contribute to the poor stature outcome in some patients.

SA471

Retarded Gene Expression for Cartilage-related Proteins During Fracture Healing in Experimental Diabetes. A. Ogasawara,* M. Yamazaki,* F. Nakajima,* S. Shimizu,* A. Nakajima,* H. Moriya.* Chiba Univercity, Chiba, Japan.

INTRODUCTION: Diabetes mellitus (DM) is a condition which clinicallyÅ@impairs fracture repair. Little has been analyzed, however, how DM condition influences the gene expression in chondrogenesis during fracture healing. In this study, we produced the standardized fracture in experimental DM rats, and analyzed the spatial and temporal expression of genes encoding cartilage-related proteins by means of in situ hybridization and northern blot analysis.MATERIALS AND METHODS: Fracture model: A closed middiaphyseal fracture was created after intramedullary pinning of the right femur in both streptozotocin induced DM rats and control rats. Animals were euthanized at 4, 7, 14, 21 and 28 days postoperatively, and the fractured femora were fixed, decalcified and embedded in paraffin. Measurement of cartilage area: Sections were stained with toluidine blue, and the cartilage area showing metachromasia was calculated using a public domain NIH image program. In situ hybridization: Sections were hybridized with DIG-labeled cRNA probes for pro-a1(II) collagen (COL2A1), pro-a1(X) collagen (COL10A1) and osteopontin (OP). Northern blot analysis: Total RNAs were extracted from DM and control calluses, and the mRNA levels were determined using 32P-labeled probes for COL2A1 and COL10A1. RESULTS: In the soft calluses of DM rats, the cartilage tissues were apparently smaller than those of control rats. In both DM and control rats, COL2A1 signal was detected in proliferative chondrocytes from day 4, and COL10A1 signal in hypertrophic chondrocytes from day 7. By quantification, expression of COL10A1 reached the peak value on day 14 in both types of rats. But expression of COL2A1 in DM rats reached peak level later than in control rats. In DM rats the amounts of COL2A1 and COL10A1 were markedly decreased on days 21. OP signal was detected in terminally differentiated chondrocytes at the front of endochondral ossification. In DM rats, the number of OP mRNA expressing cells was decreased and the structure of primary spongiosae was immature.DISCUSSION: The results demonstrated that the diabetic state suppressed the gene expression for cartilage extracellular matrices and differentiation of chondrocytes in fracture calluses. Especially, the matrix synthetic activity was apparently decreased in later stages of fracture healing. The diabetic state also suppressed the replacement of hypertrophic chondrocytes by primary spongiosae. We suggest that, in DM condition, the retarded gene expression for the cartilage-related proteins in later stages of fracture healing plays some important role(s) on the impairment of fracture repair.

SA472

Risedronate But Not Alendronate Slows Disease Progression in the Guinea Pig Model of Primary Osteoarthritis. J. M. Meyer, R. W. Farmer,* M. C. Prenger.* Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Most animal models of osteoarthritis involve chemical or surgical initiation of the disease, although the majority of human OA is considered primary. The Duncan-Hartley guinea pig is a model of primary OA and mimics human disease in many aspects. Cartilage lesions begin at about 3 months of age or 750g weight. They are bilateral and begin primarily on the medial tibial plateau (MTP). Chondrocyte cloning, osteophytes and tidemark duplication can also be seen. Disease severity progresses as the animals age and gain weight. This may be a useful model for testing potential structure modifying OA drugs. Animals were randomized into the treatment groups shown in Table 1. The bisphosphonates risedronate (Actonelä) and alendronate (Fosamax™) or sterile isotonic saline (vehicle control) were administered via subcutaneous injection 5 consecutive days/week for 12 months. At sacrifice, the left tibia of the stifle joint was disarticulated and stained with Evan's blue dye. Joints were then placed in 10% neutral buffered formalin and digitally photographed. These images were analyzed using a BDS Image Analysis System (Oncor Image, Gaithersburg, MD). Lesion, MTP and osteophyte areas were manually outlined by a single blinded grader, and area calculated by the software program. A non-parametric Friedman's rank sum test was used to compare treatment groups to control. Median scores are reported.

Treatment & Dose (mg/kg)	Lesion area mm^2	% Lesion area	Osteophyte area mm^2	% Osteophyte area
Vehicle ---	8.3	35.2	12.1	42.8
Risedronate 0.004	8.6	36.1	9.5	41.1
Risedronate 0.012	8.4	28.4*	8.6	34.1*
Risedronate 0.03	6.2*	26.9*	7.2*	24.4*
Alendronate 0.005	11.0	43.2	12.4	47.1
Alendronate 0.012	9.6	36.8	11.7	40.1
Alendronate 0.03	12.2	41.1	11.7	41.5

*=p<0.05 vs vehicle, two sided testRisedronate but not alendronate had a statistically significant effect on cartilage lesion and osteophyte size in the guinea pig model of primary OA. This is consistent with previous data (unpublished) suggesting not all bisphosphonates are effective at slowing disease progression in this model. These data suggest risedronate may be efficacious as a structure modifying drug in OA.

SA473

Prognostic Value of Serum Bone Markers and Hand Bone Densitometry in Very Early Rheumatoid Arthritis : Preliminary Results of the VERA Study. A. Daragon,[1] O. Vittecoq,*[1] M. Brazier,[2] O. Mejjad,[1] P. Fardellone,[3] K. Krzanowska,*[1] P. Boumier,[4] C. Zarnitsky,[5] A. Phan Van,[6] A. Gayet,[7] J. F. Menard,[8] X. Le Loet.[1] [1]Rheumatology, CHU, Rouen, France, [2]Biochimie, CHU, Amiens, France, [3]Rheumatology, CHU, Amiens, France, [4]Collège des Rhumatologues, Amiens, France, [5]Rheumatology, CHG, Le Havre, France, [6]Collège des Rhumatologues, Tours, France, [7]Collège des Rhumatologues, Rouen, France, [8]Biostatistiques, CHU, Rouen, France.

The aim of the study was to determine the prognostic value of serum bone markers and hand bone densitometry in very early rheumatoid arthritis (RA).From a population-based recruitment, 66 patients with very early (median duration: 4.3 months [1-6 mo]) peripheral arthritis (swelling >=2 joints for > 4 weeks) were studied. Patients were steroid and DMARD naive. After a year, patients were classified as RA or non RA. Assessments were performed at entry (T1), after 9 (T2) and 12 (T3) months. Serum bone markers were C telopeptide (sCTX) by ELISA test, free pyridinoline (Pyr) and free deoxypyridinoline (Dpyr) by HPLC. Other investigation : hand bone densitometry (DEXA), hands and feet X Rays. Prognosis was defined as the progression of radiological damage from T1 to T3 (van der Heijde modified Sharp's method).At T3, 26 patients had radiological progressive disease, and 40 no progressive disease. 50/66 patients fulfilled ACR criteria for RA (75% in non progressive group, 77% in progressive group, NS). At T1, no difference was found between progressive group and non progressive group, excepted for rheumatoid factors more frequent in progressive group ($p < 0,03$). Bone mineral density (BMD) of both hands decreased significantly from T1 to T3 in the progressive group ($p < 0,03$), whereas it remains unchanged in the non progressive group. Furthermore, sCTX, Pyr and Dpyr levels at T1 were negatively correlated to the evolution of hand BMD from T1 to T3 ($p < 0,02$, and $p < 0,01$ respectively).In patients with early RA, a structural bone damage progression after 12 months duration is associated with a decrease of hand BMD. Moreover, this decrease was negatively correlated with levels of serum bone markers at entry. These preliminary results of the VERA study need to be confirmed on a larger population.

SA474

See Friday Plenary number F474.

SA475

Osteoclast Inhibitory Lectin (OCIL), RANKL and OPG Expression at Sites of Inflammation and Joint Destruction in Rheumatoid and Collagen-Induced Arthritis. N. A. Sims, H. Zhou, T. J. Martin, M. T. Gillespie, K. W. Ng, E. Romas. St Vincent's Institute of Medical Research and Department of Medicine, University of Melbourne, Fitzroy, Victoria, Australia.

Rheumatoid arthritis (RA) has been shown recently to be associated with a high level of RANKL expression in synoviocytes, which are able to induce osteoclastogenesis in vitro. Ostoclast inhibitory lectin (OCIL) is a type II transmembrane C-type lectin also shown recently to inhibit osteoclast formation in vitro. In normal tissues, OCIL expression in normal tissue parallels that of RANKL and OPG, being highly expressed in osteoblasts and pre-hypertrophic chondrocytes.OCIL expression in rheumatoid arthritis was studied by in situ hybridisation and immunohistochemistry in human synovectomy samples and in the rat collagen-induced arthritis model (CIA). In human synovectomy samples OCIL was expressed in the synovial lining of normal, osteoarthritic (OA) and RA samples. This expression was similar to, but not identical with, RANKL and OPG which were abundant in a higher number of synovial cells. In RA samples, OCIL protein and mRNA were also expressed at high levels in TRAP positive osteoclasts at sites of erosion at the articular cartilage-bone interface. In serial sections, this expression co-localised with both RANKL and OPG mRNA expression. OCIL expression was also high in follicular lymphoid aggregates in RA samples and co-localised with RANKL and OPG in serial sections. In the CIA rat model, in which joint inflammation and destruction are induced by a single intradermal injection of type II collagen, OCIL, RANKL and OPG were also highly expressed in lymphoid aggregates at sites of inflammation. While OCIL was expressed in periosteal and trabecular osteoblasts, it was not expressed in trabecular osteoclasts in control rats or after arthritis was induced. In contrast, both immunohistochemistry and in situ hybridisation revealed high levels of OCIL in TRAP positive osteoclasts at sites of joint destruction in CIA. Osteoclastic OCIL expression co-localised with RANKL and OPG. The expression of mRNA and protein for OCIL and mRNA for RANKL and OPG all appeared to be greater in osteoclasts actively resorbing at sites of disease than in normal bone. Expression of OCIL in osteoclasts only at sites of rheumatoid joint destruction and in inflammatory tissue suggest that OCIL may be a novel target for therapeutic intervention in both the inflammation and joint destruction associated with rheumatoid arthritis.

SA476

See Friday Plenary number F476.

SA477

Post-transcriptional Regulation of Calcitonin Receptor Gene Expression in Mouse Osteoclast-Like Cells. S. Yasuda,* S. Wada,* S. Kitahama,* S. Suda,* T. Nagai,* M. Iitaka,* S. Katayama.* Fourth Department of Internal Medicine, Saitama Medical School, Iruma-gun, Japan.

Using mouse osteoclast-like cells (OCs), we have shown that short exposure to calcitonin (CT) resulted in prolonged reduction of CT binding by inhibiting de novo CT receptor (CTR) synthesis. This mechanism of CTR regulation is specific for particular target cells. Experiments to explore the molecular basis of the CTR gene expression in mouse OCs suggested that GCs up-regulate CTR by increasing transcription of CTR gene. Studies on mRNA turnover in the presence of transcriptional inhibitors suggest that the action of CT to destabilize the CTR mRNA predominates over increased transcription by GC. The mRNA stability observed in mouse OCs required ongoing transcription, suggesting the involvement of a labile protein mRNA-degrading factor. In this study, we subcloned the 3' untranslated region (UTR) of mouse CTR gene using nested PCR technique from mouse DNA library. Nuclear extracts of mouse osteoclasts srrongly degraded in vitro transcript of the 3'UTR labelled with 32P UTP, whereas the extracts did not act on the transcript of GAPDH. The treatment with RNase inhibitor and trypsin could not diminish this mRNA degrading capacity. Interestingly, the nuclear extracts from mouse hypothalamus and osteoblastic cells (MC3T3E1) did not show any degrading capacity, which may suggest that this effect is specific for particular target cells. When nuclear extracts of MC3T3E1 cells were incubated with labelled transcripts of the CTR 3'UTR, the bands were shifted to the upper portion. This may suggest that a certain nuclear factor would bind to 3'UTR of the CTR gene. It has bee shown that the AUUUA motifs, as well as other A/U-rich sequences, determine the stability of other mRNA transcripts through binding with multiple proteins in this region As we have shown, multiple copies of the AUUUA motif have been identified in the 3' UTR of CTR gene in various species. We are currently investigating the factors involved in CTR regulations and roles of the 3'UTR in the CTR gene.

SA478

Regulation of Osteoclasts by Novel Orally Bioavailable Calcitonin Conjugates: Role of Cytosolic Calcium. S. V. Komarova,*[1] L. A. Paige,*[2] S. M. Sims,*[1] S. J. Dixon.[1] [1]Department of Physiology, University of Western Ontario, London, ON, Canada, [2]Nobex Corporation, Research Triangle Park, NC, USA.

The effects of salmon calcitonin and orally bioavailable conjugates of calcitonin were tested on isolated rat osteoclasts and HEK-293 cells transfected with the calcitonin receptor. Time-lapse video microscopy was used to record osteoclast morphology and motility, and retraction was quantified as changes of planar area. The concentration of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) was monitored by spectrofluorimetry in single fura-2-loaded osteoclasts or in suspensions of indo-1-loaded HEK-293 cells. Addition of calcitonin or conjugates (CT-001 or CT-002) at 1 nM caused immediate arrest of osteoclast membrane ruffling and subsequent retraction to ~50% of initial area. The effects on retraction were concentration-dependent, with calcitonin and the conjugates causing retraction at concentrations as low as 10 pM. As a control, the same amphiphilic polymer attached to an unrelated protein was tested and shown to have no effects on osteoclast morphology and motility. Since it has been suggested previously that osteoclast retraction in response to calcitonin is mediated by Ca^{2+} signaling, we investigated the effects of calcitonin and its conjugates on $[Ca^{2+}]_i$. In osteoclasts, calcitonin-induced elevations of $[Ca^{2+}]_i$ were variable and relatively small. Therefore, we studied Ca^{2+} signaling in HEK-293 cells transfected with the C1a isoform of the calcitonin receptor. In these cells, calcitonin and the conjugates caused concentration-dependent elevations of $[Ca^{2+}]_i$. However, the rise of $[Ca^{2+}]_i$ occurred at concentrations of agonists 100 to 1000 fold higher than those required to elicit retraction. Although the rise of Ca^{2+} and retraction were quantified in different cell types, the data suggested that retraction of osteoclasts may not be due solely to rise of $[Ca^{2+}]_i$. To further investigate a requirement for Ca^{2+} in retraction, we preloaded osteoclasts with BAPTA to buffer changes of $[Ca^{2+}]_i$. Surprisingly, calcitonin (1 nM) continued to elicit retraction of osteoclasts after BAPTA treatment, although at a slower rate than in controls. Prolonged stimulation with calcitonin (3 h) resulted in comparable retraction in control and BAPTA-treated cells. We conclude that orally available calcitonin conjugates cause retraction of osteoclasts in a manner similar to that elicited by salmon calcitonin. This response is indicative of functional calcitonin receptor signaling that leads to inhibition of bone resorption. Moreover, the extent of osteoclast retraction induced by calcitonin is independent of $[Ca^{2+}]_i$, although $[Ca^{2+}]_i$ does modulate the rate of retraction.

SA479

Effects of Age and Amylin Treatment on Long Bones in Growing and Mature Mice. V. L. Ferguson, L. K. Hermann,* E. E. Smith,* T. A. Bateman, S. J. Simske. BioServe Space Technologies; University of Colorado, Boulder, CO, USA.

This study examines the effects of amylin on cortical bone properties in growing mice. Male C57BL/6J mice, aged 5- (n=30), 11- (n=36) and 40-weeks (n=45) were equally divided into three groups: baseline controls were killed at the start of the study, and vehicle (saline) and amylin (0.42 mg/kg rat amylin) treatment groups were killed on day 28. Injections were administered s.c. daily. Immediately following sacrifice, mice were weighed and the liver, thymus and spleen were removed from the treatment groups. Right femora

were measured for length and composition in all groups. Left femora were analyzed via quantitative histomorphometry at the mid-diaphysis in the 11- and 40-week old groups. Amylin treatment resulted in increased body mass (5.5%, 5.2%, and 1.3% (N.S.) for 5-, 11- and 40-week old mice, respectively), increased liver mass in the two youngest groups (10.8%, 10.4% and -0.6% (N.S.)) and decreased thymus mass (10%, 14.7% and 18.3%) in all groups from control levels. Spleen mass increased with age but was unaffected by treatment. Femur length, mass and percent mineralization were also unaffected by treatment with amylin, but increased with age. Femur cross-sectional morphology showed significant alterations with age and amylin treatment. The periosteal surface of the femur-mid-diaphysis significantly decreased in perimeter (1.3% and 3.2% in 11- and 40-week old groups) and total cross-sectional area (2.1%, 6.2%) in amylin treated animals. Cortical area tended to decrease in young animals (1.1%) and significantly decreased in old animals (7.1%) in amylin treated mice. The medullary cavity was significantly smaller in animals treated with amylin, resulting in decreased endocortical perimeter (1.7%, 2.3%) and cross-sectional area (2.9%, 5.5%), reflecting a statistically significant increase in endosteal formation compared to controls. Decreased cross-sectional area at the femur mid-diaphysis reduced the cross-sectional moment of inertia in the 11-week old (5.2%, N.S. and 2.9% for Ix and Iy, respectively) and 40-week old mice (13.8% and 16.2%), and resulted in moments that were lower than those in baseline mice. This was coupled with decreased periosteal perimeter and total cross-sectional area in both ages. Amylin did not appear to stimulate normal bone formation, and affected more mature bone at least as much as younger bone.

SA480

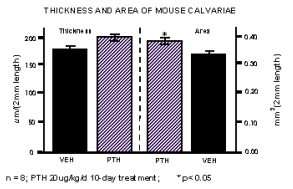
Calcitonin Induces the Transcription of Early Response Genes. M. Nakamura,[1] N. Segawa,*[2] Q. Yang,*[1] Y. Nakamura,*[1] H. Yamasaki,*[3] I. Mori,*[1] K. Kakudo.*[1] [1]Department of Pathology, Wakayama Medical University, Wakayama, Japan, [2]Department of Urology, Osaka Medical College, Osaka, Japan, [3]Department of Biology, Wakayama Medical University, Wakayama, Japan.

Calcitonin (CT), a polypeptide hormone, regulates urinary calcium excretion. The purpose of this study is to identify potential downstream gene regulatory mechanisms induced by CT and analyze their associations with the MAPK pathway. By cDNA subtraction hybridization method, we identified three immediately early (IE) genes: connective tissue growth factor (CTGF), IL-3 promoter transcriptional activator (NF-IL3A), and urokinase-type plasminogen activator (uPA). The effects of CT on the expression of IE mRNA were confirmed by Northern blot analysis. CT increased CTGF mRNA levels in a time-dependent manner, reaching a maximum after 1 hr (5.7 fold), NF-IL3A mRNA levels reaching a maximum after 1 hr (3.2 fold), and uPA mRNA levels after 5 hrs (17.5 fold). In order to exclude the possibility that CT regulates these genes expression through *de novo* protein synthesis of cytokines or transcription factors, the cells were treated for 3 hrs with CT and cycloheximide, a protein synthesis inhibitor. Addition of both CT and cycloheximide increased mRNA levels of the three genes, as compared to CT or cycloheximide alone. These data suggest that the stimulation of expression of these genes by CT is not induced by increased synthesis of regulating proteins. We also found that treatment of LLC-PK1with CT led to the phosphorylation of ERK1/2. Therefore, we examined whether the regulation of CT on the expressions of these IE genes involve ERK1/2 pathway. We demonstrated that PD98059, a MEK inhibitor, inhibited mRNA expressions of CTGF and uPA induced by CT, but had no obvious influence on the NF-IL3A expression. Our present findings suggest that the transcriptions of the CTGF, NF-IL3A and uPA are induced by CT pathway and might be important mediators of CT function in renal cells. Furthermore, we also elucidated the regulation of CTGF and uPA by CT involve RAS-ERK pathway.

SA481

Local Anabolic Effects of Parathyroid Hormone in a Mouse Calvarial Model. W. Zhao, R. J. Murrills, M. Tarby,* V. Dell,* Y. Kharode,* F. J. Bex. Bone Metabolism and Osteoporosis Research, Wyeth-Ayerst Research, Radnor, PA, USA.

PTH is well known to have anabolic effects when administered systemically but its local effects are less well documented. Female Swiss-Webster mice were injected daily directly onto the calvarium with hPTH(1-34) and bone was quantified by histomorphometry following H+E staining. Osteoblast apoptosis was visualized by TUNEL staining and CBFA expression by immunohistochemistry. An initial experiment explored various doses of PTH, using a 5-day course of injections followed by a 21-day no-treatment "chase" period. BMP-2 at 20ug/kg, a positive control, produced a clear increase in calvarial thickness. With PTH, an increase in calvarial thickness was noted at 10 and 20ug/kg, but at 40ug/kg and 80ug/kg there was no increase and at 80ug/kg there was histological evidence of increased resorption. At 20ug/kg, fewer apoptotic osteocytes and periosteal osteoblasts or stromal cells were noted whereas at 80ug/kg, an increased number of apoptotic osteoblasts were observed. CBFA expression was increased by BMP-2 in the periosteum and sagittal suture, but the effect of PTH on CBFA expression was less convincing. In a time-course study using a 5-day or 10-day course of daily injections of 20ug/kg PTH (with no "chase" period), increased periosteal cellularity was noted at 5 days and an increase in calvarial area was evident at 10 days.



The effect was most prominent on the hemicalvarium that had the injection site, indicating a local effect of the peptide. We conclude from this that PTH can, at certain doses, have a local anabolic effect when injected directly onto mouse calvaria. The effect appears to be related to an increase in osteoblast number, at least partly a consequence of a decrease in osteoblast apoptosis.</p

SA482

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SA483

In Vitro Inhibition of Bone Resorption by Human PTH(7-84). P. Divieti, M. R. John, H. Juppner, F. R. Bringhurst. Endocrine Unit, Mass General Hospital, Harvard Medical School, Boston, MA, USA.

Intact PTH from different mammalian species comprises 84 amino acids and shows high sequence conservation within both its amino (N)-terminal and carboxyl (C)-terminal regions. The N-terminal 34 amino acids are sufficient for classical actions of intact PTH in the regulation of mineral ion homeostasis and bone metabolism, which are known to be mediated through activation of the type 1 PTH/PTHrP receptor (PTH1R). C-terminal PTH (CPTH) fragments, analogous to those known to be released into blood during peripheral proteolysis of intact PTH or via direct parathyroid secretion, exert biologic effects in osteoblastic and chondrocytic cells and bind specifically to a putative CPTH receptor in such cells. Recently large CPTH fragments, minimally truncated at their N-termini and cross-reactive with most commercially available intact PTH immunoassays, were detected in normal plasma and, at higher levels, in plasma of patients with advanced renal failure. These fragments exhibit chromatographic properties similar to synthetic PTH(7-84). Moreover, hPTH(7-84) recently was shown to inhibit the calcemic actions of PTH(1-84) and PTH(1-34) in parathyroidectomized rats.To determine if hPTH(7-84) might antagonize the calcemic response to PTH(1-84) in vivo via direct effects on bone, we tested hPTH(7-84) for inhibition of in vitro assays of osteoclast formation (murine marrow cultures) and bone resorption (release of 45Ca from intact murine calvarial bone). Addition of hPTH(7-84) alone (300nM) reduced basal 45Ca release by approximately 50% (control: 17.8±5.7%; hPTH(7-84): 9.6± 1.9% ; p,< 0.001), an effect comparable to that of salmon calcitonin. HPTH(7-84) also inhibited agonist-induced bone resorption caused by a variety of agents, including intact PTH(1-84), PTH(1-34), 1,25(OH)2D3 (1,25D), prostaglandin E2 and interleukin-11. In murine marrow cultures, 1,25D (10 nM) stimulated the formation of TRAP positive cells by 13.9 fold; in the presence of 300nM PTH(7-84), this response was reduced by 65%. It is unlikely that this inhibitory effect of PTH(7-84) was due to an antagonistic effect at the PTH1R, as neither hPTH(3-34) nor [L11,DW12]PTHrP(7-36), both potent PTH1R antagonists in these systems, inhibited bone resorption induced by PTH(1-34) or 1,25D-induced osteoclast formation. We conclude that hPTH(7-84), acting via receptors (probably CPTH receptors) independent of the PTH1R, can exert a generalized antiresorptive effect that may involve both inhibition of osteoclast recruitment and reduction in formation or activation of mature osteoclasts.

SA484

See Friday Plenary number F484.

SA485

Effect of Intermittent Low Dose Treatment of Human Parathyroid Hormone (1-34) on Fracture Healing in Rats. A. Nakajima,[1] M. Yamazaki,*[1] N. Shimoji,*[2] K. Shiomi,*[2] S. Shimizu,*[1] K. Goto,[1] H. Moriya.*[1] [1]Orthopaedic Surgery, Chiba University School of Medicine, Chiba, Japan, [2]Institute for Medical Research & Development, SUNTORY Limited, Gunma, Japan.

Recent reports have shown that intermittent PTH (1-34) treatment increases callus formation and mechanical strength in healing experimental fractures. In this study, we tested whether an intermittent low dose treatment of hPTH (1-34) could increase callus formation and mechanical strength, then analyzed the molecular mechanisms of the effect by PTH (1-34). Closed mid-diaphysial fractures were created in the femora of 7-week-old male SD rats. The rats were divided into two groups: rats injected daily with 10 µg of PTH (1-34)/kg subcutaneously (PTH group), and rats injected daily with vehicle subcutaneously (vehicle group). Samples harvested at 4, 6 and 8 weeks after fracture were used for DXA and mechanical testing, and those from 2, 4, 7, 14 and 21 days after fracture were analyzed for molecular assays. It was found that at any point both BMD and ultimate load were significantly increased in the PTH group as comparison to the vehicle group ($p<0.01$). In the PTH group, the hard calluses consisted of well-developed trabeculae with less bone marrow, and the number of osteoclasts were significantly increased compared to the vehicle group. In the earlier stages, the number of PCNA-positive cells was increased in the osteoprogenitor cells of the periosteum in PTH group. Northern blot analysis demonstrated that mRNA expression levels of relatively immature osteoblast-lineage markers such as type-I collagen, ALP and osteonectin, were elevated even in the later stages of fracture healing in the PTH group. Up-regulation of IGF-I mRNA was only found in the early stage in the PTH group. The expression profiles of type-II and -X collagen, which are chondrogenic differentiation markers, showed similar results for the PTH group and vehicle group. These findings suggest that PTH stimulates the proliferation of osteoprogenitor cells, contributing to the formation of hard calluses, and that PTH modulates not only callus formation but also callus resorption, presumably accelerating callus remodeling. We speculate these molecular mechanisms lead to the increase of BMD and ultimate load of fracture calluses. In conclusion, intermittent treatment of low dose hPTH (1-34) increased callus formation and mechanical strength by simultaneously modulating osteoblast and osteoclast function.

SA486

Parathyroid Hormone Induces Cyclooxygenase-2 in Osteoblasts via a Composite NFAT/AP-1 Binding Site. D. Chikazu,[1] O. S. Voznesensky,[1] B. E. Kream,[1] X. Li,[1] H. R. Herschman,[2] C. C. Pilbeam.[1] [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Biological Chemistry, UCLA School of Medicine, Los Angeles, CA, USA.

The nuclear factor of activated T-cells (NFAT) family of transcription factors plays a central role in the regulation of cytokine gene transcription in immune cells but has not yet been implicated as a mediator of gene transcription in osteoblasts. Cyclooxygenase-2 (COX-2) is an inducible gene that is expressed not only in immune cells but also in osteoblasts. PTH, a potent transcriptional inducer of COX-2 in osteoblasts, was previously shown to act via the -150/-40 bp 5'-flanking region of the murine COX-2 gene. Sequence analysis identified three NFAT core consensus sequences (5'-GGAAA-3') at -111/-107 bp, -90/-86 bp and -77/-73 bp in the COX-2 promoter. The -77/-73 bp sequence (NFAT77) is adjacent to the AP-1 site (5'-AGAGTCA-3') at -69/-63 bp. NFAT and AP-1 (Fos/Jun) proteins interact cooperatively in other systems to mediate transcription, and the -77 to -63 bp sequence, GGAaagacagagTCA, is consistent with the minimum consensus sequence, GGA(N)9TCA, reported for NFAT/AP-1 composite sites. To determine whether the NFAT/AP-1 site at -77/-63 bp mediates PTH induction of COX-2 in osteoblasts, murine osteoblastic MC3T3-E1 cells were stably transfected with -371/+70 bp of the COX-2 5'-flanking region fused to a luciferase reporter (Pluc) or with Pluc carrying 2 bp mutations of NFAT77 (5'-ttAAA-3'), the AP-1 site (5'-AGAGTtg-3'), or both. Mutation of the AP-1 binding site, the NFAT77 site, or both NFAT77 and AP-1 decreased PTH stimulated-luciferase activity by 41%, 58%, or 92%, respectively. On electrophoretic mobility shift analysis, PTH stimulated binding to a radioactively labeled oligonucleotide spanning -85 to -57 bp of the COX-2 5'-flanking region. Binding was supershifted or inhibited by both an antibody for NFAT (specifically NFATc1) and antibodies for c-Fos / c-Jun but not by a non-specific IgG antibody. In summary, we have demonstrated that a composite NFAT/AP-1 binding site in the murine COX-2 promoter mediates PTH induction of COX-2 promoter activity in osteoblastic cells. This site is likely to be involved in prostaglandin responses to other physiological and pathological stimuli in bone.

SA487

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SA488

A New High-Affinity Parathyroid Hormone 1 Receptor Antagonist, Mouse Tuberoinfundibular Peptide (7-39). S. R. J. Hoare, T. B. Usdin.* Laboratory of Genetics, NIMH, National Institutes of Health, Bethesda, MD, USA.

The parathyroid hormone 1 (PTH1) receptor mediates the pathophysiological effects of PTH-related protein (PTHrP) in hypercalcemia of malignancy and PTH in hyperparathyroidism. A PTH1 receptor antagonist might prove useful for treating these conditions. Remarkably, one of the highest-affinity PTH1 receptor antagonists identified is an analogue of the PTH2 receptor's endogenous ligand, human/bovine tuberoinfundibular peptide of 39 residues (h/bTIP39): h/bTIP(7-39) binds with an affinity of 6.2 nM to the human PTH1 receptor. We have now prepared mouse (m) TIP(7-39), and tested it's effectiveness as an antagonist of the rat PTH1 receptor, since rodents will likely be used to test the in vivo effects of the ligand. (Cloning and sequencing of mTIP39 cDNA revealed four differences of amino-acid sequence between mTIP39 and h/bTIP39 (h/b residue in parentheses) – Arg24 (His), Asp27 (Asn), Gln31 (His) and Leu35 (Val)). mTIP(7-39) antagonized PTHrP(1-34)-stimulated cAMP accumulation via the rat PTH1 receptor with higher potency (Kb = 44nM) than h/bTIP(7-39) (210nM), PTHrP(7-34) (640nM) and bPTH(7-34) (>3000nM). mTIP(7-39) was 21-fold more potent as an antagonist of the rat PTH1 receptor than the rat PTH2 receptor. The effect of rat plasma on the bioactivity of mTIP(7-39) was tested by pre-incubation of [125I]mTIP(7-39) with plasma (50% v/v) at 37C followed by measuring binding of the radioligand to the human PTH1 receptor. Plasma pre-treatment reduced [125I]mTIP(7-39) binding with a t1/2 of 1.8 hours, an effect blocked by protease inhibitors. In radioligand binding assays mTIP(7-39) bound the human PTH1 receptor with high affinity (2.9nM), comparable with h/bTIP(7-39) (6.2nM) and greater than [D-Tryp12]bPTH(7-34) (45nM) and PTHrP(7-34) (65nM). mTIP(7-39) dissociated from the human PTH1 receptor (t1/2 of 6.3 min) much more slowly than [D-Tryp12]bPTH(7-34) (13 sec) and PTHrP(7-34) (9 sec). [125I]mTIP(7-39) also detectably bound the human PTH2 receptor, providing the first radiolabeled antagonist for this receptor. In summary, mTIP(7-39) displays higher potency for the rat PTH1 receptor than previously described antagonists, is PTH1 receptor-selective, reasonably stable in plasma, and binds with a relatively high-affinity and slow off-rate. This ligand should be useful to test the effectiveness of PTH1 receptor antagonism in rodent models of hypercalcemia.

SA489

PTH-Mediated Receptor Desensitization: Kinetics and Properties Are Influenced by Peptide Size. S. A. Morris, Q. Sun,* E. Dworakowski,* J. P. Bilezikian. College of Physicians & Surgeons, Columbia University, New York, NY, USA.

PTH receptors stimulate both adenylyl cyclase (ACA) and phospholipase C (PLC) in SaOS2 cells. In both these and other PTH responsive cells, prolonged exposure to the agonist results in loss of the ability to demonstrate PTH dependent activation of ACA, a process known as receptor desensitization. Since ACA is preferentially associated with PTH containing native residues through 29-34, we tested the hypothesis that progressive loss of amino acids at the C-terminal end of these fragments would alter patterns of receptor desensitization. PTH dependent ACA was determined in homogenates of SaOS 2 cells previously incubated with several different PTH peptides for varying time periods, and ACA in response to PTH[1-34] determined. When preincubated with PTH[1-34] at 5x10-8 M, virtually maximal (75%) receptor desensitization was observed at 2 minutes with no additional increase even after 24 hours of further preincubation. In contrast, preincubation with PTH[1-29] at 5x10-8 for 2 min was associated with only 25% receptor desensitization, with a progressive increase in magnitude reaching a maximal level of 75% only after 16 hours, ultimately identical to that achieved with PTH[1-34]. Consistent with previous reports, we determined that PTH[1-29] at any concentration demonstrates no PLC activity in contrast to PTH[1-34] which harbors clear actions on this messenger system. Accordingly, Calphostin C, a PkC inhibitor, produced an approximately 40% reduction in the magnitude of receptor desensitization observed following preincubation with PTH[1-34] for 2 min whereas this compound had no effect on the magnitude of desensitization observed with PTH[1-29] under otherwise identical conditions. H-89, a PkA inhibitor, had no influence on the magnitude of receptor desensitization following 2 min incubation with either PTH peptide. We conclude that C-terminal amino acids of PTH(1-34) participate in enhancing the kinetics of receptor desensitization, possibly through a PLC linked pathway.

Disclosures: Aventis,3.

SA490

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SA491

Bpa at Position 28 of PTHrP(1-36) Crosslinks to the Amino-terminal Extracellular Domain of the PTH/PTHrP Receptor and Thereby Assists in Predicting a Tertiary Structure for this Receptor Region. R. C. Gensure, T. J. Gardella, H. Jüppner. Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

Leucine at position 28, in the carboxy-terminal portion of PTH(1-34) (Ile in PTHrP), is critical for high affinity binding to the PTH/PTHrP receptor. We therefore prepared an analog of PTHrP(1-36) with p-benzoyl-L-phenylalanine (Bpa) substituted at position 28 (Bpa28-PTHrP(1-36)) to identify the receptor contact site of this ligand residue. When tested with LLC-PK1 cells stably transfected with human P1R (hP1R), the apparent binding affinity (IC50 8.1+/-2.4 nM) and agonist-stimulated cAMP accumulation (EC50 7.8+/-0.1 nM, Emax 152+/-8 pmol/well) for this analog were similar to those of the parent compound, indicating that substitution of this ligand residue with a larger but similarly hydrophobic amino acid derivative was well tolerated. Bpa28-PTHrP(1-36) showed weak but specific crosslinking to the hP1R. Digestive mapping with CNBr and LysC defined the crosslink interval as being located between residues 64-95 of the hP1R's amino-terminal extracellular domain. This crosslink interval lies within the region encoded by exon E2, which has been shown to be non-essential for ligand interaction. Crosslinking of this critical ligand residue to a non-essential receptor domain was not expected, and implies that proximity to this receptor region is not related to the role of residue 28 in ligand binding. Another possible role for the hydrophobic group at position 28 may be to facilitate ligand association with the cell membrane, as postulated in the two-dimensional diffusion model of ligand-receptor interaction. However, the apparent proximity between PTHrP(1-36) residue 28 and the E2-encoded P1R region does provide an important structural constraint on the receptor amino-terminal extracellular domain. The combination of these new data and previously reported mapping of P1R crosslinking sites for ligand residues 13, 23, 27 and 33, together with the recently proposed pattern of disulfide bridging in this receptor region, provides a high degree of constraint on models of the structure of the P1R's amino-terminal extracellular domain when bound to PTHrP(1-36). These constraints are easily accommodated by a putative configuration in which the amino-terminal extracellular domain assumes a spiral shape that wraps around the ligand's carboxyl-terminal helix.

SA492

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SA493

Correlation of Growth and Maturation in Roosters with Magnitude of Nongenomic Responses to 1,25(OH)2D3. B. Larsson,[1] I. Nemere.[2] [1]Nutrition and Food Sciences, Utah State University, Logan, UT, USA, [2]Nutrition and Food Sciences and The Biotechnology Center, Utah State University, Logan, UT, USA.

$1,25(OH)_2D_3$ is known to regulate calcium metabolism through a genomic mechanism mediated by nuclear receptors (nVDR). The hormone is also capable of activating the transport pathway by interacting with a membrane receptor (pmVDR). It is our hypothesis that the non-nuclear receptor is important in maintaining calcium homeostasis. In this study we investigate the correlation of growth and maturation in roosters with magnitude of non-genomic responses of $1,25(OH)_2D_3$. White leghorn roosters were raised for 7, 14, 28 and 58 wk (mean body weights; 0.56±0.02, 0.96±0.03, 1.28±0.03 and 2.53±0.05 kg respectively), prior to experiment. These age-ranges cover growth of young animals, adulthood and roosters with declining reproductive functions. Measurements of hormone responsiveness as a function of age were performed by *in vitro* perfusion studies, where the transport of ^{45}Ca from the intestinal lumen to the vasculature, in response to exogenous $1,25(OH)_2D_3$ (130 pM), was measured. ^{45}Ca in venous effluent (expressed as treated/average basal) after 40 min exposure to vehicle was 0.96±0.1, while exposure to $1,25(OH)_2D_3$ in 7, 14, 28 and 58 wk roosters resulted in values of 2.43±0.15, 1.83±0.37, 1,89±0.36 and

1.54±0.18 respectively. This decrease in intestinal Ca^{2+} transport with age indicates an involvement of the non-genomic response and the pmVDR. Further, Western analyses on isolated basal lateral membranes (BLM) showed a decreased expression of the pmVDR with increasing age, supporting the results obtained in the perfusion studies. [^{3}H]1,25$(OH)_2D_3$ binding to BLMs gave K_d values of 0.59, 1.03, 0.99 and 2.65 nM and B_{max} values of 188.9±18.2, 250±13.3, 185.9±10.8 and 640±160.1 fmol/mg protein for 7, 14, 28 and 58 wk old roosters respectively. The obtained results show a significant increase in K_d indicating a decreased affinity of the receptor for hormone with age. Increasing B_{max} values with age may be due to nonspecific binding as a result of decreased pmVDR affinity. Binding characteristics for [^{3}H]1,25$(OH)_2D_3$ to nVDR gave K_d values of 0.35, 0.38, 0.29 and 0.30 nM and B_{max} of 31.1±3.93, 36.35±5.17, 31.0±5.08 and 22.7±2.61 fmol/mg protein for 7,14, 28 and 58 wk roosters respectively. No significant changes in affinity were seen for nVDR, indicating that this pathway is not involved in the age-related changes in hormone responsiveness. In conclusion, our experiments demonstrate that there is a decrease in responsiveness to exogenous 1,25$(OH)_2D_3$ in rooster intestines as a function of age. This decrease can be explained both by a decreased affinity for 1,25$(OH)_2D_3$, and a reduced expression of pmVDR with age.

SA494

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SA495

A Novel Chromatin Remodeling Complex Function as a Transcriptional Modulator of Vitamin D Receptor (VDR). H. Kitagawa,[1] J. Yanagisawa,*[1] S. Ogawa,*[1] T. Matsumoto,[2] S. Kato.[1] [1]Institute of Molecular and Cellular Biosciences, University of Tokyo/ CREST, Tokyo, Japan, [2]The First Department of Medicine, Tokushima University, Tokushima, Japan.

Many complexes interact with nuclear receptors for their ligand-dependent transactivation through modulating chromatin structure in the promoters of a set of target genes. Distinct classes of co-regulator complexes like DRIP/TRAP complex are shown to interact with nuclear receptors in a ligand-dependent way. For such transcriptional controls, complexes to modulate chromatin structure are also considered essential. As one class of such complexes, several ATP dependent chromatin remodeling complexes have been identified, but still other unknown chromatin remodeling complexes are supposed to exist. We provide an evidence here to support possible existence of a novel complex by biochemical purification of complexes associated with VDR. Using GST fused VDR ligand binding domain (LBD) as a bait, we purified nuclear complexes associated with liganded VDR-LBD from HeLa nuclear extracts and identified complex components by mass fingerprinting method and Western blotting with specific antibodies to detect known componets in the chromatin remodeling complexes and co-activator complexes. The components of DRIP/TRAP complex were included, confirming importance of this complex for VDR transactivation. We found that some of the components of the novel complex harbor several conserved motifs which are mapped in essential components of a known ATP dependent chromatin remodeling complex. We further showed that one component of this complex interacts with VDR in ligand independent way by co-immunoprecipitation and can activate ligand-dependent transactivation of VDR by transient transfection assay. From these results, we suppose that this complex is a novel ATP dependent chromatin remodeling complex which associates with VDR and have some important role in the target gene regulation of VDR. Now we are trying to identify all components of the complex and explain how they function in the VDR transactivation by an in vitro chromatin assay.

SA496

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SA498

Calcitriol Increases VDR and CaR Expression in Peripheral Blood Mononuclear Cells (PBMC) and Decreases Monocytes Apoptosis. J. M. Quesada-Gomez,[1] F. Luque Recio,[1] G. Dorado.*[2] [1]UMM, Hospital Reina Sofia, Cordoba, Spain, [2]Departamento de Biología Molecular, Universidad Córdoba, Córdoba, Spain.

In postmenopausal osteoporotic women in spite of normal serum calcitriol levels has been reported a decrease in intestinal calcium absorption. Intestinal calcitriol receptors (VDR) are difficult to study on clinical settings, but VDR have been found in PBMC. PBMC seem to represent well other target tissues of vitamin D, and are also potential targets for the immunomodulatory actions of calcitriol.Vitamin D insufficiency has been reported in elderly subject with osteoporotic hip fracture, with a decrease in the intestinal calcium absorption. This vitamin D insufficiency through a modification of VDR could influence the intestinal calcium absorption and immune response.Purpose of the study: In elderly patients with hip fracture and a vitamin D insufficient status to evaluate baseline VDR&CaR expression in PBMC and the effect of calcitriol on VDR/CaR regulation and on monocyte apoptosis. We study 22 elderly subjects with hip fractures and controls, pre and after calcitriol treatment (IV administration during a week of 2 mg calcitriol each other day. Serum calcium, phosphorus and alkaline phosphatases were determined by standard autoanalyser. PTH was measured using the allegro inmunoradiometric assay (Nichols Institute. San Juan Capistrano. CA. USA)25OHD3 and Calcitriol was determined by RIA (modified Reinhart method) PBMC were separated by centrifugation on a Ficoll-Hypaque discontinous gradient (1.076).Apoptosis were assessed by flow cytometry (FACscan, Beckton Dickinson). Apoptotic cells are characterized by hypodiploid DNA peak. mRNA was isolated (Trizol Reagent,Life-Technologies) and the amount of VDRand CaR mRNA were studied by quantitative RT-PCR. The quantitation of the different amplicons were accomplished by laser induced fluorescence (ABI 373 A Strech Sequencer from P-E Biosystems).The study confirms vitamin D insufficiency in elderly subjects with hip fractures.In PBMC calcitriol treatment increase VDR/actine from 1.43 to 1.61($p<0.01$)and CaR mRNA from 0.18 to 0.25 ($p<0.05$), but decreases apoptosis in monocytes from 3 to 0.1%($p<0.001$)Vitamin D insufficiency, a determinant factor in the elderly hip fracture, also could contributes to decreased VDR expression and CaR. Calcitriol treatment increases VDR and CaR expression in PBMC and decreases monocytes apoptosis. These results support the role of vitamin D endocrine system as a regulator of the immune response and are relevant to explain the immune dysfunction in elderly subjects with hip fracture.

SA499

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SA500

Vitamin D3 Dimer: A Potential VDR-Inhibitory Molecule. A. Gacio-Fernandez,*[1] N. Swamy,*[2] R. Ray.[2] [1]Universidad de Santiago de Compostela, Santiago de Compostela, Spain, [2]Medicine, Boston University School of Medicine, Boston, MA, USA.

Vitamin D receptor (VDR) binds to its cognate ligand, 1,25-Dihydroxyvitamin D3 [1,25(OH)2 D3] with high fidelity, followed by the heterodimerization of holo-VDR with RXR to bring about the transcription regulation of vitamin D dependent genes. According to this mechanism VDR-1,25(OH)2 D3-RXR is a functional unit; and any disruption in the interaction among its components would result in impairment of gene-regulation by 1,25(OH)2 D3. We hypothesized that a dimer of 1,25(OH)2 D3, in which two 1,25(OH)2 D3 molecules are coupled via a long tether, might bind with low affinity to two VDRs, and this might impair the binding of RXR by steric interaction between VDR and RXR. Such a process would bring about inhibition of VDR-mediated transcriptional regulation. Such a molecule may hold promise for treating diseases like idiopathic hypercalcuria where VDR is expressed at an elevated level in intestine and kidney, thus resulting in hypercalemia and spontaneous formation of kidney stones at normal 1,25(OH)2 D3 levels. In the present study we synthesized such a dimer (of 1,25(OH)2 D3), and carried out VDR-binding studies. These results will be discussed.

SA501

See Friday Plenary number F501.

SA502

Estratriene-3-ol, an Activator of Non-genomic Estrogen-Like Signaling (ANGELS), Does Not Stimulate Bone Growth in Ovariectomized rats. J. F. Whitfield,*[1] P. Morley,*[2] G. Willick,*[2] J. Barbier,*[2] S. MacLean,*[3] V. Ross.*[2] [1]Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, Canada, [2]National Research Council of Canada, Ottawa, ON, Canada, [3]National Research Council of Canada, Ottawa, ON, Canada.

Osteoblasts can respond to estrogens through both nuclear 'genomic' and 'non-genomic' surface receptors. When activated, these surface receptors, like PTH, cause an influx of Ca^{2+} and a flurry of kinase activities by stimulating adenylyl cyclase and phospholipase-C. Because of the similarity between their signaling and the osteogenic PTHs, estrogens that have a preference for non-genomic rather than genomic receptors (ANGELS) could be anabolic agents rather than just antiresorptives and thus be ideal candidates for treating osteoporosis. Indeed Manolagas [(1)] and Manolagas et al [(2)] have reported that one of these estrogens, EOL (estratriene-3-ol), is as potent a stimulator of bone growth in intact and OVXed (ovariectomized) mice as PTH-(1-34). We have compared the abilities of EOL and Ostabolin-C™ ([Leu^{27}]cycloGlu^{22}-Lys^{26} hPTH-(1-31)NH_2) to stimulate bone growth in 3-months-old OVX Sprague-Dawley rats. We injected sc. 0.1 mg of EOL (in sesame oil)/g of body weight every other day and/or 0.8nmole of Ostabolin-C/100 g of body weight once every day starting at the end of the 2nd week and ending at the end of the 6th week (8th week after OVX). This dose of EOL was high enough to reduce the OVX-induced weight gain to the weight gain in sham-operated control rats and to drop the Ostabolin-C™-stimulated serum TRAP (tartrate-resistant acid phosphatase) activity to a value below the level in the untreated sham controls. OVX dropped the BV/TV in the distal femurs to 37.1% of the sham value by 8 weeks ($p<0.01$). EOL injections did not significantly stop the BV/TV drop, but Ostabolin-C™ kept the BV/TV value at the sham value. Neither OVX nor treating the OVX rats with EOL affected the mean trabecular thickness while Ostabolin-C™ increased it from the sham value of 72.3 ± 2.2 mm to 121.7 ± 7.1µm ($p<0.01$). Thus, while EOL can stimulate bone growth in OVX mice as effectively as PTH[1], it cannot stimulate bone growth when given in a dose that otherwise fully replaces estrogen functions in OVX rats. 1. S.C. Manolagas. Advances in the treatment of

osteoporosis. Medscape Endocrinology Journal, 1, n10, www.Medscape.com , 1999.
2. S. C. Manolagas, R.S. Weinstein, T. Bellido, D.L. Bodenner, R. L. Jilka,,A.M. Parfitt. J Bone Mineral Res. 14, S180, 1999

SA503

See Friday Plenary number F503.

SA504

Effects of Estrogen Receptor α/β Heterodimers and Steroid Receptor Coactivator Overexpression on Transcription in Osteoblasts. D. G. Monroe,[1] D. J. Rickard,[1] M. Subramaniam,[1] B. L. Riggs,[2] S. Khosla,[2] T. C. Spelsberg.[1] [1]Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA, [2]Endocrinology, Mayo Clinic, Rochester, MN, USA.

Estrogen (E) is a major sex steroid that affects the growth, maintenance and homeostasis of the skeleton. Two isoforms of estrogen receptor (ERα and ERβ) have been discovered which mediate the transcriptional effects of E. Although studies in our laboratory and others have demonstrated that endogenous ERα and ERβ are present and functional in some human osteoblast (OB) cell lines, there are few reports on the differential regulation of gene transcription by ERα and ERβ acting either as homodimers or as heterodimers. Recent gene deletion experiments in mice suggest that the ERα and ERβ isoforms may have antagonistic actions on bone. Antagonism by ERα and ERβ could be caused by differential action of ERα/β heterodimers acting through different DNA elements in gene promoters or by the differential recruitment of steroid receptor coactivators (SRCs). We have investigated the effects of ER homodimers (ERα/α and ERβ/β) in cells transfected with ERα or ERβ alone, ER heterodimers (ERα/β) in cells transfected with both receptors, and the effects of SRC overexpression on E-dependent transcription in OBs. Using FLAG-tagged ERα and ERβ expression constructs that express functional and quantifiable receptors when transiently transfected into OBs, our data demonstrate that following E treatment of the cells, ERα/β displays an antagonistic effect on transcription of a cotransfected synthetic ERE-reporter construct in both normal human OBs and MG63 osteosarcomas, compared to either ER isoform alone. Interestingly, the data also demonstrate that coexpression of SRC1 in OBs preferentially potentiates ERβ/β signaling, whereas the coexpression of SRC2 preferentially potentiates ERα/α signaling. Coexpression of SRC3 has no differential effect on ERα/α and ERβ/β. These experiments suggest that in OBs, individual SRC family members differentially enhance transactivation by the two ER isoforms. Studies are currently underway to elucidate the 1) molecular mechanisms of ERα/β antagonism at the level of ER binding to elements in endogenous gene promoters, 2) the relative proportion of ERα/β heterodimers versus homodimers at different ratios of transfected ERα and ERβ, and 3) the preferential association of SRCs with a particular ER dimer in human OB cells. We conclude that ERβ, acting through heterodimer formation with ERα, decreases the transcriptional responsiveness of OBs to E and that SRCs may play a role in mediating the ER isoform-specific transcriptional activities.

SA505

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SA506

Transcriptional Profiling of Estrogen Induced Osteogenic Differentiation of Mouse Marrow Mesenchyml Stem Cells (MSCs) In Vitro. S. Zhou,[1] S. McLarney,*[2] B. Komm,*[2] P. Bodine,*[2] D. Gazit.[1] [1]Molecular Pathology, Hebrew University-Hadassah Medical and Gene Therapy Center, Jerusalem, Israel, [2]Women's Health Research Institute, Wyeth-Ayerst Research, Radnor, PA, USA.

We have previously reported that in vitro treatment of MSCs from ovariectomized ICR mice with 100 nM 17b-estradiol (17b-E2) for 8 days promoted osteoblastic differentiation. Under these conditions, mRNA expression of the osteoblastic markers Cbfa1, alkaline phosphatase (ALP), type I collagen, transforming growth factor (TGF)-b1, bone morphogenetic protein (BMP-2) and estrogen receptor (ER)-a are increased with 17b-E2 treatment, while the expression of ER-b message is suppressed. We hypothesized that transcriptional profiling of differentiating MSCs induced by 17b-E2, may lead to the discovery of novel genes, which may be pivotal in the anabolic effect of estrogen in osteogenic tissues. We performed robotic high-throughput differential display polymerase chain reaction (RADE) and gene chip analysis with RNA samples isolated from MSCs that were treated with 17b-E2 as described above. From 3 separate experiments, we identified the following estrogen regulated genes (up = up regulated, down = down regulated). From RADE we identified a6-intergrin (up), integral membrane protein CII-3 (up), proteosome regulator PA28 (up) and 4 novel cDNAs (ESTs). From gene chip analysis, we identified ALP (up), the calcium-binding proteins MRP-8 and - 14 (both down), transglutaminase (TG) (up) and the adipocyte marker adipsin (down). All of these results were subsequently confirmed by RT-PCR analysis of new RNA samples. Although the function of most of these genes in osteoblast differentiation is as yet unknown, the up-regulation of ALP and down-regulation of adipsin is consistent with 17b-E2 promoting osteogenesis while suppressing adipogenesis. The novel ESTs are currently being characterized in order to obtain full-length cDNAs, human analogues, and for functional analysis. These ESTs may play an important role in the anabolic effects of estrogens on the murine skeleton. Thus, this approach has the potential to greatly expand our understanding of the molecular mechanisms by which estrogens regulate skeletal homeostasis, and to identify novel gene targets for estrogen action in bone.

Disclosures: Wyeth-Ayerst Research,3.

SA507

DHEA Stimulates Osteoblast Differentiation in Human Marrow Cultures. J. Simon,[1] A. Mascarenas,[1] M. S. LeBoff,[2] J. Glowacki.[1] [1]Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA, [2]Endocrinology, Brigham and Women's Hospital, Boston, MA, USA.

Dehydroepiandrosterone (DHEA) is an adrenal steroid that circulates in high concentrations in humans. Previously, we and others showed DHEA to have anti-osteolytic actions. DHEA has been reported to stimulate osteoblast proliferation and activity in vitro, but little is known about its effects on osteoblast differentiation. Bone marrow stromal cells include a subset of cells capable of osteoblastic differentiation, a process known to be stimulated by 1,25-dihydroxyvitamin D (1,25D). This study tests the hypothesis that DHEA promotes the differentiation of osteoblasts from human marrow precursors/progenitors, in the presence and absence of 1,25D. Human marrow from elderly subjects (62-79 y) and a line of human marrow stromal cells (KM101) were used in a 96-well assay for development of alkaline phosphatase (AlkP)-positive cells. Fresh marrow was obtained from subjects undergoing total hip replacement for non-inflammatory osteoarthritis. Low-density mononuclear cells were precultured for collection of adherent cells. Both the marrow-derived cells and the KM101 cells were cultured in phenol red-free α Minimum Essential Medium with 10% charcoal/dextran-stripped fetal bovine serum, 5 mM b-glycerophosphate, 0.05 mM L-ascorbic phosphate, 100 U/ml penicillin, 100 µg/ml streptomycin, and 292 mg/ml L-glutamine. AlkP activity was measured colorimetrically after 6 d for human bone marrow and after 4 d for KM101 cells. Constitutive AlkP activity in human marrow stroma was low. DHEA significantly increased AlkP activity in a dose-dependent manner for all samples to 242% at 10 nM ($p<0.0001$). Comparison of the effects of 10 nM DHEA, 17-estradiol (E2), or dihydrotestosterone (DHT) showed greatest stimulation by DHEA. In KM101 cells, DHEA increased AlkP activity in a dose-dependent manner to 311% at 10 nM ($p<0.0001$). Further, DHEA stimulated AlkP to the greatest extent at 350%, with DHT at 273% and E2 at 147%. 1,25D stimulated AlkP in marrow and in KM101 cells in a dose-dependent manner. Combinations of DHEA and 1,25D showed greater AlkP than either alone, but combinations of 1,25 with DHT or E2 were not greater than with 1,25D alone. In sum, DHEA is more potent than DHT or E2 in promoting osteoblast differentiation in human marrow stromocytes and is unique in enhancing 1,25D's stimulation. Thus, DHEA or its metabolites may be useful for treating bone diseases because of its anabolic and anti-osteolytic actions. Supported by a grant from the Dept. of Defense (US Army - Bone Health and Military Readiness)

SA508

Synthesis of 1,25-dihydroxyvitamin D_3 by Human Endothelial Cells Is Regulated by Inflammatory Cytokines: A Novel Autocrine Determinant of Vascular Cell Adhesion. D. Zehnder,* R. Bland,* D. C. Wheeler,* P. M. Stewart,* M. Hewison. Division of Medical Sciences, The University of Birmingham, Birmingham, United Kingdom.

Recent studies have shown that increased circulating concentrations of active vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25D_3$), reduce the risk of coronary calcification of atherosclerotic vessels. Furthermore, low $1,25D_3$ serum levels in patients with renal failure appear to increase vascular calcification. The precise mechanism for this remains unclear, although data have highlighted a possible role for $1,25D_3$ as a modulator of cell adhesion and angiogenesis. Previous studies have demonstrated the presence of vitamin D receptors and the enzyme 1α-hydroxylase (1-HYD) in bovine endothelial cells. Here we have used immunohistochemical, and in situ hybridization analyses to show for the first time the expression of 1-HYD mRNA and protein in human endothelial cells from kidney, spleen and lymph nodes. RT-PCR and Western analyses using primary cultures of human umbilical vein endothelial cells (HUVEC) confirmed expression of mRNA and protein corresponding to the reported renal 1-HYD. Enzyme activity assays in HUVEC using $3H\text{-}25D_3$ as substrate showed basal conversion of 318 ± 56 (±SD) fmoles $1,25D_3$/hr/mg protein which increased 3-4-fold following 24 hr treatment with TNFalpha (1054 ±166) or lipopolysaccharide (1381 ±88). Forskolin (554 ±56) increased only slightly the production of $1,25D_3$, and incubation with $1,25D_3$ itself (475 ±111) did not suppress 1-HYD activity, as is classically observed with the renal enzyme. Functional analyses showed that exogenously added $1,25D_3$ or precursor $25D_3$ significantly increased monocyte adhesion to HUVEC in a similar fashion to inflammatory agents such as TNFα; parallel ELISA studies revealed that this was not due to increased expression of ICAM-1 or VCAM-1. These data suggest that vitamin D metabolism and function in human endothelia is similar to that described previously for macrophages, with inflammatory cytokines showing greater up-regulation of endothelial 1-HYD activity than classical calcitropic regulators. We therefore propose that endothelial cell 1-HYD acts as a novel autocrine/paracrine immunomodulatory mechanism. In particular, the synthesis of $1,25D_3$ by blood vessels during inflammation may play an important role in leukocyte adhesion. Further analysis of this process may help to elucidate a mechanism for the impact of vitamin D on cardiovascular disease.

SA509

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SA511

Rapid Effects of 25-Hydroxyvitamin D on Calcium Uptake by Isolated Chick Enterocytes. R. Phadnis,*[1] I. Nemere.[2] [1]Nutrition and Food Sciences, Utah State University, Logan, UT, USA, [2]Nutrition and Food Sciences, and the Biotechnology Center, Utah State University, Logan, UT, USA.

25-Hydroxyvtiamin D_3 [25(OH)D_3]is a metabolite of vitamin D_3 that has long been considered to be an inactive precursor; consequently very few studies have addressed its biological activity. However it is known that 100 nM 25(OH)D_3 increases calcium transport in the perfused duodenal loop of the chicken to 200% of controls within 20 minutes. It is our hypothesis that 25(OH)D_3 may be a hormonally active metabolite and its effects can be studied in isolated chick enterocytes.In the current work, we investigated the time course of ^{45}Ca uptake in isolated intestinal cells (from 7 week chicks) as influenced by a range of 25(OH)D_3 concentrations. After establishing the basal uptake of ^{45}Ca for 5 minutes, cells were treated with vehicle (ethanol) or 25 nM, 50 nM, 100 nM or 300 nM 25(OH)D_3 and samples were taken at T=1,3,5,7 and 10 min. In the first series of studies, 100 nM 25(OH)D_3 decreased enterocyte ^{45}Ca to 54% of controls within 1 min (P<0.001), and 70% of controls at T=3, 5, and 7 min (P<0.01 to <0.05, relative to controls). These results suggested that 25(OH)D_3 might stimulate the extrusion of ^{45}Ca from the cell. Comparing the 7-min time point, 25(OH)D_3 appeared to yield a biphasic dose-response curve with values of ^{45}Ca observed to be 99 % (*NS*, not significant), 75% (P<0.05), 70% (P<0.01), and 80% (*NS*) of controls for 25 nM, 50 nM, 100 nM, and 300 nM metabolite, respectively. In an additional series of experiments, we found that physiological levels of 24,25-dihydroxyvitamin D_3 [24,25(OH)$_2$$D_3$, 6.5 nM]inhibited the actions of 100 nM 25(OH)D_3 in isolated chick enterocytes. Similar time course studies with isolated enterocytes from 14 week and 28 week chickens treated with 100 nM 25(OH)D_3 showed decreased responsiveness. Comparisons at T=1 min revealed ^{45}Ca levels in 7 week, 14 week and 28 week to be 54% (P<0.001), 83% (*NS*) and 80% (*NS*) of controls, respectively. We then investigated the effect of pharmacological agonists for selected signal transduction pathways. Experiments with the calcium channel activator, BAYK 8644 (2 υM), and protein kinase (PK) A activator, forskolin (20 υM), revealed enhanced levels of ^{45}Ca to 132% and 140% of controls, respectively (each, P<0.05 at T = 10). These pathways are therefore not likely to be the major ones activated by 25(OH)D_3. In conclusion, our experiments demonstrate that 25(OH)D_3 is biologically active in isolated chick enterocytes with relation to calcium uptake and that 24,25(OH)$_2$$D_3$ inhibits the action of this metabolite. There is a decrease in the response to exogenous 25(OH)D_3 as a function of age and the metabolite does not act primarily through activation of calcium channels or the PK A pathway.

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SA514

Effect of 1,25-Dihydroxyvitamin D on Intestinal Cell Phosphate Uptake. B. Zhao,*[1] I. Nemere.[2] [1]Nutrition and Food Sciences, Utah State University, Logan, UT, USA, [2]Nutrition and Food Sciences and the Biotechnology Center, Utah State University, Logan, UT, USA.

1,25-dihydroxyvitamin D_3 [1,25(OH) $_2$$D_3$] promotes phosphate transport in the perfused duodenal loop (Treated/Av Basal at T = 40min,1.82 ± 0.42, relative to controls, 1.11 ± 0.21). It is our hypothesis that direct effects of 1,25(OH) $_2$$D_3$ can be observed in isolated intestinal cells by either increases in uptake or extrusion. In this study we used isolated enterocytes to investigate (1) A range of 1,25(OH)$_2$$D_3$ concentrations to identify an optimal time and dose for 1,25(OH)$_2$$D_3$ mediated changes in ^{32}P uptake; (2) The effect of a combination of 1,25(OH)$_2$$D_3$ at optimal concentration and 24,25(OH)$_2$$D_3$ to determine whether the latter seco-steroid exerts an inhibitory effect on phosphate uptake; (3) Activators of protein kinase A, protein kinase C, and calcium channels for their effects on phosphate uptake in cells to obtain insights regarding signal transduction pathways; (4) The effect of age as a function of responsiveness to 1,25(OH)$_2$$D_3$. In time course studies, with 4-8 wk chicks, 130 pM 1,25(OH)$_2$$D_3$ increased ^{32}P uptake in enterocytes to 157% of controls after 10 min, while 300 pM 1,25(OH)$_2$$D_3$ produced an increase of 122% of controls (each R<0.05). 24,25(OH)$_2$$D_3$ exerted an inhibitory effect on phosphate uptake by 130 pM 1,25(OH) $_2$$D_3$. For signal transduction studies, isolated enterocytes were incubated with 20 µM forskolin, 100 nM phorbol ester or 2 µM BAY K 8644. Enhanced ^{32}P levels relative to controls were found for phorbol ester (150% of controls at T = 7 min, P<0.05) and BAY K 8644 (127% of controls at T = 7 min, P<0.05) but not for forskolin. Both BAY K8644 and phorbol ester, but not forskolin, also stimulated transport in perfused duodenal loops. These results suggest that PKC and calcium channel signal transduction pathways may be involved in uptake (at the brush border). For aging studies, white leghorn roosters were raised for 7, 14, and 28 weeks prior to experiment. These ages cover growth (a period of rapid bone formation) and adulthood when demand for bone mineralization decreases. These studies showed a 1,25(OH)$_2$$D_3$ mediated increase in ^{32}P uptake in isolated cells at 7wk, but not at 14 or 28 wk. In conclusion, our experiments demonstrate that 1,25(OH)$_2$$D_3$ increases phosphate uptake in isolated intestinal cells; 24,25(OH)$_2$$D_3$ exerts an inhibitory effect on phosphate uptake by 1,25(OH) $_2$$D_3$. PKC and calcium channel signal transduction pathway maybe involved in uptake (at the brush border). And that there is a decrease in 1,25(OH)$_2$$D_3$ mediated ^{32}P uptake in isolated intestinal cells with age.

SA515

See Friday Plenary number F515.

SA516

Delineation of Vitamin D Deficiency Based on Pth and Muscle Function Tests: What Is the Lower Limit for 25(oh)d? H. Glerup,[1] E. Eriksen.[2] [1]Dept V, Aarhus University Hospital, Aarhus, Denmark, [2]Dept C, Aarhus University Hospital, Aarhus, Denmark.

The diagnosis of vitamin D deficiency is primarily based on the assessment of serum (S) 25(OH)D. Which cutoff to use is, however, still subject to debate. In order to approach this problem further, we tested muscle function and measured S-PTH, S-25(OH)D, and S-1,25(OH)2D in 102 subjects with varying degrees of vitamin D deficiency. The first subjects with secondary hyperparathyroidism were detected at S-25(OH)D=25 nmol/l. Some patients, however, displayed normal PTH levels (< 7.6 pmol/l) even at 25(OH)D levels down to 5 nmol/l. At S-25(OH)D < 5 nmol/l all subjects exhibited secondary hyperparathyroidism. Muscle testing consisted of a standardized assessment of maximal voluntary contraction (MVC, newton) and muscle contractility after electrical stimulation (twitch, newton). The first abnormal values for MVC and twitch (defined as values below the 95% confidence limits for normals) were already detectable at S-25(OH)D=60 nmol/l, and 50% of subjects displayed abnormal MVC- and twitch-values at S-25(OH)D < 50 nmol/l. A few patients, however, exhibited normal muscle function even at S-25(OH)D< 5nmol/l. At S-25(OH)D < 40 nmol/l twitch correlated significantly to S-25(OH)D (r=0.43, p 40 nmol/l was r = -0.01. MVC at S-25(OH)D < 40 nmol/l the correlated to S-25(OH)D (r = 0.23, p 40 nmol/l. S-1,25(OH)2D did not correlate to neither MVC nor twitch. In conclusion, S-PTH is a rather insensitive marker of vitamin D deficiency. The first cases of elevated PTH levels were not detectable until S-25(OH)D was below 25 nmol/l, while muscle function suffers significantly at S-25(OH)D < 40 nmol/l. Based on these results we therefore suggest that individuals exhibiting S-25(OH)D < 40 nmol/l should be considered vitamin D deficient.

SA517

See Friday Plenary number F517.

SA518

Seasonal Variations of 250H Vitamin D in Young Adults and Elder Outpatients From an Overpopulated City. L. Plantalech,*[1] J. Fassi,*[1] M. F. Russo Picasso,*[1] M. Glerean,*[1] L. Camera.*[2] [1]Division of Endocrinology, Hospital Italiano, Buenos Aires, Argentina, [2]Department of Internal Medicine, Hospital Italiano, Buenos Aires, Argentina.

The aim of this study wasto assess the level of 250h vitamin D (250HD) and to determine winter (W) and summer (S) prevalence of hypovitaminosis in young (Y) and elder (E) oupatients from the city of Buenos Aires. We studied 49 E (37 women) and 44 Y (26 women) during W (June to September) and 34 E (25 women) and 32 Y (30 women) in S (December to April) were analyzed. Ages x 71.8 ± 6.5 and 29,8 ± 6,5 years old respectively, without any medication that might affect bone metabolism. Serum levels resulted in 250HD, middle molecular PTH (vn 20-100 pg/ml), Calcemia, Alkaline Phosphatase (APH) (vn:< 190Ul/l), tartrate-ressistant Acid Phosphatase (TRAP) (vn:1.8 -4.4 Ul/l). Patients were subdivided according to 250HD by McKenna criterium (Osteop. Int.:1998:Supl:8:3). The results are defined as x ± sd and analized with Student´s t test.(Table 1). Calcemia was normal in both groups (E in W and S 9,65 ± 0,4 mg/dl and 9,5 ± 0,53 mg/dl respectively ; Y in W and S 9,5 ± 0,7 mg/dl and 9,6 ± 0,5 mg/dl respectively)

	E W	E S	p	Y W	Y S	p
25OH D ng/ml	17,30 ±7,5	28,6 ±10,1	<0,001	17,1± 8,1	32,5± 12,8	<0,001
PTH pg/ml	75,2± 43,2*	63,6± 34,8 **	ns	37,6± 17,9 *	28,9± 18,7 **	ns
APH UI/l	134,7 ±34,3*	101,3± 131***	<0,001	96,2± 21,1 *	84,0± 27,1***	0,05
TRAP UI/l	3,90± 0,72*	2,7 ± 0,84	<0,001	3,20± 0,9 *	2,50± 0,6	<0,001

* p E-Y in W < 0,001 ; ** p E-Y in S < 0,001 ; *** p E-Y in S =0,007 Thirty one E analyzed in W and 20.5% analyzed in S showed secondary hyperparathyroidism.

25OHD	E in W	E in S	Y in W	Y in S
Desirable.>40ng/ml	2%	20,5%	2,3%	28,1%
Hypovitaminosis D :40-20ng/ml	36,7%	64,8%	27,3%	56,2%

Vitamin D Insufficiency 20-10 ng/ml	51%	14,7%	54,5%	15,6%
Vitamin D deficiency:<10ng/ml	14,2%	0%	15,9%	0%

A high prevalence of hypovitamin D during summer and insufficiency during winter are found in both groups. During W an increase of bone turnover was observed, higher in elders without important changes of PTH. Elders showed higher levels of PTH than young adults, therefore, secondary parathyroidism was found. Big cities lifestyle would determine this observation.

SA519

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SA521

VitaminD Regulates Phex Expression During Embryonic Development: Study in VitaminD Receptor Null Mice. M. Shinohara,[1] H. Tanaka,[1] S. Kato,[2] Y. Seino.[1] [1]Department of Pediatric Medicine, Okayama University, Okayama, Japan, [2]Institute of Molecular and Cellular Biosciences, Tokyo University, Tokyo, Japan.

Phex a new gene whose mutation causes X-linked hypophosphatemic vitamin D resistant rickets (XLH). The mechanism how Phex mutation causes hypophosphatemia is still unclarified. Moreover Phex gene expression shows clear age dependence. Namely, Phex expression is high in calvaria from embryo when ossification is very active and become low with aging., which may be related to the age dependent change in serum phosphorus concentration. However, the mechanism of this developmental regulation of phex has not been clarified. It was reported that 1,25-$(OH)_2D_3$ down-regulates Phex expression of mouse osteoblast in vitro. The aim of this study is to clarify the role of vitamin D in the regulation of phex gene expression in vivo. To this aid, we examined phex mRNA expression in vitamin D receptor knockout mice (VDRKO) at different age. The mice were bred by mating hetero type males and females in our animal facilities. The genotypes of the fetus were identified by performing multiplex PCR for exon 2 of VDR gene and neomycin resistant gene in DNA extracted from fetal liver. The calvaria, lung, liver and brain were taken from VDRKO and its brethren wild-type mice(WT) at embryonic 18.5 days , newborn(0 day), 2weeks, and 4 weeks after birth. Total RNA for RT-PCR was extracted from each tissue by AGPC method. Using the reverse transcribed product, quantitative PCR was done on Phex. and GAPDH as an internal control by SYBR Green technology in Light Cycler whose lower detection limit is 10 copies. Phex expression in calvaria was highest in embryo and decreased rapidly after birth in both mice. In 18.5 day emdryonic calvaria, phex expression in VDRKO mice were about three times to five times higher than that in WT mice. And the similar results were obtained at 0 day. But at 2 weeks after birth, there was no significant difference between VDRKO and WT. Moreover, at 4 weeks after birth, Phex expression itself is hardly detectable in both mice. On the other hand, the expression of phex gene in lung showed contradictory pattern. Phex expression in WT lung was three fold higher than that in VDRKO lung. These results indicate that vitamin D receptor is involved in the regulating system of Phex gene in embryonic development. And the regulatory mechanism may be bone specific. Vitamin D is an important regulator of Phex gene during embryonic development.

SA522

See Friday Plenary number F522.

SA523

Dietary P Regulation of Renal 25-Hydroxyvitamin D3 1 Alpha-Hydroxylase (1-OHase) Protein and mRNA Expression in Young and Adult Rats. M. Wong,*[1] M. Favus,[2] W. Lai,*[1] T. Chau.*[1] [1]Department of Applied Biology And Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, Hong Kong Special Administrative Region of China, [2]Department of Medicine, The University of Chicago, Chicago, IL, USA.

The increase in renal 1,25(OH)2D3 production in response to dietary phosphorus restriction (LPD) was lost in 12 weeks old adult rats. The present study is undertaken to investigate if the loss of LPD stimulation of renal 1,25(OH)2D3 production is due to a decrease in renal 1-OHase protein and mRNA expression with age. Young (150-175g, 6 weeks old) and adult (325-350g, 12 weeks old) male Sprague Dawley rats were fed either normal (0.6% Ca, 0.6% P) or low P (0.6% Ca, 0.1%P). The expression level of renal 1-OHase protein in renal proximal tubules isolated from young and adult rats were characterized by immunoblotting using sheep anti-human 1-OHase antibody. The mRNA expression level of renal 1-OHase and glyceraldehydes-3 P dehydrogenase (GAPDH) were studied using reverse-transcriptase polymerase chain reaction (RT-PCR). 1-OHase protein and mRNA expression level increased upon LPD feeding in young rats ($p< 0.05$ and $p< 0.01$, respectively). Adult rats had higher basal 1-OHase protein expression than young rats ($p< 0.0001$) but did not increase 1-OHase protein expression in response to LPD. Renal 1-OHase mRNA expression in adult rats was lower than that of the younger rats and its upregulation by LPD was lost in adult rats. The results indicated that the inability to increase renal 1-OHase mRNA and protein expression in adult rats might be partly responsible for their blunted response of renal 1,25(OH)2D3 production during LPD.

SA524

See Friday Plenary number F524.

SA525

Serum 25-OH Vitamin D3 in Chronic Rheumatic Patients. O. Di Munno,*[1] A. Delle Sedie,*[1] M. Mazzantini,*[1] S. Frigelli,*[1] M. R. Metelli.*[2] [1]Internal Medicine, University of Pisa, Pisa, Italy, [2]Experimental Pathology and Biotechnology, University of Pisa, Pisa, Italy.

Serum 25-OH vitamin D3 (25-OH D3) levels play a fundamental role in bone mineralization. Vitamin D3 deficiency is a underestimated problem. Low levels of 25-OH D3 (i.e. < 12 ng/mL) have been documented in both general population and internistic hospitalized patients. A recent Italian study on 799 postmenopausal women, who had been referred to 43 Centers for Osteoporosis (February-March) for their first densitometric test, showed that 74% had 25-OH D3 < 12 ng/mL. Aim of our study is the assessment of 25-OH D3 in patients with chronic rheumatic diseases, referred for hospitalization to the Rheumatologic Unit of the University of Pisa. Exclusion criteria were vitamin D supplementation in the previous 6 months and concomitant diseases or drugs that could interfere with bone metabolism, with the exception of glucocorticoids. We measured 25-OH D3 serum levels (25-Hydroxyvitamin D 125I RIA Kit, DiaSorin), 1,25-(OH)2 D3 (Gamma-B 1,25-Dihydroxy Vitamin D RIA Kit, Immunodiagnostic Systems), PTH (Intact PTH IRMA Kit, Nichols Institute Diagnostics), Osteocalcin (ELSA-OST-NAT, CIS Bio International), bone Alkaline Phosphatase (Ostase, IRMA, Beckman Coulter), N-telopeptide of type I collagen (NTx) (Osteomark NTx serum enzyme-linked immunoassay, EIA, Ostex). So far we have examined (November-May) 100 patients (78 females, 22 males), aged 58.8 years (range 22-82), affected with rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, osteoarthritis, systemic lupus erithematosus, polimyositis, Sjögren syndrome, polimialgia rheumatica and vasculitis. Mean 25-OH D3 values were 14.9±10 ng/mL (range 0.3-69.1); 44 patients (44%) had 25-OH D3 < 12 ng/mL and 13 of them had PTH above the upper normal limit; 18 patients (18%) had values < 6 ng/mL,. Significant correlations were found between 25-OH D3 and PTH ($r=-0.27$, $p<0.01$), age and PTH ($r=0.45$, $p<0.0001$), NTx and PTH ($r=0.39$, $p<0.005$), and age and NTx ($r=0.41$, $p<0.002$). Our preliminary data show that in chronic rheumatic patients serum levels of 25-OH D3 are frequently low. Many factors may play a role in such patients, including age, low sun exposure and low-fat diet. Present data show also that serum levels of 25-OH D3 are an important parameter to be evaluated in the study of bone turnover, particularly in the case of densitometric evidence of reduced bone mass.

SA526

Post-Maturational Decline in Intestinal Calcium Transport in C57BL/6 Mice. H. J. Armbrecht, M. A. Boltz,* T. L. Hodam.* Geriatric Center, St. Louis VA Medical Center, St. Louis, MO, USA.

Studies have suggested that mice undergo bone loss with age. Intestinal absorption of dietary Ca is an important factor in maintaining bone mass in humans. Therefore, the purpose of this study was to determine whether there were age-related changes in Ca transport by the small intestine of the mouse. Intestinal Ca transport was measured in C57BL/6 female mice aged 2, 6, 12, and 24 months old. Mice were fed regular rodent chow. Transport was measured in the duodenum, since this is the site of active Ca absorption. Duodenal segments (5 cm) were everted, filled with buffered saline, and tied off into sacs. The sacs were incubated in vitro for one hour in buffered saline containing 0.25 mM Ca with radiolabeled Ca on the outside of the sac (mucosal side) only. The amount of radioactivity that moved into the sac (serosal side) during the one hour incubation was taken as a measure of Ca transport. There was no change in Ca transport between 2 and 6 months of age. However, Ca transport declined in an almost linear fashion between 6 and 24 months of age. It declined 24% between 6 and 12 months of age and 19% between 12 and 24 months for an overall decline of 43%. In parallel experiments, the mRNA levels of calbindin D-9k, the vitamin D-dependent Ca binding protein, were measured by ribonuclease protection assay. Duodenal mRNA levels did not change over this age range. Using pooled plasma samples, intact plasma PTH showed an overall increase with age, and plasma 1,25(OH)2D levels did not change with age. These studies demonstrate that Ca absorption declines markedly from midlife onward in the mouse. This is unlike the rat, where the decline in Ca absorption is mostly maturational. The decline in the mouse is not explainable by a decline in calbindin mRNA levels or by a decline in serum 1,25(OH)2D. This suggests that it may be vitamin D-independent. The age-related rise in plasma PTH in the mouse may be in response to the decline in Ca absorption. This rise may contribute to the loss of bone reported in the mouse.

SA527

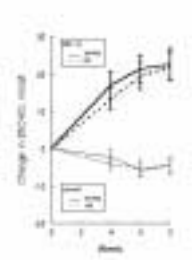
See Friday Plenary number F527.

SA528

Plasma 25-Hydroxyvitamin D Responses of Young and Old Men to Supplementation with 800 IU/day of Vitamin D. S. S. Harris,* B. Dawson-Hughes.* Jean Mayer USDA Human Nutrition Research Center, Tufts University, Boston, MA, USA.

We recently reported that, compared with young men, older men had smaller plasma 25-hydroxyvitamin D (25OHD) responses to supplementation with 1800 IU/d of vitamin D for 3 weeks. The present study was conducted to determine whether this age difference would occur in men given a smaller vitamin D dose (800 IU/d) and followed for a longer time (8 weeks). Twenty-six young (18-35) and 26 old (62-79) white men were randomized to 800 IU/d [20 µg] cholecalciferol or to no intervention. Subjects lived in the greater Boston area, had no medical conditions known to affect vitamin D absorption or metabolism,

and had usual vitamin D intakes ≤200 IU/d. Two control subjects were excluded because of southern travel during the study. Study visits were conducted in February through April to minimize effects of sun exposure on 25OHD. Plasma 25OHD was measured by competitive protein binding assay. Baseline 25OHD did not differ by age in the supplemented men (60±16[SD]nmol/L in young, 62±16 in old, P=0.797) or in the control group (50±17 nmol/L in young, 54±18 in old, P=0.514). Changes in 25OHD during the study are shown in the Figure:

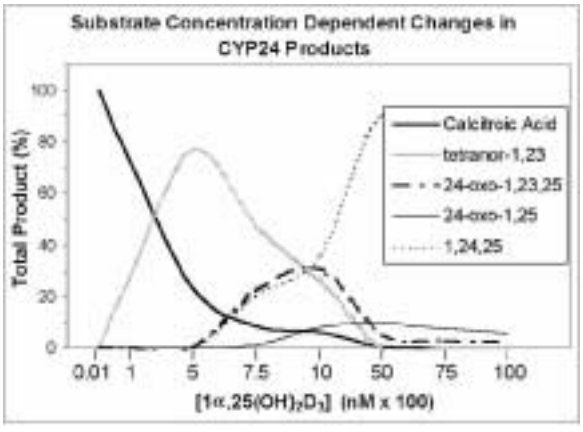


By the end of the study, the 25OHD concentrations of the young and old supplemented men had risen significantly (P<0.001) and nearly identically (22±15 nmol/L in young and 22±13 in old, P=0.948). In contrast, there were modest and similar decreases in 25OHD of the young and old controls. We conclude that young and old men have similar increases in plasma 25OHD during eight weeks of daily oral supplementation with 800 IU of cholecalciferol

SA529

LC-MS Based Enzyme Kinetic Analysis of CYP24 Confirms That 1α,25(OH)$_2$D$_3$ Is a Physiologically Relevant Substrate. M. Kaufmann,* S. Masuda,* G. Jones. Biochemistry, Queen's University, Kingston, ON, Canada.

1α,25(OH)$_2$D$_3$ is metabolized in vitamin D target cells by a 5-step, inducible, 24-oxidation pathway catalyzed by CYP24 culminating in the excretory form, calcitroic acid. Recent reports [Taniguchi *et al.*, JBMR 16: 57. 2001] have challenged this hypothesis claiming that the apparent K_m for 1α,25(OH)$_2$D$_3$ was 20μM when they used a new assay procedure based upon 1α,24,25(OH)$_3$D$_3$ production. The objective of this investigation was to study the catabolism of [1β-^{3}H]1α,25(OH)$_2$D$_3$ by CYP24 in the human keratinocyte cell line, HPK1A-*ras* over a broad substrate concentration range from 1nM to 10μM and assess activity based upon both *substrate loss* and *total* product formation using a combination of diode array, ^{3}H and LC-MS detection. Metabolite quantitation was based upon UV_{265} or [^{3}H] measurements while peak identification was based upon LC-MS using both positive and negative electrospray modes. Mass spectra for all vitamin D catabolites featured characteristic molecular ions (MH^+ or MH^-), their dehydration products and sodium adducts. Studies of substrate disappearance reveal highly efficient metabolism of 1α,25(OH)$_2$D$_3$ in the 1-100nM range whereas above 100nM intermediates start to accumulate. Studies revealed an altered distribution pattern (see figure below) of the intermediates of C-24 oxidation pathway of 1α,25(OH)$_2$D$_3$ across the substrate concentration range used, such that at low substrate concentration 1-100nM, calcitroic acid [MH^-=373] was the major product whereas at higher substrate concentrations, more proximal pathway intermediates predominated. At 100nM-1μM substrate, tetranor-1α,23(OH)$_2$D$_3$ [MH^+=361] and 24-oxo-1α,23,25(OH)$_3$D$_3$ [MH^+=447] were maximally produced and at 1-10μM both 1α,24,25(OH)$_3$D$_3$ [MH^+=433] and 24-oxo-1α,25(OH)$_2$D$_3$ [MH^+=431] were the major products. Above 1μM substrate 1α,23,25(OH)$_3$D$_3$ [MH^+=433] was also detected as a major product. Thus using LC-MS techniques, we have shown that CYP24 efficiently metabolises1α,25(OH)$_2$D$_3$ to calcitroic acid via a saturable pathway operating down to the picomolar range. Our data shows that methods which purport to measure CYP24 activity solely based upon 1α,24,25(OH)$_3$D$_3$ production underestimate total enzyme activity especially at low substrate concentrations. We conclude that one of the physiological roles of CYP24 is target cell catabolism of 1α,25(OH)$_2$D$_3$.



SA530

Bone Cyp27b1 mRNA Is Regulated by Dietary Calcium and not Vitamin D Status. P. H. Anderson,*[1] S. Iida,*[2] A. J. Moore,*[2] M. Cochran,*[2] P. D. O'Loughlin,*[2] B. K. May,*[3] H. A. Morris.[2] [1]Department of Physiology, University of Adelaide, Adelaide, Australia, [2]Institute of Medical and Veterinary Science, Adelaide, Australia, [3]Department of Biochemistry, University of Adelaide, Adelaide, Australia.

Regulation of renal 1,25-dihydroxyvitamin D3 (1,25D) synthesis by 25-hydroxyvitamin D-1alpha-hydroxylase (Cyp27b1) has been identified for 20 years, however, regulation of bone cell Cyp27b1 activity remains largely unknown. We have compared the effects of vitamin D status and dietary calcium (Ca) on Cyp27b1 mRNA expression levels in kidney and bone tissues. Vitamin D-deplete female Sprague-Dawley rats were raised on a vitamin D-deficient, 1% Ca diet and housed in a UV-free environment from birth. Six month old vitamin D-replete and vitamin D-deplete rats were fed either the 1% Ca diet (high) or a 0.1% Ca diet (low) for 3 months. Total RNA was extracted from one femur and kidneys by phenol/chloroform extraction. Real-Time RT-PCR quantified mRNA for Cyp27b1. The second femur was processed for histology and in situ hybridisation for Cyp27b1. Vitamin D-deplete rats on a low Ca diet developed hypocalcaemia, secondary hyperparathyroidism and showed a significant increase in osteoid consistent with osteomalacia. In these rats, kidney Cyp27b1 mRNA levels were increased 100-fold compared with kidneys levels in vitamin D-replete rats fed a low Ca diet($p<0.001$). Bone Cyp27b1 mRNA levels were similar to low Ca/vitamin D-replete kidney levels but unlike kidney CYP27b1, were unaffected by vitamin D depletion. In rats on a high Ca diet, bone Cyp27b1 mRNA levels were increased 5-fold independent of vitamin D status ($p<0.01$), while kidney Cyp27b1 mRNA levels were slightly suppressed. Additionally, there was a strong correlation between bone mRNA levels for Cyp27b1 and the catabolic enzyme for 1,25D, 25-hydroxyvitamin D-24-hydroxylase (CYP24). There was, however, no association between bone Cyp27b1 and kidney CYP24. Most interestingly, the high-Ca challenge in low-vitamin D rats normalised serum Ca and PTH levels as well as reduced osteoid and normalised mineralisation lag time. Preliminary identification of bone Cyp27b1 by in situ hybridisation indicates that the majority of the signal-positive cells are bone marrow derived normoblast-type, haematopoietic progenitor cells. It is evident from these rat studies that unlike kidney Cyp27b1 mRNA, bone Cyp27b1 levels are up-regulated by dietary calcium and unaffected by vitamin D depletion. This up-regulation of bone Cyp27b1 is associated with correction of the mineralisation defect and bone structural changes.

SA531

C-3 Epimerization Pathway is a Common Metabolic Pathway of Vitamin D Metabolites and Inversely Interacts with C-24 Oxydation Pathway in Mammalian Cells. T. Okano,[1] M. Kamao,*[1] S. Tatematsu,*[1] S. Hatakeyama,*[2] K. Ozono,[3] N. Kubodera.*[4] [1]Kobe Pharmaceutical University, Kobe, Japan, [2]Nagasaki University, Nagasaki, Japan, [3]Osaka Medical Center for Maternal and Child Health, Osaka, Japan, [4]Cugai Pharmaceutical Co. Ltd., Tokyo, Japan.

1α,25-Dihydroxyvitamin D_3 [1α,25-D_3] is metabolized into its epimer of hydroxyl group at C-3 of the A-ring beside side-chain cleaved products through the C-24 and C-23 oxidation pathways. In the present study, we investigated the C-3 epimerization of 25-hydroxyvitamin D_3 [25-D_3] and 24*R*,25-dihydroxyvitamin D_3 [24,25-D_3] in cell culture system to clarify whether C-3 epimerization pathway is common to vitamin D compounds. In addition, we examined further metabolism of 3-epi-25-D_3 and 3-epi-1α,25-D_3.Ten μM of 25-D_3, 24,25-D_3 or 1α,25-D_3 were incubated with UMR-106 (rat osteosarcoma), MG-63 (human osteosarcoma), Caco-2 (human colon adenocarcinoma), HepG2 (human hepatoblastoma) and LLC-PK_1 (porcine kidney) cells for 48hr. Metabolites were purified by HPLC and structural analyses were performed by ^{1}H-NMR and LC-MS. Metabolism of 3-epi-25-D_3 and 3-epi-1α,25-D_3 were examined by the same method.In all the cells tested, 25-D_3 and 24,25-D_3 were metabolized into their C-3 epimers. Although the differences existed in the amounts of the products. UMR-106, MG-63, Caco-2 and HepG2 cells generated 3-epi-25-D_3 from 25-D_3 as a major metabolite. In contrast, LLC-PK_1 cells produced 24,25-D_3 from 25-D_3 predominantly. C-3 epimerization occurred more preferentially in 25-D_3 and 1α,25-D_3 than 24,25-D_3. These findings suggest that putative C-3 epimerase can recognize side-chain structure of vitamin D_3. Biological activities of each C-3 epimer of 25-D_3, 24,25-D_3 and 1α,25-D_3 with respect to vitamin D receptor binding, transcriptional activity, regulatory activities of human leukemia (HL-60) cell proliferation and differentiation were lower than those of 25-D_3, 24,25-D_3 and 1α,25-D_3, respectively. 3-epi-25-D_3 and 3-epi-1α,25-D_3 were metabolized into their C-24 hydroxylated metabolites, 3-epi-24,25-D_3 and 3-epi-1α,24,25-D_3. Interestingly, an inverse relationship between the production rates of C-3 epimer and 24-hydroxylated metabolites was observed in 25-D_3 and 1α,25-D_3. Our results suggest that the C-3 epimerization pathway is involved in metabolism of vitamin D_3 widely and C-3 epimers are further metabolized through the C-24 oxidation pathway similar to their parent compounds.

SA532

Targeted Ablation of the 25-Hydroxyvitamin D 1a-Hydroxylase Enzyme: Evidence for Skeletal, Reproductive and Immune Dysfunction. D. K. Panda,[1] D. Miao,[1] M. L. Tremblay,*[2] J. Sirois,*[2] G. N. Hendy,[1] D. Goltzman.[1] [1]Dept. of Medicine, McGill University, Montreal, PQ, Canada, [2]Dept. of Biochemistry, McGill University, Montreal, PQ, Canada.

The active form of vitamin D, 1α,25-dihydroxyvitamin D [1α,25(OH)$_2$D], is synthesized from its precursor 25 hydroxyvitamin D [25(OH)D] via the catalytic action of the 25(OH)D-1α-hydroxylase [1α(OH)ase] enzyme. Many roles in cell growth and differentiation have been attributed to 1,25(OH)$_2$D, including a central role in calcium homeostasis and skeletal metabolism. To investigate the in vivo functions of 1,25(OH)$_2$D and the molecular basis of its actions we developed a mouse model deficient in 1α(OH)ase by targeted ablation of the hormone-binding and heme-binding domains of the1α(OH)ase gene. After weaning, mice developed hypocalcemia, secondary hyperparathyroidism, retarded growth and skeletal abnormalities characteristic of rickets. These abnormalities are similar to those described in humans with the genetic disorder vitamin D dependent ricket type1 (VDDR-1). Altered non-collagenous matrix protein expression and reduced numbers of osteoclasts were also observed in bone. Female mutant mice were infertile and exhibited uterine hypoplasia and absent corpora lutea. Furthermore histologically enlarged lymph

nodes in the vicinity of the thyroid gland and reduction in CD4- and CD8- positive peripheral T lymphocytes were observed. Alopecia, reported in vitamin D receptor (VDR)-deficient mice and in humans with VDDR-11, was not seen. The findings establish a critical role for the 1α(OH)ase enzyme in mineral and skeletal homeostasis as well as in female reproduction and also point to an important role in regulating immune function.

SU001

The Increased Bone Mass for Age Observed in Childhood Obesity Is Due to Increased Height and Bone Width, but not Increased Bone Density. M. B. Leonard,* B. S. Zemel, A. M. Tershakovec.* Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA.

Obesity has been associated with increased areal-BMD as measured by DXA, and with decreased fracture risk. Because DXA is a projectional technique, it does not distinguish between large bones (increased patient height, or increased bone width) vs. increased volumetric-BMD as an explanation for increased areal-BMD. The purpose of this study was to evaluate these alternative explanations for the increased bone mass observed in obese children compared with healthy controls. Whole body (WB) and lumbar spine (LS) DXA scans (Hologic, Inc, QDR-2000) were performed in 96 healthy controls and in 67 obese children, ages 4-21 yr. The obese children were recruited from the weight management clinic and were otherwise healthy. Pubertal stage was classified according to Tanner. Weight (Wt) and height (Ht) were converted to age, gender-specific z-scores, using NCHS data. Models for bone parameters were constructed using the healthy controls, and then a dummy variable for the obese group was entered to test for group effects. DXA WB bone area adjusted for patient height was used as an estimate of bone width, while LS BMC adjusted for LS bone area was used as an estimate of bone density (Molgaard, et al, Arch Dis Child 1997). All models were adjusted for gender, race and pubertal stage. The obese subjects had significantly increased Ht-z and Wt-z scores compared with healthy controls (all $p < 0.001$). WB BMC for age was significantly increased in the obese subjects, as expected. WB BMC, adjusting for patient Ht, was still significantly increased in the obese, suggesting that the increased bone mass is not entirely due to tall stature. WB bone area for height was also significantly increased in the obese, suggesting markedly increased bone width for patient height (i.e. broad bones). Finally, LS BMC, adjusting for bone area was not significantly different between healthy controls and obese.

	Ht-z Mean (SD)	Wt-z Mean (SD)	WB-BMC for Ht*	WB-area for Ht*	LS BMC for area*
Control	0.2 (1.0)	0.2 (1.0)	-	-	-
Obese	1.2 (1.1)	4.4 (1.9)	↑ p<0.001	↑ p<0.001	NS

*Decreased compared to healthy controls, adjusted for puberty, gender, and race

SU002

Bone Turnover During Adolescence and Young Adulthood. Y. M. Henry, A. C. Eagleton, D. Fatayerji,* R. Eastell. Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Biochemical markers of bone turnover may help in understanding the physiology of skeletal growth and maturation. Bone turnover markers are elevated during puberty but it is unclear when they are maximal. Bone markers stabilise during young adulthood but the time when minimal levels are achieved remains ambiguous. Therefore, the aims of the present study were to examine the effects of age and gender on different biochemical markers of bone turnover and to study the effect of puberty on bone markers in children. One hundred and thirty two healthy Caucasian children (63 boys and 69 girls, ages 11 to 19 years) and 134 healthy Caucasian adults (66 men and 68 premenopausal women, ages 20 to 50 years) were studied. Bone ALP, OC and PINP were measured as indices of bone formation, and NTX and i-free DPD as indices of bone resorption. Bone resorption markers were expressed as a ratio to urinary creatinine. One-way ANOVA followed by a Scheffé test were used to test the significance of the difference between results at various pubertal stages and between the decades (20 to 50 years) in which adult levels were achieved. The magnitude of the pubertal increase was estimated by expressing mean level in Tanner stages II and III in girls and III and IV in boys as a ratio to the mature adult (MA) reference range (30 to 50 years). We observed that all markers were maximal at mid-puberty (M-P), Tanner stages II and III in girls, and IV in boys. During young adulthood bone markers were minimal in women between 20 to 50 years but later in men during 30 to 50 years.

Marker	M-P Mean[1]	MA Mean[1]	Female Ratio	M-P Mean[2]	MA Mean[2]	Male Ratio
OC, ng/ml	104	26.1	4.0	140	21.7	6.4
PINP, ng/ml	481	43.5	11.1	728	46.1	15.8
Bone ALP, µg/l	50.4	9.16	5.5	75.8	12.3	6.2
NTX/Cr	328	29.9	11.0	438	41.3	10.6
i-free DPD/Cr	22.7	5.99	3.8	26.2	3.5	7.5

[1] Females. [2] Males.

To conclude: (1) all markers were elevated in children (boys more than girls) compared to adults, and were maximal at mid-puberty followed by a decline in late puberty. This pattern of change parallels growth height velocity; (2) the magnitude of the increase was specific for each gender and marker because they reflect growth, modelling and remodelling of trabecular and cortical bone and (3) adult reference ranges should take into account the later maturation of the male skeleton.

SU003

Alendronate in the Treatment of Osteogenesis Imperfecta. V. Vyskocil.[1] [1]Charles University Hospital, Plzen, Czech Republic.

Osteogenesis imperfecta is relatively rare disease which causes fractures. Previous therapeutic procedures consisting of calcitonin and vitamin D had only limited effect on the bone density. According to our results the increase in BMD was only 4,6% per year. They had no significant effect on the number of fractures. Out of these reasons we started (after agreement with parents of our patients) treatment with bisphosphonates. We evaluated 30 children with osteoporosis imperfecta (mean age 13,7) treated orally with bisphosphonates. During one year therapy the children older than 10 years were given 10 mg of alendronate daily and children from 4 to 10 years used 5 mg daily. The Z-score of L1-L4 DXA at the onset of therapy was - 2,56 in all our patients a they had more than 2 fractures per yer. After one year of alendronate therapy we have noticed significant increase in spinal bone density 14,5 %, other location of measurement (hip, forearm) were not evaluated because of frequent deformities. During the first year of the therapy we observed only one fracture caused by adequate trauma (ankle fracture due to skiing). No adverse events occured in children treated with alendronate. There were no signs of gastrointestinal inconvenience and no negative influence on bone evaluated by X.rays in our patients. We recommend to continue the alendronate therapy of these children.

SU004

Bone Size Corrections: Application to Children Born Preterm. C. M. Smith,[1] R. C. Coombs,*[2] A. T. Gibson,*[2] R. Eastell.[1] [1]Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom, [2]The Jessop Hospital for Women, Sheffield, United Kingdom.

Several methods have been proposed to correct bone mass for bone size. Our aim was to evaluate the spine bone mineral content (BMC) status of children who were born preterm, by separating BMC into its two components, volume and volumetric density. Fifty prepubertal children (25 preterm, 25 age and sex matched term controls) had posterior-antero lumbar spine BMC and bone area (BA) measured using dual energy x-ray absorptiometry (DXA, Hologic QDR 4500A). Bone mineral apparent density (BMAD) was calculated using the equation BMC/Bone Volume (BV). BV was calculated using the formula $BA^{1.5}$ (Carter, 1992). BV was adjusted for body size by dividing it by height (BV:Ht). A z-score was calculated for all these variables based on their mean and standard deviation in the term group.

	Preterm mean (SD)	Term mean (SD)	p	Preterm z-score
BMC, g	12.3 (2.87)	14.5 (4.18)	<0.01	-0.80
BMAD, mg/cm^3	82.0 (8.96)	89.1 (9.68)	<0.05	-0.73
BV, cm^3	149.9 (33.9)	161.3 (37.6)	<0.05	-0.37
Height, cm	104.9 (8.84)	110.43 (9.85)	<0.01	-0.44
BV:Ht, cm^3/cm	1.41 (0.23)	1.44 (0.24)	0.43	0.46

Bone mineral content was reduced in the preterm group. This reduction resulted from a reduction in both bone volume and volumetric bone density. However, bone volume was appropriate for height in the preterm group. Thus, children born preterm have a bone volume that is low for their age but not for their size. They also have a reduced volumetric bone density. If these deficits are not recovered during puberty this could result in a low peak bone mass.

SU005

The Bone Mass Pattern in 9-Year Old South African Black Children Is Similar to That Found in Their Mothers, but Is Different to Those in African-Americans. S. A. Norris,[1] L. Vidulich,*[1] N. Cameron,*[2] J. M. Pettifor.[3] [1]Mineral Metabolism Research Unit, Johannesburg, South Africa, [2]University of Loughborough, Loughborough, United Kingdom, [3]Paediatrics, University of the Witwatersrand, Johannesburg, South Africa.

In the USA, ethnic bone mass and body composition patterns are evident before puberty and persist through adulthood; African-Americans have similar height to that of Caucasian-Americans, but have greater site-specific bone mineral density (BMD, g/cm^2), total bone mineral content (g), lean body mass (g), and body fat (g). We investigated ethnic differences in site-specific BMD, bone mineral content (BMC, g), bone area (BA, cm^2) and body composition of a cohort of prepubertal (aged 9) white (males=47; females=52) and black (males=159; females=139) children and their mothers living in Johannesburg using DXA (QDR-4500). Black children were significantly shorter than white children, and black mothers were shorter than white mothers. Correcting for body size, there were no ethnic BMD, BMC or BA differences at the lumbar spine or radius for both children and mothers, however, black children and mothers had greater hip BMD associated with decreased bone area and similar or greater BMC. Furthermore, there were no significant ethnic differences in body composition for both children and mothers. This study confirms earlier South African findings that there is no ethnic difference in radial bone mass after adjusting for body size in prepubertal children, and is similar to findings from premenopausal women in whom no ethnic differences at the lumbar spine and radial sites were found, but where ethnic differences of the hip was evident with black women having greater hip BMD than white women. These results might explain the lower hip fracture rate in postmenopausal black women, and may suggest that black South African women are not protected against vertebral fractures as anticipated. This study further highlights that ethnic bone mass and body composition differences found between black and white children and

adults in the USA cannot be assumed to be true for other countries. Unadjusted means ± SD

Variable	White males	Black males	White females	Black females
Child hip BMD	0.704 ± 0.079	0.747 ± 0.114 [a]	0.609 ± 0.065	0.670 ± 0.079 [b]
Maternal hip BMD			0.904 ± 0.123	0.964 ± 0.121 [b]
Child spine BMD	0.549 ± 0.052	0.535 ± 0.054	0.528 ± 0.065	0.555 ± 0.083
Maternal spine BMD			1.017 ± 0.131	1.008 ± 0.110
Child radial BMD	0.407 ± 0.030	0.397 ± 0.047	0.386 ± 0.031	0.379 ± 0.048
Maternal radial BMD			0.565 ± 0.045	0.561 ± 0.049

(a) p<0.001, (b) p<0.0001

SU006

Calcium Absorption and Bone Mass in Lactating African-American Adolescents. K. O. O'Brien,*[1] A. N. Geer,*[1] F. R. Witter.*[2] [1]Center for Human Nutrition, Johns Hopkins School of Public Health, Baltimore, MD, USA, [2]Gynecology and Obstetrics, Johns Hopkins School of Medicine, Balitmore, MD, USA.

The impact of adolescent pregnancy and lactation on bone mineral density and the ability of adolescents to modify calcium absorption in response to these increased requirements are largely unknown. We addressed this issue by measuring calcium (Ca) absorption during the third trimester of pregnancy (34-36 wks gestation) and again 3-4 weeks postpartum in a group of 14 African-American adolescents (ages 16.3 ± 1.5 y), 8 of whom were breastfeeding their infants during the second study. Total body and lumbar spine bone mineral content were measured in each adolescent 3-4 weeks post-delivery using DXA (Hologic QDR 4500A). Ca absorption was determined during each study following the administration of oral (^{44}Ca or ^{46}Ca) and intravenous (^{42}Ca) stable Ca isotopes. Cumulative excretion of the oral to the intravenous isotope in a 24-hr urine collection post-dosing was used to determine Ca absorption. Percent Ca absorption (-21.4%, p<0.0001, paired t-test) and urinary Ca excretion (-190 mg/d, p<0.0001) were significantly decreased in these adolescents 3-4 weeks post-delivery. A total of 36% of this population (5/14) had evidence of osteopenia (lumbar spine z-scores < -1, n=3) or osteoporosis (lumbar spine z-scores < -2, n=2) 3-4 weeks following the delivery of their infants. Lumbar spine z-scores were on average 0.770 SD lower in lactating compared to non-lactating adolescents (-0.489, n=8 vs. +0.282, n=6) although the magnitude of this difference did not reach statistical significance in this population. Further studies are needed to address the ability of adolescents to regain bone mineral conent once menses resume.

SU007

Analysis of Skeletal Development on the Basis of Body Mass and Bone Resorption. D. L. DeMoss,[1] W. D. Geng,*[2] G. L. Wright.*[2] [1]Biology, Morehead State University, Morehead, KY, USA, [2]Physiology, Marshall University School of Medicine, Huntington, WV, USA.

Regression analysis was utilized to define the relationships among body weight, whole skeleton bone resorption (^{3}H-tetracycline method), and the development of skeletal mass. The results indicated that skeletal development (% body weight) of slowly growing, 24-week-old rats consisted of two major components, one directly ($r = 0.985$, $P < 0.001$) and one inversely ($r = 0.977$, $P < 0.001$) related to body weight. It was further noted that the skeletal resorption rate was inversely correlated to body weight ($r = 0.879$, $P < 0.05$) and directly related to skeletal development ($r = 0.865$, $P < 0.05$), suggesting that whole skeleton bone resorption and formation were highly correlated in the slowly growing animal. A third small component of skeletal development identified in the analysis of data from 24-week-old animals showed no direct relationship to either body weight or resorptive activity. The model presented enables the separation of skeleton mass into major components, which may represent mechanically and metabolically driven bone formation.

SU008

Bone Mineral Mass in Overweight and Obese Children: Diminished or Enhanced? K. J. Ellis,* R. J. Shypailo,* W. W. Wong,* S. A. Abrams. Children's Nutrition Research Center, Dept. Pediatrics, Baylor College of Medicine, Houston, TX, USA.

The purpose of this study was to determine if overweight and obese children have enhanced or diminished bone mineral mass. We have examined whole-body bone mineral content (BMC) in 918 healthy children (ages 5 -18 y) representing a full range of body fatness. Dual-energy x-ray absorptiometry (DXA) was used to obtain whole-body BMC, body fatness (%Fat), and lean tissue mass (LTM). The %Fat cutpoints for 'overweight' and 'obese' status were 25% and 30%, respectively. A reference model for BMC in children (Ellis et al, J Bone Min Res 2001; 16: in press) was used to obtain a standardized Z-score rating for each child. Additional statistical analyses included linear regression, analysis of variance, and unpaired t-test. There were ethnic and gender differences for BMC vs body weight (Wt) and height (Ht), but not for BMC vs LTM. BMC, adjusted for height (Ht), was increased for children with an 'obese' classification.

	Normal (<25%)	Overweight (25%-30%)	Obese (>30%)
N = male (female)	318 (188)	37 (96)	66 (160)
BMC (g)	1480 (1242)	1556 (1504)	1607 (1616)
BMC/Ht (g/cm)	9.55 (8.36)	10.11 (9.74)	10.36 (10.50)
BMD (g/cm2)	0.944 (0.984)	0.951 (0.939)	0.944 (0.965)

Furthermore, when evaluated using a Z-score model, the obese and overweight children usually had positive Z scores indicating above average BMC values. Overweight and obese children also tended to have a higher Tanner rating than leaner children of the same age, indicating possible hormonal influence, and not obesity *per se*. We conclude: (1) the lower bone mineral mass previously reported for overweight and obese children (Goulding et al, Int J Obs 2000; 24: 627; Goulding et al, J Bone Min Res 1998;83: 3469; Weiler et al, Bone 2000; 27: 203) was not evident when the anthropometric reference index was height; and (2) obese children have increased BMC compared with leaner children, when adjusted for Ht, age, gender, and ethnicity.

SU009

Osteoporosis Prevention in Young Women: Application of the Health Belief Model. L. S. Wallace. The University of Texas at Tyler, Tyler, TX, USA.

The purpose of this study was to examine personal characteristics and Health Belief Model (HBM) constructs associated with the practice of osteoporosis protective behaviors among a random sample of nontraditional college women. Two hundred and seventy-three women (mean age=24.81±10.23 years, mean BMI=24.81±6.56, 87.7% Caucasian) completed a valid and reliable written mail questionnaire assessing osteoporosis knowledge, HBM constructs, weight-bearing exercise (EX), and dietary calcium intake (CA). On average, women were able to correctly answer 65% of questions assessing general osteoporosis knowledge (e.g., risk factors, preventive measures). Women reported an average of 2.36±2.03 days of weight-bearing activity per week. Current daily calcium was 997.97±517.78 mg (median=922.0, range=46.7-3595.3 mg). Adequate (=) CA was defined as the consumption of >1200 mg/day, while adequate EX was defined as > 90 min/wk. Those falling below the adequate criterion were considered low (Ø) for both CA and EX. A high proportion of women did not meet current guidelines for EX (50.7%) or CA (67.8%). Subjects were classified into one of four categories based on their current calcium intake and weight-bearing exercise participation. The following CA and EX categories emerged: 1) ØCA/ØEX (n=100, 38.9%); 2) ØCA/=EX (n=81, 31.5%); 3) =CA/ØEX (n=38, 14.8%); and, 4) =CA/=EX (n=38, 14.8%). Those in =CA/=EX and ØCA/=EX had significantly higher health motivation and exercise self-efficacy than those in =CA/ØEX or ØCA/ØEX. Multiple stepwise discriminant analysis revealed exercise self-efficacy, barriers to exercise, and perceived susceptibility to osteoporosis as significant predictors of CA/EX category. On the basis of these data, it was concluded that a need exists to increase awareness of osteoporosis risk factors and encourage young women to meet recommended guidelines for both calcium intake and weight-bearing activity. Intervention activities should be developed to foster increased self-efficacy and decreased barriers to calcium intake and physical activity. Many young women are at risk of developing osteoporosis later in life, and more education on this subject is warranted.

SU010

Precision of DXA Measurements of the Spine and Total Hip in Adolescents. C. G. Miller,[1] J. Pak,*[2] J. N. Caminis.[3] [1]Bio-Imaging Technologies Inc., Newtown, USA, [2]Medical Affairs, Roche Laboratories inc., Nutley, USA, [3]Roche Laboratories Inc., Nutley, USA.

Non-invasive techniques such as DXA has made it possible to determine, with very low radiation exposure, bone mineral accumulation, at various skeletal sites during childhood and adolescence. Currently, there are no published data for total hip cross-sectional data in normal pediatric populations. The purpose of this is to evaluate the contribution of triplicate DXA measurements to precision in adolescent patients between 12 and 18 years of age. 204 children (mean age 16.55 +/- 1.3 years) (approximately 66% male) were enrolled in this trial at 19 centers. All subjects and their guardians signed informed consent, which had been approved by each local IRB. Each subject had 3 AP spine (L1 to L4) and 3 left hip DXA measurements taken within 24 hours (1 subject had right femur measurements). All measurements were obtained on GE Lunar densitometers. The %cv's for each site were then calculated as the mean of the individual's precision.The mean, minimum, maximum and median precision (%cv) for the AP Spine was 1.31%, 0.715, 2.09, 1.29 and for Total Hip was 0.653, 0.259, 1.551, 0.629, respectively. The %cv obtained in this group of pediatric subjects was less than 1% at the spine at 18 of 19 sites. The Total Hip precision (%cv), while expectedly poorer, was still very small and demonstrates the ability to obtain good data on pediatric subjects. This level of precision enables the detection of most clinically significant changes in this population. These are the first published data of Total Hip BMD in these age groups on an axial densitometer. This is important with the increasing, regulatory mandated need for more pharmaceutical studies in pediatric populations. Pediatric BMD studies are not only possible but can be conducted ethically with precision and with minimal radiation exposure (approximately 3 to 3.7 micro Sv per scan per anatomical site).

Disclosures: Bio-Imaging Technologies Inc,3; Roche Laboratories Inc.,3.

SU011

Oral Contraceptive Inhibition of Bone Growth in Young Female Rats: The Role of Androgens. T. Register,[1] M. J. Jayo.[2] [1]Comparative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA, [2]Pathology Associates, Charles River Laboratories, Advance, NC, USA.

Previous studies demonstrated that treatment of intact young adult female monkeys with an oral contraceptive (OC) resulted in inhibition of normal gains in whole body and lumbar spine bone mineral content and density (Register et al., Osteoporosis Intl 1997;7:348). OC treatment also caused a marked suppression of serum testosterone and androstenedione levels. The purpose of the present study was to determine whether hypoandrogenemia might explain the inhibitory effects of OC on bone metabolism and the attainment of peak bone mass in young adult females. Intact, 70-day old adolescent/young adult virgin female Sprague-Dawley rats were treated with placebo (Control), an OC containing ethinyl estradiol and levonorgestrel (OC), OC supplemented with the aromatization-resistant androgen methyltestosterone (OC+MT), or the anti-androgen bicalutamide (BC) for 15 weeks. OC treatment inhibited gains in bone mass relative to Controls, similar to the previous findings in cynomolgus monkeys, and also caused a reduction in tibial length. Addition of MT to the OC treatment did not prevent the adverse effects of OCs on the bone mass or tibial length, suggesting that hypoandrogenemia was not solely responsible for the OC effects. Anti-androgen (BC) treatment had no effect on the growing skeleton of young rats compared to Controls, suggesting that androgens may have a limited involvement in acquisition of bone mass in young adult females. Histomorphometry of the cancellous compartment of proximal tibias indicated that the OC and OC+MT groups had reduced bone surface, non-labeled surface, and trabecular number, and increased trabecular separation relative to Control and BC groups. Histomorphometric data suggested that double-labeled surface perimeter, bone formation rate/bone surface ratio, and mineralizing surface were higher in the OC+MT group compared to the OC group. Nevertheless, OC and OC+MT groups were not different from one another in bone density or tibial lengths. No treatment effects on the cortical compartment were observed. Taken together, these results suggest that while androgens may influence bone metabolism, hypoandrogenemia was not the underlying cause of OC inhibition of bone mineral acquisition in this study.

SU012

Bone Histomorphometry in Older Men. A. M. Kenny,*[1] K. M. Prestwood,[1] M. Gunness,[2] L. G. Raisz.[3] [1]Center on Aging, University of Connecticut, Farmington, CT, USA, [2]Portland Veterans Administration Hospital, Portland, OR, USA, [3]General Clinical Research Center, University of Connecticut, Farmington, CT, USA.

Osteoporosis is a significant problem in the aging male, resulting in 30% of hip fractures that occur worldwide. While increasing evidence suggests that estrogen and testosterone are important to skeletal integrity in men, few studies have related these hormones to bone architecture in older men. We obtained bone biopsy samples from 28 older men (x= 72.3 ± 4.6, range 65-82 years) to assess histomorphometric parameters and correlated them with sex and calcium regulating hormone levels, markers of bone turnover, lumbar spine, femoral and total bone mineral density, calcium intake, physical function, muscle strength and power. Mean static and dynamic parameters of bone histomorphometry and normative numbers are outlined in the table. Bioavailable testosterone levels correlated positively with cancellous bone volume (BV/TV) [r=.52, p=.006] and trabecular thickness (TbTh)[r=.51, p=.007]. Using linear regression analysis, bioavailable testosterone predicted BV/TV and TbTh (R2=.25, p<.01). Bone formation rates (BFR/BV, BFR/TV, BFR/BS) and mineralizing surface (MS/BS) were inversely correlated with calcium intake (r= -.38 to-.48, p<.05) and estrogen/SHBG levels (r= -.47 to-.59, p<.05) and positively correlated with muscle power (r=.44 to.55, p<.05). Using linear regression analysis with calcium intake, muscle power and estrogen as independent variables, muscle power predicted MS/BS (R2=.40, p<.012), estrogen predicted BFR/BS (R2 =.47, p=.007), calcium intake and muscle power predicted BFR/BV (R2=.68, p=.002) and estrogen and muscle power predicted BFR/TV (R2=.66, p=.003). No significant associations were found between parameters of bone histomorphometry and parathyroid hormone, markers of bone turnover, bone mineral density or physical activity. Conclusion: In healthy older men, bioavailable testosterone levels predicted cancellous bone volume and trabecular thickness. Increased mineralization surface and bone formation rates are associated with lower calcium intake, lower estrogen levels and increased muscle power.

Disclosures: SmithKline Beecham,2; Novartis,8; Mission Pharmaceutical,2.

SU013

Effect of Total Knee Arthroplasty on Patients' Bone Quaity: Five to Ten Years Followup. Y. Ishii,*[1] Y. Matsuda,*[1] S. Sakata,*[2] K. Ichimura.*[3] [1]Ishii Orthopaedic & Rehabilitation Clinic, Gyoda, Japan, [2]Sado General Hospital, Kanai Sado, Japan, [3]Toyama Kyoritsu Hospital, Toyama, Japan.

Introduction: Poor bone quality is considered as a major risk factor for hip fracture in the aged population. Eighty seven percent of patients with hip fracture are reported over 65 years old. Patients aged over 65 years who suffer from osteoarthritis of the knee are good candidates for total knee arthroplasty (TKA). The purpose of this study is to compare the bone quality between patients who underwent TKA, patients who suffered from hip fracture, and age matched controls with ultrasonographic heel measurement.Materials and Methods: Characteristics of three groups shows as follows. Each group shows ,heels/ patients,Gender, and average age respectively. Group A; TKA, 70/ 57, Female 48;Male 9,76±7years. Group B;Hip Fracture,107/104, Female 86;Male 18, 83±9years. Group C; Controls, 70/ 70, Female 59;Male 11, 76±6yrs. In TKA patients, the mean follow-up was 92 months (range, 62 to 113), and all patients had the preoperative diagnosis of osteoarthritis. The average HSS Score was 93±7. Broadband Ultrasound Attenuation (BUA; dB/MHz) through the os calcis was measured to access the bone quality of patients. Group A and B were evaluated on the affected side. ANOVA (Scheffe's methods) were used for statistical analysis.Results: There were no TKA patients who suffered from hip fracture during follow-up period. BUA of Group A, B, and C showed 42.2±15.5, 22.9±13.3 and 40.2±14.9 respectively. Group B showed significant differences compared to both A and C (p<0.0001). Although TKA group had better BUA than controls, there is no significant difference between both groups (p=0.712). Discussion and Conclusion: The results suggest that the increase in activity level after artificial replacement of arthritic joint might improve the bone quality, because of pain relief. BUA is reported to be a better discriminator of hip fracture than dual-energy x-ray absorptiometry. Early TKA might be protective against later hip fractures, by allowing increased mobility and improved bone quality.

SU014

Gender and Age-Related Effects on Bone Formation in a Human Osteoblast Implant Culture System. H. Zhang, G. Gronowicz. Orthopaedics, UCONN Health Center, Farmington, CT, USA.

Gender-related differences are found in bone geometry and strength, especially in the older population. Women also have a higher fracture rate compared to men. Implants are used extensively in orthopedic surgery and dentistry, particularly in the elderly. To investigate if gender and age affect bone formation on implant materials, we examined the response of primary human osteoblasts (HOBs) to orthopaedic Ti-6Al-4V (Tiv) implant disks (Zimmer, Warsaw, IN) by using a novel in vitro osteoblast/implant culture system. HOBs were cultured from bone chips obtained in corrective orthopedic surgery from healthy men and women; Young (Y) <15, Middle (M) = 30-50 and Old (O) >60 years old (4-6 patients per group). These cells were identified as HOBs by their ability to produce a1(I) procollagen and osteocalcin mRNA and high levels of alkaline phosphatase message and protein. HOB attachment to Tiv was examined at 4 h. HOB from female patients showed an age-related decrease in adhesion, with O females showing statistically lower attachment of cells (31.1%) compared to O men. No differences were found between females and males in Y or M groups. Collagen synthesis, assayed by ^{3}H-proline incorporation at 24 h, demonstrated a small but significant age-related decrease in both genders in the O group vs. Y and M groups. Cell proliferation determined by ^{3}H-thymidine incorporation did not show any differences at 24 h of culture. Mineralization, determined by biochemical and morphological calcium content assays, demonstrated age-dependent decreases in female patients (O<M<Y) but no differences in male patients' HOBs at 4 wks of culture. In the O group, females had significantly less calcification (26.5%) compared to males. DNA content in each group was similar. In conclusion, female patients demonstrated a significantly decreased ability for their osteoblasts to attach to implants, to synthesize collagen and to mineralize matrix in response to implant materials. However, HOBs from male patients demonstrated no significant changes among different age groups. These results suggest that elderly female patients may have decreased osseointegration of their implants. In addition, we have developed a reproducible human system in which to study age- and gender-related changes in the osteoblast's ability to form bone.

SU015

Mechanisms of the Sequential Occurrence of Two Types of Bone Loss in the Lumbar Vertebra of Male Sprague Dawley Rats During Aging. L. Wang, J. Banu, D. N. Kalu. Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

We previously demonstrated that the intact male Sprague Dawley (SD) rat experiences age-related bone loss that has the characteristics of the bone loss that occurs in aging men (L. Wang, et al. Bone: 2001, in press). In this study, male SD rats aged 3, 6, 8, 9, 10, 11, 12, 15, 18, 21, 24 and 27 months were studied at the L4 vertebral bone using peripheral quantitative computed tomography, histomorphometry and biochemical analyses. From 9 to 21 months of age, cancellous bone mineral content (BMC) decreased by 30% (p<0.0001) and cancellous bone mineral density (BMD) decreased by 35.2% (p<0.0001). Vertebral trabecular bone thickness and trabecular number decreased significantly during this period. From 3 months of age and on, mineral apposition rate (MAR) and bone formation rate (BFR) in the male SD rats decreased rapidly until 9 months of age. From 9 to 24 months of age, MAR and BFR decreased further and remained at the much lower level than the levels for 3-month-old rats (p0.05) and cancellous BMD had decreased by 10.7% (p>0.05) compared to the levels at 21 months of age. However, cancellous BFR increased significantly in the 27-month-old male SD rats compared with the level for 9-month-old rats (p<0.05). Osteocalcin and Dpd also increased at 24 and 27 months of age, but the increase was significant only at 27 months of age (p<0.05). In conclusion, it was found in this study that decreased bone formation rate preceded increased bone resorption rate in male SD rats from 9 to 27 months of age while bone mass decreased progressively. Decreased bone formation appeared to contribute primarily to the early phase of age-related bone loss while the bone loss that occurred later in the life of the male SD rats was possibly due primarily to increased bone resorption.

SU016

Risk Factors for Low Bone Mineral Density and the Six-Year Rate of Bone Change Among Pre- and Perimenopausal Women. K. E. Bainbridge,* M. F. Sowers. Epidemiology Department, University of Michigan, Ann Arbor, MI, USA.

The purpose of this population-based prospective cohort study was to determine risk factors for pre- and perimenopausal bone mineral density (BMD) as well as the rate of bone loss among women aged 24-50 years. The specific aims were to 1) estimate measures of association between anthropometric, reproductive, dietary, lifestyle, and medical history variables with BMD, and 2) evaluate which of these variables are associated with the rate

of BMD change (either gain or loss). We followed 614 women, between the ages of 24-44 at baseline, for six years beginning in 1992/93. BMD measurements of the lumbar spine (L_{2-4}) and the femoral neck were obtained using dual x-ray absorptiometry (DXA) during up to five physical examinations conducted between 1992/93 and 1998/99. Anthropometric measurements were also obtained during these examinations. Dietary information was collected over four consecutive years from 1992/93 to 1995/96 using the NCI Health, History, and Habits Questionnaire. History of chronic illness, medication use, and family history of osteoporosis as well as physical activity, alcohol use, and smoking behavior were assessed through interviews or self-administered questionnaires. Reproductive factors were also assessed including self-reported frequency of menstrual bleeds per year from which menopausal status was derived. Linear mixed models were developed to identify independent risk factors for BMD at a given time while simultaneously identifying risk factors for BMD change over the six years. Body weight was positively associated with BMD (p = .0001) and with bone change (.0001) at both the lumbar spine and femoral neck. At the lumbar spine, history of fracture at any skeletal site (p = .005) and surgical menopause (p = .03) were associated with lower BMD. Natural and surgical menopause and reproductive cancers were associated with greater lumbar spine bone loss (p = .0001, for each). At the femoral neck, family history of osteoporosis was associated with lower BMD (p = .01). Alcohol consumption (p = .0003) and high school sports participation (p = .002) were associated with greater BMD. Surgical menopause (p = .007) and reproductive cancers (p = .0008) were associated with greater femoral neck bone loss. Calcium intake, smoking, and current physical activity were not associated with BMD or bone loss at either skeletal site. Risk factors that are associated with BMD at a given point in time are not necessarily associated with rate of BMD change.

SU017

Effect of Orchidectomy and Salmon Calcitonin in Fracture Healing on Cortical Bone from Rat Femur. Bone Mass and Mechanical Strength. J. C. Koulouris,* I. Dontas, G. Trovas, E. Kataxaki, P. Raptou, G. P. Lyritis. Laboratory for the Research of the Musculoskeletal System, Athens, Greece.

Aim of this study is to investigate changes in bone mass and biomecanical competence in relation to effect of salmon Calcitonin (sCT) in healing of fracture in normal, orchidectomized and orchidectomized treated wirh sCT rats. We used 56 male Wistar rats. We orchidectomized 28 rats in the age of 2 months and we performed a half-osteotomy(OT) of the diaphysis of the femurin the age of 3 months. We divided the rats to 8 groups of 7 animals. A(2 weeks) a (4 weeks) nonorchidectomized, B(2 wks) b(4 wks)nonorchidectomized+sCT,G(2wks)g(4wks)orcidectomized,D(2wks)d(4 wks) orchidectomized+sCT. We treated the groups(B,b,D,d)immediately after OT with 5UIsCT subcutaneously daily. Rats of groups a,b,g,d were killed 2 weeks and those of A,B,C,D 4 weeks after OT. At the fracture callus we assesed the corticalBMD,TOTALBMD,CORTICALBMD,IPcort.area (polar moment of inertia) with pQCT (Stratec-960).

	2 WEEKS				4 WEEKS		
CORTICAL BMD	TOTAL BMD	CORTICAL CONTENT	IP cortical area	CORTICAL BMD	TOTAL BMD	CORTICAL CONTENT	IP cortical area
b>a (p=0.01)	ns	ns	ns	B>A (p=0.002)	B>A (p=0.01)	ns	ns
d vs g ns	ns	ns	ns	D vs G ns	ns	D>G (p=0.002	D>G (p=0.001)
				A vs G ns	ns	ns	A>G (p=0.006)

We concluded that treating fracture with sCT for 4 weeks improved the parameters of pQCT (content, density) and mecanicalbone strenght (polar moment of inertia) in the callus for the rats of group D compared with group g

SU018

Rate of Bone Loss in Perimenopausal and Early Postmenopausal Women: Role of Body Composition, Hormonal History and Environnemental Factors. S. Malochet,*[1] J. M. Ristori.[2] [1]Rheumatology, CHU Clermont Ferrand, Clermont Ferrand, France, [2]Departement of Rheumatology, CHU de Clermont Ferrand, Clermont Ferrand, France.

The object of this study was to evaluate the role of body composition, hormonal history and environnemental factors on the rapid bone loss that occure in perimenopausal and in early postmenopausal period. We espacially study the impact of food that are rich in polyphenols wich could influence bone metabolism (fruit, vegetable, red wine an tea).Sixty five women (7 pre-, 22peri-, 36 postmenopausal between 12 and 36 month since their last menstrual period), aged 43-58 years participated in a longitudinal study of bone mineral density (BMD). BMD was measured by dual-energy X-ray absorptiometry (DXA) at the lumbar spine and four femur sites and the time between the two bone scans was on average 26.4 months. Rate of bone loss was expressed as a percentage of the baseline value per year and as the bone mineral density slope in grams per cm ^{2} per year.At the time of the second bone density scan, 51 women were postmenopausal and 14 perimenopausal, and 71% of the postmenopausal women had taken Hormone Replacement Therapy (HRT) since the first measurement.Baseline BMD measures were highly predictive of the follow-up BMD values (r^2 from 0.87 to 0.95). The annual percentage change in BMD of the whole group was different compared with zero at the lumbar spine (-0.5, p=0.02), the femoral neck (-0.8, p=0.001) and the Wards'triangle (-1.5, p<0.001).Factors significantly associated with slower rate of bone loss were : longer time since last menstrual period at the spine, the trochanter and intertrochanteric site; the use of HRT at all sites except the femoral neck; shorter duration of contraceptive use and of breast-feeding only at the trochanter; greater weigth at the trochanter for the whole group and at the intertrochanteric site for the women without HRT; greater body mass index at the intertrochanteric site only in women without HRT; red wine intake at the femoral neck and at the Ward's triangle only in women without HRT. Factors significantly associated with greater rate of bone loss were smoking at the femoral neck and regular physical activity at the Ward's triangle. Calcium, fruit and vegetables, coffee and tea intake were not related to bone mass change.In conclusion, HRT seems to have the greater protective effect on the rate of bone loss . These data suggest that body composition and environnemental factors exert only little effect on the rapid bone loss in perimenopausal and early postmenopausal women.

SU019

Effects of Menopause on Femoral and Vertebral Bone Loss: A 5-year Prospective Population Based Cohort Study. J. Sirola,*[1] R. Honkanen,*[1] H. Kröger,[2] M. Tuppurainen,*[3] J. S. Jurvelin,*[4] S. Saarikoski.*[3] [1]Research Institute of Public Health, University of Kuopio, KUOPIO, Finland, [2]Dpt. of Surgery, Kuopio University Hospital, KUOPIO, Finland, [3]Dpt. of Obstetrics and Gynaecology, Kuopio University Hospital, KUOPIO, Finland, [4]Dpt. of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, KUOPIO, Finland.

This longitudinal study investigated the effects of natural menopause on vertebral and femoral bone mineral density (BMD) . The study population, 409 women aged 47-56 years, was selected from a random sample (n=2025) of the Osteoporosis Risk Factor and Prevention study population (n=14 220) Kuopio, Finland. Spinal (L) and three areas of femoral (neck (N), Ward's triangle (W) and trochanter (T)) BMDs were measured with dual X-ray absorptiometry at baseline (BL) in 1989 and at five year in 1994. Characteristics were obtained with postal inquiries. Pre- and postmenopause definitions were based on amennorhea of under or over 12 months, respectively. Of the 409 women 13 were premenopausal at both measurements, 116 perimenopausal (premenopausal at BL, postmenopausal at 5-year), 172 early postmenopausal (under 5 years postmenopausal at BL) and 108 late postmenopausal (over 5 years postmenopausal at BL). Table presents the mean annual BMD loss (%) (95% CI) according to menopausal status. The greatest annual bone loss was seen in all four areas in perimenopausal women. The annual bone loss was decreased in early and late postmenopausal women being close at the premenopausals' level. These differences were found significant between the pre- and perimenopausal as well as peri- and early postmenopausal in all areas and between early post- and late postmenopausal in lumbar spine.In linear regression model a significant positive correlation was seen in all areas between the annual BMD loss and the duration of menopause (p<0.001 (L), p=0.008 (N), p=0.006 (W), p=0.007 (T)). In conclusion after an acceleration phase of bone loss during menopausal years the bone loss rate is gradually decreased. Thus, the preventive hormonal therapy should be initiated as early as possible at the beginning of the menopause.

	L	N	W	T
Pre	0.30(-0.19, 0.79)	-0.30(-0.77, 0.18)	0.04(-0.70, 0.78)	0.76(0.15, 1.37)
Peri	-1.22(-1.38,-1.05)	-0.87(-1.03,-0.71)	-1.14(-1.39,-0.89)	-0.36(-0.56,-0.15)
Early post	-0.50(-0.64,-0.37)	-0.55(-0.68,-0.42)	-0.40(-0.60,-0.20)	0.22(0.05, 0.39)
Late post	-0.13(-0.30, 0.04)	-0.58(-0.75,-0.24)	-0.49(-0.75,-0.24)	0.16(-0.05, 0.37)

SU020

Activation of PPARγ2 by Rosiglitazone Causes Bone Loss Associated with Increased Marrow Adiposity and Decreased Osteoblast Number in Mice. R. L. Jilka, B. Lecka-Czernik, A. A. Ali, C. E. O'Brien,* R. S. Weinstein, S. C. Manolagas. Div. Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. of Arkansas for Med. Sci., Little Rock, AR, USA.

The osteoblast deficit that characterizes age-related bone loss is associated with increased marrow adiposity. Osteoblasts and marrow adipocytes are derived from a common mesenchymal progenitor, and lineage selection is governed in part by specific transcription factors - Cbfa1 for osteoblasts and PPARγ2 for adipocytes. Previous studies have shown that the PPARγ2 ligand rosiglitazone irreversibly blocks osteoblast differentiation while promoting adipocyte differentiation of a murine marrow-derived osteoblastic cell line expressing PPARγ2. Addition of rosiglitazone to murine bone marrow cultures also inhibited osteoblast differentiation, and promoted adipocyte differentiation, within the fibroblastic colonies that develop from their common mesenchymal progenitor, CFU-F. These findings suggest that age-related bone loss may be due to increased synthesis and/or activation of PPARγ2 in these early progenitors and/or their uncommitted progeny. Therefore, we examined the effect of rosiglitazone on the skeleton of adult (5 month old) Swiss-Webster mice. The animals were pair fed with normal rodent chow, or chow containing rosiglitazone, such that those receiving the drug ingested 25 µg per gram body weight per day - a dose previously shown to stimulate formation of peripheral adipose tissue in rats. None of the animals exhibited changes in food intake or weight during the 28-day course of the experiment. As expected, mice receiving rosiglitazone exhibited a 3-fold increase in the weight of interscapular brown fat. More important, the ligand caused a 7.3 ± 3.8% reduction in BMD. Histomorphometric studies revealed a reduction in cancellous bone area that was associated with a decrease in bone formation rate and osteoblast number; and an increase in the number, but not diameter, of marrow adipocytes. Consistent with the latter, there was a significant increase in the expression of mRNA for aP2, an adipocyte-specific protein, in tibiae of rosiglitazone-treated mice. However, rosiglitazone had no effect on the number of CFU-F, as detected in ex-vivo marrow cultures. Thus, activation of PPARγ2 reduces cancellous osteoblast number by acting on the differentiation rather than the replication of uncommitted progenitors of osteoblasts and adipocytes. These findings

support the contention that the decline in osteoblast number, and the bone loss, that occurs with aging may be due in part to activation of PPARγ2, resulting in the development of adipocytes at the expense of osteoblasts.

SU021

The Role of Reproductive Factors in Bone Mineral Density: Clues from Medieval Female Skeletons. S. C. Agarwal,[1] M. D. Grynpas.[2] [1]Department of Anthropology and Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, University of Toronto, Toronto, ON, Canada, [2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada.

It is generally agreed that pregnancy and lactation are high bone turnover states. However, the effects of parity and lactation on the maternal skeleton, and their contribution to bone fragility in post-reproductive years, is not fully understood. From an evolutionary perspective, it seems maladaptive that the female skeleton be incapable of efficient bone maintenance under the normal conditions of pregnancy and lactation. Human females do share similar reproductive patterns with our genetically closest non-human primate relatives. For example, life cycles of females from non-industrialized societies are typically characterized by late menarche, frequent pregnancies, prolonged lactation, and early menopause. However, reproductive patterns have changed substantially within the last century, causing a dramatic shift in the hormonal milieu of modern western females. The study of female skeletons in historical past populations gives us a unique opportunity to examine the skeletal effects of reproductive patterns that are more consistent with those found throughout the majority of female evolution. A study was made of bone mineral density (BMD) in British medieval skeletons excavated from the historic village, Wharram Percy, located in North Yorkshire, England. The burials represent primarily ordinary peasants with reproductive practices that would have included high parity and prolonged periods of lactation. A total of 55 individuals (m=24, f=31) were examined that were aged with established age determination techniques and divided in three age categories (18-29yrs, 30-49yrs, 50+yrs). 5mm thick coronal sections were removed from the fourth lumbar vertebrae and scanned in a specialized DEXA scanner (PIXImus, GE Lunar Corp.) where BMD could be measured specifically for the trabecular region of the section. Significant differences in female BMD were found between the youngest and two later age groups (ANOVA, $p<0.05$). Medieval females specifically showed significant decrease in bone mineral density at an early age, and no significant change between middle and old age. Further, the female skeletons showed few fragility fractures. Although the males demonstrated more typical age-related patterns of bone loss, the patterns of bone loss in the females contrast with those seen modern populations. We hypothesize that the patterns of bone loss found in females from archaeological populations may reflect the many changes that have occurred in reproductive behavior.

SU022

Changes of the Femur Trochanter in Women With Aging. H. Barden,[1] R. Mazess.[2] [1]GE Medical Systems - Lunar, Madison, WI, USA, [2]Department of Medical Physics, University of Wisconsin, Madison, WI, USA.

We have previously reported reference data on about 11,000 to 12,000 adult white women for spine and femur [1]. Those results showed femur neck area was constant from young adulthood through old age, but that trochanteric area increased by 20% from youth to age 80. As a consequence, the area of the "total" femur site increased about 5%. Trochanteric BMC actually increased from age 20 to age 55 (by 15%), and by age 80 it had lost this "premenopausal" gain and was identical to the BMC at age 20. The slow diminution of BMD at the trochanter from young adulthood to old age (only -15%) masks large and distinctive changes in bone area and BMC. We examined the interrelationship of trochanteric BMC and area in a large US subsample (n=1854) of the above database. Conclusion: Peak BMC at the trochanter is not reached until age 55, and peak area until age 80. The increase of trochanteric bone area and BMC may offer a biological protection against impact forces from falls. Patients with hip fracture have a smaller trochanteric area and BMC.

		Neck			Trochanter		
Age	n	BMD (g/cm^2)	BMC (g)	Area (cm^2)	BMD (g/cm^2)	BMC (g)	Area (cm^2)
20-29	81	0.983	4.69	4.79	0.787	8.57	10.76
30-39	118	0.970	4.59	4.74	0.780	9.27	11.77
40-49	286	0.936	4.37	4.67	0.774	9.60	12.33
50-59	443	0.880	4.12	4.67	0.749	9.87	13.10
60-69	454	0.814	3.80	4.67	0.712	9.70	13.53
70-79	374	0.744	3.48	4.66	0.658	9.24	13.88
80-89	98	0.675	3.15	4.65	0.597	8.47	13.88

REFERENCE Mazess RB, Barden H. (1999) Bone density of the spine and femur in adult white females. *Calcif Tissue Int* 65:91-99.

Disclosures: GE Medical Systems Lunar,3.

SU023

Differences in the Ratio of Trochanteric BMD to Femoral Neck BMD in Women Across the Lifespan. K. B. Gunter,*[1] J. M. Shaw,[2] C. M. Snow.[1] [1]Exercise and Sport Science, Oregon State University, Corvallis, OR, USA, [2]Exercise and Sport Science, University of Utah, Salt Lake, UT, USA.

Approximately 80-90% of all hip fractures occur at either the femoral neck or the trochanteric regions of the hip. Trochanteric fractures are associated with greater blood loss and higher mortality rates than fractures at the femoral neck and it has been reported that lower trochanteric BMD or a higher femoral neck BMD is associated with an increased risk of trochanteric hip fractures in the elderly. This indicates that a higher ratio of trochanteric to femoral neck BMD may provide protection against fractures. Our aim was to examine BMD changes at the femoral neck and trochanteric regions of the hip with age. We examined the ratio of trochanteric BMD to femoral neck BMD in 308 women ranging in age from 19-91 y. Participants were categorized by age into 5 groups (Group 1= 18-28; Group 2= 29-45; Group 3= 46-60; Group 4= 61-70; Group 5= 71 and older). Bone mineral density of the proximal femur was assessed by DXA and reported as mean +/- standard error (Table 1). We found the ratio of trochanteric to femoral neck BMD (TR/FN BMD) increased with each age category with significant differences between the oldest 2 age groups and the youngest 3 age groups ($p<.03$) (Table 1). In multiple regression analyses age was a significant predictor of TR/FN BMD ($r=.42$; adjusted $R2=.17$). In the older age categories estrogen use resulted in a significant differences in the ratio among 46-60 year olds. In this age category, the TR/FN ratio was .895 in estrogen users compared to .847 in non-estrogen users ($p=.009$). There were no differences in the TR/FN ratio between estrogen and non-estrogen users in the oldest age categories (4 and 5).

Table 1

Group	FN*	TR*	Ratio*
1 (18-28)	.926 (.020)	.734 (.017)	.796 (.715)
2 (29-45)	.849 (.011)	.717 (.010)	.848 (.008)
3 (46-60)	.769 (.013)	.662 (.011)	.874 (.010)
4 (61-70)	.691 (.015)	.612 (.013)	.891 (.012)
5 (71+)	.635 (.013)	.582 (.011)	.918 (.010)

Our results demonstrate a discordant loss of bone density at the femoral neck and trochanteric regions of the hip with age. The implications of these findings warrant further investigation.

SU024

Differential Response to BMPs in Immortalized Tendon-Derived Cell Lines Which Express Scleraxis/Six1 Genes and Form Hard Connective Tissues in Ovo. R. Salingcarnboriboon,[1] Y. Maeda,[1] H. Yoshitake,*[1] M. Takamoto,[1] K. Tsuji,[1] A. Nifuji,[1] V. Rosen,[2] M. Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Genetics Institute, Cambridge, MA, USA.

The development of musculoskeletal system requires coupled development of distinct types of tissues, including muscle, tendon, cartilage, and bone. Until now, molecular biology aspects of muscle, cartilage and bone have been extensively studied. However, both cell and molecular biology of tendon has not been well understood. The purpose of this study is to construct and characterize the tendon cell lines to be utilized as a model for further studies on tendon biology and also to understand the mechanism that differentiates these cells from the cells in osteoblastic and chondrocytic lineages. Three lines of tendon-derived cells (E4, G11, and D6) were cloned from the Achilles tendon of the transgenic mice harboring SV40 large T antigen gene according to limiting dilution technique. The cells were further cultured in alpha-MEM supplemented with 0.5% FBS, at 33°C, under 5% CO2 atmosphere for over one year. The proliferation of these 3 lines was significantly increased by the treatment with bFGF ($p<0.01$). Proliferation of E4 and G11 cells was also enhanced by TGF-beta ($p<0.01$) whereas BMP2 did not show any significant effect on proliferation. D6 cells grew at the slowest rate in 0.1% serum but it grew faster than the other two cell lines at 10% serum. Scleraxis and Six1, markers of tendon cells, were expressed in these cell lines. Northern blot analysis indicated the expression of osteopontin and type I collagen mRNAs, but not osteocalcin and alkaline phosphatase mRNAs, markers for osteogenic cells. However, interestingly, these cells did express all these genes when examined by a more sensitive technique such as RT-PCR. Alkaline phosphatase levels were enhanced by 100-300 % in all the tendon cell lines after the treatment with BMP2. D6 cells were characterized further to examine their behavior both in vitro and in vivo. Unlike BMP2, BMP12 and BMP13 did not show any significant effect on proliferation and alkaline phosphatase expression in D6 cells. Interestingly, dexamethasone suppressed the growth of D6 cells (about 40%) while it still significantly induced alkaline phosphatase expression ($p<0.05$). Formation of hard connective tissue mass was observed when D6 cell pellets were cultured on chorioallantoic membrane (CAM) in ovo. These observations indicated that D6 cells could serve as a tendon cell line and they possess capabilities to differentiate into osteoblasts/chondrocytes suggesting that these cells contain stem cell like features as common progenitors.

SU025

Regulation of BMP Expression by Thyroid Hormone in Differentiating Chondrocytes. M. C. Stewart, Y. Wang. Orthopaedics, Case Western Reserve University, Cleveland, OH, USA.

The induction of hypertrophic differentiation in chondrocytes by thyroid hormone is believed to be mediated through autocrine expression of BMPs. This study was conducted to identify the specific BMP family members that are upregulated in differentiating mammalian chondrocytes, in response to thyroid hormone.Chondrocytes were isolated from the proximal humeral epiphyses of neonatal rats and cultured under non-adherent conditions in defined, serum-free medium in the presence or absence of 100 ng thyroid hormone (T3)/ml of culture medium. Total RNA was isolated from samples on days 1 through 7. Changes in gene expression were monitored by semi-quantitative RT-PCR. Induction of the hypertrophic phenotype was monitored by expression of collagen type X (ColX) and ALP. Specific primers were developed to assess expression of BMPs 2, 3, 4, 5, 6 and 7. Expression was standardized to that of beta actin and GAPDH.Collagen type X expression was not detected in Control samples at any stage but was evident by Day 4 in T3-treated samples. ALP expression was detected in all samples, but expression was increased threefold in T3-treated samples after Day 5. In Control cultures, BMP-2 expression declined over time. However, T3 treatment maintained BMP-2 expression throughout the experiment. BMP-2 expression was 2-3 fold higher in T3-treated samples from Days 4-7. BMP-6 expression was stable in Control cultures but was increased approximately twofold in T3-treated samples from Days 4-7. This increase corresponded to, but did not preceed, the induction of hypertrophic-specific markers. BMP-7 expression declined throughout the course of the experiment and was not affected by T3 treatment. BMP-4 expression was not infuenced by T3. BMP-4 expression showed a transient increase from days 1-3, then dropped through the second half of the experiment in both Control and T3-treated samples. Expression of BMPs 3 and 5 were not affected by T3 treatment, nor did the levels of expression alter during the course of the experiment.These findings indicate that BMPs -2 and -6 are specifically responsive to thyroid hormone in differentiating chondrocytes. Direct treatment of chondrocytes with rhBMP-2 (50 ng/ml) resulted in strong induction of ColX and ALP expression. In contrast, there was minimal induction of hypertrophic markers in response to BMP-6. These data suggest that BMP-2 is a primary autocrine mediator of hypertrophic differentiation in mammalian chondrocytes, in response to thyroid hormone.

SU026

Dysregulation of BMP Receptor IA in Fibrodysplasia Ossificans Progressiva. L. Serrano de la Peña,* P. C. Billings,* J. Ahn,* F. S. Kaplan, E. M. Shore. Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, USA.

Fibrodysplasia ossificans progressiva (FOP) is an autosomal dominant disorder of heterotopic ossification. The gene responsible for FOP has not yet been identified. The first molecular insights into the disorder arose from observations of increased BMP4 mRNA and protein expression in lymphoblastoid cell lines (LCLs) and pre-osseous fibroproliferative lesional cells obtained from FOP patients. These findings suggested that altered BMP4 regulation and/or signal transduction pathways in affected individuals could induce ectopic bone formation. In this study, we examined specific components of the BMP signal transduction pathway. Using Fluorescence Activated Cell Sorting (FACS) analysis, we observed that levels of BMP receptor type IA (BMPRIA) are 10 fold higher on the surface of FOP LCLs compared with LCLs from unaffected individuals. This surprising observation of overexpression of both ligand and receptor, has led us to question the integrity of the BMP4 signaling pathway in FOP cells. The activation of BMPRIA by phosphorylation in BMP4-treated and untreated cells was examined by BMPRIA immunoprecipitation followed by immmunodetection of phosphothreonine. In the absence of BMP4 stimulation, BMPRIA is endogenously phosphorylated at a relatively high level in FOP cells, in contrast to normal cells which show little phosphorylation. Following BMP4 stimulation, the level of BMPRIA phosphorylation does not change in FOP cells, but increases in normal cells. We are currently examining whether the apparent chronic activation of the BMP4 pathway in FOP cells results from endogenous over-production of BMP4 protein, or from defects in a BMP receptor(s) and/or elements of the downstream signaling pathway. Our findings demonstrating overexpression and basal hyperphosphorylation of BMPR1A, together with insensitivity to exogenous BMP4 protein, provide further evidence of alterations in the BMP4 pathway in the pathogenesis of FOP.

SU027

BMP-7 Induces Both Chondrogenesis and Osteogenesis in the C3H10T1/2 Mesenchymal Stem Cell Line. C. M. Shea,*[1] G. L. Barnes,[2] T. A. Einhorn,[2] L. C. Gerstenfeld.[2] [1]Department of Periodontology, Harvard School of Dental Medicine, Boston, MA, USA, [2]Department of Orthopaedic Surgery, Boston University Medical Center, Boston, MA, USA.

Bone morphogenetic proteins (BMPs) have been shown to be instrumental in promoting cellular differentiation of mesenchymal stem cells into skeletal lineages. In order to elucidate the temporal pattern of differentiation from uncommitted mesenchymal to mature skeletal cell, C3H10T1/2 mesenchymal stem cells were treated with BMP-7 and examined for transcription factor and matrix gene expression and transcription factor DNA-binding by RNase Protection Assays (RPAs) or Electromobility Shift Assays (EMSAs) respectively. C3H10T1/2 cells were treated with rmBMP-7 at 0, 80, or 250 ng/ml in either the nutrient-rich growth media BGJb or DMEM. Additional experiments independently analyzed the effect of ascorbic acid supplementation. The results of these studies indicate that the mesenchymal stem cell line C3H10T1/2 can be induced to undergo both chondrogenesis and osteogenesis when treated with BMP-7. Furthermore, this induction is dose-dependent and occurs in a temporal pattern with chondrogenic differentiation preceding osteogenesis as determined by the expression of matrix-related genes and skeletal cell-associated transcription factors. These studies also demonstrate a default to the adipogenic phenotype upon removal of BMP-7 from the culture media demonstrating a role for BMPs in the maintenance of the skeletal cellular phenotype. Finally, our results demonstrate that the nutrient-rich media BGJb enhances both chondrogenic and osteogenic gene expression in BMP-7 treated cells by approximately two-fold and suggest that additional environmental factors may enhance BMP-7 induced chondrogenic and osteogenic differentiation.

Disclosures: Stryker Biologicals,2.

SU028

Prostate Cancer Cells Induce Osteoblastogenesis Through Bone Morphogenetic Proteins. J. Dai,*[1] J. Zhang,[2] D. Lin,*[3] E. Keller.[3] [1]Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor,, MI, USA, [2]Department of Pathology, University of Michigan, Ann Arbor, MI, USA, [3]Unit for Laboratory Animal Medicine and Department of Pathology, University of Michigan, Ann Arbor, MI, USA.

Human prostate cancer (CaP) frequently metastasizes to bone resulting in osteoblastic metastases at the site of metastasis. The exact factors mediating the osteoblastic response remain unknown. Bone morphogenetic proteins (BMPs), the members of the transforming growth factor-ß (TGF- ß) superfamily, play an important role in regulating the osteoblast differentiation and function. Accordingly, the present study was designed to explore if BMPs contribute to formation of osteoblastic metastases. To determine if prostate cancer tissue expressed BMPs, we performed immunohistology. BMPs-2, -4, -6, and -7 were expressed in prostate cancer tissue. To define if prostate cancer cells express functional BMPs, we evaluated if a bone metastatic LNCaP-derivative prostate cancer cell line, C4-2B, produces osteoblastic factors. C4-2B cells were grown for 48 h both the C4-2B cells and their conditioned media (CM) were collected. Western analysis revealed that C4-2B cells expressed BMP-2, -4, -6 and -7. To determine the production of functional osteoblastic activity by C4-2B cells, MC3T3-E1 cells, murine osteoprecursor cell line, were incubated in C4-2B CM or control media. CM had no effect on cell proliferation, but increased alkaline phosphatase (ALPase) activity and mineralized nodule formation (von Kossa positive). To determine if these effects were mediated through BMPs, noggin, a factor known to block BMPs, was added to the cultures in addition to the C4-2B CM. The presence of noggin inhibited the ALPase activity and mineralized nodule formation. Finally, a previous study had suggested that BMP-7 is regulated by androgens. Thus, to determine if androgens regulate BMP production in prostate cancer, we tested the ability of dihydrotestosterone (DHT) to regulate BMP-7 mRNA levels. In the androgen-dependent LNCaP cells line, DHT reduced BMP-7 mRNA expression. However, in the androgen-independent C4-2B cell line, DHT had no effect on BMP-7 expression. These data suggest that C4-2B BMP-7 expression is dysregulated in C4-2B cells and the increased production of BMPs, leads to osteoblastic activity. These findings suggest a possible mechanism through which prostate cancer cells produce osteoblastic lesions.

SU029

Impact of rhBMP-2 on Prognostic Risk Factors for Delayed Fracture Healing and Related Secondary Interventions in Open Tibial Shaft Fractures. M. F. Swiontkowski,*[1] P. Peeters,*[2] P. Bacquet,*[2] R. Mallick,*[3] C. Csimma,*[4] A. Valentin-Opran,*[4] S. Govender.*[5] [1]Department of Orthopaedic Surgery, MMC # 492, University of Minnesota, Minneapolis, MN, USA, [2]Quintiles, Levallois Perret Cedex, France, [3]Wyeth-Ayerst Research, Radnor, PA, USA, [4]Genetics Institute, Cambridge, MA, USA, [5]Department of Orthopaedics, University of Natal, Durban, South Africa.

Open tibial fractures are associated with delayed fracture healing (DH) and a correspondingly high rate of secondary interventions (SI) to accelerate healing. We evaluated the relative impact of risk factors for DH and associated SI, and assessed potential risk reduction with recombinant human bone morphogenetic protein-2 (rhBMP-2; dibotermin alfa).Data were analyzed for 450 open tibial fracture patients who were randomly assigned to receive rhBMP-2 (1.50 mg/mL or 0.75 mg/mL) plus standard of care (SOC; reamed or unreamed intramedullary [IM] nail fixation), or SOC alone. Multivariate logit models were estimated to evaluate relative importance of risk factors and generate corresponding predicted risks for the 6-month rate of clinically determined DH and the 12-month rate of SI (invasive and non-invasive).Predicted 6-month DH risks and 12-month SI risks varied considerably between risk factor combinations, but were consistently lower in the 1.50 mg/mL rhBMP-2 group than in the SOC group (Table I). Independent of risk factors, the 1.50 mg/mL rhBMP-2 group, compared with the SOC group, was more likely (adjusted odds ratio [OR] = 1.83; P = .005) to attain 6-month fracture healing and less likely (OR = 0.60; P = .048) to require SI.

Predicted Risk By Treatment Group

Risk Factors	SOC	rhBMP-2 (1.50 mg/mL)
	DH (predicted risk)	
Gustilo-Anderson (G-A) IIIA/B, unreamed IM nails	69%	55%
G-A I/II, reamed IM nails	33%	21%
	SI (predicted risk)	
G-A IIIA/B, unreamed IM nails, multiple fractures	51%	39%

G-A I/II, reamed IM nails, single fractures	11%	7%

Gustilo-Anderson IIIA/B fracture grade and unreamed IM nail fixation were significant risk factors for 6-month DH. Multiple fractures were an additional risk factor for SI. rhBMP-2 treatment was associated with reduced risk across all groups.Prognostic modeling to identify risk factors for DH and associated SI may facilitate optimal risk management in fractures through new treatments such as rhBMP-2

Disclosures: Pascale Peeters, MD,5; Patrice Bacquet, PhD,5; Rajiv Mallick, MD,1,3.

SU030

Transcriptional Regulation Of The Cloned Human Cbfa1 Gene Promoter By Bone Morphogenetic Protein-7. L. Tou,* N. Quibria,* J. M. Alexander. BIDMC/Harvard Medical School, Boston, MA, USA.

It is well established that core binding factor cbfa1 is required for osteoblast recruitment and differentiation from mesenchymal stem cells. However, little is known regarding the effects of osteogenic factors such as bone morphogenetic proteins (BMPs) on transcriptional regulation of the Cbfa1 gene. BMP-7 is a member of the transforming growth factor beta (TGF-b) superfamily and induces osteoblast differentiation from mesenchymal precursor stem cells in vitro and bone formation in vivo. This study examines the effects of BMP-7 on markers of osteoblast differentiation and specifically on human cbfa1 gene transcription. Recombinant human BMP-7 induced both alkaline phosphatase (ALP) and cbfa1 mRNA and protein biosynthesis in a mouse C2C12 myoblast cell line. To further understand the mechanisms of human cbfa1transcriptional regulation by BMP-7, we cloned 3.0 kb of the human cbfa1 gene 5'-upstream flanking region and created a promoter deletion series of cbfa1 in luciferase-based reporter vectors (cbfa1/Luc). Sequence data revealed 6 copies of the osteoblastic cis-acting element (OSE2) in the proximal promoter region. In C2C12 cells transiently transfected with a 1.2 kb cbfa1/Luc DNA construct, transcriptional activity of cbfa1 was upregulated by 2-fold after 24 hours of BMP-7 treatment. Electromobility shift assays with C2C12 cellular extracts indicate that BMP-7 increases binding of OSE2 promoter sequences. Supershift assays with anti-cbfa1 antibodies demonstrate that cbfa1 is part of the nucleoprotein complex binding OSE2. Co-transfection studies with both a cbfa1 cDNA expression vector and cbfa1/Luc decreased basal transcription level of cbfa1/Luc, while BMP-7 reversed cbfa1-induced repression in C2C12 cells. These data suggest that cbfa1 can attenuate its own transcription in a classic negative feedback manner, and this repression is reversible by BMP-7. Together, transfection and gel shift studies indicate that BMP-7 can upregulate cbfa1 gene expression in C2C12 myoblast cells, and that cbfa1 may bind to OSE2 elements within its own promoter to suppress cbfa1 gene transcription in a classic negative feedback loop fashion.

SU031

Development of a Standardized System for Assessing the Osteogenic Activity of BMP Gene Transduced Mouse Embryonic Fibroblast. S. Yang,* D. Wang,* D. Wei,* R. Franceschi. University of Michigan, Ann Arbor, MI, USA.

Gene therapy represents a new alternative for regenerating bone. Previous studies showed that adenoviruses expressing BMPs can induce ectopic bone formation at subcutaneous and intramuscular sites and repair bony defects. An osteogenic response was observed regardless of whether virus was directly implanted in experimental animals or used to transduce dermal fibroblasts before implantation (Franceschi et al. J Cell Biochem 78:476, 2000; Krebsbach et al., Human Gene Therapy 11:1201, 2000). However, lack of a standardized system for comparing the osteogenic activity of different virus constructs prevents clear comparisons between various BMPs and other osteogenic factors. To provide such a system, we used an inbred mouse strain (C57BL6 mice) and a clonal (syngeneic) embryoic fibroblast cell line derived from this strain (BLK cells). Cells were transduced with control (lacZ) adenovirus or adenovirus expressing BMPs 2, 4, or 7 as well as constitutively active BMP receptors 1A and 1B. In this study, we investigated the effect of different BLK cell numbers (105,106,2x106, 5x106, 107), different adenoviral titres (500, 1000, 1500, 2000, 2500pfu/cell) and different times (2, 3, 4, 6, 8 weeks) on bone formation with Ad-BMP7. After transduction, cells were suspended in a collagen hydrogel carrier (type I rat tail, 3mg/ml) and implanted into subcutaneous sites of mice. Histologically distinguishable bone begins to form at the edge of the collagen implant as early as 2 weeks following cell implantation. After 3 weeks, mature bone marrow elements were detected. The optimal time for forming bone was 3 to 4 weeks based on assay of implant alkaline phosphatase, calcium and phosphate. Increasing transduced cell number also increased bone formation. Using a saturating adenovirus titer (1000 pfu/cell), the weight of bone in 107cells groups was 27.3, 20.5, 6.3, 3.5 times of those in 105, 106, 2x106, 5x106 cells groups. When cell number was held constant, an adenovirus titer of 500 pfu/cell was best for bone formation with higher titer being toxic to cells. To follow the fate of virus-transduced cells in vivo, we generated BLK cell stable clones carrying a lacZ reporter gene. These cells are being used to assess the degree to which implanted cells contribute to the new bone formed which is known to be composed of both implanted and host cells. These studies validate use of the BLK/C57BL6 system for quantitatively evaluating the osteogenic activiy of various adenovirus constructs.

SU032

Discovery of an Effective Natural Product Carrier of Demineralized Bone Matrix. A. Fujiwara,*[1] M. P. Tippen,*[2] A. J. Marchosky,*[3] W. J. Maloney,*[1] J. Feder,*[2] K. A. Hruska,[4] H. D. Adkisson.[2] [1]Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO, USA, [2]Isto Technologies, Inc., St. Louis, MO, USA, [3]Neurosurgery, St. Lukes' Hospital, St. Louis, MO, USA, [4]Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA.

Introduction: Failure of some commercial demineralized bone matrix (DBM) products to repair large bone defects has raised concerns regarding variable efficacy and possible allograft migration. This study compared the ability of DBM formulated with either high molecular weight hyaluronic acid (HMWHA) or polymerized alginate to heal 5mm femoral gap defects in rats. Methods: Fourty 5mm defects were created in twenty female athymic nude rats aged 12-16 wks. Five treatment groups were randomly assigned: Group 1 (negative control), untreated defects; Group 2 (positive control), fresh human cancellous bone chips; Group 3, DBM alone (AlloSource, Englewood, CO); Group 4, DBM in 14 mg/ml HMWHA (Healon GV); and Group 5, DBM in 3% alginate in saline. Alginate grafts were polymerized in situ with calcium. All DBM was from a single donor and delivered in carrier at the same dose. Animals were euthanized at 12 wks for radiographic and histomorphometric analysis of bone healing. A three-point scale was adopted for radiographic scoring (0 = no bone formation; 1 = partial bone formation; 2 = complete union). Percent new bone formation was expressed as percent of total bone (DBM + new bone) observed in the defect. Data were treated statistically for determination of variance between groups.Results: Bilateral surgery was well tolerated; rats demonstrated increased weight gain over the 12-week evaluation period. Fourteen (2 in Group 2, 6 in Group 3, and 6 in Group 4) of 40 defects demonstrated complete radiographic healing. Histomorphometric analysis showed 48.3 ± 8.5 % new bone in Group 4 defects vs. 37.5 ± 12.1 in Group 3. Alginate/DBM produced only 1.6 ± 2.2 % new bone, with little evidence of osteogenic/chondrogenic differentiation. Histologic evidence for chondrogenic differentiation to bone was greater in Group 4 vs. Group 3 treated defects. Histomorphometric analysis of new bone formation showed statistical significance between Group 1 vs. 5 ($p<0.05$), Group 2 vs. Groups 1 and 5 ($p<0.001$), and Group 4 vs. Group 2 ($p<0.05$). No significant difference in % new bone formation was observed between either Group 4 vs. Group 3 or Group 3 vs. Group 2. Discussion and Conclusions: HMWHA displayed good handling characteristics and supported osteoinductive growth when combined with DBM. Although alginate has been used successfully to culture osteoblasts and chondrocytes, in vivo use for bone grafting is contraindicated because it limits migration of osteogenic precursor cells.

Disclosures: Isto Technologies, Inc.,1,3.

SU033

SOST, a BMP Antagonist Expressed in Mature Osteoblasts, Regulates Bone Formation. M. S. Kung Sutherland, J. Geoghegan,* D. G. Winkler,* T. Hayes,* R. J. Ursino,* K. Staehling-Hampton,* L. Zhao,* J. A. Latham. Celltech R&D, Inc., Bothell, WA, USA.

We previously reported that null mutations in the SOST gene are associated with the sclerosteosis phenotype typified by excess deposition of bone (Amer J Hum Genet 2001, 68: 577-589). To further characterize the SOST gene product, Sclerostin, human mesenchymal cells were used to evaluate Sclerostin expression and to determine its biological activity. Human mesenchymal cells were cultured to induce the formation of osteoblastic or chondrocytic cells. Sclerostin was expressed in committed and mature osteoblasts but not in chondrocytes. We evaluated the biological function of Sclerostin by measuring alkaline phosphatase activity, synthesis of collagen type I and mineralization after the addition of partially-purified preparations of baculoviral-expressed Sclerostin protein. Sclerostin significantly decreased the activities and levels of these osteoblastic markers. In contrast, alkaline phosphatase activity, synthesis of collagen type I and mineralization were increased upon treatment of osteoblastic cells with anti-Sclerostin monoclonal antibodies. In a rodent culture model, the mouse mesenchymal C3H10T1/2 cells, Sclerostin displayed a dose-dependent decrease in BMP-6-induced alkaline phosphatase activity. These findings support the premise that Sclerostin plays an important role in controlling osteoblast function.

SU034

Sclerostin, the Protein Product of the Sclerosteosis Gene (SOST) and a Key Regulator of Bone Matrix Formation, Binds to BMPs and Antagonizes their Function. D. G. Winkler, T. Hayes,* J. Geoghegan,* J. Skonier,* R. Ursino,* M. Kung Sutherland, J. Latham.* Gene Function and Target Validation, Celltech R & D, Inc., Bothell, WA, USA.

Null mutations in the SOST gene in humans results in Sclerosteosis. This disease causes an increased bone deposition throughout life. We have expressed and partially purified Sclerostin from SF9 cells. Sequence analysis of Sclerostin has established homology to the DAN family of BMP antagonists. We tested the ability of Sclerostin to bind to BMPs directly, and found that the protein binds to a subset of BMPs with high affinity. This interaction antagonizes BMP induced akaline phosphatase in C3H10T1/2 cells. We hypothesize that blocking the Sclerostin /BMP interaction may increase bone deposition and could lead to anabolic treatments for osteoporosis.

Disclosures: Celltech R&D,3.

SU035

Delivering BMPs by Thermoreversible Biomaterials for Increased Local Retention. H. Uludag,* T. Gao,* N. Kousinioris.* University of Alberta, Edmonton, AB, Canada.

This study was conducted to investigate the feasibility of enhancing local retention of BMPs by thermoreversible biomaterials. The latter exhibit temperature dependent solubility, where the biomaterials are water-soluble at a low temperature but become insoluble at

the physiological temperature. In that way, the biomaterials can be formulated with BMPs as a liquid at a low temperature (4 oC), but become insoluble and retain the protein in body. An enhanced retention of BMPs is expected to result in an enhanced osteoinductive activity. To investigate the feasibility of this approach, we prepared several thermoreversible biomaterials with predictable changes in their properties. Among the factors investigated were: presence/absence of protein reactive N-acryloxysuccinimide (NASI) group in the biomaterials, the lower critical solution temperature (LCST: solubility transition temperature) and molecular weight (MW) of the biomaterials. All biomaterials were based on N-isopropyacrylamide (NiPAM) and were synthesized by a free-radical polymerization process. The LCST of the biomaterials were controlled by incorporating alkylmethacrylates into the biomaterials. The MW was controlled by the choice of the polymerization conditions. The biomaterials were characterized as described elsewhere [Uludag et al., (2001) Biotech. Bioeng., in press.]. To determine the BMP retention, BMP-2 was 125I-labeled and either implanted (with a collagen sponge) or injected intramuscularly into Sprague-Dawley rats. Rats were periodically sacrificed and the radioactive count at the injected site was determined.To explore the LCST effect on BMP-2 retention, biomaterials with high (~25 oC) or low (~15 oC) LCST was used. The rhBMP-2 retention was similar on day 1 (40-50%, irrespective on the biomaterials' LCST and the animal model. By day 7, differences among the biomaterials were not that significant in the implant model, whereas biomaterials with low LCST gave an increased retention in the injection model. Incorporating protein-reactive NASI groups into the biomaterials further improved the retention of BMP-2 (irrespective of LCST). To determine the MW effect, the biomaterials were chosen where the MW was either ~50 kD or 400 kD. Biomaterials with a higher MW gave a higher retention of BMP-2 after 14 days. Taken together, the results indicated that delivering BMP-2 with thermoreversible biomaterials increased the protein retention as much as 200-fold in the chosen animal models. This was achieved by physical entrapment as well as chemical conjugation of the protein within the biomaterials. Studies to correlate the retention results with bone tissue induction are currently underway.

Disclosures: Genetics Institute Inc,2.

SU036

Bone Morphogenetic Protein-2 Enhances Statement of the Inorganic Phosphate (Pi) Transporter Pit-1 and Matrix Mineralization in Osteoblast-like Cells. A. Suzuki,[1] I. Sato,*[1] J. Guicheux,*[2] A. Kakita,*[1] Y. Miura,*[1] Y. Oiso,*[1] J. Bonjour,[3] J. Caverzasio.[3] [1]1st Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan, [2]INSERM 99-03, School of Dental Surgery, Nantes, France, [3]Division of Bone Diseases, Department of Internal Medicine, University Hospital of Geneva, Geneva, Switzerland.

Bone morphogenetic proteins (BMPs) play an important role in the development of bone and cartilage. BMP-2 is produced by osteogenic cells including osteoblasts and stimulates the differentiation of preosteoblasts and the activity of osteogenic cells. Inorganic phosphate (Pi) is an important element for the calcification of the bone matrix. Previous in vitro studies in osteoblast-like cells suggested a possible role of the Pi transport system Pit-1 in initial events of matrix mineralization and recent observations indicate that BMP-2 selectively enhances statement of Pit-1 in MC3T3-E1 cells. The aim of the present study was to investigate the role of Pit-1 in matrix mineralization induced by BMP-2 in MC3T3-E1 cells. BMP-2 time- and dose-dependently stimulated Na-dependent Pi transport in confluent MC3T3-E1 cells. This response was preceded by an increased statement of mRNA encoding Pit-1 after 2 hours BMP-2 exposure. In addition to its effect on Pi transport, BMP-2 dose-dependently enhanced both ALP activity and the formation of mineralized bone nodules in differentiating MC3T3-E1 cells in presence of 5 mM beta-glycerophosphate (13 x) in the culture medium. This latter effect of BMP-2 was also observed in MC3T3-E1 cells cultured in presence of a high phosphate containing (2.5 mM) medium. Phosphonoformic acid (0.1 mM), a selective inhibitor of Pi transport, suppressed BMP-2-induced enhancement of mineralization . In conclusion, the results of this study indicate that associated with the stimulation of Pit-1 statement in osteoblast-like cells, BMP-2 enhances bone matrix mineralization and that this effect is prevented by a selective inhibitor of Pi transport further suggesting a role of Pit-1 in initial events of bone matrix calcification.

SU037

Is Calcification closely Associated with Ossification during Atherogenesis? H. H. T. Hsu,[1] O. Tawfik,*[1] F. Sun.*[2] [1]Pathology, University of Kansas Medical Center, Kansas City, KS, USA, [2]University of Kansas Medical Center, Kansas City, KS, USA.

Several studies demonstrated that specific bone makers contributing to the regulation of calcification are present in atherosclerotic lesions. These findings are consistent with a number of clinical observations that ossification and calcification can occur in atherosclerosis. To determine whether calcification is a result of ossification during high lipid-induced atherosclerosis, a rabbit model was used. Rabbits were fed 0.25% high cholesterol and 1% peanut oil for up to 6 months. As a result of 3 months of dietary interventions, rabbits developed characteristic atherosclerotic lesions without Alizarin red positive calcification. At 6-month period, calcification was predominantly present in the area between intima and media. H & E histochemical staining indicates that neither bone cells nor chondrocytes were present throughout the entire period of the study. The fatty streaks and calcification were more abundant in proximal regions of the aorta exiting from the heart and became progressively less to the distal part of aortas. The structural irregularity of the ascending aorta close to the heart caused by the high lipid diet are more subjected to the impact of blood flow than are the distal part of aortas. This may contribute to high incidence of calcification since calcification is frequently associated with tissue injury. Thus, it is concluded that aortic calcification induced by high cholesterol diet is in part a result of local damage caused by the impact of blood flow to the abnormal aortic structure rather than a mere end result of ossification.

SU038

Environmental Fluoride Exposure, Bone Quality, and Osteoarthritis. D. Chachra,*[1] H. Limeback,*[1] D. Zukor,*[2] M. Schwartz,*[2] A. E. Gross,*[1] C. H. Hutchison,*[1] M. D. Grynpas.[1] [1]Mount Sinai Hospital and University of Toronto, Toronto, Canada, [2]Jewish General Hospital and McGill University, Montreal, Canada.

The purpose of this study was to characterize bone quality with respect to environmental fluoride exposure. Femoral heads were obtained at hip arthroplasty from 92 individuals, residing in regions with fluoridated (n=53) or non-fluoridated (n=39) municipal water. The femoral heads were primarily removed because of osteoarthritis (75) or osteoporosis (9). The fluoride content of a cancellous core from the centre of the femoral head was measured by neutron activation analysis. This sample was tested in compression. Image analysis and backscattered electron imaging (BSE) were performed on samples from the superior (weightbearing) and inferior (nonweightbearing) surfaces of the femoral head. The microhardness was determined at four sites. The fluoride content of the cancellous bone increased logarithmically with age ($R2=0.09$, $p<0.005$). The mechanical properties did not vary with the fluoride content, independently of age. The amount and connectivity of the cancellous bone were not related to the fluoride content. However, more bone was present at the superior site than at the inferior site of osteoarthritic specimens ($p<0.05$); this was not observed in the non-osteoarthritic specimens. Similarly, the amount of bone present at the superior, but not the inferior, surface of the osteoarthritic specimens was greater than that of the non-osteoarthritic specimens. These data indicate increased local bone formation associated with osteoarthritis. The degree of mineralization was lower for the superior surface of osteoarthritic samples than the inferior surface or the superior surface of the non-osteoarthritic specimens ($p<0.05$). However, for the osteoarthritic samples, the degree of mineralization of both the subchondral and the cancellous bone increased with the fluoride content ($R2=0.137$ and $R2=0.145$, respectively; $p<0.05$) at the superior surface. Consistent with this, the microhardness of the subchondral and cancellous bone at this site also increased with the fluoride content ($R2=0.414$, $R2=0.138$, respectively; $p<0.05$). These relationships were not observed at the inferior surface. In conclusion, fluoride incorporation due to environmental fluoride exposure does not have a systemic effect on bone quality. However, incorporated fluoride is positively associated with the degree of mineralization of bone in regions affected by osteoarthritis. This suggests that exposure to environmental fluoride and its subsequent incorporation may influence bone remodeling in the subchondral bone of osteoarthritic patients.

SU039

In Situ Identification of a Ca-Organic Phosphate Complex by 31P Solid State NMR Spectroscopy at Onset of Mineralization. Y. Wu,*[1] J. L. Ackerman,*[2] M. J. Glimcher.[1] [1]Orthopaedic Surgery, Harvard Medical School and Children's Hospital, Boston, MA, USA, [2]Dept of Radiology, Massachusetts General Hospital, Boston, MA, USA.

To investigate proposals that phosphoproteins play a critical role in calcification of bone, we have developed 31P solid state NMR spectroscopy techniques which permit the direct identification of Ca-free phosphoproteins, phosphoproteins complexed with Ca and Ca-P apatite crystals in intact, 8-19 d embryonic chick bone. Ca-free and Ca-complexed phosvitin and beta-casein were used as standards. To distinguish the NMR characteristics of Ca-free phosphoprotein from phosphoproteins complexed with Ca, we introduced a new parameter, δ, the sideband pattern index, which clearly distinguishes Ca-free from the Ca-complexed phosphoproteins. However it was not possible to accomplish this in moderately calcified embryonic bone because the 31P NMR spectra were overwhelmed by the mineral. Similarly, it was not possible to identify the rare organic spin species in the earliest stages of mineralization because the 31P NMR spectra were essentially identical to those of standard Ca-free phosphoproteins. The problems were solved by using a differential cross polarization (DCP) 31P NMR technique and the new index parameter δ. DCP 31P NMR of 8 day chick embryo bone generated a sideband pattern identifying the very major component as phosphoprotein (phosphoryl groups) not complexed with Ca. However, a very small signal was also generated by a Ca-phosphoprotein complex coincident with the detection of a trace of apatitic mineral phase. Additional mineral phase was identified in 10 and 12 day chick embryo bone. Phosphoproteins synthesized and excreted into the extracellular organic matrix in the very early stages of ossification are for most part not complexed with Ca. At the onset of mineralization, very few molecules of Ca-complexed phosphoproteins are detected at a time when a trace of a Ca-P mineral phase of apatite is also observed. These are the first structural data of in situ bone which support the critical role for phosphoproteins in the initiation of calcification.

SU040

Osteoinductive Potential of DBM/collagen Product Is Affected by Product Preparation Rather Than Collagen Matrix Preparation. L. Masinaei,*[1] K. Crouch,*[1] D. Softic,*[2] A. Wilson,*[2] L. Wolfinbarger.[2] [1]Center for Biotechnology, Norfolk, VA, USA, [2]LifeNet, Virginia Beach, VA, USA.

Over the past few years, human collagen has been used as a carrier for demineralized bone matrix (DBM) to improve the handling characteristics and maintain the osteoinductive potential of DBM. The purpose of this study was to determine the appropriate techniques for preparation of a collagen/DBM product that would fulfill the above criteria. The study was divided into two phases. In the first phase, different collagen preparation methods were examined and factors such as extraction acid (0.5N Acetic acid, 0.1N HCl, 0.01N HCl, and 2N Citric Acid), extraction time (1hr, 2hr, and 24hr) and heat (65°C, 50°C, and 25°C) were varied. The DBM was manually mixed with the collagen matrix to produce

composite products. The maximum amount of DBM for any one product was 27% by weight. A total of 11 different products were produced by these variations. Enough material to contain 20mg of DBM was implanted into gluteal muscle pouches of male athymic mice (three mice/product). The materials were explanted after 28 days and analyzed for new bone formation as shown by histomorphometric analysis and remineralization as shown by calcium deposition. All the results from the experimental groups were compared to a positive DBM control. In phase two of the study, the best method of collagen preparation (extraction of tendon with 0.5M Acetic Acid, pH1.67 for 2 hours at 25ºC) was used to produce the collagen matrix for all the remaining implantation studies and the methods of addition of bone to the collagen were varied. Different methods of mixing bone with the collagen (homogenization of the bone/collagen matrix, dehydration of the product at 40ºC for 24hr, and rehydration of sample), weight:weight ratio of DBM to collagen (40% DBM, 50% DBM, and 60% DBM), and different solutions for hydration of the product (saline and glycerol) were changed for producing the samples. A total of 12 products were produced and assayed as described for phase one. The products from the first phase formed less bone and deposited less calcium than a positive DBM control and did not have the desired handling characteristics. The products from the second phase provided for better sample handling characteristics and were at least as osteoinductive as the positive control. The important factors that should be taken in consideration for producing a successful collagen/DBM matrix are bone to collagen ratio and the method of mixing bone with collagen.

Disclosures: LifeNet,3.

SU041

Bone Density Changes in Pamidronate Treatment of Paget's Disease: Enhanced Increase in the Nonpagetic Spine with Calcium plus Calcitriol Supplement. R. I. Price,[1] L. Ward,*[1] D. H. Gutteridge,[1] R. W. Retallack,[1] G. O. Stewart,[2] R. L. Prince.[1] [1]Sir Charles Gairdner Hospital, Perth, Australia, [2]Fremantle Hospital, Perth, Australia.

We reported (Price et al [1993]) that intravenous pamidronate disodium (ivAPD) was associated with forearm bone loss, arising from secondary hyperparathyroidism. We also showed (Stewart et al [1999]) that the loss was abolished by calcium (Ca) plus calcitriol (1,25D) supplement (SUP). This study reports effects on bone mineral density (BMD) of ivAPD treatment, with and without SUP, in the lumbar spine (LS), hip and whole body (WB). Forty-nine patients were stratified by disease severity into; (i) a "moderate" (M, n=27) group with Hyp_E = 5-10µmol L GF, and; (ii) "severe" (S, n=22) with Hyp_E> 10mmol/L GF. Patients were randomised within-group to Ca plus 1,25D SUP or unsupplemented (UNSUP). S received 360 mg ivAPD as 6 doses of 60mg once-weekly; M received 240 mg as 4 weekly doses of 60mg. SUP received oral Ca 1.0-1.2 g/day for 6 months (mo) plus 1,25D 0.25mg twice daily for 4 weeks, starting at first ivAPD infusion. BMD was measured at 0, 3, 6, 12 & 24 mo using DXA (Hologic QDR2000 array) on the LS, hip and WB. Pagetic bone was assessed separately in the spine and hip. Only patients completing the 24 mo study were included in the analysis. WB BMD of all groups rose significantly (sig.) by 6 mo, notably in S-SUP (2.2-8.9% [(95%CI], p<0.005). All groups were elevated at 12 mo, but only M-SUP was still sig. above baseline at 24 mo (1.1-4.4%, p<0.005). SUP and UNSUP in S or M were not sig. different at any time. Spinal Pagetic BMD rose dramatically by 3 mo (see Table), but SUP and UNSUP were not different at any time in S or M. Indeed, in the combined M plus S groups, Pagetic LS BMD of SUP and UNSUP were elevated equally at 24 mo (see Table). SUP LS BMD in nonpagetic vertebrae rose mildly compared with Pagetic, but markedly compared with UNSUP, as shown below (95%CI, in units of % increase in LS BMD. * = p<0.05 , SUP vs UNSUP at same time point).

SPINE	UNSUP(M+S)	UNSUP(M+S)	SUP(M+S)	SUP(M+S)
	3mo	24mo	3mo	24mo
Pagetic	5.2-23.3 (n=9)	-1.40-18.9	10.5-25.2 (n=14)	1.7-16.6
Nonpag	-1.5-1.7 (n=15)	-0.3-4.41	0.4 -13.6 (n=11) *	-0.1-10.3

Hip Pagetic BMD responded similarly to the spine, with increases about one half. Nonpagetic hip BMD showed neither a treatment nor SUP effect. We conclude that (i) UNSUP bone loss is confined to the forearm; (ii) Ca plus1,25D SUP does not enhance Pagetic BMD or its retention at 24 mo, relative to UNSUP; (iii) SUP markedly and rapidly increases BMD in the nonpagetic spine, relative to UNSUP, suggesting a role for SUP in treatments for osteoporosis using parathyroid hormone. Price R et al (1993) J Bone Miner Res 8:210; Stewart G et al (1999) Bone 24:139.

SU042

Activation of MEK1 Inhibits Hypertrophic Differentiation of Chondrocytes. N. Ogata,[1] T. Aikawa,[1] K. Seki,[1] S. Tanaka,[2] G. V. Segre,[1] K. Lee.[1] [1]Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [2]Orthopaedic Surgery, University of Tokyo, Tokyo, Japan.

The mechanisms through which activation of the PTHrP receptor (PTHrPR), a G-protein coupled receptor, and FGF receptor (FGFR), a tyrosine kinase receptor, inhibit chondrocytic differentiation are yet to be clarified. Since both PTHrPR and FGFR are known to activate mitogen-activated protein kinases (MAPK), we hypothesized that inhibitory effects of these two receptors on chondrocytic differentiation are mediated through MAPK activation. To test this hypothesis, we first established a rapid and sensitive assay system for hypertrophic differentiation of chondrocytes. When primary chondrocytes, derived from newborn mouse ribs, and ATDC5 cells, mouse embryonal carcinoma cells, were cultured in a matrix of alginate beads, hypertrophic differentiation was dramatically accelerated compared to conventional monolayer culture. Type X collagen mRNA expression in primary chondrocytes and ATDC5 cells started by 60h and 24 h, respectively, and progressively increased to 25 days in alginate matrix culture, whereas its expression became detectable after 10 days, and levels of its expression remained low even after several weeks in monolayer culture. When ATDC5 cells in alginate beads were treated with either PTHrP or FGF2, Type X collagen mRNA expression was dramatically reduced compared to vehicle control. Moreover, PTHrP and FGF2 activated ERK1/2 in ATDC5 cells in alginate matrix culture. We then infected ATDC5 cells with a replication-defective adenovirus carrying constitutively-active MAPK kinase, MEK1 (CA-MEK virus). CA-MEK virus transfection not only activated ERK1/2 in ATDC-5 cells efficiently, but also suppressed Type X collagen mRNA expression completely within 3 days. These results suggest that MAPK activation is involved in the signaling pathways by which both PTHrPR and FGFR inhibit hypertrophic differentiation, and show that the combination of a three-dimension culture that drives chondrocytic differentiation and a highly efficient transfection method utilizing adenovirus carriers is a powerful approach to clarify the intracellular mechanisms through which chondrocytic differentiation is regulated.

SU043

Regulation of Chondrocyte Differentiation by Snail-Related Transcriptional Repressors in Endochondral Bone Development. K. Seki,*[1] T. Aikawa,[1] N. Ogata,[1] T. Fujimori,*[2] G. V. Segre,[1] K. Lee.[1] [1]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [2]Department of Pathology and Tumor Biology, Kyoto University, Kyoto, Japan.

Snail, a zinc-finger transcriptional repressor, was first identified in Drosophila as a determinant of mesoderm formation. Snail-related genes are highly conserved among species, and have been suggested to play important roles in cell differentiation, cell cycle regulation, apoptosis, and cell motility. Moreover, mouse Snail may be an effector gene for PTHrP signaling in early development; PTHrP stimulates proliferation and differentiation of the inner cell mass cultured in vitro and PTHrP up-regulate mSnail expression during differentiation of F9 cells the in vitro. To examine whether mouse Snail-related transcriptional repressors; mSnail and mSlug, play roles in endochondral bone development and whether PTHrP affects their functions, we first analyzed the expression of mSnail and mSlug in developing endochondral bone in mouse fetuses. In the growth plate in E16 mouse fetuses, transcripts for both mSnail and mSlug were highly expressed in hypertrophic chondrocytes, and also they were expressed at much lower levels in cells in the proliferating zone. Then we tested the effects of PTHrP and N-SHH on mSnail mRNA expression in chondrocytes isolated from ribs of newborn mice in three-dimension culture. Whereas PTHrP increased mSnail mRNA expression after 6-day treatment, N-SHH dramatically decreased its expression. Then we test the effects of transient overexpression of mSnail and mSlug on chondrocyte differentiation in ATDC5 cells; Type II collagen mRNA expression was dramatically decreased in 48 hours, whereas Type X collagen mRNA expression was unaffected by both of them. These data suggest that mSnail and mSlug are involved in chondrocyte differentiation, that they regulate Type II collagen expression, and that the expression of mSnail is regulated by growth/differentiation factors such as PTHrP and IHH.

SU044

Chondrogenesis Versus Myogenesis: Coordination by Wnts and BMPs. A. D. Weston,*[1] T. M. Underhill.[2] [1]Physiology, The University of Western Ontario, London, ON, Canada, [2]Physiology and Oral Biology, The University of Western Ontario, London, ON, Canada.

During development myogenesis and chondrogenesis are regulated by many common signaling pathways. The embryonic mouse limb provides an ideal model system for studying these factors. Precursors giving rise to limb musculature originate from somitic mesoderm which migrates into the limb bud whereas cells in the distal tip of the developing limb, derived from lateral plate mesoderm, give rise to the cartilaginous template. In the present study we demonstrate that myogenic cells of the limb have chondrogenic capacity and that this potential is normally suppressed by factors emanating from the ectoderm. In the absence of ectoderm myogenic cells of the limb acquire a chondrogenic phenotype as indicated by diminished expression of myosin heavy chain in matrix-producing chondrocytes. Similarly, following transfection of a myogenin-LacZ reporter to follow cells of the myogenic lineage, numerous LacZ-expressing cells displaying a chondrocyte phenotype were identified. In addition, when mixed with primary limb mesenchyme, retrovirally-tagged G8 myoblasts from an established myogenic cell line (ATCC) will lose their muscle phenotype and contribute to cartilage nodules. This conversion of muscle cells to chondroblasts is blocked when cells are cultured with limb ectoderm or with pluripotent C3H10T1/2 cells that stably express Wnt3a, a factor that is normally secreted by ectoderm. Wnt3a inhibits cartilage formation, demonstrated by a dramatic decrease in cartilage nodule formation in primary limb cultures, even in the presence of bone morphogenetic protein 4 (BMP-4). In addition, transient expression of Wnt3a decreases the activity of a Sox9 luciferase reporter and attenuates BMP-mediated induction of the reporter. In contrast, Wnt3a promotes myogenesis as indicated by an increase in the activity of luciferase reporters for the muscle-specific genes, myogenin, myoD, and cardiac actin. Wnt3a also dramatically enhances the appearance of myocytes in primary cultures. Wnt5a, normally expressed in the distal mesenchyme of the limb, has effects opposite to Wnt3a in that it promotes chondrogenesis. BMP-4 can augment the myogenic effects of Wnt3a while it enhances the chondrogenic effects of Wnt5a in a synergistic manner. Taken together, our results suggest a model whereby the fate of chondrogenic and myogenic cell populations is coordinated by the Wnt and BMP signaling pathways. Specifically, the BMPs are required for myoblast and chondroblast differentiation, however, the Wnts determine the eventual phenotype of muscle and skeletal progenitors.

SU045

See Friday Plenary number F036.

SU046

Leptin Has Direct Effects on the Metabolism of Chondrocytes. T. Koike,[1] R. Nakajima,*[2] H. Inada,*[2] T. Yamano,*[2] K. Uehara,*[1] M. Tomita,*[1] Y. Yamano,*[1] K. Inui.[1] [1]Orthopaedic Surgery, Osaka City University Medical School, Osaka, Japan, [2]Pediatrics, Osaka City University Medical School, Osaka, Japan.

The mouse Obese gene product, leptin, is a peptide hormone secreted by adipose tissue. Previous studies demonstrated that leptin is important in the regulation of body weight and fat deposition, and that it inhibits bone formation through a hypothalamic relay. It was also reported that high levels of leptin and its receptor (both mRNA and protein) are expressed in the fetal bone and cartilage. These observations suggest that leptin might have paracrine or autocrine effects on endochondral bone formation. The goal of this study is to test the hypotheses that leptin plays a direct role in regulating growth of growth-plate cartilage. We examined the effects of leptin on the growth and differentiation of rabbit costal chondrocytes in monolayer culture, and on the total growth of mouse limbs in explant culture. Chondrocytes were isolated from growth plates of ribs of 4-wk-old rabbits and seeded at 10,000 per 96-well plates in alpha MEM supplemented with 10% FCS. After 3 to 7 days, cultures were exposed to increasing concentrations of leptin. After 21 h, the cells were labeled with [3H]thymidine for 3 h. Leptin (5-10 ng/ml) increased [3H]thymidine incorporation into DNA by rabbit growth-plate chondrocytes at the sparse phase of growth in monolayer culture by two times fold. But it did not stimulate the growth of chondrocytes at the confluent phase. On the other hand, leptin stimulated the synthesis of alkaline phosphatase by chondrocytes at the confluent stage of growth. In addition, to examine the effects of leptin on the growth of fetal cartilage, we compared morphological changes of limbs of wild-type mice (ED 16.5) which were cultured under serum-free conditions with or without leptin for five days.When mouse hindlimb were treated by leptin (100 ng/ml) for five days in organ cultures, the size of the growth plate became smaller than control (a pair of hindlimbs from same fetus was cultured as a counterpart each other). However, the proportion among resting, proliferating and hypertrophic chondrocytes was not altered. These data demonstrate that leptin has direct effects on the growth of chondrocytes besides an endocrine effect.

SU047

Cyclin D1-Independent Skeletal Growth. Z. Ali,*[1] R. Seerattan,*[2] P. A. LuValle.[1] [1]Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada, [2]Department of Surgery, University of Calgary, Calgary, AB, Canada.

Endochondral bone ossification is controlled by the coordination of proliferation and differentiation of chondrocytes in the growth plate. The D-type cyclins play a major role in the control of cell cycle proliferation. Published data suggest that cyclin D1-deficient mice display significant disturbances in proliferation that are restricted to neurological, retinal and mammary gland tissues. Our studies show that cyclin D1 expression is rapidly induced by mitogenic stimuli in chondrocytes and necessary for chondrocyte proliferation in vitro. We postulated that the reduced size of the cyclin D1-nullizygous mice was related to a skeletal growth deficiency.Paraffin sections of growth plates from 3 and 6 week old wild type, cyclin D1 heterozygous and nullizygous mice were compared to analyze the role of cyclin D1 in skeletal growth in vivo. CyclinD1-deficient growth plates were considerably reduced in size compared to either heterozygous or wild-type. Regardless of age, the decrease in growth plate size was 40%. The proliferating zone in cyclin D1-deficient mice was reduced by 35% at 3 weeks and by 50% at 6 weeks. While cyclin D1-deficient mice are significantly smaller in size than normal littermates after birth, the disparity in size diminishes as the mice mature, suggesting that cyclin D1-deficient mice continue to grow after normal littermates have ceased growth. Tibiae lengths of cyclin D1-deficient- and normal littermates were measured from 3 to 16 weeks at one week intervals. Size differences between the two populations decreased with time, such that at 16 weeks of age the disparity in size was almost insignificant. Growth in these animals occurs in the absence of cyclin D1, suggesting that other cyclin family members are compensating. We analyzed cyclins D2 and D3 protein levels in chondrocytes isolated from normal and cyclin D1-deficient mice littermates and found that these cyclins were upregulated. These data suggest that cyclin D1-independent skeletal growth is mediated by other cyclin D family members. Experiments are underway to determine the effects of mitogenic signals on the activities of cell cycle components in cyclin D1-deficient primary chondrocytes and wild-type controls.

SU048

Molecular Mechanisms Underlying the Function of Retinoid Signaling in Chondrogenesis. T. M. Underhill,[1] A. D. Weston.[2] [1]School of Dentistry, The University of Western Ontario, London, ON, Canada, [2]Physiology, The University of Western Ontario, London, ON, Canada.

Patterning of the appendicular skeleton relies on the convergence of multiple signaling pathways to coordinate the condensation and differentiation of chondroprogenitors. Phenotypic changes that are associated with chondroblast differentiation have been well characterized, however, the mechanisms underlying commitment and differentiation of precartilaginous cells remain poorly defined. Previously we used a transgenic mouse model to further define the role of retinoid signaling in skeletogenesis. Mice that ectopically express a weak constitutively active form of the retinoic acid receptor, RARa, in the developing limb bud present with severe skeletal malformations due to transgene-mediated inhibition of chondroblast differentiation (Weston et al., 2000, J. Cell Biol., 148:679). Consistent with these observations, antagonism of RAR-mediated signaling in limb mesenchymal cultures causes an early increase in collagen type II (col II) expression that is preceded by an increase in Sox9 expression. Moreover, the activity of a luciferase reporter construct containing four repeats of a Sox9 binding sequence from the col II gene is increased several fold in mesenchymal cultures treated with an RAR-selective antagonist or co-transfected with a dominant-negative RARa (dnRAR). Interestingly, the histone deacetylase inhibitor trichostatin A (TSA) inhibits formation of cartilage nodules and reduces activation of a Sox9 reporter in response to RAR antagonists, further suggesting a requirement for gene repression during chondroblast differentiation. To delineate the mechanisms whereby a loss in RAR activity promotes chondrogenesis the status of multiple signal transduction pathways was profiled using luciferase reporters containing cis-acting enhancer elements. Inhibition of RAR activity by expression of a dnRAR resulted in ~5-fold activation of both a cAMP response element (CRE) reporter and an Activator Protein-1 (AP-1) reporter. A common pathway through which both of these enhancers are activated is the p38 MAPK signaling pathway. Treatment of primary cultures with the p38 inhibitors SB 202190 or SB 203580 potently blocks the formation of cartilage and attenuates the chondrogenic response to a dnRAR and RAR antagonists in a dose-dependent manner. Taken together, our results indicate that the p38 MAPK signaling pathway may function downstream of retinoid signaling to regulate chondroblast differentiation. These results will be presented along with additional findings that provide a molecular framework for understanding the action of retinoid signaling in chondrogenesis.

SU049

Bone Formation under Mechanical Stress Is Suppressed in the Absence of Osteopontin. M. Morinobu,[1] M. Ishijima,[1] S. Rittling,[2] K. Tsuji,[1] H. Yamamoto,*[3] A. NIfuji,[1] D. T. Denhardt,*[4] M. Noda.[1] [1]Dept. of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Rutgers University, Piscataway, NJ, USA, [3]Ehime University, Matsuyama, Japan, [4]Rutgers University, Piscataway, USA.

Mechanical stress is an important factor to control bone remodeling. Osteopontin is one of the noncollagenous proteins abundant in bone matrix and containing RGD motif to act as a cell attachment protein. Osteopontin expression has been shown to be induced by mechanical stress in osteoblasts, and lack of osteopontin suppressed unloading-induced enhancement of bone resorption and of reduction in bone formation. However, the function of osteopontin in osteoblasts in bone formation under stress remains unclear. The purpose of this study is to examine the role of osteopontin in the osteogenesis under the continuous tensile stress in vivo using mice calvarial sutures. We applied mechanical force onto the sagittal sutures of mice using orthodontic wire in vivo and examined bone formation by microCT, soft X-ray and histology. Application of the mechanical force resulted in the opening of the sagittal suture in the mice. After one week of the stretching, bone formation was clealy observed within the sutures under the stretching mechanical stress. The inner edges of the parietal bones started to form multiple parallel spikes towards the center of the sagittal sutures in a direction perpendicular to the sutures. Histological examination revealed the presence of plumpy osteoblasts on the spikes vigorously forming bone. In addition, osteoclasts positive for TRAP were also observed in the bone at the interface region between preexisting parietal bone and newly formed bone under mechanical stress in the sutures. RT-PCR analysis indicated expression of type 2 Cbfa1/Runx2 mRNA in the cells in the stretched sutures. In addition, type I collagen mRNA was also expressed in the suture cells under the stretching mechanical stimuli. These RNAs were also subjected to micro-array analysis. After four weeks of the application of the mechanical force, bone formation in the stretched sutures was increased in the wild type mice to fill the gap between the stretched parietal bones. In contrast, a significant reduction in the bone formation to fill the gap of the stretched sutures was observed in osteopontin-deficient mice. These observations indicated that osteopontin is required for the efficient bone formation in the sutures subjected to the tensile mechanical force.

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Sensitivity to Ion-pair Induced Apoptosis in Chondrocytes Is Maturation Dependent. C. S. Adams,* C. M. Teixeira,* K. D. Mansfield,* I. M. Shapiro,* B. Snyder.* Biochemistry, University of Pennsylvania, Philadelphia, PA, USA.

In previous studies, we have shown that terminal differentiation of epiphyseal chondrocytes is accompanied by a loss of thiol reserve and an increased sensitivity to apoptotic stimuli. To determine the mechanism of the apoptotic response, chick tibial chondrocytes and sternal chondrocytes were treated with 10-35 nM retinoic acid (RA) until almost confluent. Apoptosis was then induced by treating the hypertrophic chondrocytes with Ca^{2+} and phosphate (Pi) ion pair. Intracellular glutathione (GSH) was determined using a fluorescent probe and by biochemical assays. Since, GSH depletion is linked to generation of reactive oxygen species (ROS), we also probed the maturing cells with the fluorescent probe, hydroethidine. Following treatment with the retinoid, chondrocytes displayed an increased sensitivity to the ion pair apoptogen. Thus, cells treated with 35 nM RA for 10 days were killed by 4 mM Pi; low levels of apoptosis were seen in cells that had not been exposed to the retinoid or were treated with RA for just 5 days. When probed with hydroethidine, RA-treated chondrocytes evidenced an increase in intracellular ROS. ROS production was greatest in those chondrocytes treated with 35 nM RA. We noted that in parallel with the rise in ROS levels, there was a decrease in intracellular GSH. Next, we modulated GSH levels with 5 mM N-acetylcysteine (NAC) and 500 µM buthionine sulfoximine (BSO). We noted that depletion of GSH raised ROS levels and sensitized the cells to the ion pair apoptogen. On the other hand, NAC maintained the level of GSH and protected the cells from ROS generation and apoptosis. Since mitochondria serve as a site for ROS generation, we probed cultured chondrocytes and growth plate cells for changes in mitochondrial activity. We noted that there was a maturation-dependent change in the distribution of mitochondria within tibial chondrocytes. Compared to hypertrophic chondrocytes, proliferating chondrocytes demonstrated a heterogenous distribution of mitochondria. Findings

from this study show that, as chondrocytes become terminally differentiated, the change in mitochondrial function is accompanied by a loss of thiol reserve and an increase in the generation of ROS. The accumulation of ROS probably sensitizes the chondrocyte to the presence of ion pairs which serve to activate the apoptotic pathway.

SU051

Murine Costochondral Cell Cultures Yield Cells to Be Implanted to Regenerate Hyaline Cartilaginous Matrix in Articular Cartilage Defects in Mouse Knee Joints. K. Yagi,[1] M. Takamoto,[1] K. Tsuji,[1] K. Shinomiya,*[2] A. Nifuji,[1] M. Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Tokyo Medical and Dental University, Tokyo, Japan.

Ariticular cartilage defect is a serious clinical issue which remains to be solved as cartilage is one of the most difficult tissues to regenerate after loss due to diseases such as osteoarthritis and injury. To elucidate the molecular bases of such difficulty in regeneration of cartilage requires in vivo system to study the function of molecules with regard to their contribution to the process of regeneration and repair in vivo. Recent development of reverse genetics provides opportunity to analyze the function of genes in vivo. The animals used for such reverse genetics are almost exclusively mice but mouse system to study the regeneration and repair of the articular cartilage has not yet been established. Costochondral tissue has been used as a useful source for the chondrocytes and possibly their precursors to study in vitro. Therefore, we examined the behavior of mouse costochondral cells to see their capability to regenerate cartilaginous tissues in the articular cartilage defects made in mouse knee joints. Implantation was conducted by using costochondral cells isolated form new born mouse ribs. The cells were cultured for one week and northern analyses indicated expression of abundant type II procollagen mRNA. These cells were then implanted into the defects made in the articular cartilage of the distal end of the femur. On week after the implantation, cell mass was observed in the defect while no obvious formation of cartilage was detectable. Six weeks after the implantation, hyaline cartilage was formed in the defect where the cells were implanted. These results indicated that mouse articular cartilage defect system could be used to examine the function of genes by using costochondral cells obtained from genetically engineered knock out or transgenic mice.

SU052

Promoter Activity Determinant of Human Connective Tissue Growth Factor (CTGF/Hcs24) Gene in a Human Chondrocytic Cell Line, HCS-2/8. T. Eguchi,*[1] S. Kubota,*[1] S. Kondo,*[1] T. Shimo,*[1] T. Nakanishi,*[1] T. Kuboki,*[2] H. Yatani,*[2] M. Takigawa.[1] [1]Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan, [2]Department of Fixed Prosthodontics, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan.

CTGF/Hcs24, which is a chondrocyte-derived multi-functional growth factor, is specifically expressed in hypertrophic zone of endochondral growth plate. HCS-2/8, which is a human chondrosarcoma-derived chondrocytic cell line, also highly express CTGF/Hcs24. In this study, we performed molecular biological examination about regulatory mechanism of CTGF gene expression in chondrocytes, utilizing HCS-2/8. 1) RT-PCR and ELISA revealed that HCS-2/8 express higher level of CTGF/Hcs24 mRNA and protein molecules than other cell lines. 2) Transient expression assay with a luciferase reporter gene driven by the CTGF promoter revealed that CTGF/Hcs24 promoter activity in HCS-2/8 was 6-8 fold higher than that in HeLa. 3) CTGF/Hcs24 3'-UTR showed significant repressive effect for gene expression in HCS-2/8, as previously observed in several fibroblastic cells. 4) Deletion mutant analysis of CTGF/Hcs24 promoter mapped the region responsible for the high transcription activity down to the gene segment between -202 and -88 (numbered from the transcription initiation site). 5) Point mutation in the TGF-β response element in the corresponding region completely abolished the TGF-β responsiveness, but caused no more than 0.7-fold decrease in the basal transcription activity in HCS-2/8 cells.These results suggest that signaling pathway from TGF-β stimulation to CTGF transcription via TGF-β response element is actually functional in HCS-2/8. However, limited effect of point mutation to basal promoter activity suggests the involvement of other cis-element(s) and transcription factor(s) that are independent from TGF-β signaling. Although it is unclear whether this mode of CTGF gene activation operates in physiological hypertrophic zone of cartilage, or is attributed to one of the malignant characters of chondrosarcoma, the stronger expression of CTGF/Hcs24 in HCS-2/8 than in the other malignant tumor-derived cell lines suggests chondrocyte specificity of the phenomena observed in this study.

SU053

Expression of Connective Tissue Growth Factor/Hypertrophic Chrondrocyte-Specific Gene Product 24 (CTGF/Hcs24) During Fracture Healing. E. Nakata,*[1] T. Nakanishi,*[1] A. Kawai,*[2] K. Asaumi,*[2] T. Nishida,*[1] H. Inoue,*[2] M. Takigawa.*[1] [1]Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan, [2]Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan.

Localization and expression of connective tissue growth factor/hypertrophic chondrocyte-specific gene product 24 (CTGF/Hcs24) during fracture healing in mouse ribs were investigated. The eighth rib of 5 weeks old male ICR mice were fractured with scissors. To prepare samples for immunostaining and in situ hybridization, a total of 50 mice were fixed at 0, 2, 8, 14, and 20 days. In situ hybridization demonstrated that CTGF/Hcs24 mRNA was remarkably expressed especially in hypertrophic chondrocytes and proliferating chondrocytes in the regions of regenerating cartilage on days 8 and 14 after the fracture. CTGF/Hcs24 mRNA was also expressed in proliferating periosteal cells in the vicinity of the fracture sites on day 2, and in fibrous tissue around the callus on days 8 and 14. Immunostaining showed that CTGF/Hcs24 was localized in hypertrophic chondrocytes and proliferating chondrocytes in the regions of regenerating cartilage, and in active osteoblasts, immature osteocytes in the regions of intramembranous ossification. Although CTGF/Hcs24 was abundantly present in the proliferating and differentiating cells (on days 8 and 14), the immunostaining decreased as they differentiate torward bone formation (on day 20). CTGF/Hcs24 was also detected in fibroblasts, fibrous tissues, vascular endothelial cells, periosteal cells, and skeletal muscle around the fracture sites. Our results suggest that CTGF/Hcs24 is an important regulator in chondrogenesis, osteogenesis and angiogenesis in vivo, and promotes intramembranous ossification and endochondral ossification in the processes of fracture healing.

SU054

C-Jun, ATF-2, CREB and C/EBP Mediate the Sustained Upregulation of Integrin β5 Subunit by TGF-β in Osteoblasts. C. F. Lai,[1] X. Feng,[2] F. P. Ross,[3] S. L. Cheng.[1] [1]Div. of Bone and Mineral Diseases, Dept. of Medicine, Washington University School of Medicine, St. Louis, MO, USA, [2]Dept. of Pathology, University of Alabama, Birmingham, AL, USA, [3]Dept. of Pathology, Washington University School of Medicine, St. Louis, MO, USA.

We reported previously that interactions between Smads and Sp1/Sp3 proteins, the latter binding to -53/-48 of the β5 promoter, mediate rapid TGF-β upregulation of β5 in osteoblasts. Sp1/Sp3 binding to -53/-48 wanes after 24 h, while Northern and Western blot analyses reveal TGF-β increases β5 mRNA and protein up to 72 h. To identify the mechanisms mediating sustained TGF-β enhancement of β5 expression, we transfected MC3T3-E1 cells with luciferase (luc) reporter constructs linked to -310/+110, -274/+110 and -63/+110 regions of the β5 promoter. Exposure to TGF-β for 24 h increased the luc activities of all three constructs 2 X. After 48 h, however, the promoter activity of -310/+110 was further increased to 4 X, while those of -274/+110 and -63/+110 remained unchanged. These findings suggested that a responsive element(s) located between -310 and -275 mediated the sustained effect of TGF-β on β5 expression. Since two putative CRE/AP-1 sites (TGAC) were located in this region, we analyzed promoter activities after mutating each TGAC site and demonstrated that the 5' TGAC site played a major role in long-term stimulation of β5 promoter by TGF-β. EMSA was employed to confirm the importance of the 5' TGAC site and to identify nuclear proteins binding to the -310/-275 probe. Since three specific bands were obtained in EMSA and the consensus CRE oligonucleotide, but not that of AP-1, obliterated or diminished these bands, multiple CREB family transcription factors might be involved. Supershifting antibodies revealed that the top band was formed by c-Jun and ATF-2, the second CREB, and the bottom band C/EBP. Turning to mechanism, TGF-β stimulated the activities of c-Jun and CREB without altering their total protein levels, while upregulated both total and phosphorylated ATF-2. Phosphorylation of ATF-2 and C-Jun was paralleled by enhanced JNK activity. Consistent with the latter finding, transfection with a MEKK plasmid increased -310/+110-luc activity 2 X without altering that of -63/+110-luc. Finally, TGF-β stimulated the β, but not the δ, isoform of C/EBP. In conclusion, TGF-β upregulates long-term expression of the integrin β5 subunit by a mechanism involving enhanced activities of c-Jun, ATF-2, CREB and C/EBP β, which transactivate a TGAC site in -310/-275 of β5 promoter.

SU055

Expression Analysis of Osteoblast Differentiation: Stage-restricted and Distinct mRNA Expression Profiles. K. M. Wiren,[1] A. Evans,*[2] X. W. Zhang.[3] [1]VA Medical Center and OHSU, Portland, OR, USA, [2]VA Medical Center, Portland, OR, USA, [3]OHSU, Portland, OR, USA.

Current understanding of the mechanisms that underlie osteoblast differentiation is incomplete. In order to better understand the process of coordinate regulation of osteoblast differentiation, we have systematically investigated gene expression patterns during osteoblastic differentiation using microarray analysis. Two osteoblastic model systems were employed in the analysis: a differentiating osteoblastic model system using normal rat primary osteoblastic cultures derived from calvaria by collagenase digestion and ROS 17/2.8 osteoblastic osteosarcoma cells. Normal rat calvarial cultures were grown to confluence, and then switched to differentiation medium containing ascorbic acid and beta-glycerol phosphate. These cultures progress through a time-dependent ordered developmental sequence leading to bone nodule formation by 25 days, and thus represent an in vitro model of bone development. Total RNA was isolated at various time-points during proliferation (day 5), matrix maturation phase (day 14) and mineralization stage (day 26); and from confluent ROS 17/2.8 cultures. Complex RNA probes were generated from total RNA by linear reverse transcription in the presence of 33PdCTP. Gene expression was characterized by hybridization with nylon membranes spotted with ~4400 gene sequences using "Named Genes" GeneFilters from Research Genetics. Spot images were acquired by high-resolution phosphorimage analysis with spot intensity and gene identification determined using Pathways v3 software. Data was normalized to all data points to control for variation in RNA concentrations and variability during reverse transcription for the generation of the complex RNA probe. Clustering analysis was used to generate associations between clones. Hierarchical clustering was performed with Cluster software to produce dendrograms, and the Kmeans algorithim was used for partitional clustering to associate genes using Pathways software. Stage-specific and distinct patterns of gene expression were observed for the normal differentiating osteoblasts. Interestingly, gene expression patterns observed in confluent ROS 17/2.8 cells show highest similarity to the mineralization stage of the normal osteoblast cultures. These results highlight important transcriptional changes that influence osteoblast differentiation and mineralization, ultimately regulating osteoblast function.

SU056

Identification of Estrogen Regulated Genes During Fracture Healing Using DNA Microarray. H. Hatano,* H. J. Siegel,* J. T. Bronk,* G. Sarkar, R. T. Turner, M. E. Bolander. Orthopedic Research, Mayo Clinic and Foundation, Rochester, MN, USA.

Estrogen deficiency impairs fracture healing, reduces expression of cartilage-related genes in the callus, and decreases the strength of the healing fracture, while treatment of ovariectomized (OVX) rats with estrogen (17-beta estradiol, E2) during fracture repair strengthens the callus and up-regulates expression of cartilage matrix proteins. These data indicate that estrogen deficiency influences fracture repair by inhibiting chondrogenesis and, subsequently, endochondral ossification. Our objective was to identify genes directly or indirectly targeted by estrogen during fracture healing. To identify these genes we evaluated gene expression in calluses from 3 groups of rats, sham, OVX and OVX+E2, by DNA microarrays. Fifteen female rats (3 month old) underwent OVX (N= 10) or sham operation (N=5). Femurs were fractured 1 week later. Five OVX rats were given daily subcutaneous injections of estrogen (200ug/Kg), starting the day of fracture. Calluses were harvested for RNA extraction 10 days after fracture. Expression levels for the 5,531 genes on the Research Genetics rat DNA microarray were analyzed in pooled RNA samples from the calluses in each group. Differentially expressed genes were identified by hierarchical clustering and confirmed by RT-PCR. Four genes (collagen type 2 (COL2), superoxide dismutase (SOD), plasminogen activator (u-PA) and ptk-3) and 43 ESTs were down-regulated in calluses from OVX animals but restored with estrogen treatment. We previously reported up-regulation of COL2 in fracture calluses from OVX rats after estrogen supplementation; the present results support that observation. The other genes are potentially important in fracture healing: u-PA, a serine proteinase, converts plsminogen into plasmin, which degrades extracellular matrix during remodeling and also activates TGF-ß: SOD scavenges oxygen free radicals, toxic entities which are normally produced within cells; Ptk-3 is transcriptionally activated by DNA damage. As fracture repair involves multiple metabolic processes, regulation of expression for these genes could have significant effects on the progression of healing. COL2 is a major extracellular matrix component of fracture callus cartilage, but to the best of our knowledge this is the first report implicating SOD, u-PA and ptk-3 in fracture healing. As we have not characterized the function of the 43 differentially expressed EST's, and as we have evaluated approximately 15% of the total number of genes, our data suggests that other genes regulated by estrogen during fracture repair remain to be identified.

SU057

Gene Expression in Human Cancellous Bone Is Similar between Skeletal Sites. J. S. Kuliwaba,*[1] D. M. Findlay,[2] G. J. Atkins,*[2] M. R. Forwood,[3] N. L. Fazzalari.*[1] [1]Div of Tissue Pathology, Institute of Medical and Veterinary Science; Dept of Pathology, University of Adelaide, Adelaide, SA, Australia, [2]Dept of Orthpaedics & Trauma, University of Adelaide, Adelaide, SA, Australia, [3]Dept of Anatomical Sciences, University of Queensland, Brisbane, QLD, Australia.

The mechanisms that lead to the structure and turnover of cancellous bone are complex, involving both mechanical and chemical inputs. Our previous work has shown strong associations between the ratio of RANKL/OPG mRNA and histomorphometric indices of bone turnover at the intertrochanteric (IT) region of the human proximal femur in a control cohort, but not in an age-matched osteoarthritic group (1). In this study we have examined the expression of mRNA encoding a number of bone cell markers and regulatory molecules in several skeletal sites: iliac crest (IC), femoral neck (FN) and IT cancellous bone. These bone samples were obtained from 10 routine autopsy cases (6 women, 57-85 years; 4 men, 42-84 years; postmortem interval (PMI) 16-108 hours). Total RNA was isolated from each bone sample for semi-quantitative RT-PCR analysis of RANKL, osteoprotegerin (OPG), RANK, IL-6, TRAP, osteocalcin (OCN) and osteopontin (OPN) mRNA expression. The relative ratios of the amplified products, with respect to GAPDH, were determined. We were interested to find no significant differences, either within individuals or in the mean data, in the gene expression pattern between the three skeletal sites (Anova single factor analysis):

Ratio	***IC***	***FN***
RANKL/GAPDH	0.41±0.26	0.61±0.70
OPG/GAPDH	0.34±0.23	0.43±0.24
RANKL/OPG	1.24±0.29	1.31±0.87
RANK/GAPDH	0.16±0.06	0.14±0.04
IL-6/GAPDH	0.99±1.09	1.26±0.91
TRAP/GAPDH	0.35±0.10	0.39±0.13
OCN/GAPDH	0.95±0.49	1.18±1.01
OPN/GAPDH	0.36±0.11	0.38±0.26

Values are mean ± SD

There was no evidence that mRNA levels were influenced by the PMI. Although differences in bone turnover have been reported between these skeletal sites, we did not observe significant differences in gene expression of the above skeletal factors between the IC, FN and IT sites. It will be informative to perform a similar analysis with osteoarthritic samples to determine whether the altered gene expression we have found at the proximal femur, compared to controls (1,2), reflects the local joint disease or suggests a wider skeletal difference in bone turnover. (1) Fazzalari et al, *JBMR*, In Press, 2001 (2) Kuliwaba et al, *JBMR* 15:332-41, 2000

SU058

Cart1, Paired-like Homeoprotein That Involved in Cartilage Development, Forms a Transcriptional Machinery With P300/CBP Through a Highly Conserved Homeodomain. T. Iioka,*[1] K. Furukawa,*[1] H. Shindo,*[1] S. Yamashita,*[2] T. Tsukazaki.*[3] [1]Department of Orthopaedic Surgery, Nagasaki University School of Medicine, Nagasaki, Japan, [2]Department of Nature Medicine, Atomic Bomb Disease Institute, Nagasaki University School of Medicine, Nagasaki, Japan, [3]Department of First Anatomy, Nagasaki University School of Medicine, Nagasaki, Japan.

Cartilage homeoprotein-1 (Cart1) is a paired-like homeoprotein that expressed selectively in precursors of chondrocytes. So far physiological role of Cart1 in cartilage development is unknown, but recent studies revealed that Cart1 forms a homodimer and binds directly to DNA. P300/CBP is an important cofactor of transcription which has intrinsic acetyltansferase activity and forms a complex with many transcriptional factors. In this study, we investigated the possible involvement of P300/CBP for Cart1 dependent transcriptional activity. In luciferase assay using a putative Cart1 binding site, P300/CBP stimulated Cart1 transcriptional activity by a dose dependent manner, and this transactivation was reduced by coexpression of E1A or Tax, virus- derived suppressors for P300/CBP. To investigate in vivo interaction, Flag-tagged Cart1 and HA-tagged P300 were transiently transfected to cos7 cells, and then performed immunoprecipitation after metabolic labeling. P300 was co-immunoprecipitated by Cart1. Interaction domains of each protein were determined by Yeast Two Hybrid systems. Cart1 bound with P300 through a homeodomain. On the other hand, interaction domain of P300 for Cart1 binding was 1-146a.a. residues in amino acid terminal. Overexpression of this truncated P300 protein abolished Cart1 transcriptional activity transactivated by full- length P300. This region of P300 contains binding site for nuclear receptor, but mutagenesis studies revealed no involvement of this region for homeodomain binding. Furthermore, these proteins were colocalized in the nuclei by confocal microscopic study. Our results suggest that P300/CBP is involved in a transcriptional machinery of Cart1, although target genes for Cart1 during embryonic development is still unclear. Moreover, many other paired-like proteins that involved in embryonic development may be also regulated by P300/CBP though their homeodomain.

SU059

Functional Domain of the Cartilage Homeoprotein 1 (CART-1) Transcriptional Factor and Relation Between Dimerization and Transactivation. K. Furukawa,[1] T. Iioka,*[1] M. Morishita,[2] H. Shindou,*[1] S. Yamashita,*[2] T. Tsukazaki.[3] [1]Department of Orthopaedic Surgery, Nagasaki University School of Medicine, Nagasaki, Japan, [2]Department of Nature Medicine, Atomic Bomb Disease Institute, Nagasaki, Japan, [3]Department of First Anatomy, Nagasaki University School of Medicine, Nagasaki, Japan.

Paired-like homeoproteins play an important role in mammalian development as transcriptional factors, in which CART-1 is expressed selectively in neural tubes and craniofascial and limb bud mesenchymal cells. Although phenotype of CART-1 alone deficient mice shows the defect of head formation, it is also speculated that CART-1 plays a crucial role for chondrogenesis. Here we investigated molecular property of CART-1 involved transcriptional activation. Deletion analysis combined with Gal4 assay mapped the activation domain of CART-1 at the central portion of the C-terminal domain, and N-terminal domain was required for the full transactivation. In immunofluorescent staining, two separate nuclear localization signals (NLS) were identified beside the homeodomain, and introduction of the mutation into either NLS completely abolished the transcriptional activity. Transcriptional activity of CART-1 was enhanced in several cell lines by the copy number of Cart-1 binding sites, but in RCS cells which expresses abundant CART-1, the activity was not enhanced remarkably. RT-PCR analysis identified in RCS two specific splicing variants (Cart-1beta and Cart-1gamma) in addition to wild type, Cart-1alpha. Predicted amino acid sequence of Cart-1beta lacks C-terminal half following the end of homeodomain due to the frame shift. In contrast, Cart-1gamma misses 43 aa around the junction of C-terminal end of the homeodomain. Since these mutants were unable to bind with the consensus DNA sequence, we hypothesized that weak responsiveness in RCS was due to endogenous expression of these isoforms. In two hybrid assay, CART-1alpha bound with beta and gamma isoforms, while CART-1beta and gamma did not interact each other. In luciferase assay, CART-1beta and gamma inhibited transcriptional activity of CART-1alpha dose-dependently. These competition could be demonstrated by cotransfection of N- or C- terminal domain. Furthermore, we could obtained the evidence of direct protein-protein interaction of wild CART-1 in vivo condition. These results indicate that dimerization of CART-1 through direct interaction is required for DNA binding and subsequent transcriptional activation.

SU060

Expression Profiling of Genes Involved in Periprosthetic Bone Loss Using cDNA Microarray Technology. S. Kim,*[1] M. Kim,*[2] J. Jeong,*[3] H. Si,*[3] J. Kim,*[3] H. Kim,*[3] J. Choi.*[3] [1]Dept. of Orthopedic Surgery, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, [2]Dept. of Immunology, School of Medicine, Kyungpook Natl. Univ., Daegu, Republic of Korea, [3]Dept. of Biochemistry, School of Medicine, Kyungpook National University, Daegu, Republic of Korea.

Periprosthetic bone loss is an important contributory factor for aseptic loosening of total joint replacements. In aseptic loosening, macrophage response to biomaterial wear parti-

cles is commonly found in arthroplasty tissues. It has been shown that osteoclast precursor cells are present in the wear particle-associated macrophage infiltrate found in the tissue surrounding loose implants and that these cells are capable of differentiating into osteoclastic bone-resorbing cells. As the CD14 marker is strongly expressed on monocytes, the putative osteoclast precusor in peripheral blood, we have selected CD14(+) cells from both peripheral blood (PB) and tissue membrane surrounding loose implants (TM) to examine their expression profile of bone resorption related genes involved in aseptic loosening. Poly A(+) RNA from the two groups was reverse transcribed and labeled with different fluorescent probes (either Cy3-dUTP for PB or Cy5-dUTP for TM). The resulting fluorescent-labeled cDNA mixture was allowed to hybridized to the 3K human cDNA chip which included 3000 unigenes obtained from mesenchymal stromal cell cDNA library. The fluorescent signal on the cDNA gene chip was detected and quantified. The results showed that about 250 genes among 3000 unigenes were upregulated more than two fold and bone resorption related genes, particularly cathepsin B, D, O, and K were highly expressed in the arthroplasty tissues. This was confirmed by RT-PCR. These results indicate that the release of cathepsins by activated cells in the arthroplasty membrane is likely to contribute to pathological bone resorption associated with aseptic loosening by stimulating differentiation of mononuclear phagocyte into mature bone-resorbing cells.

Disclosures: IBEC of KOSEF of Korean government.,2.

SU061

Mutations Affecting Bone Formation and Osteoblast Function in the Zebrafish, Danio Rerio. T. Trowe,*[1] K. Schlombs,*[1] C. Kaps,*[1] A. Kirchner,*[1] U. Hagner,*[1] H. Maischein,*[2] Tübingen Screen Consortium.[3] [1]Artemis Pharmaceuticals, Tübingen, Germany, [2]Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany, [3]Artemis Pharmaceuticals and Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany.

Bone is formed and remodelled by two distinct cell types, the osteoblast (bone-forming cell) and the osteoclast (bone-resorbing cell). In bone remodelling, the processes of bone resorption and bone formation are coupled in space and time, so that the overall bone mass remains constant even though 20% of the adult bone surface is constantly "under construction",. In osteoporosis, this balance is shifted towards resorption. It remains unclear how resorption and formation are regulated, largely due to the fact that the biology of osteoblast formation and function is only poorly understood.In order to understand these processes better, we have undertaken a genetic, whole organism approach in the zebrafish. We screened the progeny of more than 3000 mutagenized families by day 9 using alizarin red, which stains mineralized bone, and discovered 200 mutants defective in bone formation. 60 mutants lack bone formation completely and a further 15 mutants show a general increase in bone formation. The remaining 125 mutants have less severe defects affecting either only individual bones or affecting perichondral but not dermal bones. By analysing bone development of wild type zebrafish, we found that although alkaline phosphatase positive osteoblasts appear by day three, TRAP-positive osteoclasts appear only around day 14, Therefore, all mutant phenotypes must stem from defects in either osteoblast formation and function or bone mineralisation. Characterisation of the mutants by analysing alkaline phosphatase and collagen 1 expression will differentiate between those affecting mineralisation and those affecting various stages of osteoblast formation. Cloning these mutants will discover novel genes involved in osteoblast differentiation and provide a much better understanding of the regulation of bone mass. Therefore, these mutants set the basis for defining new therapeutic entry points for osteoporosis and other diseases affecting bone.

Disclosures: Artemis Pharmaceuticals,3.

SU062

Chondroclasts in Growing Long Bones of Young Rats - Relationship to Potential Adhesion Proteins in the Matrix. J. Nordahl,*[1] K. Hultenby,*[1] S. Mengarelli-Widholm,*[1] D. Heinegård,[2] F. P. Reinholt.*[3] [1]Div. of Pathology, Karolinska Institutet, Huddinge Hospital, Huddinge, Sweden, [2]Dept. of Cell and Molecular Biology, University of Lund, Lund, Sweden, [3]Institute of Pathology, University of Oslo, National Hospital, Oslo, Norway.

During endochondral bone growth, mineralized cartilage close to the calcification zone of the epiphyseal growth plate is degraded by chondroclasts, which are morphologically resembling bone resorbing osteoclasts in metaphyseal bone. However, functional properties of chondroclasts have this far been paid less attention to. Previous studies have revealed that adhesion between osteoclasts and bone matrix, preceeding matrix resorption, is probably mediated by osteopontin, referring to its localization close to the clear zone of osteoclasts. The aim of the present study was to elucidate mechanisms characteristic for the cartilage resorption process. Proximal tibias from young rats were used. Immunohistochemistry at the ultrastructural level was performed using antibodies against two matrix proteins; osteopontin (OPN) and bone sialoprotein (BSP), with colloidal gold as marker. The investigated proteins are potentially mediating cell-matrix adhesion through interaction between a specific amino acid sequence (Arg-Gly-Asp) and integrin receptors on the cell membrane. Distribution of proteins was estimated from the immunolabeling levels among predetermined compartments in the tissues. For statistical evaluation we used Analysis of variance (ANOVA). There were distinctly different distribution patterns between the proteins; BSP showed accumulation of marker at matrix surfaces facing the clear zone of chondroclasts, while OPN was concentrated outside ruffled borders. The localization of BSP at the clear zone indicates a possible role as an adhesion protein in the interaction between chondroclasts and their adjacent cartilage matrix. This differs from the interaction between osteoclasts and bone matrix, where OPN is the likely ligand. OPN labeling outside chondroclast ruffled borders might be a result of unmasking of antigenic epitopes during degradation of matrix components. In summary, our results indicate different mechanisms in mineralized cartilage resorption by chondroclasts compared to the osteoclast degrading activity in metaphyseal bone.

SU063

Role of β-estradiol in Bone Matrix Protein Production and Proliferation in Vascular Smooth Muscle Cells. M. Ruan,[1] V. Miller,*[2] L. A. Fitzpatrick.[1] [1]Endocrine Research Unit, Department of Medicine, Mayo Clinic, Rochester, MN, USA, [2]Department of Surgery, Mayo Clinic, Rochester, MN, USA.

Epidemiological studies indicate a reduced relative risk of cardiovascular disease associated with estrogen replacement therapy. However, the mechanisms by which estrogen may alter cardiovascular risk are controversial and not well established. VSMCs are the major cell type in atherosclerotic plaques and proliferation of VSMCs is involved in the formation of atherosclerotic plaque. We isolated VSMCs from the coronary arteries of sexually mature pigs to determine the interactions of β-estradiol and matrix proteins in atherosclerotic plaque development. β-estradiol, at physiologic concentrations, inhibits the proliferation of VSMCs from female pigs but has no effect on the VSMCs from male or oophorectomized pigs. Western analysis revealed that coronary VSMCs from all 3 pigs have equal amounts of α and β estrogen receptors.Vascular calcification commonly occurs in the atherosclerotic lesions of coronary artery disease. VSMCs synthesize bone matrix proteins, which are involved in plaque formation and calcification. The presence of hydroxyapatite, bone matrix vesicles and bone morphogenic proteins in arterial plaque implies that vascular calcification is analogous to bone ossification. Type I collagen is associated with bone formation and is the principle collagen found in atherosclerotic plaque. To assess the effect of estrogen on regulation of bone matrix proteins in VSMCs, we treated VSMCs with β-estradiol at 10^{-6}, 10^{-7} or 10^{-9}M for 48-72 hrs. Northern blot analysis was used to measure expression of type I collagen, osteopontin and osteonectin. β-estradiol at 10^{-7}M and 10^{-9}M stimulated type I collagen expression 2 to 3 times compared to vehicle control. Slight upregulation of osteopontin and osteonectin was also observed in β-estradiol (10^{-7}M and 10^{-9}M) treated samples. Thus, estradiol is an important regulator of cell proliferation and matrix protein production in porcine coronary artery VSMCs. These findings provide insight into the mechanistic role of β-estradiol in the development of the ossification process in atherosclerotic coronary arteries. Coronary artery calcification is associated with stabilization of lipid-laden plaques. Thus, β-estradiol may provide long-term protection in coronary arteries through its enhancement of proliferation of cells that produce type I collagen, the structural framework for ossification.

SU064

Mechanisms of Magnesium-Stimulated Adhesion of Osteoblastic Cells to Commonly Used Orthopaedic Implants. H. Zreiqat,*[1] M. E. Shakibaei,*[2] P. Evans,[3] C. Knabe,*[4] C. R. Howlett.*[1] [1]School of Pathology, University of New South Wales, Sydney, Australia, [2]Anatomy, Free University of Berlin, Berlin, Germany, [3]Ion Implantation, ANSTO, Sydney, Australia, [4]Experimental Dentistry, Free University of Berlin, Berlin, Germany.

Chemical modification of a biomaterial surface may improve long-term survival of prosthetic devices. We demonstrated that modification of bioceramics with magnesium ions (Mg+2) enhanced the initial adhesion, proliferation and differentiation of human bone-derived cells (HBDC). A key factor in determining cellular interaction with biomaterials is their attachment sites to integrins; important signaling receptors that play an important role in bone remodelling at the medical device/skeletal tissue interface. We sought to determine the mechanism(s) by which Mg+2 incorporated into bioceramic surfaces affect bone remodelling. To identify which mechanisms enhance/maximize bone cell adhesion to biomaterials we attempt to define the signal transduction pathways involved in bone cell adhesion to Mg+2-modified bioceramics.HBDC were grown on tissue culture plastic, alumina oxide, alumina oxide modified with Mg+2 and hydroxyapatite for 1 hour. Integrins involved in ligand-receptor binding were identified using blocking monoclonal antibodies against different a and b subunits. We demonstrated an enhanced integrin expression level when HBDC were cultured on the Mg2+-modified bioceramic. HBDC adhesion was inhibited when the fibronectin-receptor a5b1 was blocked and not the vitronectin-receptor avb3. We observed redistribution of a5b1 integrin receptor with maximal localization at points of contact when HBDC were cultured on Mg2+-modified bioceramic, compared to a diffuse pattern when grown on the native bioceramic. These results suggest that a5b1 is a major receptor utilized by HBDC for attachment to the underlying substrata and may initiate signal transduction both alone and with other cell surface receptors. Tyrosine phosphorylation of intracellular proteins was revealed by immunoblotting with anti-phosphotyrosine antibodies. Results revealed an apparent increase in tyrosine phosphorylation and Shc proteins when cells were grown on Mg2+-modified bioceramic compared to tissue culture plastic, native bioceramic, and hydroxyapatite, suggesting that phosphorylation of signalling proteins is augmented by binding to specific biomaterial surfaces. These results suggest that modifying biomaterials with Mg2+ resulted in a functional significance in cell-cell contact and cell matrix interaction. Mg2+ incorporation into bioceramic surfaces may lead to the maintenance of mature and healthy bone at the device/ skeletal tissue interface.

SU065

Bone Cell Adhesion and pFAK Activation by Titanium and Hydroxyapatite Surfaces. R. Landesberg,[1] S. Zou,*[1] A. Ota,*[1] R. LeGeros,[2] J. LeGeros,[2] R. W. Katz.[1] [1]Columbia University, NY, NY, USA, [2]New York University, NY, USA.

This study examined the ability of bone cell lines (ROS 17/2.8, MC3T3E1, and rat neonatal calvarial osteoblasts) to adhere and activate focal adhesion kinase (pFAK) when exposed to four different implant surfaces (titanium alloy, TiA, commercially pure titanium, CpTi, hydroxyapatite, HA, and plasma-sprayed hydroxyapatite coated, PS). The effect of protein precoating on binding and pFAK upregulation was also evaluated. 13

mm implant discs were used. CpTi and TiA discs were passivated in 30% nitric acid for 30 min. All discs were autoclaved prior to use. Discs were coated with 55% calf serum, bovine vitronectin, bovine fibronectin, and media alone at 37°C for 2 hrs, washed, and placed in 24 well plates. Bone cells at 250,000 cells/well were allowed to bind for 2 hrs at 37°C in low serum conditions. For binding experiments the nonadherent cells were removed and the discs were washed. The cells were removed with trypsin and counted with a hemacytometer. Results were expressed as a ratio of cells bound to implant/cells bound to tissue culture plastic. For FAK experiments the nonadherent cells were removed and retained. The adherent cells were removed by sonication into lysis buffer and combined with the nonadherent cells. pFAK was examined by western blot analysis. Loading amounts were normalized by analysis of total ERK. Blots were quantitated by densitometry. TiA and HA surfaces had significantly less binding in the absence of a protein coating. The PS surface had low cell binding regardless of pretreatment. When coated with 55% serum CP and HA showed the largest ratio of cell adhesion (relative to TC 45, .41) followed by TiA (.31) and PS (.20). When the implants were precoated with serum proteins, serum showed a small but significantly greater amount of binding than VN or FN. The activation of pFAK was similar to the binding results in that serum>FN=VN>media on the TiA, HA, and PS surfaces. The activation of pFAK on CpTi discs however, was not affected by the coating. In conclusion, bone cell binding and subsequent activation of pFAK on implant surfaces is serum protein dependent for TiA and HA. Cell adhesion to CpTi and pFAK upregulation is independent of cell coating. Cell binding to the PS surface is poor and serum protein independent, however, the activation of pFAK is serum dependent. When compared with all other surfaces, PS shows a greater level of pFAK activation when expressed on a per cell basis. This *in vitro* system can be used to evaluate and develop better implant surfaces that speed the healing and promote the quality of implant integration.

SU066

Responsiveness of Ameloblasts to Extracellular Ca and EGF Is Similar to That of Keratinocytes. T. Suzawa, T. Katagiri, N. Udagawa, R. Kamijo, N. Takahashi. Biochemistry, School of Dentistry, Showa University, Tokyo, Japan.

Ameloblasts derived from inner dental epithelial cells form calcified enamel. When inner dental epithelial cells differentiate into ameloblasts, they start to secrete enamel matrix proteins such as amelogenin and ameloblastin. Although the differentiation of ameloblasts seems to be tightly regulated by the interaction with odontoblasts, dentine forming cells, the regulatory mechanism of ameloblast differentiation is still not know. In the present study, we studied characteristics of ameloblasts obtained from mouse mandibular incisors in comparison with those of epithelial keratinocytes. Primary ameloblasts were isolated from mandibular incisors of 7-day-old ddY mice, and keratinocytes from skins of newborn ddY mice. RT-PCR analysis showed that primary ameloblasts in culture expressed amelogenin mRNA, but keratinocytes did not. Immunological staining showed that both ameloblasts and keratinocytes expressed cytokeratin 14, a marker protein of epitherial cells. MTS assay and BrdU labeling assay clearly showed that EGF (epidermal growth factor) stimulated proliferation of both types of epitherial cells. However, the expression of amelogenin mRNA in ameloblasts was decreased by adding EGF. Similarly, EGF suppressed the expression of mRNA of transglutaminase 1, a differentiation marker, in keratinocytes. In contrast, amelogenin expression by ameloblasts and transglutaminase 1 expression by keratinocytes were up-regulated by cultivation of respective cells in the medium containing high Ca concentrations. In addition, treatment of both types of epitherial cells with high Ca concentrations induced morphological changes from the small flat shape to the large cuboidal shape. Thus, both ameloblasts and keratinocytes differentiated into more mature cells in response to high extracellular Ca, and proliferated in response to EGF. Thus, responsiveness of ameloblasts to several stimuli was quite similar to that of kelatinocytes. Differentiation of kelatinocytes in skin organization is known to be regulated by epithelial-mesenchymal interaction. This suggests that the differentiation of ameloblasts is also regulated by the interaction with mesenchymal odontoblasts during odontogenesis.

SU067

Osteocalcin Released During Bone Resorption in vitro. K. K. Ivaska,* T. J. Heino,* T. A. Hentunen, H. K. Väänänen. Department of Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland.

Osteocalcin is the most abundant non-collagenous protein in bone matrix. It is produced by bone-forming osteoblasts and considered as a marker of bone formation. However, osteocalcin incorporated into bone matrix must also be released during osteoclastic bone resorption. In this study we have used different osteocalcin immunoassays for detecting osteocalcin molecules of resorptive origin in osteoclast cultures.Rat or mouse primary osteoclasts from long bones or human osteoclasts differentiated from peripheral blood mononuclear cells (PBMC) were cultured on bovine bone slices and osteocalcin in the conditioned medium was measured with several in-house immunoassays for different molecular forms of osteocalcin. The rodent cultures were performed using serum depleted of osteocalcin but normal serum was used in human osteoclast cultures in which the resorption activity was higher. The possibility of biosynthetic osteocalcin in rodent cultures was excluded by using assays which do not recognize osteocalcin of rodent origin. In human PBMC cultures the exclusion was made with additional assays specific for human osteocalcin. These human specific assays were not able to detect osteocalcin in the conditioned medium thus confirming the resorptive origin of osteocalcin measured with other assays. Immunoreactive osteocalcin was released from bone matrix to the medium during osteoclast cultures. Osteocalcin levels in the medium increased when stimulators of bone resorption, such as 1,25(OH)2D3 or PTH, were added in the cultures. Bafilomycin A1, a specific vacuolar type proton ATPase inhibitor, blocked the release of osteocalcin into medium. Osteocalcin assays were also useful in monitoring the inhibitory effect of estradiol on human osteoclasts. The concentration of C-terminal telopeptide of type I collagen (CTx), a well-known bone resorption marker in vitro, responded to various treatments in a similar way than concentration of osteocalcin and a correlation between these markers was observed. Osteocalcin released during bone resorption was also isolated and fractionated from the conditioned medium and it consisted of at least three different molecular forms. Interestingly, the most predominant one seemed to be the intact molecule.We conclude that immunodetectable osteocalcin is released from bone matrix during bone resorption. Thus, osteocalcin may be a useful marker for monitoring bone resorption rate in rat and mouse primary osteoclast cultures and in human PBMC cultures. It remains to be evaluated if these in vitro results have significance also in vivo and if serum intact osteocalcin contains molecules derived from bone resorption.

SU068

Revascularization during Bone Regeneration in a Sheep Model of Maxillary Distraction. D. Lewinson,[1] A. Rachmiel,*[2] P. Shenzer.*[1] [1]Anatomy and Cell Biology, Technion-Israel Institute of Technology, Faculty of Medicine, Haifa, Israel, [2]Maxillofacial Surgery, Rambam Medical Center, Haifa, Israel.

The purpose of the study was to clarify the process of revascularization during membranous bone regeneration that is generated by cyclical stress. Following total osteotomy and mounting of a distraction device, we studied biopsies removed from regeneration tissue of maxillary bone in sheep during 18 days of daily distraction. Histological, ultrastructural, and immunohistochemical methods were used. By 10 days of distraction, newly formed delicate bone trabeculae could be observed growing from the edges of the cut bone and recruiting preosteogenic cells from the distraction tissue. Islands of mesenchymal tissue displaying proliferation potential, remained in the central area of the distraction gap even following 15 days of distraction. Three different modes and sites of revascularization were observed: 1) blood vessels, mostly large sinusoids, were penetrating from the old bone into intertrabecular spaces and parallel to the growing bone trabeculae; 2) sprouts of capillaries occupied mainly the central zone of the distracted tissue. These arose by angiogenesis from periosteal and mucosal surfaces; 3) cellular colonies of vascular nature, appearing mostly in adjacent paracentral and less vascularized areas, seem to give rise to new microvessels by a novel in-situ vasculogenesis process. These colonies expressed markers of endothelial cells as demonstrated by immunohistochemistry and were encapsulated in basement membrane seen by transmission electron microscopy. Preliminary studies point to the possibility that endothelial progenitor cells from the circulation might participate in this novel vasculogenesis process. In summary, we describe a novel mode of vasculogenesis during membranous bone distraction in a large mammalian animal model.

SU069

Decreased Bone Formation Accompanies Aging in a Mouse Model of Distraction Osteogenesis. J. Aronson,[1] L. Liu,[1] Z. Liu,[1] G. G. Gao,*[1] D. S. Perrien,*[2] R. A. Skinner,*[3] K. D. Morris,*[2] L. J. Suva,[3] C. K. Lumpkin.[1] [1]Dept of Pediatrics and Orthopaedics, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Arkansas Children's Hosptial Research Institute, Little Rock, AR, USA, [3]Dept of Orthopaedic Surgery, Center for Orthopaedic Research, UAMS, Little Rock, AR, USA.

Distraction osteogenesis (DO) is a unique clinical method for the stimulation of new bone formation and subsequent bone lengthening. DO can be considered a variant of fracture healing, which stretches the biological repair process to its natural limits. In other species (dog, rabbit and rat), DO reflects the clinical situation in which older DO patients demonstrate significant delays in mineralization. Given the considerable value of mouse genetics for studying the mechanism(s) of bone formation, we have developed a reliable murine DO model and utilized it to investigate the effect of age on bone formation. Young (4-month) and old (12-month) CB57BL/6 male mice (n=5 per group) underwent DO. External fixators were placed on the left tibia and mid-diaphyseal tibial osteotomies were performed immediately following fixator placement. Fracture of the fibulae was induced in a similar manner. Distraction began seven days after surgery at 0.075 mm b.i.d. (0.15 mm/day) for 14 days resulting in a total lengthening of 2.1 mm. Following distraction, the mice were sacrificed and distracted tibiae removed for high-resolution radiography and histological evaluation. Analysis of radiographs and representative histological sections was performed by video microscopy, and the relative areas of mineralization and bone column formation in the distraction gap were calculated. Mineralization of the distraction gap (as assessed by radiographic analysis) demonstrated a significant decrease in the mineralized area of distraction gaps of old versus young mice. The percentage mineralized gap area in young mice was 51.4% ± 5.4 compared with 33.5% ± 4.8 in old mice ($p<0.039$). Histological analysis of representative specimens confirmed the bone formation observed in the individual radiographs. The distraction gap appeared to contain numerous cuboidal osteoblasts and an absence of osteoclasts and chondrocytes. Endosteal new bone was predominantly intramembraneous and appeared highly oriented toward the distraction axis. These results suggest that 12-month-old mice have a relative deficit in endosteal bone formation compared to younger adult mice. The application of this murine DO model to genetically manipulated mice may provide critical insights into the mechanisms of bone formation, repair, and regeneration in a geriatric setting.

SU070

Regional Microstructural Heterogeneity in the Turkey Ulna: Implications for Understanding Fluid Flow Dynamics During Functional Loading. K. J. Hunt,*[1] J. G. Skedros.[2] [1]Orthopaedics, Univ. Utah, Salt Lake City, UT, USA, [2]Univ. Utah, Salt Lake City, UT, USA.

Recent evidence suggests that the mechano-sensitivity of osteocytes is mediated by fluid-flow through bone's lacunar-canalicular porosity. This idea has been examined in an analytical model of the turkey ulna [Srinivasan & Gross, Med. Eng. & Phys., 2000]. During normal loading this bone experiences circumferential strain gradients that are highest

along the neutral axis, which typically traverses the cranial-caudal cortices. Additionally, regional differences in fluid-flow dynamics within the turkey ulna have been described. Intercortical and transcortical pressure gradients and fluid flux are largely dependent on matrix porosity. We speculate that heterogeneities in osteocyte lacuna density and non-lacuna porosity, in addition to other material characteristics, might be important considerations in understanding fluid-flow and related strain dynamics. A transverse segment was cut at mid-diaphysis of 11 skeletally mature domestic turkeys, and four 200X backscattered electron images (two endocortical and two pericortical; excluding circumferential lamellae) were obtained from cortical octants: D, D-Cr, Cr, D-Cd, Cd, V-Cd, V, V-Cr (D = dorsal, Cr = cranial, Cd = caudal, V = ventral). These images were examined for osteocyte lacuna population densities and non-lacuna porosity (primary and secondary canals, vascular channels). Secondary osteon population densities were quantified in cortical quadrants (D, V, Cr, Cd). Octant comparisons demonstrated more lacunae in the Cr and Cd cortices compared to the other locations ($p<0.001$) [Means: Cr 1,316.6/mm2; Cd 1,388.0; range in other regions: D-Cd 966.7 to V-Cr 1,100.1]. There was relatively greater porosity in Cd, V-Cd, and D-Cd regions ($p<0.05$). However, non-lacuna porosity and lacuna density were not correlated ($r = 0.008$). Quadrant comparisons showed significantly more secondary osteons in the caudal cortex. Previous data have shown that this region has significantly greater thickness and lower mineralization (%ash). Pericortical-endocortical comparisons showed more lacunae in the pericortical region (1,234.4 vs. 1,170.1, $p=0.05$) and greater non-lacunar porosity in the endocortical region ($p = 0.06$). These data demonstrate significant regional microstructural heterogeneity. In the context of fluid-flow analyses, it is important to recognize that regional variations in lacuna and non-lacuna porosities might not be correlated. These are important considerations in analytical models examining strains and fluid flow induced by anisotropy and architectural changes between the continuum and microstructural levels of bone matrix morphology.

SU071

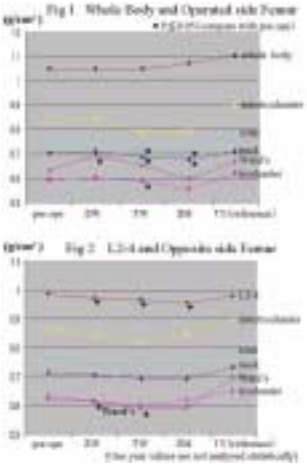
Interplay of Nitric Oxide Synthase (NOS), Cyclooxygenase (COX) and Lipoxygenase (LOX) Pathways in Response to Mechanical Compression of Articular Cartilage. B. Fermor,*[1] H. Bodduluri,[2] J. B. Weinberg,[3] D. S. Pisetsky,*[3] C. Fink,*[1] F. Guilak.*[1] [1]Duke University, Durham, NC, USA, [2]University of Louisville, Louisville, KY, USA, [3]VA and Duke University Medical Centers, Durham, NC, USA.

Mechanical signals play important roles in regulating the homeostasis of articular cartilage. Under abnormal conditions, however, mechanical stress maybe a critical factor in the onset and progression of arthritis. The pathways involved in mechanotransduction are not fully understood but are associated with increased nitric oxide (NO) production via NOS2. NOS inhibitors can prevent the onset and duration of arthritis in experimental models but their influence on other inflammatory mediators such as prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) (derived from the enzymes COX2 and LOX) are not fully understood. To test the hypothesis that physiological levels of mechanical stress may induce the formation of PGE2 or LTB4, articular cartilage explants from 2 year old female pigs were subjected to dynamic mechanical compression at 0.1 MPa, 0.5 Hz. This mechanical regimen has previously been shown to be associated with increased proteoglycan synthesis. Effects of the NOS2 selective inhibitor 1400W or the COX2 selective inhibitor NS398 were also tested. PGE2 and LTB4 levels in the media were measured by radioimmunoassay and expressed as percentage of control. COX1, COX2 and LOX proteins were determined by immunoblot.A 12-fold increase ($p <0.05$) in PGE2 occurred in response to mechanical compression which increased to 40 fold in the presence of 1400W ($p <0.001$). COX2 but not COX1 protein was detected. Mechanical compression plus NS398 diminished the PGE2 and NO induced by mechanical compression alone. LTB4 was not detected in response to compression alone but a 300-fold increase ($p < 0.001$) in LTB4 occurred in response to compression in the presence of 1400W, with increased LOX protein. These data indicate that inducible NO has an important negative feedback role in the COX and LOX pathways in articular cartilage. These findings may have important implications regarding the pathogenesis of arthritis and the development of new biophysical and pharmacological interventions for arthritis.

SU072

Temporary Increment of BMD after Rotational Acetabular Osteotomy. T. Miyajima,[1] K. Nemoto,[2] S. Ninomiya,*[1] A. Itabasi.[2] [1]Orthopedic surgery, Saitama Medical School, Saitama, Japan, [2]Clinical Laboratory Medicine, Saitama Medical School, Saitama, Japan.

Rotational acetabular osteotomy (RAO) is designed to correct a dysplastic acetabulum in adolescents and adults. This surgery requires the patients to keep complete bed rest for a period. We performed a longitudinal evaluation of bone mineral density (BMD) with dual energy X-ray absorptiometry (DXA, Hollogic QDR-4500) for the patient after RAO. Sixty-nine cases of RAO were performed between June 28, 1999 and March 12, 2001. BMD was examined in thirty-one patients. All patients were women with average age of 40.8 (21-55) and osteoporotic patients were not included. No patient underwent an operation on the ipsilateral lower extremity. For the first two weeks after surgery, patients kept a bed rest, and then they were permitted to ride on a wheel chair. For five weeks after surgery, weight bearing on the operated side was forbidden. Only one operator performed BMD measurement on the bilateral proximal aspect of the femur, L2-4 lumbar spine, and whole body, before the surgery and three weeks, seven weeks, three months after surgery. Nine cases in one year after the surgery were added to the investigation as a reference value. The results are summarized in the figure. In the operated side femur, BMD of Ward's triangle increased statistically significantly ($p\leq0.05$) and all other ROIs tended to increase after 3 weeks compared with those before surgery. Then they decreased significantly from 7 weeks to 3 months gradually, almost restored to the baseline after one year. The opposite side BMD showed decrease for the first 3 months, and then BMD reversed to increase for one year, especially in the Ward's triangle BMD showed significant and drastic change. L2-4BMD decreased at the time of 3, 7 weeks and 3 months significantly, and recovered to the baseline after one year while whole body BMD increased for one year except first 7 weeks without any significant difference. Increment of BMD was observed only in the operated side femur. The reason of increment is unclear but following hypothesis has been speculated; 1) Various kind of local osteoblastic factors secreted from the callus of the osteotomy site plays an osteogenetic role. 2) Surgical invasion with vascular obstruction inhibits osteoclast recruitment that results in osteogenesis. Further examinations including animal experiment would be necessary.



SU073

Ontogeny of Cancellous Bone Anisotropy in a Natural "Trajectorial" Structure: Genetics or Epigenetics? J. G. Skedros,[1] J. H. Brady.*[2] [1]Orthopaedics, Univ. Utah, Salt Lake City, UT, USA, [2]Univ. Utah, Salt Lake City, USA.

Since the late 1800s, preferred orientations of cancellous bone trabeculae have been interpreted as adaptations for principal tension and compression stress trajectories produced by habitual bending. During Wolff's formulation of the trajectorial theory of cancellous bone architecture (Wolff's "law" in a strict sense), he often used the human proximal femur as an example of a trajectorial structure. Many other bones exhibiting arched trabeculae have been used in this context (e.g., calcaneus, proximal tibia, metatarsals). Such patterns are commonly used to infer local loading history in extant and extinct animals. However, recent authors who have reviewed the historical use of the trajectorial hypothesis suggest that principal tension and compression strains may not be important in the formation of these distinctive trabecular anisotropies. To examine this possibility, we studied lateral radiographs of an ontogenetic series of sheep and deer calcanei (in vivo "tension/compression" bones; n=15 each), ranging from fetus to adult, for the presence of arched trabeculae (as seen clearly in mature bones). Their presence or absence was also considered in the context of predominant collagen fiber orientation (CFO) in mid-diaphyseal transverse sections (100micron) of the cranial ("compression" = C) and caudal ("tension" = T) cortices of the deer bones. We hypothesized that: 1) fetal bones lack obvious arched trabecular patterns, and 2) the temporal appearance of this anisotropy correlates with strain-mode-specific (T/C) CFO differences. All fetal bones showed the presence of obvious arched trabeculae. However, fetuses and fawns lacked cranial-caudal CFO differences. Since predominant CFO is sensitive in detecting a T/C distribution, it seems unlikely that the trabecular core (relatively lower strains) exhibits strain-mode-specific adaptation when the contiguous/adjacent cortices (higher strains) do not. The arched trabecular tracts may be strongly influenced by the orientation of the epiphyseal growth plate -- the caudal tract and growth plate have the same orientation. The cranial tract's orientation may then represent the programmed deposition of bony trabeculae that tend to maintain a consistent orientation with respect to the plate, and hence form quasi-orthogonal "intersections" with the caudal tract. Arched trabeculae in proximal femora, which strongly resemble those in the calcanei, may follow the same construction rules. This interpretation suggests that a genetically driven developmental program may be relatively more important than epigenetic mechanical stimuli in the initial construction of trabecular patterns in mammalian limb bone epiphyses.

SU074

Role of Bone Formation in Fracture Healing. T. Beil, J. M. Rueger, M. Amling. Trauma Surgery, Hamburg University, Hamburg, Germany.

Fractures are the major clinical problem associated with almost any bone disease. Although our means of therapeutic intervention have been significantly improved through the last decades, the biology of fracture healing remains poorly understood. Given the recently discovered hypothalamic control of bone formation, and thereby its accessibility to external modulation, we reasoned that bone formation would be a possible target for therapeutic intervention to enhance fracture healing in general and improve skeletal repair in cases of delayed- or non-union. Therefore, the aim of this study was to elucidate the specific role of bone formation on fracture repair. Here we compared the process of fracture healing in three mouse models with genetically determined differences in bone formation rate. More specifically, we took advantage of the fact that the ob/ob and the db/db mice, that are both deficient in leptin signaling, have a genetically determined two to three fold increase in bone formation rate in comparison to wildtype mice. To make genetic mouse models accessible to such studies, we modified the tibial fracture model, first described by Einhorn, and developed a standardized closed fracture model of the femur. This model has

the advantage of improved diaphyseal fracture reproduceability (no fibula) and better accessibility to subsequent biomechanical testing. Already radiological analysis and callus morphology demonstrated significant differences in fracture repair dependant on bone formation rate. Furthermore, we will study the qualitative and quantitative micromorphology using histology and histomorphometry. The results of these experiments will be presented at the meeting. To our knowledge this is the first report of a significant influence of bone formation on fracture healing. Therefore these data suggest a possible new approach to enhance fracture repair by targeted modulation of bone formation.

SU075

Osteocyte-Bone Lining Cells System Responds to Cyclic Loading in a Dose-Dependent Manner. A. Rubinacci,*[1] M. Covini,*[2] C. Bisogni,*[2] I. Villa,*[1] M. Galli,*[2] C. Palumbo,*[3] M. Ferretti,*[3] A. Ardizzoni,*[3] G. Marotti.*[3] [1]Bone Metabolic Unit, Scientific Institute H San Raffaele, Milano, Italy, [2]Dept of Bioengineering, Politecnico, Milano, Italy, [3]Dept of Morphological Sciences, University of Modena, Modena, Italy.

The mutual interaction between osteocyte-bone lining cells system (OBLCS) and the surrounding bone extracellular fluid (BECF) which fills the continuous network of lacuno-canalicular microcavities plays a role in mechanotransduction. Since BECF could be modified by the application of a load via streaming potential and stretch activated cation channels mechanisms and since OBLCS is able to respond to axial loading by increasing ion exchanges at the BECF/ECF (systemic extracellular fluid) interface, it is likely that the response of OBLCS to mechanical strain could vary depending on the characteristics (amplitude and frequency) of the load applied.To verify this hypothesis, metatarsal bones of weanling mice were subjected ex vivo, immersed in ECF medium, to axial cyclic loading for two minutes by respectively varying the loading parameters, amplitude and frequency. The electric (ionic) currents at the bone-medium interface were monitored by a voltage-sensitive two-dimensional vibrating probe system before and after loading. By varying the load from 0.7 g to 12 g without changing neither the loading frequency (1Hz) nor the time (2'), the increment in current density was dependent upon the applied loads reaching a plateau at 8 g. Post load current density decreased following different time dependent exponential decays to different asyntotic values depending upon the applied load. By varying the loading frequency from static load to 2 Hz with constant load (5 g) and time, the increment in current density was dependent on the applied frequencies reaching a plateau at 1.5 Hz. Post load current density decreased following different time dependent exponential decays to different asyntotic values depending upon the applied frequency. Static load did not induce any change in the current density. The post load increment in current density was associated to the total energy transferred to bone during the entire loading cycle, as defined by the loading parameters. Post load current density in the dead bones was significantly lower than in the living ones, linearly decayed to background level and did not show any relationship with the applied load. This study showed that OBLCS responds to cyclic loading depending on the applied loading parameters in a dose dependent manner. The higher is the load related perturbation of BECF the faster is the restoration of the preload conditions by OBLCS. Static load did not elicit any detectable OBLCS response.

SU076

BMD Changes up to 3 Years Following Treatment with Zoladex or CMF in Pre-/Perimenopausal Women with Early Breast Cancer Participating in the ZEBRA Study. I. Fogelman, G. M. Blake. Guy's, King's and St Thomas' Hospital Medical School, London, United Kingdom.

The large (n=1640), multicentre, randomized ZEBRA (Zoladex Early Breast Cancer Research Association) study has previously reported that Zoladex (3.6 mg every 28 days for 2 years) is as effective as cyclophosphamide/ methotrexate/5-fluorouracil (CMF; 6 x 28-day cycles) in pre-/perimenopausal patients with estrogen receptor positive early breast cancer. In a protocolled sub-study, bone mineral density (BMD) of the lumbar spine (L2–L4) and neck of femur were assessed by dual-energy X-ray absorptiometry at baseline then annually for up to 5 years. Patients with a baseline and at least one post-baseline measurement at the same site in a protocolled time window were included in the analysis. In total, 96 selected patients from eight centers (Zoladex, n=53; CMF, n=43) were included in the analysis of data to 3 years follow-up. Demographic characteristics and baseline BMD data were well balanced. Mean percentage BMD losses for Zoladex and CMF were 8.2 vs. 4.5 (p=0.00008) at 1 year and 10.5 vs. 6.5 (p=0.0005) at 2 years for lumbar spine, and 4.5 vs. 4.4 (p=0.70) at 1 year and 6.4 vs. 4.5 (p=0.04) at 2 years for neck of femur. After 3 years (i.e. 1 year after cessation of Zoladex) partial recovery of BMD was observed in the Zoladex group, whereas losses persisted in the CMF group overall (lumbar spine: 6.2 with Zoladex [n=29] vs. 7.2 with CMF [n=26], p=0.26; neck of femur: 3.1 with Zoladex [n=30] vs. 4.6 with CMF [n=26], p=0.48). As a result, no significant differences in BMD were observed between the two groups at 3 years. All Zoladex patients in the BMD sub-study became amenorrhoeic while receiving treatment compared with 63% of the CMF group at 48 weeks and 69% at 2 years. Menses returned in the majority of Zoladex patients after cessation of therapy, whereas amenorrhoea was permanent in most CMF patients. In the CMF group, based on amenorrhoea status at 48 weeks, mean percentage BMD losses at the lumbar spine were greater for amenorrhoeic than non-amenorrhoeic patients (2 years: 9.7 [n=18] vs. 2.0 [n=10]). In summary, ovarian suppression resulting in amenorrhoea was closely related to BMD loss in both groups, with the partial recovery of BMD in the Zoladex group associated with return of ovarian function in the majority of patients. Longer term follow-up, including analysis of data to 5 years follow-up, is planned to determine the degree of potential recovery of BMD with Zoladex and whether there is continuing progressive bone loss with CMF.

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SU077

Carboxyl Terminal Trimer of Collagen type I (C3) Induces Directional Migration and Metalloproteinase-2 Activation in Tumor Cells. D. Palmieri,* S. Poggi,* V. Ulivi,* P. Manduca. DOBIG, University of Genova, Genova, Italy.

We have previously shown that the agent secreted in the conditioned medium (CM) from mature osteoblasts and inducing specifically directional migration in endothelial cells, is the carboxyl trimer of procollagen type I (C3, Palmieri et al, 2000 J.Biol.Chem. 275, 32658). Endothelial cells directional migration is dependent on G0/i proteins activity, integrin b1 and b3 and MMP-2 and –9 functionality. Also tumor cells (breast and prostatic carcinoma and melanoma) are chemoattracted by osteoblast CM (Giunciuglio et al. 1995, Cancer letters 97 , 69 and Festuccia et al. 1999, Oncol.Res.11,17). We here show that purified C3 is responsible for the chemoactivity of CM on tumor cells. Chemotaxis is induced with purified C3 and is inhibited by antibodies against C3 chains a1 and a2, by PTX and by antibodies against MMP-2, –9 and uPA.The directional migration induced by C3 in Boyden chambers is concomitant to the induction of MMP-2 and to the activation of MMP-2 in two lines of breast carcinoma cells (BCC) and one of human melanoma. Secreted, free uPA and tPA are constitutively produced by BCC and are not quantitatively changed in migrating cells. The induction of MMP-2 and –14 and the activation of MMP-2 occurs increasingly in time upon exposure of not migratory (adhering to the culture dish) BCC to C3. In these conditions no significant changes occur in the free uPA and tPA levels. C3 is not a mitogen for BCC.C3 is identified by these experiments as the agent produced by mature osteoblasts capable to induce directional migration of both endothelial and carcinoma cells. It is the only agent presently known that induces migration targeting specifically both cell types. C3 production by Collagen type I producing stroma might therefore play a role in the promotion of tumor progression, by affecting tumor cells pericellular proteolysis and their spatial convergence with endothelial cells.

SU078

Disruption of Cell-Cell Contact with Stromal Cells Using Anti-α4integrin Antibody Enhances Sensitivity of Myeloma Cells to Melphalan in Vitro and in Vivo. N. Shimizu,[1] P. J. Williams,*[1] M. Niewolna,*[1] B. Story,*[1] R. Lobb,*[2] G. Mundy,[1] T. Yoneda.[1] [1]Div Endocrinol, Univ TX Hlth Sci Ctr, San Antonio, TX, USA, [2]Biogen, Cambridge, MA, USA.

Multiple myeloma is a B-cell malignancy that has strong predilection for colonizing the bone marrow and is associated with severe osteoclastic bone resorption. Although the precise mechanism of preferential colonization of myeloma cells to bone marrow still needs to be elucidated, it has been suggested that cell-cell contact of myeloma cells with stromal cells via α4β1 integrin and VCAM-1 facilitates their arrest, proliferation, survival and production of osteoclast activating factors in the bone marrow cavity. Therefore, interference with stromal cell/myeloma cell interactions is a potential adjuvant intervention point to enhance the efficacy of anti-cancer agents in myeloma bone disease. Here, we studied the effects of a neutralizing antibody to α4 integrin (α4Ab) which disrupts stromal cells/ myeloma cell interactions on the sensitivity of myeloma to melphalan, one of the most widely-used chemotherapeutic agent for myeloma, using the 5TGM1 mouse myeloma cells that reproducibly cause extensive osteolysis in tumor-bearing animals. The α4Ab (100-200μg/mouse, ip, 2 or 3 times a week) and melphalan (50, 100, 200μg/mouse, ip, once a week) were administered following inoculation of 5TGM1 cells in the tail vein in male xid-nu-bg mice. Melphalan alone at doses of 50 and 100μg/mouse failed to suppress serum IgG2 levels, a systemic indicator of myeloma tumor burden, whereas serum IgG2 levels were significantly suppressed by 200μg/mouse melphalan. On the other hand, combined treatment with melphalan (50 or 100μg/mouse) and α4Ab suppressed serum IgG2 levels to a greater extent than melphalan (200μg/mouse) alone. Consistent with these results, histomorphometric examination revealed that melphalan (50 or 100μg/mouse) combined with α4Ab significantly decreased 5TGM1 tumor burden in bone compared with melphalan (200μg/mouse) alone. To study the role of cell-cell contact of 5TGM1 cells with marrow stromal cells in the sensitivity to melphalan, the effects of melphalan on 5TGM1 cells cultured in contact with the ST2 mouse marrow stromal cells were examined. We found that 5TGM1 cells cultured on ST2 cells exhibited increased survival and reduced apoptosis in the presence of melphalan compared with 5TGM1 cells cultured on tissue culture plates. In summary, our data show that α4Ab enhances the sensitivity of 5TGM1 cells to melphalan and suggest that disruption of stromal cell/myeloma cell interactions using α4Ab is an effective adjuvant therapy, allowing dosage reduction of chemotherapeutic agents and thereby lowering the risk of adverse effects.

SU079

Bone Metastases Decrease and Liver Metastases Increase with Age in a Model of Breast Cancer. T. Yoneda, P. J. Williams,* M. Niewolna,* B. Story.* Endocrinology, University of Texas, San Antonio, TX, USA.

It has long been noted that the frequency of breast tumors increases with age. Although human and animal studies have not definitively demonstrated whether dissemination of breast cancer cells to distant target sites increases or decreases with age, metastasis is likely influenced by local host factors such as fibrosis, angiogenesis and immune response that may change with age. Likewise, bone metastasis is also affected by local bone turnover rate. To examine the relationship between age and tumor behavior, we took an approach in which tumor formation in the orthotopic site and distant metastasis to the bone, lung and liver in breast tumor were examined in female Balb/c mice at 1, 6 and 18-24 month-old. Preliminary histologic examination using TRAP staining demonstrated that osteoclast number/trabecular bone surface was decreased in an age-dependent manner in these mice, suggesting impaired bone turnover rate in aged mice. Mice were inoculated with the 4T1 mouse mammary tumor cells which stably expressed firefly luciferase in the orthotopic mammary fat pad. 4T1 cells inoculated in the orthotopic site consistently grew as a func-

tion of time in all mice. However, the orthotopic tumors were much smaller in 18-24 than 1 and 6 month-old mice. Histologic examination revealed that 78% of 1-month-old mice had prominent bone metastases with osteoclastic bone resorption, while only 6% of 6 and 18-24-month-old mice showed bone metastases, suggesting that bone metastases are dependent on bone turnover rate. To our surprise, liver metastases as determined by luciferase activity were significantly increased in 18-24 month-old mice. To examine whether tumor-associated angiogenesis plays a role in these differences in metastasis, the microvessel density in the metastases in bone, liver and lung was determined using immunocytochemical staining with anti-factor VIII antibody. We found that the microvessel density in the metastases in these organs was not significantly different between mice at different age. Since 4T1 cells are positive for estrogen receptors, it was possible that estrogen influenced 4T1 tumor behavior. ELISA, however, showed that serum estrogen levels were not decreased with age in these mice. In summary, our results suggest that bone metastasis in 4T1 breast cancer is diminished with age due to decreased bone turnover rate. On the other hand, liver metastasis is increased with age by local mechanisms other than angiogenesis. The results also suggest that there is no correlation between orthotopic tumor formation and tumor burden in secondary sites. These results indicate important differences in tumor cell behavior at different sites in different age groups.

SU080

RANK Ligand Expression at Bone Metastatic Sites of Malignant Tumor: Analysis of Autopsy Cases. R. Kitazawa, S. Kitazawa. Division of Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan.

RANK ligand (RANKL) has been identified as a prerequisite to osteoclastogenesis. Most of the cancer-derived bone resorbing factors are now thought to promote bone metastasis by upregulating RANKL expression on osteoblasts. On the other hand, recent reports suggest that RANKL produced by tumor cells might also participate in the development of osteolytic lesions, although details of such pathogenesis remain to be clarified. We therefore examined RANKL expression on the tissues of bone metastasis from malignant tumors obtained at autopsy. Bone metastasis was found in 48 of 168 malignant cases (28.6%). The histology of primary tumors, the mode of bone metastatic lesions and serum calcium levels were analyzed. RANKL expression was assessed by immunohistochemistry with a polyclonal anti-human RANKL antibody and by in situ hybridization with digoxigenin-labeled single stranded DNA probe generated by unidirectional PCR. The 48 cases included 20 with lung cancer (9 adenocarcinoma, 6 small cell carcinoma and 5 squamous cell carcinoma), 7 with oral- pharyngeal cancer, 5 with hepatic cancer and 4 with multiple myeloma; the mode of bone metastasis was osteolytic (32; 66.6%), osteoblastic (2; 4.2%), mixed (2; 4.2%), with intertrabecular pattern (8; 16.7%) and with minor involvement of bone marrow (4; 8.3%). Hypercalcemia (>10.5 mg/dl) was found in 21 cases (43.7%), all of which were associated with osteolytic bone lesions. The incidence of hypercalcemia in the bone metastatic cases was 100% in multiple myeloma (4/4), 85% in oral-pharyngeal cancer (6/7) and 55% in lung cancer (11/20). Among the histological subtypes of lung cancer, hypercalcemia was predominant in squamous cell carcinoma (80%). Immunohistochemically, RANKL expression was detected on osteoblasts/ bone lining cells and, in part, on osteoclasts of the trabecular bone. Overexpression of RANKL, however, was not demonstrated at sites of osteolytic lesions. RANKL expression on tumor cells was immunohistochemically detected in 3 cases with severe hypercalcemia (>16 mg/dl), 2 of which, multiple myeloma and oral squamous cell carcinoma, showed RANKL mRNA expression assessed by in situ hybridization. These data suggested that tumor-derived RANKL may, in part, participate in the development of osteolytic lesions and hypercalcemia.

SU081

High Extracellular Calcium Concentrations Modulate Cell Growth and Estrogen Receptor in MCF-7 Breast Cancer Cells. F. Journé,* J. Dumon,* N. Kheddoumi,* I. Laïos,* G. Leclercq,* J. Body. Laboratory of Endocrinology and Breast Cancer Research, Free University of Brussels, Brussels, Belgium.

Bone tissue is the most common metastatic site for breast cancer, especially for estrogen receptor (ER)-positive cells. Increased osteoclast-mediated bone resorption leads to the release of growth factors but also of large quantities of Ca++, whose effects on breast cancer cells have been little studied. The effects of high extracellular Ca++ concentration ([Ca++]) on tumor cell growth and on ER regulation could, however, be of prominent importance. We examined the MCF-7 cell growth and their ER after a 24-hr incubation with increasing [Ca++]. We tested two ranges of concentrations: 0 to 3 and 5 to 20 mM whose upper limits correspond, respectively, to [Ca++] which can be observed in the serum of cancer patients and in the skeletal microenvironment (40 mM has been reported at resorption sites). We evaluated cell growth by the MTT test. We observed a weak (10%) and progressive decrease of the metabolic activity of tumor cells when [Ca++] was increased from 0 to 10 mM. In the presence of 15 and 20 mM of Ca++, a decrease of 50% was observed, corresponding to a similar decrease in cell population through apoptotic and necrotic processes. At the same high concentrations, Mg++ failed to show any effects, suggesting that Ca++ effects are specific. We then examined the ER regulation and activity after addition of Ca++. We showed by Western blot that high [Ca++] (15 and 20 mM) induced a near complete disappearance of the ER protein. No decrease of the ER expression was observed with the same high [Mg++]. We further investigated the ER synthesis and degradation levels using in situ labelling of cellular proteins with [35S]-cysteine, ER immunoprecipitation, SDS-PAGE and autoradiography. We found that 20 mM of Ca++ decreased ER synthesis by 80% and increased its degradation rate by 34%, both explaining the decrease of ER protein expression. Moreover, in MVLN cells (MCF-7 cells stably transfected with the ERE cloned upstream the luciferase reporter gene), we observed that Ca++ led to a dose-related increase of ER transcriptional activity which reached 150% of control values with a 20 mM [Ca++]. These results show that Ca++ may act as an "estrogen-like" compound on ER regulation, acting most probably onto the membrane, may be through specific Ca++ receptors, since increase of intracellular [Ca++] by the ionophore A23187 failed to show similar effects. These data suggest that Ca++ released during the process of metastatic bone resorption could modulate breast cancer cells growth and the function of ER. Our observations could be relevant to the pathogenesis and the therapy of breast cancer-induced osteolysis.

SU082

Bioluminescent Reporter Imaging of Cancer Metastasis to Bone Marrow and Bone: A Mouse Model of Minimal Residual Disease. A. Wetterwald,*[1] E. Gautschi,*[1] G. van der Pluijm,[2] J. Buijs,*[2] M. Karperien,[2] B. Sijmons,*[2] C. Löwik,[2] G. Thalmann,*[1] M. Cecchini.[1] [1]Gene Therapy Lab., Dept. of Clinical Research and Urology, University of Bern, Inselspital, Bern, Switzerland, [2]Endocrinology, Leiden University Medical Center, Leiden, The Netherlands.

Mortality in cancer patients is increasingly linked to metastatic disease. Bone is the second most common site of overt metastasis. Furthermore, bone marrow is a relatively early site of micrometastatic spread that cannot be detected by conventional staging methods nor influenced by current treatment. Thus, there is the need to develop new therapeutic strategies to be tested in experimental animal models able to mimic the micrometastatic spread. For this, non-invasive and sensitive methods able to detect directly early stages of metastatic growth are needed. We describe here the use of a CCD camera connected to the Argus-20 image processor (C-2400-47/VIM, Hamamatsu) to monitor bone metastasis in vivo by cancer cells stably transfected with the luciferase reporter gene (Bioluminescent Reporter Imaging, BRI).The human mammary carcinoma cell line MDA-MB-231 were stably transfected with the pCMV plasmid containing the firefly luciferase gene (MDA-231/Luc+). BALB/c nu/nu mice were injected into the left cardiac ventricle with MDA-231/Luc+ cells and development of bone metastases was monitored radiographically and by BRI at weekly intervals. Distinct photon emission localized in long bones and spine was first detected 24 days after intracardiac injection. At the same time point, no osteolytic lesions were detectable by radiography. Instead, first osteolytic lesions were seen on radiographs not earlier than 35 days after injection of tumor cells and they were representing only a minority of the bone metastatic sites identified by BRI at the same time point. In order to determine the minimal number of cells detectable by BRI, different amounts of cells were implanted intra-osseously. The lowest detection limit in bone was 20'000 cells. For comparison, as little as 1000 cells were detected after subcutaneous implantation.Advantages of BRI are early detection of minimal bone metastatic lesions, possibility to follow the kinetics of tumor growth in the same animal, and direct quantification of the tumor burden for each metastatic site. This method will be extremely useful to follow the natural history of minimal residual disease and the development of metastases in living animals, and to verify the efficacy of novel therapeutic approaches (e.g. anti-angiogenic agents) aimed at repression of the initial stages of the metastatic disease.

SU083

Myeloma Cells Express and Release RANK-Ligand and Promote the Formation of Osteoclast-like Cells. P. Boissy,*[1] T. Plesner,*[2] M. Dartell,*[1] K. Norrild,*[3] N. Hastrup,*[3] M. Rasmussen,*[3] E. Gaarsdal,*[3] J. M. Delaissé,[1] D. M. Anderson.[4] [1]OSTEOPRO A/S, Herley, Denmark, [2]Department of Hematology, Vejle Hospital, Vejle, Denmark, [3]Department of Hematology and Pathology, Herlev Hospital, Herlev, Denmark, [4]Immunex Corporation, Seattle, WA, USA.

Osteolytic lesions are common symptoms of patients with Multiple Myeloma and contribute significantly to their morbidity. This excessive bone degradation is caused by an increase in osteoclast formation and activity. Until now, it was considered that such an effect of myeloma cells was mediated through neighboring bone marrow stromal cells which, after induction express RANKL, a pivotal transmembrane factor for osteoclast differentiation and activation. Since previous studies have reported the expression of RANKL by myeloid cells like activated T cells, we investigated whether it may also be detected in myeloma cells and whether those cells may promote directly osteoclast differentiation. By immunostaining, we detected RANKL in plasma cells of bone marrow biopsies from all of 19 patients with multiple myeloma as well as in 8/8 human myeloma cell lines. Analysis by flow cytometry confirmed the presence of RANKL in cytoplasm of myeloma cell lines whereas at the cell surface, it was faintly detected. Furthermore, ELISA as well as western blot experiments using both anti-RANKL antibodies and RANK-Fc fusion protein, also revealed that a soluble form of this factor can be found in the culture medium of these cancer cells. Finally, to determine whether RANKL produced by myeloma cells was functional, we studied osteoclast differentiation in culture of human peripheral blood monocytes only maintained in presence of M-CSF. Treatment of cells with conditioned medium from fresh myeloma cell line cultures strongly promoted the formation of TRAP positive multinucleated osteoclast-like cells whereas co-treatment at the same time with OPG, the decoy receptor of RANKL, reduced significantly this effect. Our study strongly suggests that during bone destruction induced by multiple myeloma, myeloma cells could be involved directly in osteoclast differentiation by expressing RANKL. These observations may provide basis for new strategies for treatment and prevention of osteolysis in myeloma.

SU084

Differentially Expressed Genes in Human Giant Cell Tumor of Bone. M. Wuelling,* G. Delling, E. A. Kaiser. Bone Pathology, Center for Biomechanics, Hamburg, Germany.

The Giant Cell Tumor (GCT) is a unique model for the hematopoietic-stromal cell interaction in human bone. There is evidence that the stromal cells in GCT foster accumulation, size and activity of the giant cells albeit hardly any information about the properties of the stromal cells are available. Further, the GCT is of intermediary malignancy, but so

far no genes have been identified that contribute for the transformation. Therefore, we initially characterized the origin of the GCT neoplastic cells with the intention to define the corresponding control cell type for differential gene expression analysis utilizing cDNA techniques (Tumor cDNA Array, Clontech). We compared the expression profile of commercially available mesenchymal stem cells (MSC) and normal human bone marrow stromal cells with the selected and enriched neoplastic cells of four GCTs. From resected GCT all cell types were isolated and with progressing passage monocytic and giant cells were lost, augmenting the stromal component of GCT.From passage three RNA was prepared, radioactively labeled during reverse transcription and used to probe the Atlas Cancer 1.2 Array with 1176 genes that are significant in tumor development. Results were analyzed using a phosphorimager and the Atlas Image 2.0 software and differently regulated genes were selected for further analysis.Hybridization results reveal regulation of distinct genes in the expression pattern comparison of MSCs and the stromal cells of GCTs, validating the choice of MSCs as control cells. We found the expression of the Metalloproteinase 3 Inhibitor and some extracellular matrix proteins downregulated, whereas the expression of receptor proteins were increase in the GCT stromal cells. These are genes that are important in the regulation of matrix degradation and proliferation/differentiation and may be responsible for the GCT expansion and growth. Further in vitro studies and in situ hybridization studies are currently being conducted to determine their direct or indirect role of these genes role in GCT development.

SU085

Scanning Electron Microscopy Reveals Directional Responses of Breast Cancer Cells to Osteonectin. C. V. Gay,[1] A. M. Mastro,*[1] D. R. Welch.*[2] [1]Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA, [2]Jake Gittlen Cancer Research Institute, The Pennsylvania State University, University Park, PA, USA.

Osteonectin, a bone derived peptide, has been shown to be a powerful chemoattractant for prostate cancer cells (Jacob *et al.*, Cancer Res. 59:4453-4457, 1999). Because bone is also a preferred site for breast cancer metastasis, we compared breast cancer cell morphology on osteonectin coated bone wafers by scanning electron microscopy. Devitalized bovine cortical bone slices were coated with 1 µg/ml osteonectin derived from bovine bone or with vehicle and air dried. Metastatic MDA-MB-231 and MDA-MB-435 breast cancer cells ($1x10^4$) were placed on ~0.5 cm^2 bone surfaces in 1 ml DME culture medium + 5% fetal bovine serum for 1 week. The preparations were sequentially fixed with 2.5% glutaraldehyde in cacodylate buffer and 1% osmium tetroxide, followed by dehydration, critical point drying with liquid CO_2 and sputter coating with gold-palladium. Scanning electron microscopy revealed numerous microvillar-like cell processes on cell surfaces facing the osteonectin coated bone surfaces. These cells were thick and amoeba-like and also had pseudopods stretching along the bone surface. In the absence of osteonectin the cells remained flat and had few microvillar processes and pseudopods. This pronounced effect of osteonectin on cell shape and localized formation of cell processes indicates that breast cancer cells have or develop receptors and signal pathways needed to respond to osteonectin in a directional manner. This study provides evidence that osteonectin is chemotactic for breast cancer cells.

SU086

Activators of NonGenotropic Estrogen-like Signaling (ANGELS) are Devoid of the Mitogenic Effects of Estrogen on MCF-7 Breast Cancer Cells. A. T. Mancino, Y. Wen,* L. Han, T. Bellido, S. Kousteni, S. C. Manolagas. Division of Endocrinology & Metabolism and Department of Surgery, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

In MCF-7 breast carcinoma cells, estrogens acting through the estrogen receptor (ER) rapidly induce ERK activation as well as cell proliferation. However, several studies have shown that the mitogenic effect of estrogens in MCF-7 cells is due to increased transcription of the immediate response genes c-fos, c-myc and pS2, is mediated by cyclins, and is independent of ERK activation. ER-mediated rapid nongenotropic MAPK activation can be functionally dissociated from the transcriptional activity of the ER; and this can be accomplished using synthetic ligands. To assess the relative contribution of nongenotropic, and in particular MAPK activation, versus genotropic actions of the ER to the estrogen-dependent growth of cancer cells, we have compared the effects of 17β-estradiol (E_2) on breast cancer cell proliferation to the effects of two such synthetic ligands: an estren which has no transcriptional activity via the ER but is a potent inducer of ERK activation, and a pyrazole with strong transcriptional activity but no effect on ERK activation. In these experiments, proliferation was assayed by ^{3}H-Thymidine uptake or by the reduction of MTT to the formazan product. MCF-7, or ER-negative MDA-MB-231 cells, maintained in 10% FCS, were transferred to serum-free medium containing 10^{-8} M ICI 182,780. 96 hrs later, the ICI-containing medium was removed and cultures were continued for another 48 (for the ^{3}H-Thymidine assay) or 72 hrs (for the MTT assay) in the presence of 10^{-7} M of the test compounds or vehicle. Like E_2, pyrazol stimulated proliferation of MCF-7 cells, albeit somewhat less potently (6- vs 4-fold) compared to vehicle. In sharp contrast, estren had no effect on MCF-7 cell proliferation. None of the compounds induced proliferation in the ER negative MDA-MB-231 cells. Consistent with the differential effects of the estren vs the pyrazole, the proliferative effect of E_2 and pyrazole was not influenced by the specific inhibitor of ERK phosphorylation, PD98059 (10 µ M), indicating that these actions were independent of ERK phosphorylation, but dependent on the classical genotropic action of the activated ER. These results support the contention that ANGELS may not only be an advantageous class of pharmacotherapeutic agents for the management of osteopenic states during post-reproductive life (true anabolic as opposed to antiresorptive), but may also circumvent some of the adverse effects of classical estrogen on breast cancer.

SU087

Chromosome 18 Suppresses Prostate Cancer Metastases. S. S. Padalecki, T. L. Johnson-Pais,* K. S. Weldon,* X. Reveles,* C. L. Buller,* B. Grubbs,* Y. Cui,* J. J. Yin, M. Dallas,* R. J. Leach,* T. A. Guise. U.T. Health Science Center, San Antonio, TX, USA.

The skeleton is the most common site of prostate cancer metastases. Most patients with advanced disease have bone metastases, and these lesions contribute significantly to the morbidity and mortality of the disease. Previous data have shown that the prostate cancer cell line, PC-3, metastasizes to bone in nude mice following inoculation of tumor cells into the left cardiac ventricle. Loss of heterozygosity (LOH) data in humans has shown that there are two distinct regions of loss on chromosome 18 associated with the progression of prostate cancer. Analysis of PC-3 DNA indicates that PC-3 harbors a stretch of extended homozygosity in one of these regions, indicating possible LOH events in this region. To investigate the functional significance of chromosome 18q LOH in prostate cancer metastasis to bone, we utilized the technique of microcell-mediated chromosome transfer to introduce an intact chromosome 18 into PC-3 cells (PC-3 hybrids). Two of the resulting hybrid cell lines were compared to the PC-3 parental cell line *in vitro* and *in vivo*. To assess the ability of the cells to metastasize to bone, mice underwent tumor inoculation with PC-3 cells or the resulting hybrids via cardiac injection. Following administration of the tumor cells or hybrids, mice were regularly monitored for the evidence of bone metastases by Faxitron X-ray. Upon sacrifice, long bones, spine, calvaria and soft tissues were harvested and processed for histomorphometric analysis. Bone histomorphometric analysis was performed on samples to measure tumor burden, total bone area and osteoclast number at the tumor-bone interface (an indicator of tumor-induced osteolysis). The hybrid cell lines containing intact copies of chromosome 18 exhibited a substantial reduction in anchorage-dependent and –independent growth *in vitro*. These PC-3 hybrids made smaller tumors in nude mice following subcutaneous injection compared to PC-3 parental cells. Mice inoculated into the left cardiac ventricle with PC-3 hybrids had significantly fewer osteolytic bone metastases and dramatically improved survival compared with control PC-3 cells. In addition, the introduction of an intact copy of chromosome 18 significantly reduced tumor burden in non-bone sites. Histomorphometric analysis of bone revealed that mice bearing PC-3 hybrids had less tumor burden in bone. Taken together, these data suggest that chromosome 18 has a functional role in prostate cancer to suppress growth and metastases. Identification of the responsible genes may lead to molecular targets for drug discovery.

SU088

Parathyroid Hormone Related Protein Inhibits All-Retinoic Acid-induced Apoptosis in Prostate Cancer Cell Lines. F. C. Asadi,* W. Zheng,* R. Zariffard,* S. C. Kukreja. Medicine, VA Chicago-West Side/Univ. of Illinois, Chicago, IL, USA.

Previous studies have demonstrated that parathyroid hormone related protein (PTHrP)levels are high in prostate cancer and that PTHrP expression is greater in poorly differentiated tumors as compared to that in well differentiated tumors. In the advanced stages, prostate cancer cells become androgen-independent and resist apoptosis. PTHrP has been demonstrated to be an intracellular regulator of cell cycle progression and apoptosis. In the present study, we evaluated the effects of PTHrP (1-34) on All-trans retinoic acid (tRA)-induced apoptosis in an androgen-responsive (LnCaP) and androgen-unresponsive (C4-2 LnCaP) cell lines of human prostate cancer.The effects of dihydrotestosterone (DHT) or PTHrP on tRA-induced apoptosis and viability were tested in cells cultured under serum free conditions for 72 hours. Cell viability was tested by MTS Proliferation assay and apoptosis by intracellular DNA fragmentation analysis and caspase-3 activity assay. In both cell lines, tRA (5uM) treatment reduced cell viability. DHT (10nM) treatment had no effect, whereas PTHrP (1-34)(100nM) treatment protected against this tRA-induced loss in cell viability. Similarly, PTHrP had a protective effect against tRA-induced DNA fragmentation and caspase-3 activation in both cell lines, whereas addition of DHT had no effect on the tRA-induced changes. These results demonstrate that PTHrP (1-34) has protective effects against tRA-induced apoptosis in both androgen-responsive and androgen-unresponsive prostate cancer cell lines. Further studies to evaluate the effects of PTHrP-neutralization on apoptosis are warranted.

SU089

Androgens Regulate Parathyroid Hormone Related Peptide (PTHrP) Production in Human Prostate Cancer Cells. H. Pizzi,* L. Carpio,* D. Goltzman, S. A. Rabbani. Department of Medicine, McGill University Health Center, McGill University, Montreal, PQ, Canada.

Androgen insensitive prostate cancer is known to be more aggressive than androgen responsive cancer. As well, higher PTHrP expression has been found in neoplastic versus hyperplastic prostatic lesions, and PTHrP overproduction has been associated with other advanced neoplasms. We therefore assessed the ability of androgens to regulate PTHrP in prostate cancer by using androgen insensitive PC-3 prostate cancer cells and cells transfected with a functional human androgen receptor (PC-3T). PC-3T cells grew more slowly in vitro in whole serum or in charcoal treated stripped serum supplemented with dihydrotestosterone (DHT) than in stripped serum alone. Additionally, in PC-3T cells, whole serum or DHT in stripped serum inhibited PTHrP mRNA level and immunoreactive PTHrP production. The DHT effect could be blocked by the androgen receptor antagonist, flutamide. In cells transiently transfected with a PTHrP promoter- luciferase reporter construct, DHT induced a 30% decrease in luciferase activity. Subsequent analysis of PTHrP promoter deletion constructs suggested the presence of a putative ARE. PC-3T cells injected subcutaneously into athymic nude mice developed palpable tumors later (4 weeks) than did PC-3 cells (2 weeks) but castration of the host animals increased PC-3T cells growth. PTHRP mRNA levels were higher in tumors of PC-3 cells than of PC-3T cells and castra-

tion enhanced PTHrP mRNA expression in PC-3T tumors. These results indicate that prostate tumor cell growth correlates inversely with androgen sensitivity and directly with PTHrP production both in vitro and in vivo in this model, that androgens can directly regulate PTHrP production, and that the androgen effect is mediated by transcriptional regulation via the androgen receptor.

SU090

Expression and Activity of Cathepsin K in Prostate Cancer. K. D. Brubaker,[1] R. Thomas,[2] R. L. Vessella,*[1] E. Corey.[1] [1]Department of Urology, University of Washington, Seattle, WA, USA, [2]Bracco Research USA, Princeton, NJ, USA.

Prostate cancer (CaP) bone metastases are generally osteoblastic in nature, while breast cancer bone metastases are osteolytic. Even though CaP bone metastases are osteoblastic, markers of bone resorption are elevated in patients with CaP bone metastases. It has been reported that tumor cells need a site of resorption for attachment in bone. Osteoclasts create these sites of attachment through lysis and degradation of the extracellular matrix and mineralized bone. Cathepsin K (cat K) is a cysteine protease that is critical for osteoclast-mediated collagen degradation. Cat K expression has been reported in other cell types, such as breast cancer and lung epithelial cells, in addition to osteoclasts. This study was undertaken to determine whether CaP cells express cat K as a possible participant in the establishment of CaP bone metastases. RT-PCR demonstrated the expression of cat K mRNA in all CaP cell lines tested (DU 145, PC-3, N-PC-3, B-PC-3, LNCaP, C4 and C4-2). Non-radioactive in situ hybridization showed the presence of cat K mRNA in normal prostate glands and CaP. Immunohistochemistry with a chicken polyclonal antibody raised against amino acids 266-275 of human cat K demonstrated immunoreactivity in CaP and CaP metastases, while normal prostate epithelium was negative. The presence of cat K activity was demonstrated in CaP cell lines, PC-3, DU 145 and LNCaP, using Z-GPR-MCA as a substrate. Osteoclasts generated from murine bone marrow were used as a positive control. Since Z-GPR-MCA can be cleaved by cathepsin B (cat B), but with much less specificity, the cat B inhibitor CA-074 was used to block this activity. Approximately 40% of the activity was lost with CA-074 treatment. We found that cat K activity was approximately 10 fold higher in LNCaP than in PC-3. In conclusion, we have shown that CaP cells express cat K which has proteolytic activity, therefore we hypothesize that cat K could be involved in the establishment of bone metastases by initiating or enhancing degradation of collagen, and that this may represent a priming step for the establishment of tumor cells in the bone milieu.

SU091

Bone Morphogenetic Protein Receptors IA, IB and II mRNA and Protein Expression in Prostate Cancer. K. D. Brubaker,[1] R. Thomas,[2] R. L. Vessella.*[1] [1]Department of Urology, University of Washington, Seattle, WA, USA, [2]Bracco Research USA, Princeton, NJ, USA.

Skeletal metastases are the most common cause of morbidity in men with advanced prostate cancer (CaP) and are usually osteoblastic. Bone morphogenetic proteins (BMPs) can induce bone formation at ectopic sites and our previous studies demonstrate that high levels of BMPs -4 and -7 are present in CaP osseous metastases, suggesting a role in the formation of osteoblastic lesions. BMPs signal through a complex of type I and II receptors, which initiates a cascade of events that regulates many processes, including osteoblast differentiation. In this study we characterized the expression of BMP receptors IA, IB and II in CaP progression, including osseous metastases, to determine the potential for autocrine effects of CaP-expressed BMPs in addition to paracrine effects on osteoblasts.Antibodies against BMPRs IA, IB and II were used to determine protein expression in prostatic tissues and cell lines by Western blotting and immunohistochemistry (IHC). Western blotting demonstrated BMPR II in all CaP cell lines, DU 145, PC-3, LNCaP and LNCaP sublines, C-4 and C4-2B, and normal/CaP tissues, whereas BMPR IB was only detected in LNCaP and its sublines and normal/CaP tissues. BMPR IA was only detected in normal/CaP tissues. IHC (see table) revealed BMPR IA and II expression in benign prostatic tissues (10/10), while BMPR IB was present in 2/10 samples. In low grade primary CaP, BMPR IB was absent, while BMPR IA and BMPR II were present (10/10). BMPR IB was present in 6/10 high grade CaP, while BMPR IA was observed in only 1/10 high grade samples. BMPR II was observed in 7/10 high grade CaP. BMPR IB was observed in 3/9 osseous metastases, while BMPR II was detected in 7/9 osseous metastases. BMPR IA was not present in osseous metastases, except for staining of bone marrow cells and cells lining bone. All three receptors were absent in non-osseous metastases (n=3).

BMP Receptor Protein Expression in Prostatic Tissues

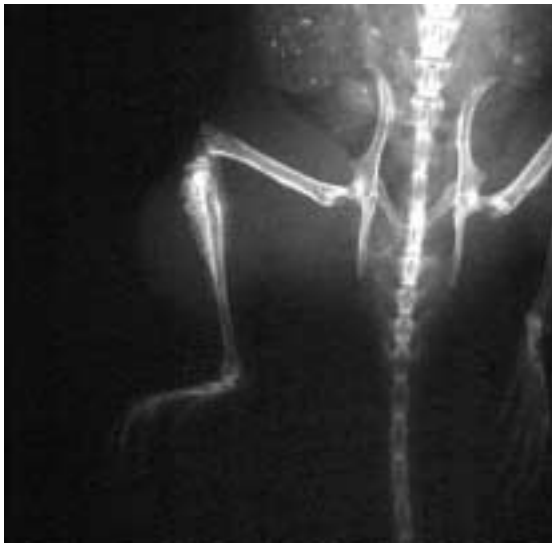
	Benign n=10	Low Grade n=10	High Grade n=10	Non-Osseous Mets n=3	Osseous Mets n=9
No Receptors	0	0	3	3	2
IA + II	8	10	1	0	0
IB + II	0	0	6	0	3
IA + IB + II	2	0	0	0	0
II	0	0	0	0	4

In situ hybridization studies revealed expression of all three receptors in all tissues tested, with higher levels in primary CaP and metastases, as compared to normal tissues. In summary, we have demonstrated the presence of BMP receptors in prostatic tissues from various stages of cancer progression. The differences in expression of BMPRs IA and IB in low and high grade CaP, suggests varying roles for these type I receptors in the prostate. This study also reveals the potential for autocrine signaling through CaP-expressed BMPs in osseous metastases, in addition to their effects on osteoblasts

SU092

Differences in the Cytokine Profiles Associated with Prostate Cancer Cell Induced Osteoblastic and Osteolytic Lesions in Bone. Y. Lee,*[1] E. Schwarz,*[2] M. Davies,*[1] M. Jo,*[1] X. Zhang,*[1] J. Wu,*[1] J. Lieberman.[1] [1]Orthopaedic Surgery, UCLA School of Medicine, Los Angeles, CA, USA, [2]Department of Orthopaedic Surgery, University of Rochester Medical Center, Rochester, NY, USA.

Prostate cancer metastases to bone are associated with the development of osteoblastic lesions. The pathophysiology of the formation of osteoblastic metastases remains poorly understood. Two different prostate cancer cell lines were injected into the tibias of SCID mice to study the cytokine production associated with the formation of osteolytic and osteoblastic lesions in bone.PC-3 and LAPC-9 cells were isolated in cell suspension from tumors. Tumor cells (1x105) were injected into the intramedullary canal of the tibias of SCID mice. Animals were sacrificed at 1, 2, 4, and 6 weeks after injection. Radiographs of the mouse tibias demonstrated that the PC-3 cells were associated with the formation of lytic lesions whereas the LAPC-9 cell injections formed osteoblastic lesions in bone (See figure). Histological analysis of the PC-3 treated tibias showed cortical erosion occurring at two weeks with significant bony destruction occurring at six weeks. Immunostains showed moderate interleukin-1 (IL-1) activity, minimal interleukin-6 (IL-6) and tumor necrosis factor-a (TNF-a) activity, and mild OPG expression. Tartrate-resistant acid phosphatase (TRAP) stains revealed abundant TRAP positive cells (osteoclasts) lining the intramedullary canal. Histological analysis of the mice injected with LAPC-9 cells showed abundant bone formation in the intramedullary canal at six weeks. Immunostains showed moderate IL-6 activity, minimal IL-1 and TNF-a activity, and abundant OPG expression. TRAP stains revealed very few osteoclast-like cells. PCR data revealed that PC-3 cells produced RANKL but LAPC-9 cells did not. This data suggests that the different cytokines produced by prostate tumors may determine what types of lesions are formed in bone. IL-1 and RANKL have been shown to activate NF-kappaB, which is important for osteoclast survival and activation. IL-6 in turn has been shown to be associated with osteoblast activity and OPG can inhibit osteoclast activiation. Further study of the cytokine production of prostate cancer cells is necessary to elucidate the pathophysiology of metastatic osteoblastic lesions in bone.



SU093

Prostate Cancer Cells Secrete Interleukin-18 and Inhibit Osteoclast Formation. B. L. Hill, X. Sun, X. Li,* S. Choi, G. D. Roodman, G. R. Mundy, J. M. Chirgwin. Medicine, U TX Hlth Sci Ctr, San Antonio, TX, USA.

Bone metastases in prostate cancer are predominantly osteoblastic, although the tumor cells frequently express the osteolytic factor, PTHrP. This suggested to us that prostate cancer cells might also express factors which inhibit osteoclast formation. We found that three of four prostate cancer cell lines (PC3, DU145, TSU-Pr1) inhibited TRAP+ multi-nucleated cell (MNC) formation in co-culture with bone marrow cells stimulated by 10nM 1,25-dihydroxyvitamin D3 (vitD3). Similar inhibition was seen in co-culture experiments with prostate cancer cells plus the marrow stromal cell line ST2 and spleen non-adherent cells as a source of osteoclast precursors. Similar results were obtained when ST2 cells were substituted with primary mouse calvarial osteoblasts, when co-culture was replaced by the addition of 20% v/v serum-free conditioned medium (CM) from prostate cancer cells, or when the positive stimulus was 10nM PTHrP1-34. The prostate cancer cell lines were screened by RT-PCR for expression of factors active on bone cells. The breast cancer cell line MDA-MB-231 did not inhibit osteoclast formation in these experiments and was used as a negative control for the RT-PCR analysis. None of the cell lines expressed osteoprotegerin, interferon-gamma, or platelet-derived growth factor A or B chains.We found the prostate cell lines expressed mRNAs for several stimulators of osteoclast formation (PTHrP, RANK ligand) as well as for two potential inhibitors: fibroblast growth factor-2 (FGF-2) and interleukin-18 (IL-18). When protein secretion of the two inhibitors was

tested by ELISA of CM, we found that prostate cancer cells secreted only IL-18, a potent inhibitor of osteoclast formation. The cells did not express mRNA for the inhibitory IL-18 binding proteins. PC3 cells secreted 500pg/ml IL-18/105 cells/48hrs. Under these same conditions DU145 and TSU-Pr1 cells made 100-200pg IL-18. MDA-MB-231, 3 other breast cancer cell lines, and LNCaP prostate cells, made 20pg/ml or less. When 100ng/ml IL-18-neutralizing antibody was added to PC3 conditioned medium, twice as many TRAP+ MNC were formed in the presence of the medium (10%v/v) than in the presence of medium not treated with the antibody. 10ng/ml of antibody completely relieved the 50% inhibition of TRAP+ MNC formation caused by addition of 20%v/v PC3 CM to mouse bone marrow cultures treated with 10nM vitD3. The results suggest that prostate cancers metastatic to bone may regulate bone resorption by producing inhibitors of osteoclast formation such as interleukin-18, which counter the effects of osteolytic factors such as PTHrP.

SU094

Osteoprotegerin Production by Prostate Cancer Cell Lines. H. Penno,*[1] C. Silfverswärd,*[1] A. Frost,[1] H. Brändström,*[2] O. Nilsson,*[1] Ö. Ljunggren.[2] [1]Department of Surgical Sciences, Uppsala, Sweden, [2]Department of Medical Sciences, Uppsala, Sweden.

Osteoprotegerin (OPG), a member of the tumor necrosis receptor family, is produced by various tissues in the human body, and by malignant cell lineages, i.e. giant cell tumor of the bone and osteosarcoma. OPG inhibits osteoclast differentiation and activity. Since the metastasis of prostate cancer to the bone have an osteosclerotic effect, the production of OPG by these cells is of interest, and also if this production is under the regulation of various cytokines or hormones. We have investigated whether the prostate cancer cel lines LNCaP, PC-3 and DU-145 produce and secrete OPG. OPG transcripts were detected by RT-PCR in all cell lines. An ELISA was developed for measuring OPG in culture media. The highest concentration of OPG was detected in the culture media of DU-145, followed by PC-3 and LNCaP. In all three lineages, treatment with tumor necrosis factor-alpha dose dependently (5-5000 pM) induced elevated OPG secretion. Treatment with interleukin-1 in increasing concentrations (5-5000 pM) stimulated OPG secretion in PC-3 and in LNCaP but had no effect in DU-145 cells. Dexamethasone (100 pM) treatment decreased OPG production in DU-145, but did not affect OPG production in PC-3 or LNCaP cells. In conclusion OPG production by different lineages of prostate cancer cells could be measured by ELISA and the production could be stimulated or inhibited by treatment with cytokines and dexamethasone to various degrees.

SU095

Androgen Deprivation Therapy Causes Bone Loss Predominantly at the Radius. P. S. Coates,* J. Wagner,* J. Ribich,* D. L. Trump,* J. B. Nelson,* S. L. Greenspan. University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

Androgen deprivation is the most effective systemic therapy for prostate cancer but is a major risk factor for osteoporosis in men. To examine if there is a differential effect on the site and rate of bone loss in men on androgen deprivation, we will follow 180 men with prostate cancer and 45 healthy controls prospectively over 2 years. Bone mass and body composition were measured with a Hologic QDR-4500A densitometer. Results from baseline evaluation in 49 men with prostate cancer (table, mean ±SD): including men with 1) no androgen deprivation =-AnDep, 2) androgen deprivation >6months =+AnDep (mean 26 months), 3) advanced cancer on androgen deprivation (mean 37 months) plus other systemic therapy =++AnDep. There were no differences in age, height or weight, but a trend for decreased lean mass and increased fat mass in men +AnDep or ++AnDep. Bone mass at all radial sites was lower in both groups on +AnDep vs. -AnDep with a similar trend at the spine but not the hip. There was a significant negative correlation between duration of androgen deprivation and BMD at all radial sites ($p<0.001$), femoral neck ($p<0.01$) and total hip ($p=0.01$) but not the spine ($p=0.19$). Age and body composition were not related to duration of androgen deprivation. We conclude that androgen deprivation has a greater impact on radial bone than at other skeletal sites.

	-AnDep (N=18)	+AnDep (N=20)	++AnDep (N=11)
Age (years)	67 ± 7	69 ± 10	69 ± 8
PSA (ng/ml)	2.5 ± 3	13.9 ± 26	67.7 ± 79*
% body fat	26 ± 7	29 ± 4	31 ± 7
Distal radius BMD, g/cm^2	0.764 ± 0.07	0.696 ± 0.11*	0.673 ± 0.09**
Ultradistal radius BMD, g/cm^2	0.487 ± 0.08	0.431 ± 0.09	0.411 ± 0.07*
Total radius BMD, g/cm^2	0.638 ± 0.07	0.575 ± 0.11	0.553 ± 0.08**
PA spine BMD, g/cm^2	1.099 ± 0.25	1.040 ± 0.24	1.028 ± 0.24
Total hip BMD, g/cm^2	0.973 ± 0.21	0.900 ± 0.19	0.965 ± 0.13
Femoral neck BMD, g/cm^2	0.797 ± 0.17	0.686 ± 0.16	0.759 ± 0.15

*p<0.05, **p<0.01, -AnDep vs. +AnDep or ++AnDep

SU096

Androgen-independent Induction of Prostate-Specific Antigen in Prostate Cancer Cells by Factors Secreted from Osteoblast-like Cells. T. S. Y. Kim,*[1] N. Bruchovsky,*[1] G. McAlinden,*[2] C. P. Duncan,*[2] E. C. Jones,*[3] D. D. Schnabel,*[4] H. U. Schweikert,*[4] M. D. Sadar.*[1] [1]Cancer Endocrinology, BC Cancer Agency, Vancouver, BC, Canada, [2]Orthopaedic Surgery, Vancouver General Hospital, Vancouver, Canada, [3]Clinical Pathology, Vancouver General Hospital, Vancouver, Canada, [4]University of Bonn, Bonn, Germany.

Carcinoma of the prostate has a propensity to form osteoblastic lesions. Osteoblasts may reciprocate paracrine factors that stimulate proliferation and hormonal progression of prostate cancer cells to the terminal androgen-independent stage of the disease. Progression to androgen-independence is monitored by increasing levels of prostate-specific antigen (PSA) in the serum of patients with prostate cancer being treated with androgen ablation therapy. The symbiotic interaction between prostate cancer cells and osteoblasts was studied by examining the effect of osteoblast-conditioned medium on LNCaP prostate cancer cells. To do this, human osteoblasts were derived from trabecular bone explants from the femoral head and cultured in serum-free media to obtain the osteoblast-conditioned media. The expression of PSA and stimulation of the androgen responsive reporter, pARR3-tk-Luc, was measured in LNCaP cells. Addition of osteoblast-conditioned medium to LNCaP cells increased the expression of PSA and increased proliferation. Osteoblast-conditioned medium stimulated the androgen-responsive reporters, p6.1kb-PSA-Luc and pARR3-tk-Luc. Stimulation of pARR3tk-Luc, which contains three tandem repeats of the rat probasin androgen response region, was abrogated by the anti-androgen, bicalutamide. This suggests that the androgen receptor may be activated in LNCaP cells by factors secreted from osteoblasts. Bicalutamide did not block the induction of either PSA mRNA or secreted PSA protein from LNCaP cells exposed to osteoblast-conditioned medium. In addition to the effects on gene expression, osteoblast-conditioned medium also induced changes to the LNCaP cell morphology by developing longer dendritic processes and smaller cell bodies to resemble neuroendocrine cells. Taken together these results suggest that osteoblasts secrete paracrine factors that differentiate LNCaP cells into the more aggressive neuroendocrine-like morphology with an increase in PSA gene expression that was not attenuated by bicalutamide. This suggests that bicalutamide may not be an effective therapeutic for patients with bone metastases due to its inability to prevent androgen-independent expression of PSA and possibly concomitant proliferation of prostate cancer cells.

SU097

Contrasting Anabolic Responses of MC3T3-E1 Cells and Neonatal Mouse Calvaria to LNCaP Prostate Cancer Cell Conditioned Media. R. S. Bhattacharyya,* P. H. Stern. Molecular Pharmacology and Biological Chemistry, Northwestern University, Chicago, IL, USA.

Skeletal metastases are a predominant consequence of late stage prostate cancer causing debilitating pain and immobility. These metastases are primarily osteoblastic and are characterized by irregular new bone formation. It has been hypothesized that a soluble factor(s) produced by the prostate cancer cells is responsible for mediating this anabolic response on bone. Our studies have been directed at characterizing the responses elicited by conditioned media from prostate cancer cells on bone. Previous studies from our laboratory demonstrated that incubation of neonatal mouse calvaria with LNCaP conditioned media (CM) stimulates ^{3}H-thymidine incorporation, with smaller effects on ^{14}C-proline incorporation. Inhibiting the MAP kinase signaling pathway with the MEK inhibitor PD98059 decreased the effects of the LNCaP CM on ^{3}H-thymidine incorporation and enhanced the effects on ^{14}C-proline incorporation in the calvaria. In the current studies we examined the effects of these same treatments in MC3T3-E1 osteoblastic cells. Cells were cultured for 72 hr and labeled during the final 2 hr with ^{3}H-thymidine and ^{14}C-proline in order to assess changes in cell proliferation and protein/collagen synthesis, respectively. A different pattern of responses was observed in the cells as compared to the calvaria, in that LNCaP CM consistently produced greater effects on ^{14}C-proline than on ^{3}H-thymidine in the MC3T3-E1 cell cultures, even when cells were plated at low densities or incubated in serum-free (0.1% bovine serum albumin) medium in which growth was slower. The effects of inhibiting the MAP kinase signaling pathway were also different in the cells, in that PD98059 and the more selective MEK inhibitor U0126 inhibited the LNCaP CM-stimulated ^{14}C-proline incorporation. An exception was found when cells were incubated in the serum-free medium, in which case the response was increased by PD98059. The results indicate that although the LNCaP CM has anabolic effects on bone cells in both the calvaria cultures and the MC3T3-E1 cells, the tissue environment may determine whether a proliferative response or an increase in matrix synthesis is the predominant effect elicited by the prostate cancer cell CM. Furthermore, inhibiting the MAP kinase signaling pathway not only affects the magnitude of the anabolic response to the prostate cancer cell CM but can also either inhibit or enhance it, depending upon whether the CM is added to the calvarial cultures or the MC3T3-E1 cells, suggesting that MAP kinases can play unique roles in these different systems.

SU098

DXA Report Influences Patient Compliance in Filling Prescriptions for Osteoporosis. B. Greenwald,*[1] A. Bardwell,[2] M. Greenwald,[3] J. Malinak,*[4] R. Rude,[5] S. Silverman.[6] [1]Desert Medical Advances, Palm Desert, CA, USA, [2]Santa Fe, New Mexico, USA, [3]Palm Desert, CA, USA, [4]La Mesa, CA, USA, [5]U Southern CA, Los Angeles, USA, [6]Osteoporosis Medical Center, UCLA, Los Angeles, USA.

A survey with 144 post-menopausal women with no prior treatment for osteoporosis was conducted between April-June 2000 at seven centers across the Southwestern U.S.

Women referred by a primary physician to a bone density testing center were given their report form and asked to complete a questionnaire regarding what action was initiated by their primary physician within the next 30 days. Each woman in the survey had to have a different primary physician. Therefore the results reflect the action taken by 144 different primary physicians and patients. The average age was 67 (64.8-69.7). Insurance carriers were private (54%), welfare (12%), or managed care (34%). Insurance type did not affect follow up with the primary physician. Patients and the primary physician were randomly given the bone density results as the traditional T-score report or an absolute fracture report. More women followed up with their physician, filled a new prescription, and followed therapy when the absolute fracture report form was used ($p<0.001$ in all categories). No difference in age, degree of bone loss, or type of insurance was found between groups.

Report type	Discussed with MD	Filled new Rx	Followed advice (includes calcium)
Fracture report	92% *	72% *	78% *
T-score report	81%	61%	64%

*$p<0.001$

Conversely, 14 of 36 (39%) prescriptions were not filled by the patient after the T-score report and 12 of 42 (28%) prescriptions were not filled by the patient after an absolute fracture report ($p<0.02$). The primary physicians increased the number of prescriptions written from 69% (T-score report) to 84% (fracture report). Patients improved compliance from 64% to 78% by using an absolute fracture report for bone measurement reporting

SU099

Short-term In Vivo Repeatability of Digital X-ray Radiogrammetry and Dual Energy X-ray Absorptiometry. O. Johnell, K. Önnby,* I. Redlund-Johnell.* UMAS, Malmø, Sweden.

In the present study a bone densitometer using the Digital X-ray Radiogrammetry (DXR) technology (Pronosco X-posure System^{TM}) was compared to the established DXA technology with respect to reproducibility. The study used a balanced design with each participant having 3 radiographs taken and 3 DXA-scans performed with repositioning of the hand and forearm in between each capture. The DXA scanner used was the Lunar DPX-L scanner (Region 10%). The radiographs were analysed using the X-posure system at UMAS, Malmø. The radiographs and the DXA scans were performed at baseline and at a 8 month follow up visit. A total of 20 women were studied, 19 were post-menopausal women above the age of 50 years at baseline 14 women completed the 8 month follow-up visit. The short-term precision errors were calculated as root-mean-square averages of standard deviations of repeated measurements and expressed as the coefficient of variation (CV) with the corresponding 90% confidence intervals. The individual change in BMD between baseline and the follow-up visit was calculated and made relative to the CV%. The mean age of the women at baseline was 67.5 years, and the mean DXR-BMD at baseline was 0.489 g/cm^2 (SD=0.0747 g/cm^2), the mean DXR-MCI at baseline was 0.385 (SD=0.0865), and the mean DXA BMD was 0.325 g/cm^2 (SD=0.0847 g/cm^2). For the 14 women measured at the 8 month interval, the mean values at baseline and the follow-up visit were: DXR-BMD: 0.504 g/cm^2 to 0,501 g/cm^2, DXR-MCI: 0.408 to 0.405, and DXA-BMD: 0.346 g/cm^2 to 0.344 g/cm^2. The CV% (95%CI) for the measurements at baseline and at the 8-month follow-up are given in the Table:

	CV% Baseline	CV% follow-up	Change	% change/CV%
DXR-BMD	0.52 (0.44 - 0.64)	0.61 (0.50 - 0.79)	0.59%	1.044
DXR-MCI	0.60 (0.51 - 0.73)	0.71 (0.59 - 0.91	0.69%	1.053
DXA-BMD	3.53 (2.93 - 4.50)	3.18 (2.61 - 4.09)	0.78%	0.232

In conclusion, the present study shows that the short-term *in vivo* precision error of the DXR technology is better than the precision error found for the DXA technology.

Disclosures: Pronocso A/S,2.

SU100

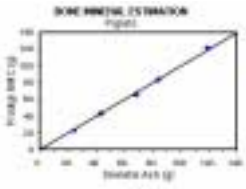
Body Composition Measurements on Small Animals with the GE Lunar PRODIGY. R. H. Nord,[1] T. D. Crenshaw,[2] D. K. Schneider,[2] L. M. Freeman.*[3] [1]Research & Development, GE Medical Systems - Lunar, Madison, WI, USA, [2]Dept. of Animal Sciences, University of Wisconsin, Madison, WI, USA, [3]School of Veterinary Medicine, Tufts University, North Grafton, MA, USA.

The purpose of this study was to evaluate a new scan mode on the GE Lunar PRODIGY bone densitometer for small animal body composition. Two sub-studies contributed to this evaluation effort: (1) Six piglets ranging in weight from 1.8 to 6 kg were killed and then scanned the same morning on a PRODIGY densitometer at GE Lunar. They were then taken to the Univ. of Wisconsin Dept. of Animal Science where they were chemically analyzed. Pigs were dissected to separate the skeleton from the soft tissue and viscera contents. Each fraction was homogenized and then analyzed to determine water, protein, fat, and ash content. Bone mineral content (BMC) was taken to be equal to the skeletal ash mass. Fat mass was the total fat from all fractions. Lean mass was calculated as live weight less BMC and Fat mass. (2) Twenty-six rhesus monkeys ranging from 2.1 to 4.7 kg were carefully weighed at New England Regional Primate Center. They were then scanned on a PRODIGY densitometer. Some of the animals were re-measured at 2-4 week intervals, over a 4 month period during which some of their weights were changing. Carcase analysis of the pigs provided reference values for all of the DXA body composition quantities: BMC, Fat mass, Lean mass, Total body mass, and tissue %Fat. The monkeys provided reference data on Total body mass only. Regressions of PRODIGY measurements vs reference values showed very good correlation and agreement.

		n	Rsquared	Mean value	Standard error	% of mean
	BMC	5	1.00	68 g	1.7 g	2.5 %
	Fas Mass	5	0.99	439 g	41.1 g	1.1 % *
PIGS	Lean Mass	5	1.00	3104 g	53.2 g	1.5 % *
	Total Mass	5	1.00	3611 g	12.8 g	0.4 %
	% Fat	5	0.96	10.6 %	1.1 %	
MONKEYS	Total Mass	95	1.00	2896 g	29.9 g	1.0 %

* % of mean total body mass

Average PRODIGY values differed from average chemical analysis values by -1.1% in BMC, -3.7% in Fat mass, 0.6% in Lean mass, 0.1% in Total mass, and -0.3% in %Fat.



We conclude that the PRODIGY DXA densitometer will be a useful tool in estimating the body composition of animals in the range of 1.5 to 6 k

Disclosures: GE Medical Systems -- Lunar,3.

SU101

Community Awareness Bone Density Testing Program (CABDTP): Report of 50,000 Cases and 5 Years Experience. E. N. Schwartz, R. Kagan,* B. Tracewell,* D. M. Steinberg.* Foundation for Osteoporosis Research and Education, Oakland, CA, USA.

We have previously reported on several aspects of our CABDTP (ISCD 1998 and ASBMR, 1998 and 1999). We now report the results of over 50,000 forearm scans performed on Norland pDEXA@ Densitometers.Beginning with one machine, one technologist and one registrar scanning at 10 stores in a pilot program in April, 1996, the staff, at present, consists of 10 densitometry technologists, 13 registrars and a program manager; scanning at more than 240 pharmacies in N. California and Nevada, worksites, health clubs and other venues.Previous data have shown that in Caucasian women 42.1% had a T-Score more than 1 SD below peak adult bone mass (PABM), 15.9% were more than 2 SD below PABM and 9% are more than 2.5 SD below PABM; for Asian women, their percentages were 53.5%, 25.7% and 14.3% respectively; for Hispanic women 45.8%, 20.3% and 12.2% respectively; for African-American women 40.6%, 17.4% and 5.7% respectively; for men of all ethnicities, 44.6%, 20.1% and 10.9% respectively.The latest analysis of the data in these groups will be presented.

SU102

BMD Treatment Thresholds: Should We Treat Osteopenic Women? E. Siris,[1] P. Miller,[2] T. Abbott,[3] Y. Chen,[3] K. Faulkner,[4] E. Barrett-Connor,[5] M. Berger,[3] A. Santora,[3] L. Sherwood.[3] [1]Columbia Presbyterian Medical Center, New York, NY, USA, [2]Colorado Center for Bone Research, Lakewood, CO, USA, [3]Merck & Co., Inc., West Point, NY, USA, [4]GE Medical Systems/Lunar, Madison, WI, USA, [5]University of California, San Diego, CA, USA.

Low bone mineral density (BMD) predicts increased risk for fracture. However, debate continues over the BMD threshold that defines "high risk for fracture" and should be used for treatment decision. Using data from the National Osteoporosis Risk Assessment (NORA), we compared the sensitivity (# events detected at or below threshold/total events) for detecting, prospectively, osteoporotic fractures (hip, forearm/wrist, spine, and rib combined) and hip fractures alone at different BMD (expressed as T-score) thresholds. Each participant had BMD measured at one site (forearm, finger, or heel). Fractures occurring in the next year were identified by self-reported. This analysis is limited to 131,126 Caucasian women with heel SXA (Osteoanalyzer, Dove) or forearm (pDEXA, Norland) measures who responded to follow-up survey. During 1-year follow-up, 2005 osteoporotic fractures were reported, of which 353 were hip fractures. Using a threshold of T-scores -2.5 and below, only 18% of osteoporotic fractures and 27% of hip fractures were captured. As the threshold was increased to T-scores -1.0 and below, sensitivity increased to 70% and 79% for osteoporotic and hip fractures, respectively. Using the NOF guidelines of T-scores -2.0 and below or T-scores between -1.5 and -2.0 with a risk factor, about 50% of the fractures were captured. In conclusion, limiting treatment interventions to women with very low T-scores misses a significant portion of fractures that might be preventable, and does relatively little to address the societal burden of fractures.

SU103

T-Score Thresholds Based on Equal Fracture Risk: Are They a Viable Paradigm For Interpreting Peripheral BMD Measurements? G. M. Blake. Guy's, King's and St Thomas' Hospital Medical School, London, United Kingdom.

Over the past decade, bone density scans have assumed an essential role in the diagnosis of osteoporosis. Although BMD scans of the central skeleton remain widely used, a variety of different types of equipment for measuring peripheral sites are now available. However, a lack of consensus on how peripheral BMD results should be interpreted has proved a barrier to the more widespread use of these devices. Prospective studies of osteoporotic fractures show that hip BMD is the optimum measurement for predicting hip fracture risk with a relative risk (RR) per population standard deviation of 2.7. [1] In comparison, peripheral measurements have poorer discrimination with RR ~ 1.5. [1] On this basis it has been proposed that treatment decisions based on peripheral BMD should be made by defining equivalent T-scores at which patients' 5-year hip fracture risk is equal to the risk set by a femoral neck BMD T-score of –2.5. [2] Although this is an attractive paradigm, it is necessary to ask whether it is valid to assume that estimates of fracture risk may be quantiatively compared between techniques in the way intended. When the fracture risk curves for two techniques with RR values RR1 and RR2 are plotted together they intersect at a Z-score of Z12 = -(lnRR1 + lnRR2)/2. For RR values of 2.5 and 1.5, Z12 = -0.7. For patients with Z-scores < Z12, quantitative figures for fracture risk derived from peripheral BMDs will be systematically smaller than those derived hip BMD from because of the shallower gradient of risk curve. The opposite effect occurs with patients with Z-scores > Z12. For white women, a femoral neck T-score of -2.5 coincides with the intersection point Z12 at age 70 and results in equal percentages of women being treated at this age by both techniques. For women younger than 70, fewer patients will be treated using a peripheral BMD than hip BMD because the shallower gradient of risk curve results in a more negative Z-score being required to reach the required fracture risk threshold. In contrast, women older than 70 will be relatively over treated. We conclude that quantitative estimates of fracture risk derived from densitometry depend on the RR value of the technique as well as the skeletal status of the patient and should not be equated between techniques. Peripheral T-score thresholds set by equal fracture risk will result in the under treatment of younger patients and over treatment of older patients compared with hip BMD. Alternative methods of setting treatment thresholds such as the lowest quartile of women over age 65 provide a more logical paradigm for the wider use of peripheral devices. 1. Black DM, Palermo L, Bauer D. Osteoporosis Int. 11 (Suppl 2) S59, 20002. Black DM. Osteoporosis Int. 11 (Suppl 2) S58, 2000

SU104

Identifying Osteoporosis in Proximal Humeral fractures. P. J. Ryan,* G. Worcester.* Osteoporosis Unit, Department of Nuclear Medicine, Medway Maritime Hospital, Gillingham, Kent, United Kingdom.

There is a relative paucity of bone mineral density data with regard to proximal humeral fractures. In patients with such fractures this study examined the relative merits of hip, spine and forearm BMD for the identification of those who are osteoporotic (T <-2.5), and threshold values of forearm BMD to identify osteoporosis at the hip and spine. Patients with low trauma (fall from standing or less) fractures were referred from the Accident and Emergency department to the Osteoporosis unit as part of a programme called the Medway Fracture Initiative (MFI). Sixty four consecutive patients presenting with such fractures of the surgical humeral neck or proximal shaft were examined by DXA using a Hologic QDR 4500C. Measurements were made of the femoral neck (FN), lumbar spine (L1-L4) (LS) and ultradistal radius (UDR). Fractures of the greater tuberosity or mid shaft were excluded. Hologic reference ranges were used for spine and forearm with NHANES 111 reference range for the femoral neck. Patients were of average age 70.5 years (SD 10.4) and 80 % were female. Mean T scores were for the FN -1.87, LS -1.95 and UDR -1.93. The number of osteoporotic patients identified at the measured sites were FN 17, LS 20 and UDR 19. 2 patients were osteoporotic at FN but not UDR, but would have been identified by a UDR threshold of T <-2.0. 6 pts were osteoporotic at the LS but not UDR. Of these 1 would have been identified with a UDR threshold of T <-2.0 and 3 with a threshold of T < -1.5. In conclusion, hip, spine and forearm BMD identify a comparable number of osteoporotic patients presenting with low trauma proximal humeral fractures. With regard to identifying those with T < -2.5 at the hip, a forearm BMD of T <-2.0 appears a satisfactory threshold. To identify those with T < -2.5 at the spine a higher UDR threshold is required.

SU105

In-Vivo Cross-Calibration of a New Peripheral DXA Densitometer. R. Patel,* G. M. Blake, I. Fogelman. Guy's, King's and St Thomas' Hospital Medical School, London, United Kingdom.

The purpose of this study was to compare forearm bone mineral measurements made with the prototype of a new generation of peripheral DXA system, the Osteometer DTX-222, with the earlier Osteometer DTX-200 model. Measurements of the non-dominant forearm bone mineral content (BMC), projected area (Area) and bone mineral density (BMD) at the distal radius and ulna were compared in 89 patients (mean age: 55 years, range 29 to 85 years) referred by their primary care physician for a densitometry investigation. Fourteen volunteers (mean age: 37 years, range 20 to 58 years) had duplicate measurements with repositioning between scans to evaluate short-term precision. Linear regression and Bland-Altman plots were used to assess the agreement between the two densitometers. Precision was evaluated using the coefficient of variation (CV%). The results are summarised in the Table below. The relationship between the two devices was linear for all three variables with correlation coefficients for BMD and BMC greater than r = 0.98. None of the regression slopes were statistically significantly different from unity. However, the results showed that the intercepts for BMD and BMC were both statistically significantly different from zero ($p < 0.001$). The intercept for Area was not significantly different from zero and when regression analysis was repeated with the regression line forced through the origin the slope was 1.013 (0.003). The precision errors for the DTX-222 were slightly larger than for the DTX-200, although the differences were not statistically significant for BMD and Area. The RMSE errors from the regression analysis were consistent with the precision errors. Results of this preliminary comparison show that the DTX-222 system satisfactorily reproduced the performance of the earlier DTX-200 model.

SU106

Cut-off Values Determined for Vertebral Fracture by Peripheral Quantitative Computed Tomography in Japanese Women. I. Gorai,*[1] K. Nonaka,*[2] H. Kishimoto,[3] H. Sakata,*[4] Y. Fujii,[5] T. Fujita.[5] [1]Department of Obstetrics and Gynecology, Yokohama City University School of Medicine, Yokohama, Japan, [2]Tokyo Medical and Dental University, Tokyo, Japan, [3]San-in Rosai Hospital, Yonago, Japan, [4]Nayoro Central Orthopedic Hospital, Nayoro, Japan, [5]Calcium Research Institute, Kishiwada, Japan.

Ultradistal radius bone density was measured using peripheral quantitative computed tomography (pQCT) to determine reference values for total bone density (BD), trabecular bone density (TBD), polar strength strain index (pSSI) and other indicators in the Japanese female population, and ascertain the cut-off values of the measured indicators that could most efficiently discriminate osteoporotic subjects with vertebral fractures. A total of 5,266 healthy Japanese women aged 20 to 89 years were included in this study to determine Japanese reference values. Additionally, 621 who had undergone radiographic examination of the thoracic and lumbar spine at the time of pQCT measurement were selected to determine the cut-off values of BD, TBD, pSSI and other indicators for vertebral fractures. All the healthy subjects were divided according to age into groups each five years wide. The BD showed non-significant changes from the 20-24-year age group of to the of 45-49-year age group, and fell significantly thereafter. The TBD maintained a plateau until the 40-44-year group and decreased significantly thereafter. The pSSI did not change significantly from the 20-24 age group to that of 45-49 years, and decreased slightly in the 50-54 age group and markedly after the 55-59 age group. The cut-off values for the discrimination of vertebral fractures were obtained by the calculation of sensitivities, specificities and the area under the curves (AUC) obtained using age-adjusted receiver operating characteristics (ROC) analysis. Odds ratios and 95% confidence limits (CL) were calculated using age-adjusted logistic analysis. The cut-off values for vertebral fractures, the area under the ROC curves (AUC) and odds ratios were 270.1 mg/cm3 (-2.2SD, 66.6% of YAM), 0.689±0.025, 2.10 (1.63, 2.70) for BD, 104.8 mg/cm3 (-2.2SD, 53.5% of YAM), 0.699±0.023, 2.17 (1.69, 2.77) for TBD and 192.8 mm3 (-1.9SD, 59.8% of YAM), 0.631±0.028, 1.72 (1.34, 2.21) for pSSI, respectively. These findings suggest that ultradistal radius BMD measured using pQCT can be used to discriminate women with vertebral fractures.

SU107

An Improved Algorithm For Spine Analysis In DXA Bone Densitometry. C. C. Ruth,* L. E. Jones,* T. L. Kelly, E. von Stetton, K. E. Wilson.* Hologic, Inc., Bedford, MA, USA.

A new algorithm has been developed to eliminate the need for operator intervention in the analysis of AP spine scans with poor bone maps. This algorithm, in combination with automatic region placement[1] (One Time™ Auto Analysis, Hologic, Inc.), significantly improves ease of use by minimizing operator analysis time. The algorithm is only applied to the small percentage of spine scans that may require alteration of the default bone map (area of bone shown in the image) by the operator. In the previous method the operator may edit the default bone map by adding or deleting bone manually with the cursor, or in cases where the editing is not obvious they may choose the 'Low Density Spine Analysis'. The new algorithm identifies bone much more reliably in these scans and minimizes intervention from the operator. An independent expert operator selected 50 AP spine scans with default bone maps that required intervention, either by switching to the Low Density Spine Analysis mode or editing the bone map. The expert then analyzed the scans using the new algorithm and found that only 1 of 50 required subsequent bone map intervention. In conclusion, the algorithm successfully identified the bone maps in AP spine scans that would normally require operator intervention. This algorithm simplifies analysis, reduces subjectivity in results, and improves patient throughput by reducing analysis time. [1]C. Ruth *et. al*, "Performance of an automatic analysis algorithm for a DXA bone densitometer," Osteo. Int. 11, S81 (2000).

Disclosures: Hologic, Inc.,3.

SU108

Integral Measurement of Bone Density - "Gold Standard"? M. Neff,[1] H. Schwarz,*[2] W. Kneer,*[3] R. Kissling,*[4] M. A. Dambacher.[4] [1]Center for Osteoporosis, Zurich, Switzerland, [2]Center for Osteoporosis, Freudenstadt, Germany, [3]Center for Osteoporosis, Stockach, Germany, [4]Dept. for Rheumatology, University Clinic Balgrist, Zurich, Switzerland.

The early postmenopausal bone loss is mainly trabecular. Despite this fact the integral bone density measurement is often declared as a "gold standard" in the early recognition of osteoporosis as well as in short terms quantification of therapeutical effects. Is this to maintain? Using high resolution peripheral computed tomography (hrpQCT, Densiscan 1000, Scanco Medical AG, Bassersdorf, Switzerl., lateral spatial resolution 0.2-0.3mm, reproducibility in mixed collectives ±0.3%) we evaluated in 350 female patients between 45 and 65 years of age the volumetric trabecular and cortical bone density (vBMD) in the distal

radius of the non-dominant forearm separately as well as the integral vBMD. 51 (~15%) of these 350 female patients showed cortical and integral vBMD within the normal range according to the WHO-definition (T-score >-1): cortical (c) epiphyseal 101.2 ± 5.2%, c meta-/diaphyseal 100.3 ± 6.5% and integral T-score -0.49 ± 0.42 but a reduction in trabecular volumetric BMD (vBMD) of >20% (mean 67.4 ± 9.5%) compared with vBMD of healthy female probands between 25 and 40 years of age. The phenomenon was pronounced in the age group between 45 and 65 years. This corresponds to the clinical observations - probably due to the different endosteal surfaces of trabecular and cortical bone - that after menopause often first a loss of trabecular bone can be observed or that even in osteoporosis of the spine often normal values in the proximal femur are measured, moreover therapeutical effects (e.g. under HRT, Alendronate or Alphacalcidol) are normally seen in the trabecular bone compartment first. In addition our results show that due to the low bone mass of trabecular bone a significant loss from this compartment can be masked by an unaffected high cortical bone mass. Conclusions: 1. In spite of normal integral BMD a significant number of female patients show osteoporotic trabecular bone masses. 2. The lower the amount of trabecular bone mass at a measuring site the less is the possibility of an early recognition of osteoporosis.

SU109

Bone Loss: New Densitometric Data. M. A. Dambacher,[1] M. Neff,[2] R. Kissling,*[1] H. Radspieler,*[3] L. Qin.*[4] [1]University Clinic Balgrist, Zurich, Switzerland, [2]Center for Osteoporosis, Zurich, Switzerland, [3]Center for Osteoporosis, Munich, Germany, [4]Chinese University, Hong Kong, Hong Kong Special Administrative Region of China.

Osteoporosis is defined (cons. conf. Copenhague, Hong Kong, Amsterdam) by the key words bone density, loss of bone density and bone structures.

Our diagnosis, prophylaxis and treatment of osteoporosis is based on this definition. We use the high resolution peripheral quantitative computed tomography (HrpQCT).

Loss of bone density: The importance of time serial examinations with (HrpQCT) reproducibility in a mixed population (normals, patients with osteopenia and osteoporosis) ± 0.3%) is that they enable the detection of patients at high risk for osteoporosis and the individualisation of prophylaxis and treatment. As a consequence, we use agents that inhibit bone resorption in patients who are fast bone losers and agents that stimulate bone formation in patients who are slow bone losers with low bone mass. Our system enabled us to assess trabecular and cortical bone mass separately in the radius and tibia, and to differentiate between fast and slow bone losers (threshold: 3% of loss of trabecular bone density in the radius/year) within a few months. The annual perimenopausal losses (n=69) were -6.8±0.6% in fast losers (34%) versus -1.0±0.2% in slow losers (66%) ($p<0.001$) in the distal radius. Hong Kong data show 33% fast losers versus 67% slow losers. In a group of severe senile osteoporosis (n=23) in comparison 74% fast losers were found.These data (perimenopausal and in severe Osteoporosis) are in contrast to the current opinion.

Also we have shown that calcitonin and etidronate are more effective in fast than in slow bone losers, and that vitamin D metabolites (calcitriol or 1a-calcidol) estrogens and alendronate can halt fast bone loss. The highly sensitive HrpQCT measurement method enables us to adapt the treatment to the different forms of osteoporosis and bone turnover, increasing not only the number of successful treatments but also the compliance of the patient. Because our treatment is based on measurements, treatment modifications - especially in those who change from a slow to a fast bone-loser state - can be easily justified.

We have evaluated the fast trabecular bone loss (calculated for one year) in 11 different groups of untreated patients: pregnant women (- 8,4%), brest feeding women (- 9,5%) peri-/immediate postmenopausal women (- 7%), 70-80 yrs old (-5%), pre treatment phase of the following studies: EHDP (- 8,7%), Calcitriol (- 6,53%), Alpha-Calcidol (- 6,96%), Alendronat (- 6%), Calcium/D3 (- 5,8%), Calcitonin (- 6,3%), Nandrolon-Decanoate (-9,4%). This means, that the fast trabecular bone loss in all these different groups is between - 5%/y and - 9,5%/y.

SU110

Normative Data and Reference Curves for Differential Diagnosis Between Physiological and True Osteopenias Employing DXA. J. L. Ferretti,[1] S. L. García,[2] R. F. Capozza,*[1] G. R. Cointry,*[1] H. Plotkin,[3] E. J. A. Roldán,[4] J. R. Zanchetta.[5] [1]Centro de Estudios de Metabolismo Fosfocálcico, Facultad de Medicina, Univ. Nacional de Rosario, Rosario, Argentina, [2]Centro de Estudios Metabólicos (CEME), Santa Fe, Argentina, [3]Department of Genetics, Shriners Hospital for Children, Montréal, Quebec, Canada, [4]Dept. Farmacología Clínica, Gador SA, Buenos Aires, Argentina, [5]Instituto de Investigaciones Metabólicas (IDIM), Buenos Aires, Argentina.

The strength (and the mass) of bones is determined by the stiffness and thespatial distribution of the mineralized matrix. Both determinants areinversely related through a feedback mechanism (mechanostat theory), in turnstimulated by the strain history of the skeleton (contractions of theregional muscles). Indeed, the bone/muscle masses are linearly related,showing the same slope for any gender, age or body habitus, and are mutuallyaffected by physical activity. The setpoint of the system, geneticallydetermined, is sensitive to.systemic (disturbing) factors as hormones,drugs, etc. Therefore, "physiologic" osteopenia results from a prolongedinactivity or weightlessness (reduced input), and "true" osteopenia by agenetic or systemic disorder (shifted setpoint). When that osteopeniainvolves a mechanical compromise (bone fragility) it can be regarded as a"disuse" or a "true" osteoporosis, respectively. The DXA-BMC is the bestresource for diagnosing an osteopenia not so for an osteoporosis; due to it does not provide any information on bone tissue quality or distribution. However, DXA is able to assess all bone (BMC), fat, and lean (proportional to muscle) masses. Thus, DXA allows correlating the whole-body or regional BMC and "muscle" mass and approaching a differential diagnosis between "physiologic" and "true" osteopenias (appropriate orinappropriate bone/muscle mass proportion) as preconditions for diagnosing a "disuse" or a "true" osteoporosis. We have developed adequate reference BMC/lean-mass charts for whole-body DXA data (XR-26, Norland, Wi) in normal boys and girls (n=545), men (n=228), and pre- and post-menopausal women (n=330, 347) showing the 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, and 99 percentiles. The same were also performed for BMC values adjusted to a common, 18-kg fat mass according to the corresponding regression equations. A very simple, graphic procedure estimates any percentile and would approach a differential diagnosis between physiologic and true osteopenias according to a suitable reference limit. Appropriate factors allow the data transformation for use with different systems.

SU111

The Risk for Misclassification with Unilateral DXA-Measurement of the Proximal Femur and Heel. H. Mallmin,*[1] K. Larsson,*[2] O. Ljunggren.[2] [1]Orthopedics, Surgical Sciences/Orthopedics, Uppsala, Sweden, [2]Dept of Internal Medicine, Medical sciences, Uppsala, Sweden.

The rational for unilateral bone densitometry measurement is biological identity between left and right side. However, a side to side difference of the same magnitude or greater than the precision error indicates a biological side difference. We determined the precision error for DPX-IQ-equipment with the coefficient of variance %, (CV%) for 10 repeated scans of a Wahner/Hahn hip phantom without repositioning, and the integrated precision error for the DXA-method with the root-mean-square method (95% confidence interval, CI, g/cm2) and CV% on 14 patients for three repeated scans at the proximal femur, DPX-IQ, and at the heel, PIXI, with repositioning(Table). Table. The precision error for DXA at different anatomical sites.

Precision error estimation method	Femoral neck	Total hip	Heel
CV% hip phantom	1.8%	0.6%	na
CV%, 14 patients	2.4%	2.1%	2.1%
RMS-method 95% CI, g/cm2, 14 patients	0.037	0.034	0.023

In a consecutive study, 558 patients (478 women, 80 men), age 63 years (23-88), BMI 26 kg/m2 (16-40), we evaluated bilateral DXA-measurements of the proximal femur, DPX-IQ, and heel, PIXI, for side differences. Statistical analysis included simple regression, correlation, dependent t-test, percentage individuals with side to side differences equal to or exceeding the precision error, and percentage individuals with a relevant T-score side-to-side difference. Correlation coefficients for left vs right side were 0.92, 0.96 and 0.95 for the femoral neck(FN), total hip(TH) and the heel respectively. R-values within the same side were eg. for the left side 0.92 for FN vs TH and 0.67 for FN vs heel. At the FN 48% of the patients had a side difference of 0.037 g/cm2 or more, 46% 0.034 g/cm2 or more and 43% 0.023 g/cm2 or more, at the TH and heel respectively, indicating a biological side to side difference. A T-score side difference of 0.5 or more or 1 or more were registered for 33% of the patients and 6% of the patients at the femoral neck, 24% and 2% for total hip and 23% and 5% at the heel respectively. We conclude that although we found excellent correlation between left and right side, close to earlier reports, almost every second individual had a side to side difference equal to or outside the 95% confidence interval, introducing an obvious risk for misclassification of patients. Bilateral DXA-measurements of the proximal femurs and heels are recommended.

SU112

Use of a Hip Positioning Device Improves Precision of Hip Bone Density Measurements. V. Addesso-Dodd,[1] R. B. Staron,*[1] P. Namerow,*[2] B. Diamond,*[1] E. S. Siris,[1] J. P. Bilezikian,[1] E. Shane.[1] [1]College of Physicians & Surgeons, Columbia University, New York, NY, USA, [2]School of Public Health, Columbia University, New York, NY, USA.

Precision of bone mineral density (BMD) scans of the hip may be affected by small changes in rotation and abduction. Therefore, the precision of serial hip BMD scans, particularly at the femoral neck (FN), is worse than that of the spine. We therefore determined whether a Hip Positioning Device (HPD), that maintains the hip in a fixed position with 25° internal rotation and abduction, improves the precision error of hip BMD measurements. Hip BMD was measured in 101 postmenopausal women using a HOLOGIC QDR 4500 densitometer. Four consecutive BMD scans of the hip (2 with HPD and 2 without) were performed on the same densitometer with complete repositioning between each measurement.

Tech	N	+ HPD Precision (g/cm^2)	+ HPD CV%	- HPD Precision (g/cm^2)	- HPD CV%
DBT	67	0.017	2.46	0.017	2.40
GBT	34	0.019	2.68	0.061	8.60

We also evaluated precision at the FN by operator, categorizing technicians as Dedicated BMD Technologists (DBT; perform only BMD) and General BMD Technologists (GBT; perform BMD and other studies).

Tech	N	+ HPD Precision (g/cm^2)	+ HPD CV%	- HPD Precision (g/cm^2)	- HPD CV%
DBT	67	0.017	2.46	0.017	2.40
GBT	34	0.019	2.68	0.061	8.60

We conclude that use of the HPD is associated with improved precision at the FN and trochanteric regions, particularly when BMD is performed by general rather than by dedicated BMD technologists.

SU113

Male Reference Database for Digital X-ray Radiogrammetry for Metacarpal and Radius BMD. T. Gühring,*[1] B. Bickert,*[2] M. Arens,*[3] W. Kneer,*[4] C. Wüster.[5] [1]Univ Heidelberg, Heidelberg, Germany, [2]BG-Unfallklinik, Ludwigshafen, Germany, [3]Pronosco, Wasserburg, Germany, [4]Orthopedic Praxis, Stockach, Germany, [5]Novo Nordisk Pharma, Mainz, Germany.

The Pronosco X-posure system estimates bone mineral density (BMD) and bone geometry using digital x-ray radiogrammetry and textural analysis of digitised conventional radiographs of hand and forearm. The system calculates BMD using a weighted average of cortical and bone width measurements at the radius, ulna and second through fourth metacarpal. The output is an absolute BMD estimate called DXR-BMD and parameters of bone geometry in tubular bones. Furthermore the system calculates a parameter of cortical structure called porosity.The aim of this study was to establish a normative database for males for the Pronosco system. One hundred and eight x-rays from males attending a special clinic for hand surgery (BG-Unfallklinik Ludwigshafen, Germany) were selected in order to measure bone geometry and DXR-BMD. Men with osteological diseases or known to use osteotropic drugs on a regular basis were excluded from the analysis. Results for DXR-BMD [g/cm²] and porosity [absolute units] were as follows: 20-29 years (n=30): 0.65/3.0; 30-39 years (n=44): 0.69/3.15; 40-49 years (n=30): 0.66/3.05; 50-59 years (n=28): 0.66/4.06; 60-69 years (n=17): 0.62/4.02; 70-79 years (n=9): 0.59/4.29. The best fit polynomial function was $y = -0.0001x^2 + 0.0067x + 0.5458$ for DXR-BMD and $y = 0.006x^2 - 0.0291x+3.3478$ for porosity, linear regression analysis showed the following: $y = -0.0011x + 0.7067$ for DXR-BMD and $y = 0.0294x + 2.14933$ for porosity.Mean DXR-BMD values in males were considerably higher than in German females and higher compared to values reported from Asian investigators. They were lower than DXA-BMD as given by Hologic and Lunar and higher those given for Norland machines. These data might give the basis for the calculation of T-and Z-scores as well as absolute risk calculations or standardized T-scores

Disclosures: Novo Nordisk Pharma,3.

SU114

Peripheral Bone Mass Measurement, a Helpful Tool for the Diagnosis of Osteoporosis in Areas Where Central DXA Is Not Available. D. Picard, M. Couturier,* L. Rosenthall, J. P. Brown, J. Lévesque,* M. Dumont,* L. G. Ste-Marie, A. Tenenhouse, S. Dodin.* Quebec Osteoporosis Study Group, Quebec, Canada.

Central measurement of hip and spine bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) is the preferred method of diagnosis of osteoporosis. Peripheral measurement of bone mass could offer an alternative approach where central DXA is not readily accessible. To evaluate their utility, we determined BMD of the spine (*s*), femoral neck (*fn*) (1 Hologic, 3 Lunar), phalanx *(p*) with Schick AccuDXA, as well as proximal (*pfa*) and distal forearm (*dfa*) with Norland pDXA in 835 women aged 20 to 85 years recruited in four centers. The *s*BMD and *fn*BMD were converted to a Hologic base using the method of Genant et al. and t-score were then derived using data from the Canadian Multicentre Osteoporosis Study (Ca*M*os). The subjects completed a short questionnaire including general and medical data, lifestyle habits and hormonal status. There was a good correlation between BMD of the different anatomic regions ($r = 0.581$-0.712, $p < 0.0001$). To examine the performance of DXA in phalanx and forearm as a diagnostic test for osteoporosis, we performed ROC curves where a positive case was defined as a t-score <= -2.5 either on *s* or *fn*. The areas under the curve were not significantly different between sites and were respectively 0.870 for *p*, 0.889 for *pfa*, and 0.893 for *dfa*.

	Peripheral T-score Sensitivity	< = -2.5 Specificity	Absolute BMD cutoff values	Sensitivity	Specificity
p	39%	95%	0.436	79%	83%
pfa	75%	85%	0.703	84%	79%
dfa	42%	96%	0.280	90%	75%
pfa + dfa	77%	85%	0.641 and 0.252	84%	83%

At a cutoff t-score of -2.5 as determined by each peripheral apparatus, the sensitivity and specificity when compared to previously defined positive cases, are shown in the above table. Using absolute BMD cutoff values and combining the two forearm measurements, both sensitivity and specificity are markedly improved. At these absolute values for combined forearm measurements, the mean central T-score among the false positives is -1.94 (± 0.42) and the median is -2.00. Only three subjects with a central T-score >-1 are classified osteoporotic. The mean central T-score among the false negatives is -2.85 (± 0.31) and the median is -2.74. Only five subjects with a central T-score < -3 are missed. There is no statistical difference between the different sites. Hence, although peripheral bone mass measurements present relatively low sensitivity at a t-score cut point of -2.5, their validity is increased at other cut points. Therefore, a peripheral measurement of BMD together with a good clinical evaluation of the osteoporosis risk profile of the subject, can be an interesting tool for the diagnosis of osteoporosis in areas where central DXA is not available.

SU115

Using Forearm BMD to Find Osteoporosis at the Hip. S. R. Cummings,[1] L. Palermo,[2] D. M. Black.[2] [1]Medicine, University of California, San Francisco, CA, USA, [2]University of California, San Francisco, CA, USA.

Hip DXA is the strongest predictor of hip fracture and predictor of reduction in fracture risk with alendronate or risedronate. Treatment guidelines are based on hip BMD. Forearm BMD could be a useful initial screening test if it inexpensively predicts whether a woman will have osteoporosis by hip DXA. We measured distal forearm and femoral neck hip DXA (Hologic) in 209 healthy Caucasian women, age 60 to 79, recruited from 4 U.S.cities. Means and standard deviations were based on Hologic norms and NHANES values for femoral neck BMD. 35 (17%) had femoral neck BMD T score ≤ -2.5 while 61 (29%) had distal forearm BMD T-score ≤ -2.5. The probability that a woman has osteoporosis (T≤-2.5) at the hip increased with decreases in forearm BMD (Table). Osteoporosis at the femoral neck was uncommon (7%) if the forearm BMD T was above ≤2.5 but common (39%) if the forearm T-score was below - 2.5.

Forearm T-Score	***% (95%CI) with hip T*≤–2.5**
> -1.4	4 (0 to 8)
-1.5 to -2.4	12 (4 to 20)
-2.5 to -3.4	32 (17 to 46)
T≤–3.5	52 (32 to 73)

Referring the 39% of women with forearm T ≤-2.5 would have missed 31% of women with osteoporosis at the femoral neck. These results apply to elderly women and the Hologic forearm DXA and will differ for other age groups and devices; those devices need similar analyses. We conclude that forearm DXA may be a useful 1st screen. Women with a forearm T-score above -2.5 uncommonly have osteoporosis at the hip. Forearm BMD below -2.5 leaves uncertainty about whether the woman has osteoporosis at the hip and warrants measurement of hip BMD

SU116

Accuracy of pQCT for Density and Area Measurements of Mouse Bones. M. D. Brodt,* J. Taniguchi,* C. B. Ellis,* M. J. Silva. Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO, USA.

Peripheral quantitative computed tomography (pQCT) is increasingly used to measure the density and geometry of rat and mouse long bones. While excellent accuracy has been demonstrated for rat bones, accuracy has not been reported for specimens the size of mouse bones. Due to the small cortical thickness of mouse bones (0.1-0.5 mm) and the relatively large voxel size of pQCT scanners (0.07 mm and larger), volume averaging artifacts may introduce errors in measurements of density and area. Our objectives were: 1) to determine the accuracy of pQCT density and area measurements made on standard aluminum phantoms of varying thickness, and 2) to determine the effect of varying software threshold on the accuracy of pQCT area measurements of mouse femora. All specimens were scanned at 0.07 and 0.09 mm resolution using a commercial pQCT scanner (XCT Research M, Norland, Stratec). Aluminum Phantoms: Five aluminum tubes and one solid cylinder were used as standard phantoms. Dimensions were chosen to represent bones ranging from young mouse femora to adult rat femora (wall thickness 0.1 – 1.0 mm). Despite their uniform composition, the pQCT-measured density and area of the aluminum phantoms varied greatly with wall thickness. Errors of -70% in BMD (relative to BMD for the solid cylinder) and greater than +100% in area (relative to caliper measurements) were observed for the tube with a wall thickness of 0.1 mm. Increasing the software threshold (from 100 to 700 mg/cm^3) and decreasing the voxel size (from 0.09 to 0.07 mm) decreased but did not eliminate these errors. Mouse Femora: Femora from female C57BL/6J mice were obtained at ages 4, 8, 16 and 24 weeks (n = 5/group) and scanned at the midshaft. After scanning, femora were embedded and sectioned for histomorphometric analysis to determine reference values of bone area. The average threshold values required to match pQCT bone area with histomorphometric bone area ranged from 775 mg/cm^3 for 4-week old bones (average thickness = 0.16 mm) to 900 mg/cm^3 for 16-week bones (average thickness = 0.18 mm) ($p < 0.005$). Conclusions: 1) Differences in specimen wall thickness can lead to dramatic differences in area and density. Thus, slight differences in cortical thickness between experimental groups may introduce systematic errors in pQCT measurements. 2) pQCT can be used to accurately determine cross-sectional area of mouse bones provided that an optimal threshold is determined a priori. Validation of this threshold should be done by comparison to histomorphometric measurements and be specific to the size of the bones being analyzed. 3) Our results suggest that researchers using pQCT to study mouse bones interpret their results with some caution.

SU117

BMD Z-score Threshold for Osteoporosis in Young Patients: A Proposal. M. L. Bianchi,[1] S. Saraifogher,*[1] E. Galbiati,*[1] M. Bardare,*[2] L. Ghio,*[3] L. Morandi,*[4] A. M. Giunta.*[3] [1]Bone Metabolic Unit, Istituto Auxologico Italiano IRCCS, Milano, Italy, [2]Clinica Pediatrica I, University of Milano, Milano, Italy, [3]Clinica Pediatrica II, University of Milano, Milano, Italy, [4]Clinica Neurologica, Istituto Besta, Milano, Italy.

According to the WHO, the diagnosis of osteoporosis in post-menopausal **w**omen can be made on the basis of the decrease of bone mass below a certain T-score threshold (-2.5), even in the absence of fractures.Osteoporosis is increasingly encountered in children and adolescents affected by many chronic diseases, either as a consequence of the primary disease itself or as an effect of drug therapy (e.g. steroids). Moreover, fragility fractures seem

to be relatively infrequent in young patients even in the presence of low bone mass, apart from special conditions such as osteogenesis imperfecta. Thus, it would be very important to be able to make a diagnosis of osteoporosis before fractures, in order to implement preventive and/or therapeutic strategies (diet, mobilization, drugs), especially in view of future interventions (such as transplants in chronic renal failure or cystic fibrosis).With this study, we are tentatively suggesting a BMD Z-score threshold for the diagnosis of osteoporosis in younger patients. 705 patients (aged 2-19 years) affected by various chronic diseases potentially involving bone - excluding osteogenesis imperfecta - were studied. The Z-scores were calculated with reference to a sex/age matched group of healthy Italian subjects. In our sample, 75 patients (10.6%; Z-score –3.3 ± 2.1; range –0.9 to –5.5) had one or more fractures after minor trauma. Among them, 49 (65.3%) had a Z-score below -2; 23 (30.7%) had a Z-score between -2 and -1; 3 (4%) were over -1 (= -0.9). The first group comprised most cases of vertebral, hip or humerus fractures, as well as multiple fractures. In the last group, only a single fracture (forearm or foot) was present.If a fragility fracture can be considered diagnostic of osteoporosis, a Z-score below -2 would identify no more than two thirds of these fractures in our sample. Thus, in our opinion, a Z- score = -2 could be considered a reasonably prudent cut-off value for a diagnosis of osteoporosis in pediatric patients even in the absence of fractures.By this criterion, our sample would be characterized as follows: 294 patients (41.8%) had osteoporosis (Z-score < -2); 233 (33%) could be considered affected by osteopenia (Z-score between -2 and -1); 178 (25.2%) were normal (Z-scores > -1). If the 75 subjects with fractures were all considered as having osteoporosis, the remaining 630 patients without fractures would be characterized as follows: 245 patients (38.9%) had osteoporosis; 210 (33.3%) had osteopenia; 175 (27.8%) were normal.

SU118

Pediatric Total Body and Spine Measurements with Lunar DPX and Lunar EXPERT-XL in a Clinical Setting. G. Lien,*[1] J. Bollerslev,[2] G. A. Isaksen,*[2] K. Godang,*[2] Ø. Førre.*[1] [1]Center for Rheumatic Diseases, National Hospital, Oslo, Norway, [2]Section of Endocrinology, National Hospital, Oslo, Norway.

Two different densitometry systems representing two models, Lunar DPX and Lunar EXPERT-XL are used for osteodensitometry in our hospital. The results for adults produced on the EXPERT-XL system correlates highly significantly to results from the DPX. Thus, the reference population data for adults are the same for the two systems. For children, however, the correlation between osteodensitometry data from the two systems have not been published. The aim of this study was to determine the correlation between results for children measured on the two systems and whether the DPX reference data can be applied on results from the EXPERT.Thirty-one children (26 females, 5 males) with rheumatic disease were recruited as part of a larger study approved by the Regional Ethics Committee for Medical Research and the Norwegian Radiation Protection Authority. Informed consent was obtained from all participants. Mean age was 11,7 ± 3,6, range 6-19 years. Mean body weight was 40,5kg ± 16,4, range 16-81. Each subject was measured at the lumbar spine and total body on the Lunar DPX and the Lunar EXPERT-XL. The internal reliability of the results on the DPX and EXPERT-XL was assessed by analysis of internal consistency using Cronbach's alpha coefficient. The inter-item correlation of the lumbar spine (L2-L4) BMC was 0,99 (p<0,001, 95%CI 0,981-0,995). The inter-item correlation of the lumbar spine (L2-L4) BMD was 0,98 (p<0,001, 95%CI 0,968-0,992). In the assessment of total body the BMC inter-item correlation was 0,99 (p<0,001, 95%CI 0,986-0,996) and the BMD inter-item correlation 0,98 (p<0,001, 95%CI 0,972-0,993). Lean mass inter-item correlation was 0,99 (p<0,001, 95%CI 0,994-0,998), fat mass inter-item correlation 0,99 (p<0,001, 95%CI 0,996-0,999) and tissue % fat 0,99 (p<0,001, 95%CI 0,983-0,996). The correlations between results from the children measured on the DPX and EXPERT were highly significant. The data indicate that the reference population data for children can be equally applied on the two systems.

SU119

Performance of Simple Risk Indices for Identifying Postmenopausal Japanese Women with Osteoporosis. S. Fujiwara,[1] N. Masunari,*[1] G. Suzuki,*[1] P. D. Ross.[2] [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Merck Research Laboratories, Rahway, NJ, USA.

Risk assessment tools based on self-reported data have been reported - the purpose of such tools is to help physicians focus their efforts on patients at increased risk, and encourage appropriate use of BMD measurements. These tools include the Osteoporosis Self-assessment Tool for Asians (OSTA), SOFSURF, ORAI, and the Simple Calculated Osteoporosis Risk Evaluation (SCORE). The objective of the current study was to evaluate the performance of these indices among Japanese women in an ongoing large epidemiologic study in Japan, the Adult Health Study. The participants were ages 47-91 (mean 65) yr. There were 1127 women with spine BMD data, and 270 of these women (24%) had osteoporosis, as defined by spine BMD ≤70% of the young adult mean (a definition commonly used in Japan). An alternative definition of osteoporosis was also evaluated; 26% of all women had osteoporosis defined as spine BMD T-scores ≤-2.5. All 4 of the risk tools yielded reasonable (31-39%) specificity when sensitivity was set to approximately 90% for diagnosing osteoporosis using the 70% definition; similar results were obtained using T ≤-2.5 (specificity = 32-39% at ~90% sensitivity). The sensitivity and specificity using the OSTA index were very similar to those reported for other Asian populations using hip BMD. The OSTA is very easy to use - risk can be tabulated by age and weight, so that calculations are not necessary. Three OSTA risk categories were previously reported; using the 70% definition, the high risk subgroup (OSTA values < -4, representing 25% of all women) had a high prevalence of osteoporosis (43%), whereas the prevalence was 24% among a medium risk group (OSTA = -4 to -1, 50% of all women), and only 5% in the low risk group (OSTA ≥ 0, 25% of all women). Results were almost identical when the alternative definition of osteoporosis (spine BMD T-scores ≤-2.5) was used. These results are similar to those reported in the original Asian sample (osteoporosis prevalence = 61%, 15%, and 3%, respectively, based on hip BMD). We conclude that these risk indices developed in other populations performed well in this Japanese population. These free and simple risk assessment tools could encourage patients and clinicians to actively assess osteoporosis, and measure BMD when appropriate, before fractures occur.

SU120

Performance of Self-assessment Risk Indices for Encouraging Appropriate Use of Bone Density Measurements among Postmenopausal Women. E. Siris,[1] P. Geusens,*[2] H. Pols,*[3] C. Byrnes,*[4] J. Turpin,*[4] M. Melton,[4] P. Ross.[4] [1]Columbia University, New York, NY, USA, [2]Limburg University, Diepenbeek, Belgium, [3]Erasmus University Medical School, Rotterdam, The Netherlands, [4]Merck Research Labs, Rahway, NJ, USA.

Self-assessment risk tools could help increase awareness of osteoporosis, and encourage measurement of BMD - especially among patients at increased risk. Several risk assessment tools have been developed for postmenopausal women, including the Osteoporosis Self-assessment Tool for Asians (OSTA), SOFSURF, ORAI, and the Simple Calculated Osteoporosis Risk Evaluation (SCORE). The objective of the current study was to evaluate the performance of these indices in a sample of postmenopausal US women from the dataset originally used to develop SCORE. The original SCORE publication calculated T-scores from the manufacturer databases; therefore, we recalculated T-scores for Hologic using NHANES reference data for the current analyses. Data were available for 1102 postmenopausal women (82% were Caucasian, 5% Black, and 13% other ethnicity), ages 45-87 yr (mean = 61); 15% (n = 162) of these women had osteoporosis defined as femoral neck BMD T-scores ≤ -2.5, and 29% (n = 321) had T ≤-2.0. We chose index thresholds that identified ~90% of women with osteoporosis (~90% sensitivity), because the goal is to identify most women with osteoporosis. All four of the indices performed well, with specificity ranging from 41-59% (Table). Similar results were obtained when osteoporosis was defined as T≤ -2.0. Using the SCORE index, the prevalence of osteoporosis (T ≤ -2.5) was very high (60%) among the 8% of women with the highest index values (>15). The prevalence was much lower (2%) among the 43% of women with low index values (<7), and 18% among the remaining 49% of women with intermediate index values (7-15). Similar findings were obtained for the other indices using 3 risk categories. We conclude that all 4 risk indices performed well in this sample of postmenopausal women, although somewhat less stringent thresholds were necessary to achieve a sensitivity of 90% in this sample, compared to those reported for certain other populations. These free and simple risk assessment tools could help increase awareness among patients, and encourage the appropriate use of BMD measurements to diagnose osteoporosis before fractures occur.

Index	Sensitivity	Specificity
OSTA	89	52
ORAI	89	49
SOFSURF	92	41
SCORE	90	59

Disclosures: Merck &Co., Inc.,8.

SU121

Hologic QDR4500A: Recalibration of Fat Free Mass Measured by the QDR 4500A Using the Four Compartment Model. B. A. Blunt,[1] T. Fuerst,[2] M. Nevitt,[1] T. Lohman,[3] D. Schoeller,[4] M. Visser,*[5] T. Harris,[6] F. Tylavsky.[7] [1]Univ of Calif, San Francisco, CA, USA, [2]Synarc Inc, San Francisco, CA, USA, [3]Univ of Arizona, Tucson, AZ, USA, [4]Univ of Wisconsin, Madison, WI, USA, [5]Vrije Univ, Amsterdam, Netherlands Antilles, [6]NIH, Bethesda, MD, USA, [7]Univ of Tennessee, Memphis, TN, USA.

Several studies have found differences between body composition measured with Hologic QDR 4500A densitometers and body composition from reference methods. We developed a calibration adjustment for the 4500A to bring total body fat free mass (FFM_{DXA}) estimates in older adults into agreement with estimates obtained using the 4 compartment model (FFM_{4C}) and evaluated this adjustment in different subjects and scanners using estimates of FFM derived from total body water (TBW) measurements (FFM_{TBW}). The 4C model in the recalibration sample (sample 1) utilized body density (underwater weighing), scale weight, total body bone mineral mass (1.23*DXA BMC) and TBW (deuterium dilution space corrected for 4.1% nonaqueous distribution). In the validation subjects (samples 2-4), TBW was measured using the same method. FFM_{TBW} was calculated as TBW/0.73 in all samples. FFM_{DXA} was 3.9% higher than FFM_{4C} in sample 1, and an adjustment was derived using linear regression (Adj FFM_{DXA} = 0.964*FFM_{DXA}). After adjustment, FFM_{DXA} was closer to FFM_{TBW} in sample 1 (Table 1). In the validation samples, FFM_{DXA} was 4.1% to 10.1% higher than FFM_{TBW}. Adjusted FFM_{DXA} was no different from FFM_{TBW} in sample 2 but remained about 6% higher than FFM_{TBW} in samples 3 and 4 (Table 1). Reasons for the greater differences between FFM_{DXA} and FFM_{TBW} in samples 3 and 4 are uncertain. Validation of this adjustment for the whole body subregions is planned. Table 1. % Difference (FFM_{DXA} - FFM_{TBW}) (95%CI)

Sample (scanner #)	N	Ages (years)	Mean (SD) BMI	% Diff Before Adjust	% Diff After Adjust
1 (#1)	58	70-79	27.4 (4.5)	5.2 (4.3,6.1)	1.4 (0.6,2.2)
2 (#2)	37	18-71	31.1 (3.8)	4.1 (3.6,4.6)	0.4 NS(-0.1,0.9)

3 (#2)	140	70-79	27.5 (4.8)	10.1 (9.4,10.8)	6.2 (5.6,6.8)
4 (#3)	127	70-79	26.8 (4.8)	10.1 (9.4,10.8)	6.1 (5.4,6.8)

These data support an adjustment to the calibration of the QDR 4500A that will decrease the "leanness" of the soft tissue body composition estimates, bringing them closer to estimates obtained using the 4C and TBW reference methods.

SU122

A Proposal to Establish Comparable Diagnostic Categories for Bone Densitometry Based on Hip Fracture Risk among Caucasian Women over Age 65. D. M. Black. UC San Francisco, San Francisco, CA, USA.

For the NOF/ISCD Joint Committee on Simplification of BMD reportingIn 1992, the WHO Working Group on Osteoporosis proposed the BMD T-score concept as an epidemiologic tool for comparing the prevalence of osteoporosis across different populations. Increasingly, T-scores have become used for individual diagnosis and serve as the basis for many treatment guidelines. While T-scores were originally proposed for hip BMD, they have been applied to define thresholds for other BMD sites/techniques. However, it has become apparent that T-score thresholds from other sites are problematic and are not comparable to those from the hip. Therefore a consensus is emerging that T-scores cannot form the basis for comparability across devices. After consideration of various alternatives, our committee is proposing the following system for establishing comparable diagnostic cut-points for bone densitometry devices: 1. Index levels of hip fracture risk will be set based on age-specific 5 year risk of hip fracture risk among those with a BMD below specific T-score values (e.g. -2.5) at the femoral neck of the hip. 2. Analogous ("risk-equivalent") values for other devices will be calculated such that the hip fracture risk among those below those values is equivalent to the index risk. For example, the 5-year risk of hip fracture among 70-74 year old women with femoral neck BMD T-score (Hologic) below -2.5 at the femoral neck is 4.8%. In order to achieve that same risk, a woman of the same age would need to have Lunar spine BMD below 0.76 g/cm2 or Sahara ultrasound QUI below 58.3. To calculate these risk-equivalent values, we developed the necessary statistical models and gathered and confirmed normative data from all manufacturers. We also performed a meta-analysis of BMD and hip fracture risk since risk equivalent values could be derived only for devices/measurements for which the relationship to future hip fracture risk can be reliably estimated. In the future, values for devices will be added as data relating them to hip fracture risk become available. Another important limitation of this approach is that it must be limited to Caucasian women over age 65 for whom there is sufficient information about hip fracture risk. This model is currently being adapted for younger post-menopausal women using risk of all fractures, rather than hip fractures, to calculate risk equivalency. While a number of important issues remain, we believe this method provides a flexible framework for unified diagnoses in osteoporosis.

SU123

Frequency of Technical Errors and Inaccurate Interpretations of Bone Density Measurements. C. R. Schneyer, N. Orbach.* Division of Endocrinology, Sinai Hospital, Baltimore, MD, USA.

The purpose of this study was to assess the reliability of bone density studies performed on patients referred to an academic osteoporosis referral center. We conducted a retrospective review of records of 55 consecutive patients referred over a 3-month period in 2000. Patients included 51 women and 4 men (ages 29-81) who had been referred by physicians affiliated with a community teaching hospital. Bone density studies (DEXA) had been performed at 4 different outside radiology centers and 1 private group practice facility. During the review, we evaluated the accuracy of bone density interpretations and the reliability of comparisons with previous studies. We detected technical errors and misinterpretations in bone density measurements in 12 of the 55 patients, and most had multiple errors. These errors consisted of the following: (A) direct comparison of bone density measurements done by different methodologies (e.g. Hologic vs Lunar) (9 of 12); (B) disregard of sclerotic changes in the lumbar spine (9 of 12); (C) improper positioning of the hip during scanning (6 of 12); (D) measurements of incorrect sequences of lumbar vertebrae and comparison to measurements of correct vertebrae from previous scans (2 of 12); (E) comparison of opposite hips on sequential scans (1 of 12). Overall, 22% of the reports were erroneous, all to the detriment of the patient. Because of these errors, the reports incorrectly described reductions in bone density of up to 12% in the spine and 11% in the hip. In all cases, the primary care physician needlessly referred the patient to the osteoporosis referral center, and, in some cases, even prescribed a second anti-resorptive agent. Finally, the patients were (not unreasonably) distressed by this complication in their management. The current unreliability of bone density measurements and reports is damaging in the clinical setting as the inconsistent test results influence clinical judgment and affect patient treatment. Of considerable significance, these errors contribute to the high cost of health care. Since bone densitometry is the most valuable test available for diagnosing osteoporosis and for monitoring the efficacy of therapy, it is crucial that national standards be established to improve and preserve the reliability of these measurements.

SU124

Prediction of Hip Fragility from Radiographs: Glüer et al's 1994 Method Revisited. U. Olesen,*[1] J. Dequeker,*[2] L. Hyldstrup,[3] H. Thodberg.[1] [1]Pronosco A/S, Vedbaek, Denmark, [2]Reumatologie, Katholieke Universiteit, Leuven, Belgium, [3]Dept. of Endocrinology, Hvidovre Hospital, Hvidovre, Denmark.

In 1994 Glüer et al (JBMR 9, p.671) presented a method for prediction of hip fractures from pelvic radiographs. The study was based on SOF data and achieved a ROC area comparable to that of hip DXA. The present work is a continuation and evaluation of this radiographic method.

The study is based on 63 cadaver femurs from the BIOMED study by Dequeker et al. The excised femurs were imaged using films and were digitised in 300 dpi on a CCD scanner. From the identified contour of the femur, the caput center, the hip axis and the shaft axis were reconstructed and 172 standard points defined. 50 points were selected in the lower neck and on both sides of the shaft, and the cortical thickness was determined here using automated radiogrammetry (as in the DXR-BMD method). A principal component analysis was applied to reduce these measurements to two cortical thickness (CT) variables.The contours were aligned using Procrustes analysis, whereby a measure of the overall size of the femur is generated. A principal component analysis was then applied to yield three shape parameters describing the most important shape variation among the cases. The total hip DXA has also been measured. A number of researchers from our laboratory scored the texture of the femurs. Some used their intuition and others imitated the Singh Index. Finally an endocrinology MD scored the images.The femurs were crushed using the standard method of Bouxsein and this strength was to be predicted by the various methods alone or in combination.Results: The MD outperformed the laymen in the texture scoring, so the MD texture score is used in the results below.The following correlations to hip strength were obtained

Correlations to Hip Strength

Method	Correlation
CT + size + shape	0.67
CT + size + shape + texture	**0.82**
DXA	0.87
DXA + size	0.88
DXA + size + shape	**0.89**
DXA + size + shape + texture	0.90

The combination CT + size + shape + texture is an emulation of the radiographic method, and it is not as good as DXA, which could be explained by the different end-point and the use of excised bones. This presents a challenge for the radiographic method, which can be improved using advanced texture analysis. Finally it is seen that DXA is not the best method, since shape, size and texture of the femur contain additional relevant information.

SU125

Do μCT and SRCT Provide the Same 3D Structural Information for Trabecular Bone in the Rat Model? J. H. Kinney,*[1] T. M. Breunig,*[1] J. Kumer,[2] N. E. Lane.[2] [1]Preventive and Restorative Dental Sciences, UCSF, San Francisco, CA, USA, [2]Medicine, UCSF, San Francisco, CA, USA.

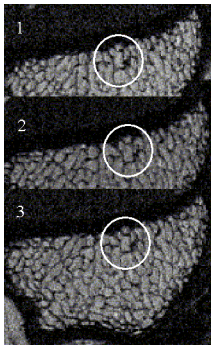
Recently, the μCT, a laboratory-based x-ray imaging technique capable of measuring the trabecular microarchitecture and structure from intact bones, has become commercially available. In addition to structural information, data generated by μCT can be used as a model for calculating the biomechanical properties of trabecular bone. The ability to non-invasively determine the 3D structure of trabecular bone and calculate its structural properties from intact specimens is an exciting technological advance. High resolution Synchrotron Radiation-CT (SRCT) has also been utilized for the past 7 years to evaluate the 3D properties of trabecular bone and to calculate its material properties. The purpose of this study was to compare these 3D imaging techniques by determining morphometric indices of trabecular bone measured with similar volume element sizes and signal-to-noise ratios in the same specimens. The right tibiae from 16 female Sprague Dawley rats, 8 months old, were imaged post mortem. The SRCT data was collected using parallel, 25 keV, monochromatic synchrotron radiation. The μCT data was collected using a Scanco μCT20 system. In the SRCT data, cortical and trabecular bone densities were the same, whereas the μCT density of trabecular bone was lower than cortical bone. The trabecular bone volume varies linearly for both systems between thresholds of 80 and 120, but with significantly different rates (μCT=0.53, SRCT=0.36). Below 80, the μCT data for trabecular bone begins to overlap the storage solution attenuation. Table 1 illustrates the differences in morphometric indices from the two systems. When a common cortical bone density was selected, the μCT underestimated all of the indices. To match the trabecular bone volume, a very low bone density threshold was required and resulted in overestimates of the other indices. Matching trabecular width, a significant feature for calculating 3D structural response in finite element models, decreased the trabecular bone volume that would be incorporated into a model. Our preliminary findings suggest that there are significant differences in the morphometric indices measured by these two systems. These differences in precision are caused by the energy correction, fan beam sampling geometry and reconstruction algorithm used by the μCT system. Further analyses will be performed to confirm or refute these findings. Table 1: Comparison of morphometric indices when the threshold is set to match cortical bone density, trabecular bone volume, or trabecular width.

Method	TB/TV (%)	Tb.W.(μm)	Connectivity	Threshold
SRCT	17	90	1672	110
μCT	9	93	1051	110
μCT	17	104	1378	80
μCT	6	90	767	120

SU126

Reproducibility of μ-MRI-based Trabecular Bone Structure Measurement. R. H. Weening, F. W. Wehrli, B. R. Gomberg, S. N. Hwang,* H. Song,* A. C. Wright.* Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA.

The practicality of μ-MRI-based "virtual bone biopsy" (VBB) for longitudinal studies hinges on the reproducibility of the derived bone structural parameters, which largely determine the size of the effect that can be detected at a given power and significance. In this work, we examine the reproducibility of VBB through measurement of trabecular bone structure in the distal radius and distal tibia. Toward this goal four healthy volunteers received three magnetic resonance imaging (MRI) scans each at both the distal tibia and distal radius (see below). The scans were performed on a standard General Electric 1.5 T clinical MRI scanner using custom-designed closely fitting wrist and ankle coils. Volunteers were scanned on separate days or by removing from the scanner and repositioning. The scan protocol included high-resolution localizers to aid in reproducible positioning. The MR data were acquired as a volume with an optimized 3-D spin-echo imaging sequence. The data were analyzed using bone volume fraction (TB/TV) mapping techniques described previously. After resolution enhancement the TB/TV maps were skeletonized and digital topological analysis (DTA) was performed. During the DTA procedure each bone voxel was classified as belonging to a surface (plate), curve (rod), or their mutual junctions. Following classification several derived structural parameters were computed, including "surface-to-curve ratio" and "erosion index." Finally, conventional parameters including thickness (TbTh), free end density, and node density were computed as well. Subject average coefficients of variation (CV's) were 3-5% or less for TB/TV in both the distal radius and distal tibia. The CV's for the DTA parameters were higher, in the neighborhood of 10%-15%. The lower reproducibility of the DTA parameters relative to TB/TV is more than offset by their significantly greater range (up to ten-fold). CV's for TbTh and node density were between 2.5% and 3.5% at the two measurement sites. The reproducibility achieved suggests that μ-MRI-based trabecular bone micromorphometry, here denoted "virtual bone biopsy," will be feasible for performing longitudinal studies to evaluate drug efficacy. Such a study, evaluating the structural implications of hormone replacement therapy, is currently in progress in the authors' laboratory.



SU127

Bone Architecture Quantification: Measures of Complexity Compared to Failure Load Results. W. Gowin,[1] P. Saparin,*[1] J. Kurths,*[2] C. Glaser,*[3] E. M. Lochmüller,*[4] D. Bürklein,*[4] F. Eckstein,[5] D. Felsenberg.[1] [1]Dept. of Radiology, Free University Berlin, Berlin, Germany, [2]Dept. of Physics, Univ. of Potsdam, Potsdam, Germany, [3]Inst. of Clin. Radiology, LMU Munich, Munich, Germany, [4]1. UFK, LMU Munich, Munich, Germany, [5]Inst. of Anatomy, LMU Munich, Munich, Germany.

The purpose of the study is to show that measures of complexity assess reliably the architectural composition of vertebral bodies.65 human lumbar specimens were obtained (42 females, 51-96 years old; 23 males, 64-93 years old) . The L3 were scanned transaxial by QCT (Siemens, Somatom Plus 4) at the midvertebral level in 10 mm slice thickness using 120 kV and 180 mAs. The Osteo-CT software (Siemens) was applied to obtain cortical and trabecular BMD. Utilizing the originally developed image processing technique, the same slices were used to calculate the mean Hounsfield Units (HU) of each vertebral body (pedicles and arch structures excluded) as well as four architectural parameters based on measures of complexity (Index of Global Ensemble [IGE], Structure Complexity Index [SCI], Trabecular Net Index [TNI], Max. L-Block). The L3 vertebrae were mechanically tested to failure in axial compression (3-segment method) by a material testing machine (Zwick 1445).The Rank-Order correlation coefficients for BMD, mean HU, and the measures of complexity compared to failure stress were calculated using MedCalc software.

Rank-Order correlation coefficients for failure stress and radiological measurements of L3 vertebrae

	Failure Stress
BMD trab.	0.601
BMD cort.	0.639
Mean HU	0.738
IGE	0.773
SCI	0.78
TNI	0.729
Max. L-Block	-0.744

The BMD of the vertebrae L3, measured either in the trabecular or cortical part, is a less reliable predictor for bone strength than the related quantity, mean Hounsfield units, obtained from non-segmented vertebral bodies. The mean HU of the trabecular bone only has a Rank-Order correlation to failure stress of 0.59. This indicates the importance to evaluate the trabecular as well as the cortical parts together when comparing those radiological measurements with biomechanical parameters.2. Structure quantification parameters based on measures of complexity correlate with failure stress very well and are significantly better than BMD (p=0.0001).3. A multiple regression analysis of four measures of complexity against failure stress resulted in r = 0.791.

SU128

3D Micro-Computed Tomography Assessment of Injectable Calcium Phosphate Biomaterial in vivo. D. von Stechow,*[1] O. Gaulthier,*[2] J. Bouler,*[3] G. Daculsi,*[3] E. Aguado,*[2] R. Müller.[4] [1]Orthopedic Biomechanics Laboratory, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA, [2]Laboratoire de Chirurgie, Ecole Nationale Vétérinaire, Nantes, France, [3]Laboratoire de Recherche sur les Matériaux d'intérêt Biologique, Faculté de Chirurgie Dentaire, Nantes, France, [4]Institute for Biomedical Engineering, ETH and University, Zürich, Switzerland.

This study investigates for the first time the in vivo performance of a composite injectable calcium phosphate bone substitute (IBS) with micro-computed tomography (μCT). The injectable biomaterial was obtained by the association of a biphasic calcium phosphate (BCP) ceramic mineral phase and a 3% solution of a cellulosic polymer (hydroxy-propyl-methyl cellulose) in a 50/50 weight ratio. The BCP particles were 200 to 500 μm in diameter. The injectable material was implanted for 6 weeks in 7x10 mm osseous cylindrical defects at the distal end of rabbit femurs. Qualitative and quantitative histological examinations were performed on both two-dimensional classical scanning electron microscopy and three-dimensional μCT. Micro-computed tomography provides a fast, reproducible, non-destructible method that gives results which are virtually identical to histology. Quantitative results of new bone formation and BCP resorption were assessed and compared for statistical purposes with variance analysis. Extensive bone colonization occurred during the 6-week implantation period and was observed with both 2D and 3D techniques. and three-dimensional approach to describe the new bone architecture. It showed that newly-formed bone was in perfect continuity with the trabecular host bone structure and demonstrated the total interconnectivity of the restored newly-formed bone network that developed inside the femoral defects. Both imaging techniques showed not only the development of bone ingrowth inside the defects but also the BCP degradation during the implantation period. This study provides the first 3D description of bone substitution using calcium phosphate bone substitute. The ability of IBS to restore the initial bone trabecular structure in rabbits 6 weeks after implantation is demonstrated. The non-destructive μCT imaging technique allowed not only a precise qualitative description of bone ingrowth but also to perform quantitative bone morphometric measurements. 3-D micro-CT imaging allows computation of additional parameters enabling us to monitor precisely the bone resorption-substituion process over time.

SU129

Digital Assessment of Radiographic Progression in Clinical Trials of Rheumatoid Arthritis Inter-reader Agreement. C. Wu.* Synarc, Inc., San Francisco, CA, USA.

Radiography of the hands and feet remains the mainstay of imaging assessment of rheumatoid arthritis (RA) in clinical trials. However, recent advances in digital image technology, including web-based electronic transfer, image edge enhancement and contrast manipulation, automated blinding of treatment and chronology data, and electronic reporting and databasing of results, are improving the speed, reliability, security and auditing capacity of these assessments. In the present study, we report the inter-reader agreement for independent, blinded assessments of radiographic progression in a multi-center clinical trial using a digital radiographic assessment system. Radiographs of the hands and feet of 29 patients with RA were acquired at baseline and after 24 months using a standardized technique and high-detail film. The radiographs were transferred to a central facility, digitized to a pixel resolution of 100 , edge-enhanced using unsharp masking, stripped of any chronological information and presented as baseline and follow-up pairs to two specially trained, experienced clinical-trials radiologists, who independently scored the images for erosions and joint-space narrowing using the Genant-modified Sharp grading scheme (1). Baseline scores were subtracted from follow-up scores to determine the progression scores for individual patients. Inter-reader agreement for radiographic progression was expressed as Pearson's correlation coefficient and intraclass correlation coefficient (ICC).Inter-reader Agreement for Radiographic ProgressionPearson's Correlation (ICC)Change in Erosion Score 0.94 (0.93)Change in Joint-Space Narrowing Score 0.97 (0.97)Change in Total Score 0.98 (0.97)As shown in the table, inter-reader agreements for change in erosion score, joint-space narrowing score and total score (erosion score + joint-space narrowing score) using the above digital assessment system in a multi-center clinical trial were all very high. These findings support the utility of centralized digital assessment of radiographic progression in clinical trials of RA.

SU130

Device for Digital Topological Analysis of Trabecular Bone Images. B. R. Gomberg, S. N. Hwang,* P. K. Saha,* H. K. Song,* F. W. Wehrli. Laboratory for Structural NMR Imaging, Radiology, University of Pennsylvania, Philadelphia, PA, USA.

The purpose of this work was to develop a system for digital topological analysis (DTA) and structural orientation measurement of three-dimensional trabecular bone images acquired in vivo in patients or ex vivo in specimens. DTA determines each voxel's unique topological class (one of 10 possible surface, curve and junction types). Fitting a plate, rod, or junction to the bone volume fraction (BV/TV) maps around each appropriately classified voxel allows derivation of structure-specific local parameters, notably plate orientation. The local topological and orientation information can be combined to derive indices of trabecular bone network integrity. Topological parameters and indices have previously been shown to correlate with biomechanical parameters and vertebral deformity status. Application of conventional (MIL, Tb.Th, Tb.N, etc.) and topology-based parameters in clinical and laboratory studies requires a system that is reproducible and efficient enough for large-scale batch processing. In vivo images were acquired on a clinical MRI scanner at the distal radius or distal tibia at 137x137x410 µm^3 voxel size in 15 minutes acquisition time by means of a custom-designed 3D spin-echo pulse sequence. In vivo DTA processing steps are: 1) manual outline of region of interest (a few minutes per dataset); 2) motion correction and Fourier reconstruction; 3) histogram deconvolution producing a BV/TV map; 4) subvoxel processing for resolution-enhancement resulting in a 69x69x102 µm^3 voxel size 3D data set; 5) binarization, skeletonization and topological classification; 6) orientation analysis yielding structural orientation histograms; 7) display of "virtual bone biopsy" for visualization. Finally, images of human bone specimens were acquired by µ-MRI and µ-CT at 80 and 22 µm^3 voxel size, respectively, and analyzed using steps 1, and 5-7. The system was built within the framework of Interactive Data Language (IDL - RSI, Boulder, CO, USA) enabling distributed processing among IDL routines, command-line programs, and a Unix-based processing subsystem. Integration of algorithms from multiple sources was implemented so as to facilitate incorporation of new analysis techniques in the future. Output concludes with a Microsoft Excel spreadsheet and Microsoft Word clinical report. Total time for analysis of 3D in vivo images was 52 and 124 min (distal radius and tibia, respectively, 1-2x10^7 voxels/scan location), and 84 min for a µ-CT images (6x10^7 voxels/scan location) on a 933 MHz Pentium III/512 MB PC. In conclusion, the system can easily analyze large amounts of data and provide useful information on trabecular bone microarchitecture.

SU131

Modeling Contributors to Bending Stiffness of Human Long Bones *in vivo*: Differentiating Factors in Weight Bearing (Tibia) vs. Non-weight Bearing (Ulna) Bones. L. E. Miller,[1] D. F. Wootten,[1] M. K. Zack,*[1] J. M. Beiseigel,*[1] S. M. Nickols-Richardson,[1] L. H. Cross,*[1] W. K. Ramp,[2] C. R. Steele,*[3] W. G. Herbert.[1] [1]Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, [2]Health Research Group, Blacksburg, VA, USA, [3]Stanford University, Stanford, CA, USA.

Dual energy x-ray absorptiometry (DXA) is widely used to evaluate bone quality at specific anatomical sites for research and clinical purposes. However, bone mineral density (BMD) and bone mineral content (BMC) have limitations for determining bone mechanical strength and fracture risk. Mechanical response tissue analysis (MRTA) is a non-invasive procedure that assesses bone stiffness using low frequency vibrations. The measured variable, EI, which is the product of Young's modulus of elasticity (E) and the cross-sectional moment of inertia (I), is a measure of the bending stiffness in long bones. Mechanical failure limits of monkey tibiae have shown that EI, in comparison to BMD, is a stronger predictor of maximum bone strength (Roberts et al., J Biomech 29: 91-98, 1996). Although McCabe et al. (J Bone Miner Res 6: 53-59, 1991) have quantified the relationship of EI vs. BMC (r = 0.59) for the ulna in young women, these relationships have not been described for the human tibia *in vivo*. Twenty-nine females (mean±SD: 20.0±1.7 yr, height 164.1±6.3 cm, weight 59.7±7.5 kg) underwent DXA, ulnar and tibial MRTA, and isokinetic strength tests. Ulnar width demonstrated a positive relationship (r = 0.46) with ulnar stiffness. Neither BMC, BMD, nor upper arm isokinetic strength were related to ulnar EI. For the tibia, the sum of fat-free leg mass and leg BMC was positively correlated (r = 0.61) with EI. In addition, tibial EI was positively correlated with tibia width, tibial BMC, total body BMC, and eccentric knee extension strength. However, using multiple regression, the sum of fat-free leg mass and leg BMC was the only predictor of tibial EI. Finally, bone stiffness values were divided into tertiles for the ulna and tibia, with comparisons made between high and low EI groups. No significant differences were noted in any variable means between ulnar EI groups when controlling for height, weight, and body fat percentage. However, when groups were separated into tertiles on the basis of tibial EI, and controlled for the same variables, significant differences were observed in fat-free leg mass (adjusted mean±SE: 7.5±0.2 vs. 6.5±0.2 kg, p<.03) and the sum of fat-free leg mass and leg BMC (7.9±0.2 vs. 6.8±0.3 kg, p<.02). These results suggest that weight-bearing bones have a dependence on regional muscle mass and BMC, whereas nonweight-bearing bones are influenced primarily by bone width.

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SU132

Tibial Measurement Reliability of Refined Mechanical Response Tissue Analysis. D. F. Wootten,[1] R. Thorne,[1] W. K. Ramp,[2] S. M. Nickols-Richardson,[1] S. Mott,*[1] S. Guill,*[1] W. G. Herbert.[1] [1]Virginia Tech, Blacksburg, VA, USA, [2]Health Research Group, Blacksburg, VA, USA.

Tibial EI_{MRTA} assessment is of central interest in our laboratory for quantification of mechanical properties in long bones resulting from various experimental exercise interventions. Previous work with tibial EI_{MRTA} by Arnaud et al (Biomechanics,13, 1984-1985, 1991) reported an intra-test (multiple measures without re-positioning of the limb) and inter-test (measures with re-positioning of the limb) coefficient of variations (CV) of 3.2% and 5-12%, respectively. Refinements (6-, 9-, and 12-parameter) in the mathematical modeling of the bone (Steele, 2000; personal communication) and overlying soft tissue (Roberts et al., J Biomech, 29, 91-98, 1996), have been developed and show promise for improving inter-day measurement reliability for tibial EI_{MRTA}. To evaluate the effectiveness of these models in improving intra-test, and inter-test we assessed measurement reliability for tibial EI_{MRTA} with 19 healthy women [20.8 $\pm$ 0.4 yr (Mean$\pm$SEM)] twice daily for three non-consecutive days. Subjects were familiarized with the measurement protocol on day one, and measurements were obtained on day three and five. On each day, five serial measurements were obtained for set 1, after which, subjects were removed from the measurement chair and re-positioned ~ 10 min later, then five serial measurements were obtained for set 2. Measurements were analyzed with each of the mathematical models and final EI values used for statistical analyses were selected based on the mathematical model that elicited the least root mean square (RMS) error for prediction of stiffness, as well as the least coefficient of variation (CV) for within trial measures. Bending stiffness (EI_{MRTA}) for day 3 was 87.7 $\pm$ 33.2 N•m^2 (mean $\pm$ SD) and 91 $\pm$ 24.7 N•m^2 for set 1 and set 2 respectively. Bending stiffness for day 5 was 85.6 $\pm$ 30.0 N•m^2, and 86.5 $\pm$ 28.9 N•m^2 for set 1 and set 2, respectively. The mean intra-test CV for EI_{MRTA} for all measurements on days 3 and 5 was $\leq$ 3.4% (range = 2.2% - 3.3%). The inter-test CV values within days 3 and 5 were 2.7%, and 0.7%, respectively. The mean of set 1 and set 2 measurements on day 1 was 89.3 N•m^2 compared to 86.1 N•m^2 on day two, a mean difference of 3.5%. The results of this study indicate that the refinements in the mathematical modeling parameters substantially improve inter-test measurement reliability. Inter-day measurement stability observed in this study suggests potential of MRTA for evaluating skeletal changes in tibial stiffness in experimental settings where interventions applied to groups may have robust effects on bone mechanical properties.

SU133

Resolution Sensitivity and Precision of Mouse Trabecular Bone Morphometry Measurements by Micro-Computed Tomographic Imaging. D. J. Adams,*[1] V. Diaz-Doran.*[2] [1]Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT, USA, [2]University of Connecticut Health Center, Farmington, CT, USA.

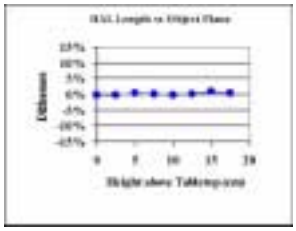
Commercial availability has popularized three-dimensional micro-computed tomographic imaging (µCT) for quantifying bone morphometry in small rodents. To aid investigators in planning and evaluating studies that include µCT quantitation of mouse bone architecture, we measured baseline sensitivity and reproducibility of mouse trabecular bone morphometric parameters at different resolution settings. Sensitivity was measured parametrically using a commercial fanbeam X-ray µCT instrument with a 7 µm focal spot (Scanco model µCT20). The sensitivity of mouse vertebral bone morphometry measurements to image array dimension and exposure time was examined for 512×512 vs 1024×1024 voxels/slice (18 µm vs 9 µm voxels) and 100 vs 350 msec/projection, respectively. Field of view was maintained at the highest resolution setting. Precision was measured by collecting five volumetric image arrays at identical geometric locations for each bone and permutation of resolution settings. Segmentation of bone from image background and selection of volumetric regions for analysis were identical throughout the series and equivalent in location to those used in standard 2D histomorphometry. Relative changes in morphometric parameters were computed as percent differences. Precision was quantified as the coefficient of variation (sd/mean × 100%, n=5) for individual trabecular parameters, including bone volume fraction (BV/TV), average trabecular thickness (Tb.Th.*), trabecular number (Tb.N*), trabecular spacing (Tb.Sp.*), bone surface area to volume fraction (BS/BV), connectivity density (Conn.D.), and the degree of anisotropy (DA). Doubling the image array dimension (a fourfold increase in voxel density) resulted in an 8.5% decrease in BV/TV, 19.2% decrease in Tb.Th.*, 41.3% increase in BS/BV, and 65.0% increase in Conn.D. A 350% increase in integration time resulted in parameter magnitude changes of less than 4%. Measurement precision was increased by doubling array dimension, but not by increasing integration time. Coefficients of variation were less than 3% for nearly all parameters, and typically less than 1%. Sensitivity of morphometric parameter magnitude to array dimension demonstrates the importance of comparing morphometric data derived only from images collected with identical array dimension (voxel size) and scan settings. Baseline precision of µCT morphometric measurements must be considered in terms of specific trabecular parameters, and demonstrates the utility of µCT to discriminate small differences in mouse trabecular bone morphometry.

SU134

Measurement of Femur Geometry (HAL) with PRODIGY Is Accurate and Unaffected by Magnification. H. Barden,[1] D. Settergren,[1] C. McClintock,[2] C. C. Johnston Jr,[2] C. H. Turner.[2] [1]GE Lunar, Madison, WI, USA, [2]Indiana University, Indianapolis, IN, USA.

Measurement of proximal femur BMD is recognized as the 'gold standard' for evaluating fracture risk at the hip. However, a positive association has also been shown between hip axis length (HAL), a measurement extending from the base of the greater trochanter along the hip axis to the inner pelvic brim, and hip fracture risk in at least six independent studies. Combining some aspects of femoral geometry with BMD may improve the estimate of hip fracture risk beyond that found for BMD alone. The accurate and precise measurement of HAL was reported previously with pencil-beam densitometers. The trend of newer instrumentation, however, has been toward faster, fan-beam systems that have the potential of producing errors on linear measurements due to magnification effects related to position of the measured object in the fan-beam. We determined the accuracy and magnification-effect of HAL measured with a narrow angle (4 degrees) fan-beam (PRODIGY, GE Lunar) densitometer. Accuracy was measured on 14 human excised femora. The effect

of magnification was determined by measuring a femur with known HAL at various heights (0 to 17.5 cm) above the tabletop. HAL measured with PRODIGY was highly correlated with HAL measured directly using calipers (r = 0.98), and showed no significant effect of magnification. The precision error (CV) of HAL measurements at all heights above the tabletop was less than 0.4%. PRODIGY provides an accurate measurement of HAL with no appreciable magnification effect.



Disclosures: Lunar,3.

SU135

Serum βCTx and TRAP in Various Disorders Affecting Bone. E. Romagnoli,*[1] M. Pacitti,*[2] V. Carnevale,*[3] A. Scillitani,*[3] S. Dionisi,*[2] F. Paglia,*[2] S. De Geronimo,*[2] J. Pepe,*[2] S. Mazzaferro,*[2] A. Ragno,*[2] M. Celi,*[2] N. Raejntroph,*[2] E. D'Erasmo,*[2] G. Mazzuoli,*[2] S. Minisola.[2] [1]Ospedale San Giovanni Battista, Rome, Italy, [2]Department of Clinical Sciences, Rome, Italy, [3]Ospedale C.S.S., San Giovanni Rotondo, Italy.

We evaluated two serum markers of bone resorption (C-terminal telopeptide of type I collagen -βCTx- and tartrate resistant acid phosphatase activity -TRAP-) in several groups of patients with the most common metabolic and endocrine disorders affecting bone. We studied: 77 patients with established osteoporosis (OP) (12 males -M- and 65 females -F-, mean age 73.1±8.9 yrs and 76.7±9.07 yrs, respectively, 60 of whom with hip, 13 with vertebral, and 4 with Colles's fractures); 44 with primary hyperparathyroidism (PHPT) (10 M and 34 F, 63.2±18.1 yrs and 57.5±14.7 yrs, respectively); 39 with chronic renal failure (CRF) (20 M and 19 F, 48.1± 13.4 yrs and 46±16 yrs, respectively), whose creatinine clearance was < 70 mL/min; 5 M with Paget's disease (PD); 3 M (66.6±9.4 yrs) with humoral hypercalcemia of malignancy (HHM) due to lung cancer; 3 M (53±11 yrs) with osteomalacia due to coeliac disease (OM); 10 F patients with post-surgical hypoparathyroidism (HPT) (57±6 yrs); 10 F with thyrotoxicosis (HT) (66±10 yrs); 10 F with Cushing's syndrome (CS) (43±16 yrs); 8 F with acromegaly (AC) (57±11 yrs). The results were compared to those of healthy subjects matched for age, sex and gonadal status. Serum βCTx was measured by a two-site ELISA and TRAP by spectrophotometric assay. In male patients mean Z-score values of βCTx and TRAP were: OP 4.4±1.9 vs 2.2±2.7; PHPT 5.5±4.9 vs 1.5±1.9; PD 15.1±5.5 vs 3.6±2.2; HHM 13.6±4.1 vs 3.6±2.9; OM 10±1.9 vs 2.7± 0.7; CRF 17.8±12.2 vs 2.4±2.3. In female patients the corresponding results were: OP 2.0±2.2 vs 0.9±1.9; PHPT 3.1±3.8 vs 0.9±3.1; HT 2.4±2.4 vs -1.1±0.7; HPT -0.8±0.3 vs -1.9±0.6; AC 1.2±0.9 vs -2±0.3; CS 1.7±1.5 vs 0.4± 0.8; CRF 8.4±10.4 vs 1.8±1.4. In each group the mean Z-score values of βCTx were significantly higher than those of TRAP ($p<0.04$). As compared to healthy controls, βCTx levels were significantly different in all groups, while TRAP in all patients but HHM, female PHPT and CS. In AC and HT TRAP Z-score values were lower while βCTx higher than in controls. In CRF creatinine clearance showed a significant inverse relation with βCTx ($r= - 0.61$, $p<0.001$) but not with TRAP. The measurement of βCTx seems to reliably estimate the resorption rate in most diseases affecting bone. βCTx and TRAP may reflect different phases of the resorption process, the first providing a superior sensitivity in the disorders studied; the results should be cautiously interpreted in patients with reduced creatinine clearance.

SU136

Replicated Diurnal Profiles of Bone Turnover Markers: Sampling Time Affects Within-Subject Reproducibility. A. C. Eagleton, R. Eastell, A. Blumsohn. Bone Metabolism Group, Clinical Sciences Division, University of Sheffield, Sheffield, United Kingdom.

Markers of bone turnover show a marked diurnal rhythm (within-day variability) which influences interpretation of these measurements. Between-day variability also influences interpretation of therapeutic response in individuals. The reproducibility of the "shape" of the diurnal profile within an individual is uncertain. The effect of sampling time on within-subject reproducibility is also unknown. The aims of this study were to investigate these issues for 3 serum-based markers of bone turnover:- serum β Crosslaps (βCTX), osteocalcin (OC) and PINP on the Roche Elecsys® 2010 automated system. Analytical precision was determined by replicate analysis of samples. Replicated diurnal profiles were determined in 20 postmenopausal women at an interval of one week (mean age 61.5, range 52-69; none taking drugs known to affect bone metabolism). Study conditions including composition and timing of meals were strictly controlled (meals at 10h00, 11h00, 13h00 and 16h00). Blood samples were taken at 8h30, 9h00, 9h30, 11h00, 12h00, 14h00, 15h00, 17h00, 18h00 and 19h00. Serum βCTX showed a marked diurnal rhythm (peak to trough difference 113%; ANOVA for time of day effect $P < 0.0001$). However the "shape" of the rhythm differed markedly between subjects but was highly reproducible within subjects. Before breakfast βCTX levels were constant but in all subjects a marked decline occurred following breakfast. In the late afternoon some but not all subjects showed a progressive rise in βCTX level. Peak to trough differences were relatively small for PINP (9%; ANOVA $P < 0.0001$) and OC (10%; ANOVA $P < 0.0001$) on a group basis, although the diurnal profile was similarly replicated within subjects. Nested ANOVA was used to determine components of variability (CVa = analytical; CVi = within-subject after accounting for CVa) at each time-point. CVa was less than 2% for OC and PINP at all timepoints. CVa was less than 7% for βCTX. Within-subject variability (CVi) for βCTX was lowest in the morning (CVi < 10%) and highest in the afternoon (CVi > 24%) at the nadir of the rhythm. There was no effect of sampling time on CVi for PINP, but for OC, CVi was somewhat lower in the afternoon (morning 9%, afternoon 5%).In conclusion: The diurnal profile of serum markers of bone turnover (particularly βCTX) is highly individual. Within-subject measurement variability expressed as CV is lowest in the morning for βCTX, and this would appear to be an optimal sampling time in clinical practice. However, the effect of sampling time on the response to antiresorptive therapy is uncertain.

SU137

First Measurements of the Calcium-41 Tracer Signal of Skeletal Turnover with a Compact Device. S. Freeman,[1] K. Wendt,*[2] P. Mueller,*[2] C. Geppert.*[2] [1]Scottish Universities Environmental Research Centre, Glasgow, United Kingdom, [2]Institut für Physik, Johannes Gutenberg-Universität, Mainz, Germany.

The ingestion and subsequent excretion of ^{41}Ca tracer results in a long-term noise-free urinary marker apparently sensitive to changes in individuals' skeletal turnover. However, tracer measurement has heretofore required the extreme sensitivity of a mass spectrometer based on a large particle accelerator. Wide adoption of the tracer technique likely requires a detection technology that can be generally deployed. In pursuance of this we have made measurements with a prototype benchtop laser resonance ionisation mass spectrometer. Its operation depends on ionising atoms of the desired isotope for analysis by very selective multi-photonic excitation. To compare the spectrometries samples were obtained from Stanford University tests of the ^{41}Ca signal and analysed. First results are in agreement with the previous accelerator measurements, attesting to the new instrument's capabilities.

SU138

The Bone Absorption Marker Deoxypyridinolin in Healthy Children and Young People of the Caucasien Race Between the Ages of 9 and 18. A. Knauerhase,*[1] C. Seelig,[1] M. Demuth,[1] C. Zingler,*[2] R. Hampel.[1] [1]Klinik für Innere Medizin, Universität Rostock, Rostock, Germany, [2]Institut für Klinische Chemie und Pathobiochemie, Universität Rostock, Rostock, Germany.

In a cross-sectional Study, the behaviour of the marker for bone absorption, deoxypyridinolin (DPD), was investigated in a large group of children and young people, together with the way this parameter correlates with body height, body mass, BMI and body surface area. In 1999 spontaneous urin samples were obtained in the morning from 1838 healthy boys and girls (10 age groups, ages 9-18). On average the children engaged actively in sports. Vegetarians, vegans, smokers, pupils with alcohol or drug problems or receiving medicines regularly were excluded. The BMI and body surface area were calculated from the body height and mass. Test persons whose body height or mass was beyond the 5th and 95th percentile were not included in the evaluation. The determination of the DPD was performed using the Pyrilinks-D-EIA-KIT, Immulite System (Biermann & Co.), in reference to the excretion of urinary creatinine. Statistics: SPSS programme, Spearman-Rho and Mann-Withney test. The maximum average amount of DPD for the boys was found in the 13 years group (20.6 +/- 8.3 nmol/mmol Crea, n=103). For the girls this amount was found in the 12 years group (20.3 +/- 8.2 nmol/mmol Crea, n=113). In the 9-11 years groups the average figure for DPD for boys and girls was only slightly below the maximum (approx. 19.0 +/- 8 nmol/mmol Crea). A sharp decline in DPD was found from the age of 15 to 16 for boys and from 14 to 15 for girls. For boys aged 18 and girls aged 17 the DPD figures were almost in the range for adults. The male test persons showed weaker (negative) correlations between DPD and body height, mass, BMI and body surface area (r=0.28) than the females. In the latter, the (negative) correlations were markedly higher, the strongest being that to the body surface area (r=0.52). Girls have significantly higher DPD amounts then boys. Boys and girls reach the maximum excretion of DPD in their 13th or 12th year, respectively. Prior to this the amounts are only slightly below the maximum. Thereafter they decline sharply for both sexes and reach the normal range for adults in their 17th-18th year. Because of the demonstrable correlations, DPD should be related to anthropometric parameters. A knowledge of the physiological course of this marker also serves to distinguish it from bone diseases in childhood and youth.

SU139

Evaluation of an Immunoassay for Type I Collagen Alpha 1 Helicoidal Peptide 620-633 as a Marker of Bone Resorption in Osteoporosis. P. Garnero,[1] P. D. Delmas.[2] [1]Inserm Unit 403 , Synarc, Lyon, France, [2]Inserm Unit 403, Lyon, France.

Type I collagen, the most abundant protein of bone matrix, consists of two alpha 1 chains and one alpha 2 chain associated in the triple helix except at the ends (telopeptides). During osteoclastic bone resorption, type I collagen is cleaved by proteinases including cathepsin K, both in the telopeptides and the helicoidal region. Currently, markers reflecting type I collagen degradation are based on the measurements of N and C-terminal crosslinking telopeptides (NTX and CTX, respectively) in urine and serum. A peptide was isolated from the urine of a patient with Paget's disease consisting of residues 620-633 of the helical region of the alpha 1 chain. An ELISA employing a mouse monoclonal antibody raised against the helical peptide was developed (Metra Helical Peptide, Quidel Corporation). This assay has no significant cross-reactivity with intact collagens type I, II and III, nor with alpha 1 (II) and alpha 2 (I) homologous peptides. The intra and inter assay coefficients of variation were found to be low at 7% and 9% respectively, and analytical dilution and recovery ranged from 94 to 115%. Urinary helical peptide levels increased from a mean of 54.6 (SD:20.9) micrograms/mmol Cr in premenopausal women (n=24) to 77.6(SD:35.8) micrograms/mmol Cr in 65 healthy untreated postmenopausal women (+42%, p=0.0012). Levels were highly correlated (r=0.77, p<0.0001) with urinary CTX

levels (Crosslaps ELISA, Osteometer Biotech). After 3 months of oral alendronate (10 mg/day) in 20 postmenopausal women with osteoporosis, urinary helical peptide decreased by 71.5% (p<0.0001), a decrease similar to that of urinary CTX (-69%, p<0.0001). After 6 months of treatment with transdermal 17 beta estradiol (50 micrograms / day) in 21 healthy postmenopausal women, urinary helical peptide decreased by 59% (p<0.0001 vs baseline), a decrease similar to that of urinary CTX (-64%), whereas no significant change was observed with placebo (-0.29%). Interestingly the percentage change of helical peptide at 6 months significantly correlated with the change of spinal bone mineral density after 2 years (r=-0.42, p=0.0014).In conclusion, this new assay for type I collagen helical peptide has demonstrated adequate analytical and clinical performance. The test was a sensitive indicator of the antiresorptive effects of bisphosphonate and estrogens and was highly correlated with urinary CTX, an established bone resorption marker. Thus this new bone resorption marker and assay should be useful for the clinical investigation of patients with osteoporosis

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Assay of Helical Peptide (α1(I)620-633) as an Indicator of Bone Collagen Resorption. F. Gossiel,* K. E. Naylor, R. A. Hannon, R. Eastell, A. Blumsohn. Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Most clinically useful biochemical markers of bone resorption are based on measurement of deoxypyridinoline or associated epitopes in the telopeptide region of type I collagen. We evaluated the performance of an assay for an epitope in the helical domain, α1(I)620-633 in urine (uHelPep) in comparison with urinary N-telopeptide (NTX). The assay is ELISA-based in a competitive format (Quidel Corp., CA).Analytical performance was satisfactory (within batch CV 5.6% at concentrations > 10ug/L; total CV <10%, linearity of dilution in water). HelPep in urine decreased by less than 12% after 2 days of storage at 4°C and by 5.1% following 9 brief freeze-thaw cycles. Excretion of uHelPep showed a large-amplitude circadian rhythm which was slightly more pronounced than that for uNTx (n=17 premenopausal women; peak to peak amplitude 79.8% of 24hr mean for uHelPep/Cr; 63.3% for uNTX/Cr; ANOVA time of day effect P < 0.001 for both analytes; P=0.058 for uHelPep vs NTx). Performance was explored in several treatment models. 1) Following alendronate therapy (10mg, n=16) in postmenopausal osteoporosis uHelPep/Cr decreased by 78%±6 SEM at 25 weeks (n = 16). This change was greater than that for NTX/Cr (71%±5 SEM; P = 0.03 vs uHelPep). 2) Following calcium supplementation (500mg/day x 6 months) uHelPep/Cr decreased by 36%±9 SEM at 25 weeks (n = 16). This change was greater than that for NTX/Cr (21%±10; P = 0.09 vs uHelPep). 3) In Paget's disease uHelPep/Cr decreased by 81%±8 SEM 14 days after i.v. pamidronate (60mg, n=8) and by 79%±8 at 6 months after oral etidronate (400mg/day, n=7). uNTX fell by 75%±10 and 66%±11 respectively. 4) 25 weeks following subcutaneous estradiol implant (25mg) in postmenopausal women (n = 21), uHelPep decreased by 54%±6 (vs 44%±5 for uNTX; P<0.05 for comparison with uHelPep/Cr). Control subjects (n=11) showed no significant response to sham implant. Within subject variability (CVi) in control subjects from this study was also slightly greater for uHelPep/Cr (24.1%) than for NTX (19.1%) in controls. The signal to noise ratio (%difference/CV) of the two analytes was therefore similar (3.0 for uHelPep/Cr and 2.9 for uNTX/Cr). In conclusion: Measurement of epitopes in the helical domain of type I collagen are likely to provide useful information about bone collagen resorption. Epitope α1(I)620-633 in urine tends to show slightly greater responsiveness than urine NTX in several clinical models.

SU141

Bone Mineral Density and Bone Turnover Markers in Women with Lupus and in Healthy Controls. A. Bongu,*[1] C. B. Langman,*[2] S. Manzi,*[3] S. Spies,*[1] R. Ramsey-Goldman.[1] [1]Northwestern University, Chicago, IL, USA, [2]Northwestern University &Childrens' Memorial Hospital, Chicago, IL, USA, [3]University of Pittsburgh, Pittsburgh, PA, USA.

Improvements in survival rates in patients with lupus have focused attention towards improving morbidity associated with disease and its treatment. Osteoporosis is an ever-increasing complication confronting lupus patients. The goal of this study was to determine if in bone mineral density (BMD) correlated with bone turnover markers (BTM) in lupus patients and in healthy, unrelated controls matched by age (± 4 years), race, and menopause status. Forty-seven pairs of lupus women and controls had BMD of the spine, hip, and wrist and the following BTM measured: 25(OH)D and $1,25(OH)_2D_3$, bone alkaline phosphatase (BAP), osteocalcin (OC), and urinary N-linked telopeptides (NTx). Descriptive statistics were used to describe patient characteristics, and Spearman correlations were used to describe relationships between each marker and BMD at each site. The mean age of the lupus women and controls was 43.5 and 43.6 years, and mean disease duration in lupus women was 9.9 years. Eighteen pairs were menopausal and 29 pairs were still menstruating. The mean age at menopause was 42.3 years for lupus and 43.9 years for the controls (p=0.05). Only lupus women reported taking corticosteroids. Mean BMD was lower at all sites in lupus women compared with controls at the hip (0.892 vs. 0.897 gm/cm^2, p=0.9), spine (0.994 vs. 1.028 gm/cm^2, p=0.2), and wrist (0.677 vs. 0.695 gm/cm^2, p=0.2), respectively. For lupus women, higher urinary NTx was correlated with lower BMD at the hip (r= -0.299, p= 0.04), spine (r= -0.323, p= 0.03), and wrist (r= -0.333, p= 0.03); higher BAP was correlated with lower BMD at the spine (r= -0.309, p= 0.04) and wrist (r= -0.532, p= 0.00); and higher OC correlated with lower BMD at the hip (r= -0.307, p= 0.04). In contrast, the only significant correlations noted in controls were between higher BAP and lower BMD at the spine (r= -0.371, p= 0.01) and wrist (r= -0.312, p= 0.04).

Median BMT Levels in Lupus Women and Controls Stratified by Menstrual Status

Bone Marker	Postmenopausal Lupus	Postmenopausal Control	Premenopausal Lupus	Premenopausal Control
NTx nM/mM	33.5	35.0	33.0	30.0
BAP U/L	16.6	17.4	13.4	13.8
OC ng/mL	6.6	7.1	7.0	6.4
25(OD)D ng/mL	12.7	12.9	13.6	13.3
$1,25(OD)_2D_3$ pg/mL	29.8	45.1	40.3	44.8

When stratified by menopause status, correlations in lupus patients between BMD and NTx, BAP, and OC were similar but not statistically significant likely due to small numbers in each group. Bone turnover markers may have a potential role in identifying lupus patients at risk for low BMD. Future studies will include longitudinal followup of subjects' BMD to assess the ability of baseline BTM to predict subsequent BMD

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Osteocalcin Does Not Improve the Short-Term Fracture Prediction by Femoral Neck BMD in Perimenopausal Women. L. Sandini,*[1] R. Honkanen,*[2] M. Tuppurainen,*[3] J. Jurvelin,*[4] J. Huopio,[1] H. Kröger.[1] [1]Department of Surgery, Kuopio University Hospital, Kuopio, Finland, [2]Public Health Research Institute, University of Kuopio, Kuopio, Finland, [3]Department of Gynecology and Obstetrics, Kuopio University Hospital, Kuopio, Finland, [4]Department of Clinical Physiology, University of Kuopio, Kuopio, Finland.

Introduction: menopause induces an increase in bone turnover and significant loss of bone. However, the incidence of fractures increases about 2 decades later, and the early identification of individuals at risk of fracture is difficult. We assessed the predictive value of serum osteocalcin in addition to BMD measurement. Methods: serum samples for osteocalcin (OC) and femoral neck BMD measurements were obtained for 1145 volunteers from the population-based Kuopio Osteporosis Risk Factor and Prevention (OSTPRE) Study between 1995 and 1997. OC was measured regardless of the time of day or of the fasting status. Data about HRT use was obtained from self-administred questionnaires. Subjects were considered HRT users if they had been on HRT for more than half of the time during each year of follow-up. Fractures were self-reported in a postal enquiry sent to all participants in may 1999. Results: Mean duration of follow-up was 4.4 yrs (range 1-52 months). Eighty subjects reported at least one fracture (wrist 33, ankle 10, lumbar spine 3, any other 34, no femoral fractures). The fractured (FR)and non-fractured (N-FR) groups did not differ in age or BMI. There was no statistically significant difference in the fraction of HRT users at inclusion between the FR (26.3%) and the N-FR (31.8%) groups. Serum OC was significantly higher in the FR group (8.3 ± 4.2 ng/ml) than in the N-FR (7.4 ± 3.3 ng/ml; p=0.045). Femoral neck BMD was lower in the FR) group (0.854 ± 0.125 g/cm^2) than in the N-FR (0.904 ± 0.129 g/cm^2; p=0.001. In a Cox regression model, after correction for age, BMI and HRT use at inclusion, serum osteocalcin was predictive of the fracture event (p=0.05). However, when HRT use during follow-up was added to the model as a time-dependent covariate, osteocalcin lost its significance (p=0.06), while none of the other predictors had a significance under p=0.12. Femoral neck BMD expressed in standard deviations of the mean, corrected for age, BMI and HRT use during follow-up was highly predictive of the fracture, (hazard ratio for each decrease of one SD: 1,57; 95%CI: 1,17-2,11; p=0.003). Adding osteocalcin to the model only marginally altered this result. Conclusion: Serum osteocalcin measured in the early postmenopausal years is predictive of fracture. However, when BMD measurement is available, OC determination does not increase the short-term predictive value of BMD in early postmenopausal women.

SU143

Validation of a New Automated Immunoassay for Measurement of Intact Osteocalcin. W. J. Fassbender,[1] B. Steinhauer,*[2] H. Stracke,[2] P. M. Schumm-Draeger,*[1] K. H. Usadel.*[1] [1]Medical Dept. I, Endocrinology, University Clinic of Frankfurt/M, Frankfurt am Main, Germany, [2]Medical Dept. III, University Clinic, Giessen, Germany.

Bone turnover is assessed indirectly by measurement of biochemical markers of bone turnover. Osteocalcin, a 49-amino-acid protein is a major noncollagenous protein of bone matrix, synthesized by osteoblasts and odontoblasts. Various assays exist for assessment of osteocalcin in serum and concentrations in the same sample may vary enormous. The used antibodies may recognize intact osteocalcin and/or circulating fragments of osteocalcin. We here describe and validate a new automated immunoassay system for measurement of intact osteocalcin (DPC IMMULITE assay) using monoclonal antibodies (mouse) against the C-terminus of osteocalcin (AA 44 - 49). Detection limit of the assay is 1.0 ng/ml. While different laboratory assays show marked clinical discordance, we evaluated our results comparatively to an established IRMA method (Nichols). We observed a highly significant correlation between both assays (r = 0.9352, p < 0.0001, n = 286) for healthy persons and also for patient samples (osteoporosis, diabetes type 1, rheumatoid arthritis). Very low inter- and intraassay covariance as well as highly significant linearity (analytical recovery near 100 %) tested by serial dilutions is demonstrated for the DPC IMMULITE intact osteocalcin assay. We conclude, that the IMMULITE assay system is an useful method for assessment of serum intact osteocalcin giving valuable results in comparison to an established non-automated assay.

SU144

Clinical Use of Automated Serum C-Telopeptide Assay (sCTX)on the Elecsys 1010, in Normal Subjects, in Osteoporosis and Paget Disease. Comparison with Urinary C-Telopeptide CrossLaps (uCTX). C. A. Casco,[1]

A. Oviedo,*[2] C. A. Mautalen.[3] [1]Laboratorio, Centro de Osteopatias Medicas, Buenos Aires, Argentina, [2]Centro de Osteopatias Medicas, Buenos Aires, Argentina, [3]Division Osteopatias-Hospital de Clinicas-Universidad de Buenos Aires, Buenos Aires, Argentina.

The aim of the present study was to examine the clinical use of sCTX, by an automated assay on the Elecsys 1010 (Roche Diagnostics), compared to the excretion of uCTX by a manual ELISA assay (Osteometer Bio Tech A/S), in controls subjects, osteoporosis and Paget Disease. We studied 64 control subjects: 22 premenopausal women (age 36 ± 9 years) , 27 postmenopausal women (age 66 ± 8 years) and 15 men (age 52 ± 18 years).The results were (mean ± SD): uCTX ug/mM Cr was: 224 ± 117, 324 ±120 and 162 ± 128 respectively, and sCTX ng/L was 245 ± 102, 330 ± 124 and 231 ± 108 , respectively. In 32 women with osteoporosis, (age 62 ±11 years) , the correlation between sCTX and uCTX by ANOVA was r: 0.87 ; p<0.0001. This group was divided by levels of uCTX . The grup I with uCTX 400 ug/mM Cr the mean ± SD of sCTX was 855 ± 348 ng/L. In 43 treated and untreated patients with Paget diseases (18 men , and 25 women; age 71± 11 years), we measure bone alkaline phosphatase (BAP), by lectine precipitation, to assess the activity of the disease. Normal values : 35 to 95 UI/L). The correlation between uCTX and sCTX by ANOVA was : r= 0.84, p100 UI/L) , uCTX was 778 ± 880 ug/mM Cr and sCTX was 936 ± 597 ng/L ; in the group II (borderline activity) (n=12) (BAP between 80-100 UI/L), uCTX was 356 ± 237 ug/mM Cr , and sCTX was 638 ± 429 ng/L, and in the group III (inactive disease) (n=21) (BAP < 80 UI/L), uCTX was 181 ± 100 ug/mM Cr and sCTX was 341 ± 181 ng/L.. In conclusion, this study suggests that sCTX by automated method is as reliable as uCTX to assess bone resorption in normal subjects and patients with osteoporosis and Paget Disease, with the advantage that it is not necessary collect urine and that the automated method is more rapid and precise than uCTX.

SU145

Hip Fracture Discrimination with Sunlight Omnisense™ - A Meta-analysis Report. M. Weiss,[1] K. K. Knapp,*[2] D. Hans.[3] [1]Endocrine Institute, "Assaf Harofeh" Medical Center, Zerifin, Israel, [2]The Twin Research abg Genetic Epidemiology Unit, St Thomas' Hospital, London, United Kingdom, [3]Nuclear Medicin Division,, Geneva University Hospital, Geneva, Switzerland.

Several cross-sectional studies of Hip fracture discrimination performed by the Sunlight OmnisenseTM 7000S (Omnisense) were published recently. A small sample size in some of these studies resulted in a relative low statistical power.To the current meta-analysis we have gathered cross-sectional data obtained by measurements at the distal 1/3 radius in 4 different study centers. The data was gathered under separate protocols and has been published or submitted for publication, elsewhere(1,2,3,4).To estimate Odds Ratio (OR) for the population aged 60-90 years we have applied the same eligibility criteria for all the data set. The population set includes 210 Hip fractured (F) and 323 aged matched non-fractured (NF). Number of F/NF per study was 30/81; 50/130; 85/71; 45/41. OR was calculated by a single logistic regression, with age and body mass index (BMI) added as cofactors. The 'center' was included as a categorical variable in the logistic regression. Interaction between cofactors and 'center' was not found to be significant and therefore omitted from the final regression.An overall OR of 1.83 (95% CI=1.50-2.22) for one SD deviation was found for the radius measurements. OR per center was Knapp(1) - OR=1.73 (95% CI=1.06-2.84), Weiss -1.86 (95% CI=1.29-2.69), Weiss -1.52 (95% CI=1.10-2.08) and Hans(4) 1.83 (95% CI=1.07-3.13). This meta analysis verifies findings observed at the separate studies, and provides a robust estimate for the OR for the Omnisense seen in separate smaller studies.References:1. Knapp KM , Blake GM, Spector TD, Fogelman I. Osteoporosis Intl 2001, In Press 2. Weiss M, Ben-Shlomo A, Hagag P, Ish-Shalom S. Osteoporosis Intl 2000 11:411-416 3. Weiss M, Segal E, Hagag P, Ish-Shalom S. Submitted. 4. Hans D, Allaoua S, Perron C, Genton L, Barada M, Delmi M, Rizzoli R, Pichard C, Slosman DO. J Bone Min Res 200;15(Suppl 1):S528.

SU146

Calcaneal Broadband Ultrasound Attenuation Changes Over a Twelve Month Period. I. P. Drysdale, H. J. Hinkley, D. Bird,* N. J. Walters.* British College of Naturopathy and Osteopathy, London, United Kingdom.

The aim of this study was to measure Broadband Ultrasound attenuation (BUA) in 'normal' subjects over a period of one year. The McCue Cubaclinical II device was employed in order to determine device reliability and ability to assess BUA changes over time.Fifteen subjects (9 female, 6 male) were scanned at weekly or monthly intervals by the same operator. Each foot was re-positioned between measurements to allow for anatomical variation. The mean of three measurements was calculated for both the left and right calcaneus. Lifestyle and exercise factors were noted and for women the position in their menstrual cycle was recorded.The coefficient of variation (CV) of repeat measures on the same day ranged from 0 to 5.3%, with a mean of 1.6%, indicating good reliability.Regression analysis was applied and six of the fifteen subjects' readings showed a significant decrease over the year (P<0.05). Slope coefficients for males ranged from -0.005 to -0.08 (P<0.0001 to 0.8) and for females, -0.006 to +0.06 (P<0.0001 to 0.85). Four female subjects readings showed a non-significant increase over the year. Ageing would be expected to show a decline in BUA values but the effect should be small over a period as short as one year. The group CV over time ranged from 2.0 to 6.8% and it was noted that the group CV for male measurements was less than that for females (P<0.0002).None of the subjects had any significant lifestyle changes over the year and although some females showed a cyclicity in their BUA values, this did not appear to be related to the menstrual cycle.

SU147

The Role of Bone Ultrasonography in the Therapeutic Follow-Up of Postmenopausal Osteoporosis. I. Grosso,* S. Casalis,* G. Isaia. Internal Medicine, University of Turin, Turin, Italy.

There is a great interest about the application of bone ultrasonography (US) techniques in the diagnosis of postmenopausal osteoporosis and, in particular, the evaluation of bone US in pharmacological follow-up of osteoporosis, which is one of the major debate issue.In order to evaluate the role of heel bone US compared with DXA in the therapeutic follow-up of patients, we decided to conduct an open study on an osteoporotic postmenopausal population.We recently examined 45 osteoporotic postmenopausal women, whose age ranged from 46 to 62 (mean 54.95±3.91 SD) that we divided into two groups of treatment (alendronate or HRT) and followed for one year. At baseline all patients underwent a lumbar and a femural densitometry (Hologic QDR 4500) and a heel (Sahara Hologic) bone ultrasonography.We report here some preliminary data after six months follow-up of 32 patients (20 on alendronate and 12 on HRT) and after one year of treatment for 24 patients (14 on alendronate and 10 on HRT).We obviously found lumbar and total femural BMD values after 6 and 12 months significantly higher than baseline ones both in the two groups pooled together and considered separately. All heel QUS parameters (SOS, BUA and Stiffness) showed a significant increase, expecially one year after therapy, in both groups (table). In particular, SOS after six months of therapy increased though in a non-significant manner; while BUA and Stiffness index, also considered after six months of therapy, grew significantly. According to the results we obtained, the heel ultrasonography seems to be as valid as DXA in long-term therapeutic follow-up of postmenopausal osteoporosis with some advantages such as the absence of ionizing radiations, the low cost of the instruments and simplicity of use. Anyway, further studies are necessary in order to support the replacement of traditional DXA with QUS.

SU148

The Utility of Heel Utrasound in Healthy, Estrogen Supplemented Postmenopausal Women. J. Smith,[1] J. O. Judge,*[1] A. Kleppinger,*[1] M. Kulldorff,*[2] L. G. Raisz.[1] [1]Medicine, Univ. of Connecticut Health Center, Farmington, CT, USA, [2]Biostatistics, Univ. of Connecticut Health Center, Farmington, CT, USA.

Quantitative Ultrasound (QUS) has been found to be a useful, inexpensive method of assessing osteoporosis (OP) and predicting hip fracture risk in elderly Caucasian women. Current NOF guidelines recommend BMD testing in all women over 65 years, including those on estrogen replacement, to identify individuals at increased fracture risk. The purpose of this study is to evaluate the agreement of QUS to central DXA in a group of healthy estrogen-supplemented postmenopausal women. We studied 149 healthy active, Caucasian women (69.4 ±4.7 years) on long-term estrogen supplementation (11.8 ± 7.8 years), adequate calcium and vitamin D and no known risk factors for bone disease. However, they were selected for osteopenia at the femoral neck at the time of enrollment into a longitudinal exercise trial. Measurements were performed at 18 or 24 months into the exercise study and included bilateral duplicated calcaneal BUA, SOS and Stiffness Index (SI) with QUS (Lunar Achilles+) and the lumbar spine (LSpine), proximal femur (Fneck, Ftotal, Ftroch) and Total Body (Tbody) bone mineral density (BMD) with DXA (GE/Lunar, DPXIQ). The mean SI valueis reported.The frequency of the diagnosis of osteopenia or osteoporosis depended on the skeletal site measured, with the lowest for the Tbody (T=-0.16± 0.92), intermediate for the SI (T= -1.03 ± 0.84) and the highest for the Fneck (T= -1.46± 0.44). Spearman correlations between QUS and DXA measurements were significant but weak in all but the TBody BMD (Table 1).

Table 1. Correlation of QUS and DXA

	Stiffness	LSpine	FNeck	FTotal	FTroch
Stiffness	1.000				
LSpine	.285*	1.000			
FNeck	.218*	.345**	1.000		
FTotal	.289**	.365**	.704**	1.000	
FTroch	.156	.246*	.536**	.865**	1.000
TBody *p<.001,**p<.001	.564**	.693**	.466**	.600**	.424**

Using WHO diagnostic criteria, 15%, 84%, and 1% were normal, osteopenic or osteoporotic by femoral neck DXA in contrast to 46%, 50% and 3% respectively by QUS. Fifty % with mild to moderate Fneck osteopenia (Tscore -2.0) and 27% with severe osteopenia (T score <-2.0 but >-2.5) were normal by QUS. Moving up the cutoff points for SI increased the sensitivity but decreased the specificity.We conclude that SI is a weak predictor of the spine and femoral BMD in healthy, exercising, estrogen-supplemented postmenopausal women over 60 years old. Further studies of the predictive value of QUS for fracture are needed before there can be widespread reliance on QUS for osteoporosis risk assessment in many women

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Prevalence of Osteoporosis in the Spanish Population by Quantitative Ultrasound. The GIUMO Study. M. Sosa,[1] P. Saavedra,*[2] GIUMO Study Group.[3] [1]Bone Metabolic Unit, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [2]Mathematics, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [3]Cooperative Group, Spain.

Introduction: Osteoporosis is a wide-world problem affecting several millions of people. The measurement of bone mineral density (BMD) is needed to diagnose the disease in absence of fractures. Several methods have been developed to measure BMD and quantitative ultrasound (QUS) at the calcaneus has been found to be a safe and reliable method for evaluating skeletal status. In this study we present the prevalence of osteoporosis, osteopenia and normal BMD values in a healthy Spanish population of both genders, according the World Health Organization criteria.Methods: Cross-sectional study. Participants were selected in a random manner from the Spanish population. Data were collected from 11 provinces. 2610 people of both genders took part in the study (1158 males and 1452 females). The range of age was from 18 to 99 years old. We determined Quantitative Ultrasound Parameters (QUS): Speed of Sound (SOS), Broadband Ultrasound Attenuation (BUA). QUI/STIFFNESS was obtained derived from a formula and bone mineral density (BMD) in a Sahara Clinical Bone Sonometer. Results: In the whole population of both genders, the prevalence of osteoporosis was 3%, but it increased with age and sex. We did not find osteoporosis between young people aged 18-30 years but in women aged 70 years and more, the prevalence of osteoporosis was 22.1%. If we considered osteoporosis as a QUS value lower than -1.8 as has been alternative proposed, the prevalence of osteoporosis rose to 21.9% in women aged more than 50 years and to 40.9 in those aged more than 70 years old.Conclusion: Osteoporosis is a disease with a high prevalence in the general population, especially among aged women. As the prevalence found was very different depending on the T-Score value used to make the diagnose (-2.5 vs. -1.8), a consensus to establish an appropriate Tscore cut level for QUS must be developed.

	18-30	31-50	51-70	>70
Normal				
Total	86.3	71.7	62.4	51.9
Males	88.3	73.1	73.6	70.8
Females	84.6	70.7	53.3	33.1
Osteopenia				
Total	13.7	27.9	33.7	35.7
Males	11.7	26.6	24.9	26.6
Females	15.4	28.7	40.8	44.8
Osteoporosis (1)				
Total	0	0.4	4	12.3
Males	0	0.3	1.5	2.6
Females	0	0.5	5.9	22.1
Osteoporosis (2)				
Total	2.4	6.7	13.8	24.7
Males	2.7	7.4	8.2	8.4
Females	2.2	6.2	21.9	40.9

1 Osteoporosis= tscore <-2.5. 2 Osteoporosis= tscore <-1.8

SU150

Quantitative Ultrasound of the Calcaneus in Relationship with Bone Density of Bone and Hip in Patients with Ankylosing Spondylitis. R. Westra,*[1] G. A. W. Bruyn,*[2] M. Aarts,*[3] S. Zaanen,*[3] T. L. T. Jansen.*[4] [1]Department of Rheumatology, Medisch Centrum Leeuwarden, Leeuwarden, The Netherlands, [2]Department of Rheumatology, Medisch Centrum Leeuwarden, Leeuwarden, The Netherlands, [3]Isala Klinieken, Zwolle, The Netherlands, [4]Medisch Centrum Leeuwarden, Leeuwarden, The Netherlands.

Aims. To evaluate the bone density at the os calcis in patients with ankylosing spondylitis (AS) with the quantitative ultrasound (QUS) method; and to compare the QUS variables with bone mineral density (BMD) of the spine and femoral neck. Methods. The subjects were adult men and women with AS. All women were post-menopausal. The Hologic Sahara Clinical Bone Sonometer was used and is a small, portable, waterless ultrasound system. Sahara measured the broadband ultrasound attenuation (BUA, in dB MHZ^{-1}) and speed of sound (SOS, in ms^{-1}) by transmission of sound through the heel. BUA and SOS were combined linearly to form the quantitative ultrasound index (QUI). Estimated heel BMD was calculated from the QUI according to manufacturers guidelines in g/cm^{-2}. The BMD in lumbar spine (anterior-posterior view) and femoral neck was also measured using dual energy X-ray absorptiometry (Hologic, USA). We also assessed whether these parameters were able to distinct long-standing disease (> 10 yrs) from short-term disease (< 10 yrs), Results. 39 patients (27 male, 12 female) were measured, aged 32-72 years, mean 51 ± 12 (SD) years. Mean BUA of the group was 83.1 ± 4.5(SEM), mean SOS was 1560.5 ± 9, mean QUI 96,7 ± 4.8, mean BMD heel 0.5245 ± 0.023, mean heel T-score -0.72 ± 0.23, mean BMD T-score spine was -1.3 ± 1.5 and mean BMD femoral neck was -1.5 ± 1.0. Pearson correlation (r) BMD heel/BMD spine was 0.48 (P<0.0005), r BMD heel/hip was 0.40 (P< 0.01); r BMD heel/spine short duration = 0.48 (NS), r BMD heel/femoral neck short duration 0.17 (NS); r BMD heel/spine long-standing disease = 0.62 (P< 0.001), r BMD heel/femoral neck long-standing disease 0.53 (P< 0.001). Discussion.BUA and VOS were not significantly corelated with femoral neck and lumbar spine BMD's. Estimated BMD heel was significantly correlated with both femoral neck and spine BMD's. BUA was significantly correlated with spine BMD in short duration but not in long-standing disease; SOS was not correlated with spine and femoral neck BMD's. Estimated heel BMD significantly correlated with BMD of the femoral neck and lumbal BMD's in patients with long-standing disease, but not in short-term disease. BUA and SOS were not able to differentiate between these two durations of disease.

SU151

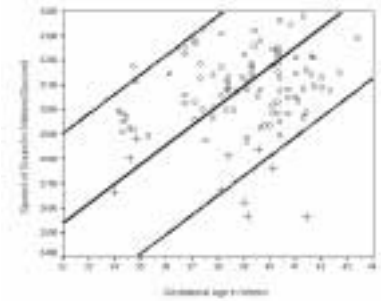
Does SOS Reflect Different Characteristics than BMD Parameters of Bone in Patients with SLE? M. Grigorian,*[1] J. A. Shepherd,[1] C. F. Njeh,[1] B. Fan,[1] X. G. Cheng,[1] H. K. Genant,[1] E. von Scheven.*[2] [1]Radiology, UCSF, San Francisco, CA, USA, [2]Pediatric Rheumatology, UCSF, San Francisco, CA, USA.

The usefulness of quantitative ultrasound to diagnose and monitor pediatric bone disease has yet to be determined. The purpose of this preliminary study is to compare, in a pediatric population, the speed of sound (SOS) measured in axial transmission mode with the bone mineral density (BMD)of common fracture sites. A total of 77 hispanic and caucasian females participated in the study up- to-date. Of those, 22 were children with Systemic Lupus Erythematosus (SLE) (aged 9 to 21 years, mean=15.5) with varied disease duration and 55 (aged 7 to 21 years, mean=13.9) were healthy volunteers. The QUS SOS (Sunlight, Omnisense) was measured on all participants at their non-dominant distal radius and tibial shaft. In addition, QCT of the spine (GE9800) and DXA of the AP, lateral spine, non-dominant femur and whole body (Hologic, QDR-4500A) were measured. A subset of participants had triplicate QUS measurements at both sites to quantify short-term precision. The CV values (%) were 0.60 and 0.35 for radius and tibia respectively, the sCV values (%) were 4.04 and 2.00 for radius and tibia respectively and the RMS SD values (m/s) were 15.8 and 9.2 for radius and tibia respectively.Both the radial and tibial SOS for the healthy group had markedly higher correlation to BMD at all sites relative to the SLE subjects (all differences were significant). The r-values comparison is given below (see the table) :Both radial and tibial SOS showed similar positive correlations with age and bone age in healthy (r = 0.68 and 0.64 respectively) and SLE (r = 0.67 and 0.71 respectively) children groups. Our preliminary results indicate that SOS of the radius and tibia can be measured with good short-term precision in a pediatric population. We also suggest that radial and tibial QUS parameters (SOS) in children with SLE may reflect changes of bone properties different from BMD.

SU152

Tibial Speed of Sound in Term and Preterm Infants. M. Yiallourides,*[1] M. Savoia,*[2] J. May,*[2] A. Emmerson,*[2] Z. Mughal.[3] [1]Faculty of Medicine, University of Manchester, Manchester, United Kingdom, [2]Department of Neonatal Medicine, Saint Mary's Hospital for Women & Children, Manchester, United Kingdom, [3]Department of Paediatric Medicine, Saint Mary's Hospital for Women & Children, Manchester, United Kingdom.

Osteopenia of prematurity is a complex bone disorder characterized by inadequate mineralization of the rapidly growing skeleton of infants who are born preterm. It is often associated with raised plasma alkaline phosphatase activity and fractures of ribs & long bones.In post-menopausal women, Quantitative Ultrasound (QUS) parameters are known to predict fracture risk independently of bone mineral density, suggesting that they must be related to some aspect of bone strength. We used the Sunlight OmnisenseTM QUS device (Sunlight Medical Ltd, Israel), to measure the speed of sound (SOS) in the axial transmission mode along the tibiae of term and preterm infants. The precision error was < 1%. The aims of this cross-sectional study were: (1)To establish normal tibial SOS values in healthy term and preterm infants (n = 95) between 34 to 43 weeks gestation. The data for these infants, with 95% confidence intervals, is displayed as [o] in the graph.(2)To measure tibial SOS in 9 infants who were born very pre-term (24 to 30 week gestation) and who had reached the corrected gestation age between 34 to 43 weeks. The data for these infants is displayed as [+] in the graph. The median (range) plasma alkaline phosphatase activity of these infants at the time of tibial SOS measurements was 1551 IU/l (549 - 2814; normal <400 IU/l). The mean tibial SOS in healthy infants (3103 ± 124 m/s) was significantly higher (p< 0.001) than in very preterm infants (2721 ± 116 m/s), when their corrected gestation age was between 34 to 43 weeks. From these data we conclude that tibial SOS measurements may allow radiation free assessment of osteopenia of prematurity.



The OmnisenseTM QUS device used in this study was on loan from Sunlight Medical Ltd, Israel

Disclosures: Sunlight Medical Ltd, Israel.,2.

SU153

Discriminating Ability of QUS Compared to DEXA in Rheumatoid Arthritis Patients With and Without Vertebral Deformities. R. E. Orstavik,*[1] G. Haugeberg,*[1] T. K. Kvien,[1] A. Hoiseth,*[2] J. Halse,[3] J. Falch.[4]

[1]Dep of Rheumatology, Diakonhjemmet Hospital, Oslo, Norway, [2]Sentrum Institute of Radiology, Oslo, Norway, [3]Osteoporosis Clinic, Oslo, Norway, [4]Aker Hospital, Oslo, Norway.

Background Previous publications have addressed the ability of quantitative ultrasound (QUS) to discriminate between postmenopausal women with and without vertebral deformities, but no studies have been performed on rheumatoid arthritis (RA) patients. The aim of this study was to compare the ability of QUS and double X-ray absorbtiometry (DEXA) to discrimintate between RA patients with and without vertebral deformities. . **Matherials and Methods** Lateral radiographs of the spine were obtained from 188 female postemopausal RA patients (mean age (SD) 62.9 (6.7) yrs, mean disease duration 16.3 (10.2) yrs). Vertebral deformities were measured by an experienced radiologist, using a standardised semiquantitative method, and classified as mild, moderate or severe. The patients underwent BMD measurements of the hip and spine by DEXA (Lunar Expert) and QUS (speed of sound (SOS), broadband ultrasound attenuation (BUA) and Stiffness (Lunar Achilles+)) of the heel. Receiver operating curve (ROC) analysis was applied to compare discriminative abilities of the different measuremnt methods and sites. **Results** Thirty-six patients (19.1%) had at least two mild or one moderate/severe deformity. Mean BMD (SD) at the femoral neck, total hip and lumbar spine (L2-L4) were 0.806 (0.140), 0.840 (0.150) and 1.057 (0.200) gm/cm2 respectively. Mean BUA, SOS and Stiffness were 99.09 (14.69) dB/MHz, 1486.1 (34.8) m/s and 62.21 (18.63).Table 1 displays the areas under the the ROC curves (AUC) for the different measurements taken, applying at least two mild or one moderate deformity as state variable.The difference between the AUCs for the BMD measurement site (femoral neck) and QUS variable (Stiffness) with greatest AUC was calculated (1), and was not statistically different ($z = 0.69$, $p = 0.49$).

Table 1

	AUC	SE	95% CI
BMD femoral neck	0.701	0.044	0.614 - 0.788
BMD total hip	0.697	0.047	0.605 – 0.789
BMD L2-L4	0.673	0.049	0.576 – 0.769
SOS	0.724	0.049	0.628 – 0.820
BUA	0.722	0.049	0.627 – 0.817
Stiffness	0.731	0.048	0.636 – 0.826

Conclusion Both DEXA and QUS measurements discriminate between subjects with and without vertebral deformities in RA. There was a small trend towards the AUCs being greater for QUS, but the the difference was not statistically significant.1. Hanley JA, McNeil BJ. A Method of Comparing the Areas under Reciever Operating Characteristics Curves Derived from the Same Cases. Radiology 148; 1983: 839-49.

SU154

Evaluation of Bone Status in Patients Undergoing Total Hip Arthroplasty for Osteoarthritis – A Quantitative Multi-Site Ultrasound Study. G. Möller, M. Akdemir.* Department of Orthopaedics, Hamburg University School of Medicine, Hamburg, Germany.

Disuse osteoporosis is a localized or generalized loss of bone mineral density after a period of inactivity or immobilization subsequent to trauma or nontraumatic conditions. Up to now limited research regarding changes in bone status in patients undergoing orthopaedic surgery has been done. Quantitative ultrasound (QUS) is accepted as a technique to assess bone changes. Recently, a new type of quantitative ultrasound device (Sunlight Omnisense, Sunlight Ultrasound Technologies Ltd., Israel) that measures speed of sound (SOS) at multiple skeletal sites was established to evaluate bone status. The goal of this study was to assess the pre- and postoperative bone status in patients undergoing hip joint surgery because of osteoarthritis. In a prospective follow-up study with 30 patients (mean age 63 years) undergoing hip arthroplasty because of monolateral hip joint osteoarthritis speed of sound (SOS) at bilateral tibial midshaft was measured pre- and 6, 12 and 26 weeks postoperatively and the T- and Z-score determined. The Omnisense ultrasound device uses axial transmission mode. The patients had no history of drugs or diseases that effect bone metabolism. Before arthroplasty, every patient had a limp; after the operation, all patients were pain-free and could walk without assistance. Preoperatively a mean 3 % relative loss of SOS in the mid tibia on the osteoarthritis side compared with the not affected side was detected. 6, 12 and 26 weeks after total hip arthroplasty operation, no significant changes in SOS on either tibial side were detected. Restoration in SOS after THA despite remobilisation was not to note during the first six month postoperatively. Preoperatively in 10 % of patients bilateral marked reduced SOS-values were detected and osteoporosis could be newly diagnosed.The results indicate that preoperative decreased weigthbearing because of osteoarthritis leads to a significant bone loss in the affected leg. Bone seems to has difficulties in adapting promptly to patients´ improved mobility after THA. Further follow-up multi-site quantitative ultrasound investigations to determine longterm bone alterations and to detect potential bone restoration are currently performed. Preoperative evaluation of bone status in patients undergoing orthopaedic surgery is important to detect and to treat osteoporotic patients and should be established routinely. Multisite quantitative ultrasound technology can be a usefool tool for assessing bone changes in Orthopaedics.

SU155

Ultrasound Signal Analysis Is Differently Influenced by Bone Loss in Patients with Primitive Hyperparathyroidism and Osteoporosis. A. Montagnani,* S. Gonnelli, C. Cepollaro,* D. Bruni,* M. Campagna,* M. Franci,* B. Lucani,* C. Gennari. Institute of Internal Medicine, University of Siena, Siena, Italy.

Hyperparathyroidism (PHPT) and osteoporosis (OP) show two different histological pattern of bone loss. High PTH serum levels mainly induce subcortical bone reabsorption, in contrast osteoporotic patients show a prevalent loss of trabeculae. Quantitative ultrasound (QUS), being influenced by bone structure other than by bone mineral density (BMD), could be differently influenced by histological pattern of bone loss due to PHPT or OP. The aim of the present study was to investigate the usefulness of QUS parameters at phalanx in discriminating PHPT and OP.We studied 40 patients with PHPT (mean age 60.4±10.8 yrs), 40 patients with OP (mean age 61.2±8.2 yrs) and 40 healthy subjects as controls (mean age:60.3±9.8 yrs). In all subjects QUS measurements were performed at phalanx with Bone Profiler (IGEA, Italy), obtaining amplitude-dependent speed of sound (AD-SoS), and other parameters characterising the QUS graphic trace: fast wave amplitude (FWA), signal dynamic (SDy), bone transmission time (BTT) and ultrasound bone profile index (UBPI). Moreover, serum calcium, phosphorus, parathyroid hormone (PTH), bone isoenzyme of alkaline phosphatase (BALP) and ionized calcium (Ca2+) were measured in a fasting blood sample of all subjects. All QUS parameters were significantly reduced both in PHPT and OP in comparison with control group, except for FWA in PHPT patients. All US signal parameters, but not AD-SoS, were significantly different between PHPT and OP groups. In PHPT patients BTT was correlated with PTH, Ca2+ and BALP levels, whereas FWA, SDy and UBPI correlated only with BALP. UBPI, BTT, FWA and BTT/FWA ratio, but not SDy, discriminated between the two groups (AUC =0.66, 0.69, 0.67 and 0.81, respectively).Our findings show that BTT, FWA and namely BTT/FWA ratio can discriminate between PHPT and OP patients presenting the same extent of bone loss. Although our data need to be confirmed in a larger sample, they suggest that QUS signal is influenced by structural characteristics of bone, other than by BMD, as previously reported by in vitro studies. These results seem to open a new prospective in the use of QUS in the diagnosis of metabolic bone diseases.

SU156

Can Heel Ultrasound Plus a Questionnaire Predict Osteoporosis in Pulmonary Clinic Patients? R. A. Adler,[1] H. L. Funkhouser,*[1] C. Holt,*[1] B. L. Elmore,*[2] C. McMurtry,[3] D. Bechard.*[3] [1]Endocrinology, McGuire Veterans Affairs Medical Center, Richmond, VA, USA, [2]School of Pharmacy, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA, USA, [3]McGuire Veterans Affairs Medical Center, Richmond, VA, USA.

Osteoporosis is an often overlooked but important side effect of glucocorticoid therapy. Patients with pulmonary diseases may use systemic or inhaled steroids and may also be at risk for osteoporosis because of decreased physical activity, smoking, and the muscle wasting of emphysema. Ideally, all such patients would be tested for osteoporosis by central DEXA (dual energy x-ray absorptiometry). We have previously reported that, in men, there is poor concordance between heel ultrasound measurements and DEXA, but others have found that a simple questionnaire can predict DEXA results in men. Thus, we prospectively studied predominantly males at a Veterans Affairs Medical Center Pulmonary Clinic to determine if the combination of heel ultrasound plus a questionnaire could predict which patients have osteoporosis by DEXA. In preliminary review of the 37 men (28 white, 10 black, 1 Asian) who have completed the study, the overall means were as follows: spine T -0.602, total hip T -0.946, femoral neck T -1.55, heel T -0.85, questionnaire score 9.8/25 points. Multiple linear regression using all possible heel parameters (Sahara) and overall questionnaire score did not predict DEXA results. Heel ultrasound BMD plus body mass index (BMI) predicted spine and femoral neck T-scores. Heel ultrasound T-score (female database) plus BMI predicted total hip and femoral neck T-scores (all $P < 0.001$). Further analysis of specific questions and refinement from additional patients will be considered, but the data suggest that heel ultrasound plus BMI will predict those patients with osteoporosis by DEXA in the setting of pulmonary disease.

SU157

The Ability of Ultrasound to Discriminate Vertebral Fractures in a Group of Postmenopausal Women with Low Bone Density. V. Camozzi,* G. Luisetto.* Medical and Surgical Sciences, University of Padua, Padua, Italy.

Ultrasound (US) technique is widely used to assess bone strength in metabolic bone disease, due to its ability to correlate not only with bone density but also with bone structure. US show a poor correlation with DEXA measurements and give additional information to assess fracture risk.In this study we evaluate several US parameters in a group of postmenopausal women with low bone mass, with or without atraumatic vertebral fractures. One hundred thirty-five women aged 50 to 85 years (70.4 ± 6.9) were studied. The inclusion criteria were: bone mineral density (BMD) measured at lumbar spine (L2-L4) and/or femoral neck with T-score lower than -2; absence of chronic disease or treatment affecting bone metabolism. Women were divided into two groups according to the presence (F+: N = 54) or absence (F-: N = 81) of vertebral fractures. The two groups did not differ by age, body size and femoral BMD. US measurements were performed at the right hand (fingers 2 to 5) using a DBM Sonic 1200 device (Igea, Carpi, Italy), and at the left heel by a Hologic Sahara densitomer (Waltham, MA, USA). The following parameters were measured: DBM Sonic: Amplitude dependent speed of sound (AD-SOS), Signal dynamic (Sdy), Fast wave amplitude (FWA), Time frame (TF), Ultrasound bone profile index (UBPI); Sahara: SOS and Broadband ultrasound attenuation (BUA). Fractured women showed US variables correlated with bone structure significantly different than those of the non fractured ones:

BUA: F+: 39.2 ± 13.4, F-: 46.8 ± 12.4 db/MHz, p<0.001; FWA: F+: 24.0 ± 5.3, F-: 25.8 ± 5.0 mV, p = 0.05; Sdy: F+: -797 ± 211, F-: -727 ± 194 mV/mcsec, p<0.05. Variables related to US velocity (SOS and AD-SOS), which mainly depend on bone calcification, were not different between the two groups. These findings suggest that US parameters related to skeletal microarchitecture are more useful than those related to bone mineralization in detecting women at risk of fracture.

SU158

The Correlation Between Ultrasound of the Calcaneus and Axial BMD in a Pediatric Population. B. Fan,[1] C. F. Njeh,[1] J. A. Shepherd,[2] M. Grigorian,*[2] X. Cheng,*[2] H. K. Genant,[2] E. Von Sheven.[3] [1]Radiology, University of California San Francisco, San Francisco, CA, USA, [2]Radiology, University of California San Francisco, San Francisco, USA, [3]Pediatric Rheumatology, University of California San Francisco, San Francisco, CA, USA.

Quantitative ultrasound measurement is a reliable method for assessment of bone mineral. For pediatric patients the short scan time and lack of ionizing radiation are important advantages over dual x-ray absorptiometry approach. The purpose of this study was to assess relationships between an imaging quantitative ultrasound (QUS) scanner, the UBIS-5000 (DMS, France), and BMD measured by DXA and QCT in a pediatric population. 72 Caucasian and Hispanic female children and adolescents (aged 7-21 years) were recruited for the study. 49 were healthy children and adolescents with a mean age of 14.8±4.29 years, and 23 were systemic lupus erythematous (SLE) patients with a mean age of 16.15±3.49 years. The non-dominant heel was measured by the UBIS 5000 according to the manufacturer's protocols. BMD was measured using QCT at the spine (GE 9800) and DXA for the AP, lateral spine and non-dominant femur (Hologic QDR-4500A, MA) in all the participants. Bone age was estimated using an X-ray of the non-dominant hand. The correlation coefficient between BUA and BMD, expressed as r, was calculated using linear regression analysis. For healthy subjects BUA correlated positively and significantly with age (r=0.61) and bone age (r=0.67) (p<0.001). The changes in BUA with age paralleled those in BMD. For the SLE patients there was no correlation with age (r=0.006) or bone age (r=-0.08). BUA was significantly correlated with all the BMD parameters (p<0.05) for healthy subjects. However, for the SLE patients the correlation was substantially reduced.

Correlation (r)	BUA_Healthy (n=49)	BUA_SLE (n=23)
AP Spine BMD	0.73	0.47
Lateral Spine BMD	0.75	0.45
Hip Total BMD	0.76	0.40
QCT_Elliptical BMD	0.57	0.35
QCT_Integral BMD	0.71	0.57

The poor correlation observed in SLE patients may be due to the influence of the disease on bone status. This study demonstrates the potential utility of BUA in pediatric bone status assessment. Further studies are needed to clarify the impact of disease on BUA

Disclosures: Diagnostic Medical Systems,2.

SU159

Establishment of Pediatric Reference Curve for Sunlight OmnisesnseTM 7000P. D. Geva,*[1] Z. Zadik,*[2] N. Cho,*[3] T. Schwarts,*[1] I. Yaniv.*[1] [1]Sunlight Medical Ltd., Tel-Aviv, Israel, [2]Pediatric Endocrinology Department, Kaplan Medical Center, Rehovot, Israel, [3]Ajou University School of Medicine, Suwon, Republic of Korea.

The adult reference curve of the Sunlight OmnisenseTM; was estimated by using the moving-average technique. This technique however seems to be limited when applied for the estimation of pediatric population curves, especially due to the sharp slopes observed during the first years of life and during adolescence. Establishing the pediatric reference curves for the Sunlight OmnisenseTM 7000P we have fitted a series of polynomials by minimizing the squared residuals. The method provides adequate curve estimation while taking into account biological considerations in applying the polynomial curves. Periods of different growth rates were modeled with different polynomials. The proposed model provides enough flexibility to allow reliable estimation from a variety of data sources .The function we propose is a composition of three polynomials over three age brackets: Infancy (age 0-5), prepuberty (age 5-10) and puberty (age 10-18). The curves were estimated separately for males and females. The fit was carried out by finding the set of parameters which minimize the sum of the squared residuals (SS(residuals)). For this procedure we used the solver of MS-Excel™. Continuity within the fragments of the pediatric curve and with the adult curve was assured by imposing a consrtaint on the connection points. In addition, the velocity is forced to be positive throughout. The estimated Standard Deviation was calculated as the square root of SS residuals divided by (n-1), and the velocity function is the first derivative of the polynomial.Two pediatric data sets of speed of sound (SOS) between ages 0-18 have been collected todate with Sunlight Omnisesnse TM 7000P in Israel (n=1085) and Korea (n=1296) for two skeletal sites: mid-shaft tibia and distal 1/3 radius. The results from the two sets are very similar. The radial is above tibial SOS, and girls appear to mature earlier. Sunlight Omnisense™ 7000P is the only bone strength assessment device that provides built-in reference curves by gender and by ethnicity from birth to adulthood. These references serves for calculation of sex,age specifics Z-scores.

SU160

The Effect of Sex on Genetic Determinants of Pre- and Post-Maturity Bone Accrual in Mice. R. J. Shmookler Reis,[1] H. Benes,[2] T. McClure,*[1] P. Kang,*[1] R. S. Weinstein,[3] R. S. Shelton,[3] R. L. Jilka,[3] S. C. Manolagas.[3] [1]Geriatrics and Medicine, University of Arkansas for Medical Sciences and Veterans Administration Medical Center, Little Rock, AR, USA, [2]Biochemistry & Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [3]Center for Osteoporosis & Metabolic Bone Diseases, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

BMD is a highly heritable trait in both humans and mice. The SAMP6 mouse displays low bone mineral density (BMD) at maturity, and age-dependent osteopenia. We identified quantitative trait loci (QTLs) associated with peak bone density in F_2 progeny of a cross between SAMP6 and a closely related strain, AKR/J. QTLs on chromosomes 2 and 11 have the highest significance for effects on BMD at 4 months, with no discernable contribution from sex or weight. In order to position these QTLs more precisely, we are constructing recombinant-inbred lines for each QTL by marker-directed backcrossing into either parental strain, selecting mice heterozygous for the introgressed allelic markers. We now have 14 recombinants at backcross generation 9, retaining varying segments of the chromosome-11 QTL. To study the effect of genotype on postmaturity changes in BMD, we calculated the ratio of 6-month to 4-month BMD for individual F_2 mice. From two independent SAMP6×AKR crosses, we have mapped significant QTLs (LOD >3.2, genome-wide p<0.05) on chromosomes 7, 18 and X. The X-chromosome QTL offers a mechanism by which sex may influence post-maturity BMD maintenance. Epigenetic effects on traits can also arise through imprinting, in which expression of an autosomal allelic variant depends on the sex of the transmitting parent. We sought evidence of imprinting by asking whether *QTL peak height* (the maximum LOD score attained) depends on the sex through which the P6 genome was transmitted. For at least one QTL, on chromosome 7, specification of the G_0 paternal strain produced a 1.5-fold increase in peak LOD score for the 6-month/4-month BMD ratio. Although these results are provisional until confirmed, the recognition of imprinting should expedite gene identification because candidate genes can be readily assessed for sex-specific transmission of DNA methylation, a known concomitant of genetic imprinting.

SU161

Linkage in Extended Pedigree Studies of Bone Phenotypes. J. A. Eisman,[1] B. Taneri,*[2] T. V. Nguyen,*[1] J. Ott.*[2] [1]Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW, Australia, [2]Rockefeller University, New York, NY, USA.

Family and twin studies have demonstrated high heritability of bone mineral density (BMD), geometry and ultrasound characteristics. However attempts to identify genes directly responsible for such effects have been relatively unproductive. Most studies have focussed on candidate genes and association approaches, which have relatively low power and are particularly susceptible to type II errors from population admixture and stratification. Linkage studies, which have been less common, generally focus on candidate genes in nuclear families, twins and discordant/concordant sibpairs. These approaches have not been as productive as animal studies using inbred strains. We hypothesised that the comparable approach in humans of examining extended pedigrees, in which bone density segregates as a trait, could more effectively identify specific loci.We used the Dubbo Osteoporosis Epidemiology Study cohort, which includes 400 husband-wife pairs, as a resource to explore this approach. Five families were initially selected based on one member having bone density in the upper 10% of the normal age-, weight- and sex-adjusted range for hip bone density and availability of large numbers of relatives. High bone density was chosen to avoid confounding by various causes of rapid bone loss and the hip site to avoid confounding by osteoarthritic spine changes.Of a total of 400 members in these five extended families, 180 individuals have had BMD assessed. BMD in the "affected" members (+1.66 Z score) appears to segregate with a dominant mode of inheritance compared with the "unaffected" mean of –0.03 Z score.&graphicAnalysis by SLINK and MSIM indicated an average maximum LOD score of 5.79, with probabilities of 44% and 68% of exceeding LOD scores of 3.00 and 2.00 respectively in this dataset.This study suggests that the extended pedigree approach is powerful and more efficient than other linkage studies, eg discordant/concordant sibpairs for which more than 1000 sibpairs may be required for traits within the normal range. The extended pedigree approach, particularly when based on existing epidemiological datasets, is a potentially powerful tool to identify genetic loci that contribute to bone density within or close to the normal range.

SU162

Genetic Distance between Candidate Gene and Bone Mineral Density-Affecting Gene Loci. G. Gong, G. Haynatzki, R. R. Recker. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

The purpose of this study is to determine whether association studies on unrelated subjects are reliable and whether they can map certain bone mineral density (BMD)-affecting genes to a definite distance to a marker locus. We searched the literature and found that polymorphisms at fifteen candidate genes had been reported to be associated with BMD. Ten of them had been found to be associated with BMD by two or more independent investigations. Five candidate genes had been reported to be associated with BMD by a single publication each. We judged whether an association is reliable based on the vote-counting meta-analysis. Negative results showing no association between polymorphisms of the estrogen receptor gene and BMD, for example, were all due to problems in study design and/or data analysis as is the case for the association between polymorphisms of vitamin D receptor (VDR) gene and BMD reported previously. It appears that results from association studies are reproducible and reliable given sound study design and appropriate data analy-

sis (e.g., adjust BMD for age, sex, environmental factors etc.). We further investigated the major ethnic populations (Caucasians, Asians, and Africans) in which an association is found. Polymorphisms of eight of the fifteen candidate genes had been adequately studied in both Caucasian and Asian populations, and all were found to be associated with BMD in both populations. This suggests that the two populations share certain common mutations causing susceptibility to osteoporosis and that these mutations had occurred before the divergence between the two populations 41,000 years ago. Polymorphisms of VDR had been studied in African Americans (in addition to Caucasians and Asians) and found to be associated with BMD in this population. This suggests that all populations in the world share a common mutation at or near the VDR gene, which had occurred before the divergence of all peoples 100,000 years ago, if spurious association due to population admixture can be ruled out. With the age of a mutation so estimated, we calculated the distances between these BMD-affecting gene loci and their respective marker loci to be less than 276 kb in associations found in Caucasian and Asian populations. The VDR-BMD gene distance is less than 90 kb. We demonstrated that these distances were overestimates because of the presence of locus heterogeneity and other factors. Conclusions: Association studies are reproducible and reliable given sound study design and appropriate data analysis. A BMD-candidate gene allelic association found in two or three major ethnic populations suggests that the distance between the two loci is very short and possibly at the candidate gene itself.

SU163

Chromosomal Loci Influencing Skeletal Strength and Architecture. D. Lang,*[1] N. A. Sharkey,*[1] G. P. Vogler,*[2] D. Blizard,*[2] D. J. Vandenbergh,*[2] L. G. Larsson,*[3] G. E. McClearn.*[2] [1]The Center for Locomotion Studies and the Department of Kinesiology, Pennsylvania State University, University Park, PA, USA, [2]The Center for Developmental and Health Genetics and the Department of Biobehavioral Health, Pennsylvania State University, University Park, PA, USA, [3]Noll Physiological Research Center and the Kinesiology Department, Pennsylvania State University, University Park, PA, USA.

Quantitative Trait Loci (QTL) analyses were performed on phenotypic variables indicative of skeletal strength and architecture in 150 day-old F_2 mice (n = 400) derived from a cross between C57BL/6 (B6) and DBA/2 (D2) inbred strains. F_1 mice (n = 19) and the F_2 cohort were used to determine the heritability for each phenotypic variable. The femur, tibia, gastrocnemius, soleus, tibialis anterior, and extensor digitorum longus were harvested from the right hindlimb. Muscle masses, bone dimensions, bone compositions, and the mechanical properties of the femoral neck and femoral and tibial diaphyses were measured. All phenotypic data was normalized to body mass index (BMI). Each F_2 mouse was genotyped for 96 anonymous markers. Sex-specific QTL analyses were performed to locate chromosomal regions (QTLs) influencing each phenotypic variable. Many sex-dependent QTLs were identified. Often times the same locus influenced muscle mass, skeletal dimensions, and skeletal mechanics, suggesting that the same gene or group of genes exerted its effects on both muscle and bone simultaneously or its action on one was transmitted to the other through a cascade of events. We hypothesize that bone strength and size are influenced indirectly by QTLs controlling muscle mass. For females QTLs for several skeletal and muscle phenotypes were found on chr. 9 (48 to 71 cM). For males similar interactions were found on chr. 1 (72.1 to 91.2 cM), chr. 6 (32.5 to 52.3 cM), and chr. 7 (19.4 to 34.5 cM). The heritability calculated from F_1 and F_2 variances fell between 0.34 and 0.91 for many of our variables and provides support for their genetic influence. The QTLs isolated using F_2 mice are currently being verified in 26 B6 X D2 RI strains of mice.

Phenotypic Trait	Female Chr.	Male Chr.	Phenotypic Trait	Female Chr.	Male Chr.
Femoral Width, Coronal	17	2, 6	Tibial Width, Coronal	13	
Femoral Width, Sagittal		1, 6	Tibial Width, Sagittal		1, 13
Femoral Stiffness		1, 17	Tibial Stiffness	9	1
Femoral Ultimate Load	3	7	Tibial Ultimate Load	9	6
Femoral Neck Diameter		6	Tibial % Mineral		8
Femoral Shear Ultimate Load	9	7	Extensor Digitorum Mass	4	7
Femur Shear Stiffness		6	Soleus Mass		16
Gastrocnemius Mass	3	1, 6, 9	Tibialis Anterior Mass	9	1, 6, 7

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Genome Screen for QTLs Underlying Normal Variation in Vertebral Structure. D. L. Koller,*[1] G. Liu,[2] M. J. Econs,[2] S. L. Hui,[2] P. M. Conneally,*[1] J. C. Christian,*[1] C. C. Johnston,[2] T. Foroud,[1] M. Peacock.[2] [1]Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Medicine, Indiana University School of Medicine, Indianapolis, IN, USA.

The lumbar vertebrae are common sites of osteoporotic fracture, which result largely from loss of strength. Components of bone strength include bone mineral density (BMD) and bone structure. We have previously reported high heritability and significant QTL linkage findings for BMD at the lumbar spine in pairs of Caucasian sisters (Koller et al, J Clin Endocrinol Metab 85:3116-20, 2000). In the present study, we examine the evidence for heritability and linkage of vertebral structure phenotypes. Five structural measures were made from lateral radiographs on 158 premenopausal Caucasian sister pairs aged 25-45 for vertebrae L1-L4. Anterior height, posterior height, and midpoint height was measured for each vertebra. Upper depth and lower depth was measured as well for each of the four vertebrae. Regression residuals of all measures were computed to correct for height and body composition. Heritabilities computed using the sibpair model ranged from 48 percent to 83 percent for all measures, and were highest for the midpoint height and upper depth measure for each of the four vertebrae (70 percent to 83 percent). Due to high Pearson correlation coefficients among these measures (0.73 to 0.82 for the same structural measure in adjacent vertebrae), we chose to focus our linkage study on the height and depth measures with highest heritability. These were midpoint height (H2=83 percent) and upper depth (H2=72 percent) of the L3 vertebra. An autosomal genome screen was then conducted using 270 microsatellite markers, with multipoint sibpair linkage analysis performed using the maximum likelihood variance estimation method in Mapmaker/SIBS. Two QTLs were identified with suggestive evidence of linkage. A LOD score of 2.3 was obtained for L3 midpoint height on chromosome 2p15 near marker D2S391. Evidence of linkage to chromosome 3q26 was also found with L3 midpoint height (LOD=2.2) between markers D3S1565 and D3S1602. No chromosomal regions were found with suggestive evidence of linkage to the L3 upper width measure. These findings suggest that novel loci on chromosomes 2 and 3 may play a role in determining vertebral structure, and possibly contribute to fracture risk independently of the QTLs for L2-L4 BMD we previously reported on chromosomes 1 and 6. This study is the first genome screen for loci underlying variation in vertebral structure, and represents an important step toward identifying genes contributing to the risk of vertebral compression fractures in the general population.

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Polymorphism at the Type I Collagen (COLIA1) and Vitamin D Receptor (VDR): Relative Risk of Osteoporosis and Fractures in Postmenopausal Women. M. Bernad,*[1] C. Gonzalez,*[2] M. Gonzalez,*[3] M. Garces,*[4] M. Escalona,*[5] J. Fernandez,*[1] L. Carreño,*[2] E. Martin-Mola,*[1] M. Martinez.[6] [1]Rheumatology, HU La Paz, Madrid, Spain, [2]Rheumatology, Gregorio Marañon H, Madrid, Spain, [3]Biochemistry, Gomez Ulla H, Madrid, Spain, [4]Jimenez Diaz Fundation, Madrid, Spain, [5]Pathological Anatomy, Gregorio Marañon H, Madrid, Spain, [6]Biochemistry, HU La Paz, Madrid, Spain.

OBJETS Twin and family studies have demonstrated that an important degree of the population variance in bone mineral density (BMD) is attributable to genetic factors. A polymorphism in the collagen type 1 1(COLIA1) and Vitamin D Receptor (VDR) gene has been associated with low bone mass and fracture incidence.METHODS We analyzed the relationship between COLIA1/VDR gene polymorphism, lumbar spine and hip BMD, T-score and fracture incidence in a population of 319 postmenopausal women classified by WHO standards: 98 non osteoporotic women (NOPW) (57.4, 6.2 yrs), 146 osteoporotic women without fracture (OPW without fracture: OPWnF) (61.2, 6.3 yrs) and 75 osteoporotic women with fracture (OPW with fracture: OPWwF). The COLIA1/VDR genotype was assessed by polymerase chain reaction and Bal I endonuclease digestion.RESULTS COLI A1 genotype frequencies for the total group were 49.2% "GG" homozygotes, 39.5% "GT" heterozygotes, and 11.3% "TT" homozygotes. We found significant differences in the percentage of homozygous "TT" between NOPW and OPW (6.1% and 13.6%, respectively). However, the incidence of genotype "TT" in OPWnF was 6.2% and 28% in OPWwF. VDR genotype frequencies for the total group were 32,8% "bb" homozygotes, 46,4% "Bb" heterozygotes, and 20,6% "BB" homozygotes. We don't found significant differences in the percentage of homozygous "BB" between NOPW and OPW (21,8% and 20%, respectively). We observed no associations between the COLIA1/VDR genotype and lumbar and hip BMD. The incidence of fractures (64 vertebral and 11 Colles) varied significantly by COLI A1 genotipe: "GG" 21.7%; "GT" 15.9%; and "TT" 58.3% (2=15.43; p<0.0001); this resulted in a fracture odds ratio of 5.96 (95% confidence interval: 2.26-15.69). Logistic regression analysis of fracture prevalence showed that for prevalent fractures, the women with "TT" group had 4.17 times the risk of the "GG" group. We observed no associations between the VDR genotype and incidence of fractures.CONCLUSIÓN. The higher incidence of fracture in postmenopausal women is independent of BMD but apparently related with "TT" expresion.

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ER alpha, AR and VDR Polymorphisms in a Longitudinal Study of Healthy Males - Association to BMD and Hormone Levels. F. Stiger,*[1] P. Gillberg,[1] H. Brändström,*[1] H. Melhus,[1] K. Michaelsson,[2] A. Kindmark.[1] [1]Department of Medical Sciences, Uppsala, Sweden, [2]Department of Orthopedics, Uppsala, Sweden.

Bone density is regulated by both environmental and genetic factors. Some candidate genes have been investigated, mostly in women, for association between allelic variations and BMD. In a population-based random sample of 248 males, aged 39-76 years, we used heel ultrasound and DXA to quantify BMD . A second DXA measurement was done at least two years from the first one. Samples for analysis of candidate genes and sex steroid hormones were obtained. Calcium intake was assessed by 14 dietary 24-h recall interviews. Three candidate genes were analyzed for microsatellite variation: the promoter region of the estrogen receptor alpha (ERa, TA-repeat), in exon 1 of the androgen receptor (AR, CAG-repeat), and in the 3'UTR of the vitamin D receptor (VDR, polyA-repeat). PCR fragments using fluorescently labelled primers were separated on an ABI 377 automated sequencer, and repeat units for each allele was determined. All genotype frequencies were in Hardy-Weinberg equilibrium. For ERa, there was a significant lower baseline BMD in the ee genotype group (e<17 repeats, E*17 repeats) vs the presence of E (4.2 % difference at the femoral neck, p=0.03). Furthermore, there was a positive association between serum estradiol levels and BMD, but only in the ee genotype group (B-estimate 0.002 and 0.002, R2 0.07 and 0.10, p=0.02 and 0.01 for femoral neck and total body, respectively). This effect modification was also evident when analyzing rate of loss at femoral neck (FN). AR

displayed a positive association with baseline BMD at all sites. B-estimates of the linear regression model were at FN 0.008, p=0.01, lumbar spine 0.009, p=0.01, total body 0.005, p=0.01, BUA 0.54, p=0.03, and SOS 2.1, p=0.006. We found also for AR, in accordance with ERa, a modifying effect by repeat length on the association between serum estradiol and BMD. The association was most apparent among those with less than 22 repeats of AR. No such interaction was evident for serum testosterone levels. Interestingly, the AR and ERa combined aa*ee haplotype showed an even more profound effect on the association between estradiol levels and FN BMD (B-estimate 0.004, p=0.03) and 24% of the variance in BMD was explained by estradiol. There was however no direct association between AR and ERa allele length and rate of loss of BMD at any site. There was no association between variation in the VDR and BMD, neither at baseline nor for rate of loss. The relationship between calcium intake and BMD was independent of VDR genotype. This study indicate important interactions on bone density in men between estrogen and androgen receptor polymorphisms and serum sexual hormone levels.

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Aromatase Gene Polymorphysm: Role of the Various Genotypes in the Estrogen Production. L. Masi,[1] L. Picariello,*[1] L. Becherini,*[1] G. Fiorelli,*[1] L. Gennari,[1] A. Falchetti,*[1] E. Colli,*[1] M. Brandi.[2] [1]Clinical Physiopathology, University of Florence, Florence, Italy, [2]Internal Medicine, University of Florence, Florence, Italy.

Genes involved in estrogen metabolism (the aromatase gene) and in estrogenic response (the estrogen receptor a gene) are possible contributors to the abnormal pathophysiological processes associated with osteoporosis. Aromatase represent the key enzyme to convert C19 steroids in the estrogens. Inactivating mutations of the aromatase gene are associated with low BMD both in women and in men. Several genes contribute to the regulation of bone and the aromatase gene is a potential candidate to be evaluated for segregation with bone metabolism and bone mass.A tetranucleotide simple tandem repeat polymorphism in intron 4 at the human aromatase gene has been recently described. We previously evaluated the distribution of this polymorphism in Italian postmenopausal women and the association of various aromatase genotypes with BMD. Allele N (containing the longer TTTA repeats) was statistically more represented in non osteoporotic women . In addition, women with NN genotype had a significant higher lumbar in comparison with opposite genotype (CC: low number of TTTA repeats). The present study was designed to evaluate a possible functional role of this polymorphism on estrogen metabolism. In particular we evaluated the difference of estrogen production in fibroblasts obtained from patient with opposite aromatase genotype. Fibroblasts were obtained from four postmenopausal women (2 with NN genotype: high number of TTTA repeats and 2 with CC genotype: low number of TTTA repeats). Women gave informed consensus and the study was approved by the IRB. Fibroblast cells were evaluated for putative products of aromatase using [3H]A (2nM) as substrates, by thin layer chromatography.In fibroblasts obtained from patients with high TTTA repeats genotype we observed that most of the [3H]A was converted into estradiol in comparison with those obtained from women with low TTTA repeats (32% vs. 5%). About 9% of estrone was obtained from both fibroblasts with high and low TTTA repeats. Finally, 4% of A was present in fibroblasts with high TTTA repeats and about 29% in fibroblasts with low TTTA repeats. These data indicate the aromatase gene pivotal in the maintenance of a sufficient amount of local estrogens in bone tissue. Interestingly, the allelic variant containing longer TTTA repeats (NN genotype) segregates with high bone mass and low bone fractures risk. Patients with allele containing longer TTTA repeats express higher aromatase activity with increased estrogen production, that should be protective for bone loss.

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Genome-wide Epidemiological Approaches for Osteoporosis Susceptible Genes in Japanese Population. H. Iwasaki,*[1] R. Ishida,*[1] H. Otsuka,*[1] S. Inoue,*[2] T. Hosoi,*[3] T. Suzuki,*[4] M. Shiraki,*[5] Y. Ezura,[1] M. Emi.[1] [1]Dept. Molecular Biology, Nippon Medical School, Institute of Gerontology, Kawasaki, Japan, [2]Dept. Geriatric Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan, [3]Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan, [4]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan, [5]Research Institute and Practice for Involutional Diseases, Nagano, Japan.

Osteoporosis is a common human disease, which is caused by the interplay of multiple genetic and environmental factors. Previously, we have shown that in Japanese, polymorphisms of several genes, such as tumor necrosis factor alpha (TNF-a), interleukin-6 (IL-6), and estrogen receptor alpha (ER-a) correlate with bone mineral density (BMD). However, there must be much more factors genetically regulating BMD, and it is impossible to point out all of them through a candidate gene approach. To define every possible determinant for BMD, we designed a genome-wide screening study using single nucleotide polymorphisms (SNPs). Three-step screening via combination of the small-scale and large-scale association studies was planned, using genomic DNA isolated from blood samples of 1200 applicants consisting of five different groups. Here, we report a set of candidate susceptible genes (n=26) selected from the first step screening in Japanese population. A hundred and ninety-two samples from two groups of the applicants (96 samples per group) were chosen, and randomly selected SNPs identified within the coding region of genes were tested. These SNPs are from the database of the Japanese Millennium Projects with the collaboration of Human Genome Center, Institute of Medical Science, The University of Tokyo and the Japan Science and Technology Corporation. Two hundred and sixteen SNPs were genotyped by now, via ddNTP primer extension method. Regression analysis between genotypes and the adjusted BMD were performed, by making correlation efficient and p-value for F-test (ANOVA) as indicators for selection (r>0.18, p<0.1). About 12 % of SNPs (n=26) were chosen as possible candidates. Since this is a rough estimation, cut off values were set loosely, and the Bonferroni approximation was not considered. These selected SNPs, including IL-3, IRKK, CD36 and PWP2, are now in the process of the second screening using another group of samples (n=303). Candidate genes passed thorough the second screening will be applied for the third step screening via micro-array hybridization method for allele typing using total 1200 samples of genomic DNA. These results would provide an important knowledge for the etiological aspect of the osteoporosis.

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VDR Genotypes Predict Serum 25-hydroxyvitamin D Concentration and Bone Mass Change in Prepubertal Finnish Girls. A. Mahonen,[1] H. Kröger,[2] C. Lamberg-Allardt,[3] J. Halleen,[4] F. Tylavsky,[5] S. Cheng.[6] [1]Department of Medical Biochemistry, University of Kuopio, Kuopio, Finland, [2]Department of Surgery, Kuopio University Hospital, Kuopio, Finland, [3]Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki, Finland, [4]Department of Anatomy, University of Turku, Turku, Finland, [5]Department of Preventive Medicine, University of Tennessee, Memphis, TN, USA, [6]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland.

In the present study, we examined the role of VDR gene alleles for bone mass, circulating 25(OH)vitamin D3 [25(OH)D] and parathyroid hormone (PTH) levels during prepubertal phase. The subjects were 10- to 13-year-old girls (n=104) with Tanner stage I-II, who enrolled in the intervention study (CALEX). Genotyping the VDR locus at TaqI and FokI polymorphic sites were performed. Serum concentrations of 25(OH)D, PTH, bone formation, and resorption markers were assessed. Bone properties of total body, hip, spine, radius, and tibia were measured at baseline and after one year follow-up using different bone assessment modalities (DXA, Prodigy, Lunar; pQCT, XCT 2000, Stratec).Tanner stage, height, and weight did not differ among girls with the different VDR genotypes. At baseline, the results indicated that the VDR TaqI genotypes (TT n=42; Tt n=47; tt n=8) significantly predicted serum 25(OH)D levels. Girls with the TT genotypes had 15% lower vitamin D levels in serum than girls with the other genotypes (p=0.026). A trend was also observed for PTH levels, TT genotypes having 2 to 12% higher concentrations compared to Tt and tt genotypes, respectively. We did not find any significant difference in bone mineral content (BMC) or areal bone mineral density (BMD) in total body, femur, or spine in the VDR genotype groups at baseline. There were no associations between studied polymorphic locuses and volumetric BMD and cross-sectional area (CSA) in radius or tibia. Neither bone formation nor resorption biomarkers did show differences among the VDR genotypes at baseline. However, VDR TaqI and FokI genotypes associated with the annual femoral neck BMC and BMD changes. Girls with the FF genotypes (n=35) gained more bone when compared with Ff (n=46), and ff (n=7) genotypes (p=0.050). Similarly, girls with the TT genotypes increased their bone mineral content more than the girls with the other genotypes (p=0.046).We conclude that the genetic variations at the VDR locus are associated with serum 25(OH)D levels and the achievement of cortical bone mass during prepubertal phase.

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The Association of Calcitonin Receptor Genotypes With Bone Mineral Density in Chinese Women of Han Ethnicity. X. W. Meng,* Z. L. Zhang,* X. Y. Zhou,* X. P. Xing,* W. B. Xia,* W. Yu.* Endocrinology, Peking Union Medical College Hospital, Beijing, China.

The aim of this study was to investigate the association of calcitonin receptor (CTR) genotypes with bone mineral density (BMD) in Chinese Han ethnic women. The CTR genotypes were determined by PCR-Restriction Fragment Length Polymorphism (RFLP) and sequence of PCR products in 95 unrelated healthy young women and 127 postmenopausal women of Han ethnicity in Beijing area. BMD was measured by duel-energy X-ray absorptiometry. In the present population, the distribution of CTR genotypes followed the Hardy-Winberg equilibrium. The frequencies of CC, TC and TT were 91.0%, 8.1% and 0.9% respectively, and the frequencies of allele C and T were 95.0% and 5.0% respectively in Chinese women. The distribution frequency of CTR genotypes were no difference by compared with 66 osteoporosis and/or fracture women and 61 normal postmenopausal women. The BMD values in lumbar spine and trochanter in TC genotype were higher than that of CC genotype (p<0.05) young women, but, the BMD values in femoral neck or Ward's triangle were no difference among CTR genotypes. We couldn't find any association of CTR genotypes with BMD in postmenopausal women. This study suggested that the frequencies of CTR genotypes in Chinese women were different from those of other ethnicity, and there was some association between CTR genotypes and BMD in Chinese young women.

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The Presence of the APOE4 Allele Is Not Associated with Prevalent or Incident Fracture in a Western Australian Population. I. M. Dick,[1] R. L. Prince,[1] A. Devine,[1] S. S. Dhaliwal,[2] A. Marangou,[1] R. N. Martins,*[3] M. Rodrigues.*[3] [1]Medicine, University of Western Australia, Perth, Australia, [2]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, Australia, [3]Surgery, University of Western Australia, Perth, Australia.

A number of conflicting studies have been published indicating either an association, or no association, of the APOE4 allele with an increased risk of fracture and/or decreased bone mineral density (BMD). The aim of this study was to examine the association of the APOE4 allele with bone mineral density (BMD), quantitative ultrasound (QUS) and prevalent and incident fracture in a Western Australian population of women aged over 70 years. The study population consists of 1500 women that comprises 6.2% of the 24800 women over the age of 70 available in the elderly population in Perth. The mean age of the population at baseline was 75.1±2.7. APOE genotyping was performed in 1136 subjects and comprises the subject group for this study. Of these, QUS was performed at baseline on 1118

subjects. At 12 months after randomization, Dual Energy X-ray Absorptiometry (DEXA) (Hologic) was performed at the hip on 950 of these subjects and at the spine in 224 subjects. Based on a T score cutoff for the total hip site of < 2 SD's below the premenopausal normal range, the subjects were classified as osteoporotic (n=322) or non-osteoporotic (n=628). Biochemical parameters of bone formation and resorption, measures of physical function (Barthel, Timed Up and Go) and balance (Rhomberg), grip strength, weight and lean body mass were measured. A total of 278 prevalent fractures resulting from minimal trauma, that had occurred prior to the commencement of the study, were reported by the subjects. A total of 85 minimal trauma incident fractures, up to 24 months after commencement of the study, were confirmed radiologically in this group. APOE genotyping indicated that 24.2% of the subjects had an APOE4 allele, with 2% being homozygous. The presence of the APOE4 allele was not associated with an increase in prevalent fractures (OR=1.13, 96%CI 0.82-1.56) or an increase in the risk of incident fracture (RR=1.06, 95% CI 0.63-1.77). There was no significant difference in BMD at any site, nor was there a difference in any of the QUS parameters, biochemical parameters, age or functional tests in the APOE4 allele group. The APOE4 allele was not associated with an increased prevalence of DEXA defined osteoporosis at the hip (OR 1.37, 95% CI 0.99-1.89). The results obtained from this study therefore do not support a role for APOE genotype in determining bone mineral density or fracture risk in a free living, elderly Caucasian female population.

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Vitamin D Receptor Genotype in Community-Dwelling Elderly Men and Women: Its Relation to Muscle Strength Results from the Cross-Sectional Analyses of the Baseline Measurements of the AIMS-Study* *Alfacalcidol Influence on Muscle Strength. L. Dukas,*[1] H. A. Bischoff,[2] L. Michiels,*[3] P. Geusens,*[4] B. Thalmann,*[1] K. Binder,*[1] A. U. Monsch,*[1] G. Boos,*[1] H. B. Stähelin.*[1] [1]Geriatric University Clinic, Basel, Switzerland, [2]Devision of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, Switzerland, [3]Biomedical Research Institute, Limburg University Center, Diepenbeek, Belgium, [4]3 Dept. of Rheumatology, Academic Hospital Maasitricht, Maastricht, Belgium.

The objective of the AIMS-Study, an ongoing randomised double-blind controlled trial, is to investigate whether supplementation of Alfacalcidol (1(OH)D) in a healthy elderly population is associated with an increase in muscle strength and a reduction in the frequency of falls. In the present analysis baseline strength measures were compared to the distribution of vitamin D receptor genotype. The subjects are 380 healthy Swiss elderly, 188 men and 192 women. Serum 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and iPTH concentration were measured by radioimmunoassay (Nichols®). For the vitamin D receptor status (VDR) the following codes were used as in the VDR publication: BB: no Bsm I restriction site in both alleles / Bb: only one allele has a Bsm I restriction site / bb: both alleles have a Bsm I restriction site. Strength measures included grip strength test, knee extensor and knee flexor strength and leg extension power. The following distribution of BB, Bb, and bb VDR status was found: 16 / 42.3 / 41.7 percent for men and 20.5 / 45.3 / 34.2 percent for women. The VDR distribution did not differ between genders ($p > 0.05$). According to the VDR status we found no difference in 25-hydroxy vitamin D and 1,25-dihydroxyvitamin D serum concentrations. Grip strength and knee flexor strength was significantly increased in the BB genotype in women, but not in men.In this study group the most frequent VDR genotype in women as well as in men was the Bb VDR genotype, followed by the bb and the BB VDR genotype. In women the BB VDR genotype was associated with muscle strength measured as grip strength and knee flexor strength.

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Gender Specific Effect of FokI Polymorphism at the VDR Gene on pQCT Parameters: Results from the InChianti Study. L. Gennari,[1] L. Becherini,*[1] A. Gozzini,*[1] R. Mansani,*[1] L. Masi,[1] A. Falchetti,*[1] C. Russo,*[2] L. Ferrucci,*[3] A. Tanini,[1] M. L. Brandi.[1] [1]Internal Medicine, University of Florence, Florence, Italy, [2]Internal Medicine, Nuovo Ospedale S. Giovanni di Dio, Florence, Italy, [3]Clinical Epidemiology, INRCA Geriatric Department, Florence, Italy.

Osteoporosis is a multifactorial disorder that recognize a substantial genetic component. Among the several candidate genes, the gene encoding for the vitamin D receptor (VDR) was the first to be proposed as a major locus for the genetic effect on bone mass. However, studies on the association between VDR polymorphisms and bone density have yelded conflicting results. In this study we analyzed the effect of Fok I restriction fragment length polymorphism (RFLP) at the VDR gene locus in a large cohort of elderly subjects from the InChianti Study. The population sample comprised 1299 subjects (746 women and 553 men) selected by using multistage sampling method from the populations of Greve in Chianti and Bagno a Ripoli, two small towns in the countryside of the Chianti area of Tuscany. Each participant underwent a detailed clinical interview, a peripheral quantitative computed tomography (pQCT) at the tibia, and peripheral blood sampling in order to evaluate bone turnover markers. In the total cohort of subjects, tibial total cross-sectional area (T-CSA) and cortical cross-sectional (C-CSA) area significantly increased with age in males but not in females. Moreover, males showed greater bone size, tibial T-CSA and C-CSA, and cortical bone density (C-BMD) with respect to females, while trabecular bone density (T-BMD) did not significantly differ between sexes. Fok I RFLP at the VDR gene was evaluated in 810 of the 1299 subjects by PCR amplification and restriction endonuclease digestion. In all the analyzed subjects no overall association between Fok I RFLP and pQCT parameters (T-CSA, C-CSA, C-BMD, T-BMD or total BMD) was observed. However, in males Fok I RFLP was significantly correlated with bone size, with 8%-11% differences in T-CSA and C-CSA between opposite FF and ff genotypes. Conversely in females Fok I polymorphism resulted weakly associated with T-BMD (P=0.04, ANCOVA), but not with C-BMD, T-CSA or C-CSA. Taken all together the results from the present study indicate that the effect of Fok I VDR polymorphism on bone is complex and gender-specific, with different implications in males with respect to females.

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Oestrogen Receptor Alpha Gene Polymorphisms and Bone Metabolism in Ambulatory Elderly Men. I. Van Pottelbergh,*[1] S. Goemaere,[1] M. Daems,*[1] R. De Muynck,*[1] A. De Paepe,*[2] J. Kaufman.[1] [1]Unit for Osteoporosis and Metabolic Bone Diseases, Ghent University Hospital, Gent, Belgium, [2]Center for Medical Genetics, Ghent University Hospital, Gent, Belgium.

In male senile bone metabolism both androgens and oestrogens appear to play a role. Since a functional loss of the oestrogen receptor alpha gene (ER) is characterized by an osteoporotic phenotype in men, the ER has been postulated as a candidate gene for osteoporosis. The first aim of the present study was to assess a modulating role of the intronic ER polymorphisms on circulating levels of sex steroids in elderly men. Furthermore, we evaluated an association between the ER polymorphisms, bone mineral density (BMD), biochemical indices of bone turnover and upper limb muscle strength in a sample of 270 healthy ambulatory men aged 71 to 86 years.The ER polymorphisms (XbaI and PvuII) were determined by restriction enzyme digestion (XbaI and PvuII, respectively) of PCR amplified gDNA, extracted from peripheral blood samples. After overnight fasting venous blood and second void urine were obtained and analysed for testosterone (T), oestradiol (E), sex hormone binding globulin, serum bone-specific alkaline phosphatase, serum intact osteocalcin, serum C-terminal type I procollagen peptide, serum and urinary C-terminal telopeptides of type I collagen and urinary deoxypyridinoline levels.The observed frequency distribution was XX 13.3%,Xx 39.5% and xx 47.2% for the XbaI and PP 20.7%, Pp 47.9% and pp31.4% for the PvuII ER polymorphism. Neither the XbaI nor the PvuII ER polymorphism were associated with serum levels of (free)E or (free)T, after correction for age and body mass index. No significant association was found between the ER polymorphisms and either BMD at the proximal femur or at the forearm (DEXA;QDR1000+,Hologic Inc), nor with the upper limb muscle strength (grip strength and isometric biceps strength) or any of the biochemical indices of bone formation and bone resorption.In conclusion, we could not establish an independent role of the XbaI and PvuII ER polymorphisms in the determination of circulating sex steroid levels, BMD, muscle strength or bone turnover in ambulatory elderly men.

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Androgen Receptor Gene (CAG)n Repeat Polymorphism and Bone Mineral Density in Elderly Men. L. Gennari,[1] L. Becherini,*[1] D. Merlotti,*[2] S. Gonnelli,[2] M. Mangeri,*[2] A. Montagnani,*[2] L. Masi,[1] A. Falchetti,*[1] B. Lucani,*[2] C. Gennari,[2] M. L. Brandi.[1] [1]Internal Medicine, University of Florence, Florence, Italy, [2]Institute of Internal Medicine, University of Siena, Siena, Italy.

Recent epidemiological studies pointed out that male osteoporosis is an increasingly important health problem. However, predictors of osteoporosis in men are not clearly defined. Androgens have long been assumed to be critical for skeletal maintenance in men, even though the extent to which the bone effects of testosterone may be attributed to its direct action via the androgen receptor (AR) rather than to its aromatization into estrogen is not well established. The human AR contains a CAG repeat polymorphism in exon 1, encoding for a poliglutamine sequence of variable length, which has been associated to differences in the androgenic activity. Within the normal range of CAG repeats, AR transactivation has been inversely correlated to the number of CAG repeats. In this study we analyzed the role of trinucleotide (CAG)n repeat polymorphism at the AR gene in 300 elderly Caucasian males (age range 55-85 years), recruited by direct mailing and followed for four years. Femoral and lumbar BMD (DEXA, Hologic QDR), bone ultrasound parameters at os calcis (Achilles, Lunar) and at phalanx (DBM Sonic, IGEA), serum estradiol, testosterone, sex hormone binding globulin, 25-OH vitamin D and bone turnover markers (urinary crosslaps and serum bone specific alkaline phosphatase) were evaluated for each man. In the recruited population, the number of CAG repeats varied from 10 to 31, with a mean repeat length of 21.28. There was no overall statistically significant association between the CAG repeats length and BMD, bone ultrasound parameters or bone turnover markers. Further analysis showed that after classification of subjects into three major CAG groups (A, B, and C), a small group of men with the highest number of repeats (group C>26 repeats) had lower FN-BMD values, higher rates of bone loss at the femoral neck and lower phalangeal thickness than those with the lowest CAG repeat length (group A<18 repeats).Taken all together, these results suggest a weak but significant contribution of AR poliglutamine repeat polymorphism to bone mass and bone size in elderly males.

SU176

Interaction Between Polymorphisms in the Methylenetetrahydrofolate Reductase (MTHFR) Gene and the Collagen Type I Alpha 1 (COLIA1) Gene Determines Bone Mineral Density and Fracture Risk. J. B. J. van Meurs,*[1] P. P. Arp,*[1] M. van der Klift,*[2] J. Witteman,*[2] A. Hofman,*[2] A. G. Uitterlinden,[1] H. A. P. Pols.[1] [1]Internal Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands, [2]Epidemiology and Biostatistics, Erasmus University Rotterdam, Rotterdam, The Netherlands.

Osteoporosis has a strong genetic component including a polymorphism in COLIA1, the gene for collagen type Iα1, the major bone matrix protein. Homocystinuria is associated with a high prevalence of osteoporosis, probably due to interference of homocysteine with collagen metabolism. We therefore examined whether part of the genetic component of osteoporosis is expressed through interaction of polymorphisms in COLIA1 and MTHFR, which is a rate-limiting enzyme in the homocysteine metabolism. We analysed the COLIA1 Sp1 G/T polymorphism and the C677T (Ala222Val) MTHFR polymorphisms

in a population-based sample of 1717 postmenopausal women (aged 55-85 years) in relation to BMD and incident fractures (mean follow-up time 6 years). Subjects were divided into four groups: [--]those without risk alleles (n=516), [+-] presence of MTHFR T-allele(s) but no COLIA1 T-allele (n=640), [-+] presence of COLIA1 T-allele(s) but no MTHFR T-allele (n=246), and [++] presence of both risk allele(s) (n=315). Compared with the [--] group, the [++] group had on average a 6% lower BMD at the femoral neck and lumbar spine ($p<0.001$). This effect was larger then the effect of MTHFR or COL1A1 alone, since the [+-] and [-+] groups both showed a decrease of approximately 1% in BMD. The differences between [++] and [--] showed an age-dependent trend ($p=0.02$) with in the age group 55-65 yrs a difference of 3% and in those above age 75 yrs a difference of 10%. Women with the [++] genotype were overrepresented among the 171 women who had incident fractures. The relative risk (RR) was 2.4 (95% CI:1.5-4.0), which was significant and larger then the (non-significant) effects of the [+-] and [-+] groups alone ([+-]: RR =1.2, 95% CI:0.8-1.9; [-+]: RR=1.3, 95%CI:0.7-2.2) The risk in the [++] group increased further to 3.3 (95% CI: 1.4-7.9) for women 70 years and older but was largely independent of BMD differences. We conclude that the combination of COLIA1 and MTHFR polymorphisms predisposes women to osteoporotic fractures. This risk is only partly explained by differences in bone mineral density, suggesting that factors, other then those reflected by BMD, determine the increased fracture risk.

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Collagen TypeI Gene Is not Associated with BMD or Stress Fracture Occurence in Elite Military Cadets. J. W. Nieves,[1] M. Zion,*[1] J. Ruffing,*[1] S. Ralston,[2] J. M. Uhorchak,*[3] S. Gordon,*[1] R. Lindsay,[1] F. Cosman.[1 1]Clinical Research Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]University of Aberdeen Medical School, Aberdeen, United Kingdom, [3]Keller Army Hospital, West Point, NY, USA.

A polymorphism affecting the Sp1 binding site in the collagen I (COLI) A1 gene has been associated with fracture and central bone density in some but not all cohorts of women and men. COLIA1 genotypes were analyzed in relation to bone density (at average age 18), markers of bone turnover, and stress fracture occurrence in a cohort of 730 Caucasian military cadets (626 male and 104 female) from the United States Military Academy at West Point. COLIA1 genotype was determined by polymerase chain reaction analysis of genomic DNA extracted from peripheral blood leukocytes. Markers of bone turnover in serum were measured by RIA for BGP, IRMA for BSAP, and ELISA for NTX. Bone density was measured at the calcaneus by Lunar PIXI and at the tibia by peripheral-QCT (Stratec/Norland). Stress fractures occurred in 62 cadets (male=40; female=22) in this sample over a 2.5-year period. These were diagnosed by an orthopedic surgeon and confirmed by x-ray or scintiscan. On analysis of genotype distribution in the entire study group we found 64% SS homozygotes, 32% Ss heterozygotes, and 4% ss homozygotes, similar results to those previously reported in other Caucasian populations. In both males and females, age, weight, height and body mass index were similar across the three genotypes. Between group comparisons by ANOVA showed that COLIA1 genotype was not significantly associated with BMD at the calcaneus or tibia or with tibial cortical thickness. COLIA1 was not associated with markers of bone turnover (NTX, BSAP, BGP) in this cohort of military cadets. In addition, stress fracture incidence in males or females was not associated with COLIA1 genotype. We conclude that COLIA1 does not appear to be an important determinant of bone density or susceptibility to stress fractures in this young healthy population. Whether this gene relates to the acquisition of peak bone mass is the subject of an ongoing longitudinal investigation in this cohort.

SU178

The BsmI Vitamin D Receptor Gene Polymorphism in Jewish Ashkenazi and Non-Ashkenazi Patients with Inflammatory Bowel Disease. R. Dresner Pollak,[1] H. H. Fidder,*[2] A. Blumenfeld,*[1] M. Idelson,*[1] Y. Chowers,*[2] R. Eliakim.*[3] [1]Hadassah University Hospital, Jerusalem, Israel, [2]Chaim Sheba Medical Center, Tel Hashomer, Israel, [3]Rambam Medical Center, Haifa, Israel.

Susceptibility to inflammatory bowel disease (IBD) has a strong genetic component. Linkage studies suggested involvement of chromosomes 16, 12, 3 and 7. The vitamin D receptor (VDR) gene maps to a region on chromosome 12 that was shown to be associated with IBD, and was suggested as a susceptibility gene for Crohn's disease (CD) in European Caucasians. The aim of this case-control study was to determine the association between the *BsmI* VDR gene polymorphism and IBD in Israeli Jewish Ashkenazi and non-Ashkenazi CD and ulcerative colitis (UC) patients. Two hundred and thirty two IBD patients, 162 CD and 70 UC patients, 87 Ashkenazi, 39 Moroccans, 45 Iraqi, and 44 patients of mixed ethnic origin were studied. The control group was comprised of Jewish Israeli healthy blood donors: 143 Ashkenazi, 75 Moroccans and 75 Iraqi. All participants were typed for the *BsmI* VDR gene polymorphism utilizing polymerase chain reaction. There were no significant differences in the frequencies of homozygotes for the *BsmI* polymorphism (*BB* genotype) or the *B* allele between CD and UC patients (0.17 vs. 0.14, chi sq.=0.3, p=0.6; 0.40 vs. 0.39, chi sq.=0.05, p=0.8, respectively). There were no significant differences in the frequencies of the *BB* genotype or the *B* allele between IBD patients and ethnically matched controls. The frequency of the *BB* genotype was significantly higher in healthy Iraqi subjects compared to Ashkenazi subjects (0.20 vs. 0.11 homozygotes, chi sq.=5.5, p=0.05). The *BsmI* VDR gene polymorphism was not found to be associated with increased susceptibility to IBD in Israeli Ashkenazi and non-Ashkenazi Jews.

SU179

Pharmacogenetic Markers of Bone Response to Hormone Replacement Terapy in Postmenopausal Women. N. Laflamme,*[1] J. Laroche,[2] S. Dodin,[3] C. Blanchet,[3] Y. Giguere,*[2] K. Morgan,*[4] F. Rousseau.[5] [1]SignalGene Inc., Montreal, PQ, Canada, [2]Centre de Recherche de l'Hôpital St-François d'Assise, Université Laval, Québec, PQ, Canada, [3]Reproductive Endocrinology Laboratory, HSFA, Québec, PQ, Canada, [4]McGill University, Montreal, PQ, Canada, [5]Biochemistry, Université Laval, Québec, PQ, Canada.

In our first study among 425 postmenopausal women (Giguere, 2000), results suggested that the effect of hormone replacement therapy (HRT) on heel bone density may be modulated by a two-locus vitamin D and estrogen receptor genotype. Those results needed to be replicated in an independent sample to be confirmed.Thus, a new sample of 596 postmenopausal French Canadian women from Quebec were recruited. Heel bone density was determined by right calcaneal quantitative ultrasound and results were expressed as age-and-weight-adjusted stiffness index (heel-SI z-score). Women were genotyped for a common BsmI polymorphism in the vitamin D receptor (VDR) gene as well as the PvuII polymorphism in the estrogen receptor (ESR1) gene. The association between heel bone density, HRT and the combined VDR/ESR1 genotype was evaluated by ANOVA.As observed previously, the strength of the association between HRT and heel-SI was different according to the VDR/ESR1 genotype ($p=0.05$). In fact, among women bearing the VDR bb/ESR PP genotype, those who received HRT for more than 5 years had a heel-SI of 1.0 SD higher ($p=0.006$) than those who received HRT for < 5 years. This may translate into a 2-fold difference in the risk of fracture. However, among women bearing other genotypes, this difference was 3 times smaller (0.3 SD). The potential confounding effects of standard risk factors for low bone density were also evaluated and did not changed our results.These findings support the hypothesis that the benefit from taking HRT on heel bone density in postmenopausal women is modulated by variation in VDR and ESR1 loci, jointly.

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Osteoprotegerin Promoter Polymorphism Is Not Associated With Bone Mass, Bone Turnover or Bone Loss in Older Men. M. Lee,[1] J. M. Zmuda,[2] J. A. Cauley,[2] R. Ferrell.*[2] [1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA.

Osteoprotegerin (OPG) is a soluble glycoprotein of the tumor necrosis factor receptor family, and may play an important role in osteoclastogenesis. Mice lacking the OPG gene develop early onset osteoporosis, and suffer from spontaneous fractures whereas OPG treatment in rats increases bone mineral density (BMD). In the present study, we assessed the association between a T/C substitution (T-950C) in the promoter region of the OPG gene and bone mass, biochemical markers of bone turnover, and bone loss in 295 Caucasian men aged 58 to 91 years. Hip BMD was measured with dual energy X-ray absorptiometry (Hologic QDR) in 1991-1992, and after an average of 7 years. Urinary excretion of cross-linked N-telopeptide of type I collagen and serum osteocalcin were determined by enzyme linked immunosorbent assay and radioimmunoassay, respectively (Endocrine Science). Genotypes frequencies were in Hardy-Weinberg equilibrium (TT, 28.5%; TC, 44.5 %; CC, 27.1%). Age, body weight, height, and total calcium and vitamin D intake were similar across genotype. Hip BMD and biochemical makers of bone turnover did not differ across OPG genotype. Heterozygous men tended to lose less bone than homozygous men, but this was statistically significant only for the inter-trochanteric region (Table). In summary, we found no consistent association between the T-950C promoter polymorphism, and BMD, markers of bone turnover, or rates of bone loss in this sample of older Caucasian men. Table. Mean annual % changes in BMD across T-950C genotype

Annual Change (%/yr)	T/T	T/C	C/C	p-value
Total Hip	-0.15	-0.03	-0.26	0.058
Femoral Neck	-0.14	-0.14	-0.25	0.570
Inter-trochanter	-0.22	-0.03	-0.33	0.010
Trochanter	- 0.04	0.05	-0.20	0.069

SU181

Vitamin D Receptor (VDR) Polimorphisms and Cardiac Rejection in Heart Transplantation. F. Luque,*[1] J. M. Quesada Gomez,[1] E. Jodar,[2] M. Gil,*[3] J. Vara,*[3] G. Martínez,*[2] G. Dorado,*[1] F. Hawkins.[2] [1]Unidad de Metabolismo Mineral, Hospital Reina Sofia, Cordoba, Spain, [2]Servicio de Endocrinología, Hospital 12 de Octubre, Madrid, Spain, [3]Servicio de Rehabilitación, Hospital 12 de Octubre, Madrid, Spain.

Vitamin D endocrine system has not only a central role in bone and calcium metabolism, but also has important general immunomodulatory actions. In recent years, this new physiological role has been studied intensively. VDR gene polymorphism influences bone loss after organ transplantation. Patients with the bb genotype are, to some extent, protected against post-transplantation bone loss. Recently It has been suggested that the VDR gene polymorphisms have a role also as a genetic determinant of transplant rejection. The purpose of this study was to investigate the potential relationships between vitamin D receptor gene polymorphisms and organ rejection after cardiac transplantation. We studied in 117 patients who had received a heart transplantation at Hospital 12 de Octubre. Madrid. Spain. Genotypic Analysis VDR polymorphisms was studied by Frozen-Start technique, loci of the vitamin D receptor gene was amplified by PCR and the resulting products were then digested by restriction endonuclease using restriction enzymes: Bsm1, cleavage in exons 8 and 9; Fok: Cleavage in exons 1c and 2; and Taq: Cleavage in exon 9. Endomyocardial biopsies were used as diagnosis of graft rejection (International Society of Heart and Lung Transplantation classification). The studies of the cellular cycle and apoptosis were assessed by flow cytometry (FACscan, Beckton Dickinson. Chi-squared test were performed to compare genotypic frecuencies expected with observed. Graft rejection were more observed in men than in women (33.3% vs 22.2%). VDR genotypes BsmI and Taq I

had not effects on graft rejection, but in men we found an association between Fok I and graft rejection (X2=7.96; p<0.05); without differences in cellular cell cycle Go/G1, S and apoptosis between genotypes. In conclusion, our findings suggest that Fok I vitamin D receptor gene polymorphism could be useful for identifying patients at risk for cardiac rejection after transplantation

SU182

Identification of a New Single Nucleotide and a New Microsatellite Polymorphism in the Human Vitamin D Receptor Gene. R. C. Foster,*[1] R. Raiford,*[1] J. C. Fleet,[2] E. Krall,[3] V. C. Henrich.[1] [1]UNC-Greensboro, Greensboro, NC, USA, [2]Purdue University, West Lafayette, IN, USA, [3]Boston University, Boston, MA, USA.

The gene for the human vitamin D receptor (VDR), a nuclear receptor responsible for the genomic effects of vitamin D, has been examined for polymorphisms that may contribute to osteoporosis. While several polymorphisms have been identified, their contribution to the variation in bone density has been controversial. Recent studies indicate that phase differences found in as little as 5 kb among African-American populations may confound attempts at linkage of certain disease states to specific VDR genotypes. Thus, the role of VDR gene polymorphisms in the genetic control of bone density or calcium metabolism requires discovery of additional polymorphisms for use as genetic markers. A goal of our research has been to identify a set of novel genetic markers across the vitamin D receptor gene that might be useful for phenotype-genotype association studies. We used two approaches for this search. Both approaches utilized 40-blinded DNA samples from men. This number is sufficient to detect a polymorphism with a frequency of 5% in the general population. In approach one, we examined exons 6 though 9 of the VDR gene for single nucleotide polymorphisms (SNP). PCR primers were designed in the introns flanking exons 6, 9 and the combined exons 7/8. Following amplification of the exons by PCR, the products were directly sequenced using a LiCor 4000 Gene Reader. No new SNPs were detected in the parts of coding region or introns that we examined. A new C/A transversion SNP was found in the 3' UTR of exon 9 (frequency = 13.7%). Next, we designed a computer program to locate possible repeated elements within genes. When we applied this program to the entire VDR gene, roughly 50 elements were predicted. Five candidates were tested by direct sequencing. We have confirmed the presence of one of these, a previously undescribed polymorphic tetranucleotide repeat within the intron between exons 2 and 3. The discovery of these novel polymorphisms in the VDR gene may permit more precise haplotyping, thereby providing greater resolution of any possible association between VDR genotype and bone density.

SU183

Insulin-like Growth Factor I (IGF-I) Infusion Increases Bone Calcium Deposition in the Growing Rat Model. Q. Zhang,*[1] M. E. Wastney,*[1] C. J. Rosen,[2] C. M. Weaver.[1] [1]Foods and Nutrition, Purdue University, West Lafayette, IN, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA.

Insulin-like growth factor I (IGF-I) is considered a powerful mediator of skeletal development during pubertal growth. This study was designed to investigate the effect of IGF-I treatment on calcium kinetics and bone parameters in the growing rat model. Female Sprague-Dawley rats at two different ages, 6 wks and 9 wks age, were used in the study to mimic early puberty and young adulthood in humans. At each age, rats were divided into two groups (n = 15 rats/group). Rats in the treatment groups received continuous rhIGF-I/IGFBP3 infusion (0.3 mg/day) by osmotic mini-pumps, while rats in control groups received PBS solution. The infusion lasted four weeks, and pumps were changed at two weeks. At the end of intervention, kinetic studies were performed. In each group, three rats received an intravenous injection of 20 uCi 45CaCl2, and five rats received an oral gavage of 25 mg calcium as calcium acetate which contained 20 uCi 45CaCl2. Sequential blood samples and complete urine and fecal samples were collected for 48 hours after dosing. The data were analyzed by compartment modeling using WinSAAM (Simulation, Analysis And Modeling). In pubertal rats, IGF-I treatment significantly increased body weight (219 ± 18 g for IGF-I treated rats vs. 169 ± 8 g for control rats, p<0.05) and femoral calcium content (493 ± 42 mg calcium/ g ash weight for IGF-I treated vs. 368 ± 24 mg calcium/ g ash weight for control rats, p<0.05). In addition, kinetic analyses demonstrated higher calcium absorption in IGF-I treated rats (26% ± 6%) compared to control rats (20% ± 3%). The transfer coefficients from soft tissue to the calcium exchangeable pool on bone were twice as high in IGF-I treated rats than in control rats. However, the effect of the treatment on young adult rats was not consistent with the pubertal rats.

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Evidence that Liver Derived IGF-I Exerts a Minimal Role in the Regulation of Peak BMD in Mice. K. Sjogren,*[1] S. Mohan,[2] H. C. M. Sheng,[2] J. L. Liu,*[3] K. Blad,*[1] O. Isaksson,*[1] J. Tornell,*[4] C. Ohlsson.[1] [1]Sahlgrenska University Hospital, Goteborg, Sweden, [2]MDC, Pettis VAMC, Loma Linda, CA, USA, [3]NIDDK, NIH, Bethesda, MD, USA, [4]University of Goteborg, Goteborg, Sweden.

Mice lacking functional IGF-I exhibit severe impairment of bone growth. An adolescent human male with a disrupted IGF-I gene had a BMD significantly less than that of healthy adolescent males. These data demonstrate that IGF-I is an important regulator of skeletal growth. IGF-I is known to act as a systemic, as well as a local, growth factor. To better understand the relative importance of systemic IGF-I versus locally expressed IGF-I, we recently developed a transgenic mouse model with the IGF-I gene deleted specifically in the liver (LI-IGF-I -/-) but normal in bone and other tissues. These mice exhibited 80% reduction in serum levels of IGF-I, grew normally up to 12 weeks of age but had disturbed carbohydrate- and lipid- metabolism. In the present study the long-term effects of liver-specific IGF-I inactivation on skeletal growth and adult bone metabolism were investigated. DEXA measurements revealed that neither total body bone mineral content (BMC, mg) nor total body BMD (mg/cm^2) was significantly different between LI-IGF-I -/- and corresponding control animals at 8 weeks of age. In addition, neither BMC nor BMD was different for individual skeletal sites such as femur, spine or cranium. At 55 weeks of age, although total body BMC and BMD were decreased by 8% and 7%, respectively, in LI-IGF-I -/- mice compared to control mice, these changes were not statistically significant. Of the individual skeletal sites analyzed, BMC (22.4±0.7 vs 26.7±0.6, P<0.05) and BMD (57.6±0.9 vs 62.3±1.6, P<0.05) were significantly decreased in the femur of LI-IGF-I-/- mice compared to control mice but not in the spine or cranium. Histomorphometric studies of trabecular bone parameters at the proximal metaphysis of tibia revealed no significant difference in trabecular area, trabecular thickness, trabecular separation or trabecular number between control and transgenic mice at 11 weeks of age. Furthermore, serum osteocalcin level was not significantly lower in LI-IGF-I-/- mice compared to control mice at 8 or 55 weeks of age. Conclusions: 1) The lack of difference in total BMC or BMD at 8 weeks of age reveal that liver derived IGF-I is not essential for bone mass accretion that occurs during pubertal growth phase. 2) Our findings are consistent with the hypothesis that liver-derived IGF-I has a minimal role compared to bone-derived IGF-I in the acquisition of peak BMD, a hypothesis which needs to be verified by disruption of the IGF-I gene in bone but not in liver.

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Lack of Evidence For Pregnancy-Induced Increase in IGF Binding Protein (IGFBP)-4 Proteolytic Activity in Mouse Serum. X. Qin, C. Sexton,* J. Ehrhardt,* D. J. Baylink, S. Mohan. MDC, Pettis VAMC, Loma Linda, CA, USA.

The bioavailability of IGFBP-4, an inhibitor of IGF actions, is regulated by the action of specific IGFBP-4 protease, which has been recently identified to be the pregnancy-associated plasma protein-A (PAPP-A). Our previous studies demonstrate that PAPP-A is the major IGFBP-4 protease in human pregnancy serum and that serum IGFBP-4 proteolytic activity is IGF-dependent and increases during pregnancy. In our efforts to understand the regulation of production and actions of PAPP-A *in vivo*, we evaluated the utility of mouse as a model in this study. We first determined if IGFBP-4 proteolytic activity is increased in mouse serum during pregnancy, as in humans. Serum from pregnant mice at day 9 and 17 was tested for IGFBP-4 proteolytic activity using recombinant mouse IGFBP-4 as substrate and compared to non-pregnant control mouse serum. To our surprise, it was found that IGFBP-4 proteolytic activity was not increased in serum during pregnancy while under identical assay conditions; whereas, IGFBP-4 proteolytic activity increased by more than 50-fold in human serum during pregnancy. Furthermore, immunodepletion of PAPPA-A or direct addition of PAPP-A antibody failed to inhibit IGFBP-4 proteolysis in pregnant mouse serum. The lack of effect of PAPP-A antibody to block IGFBP-4 proteolysis could not be explained by the inability of the antibody to bind to mouse PAPP-A, since PAPP-A antibody blocked IGFBP-4 proteolysis in mouse osteoblasts conditioned medium. To determine whether the lack of PAPP-A activity in mouse pregnancy serum is due to low expression of PAPP-A in the placenta, a major source of hPAPP-A during pregnancy, we obtained a 4 kb mouse genomic DNA sequence which contains 1 kb of the entire exon 2 coding sequence. Sequence analysis revealed 86% homology between human and mouse PAPP-A at the amino acid level. In humans, PAPP-A was highly expressed in the placenta and osteoblasts. In the mouse, the expression of PAPP-A is very low in the placenta and liver but is high in kidney, osteoblasts, and bone marrow stromal cells. Conclusions: 1) Serum IGFBP-4 proteolytic activity was not increased during mouse pregnancy; 2) PAPP-A expression is very low in the placenta during pregnancy in the mouse, unlike humans. 3) PAPP-A is expressed in both mouse and human osteoblasts; 4) While the mouse may be a suitable model for studies on osteoblast regulation of PAPP-A, it is not a suitable model for studies on pregnancy-induced increase in IGFBP-4 proteolysis that occurs in humans.

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Insulin-like Growth Factor-I Transcriptionally Activates Vascular Endothelial Growth Factor Expression in Osteoblast-like Cells Through the Hypoxia Inducible Factor-2 Alpha. N. Akeno,[1] J. Robins,*[2] M. Czyzyk-Krzeska,*[3] T. L. Clemens.[1] [1]Department of Medicine, University of Cincinnati, Cincinnati, OH, USA, [2]Department of Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA, [3]Department of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA.

Insulin-like growth factor-I (IGF-I) stimulates the expression of hypoxia-inducible genes in oxygen sensitive cells including the potent angiogenic factor vascular endothelial growth factor (VEGF). We have previously demonstrated that hypoxia transcriptionally activates VEGF expression in osteoblast-like cells by elevating the level of the basic-helix-loop-helix transcription factor Hif-2 alpha. In this study we investigated the transcriptional mechanisms and signal transduction events responsible for IGF-I induction of VEGF gene expression in osteoblast-like cells. Confluent layers of MG63 and SaOS-2 human osteoblast-like cells were exposed to IGF-I (50 ng/ml) for up to 24 hours. IGF-I induced a rapid (3h) increase (3 fold) in VEGF mRNA in both cell lines. This was accompanied by a 4-5 fold increase in the level of Hif-2 alpha protein. The level of Hif-2 alpha is known to be regulated by ubiquitination and proteosomal degradation. Consistent with this, inhibition of proteosomal degradation of Hif-2 alpha by treatment with a proteosomal inhibitor (CBZLLN) caused an increase in VEGF mRNA expression. To determine the effect of IGF-I on VEGF transcription, cells were transiently transfected with a segment of the VEGF promoter construct fused to luciferase and then exposed to IGF-I for 24 hours. IGF-I increased VEGF promoter activity by 4-5 fold. IGF-I induced activation of the VEGF promoter was greatly enhanced by co-transfection with a Hif-2 alpha but not a Hif-1 alpha construct. By contrast, IGF-I had no effect on the activity of a VEGF promoter segment containing a mutation in the hypoxia response consensus element. Transcriptional activa-

tion of VEGF by IGF-I was accompanied by a robust stimulation of Akt phosphorylation, a key downstream target of PI(3) kinase. Pretreatment of cells with the PI(3) kinase inhibitor LY-294002 largely abolished the IGF-I induced Akt phosphorylation and greatly attenuated IGF-I induction of Hif-2 alpha. These data indicate that IGF-I, by activation of the PI(3) kinase pathway, induces VEGF expression in osteoblasts through transcriptional control mechanisms common to those that activate VEGF and other hypoxia response genes. We speculate that the Hif-2 alpha transcriptional pathway affords osteoblasts an efficient means of responding to either a growth factor or oxygen deprivation in bone during normal and pathophysiological states.

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Increased Osteoblastic Differentiation in Primary Murine Osteoblasts Transduced with an IGF-I/eGFP Expressing Retrovirus. J. He,*[1] J. Jiang,[1] A. C. Lichtler,[2] B. E. Kream.[1] [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA.

Insulin-like growth factor-1 (IGF-1), an anabolic growth factor produced by cells of the osteoblast lineage, likely plays an important role in physiological bone remodeling. The application of IGF-1 as a potential therapeutic treatment for osteoporosis has been limited by the side effects of systemic administration. We have developed a bicistronic retroviral system that co-expresses epitope-tagged murine IGF-1 and the visual marker enhanced green fluorescent protein (eGFP) under control of a 2.3 kb fragment of the rat Col1a1 promoter (2.3ROSA-IGF-1/eGFP). The goal of the present study was to determine the effect of retroviral-delivered IGF-1 on the differentiation of primary murine osteoblast cultures. 2.3ROSA-IGF-1/eGFP and 2.3ROSA-GFP (control) retroviruses were generated in 293 GPG producer cells. The expression of IGF-1 and eGFP in transduced ROS 17/2.8 cells was demonstrated at both the mRNA and protein level. To test the functional consequences of IGF-1 expression on osteoblast differentiation, primary osteoblastic cells were obtained by sequential digestion of neonatal mouse calvariae and cultured for 2 days. Cells were then transduced three times with either 2.3ROSA-IGF-1/eGFP or 2.3ROSA-GFP retrovirus. Both cultures showed similar patterns of eGFP expression after viral transduction. Osteoblastic differentiation was evaluated by alkaline phosphatase (ALP) staining and the expression of bone mRNA markers after 10, 14 and 21 days of culture. 2.3ROSA-IGF-1/eGFP transduced cultures had increased ALP staining compared to 2.3ROSA-GFP transduced cultures. After 21 days, Northern blot analysis showed almost a 2-fold increase in Col1a1 mRNA, a 4.5-fold increase in bone sialoprotein mRNA and a 4-fold increase in osteocalcin mRNA in 2.3ROSA-IGF-1/eGFP transduced cultures compared to 2.3ROSA-GFP transduced cultures. In conclusion, retrovirally-delivered IGF-1 caused enhanced differentiation in primary murine calvarial osteoblast cultures. These results suggest that an osteoblast-targeted retroviral gene delivery system may be applicable to cell-based somatic gene therapy of bone.

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A Dual Gene Therapy Approach to Osteochondral Defect Repairs Using BMP-7 and IGF-1 Transduced Periosteal Cells. D. Grande,*[1] E. Tomin,[2] J. Mason,*[1] J. M. Lane.[2] [1]North Shore University Hospital, Manhasset, NY, USA, [2]Hospital for Special Surgery, New York, NY, USA.

Cells transduced with single therapeutic gene has shown promise in healing cartilage defects. This study tests the hypothesis of bone morphogenic protein-7 [BMP-7] and insulin-like growth factor-1 [IGF-1] transduced cells combined in a bilayer implant which targeted specifically to repair both bone and cartilage of an osteochondral defect. Materials and Methods: Periosteal tissue explants were harvested from the proximal tibiae of adult male NZW rabbits and cultured in D10 media. BMP-7 and IGF-1 genes cDNA fragments were generated by RT-PCR. Further subcloning generated the retroviral vector plasmid LNCX-hBMP7. Stable G418-resistant PA317 producer cell populations were generated as a source of retroviral vector particles, added to the cells at 25-50% confluence and incubated overnight at 37oC. Cells were pulsed during the log phase of in vitro growth with bromo-deoxyuridine [Brd-U]. Confluent cells were harvested and seeded onto polyglycolic acid [PGA] polymer scaffolds. Rabbits received bilateral 3 mm-diameter defects in the mid-trochlear region of the femurs. Four milimeter PGA scaffolds were placed into the defects. BMP-7 transduced cell construct was placed at the bottom and IGF-1 at the top of the defect. The contralateral knees received either empty defects or scaffolds alone. Animals were sacrificed at 6, 8, 10, or 12 weeks post-operatively. Any indication of synovitis and/or degenerative changes was graded after the examinations of the knees. Paraffin embedded five micron thick sections were cut and stained with H&E or Brd-U immunodetection kit, then assessed by the O'Driscoll method.Results: Empty controls displayed a repair with low grade average scoring. Treated defects scored significantly higher than controls over the study period with a higher percentage of hyaline cartilage and quicker restoration of the subchondral bone with continuos improvement for later interval point. Brd-U staining was positive in all cartilage and bone compartments. Brd-U positive nuclei were identified in both clusters of replicating cells within the repair tissue.Discussion: This study demonstrates the superiority of gene enhanced tissue engineering when using a two-layered graft specifically designed to address regeneration of both bone and cartilage. The implants in this study demonstrated a rapid and nearly complete repair of both types of tissue. The addition of genetically transduced stem cells produced better repair tissue than controls. Although PGA alone initiates early tissue regeneration, the viability of the repair is improved by the transduced cells.

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Age-Related Changes in Cortical and Cancellous Bones in Male Mice Overexpressing IGF-I in Muscles. J. Banu, L. Wang, D. N. Kalu. Physiology, UTHSCSA, San Antonio, TX, USA.

The aim of this study is to determine the effect of increased aging and muscle mass on bone. A colony of transgenic FVB mice that overexpresses IGF-I in muscle was established. The offsprings of transgenic animals were screened for their genotype and only the heterozygotes and wild type males were used in this study. The animals were scanned at 1.5, 2.5, 3.5, 4.5, 6, 9 and 12 months of age, using a pQCT densitometer. The tibial diaphysis, distal femoral metaphysis and the tibial muscle cross-sectional area were analyzed. Body weight and muscle area: The body weight of the wild type animals significantly increased with age up to 9 months of age. In the heterozygotes, the body weight increased significantly up to 6 months of age and it did not change significantly thereafter. The muscle area increased with age in both groups up to 6 months. At 9 months there was significant decrease in muscle area in both groups when compared to 6 months old animals. This decrease was maintained in the 12 months old animals. Tibial diaphysis: In the wild type animals, very little changes were observed with age in cortical bone mineral content (Ct. BMC), cortical bone mineral density (Ct. BMD) and cortical thickness (Ct. Th). In the heterozygotes, there were gradual, but not significant, increases in the Ct. BMC and Ct. Th upto 6 months of age. No difference was observed thereafter. Distal femoral metaphysis: The cancellous bone mineral content (Cn. BMC) and cancellous bone mineral density (Cn. BMD) did not differ significantly between 6 weeks and 6 months old wild type animals but at 9 months and 12 months Cn. BMC and Cn. BMD decreased significantly. In the heterozygote animals, the Cn. BMC and Cn. BMD decreased from 6 weeks to 12 months gradually, but not significantly.The differences between the heterozygotes and the wild type are as follows: Body weight and muscle area: The heterozygote animals maintained a significantly higher body weight and muscle area, in all the age groups studied, when compared with the wild type. Tibial diaphysis: The heterozygote animals had significantly higher Ct. BMC and Ct. Th when compared to the wild type animals.Distal femoral metaphysis: No significant differences were observed between the heterozygotes and the wild type animals in the Cn. BMC and Cn. BMD.In conclusion, male mice overexpressing IGF-I in the muscle had increased muscle area. Increase in muscle area was associated with increase in cortical bone. With age, wild type and heterozygotes lost cortical and cancellous bones.

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IGF-II Induces Apoptosis and IGF-I Prevents Glucocorticoid-induced Apoptosis in Primary Human and Rat Osteoblasts. G. Gronowicz, M. B. McCarthy,* H. Zhang. Orthopaedics, UCONN Health Center, Farmington, CT, USA.

The ability of IGF-I and IGF-II to modulate apoptosis was studied in primary rat and human osteoblast cultures. Rat osteoblasts were obtained by sequential digestion (fraction 3) with collagenase/ hyaluronidase of 20 day fetal rat calvaria. Human osteoblasts were obtained by outgrowth of cells from bone chips discarded after orthopedic procedures. At 72 h of treatment, concentrations of 0.001 to 1 nM IGF-I had no effect on apoptosis on either primary rat or human osteoblasts as determined by acridine orange/ethidium bromide and TUNEL staining. However, 0.001 to 1 nM IGF-II produced a dose-dependent increase in apoptosis in both types of cell culture. A maximal increase of 5.0-fold above control levels was found with 1 nM IGF-II. In previous work, glucocorticoids were shown to increase apoptosis and to inhibit IGF-I in osteoblasts, therefore, the effect of IGF-I on glucocorticoid-induced apoptosis was studied. Corticosterone treatment for 72 h had been shown to cause a dose-dependent increase in the number of apoptotic cells producing a maximal response with 100 nM corticosterone. Therefore, osteoblasts were treated with 100 nM corticosterone and 0.1 to 1 nM IGF-I. IGF-I was able to prevent glucocorticoid-induced apoptosis in a dose-dependent manner. In addition, apoptosis was induced by 10 μg/ml of lipopolysaccharide (LPS) administration for 72 h. IGF-I was unable to prevent LPS-induced apoptosis. To understand the mechanism for IGF-I's effect on glucocorticoid-induced apoptosis, bcl-2 and bax levels were determined by Western blot analysis at 72 h of culture of primary rat osteoblasts. Doses of corticosterone (1 to 1000 nM) decreased the bcl-2/bax ratio from 1.0 to 0.37 but the bcl-2/bax ratio did not diminish significantly with glucocorticoids and 1.0 nM IGF-I. IGF-I was able to abrogate the effect of corticosterone on bcl-2/bax in a dose-dependent manner. IGF-I alone had no visible effect on bcl-2 protein. However, IGF-II significantly decreased bcl-2 levels so that the bcl-2/bax ratio decreased to 0.7 with 1 nM IGF-II compared to a control ratio of 1.0 without IGF. Neither IGF-I nor IGF-II altered bax levels. In conclusion, IGF-II induces apoptosis in osteoblasts through a decrease in bcl-2 levels. However, IGF-I had no effect by itself on baseline apoptosis, bcl-2, or bax, but was able to prevent glucocorticoid-induced apoptosis and the down-regulation of bcl-2/bax in osteoblasts.

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TGF-β1 Regulation of Growth Plate Chondrocytes Is Mediated by Multiple Interacting Pathways. Z. Schwartz,[1] D. D. Dean,[2] E. E. Rosado,*[3] V. L. Sylvia,[2] B. D. Boyan.[2] [1]Periodontics, Hebrew U, Jerusalem, Israel, [2]Orthopaedics, U Texas HSC, San Antonio, TX, USA, [3]Periodontics, Wilford Hall Med Ctr, Lackland AFB, TX, USA.

TGF-β1 affects growth plate chondrocytes through Smad-mediated mechanisms and has been shown to increase PKC. This study determined if the physiological response of growth zone (GC) cells to TGF-β1 occurs via Type II or Type III TGF-β receptors and which receptor mediates the increase in PKC. Further, since the physiological responses to TGF-β1 were mediated by PKC, then the signal transduction pathway involved was elucidated. Confluent GC cells were treated for 24 h with TGF-β1 (0.11, 0.22, or 0.88 ng/ml) ± antibodies to TGF-β receptor II or III, or with soluble type II or III receptors. TGF-β1 stim-

ulated [^{3}H]-thymidine and [^{35}S]-sulfate incorporation as well as alkaline phosphatase (ALPase) and PKC specific activities; only anti-type II TGF-β receptor Ab and soluble type II TGF-β receptor decreased the TGF-β1 effects. This showed that TGF-β1 mediates the physiological responses of GC cells through the type II receptor. Inhibition of PKC with chelerythrine, staurosporine, or H-7 also caused a dose-dependent decrease in these parameters and inhibition of PKA with H-8 partially blocked the TGF-β1-stimulated increase in [^{3}H]-thymidine incorporation and ALPase specific activity. These results show that both PKC and PKA are involved. In addition, TGF-β1 maximally stimulated PGE_2 levels at 0.22 ng/ml and this increase was augmented by GTPγS, but not by chelerythrine, indicating that prostanoid production is mediated by a G-protein-dependent mechanism. To determine the signaling pathways involved in the TGF-β1-dependent increase in PKC, GC cells were treated for 12 h ± 0.22 ng/ml TGF-β1 in the presence/absence of inhibitors or activators of possible signal transduction pathways. TGF-β1-dependent PKC was not regulated by diacylglycerol; dioctanoylglycerol and the diacylglycerol kinase inhibitor R59022 had no effect. Arachidonic acid increased PKC in control cultures and was synergistic with TGF-β1. Thus, TGF-β1, most likely, stimulates PKC via PLA_2 and not PLC. Prostaglandins are required, as indomethacin blocked the effect of TGF-β1; Cox-1, but not Cox-2, is involved in producing the increase in PKC. Exogenous PGE_2 stimulated PKC, but not as much as TGF-β1, suggesting that PGE_2 is not sufficient for all of the prostaglandin effect. GDPβS, pertussis toxin, and cholera toxin blocked the TGF-β1-dependent increase in PKC, while GTPγS caused a further increase; thus both G_i and G_s are required. Inhibition of PKA with H-8 also partially inhibited the effect. This demonstrates that multiple signaling pathways are involved.

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1,25-Dihydroxyvitamin D Regulates the Concentrations of Transforming Growth Factor β Receptor I and II in Human Osteoblasts. D. E. Nagel,* R. Kumar. Mayo Clinic/Foundation, Rochester, MN, USA.

1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$) regulates the concentrations of transforming growth factor β (TGFβ) 2 in the osteoblasts via vitamin D response elements in the TGF β2 gene (Wu et al, Biochemistry 38:2654-2660, 1999). Others have shown that the vitamin D receptor and the Smad proteins interact to potentiate 1α,25$(OH)_2D_3$ action in cells. To determine whether 1α,25$(OH)_2D_3$ also regulates concentrations of TGF β type I and II receptors, we treated transformed human osteoblasts (hFOB cells) with 1α,25$(OH)_2D_3$ (10^{-8}M) and measured TGF β type I and II receptor mRNA concentrations using RNA-based RT-PCR methods. We found that 1α,25$(OH)_2D_3$ increased TGF β type I receptor mRNA concentrations in a time-dependent manner. 1α,25$(OH)_2D_3$ did not increase mRNA concentrations for the TGF β type II receptor. To determine the mechanisms by which such regulation occurs, we cloned the promoter region for human TGF β type I receptor (-108 to -726 bp) and TGF β type II receptor (-1670 to +36) upstream of a luciferase reporter gene in the pGL3 plasmid vector and used these chimeric plasmids to transiently transform human osteoblasts. We used the Renilla luciferase plasmid vector to correct for variability in the efficiency of transformation. The transiently transfected cells were then treated with 1α,25$(OH)_2D_3$ (10^{-8}M) for a period of 24 hours. Fire fly and Renilla luciferase activities were determined. 1α,25$(OH)_2D_3$ did not significantly increase the activity of the TGF β type I and II receptor promoters. Conclusion: 1α,25$(OH)_2D_3$ increases TGF β type I receptor concentrations in human osteoblasts. The effect does not appear to involve an increase in the transcriptional activity of the TGF receptor I promoter. The effects of 1α,25$(OH)_2D_3$ in human osteoblasts are secondary to changes not only in TGF β2 concentrations but also are secondary to changes in TGF β type I receptor expression.

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The Role of the Perichondrium in TGF-beta and Hedghog Signaling During Endochondral Bone Formation. R. Serra, J. Alvarez.* Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA.

Endochondral bone formation is complex and requires the coordination of signals from several factors synthesized by both chondrocytes and cells in the perichondrium. For example, Indian Hedgehog (Ihh), synthesized in prehypertrophic chondrocytes, is thought to act on the perichondrium to form a negative feed back loop with Parathyroid Hormone related Peptide (PTHrP) providing a mechanism for chondrocytes to sense and down-regulate their rate of differentiation. Members of the Transforming Growth Factor-beta (TGF-ß) superfamily are secreted growth factors that also regulate growth and differentiation. Expression of a dominant-negative form of the TGF-ß type II receptor in the perichondrium of transgenic mice results in increased hypertrophic differentiation suggesting a role for the perichondrium in mediating the effects of TGF-ß on the growth plate. Previously, we showed that addition of TGF-ß1 to embryonic mouse metatarsals grown in organ culture resulted in inhibition of chondrocyte hypertrophy. Furthermore, PTHrP mRNA expression was stimulated in the perichondrium after treatment with TGF-ß1 and PTHrP was shown to be required to mediate the effects of TGF-ß1 on hypertrophic differentiation. The objective of this study was to test the hypothesis that the perichondrium is required to mediate the effects of TGF-ß1 and Sonic Hedgehog (Shh) on endochondral bone formation. First, we show that similar to treatment with TGF-ß1, hypertrophic differentiation is inhibited in embryonic mouse metatarsal cultures treated with Shh conditioned medium. PTHrP mRNA is also induced in the perichondrium after Shh treatment. Furthermore, treatment with Shh stimulated TGF-ß3 mRNA expression in the perichondrium. Next, we compared chondrocyte proliferation and hypertrophic differentiation in intact and perichondrium-free metatarsals either untreated or treated with TGF-ß1 or Shh. Unlike intact cultures, hypertrophic differentiation was not inhibited by TGF-ß1 in perichondrium-free cultures. Furthermore, metatarsals in which the perichondrium was infected with an adenovirus containing a dominant-negative mutation of the TGF-ß type II receptor did not respond to TGF-ß1. In addition, hypertrophic differentiation was not inhibited by Shh in perichondrium-free cultures. These results suggest that regulation of hypertrophic differentiation by TGF-ß and Shh are dependent on the perichondrium. Since treatment with Shh resulted in an increase in TGF-ß3 mRNA expression in the perichondrium, we are currently testing the hypothesis that TGF-ß in the perichondrium mediates the effects of Shh on hypertrophic differentiation.

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Increased Bone Mineral Density in Aging Male Mice Overexpressing a Mutated Form of Latent TGFbeta Binding Protein (LTBP-1). L. F. Bonewald,*[1] K. Traianedes,*[1] S. Amano,*[1] J. Rosser,*[1] C. Barley,*[2] S. L. Dallas.[2] [1]University of Texas Health Science Center, San Antonio, TX, USA, [2]Biochemistry, University of Manchester, Manchester, United Kingdom.

LTBPs are members of the fibrillin superfamily of structural extracellular matrix proteins that bind TGFbeta and regulate its availability. LTBP1 covalently binds to latent TGFbeta prior to secretion and then targets latent TGFbeta to the extracellular matrix (ECM) for storage. However, approximately 90% of the LTBP1 produced by bone cells is not complexed to TGFbeta, suggesting an independent role. Mutations in fibrillins are associated with Marfan and related syndromes that frequently exhibit skeletal abnormalities. Several of the fibrillin-1 mutations act as dominant-negatives by covalently binding with the ECM, but preventing incorporation of the normal protein into microfibrils. In the present study, mutations in LTBP-1 were engineered to mimic this function of mutated fibrillin-1 and to determine the consequences of disruption of LTBP1 on bone formation in transgenic mice.Mutations were made in two EGF-like domains in LTBP-1 (C550R and C592R) which were homologous to mutations in fibrillin-1, (C1074R and C1117Y), associated with a severe form of Marfan's syndrome. 2T3 osteoblasts stably transfected with this construct demonstrated accelerated differentiation and mineralization. Transient transfection of the construct in 293 cells indicated that the mutated LTBP1 protein is efficiently secreted and incorporated into ECM microfibrils. Transgenic mice were generated expressing approximately 50 copies of the transgene driven by the collagen type 1 enhancer. Bone mineral density (BMD) on the spine, femur, tibia, the tibial-femoral joint (both right and left) and total body was measured using a Lunar PIXImus mouse bone densitometer on 3, 9 and 13 month old male normal and transgenic mice. No gross abnormalities in bone development and growth were observed in transgenic mice compared to control animals However, at three months, the transgenic mice showed significantly increased BMD for all eight parameters when normalized for weight. At both 9 and 13 months, the transgenic mice maintained a higher BMD compared to normal controls. In addition, whereas the normal mice began to lose BMD between 9 and 13 months, the transgenic animals lost significantly less BMD. This was true with or without weight taken into consideration (change in BMD from 9 to 13 months for normal mice = -8.4 units, transgenics= -1.9 units; change in BMD/gm for normal mice = -35.4 units, transgenics = -14.3 units). LTBP-1 may be an important regulator of bone formation, either through its role in regulation of TGFbeta or independently via its function as an ECM protein.

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Localization and Expression of TGF-b and TGF-b Type II Receptors During Fracture Haling in the Rat. K. Goto, A. Ogasawara, A. Nakajima, S. Shimizu,* F. Nakajima,* M. Yamazaki,* H. Moriya.* Orthopaedic Surgery, Chiba Univ. School of Medicine, Chiba, Japan.

Transforming growth factor-b (TGF-b) is one of the most abundant growth factor in bone, and TGF-b injection to the parietal bone of neonatal rats stimulates intramembranous ossification, whereas an injection to newborn rat femurs promotes endochondral ossification. Joyce et al. suggested that TGF-b was synthesized by osteoblasts and chodrocytes during rat bone fracture healing. TGF-b may play a critical role in bone remodeling and cartilage growth and metabolism. TGF-b type II receptor (TbR-II) is a transmembrane serine/threonine kinase, and TGF-b signals can be transduced by binding to type II receptors. This ligand-receptor complex recruits type I receptors which become phosphorylated and activated by the type II receptor intracellular kinase domain and eventually signal through Smad proteins. Therefore, the purpose of this study was to examine localization of TGF-b and TbR-II using immunohistochemistry and expression by RNase protection assay in an adult rat fracture model. A standard closed mid-diaphysial fractures were produced in the right femurs of 11-week old male S-D rats according to the method of Einhorn. Fractured femurs were dissected on day 1, 4, 7, 14, 21and 28 after the fracture. Paraffin-embedded sections were prepared for immunostainning and total RNA of the fracture site for Rnase protection assay. [Immunohistochemistry] TGF-b and TbR-II were visualized similarly in proliferative and hypertrophyic chondrocytes in the endochondral ossification area. We detected neither TGF-b nor TbR-II positive cells in the intramembranous ossification area. [Rnase protection assay] A high level of TGF-b1 mRNA expression was maintained throughout the period of fracture healing. TbR-II mRNA expression increased gradually after fracture and peaked on day 21. In this study, we have demonstrated that co-localization of TGF-b and TbR-II in proliferative and hypertrophyic chondrocytes in soft callus. A previous report showed that TGF-b1 regulates terminal differentiation of epiphyseal chondrocytes into hypertrophyic chondrocytes in vitro. These findings suggested that TGF-b1 regulates the functional differentiation of chondrocytes in an autocrine/paracrine fashion during fracture healing. Moreover, TGF-b1 gene expression is almost constant. On the other hand, there is less TbR-II expression and it changes considerably after fracture. In conclusion, we suggest that TGF-b regulates chondrocyte differentiation during the endochondral ossification process by expression of its receptors during fracture healing.

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TGF-beta-induced Repression of CBFA1 by Smad3 Decreases Cbfa1 and Osteocalcin Expression and Inhibits Osteoblast Differentiation. T. Alliston,*[1] P. Ducy,[2] L. Choy,*[1] G. Karsenty,[2] R. Derynck.*[1] [1]Growth and

Development, University of California - San Francisco, San Francisco, CA, USA, [2]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

Transforming growth factor-β (TGF-β), a secreted growth factor present at high levels in bone, inhibits many aspects of osteoblast differentiation in culture; yet, the mechanism of this inhibition remains unclear. We have studied the effects of TGF-β and its effectors, the Smads, on the expression and function of the osteoblast transcription factor CBFA1. We discovered that TGF-β inhibited the expression of the cbfa1 gene and the osteocalcin gene, whose expression is controlled by CBFA1 in osteoblast-like cell lines. This inhibition was mediated by Smad3, which physically interacts with CBFA1 and represses its transcriptional activity at the CBFA1-binding OSE2 promoter sequence. The TGF-β regulated interaction of Smad3 and CBFA1 was present at the DNA level and required CBFA1, but not Smad3, DNA binding ability for functional repression. The ability of Smad3 to repress the function of CBFA1 stands in contrast to previous observations that Smads function as transcription activators. Though inhibition of TGF-β activated gene expression has been described, no mechanism for repression of gene expression by TGF-β/Smad3 has yet been reported. Since we have learned that the repressor activity of Smad3 on CBFA1 occurred in mesenchymal, but not in epithelial cells, and depended on the promoter sequence context; we are actively seeking the cis and/or trans elements that enable this mesenchyme-specific Smad repression. The Smad3-mediated repression of CBFA1 provides a central regulatory mechanism to explain the strong inhibitory effect of TGF-β on osteoblast differentiation, since it inhibits both cbfa1 transcription and transcriptional activation of osteoblast differentiation genes by CBFA1. Accordingly, altering Smad3 signaling in stable cell lines influenced the extent of osteoblast differentiation both in the presence and absence of TGF-β, thereby implicating Smad3/TGF-β-mediated repression in autocrine regulation of osteoblast differentiation. Furthermore, we are investigating the TGF-β inhibition of CBFA1 function in vivo, as TGF-β overexpressing mice have been shown to exhibit a phenotype that resembles that observed in cbfa1 +/- transgenic mice. In summary, we show that TGF-β inhibits CBFA1 expression and function by physical interaction of CBFA1 with Smad3, providing the first report of Smad3 mediated repression. This mechanism provides insight into the inhibition of osteoblast differentiation by TGF-β in vivo.

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Testis-Specific Overexpression of SMAD4 Causes Dwarfism in Mice. D. C. Montague,[1] W. R. Hogue,*[1] R. A. Skinner,*[1] L. J. Suva,[1] T. K. Woodruff,*[2] D. Gaddy-Kurten.[1] [1]Physiology and Biophysics/Center for Orthopaedic Research, Orthopaedic Surgery, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Neurobiology and Physiology, Northwestern University, Evanston, IL, USA.

In the testis, locally produced activin modulates steroid production and reproductive function. SMAD4 is the common signaling component of the receptor serine kinase pathway activated by activin and other members of the TGFbeta superfamily. To better understand the mechanism by which activin regulates testicular function we targeted overexpression of a SMAD4-FLAG epitope tagged construct by the developmentally regulated testis-specific Mullerian inhibiting substance (MIS) promoter. Homozygous SMAD4 transgenic mice were born with normal Mendelian inheritance. SMAD4 overexpression appeared to be testis specific. Testes of transgenic mice lacked germ cells, and exhibited Leydig cell hyperplasia, and 3 week old male mice were dwarfed and had severe kyphosis. Female mice were unaffected. Therefore, we investigated the skeletal phenotype of the male SMAD4 mice by DXA, pQCT, and static histomorphometry. By DXA, bone mineral density (BMD) was diminished by 15-20% in long bones and lumbar spine. pQCT analysis of vertebra (T12 and L1) revealed a significant decrease in volumetric trabecular and total BMD of around 20-30%. BV/TV measured in the proximal tibia was significantly decreased in transgenic mice, as was osteoblast surface area, suggesting altered osteoblast function or formation. To determine if the decrease in bone mass could be explained by inappropriate expression of the transgene in bone, we measured SMAD4 expression in wild type and transgenic mouse bone and bone marrow. No differential SMAD4 or FLAG immunostaining was detected. Interestingly, penetrance of the gross phenotype was less robust in older mice (12 months old), since subsequent generations maintained dwarfism in the absence of kyphosis. In addition, the long bone and vertebral bone densities of older mice were not significantly different between the dwarfed transgenic and wild-type control mice, suggesting that the early decreased bone mass observed at 3 weeks may be restored in adult mice. These data suggest that overexpression of SMAD4 in the testis during development results in the systemic dysregulation of either bone formation and/or bone turnover. Unlike the reproductive phenotype, which progressively deteriorates with age, the skeletal phenotype appears transient. The identity of the specific testis-derived molecule(s) responsible for the dramatic and transient loss of bone in these mice remains unknown.

Disclosures: Glaxo SmithKline,1.

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Mechanically Induced Gene Expression in Osteoblasts. R. Thomas,*[1] J. Triffitt,[1] J. Kenwright.*[2] [1]Bone Laboratory, Nuffield Dept. of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford, United Kingdom, [2]Bone Laboratory, Windmill Road, Headington, Oxford, United Kingdom.

Introduction:Bone cells are highly sensitive to cyclical mechanical strain. Many studies have documented the effects of individual genes thought to be important in the mechanotransduction pathway. Modern methods of differential gene expression allows high through-put analysis of many genes simultaneously after a stimulus. Our aim was to apply cyclical strain to living human bone cells in culture, and study the differential expression of 5000 genes at 6 and 12 hours following application of the strain stimulus.Methods:Human mesenchymal stem cells were cultured and passaged onto a stretchable polyurethane membrane. Cyclical strain was applied via a computer controlled cell stretch device at 1% strain (10,000 micro-strain) for twenty minutes. 5 sets of samples (3 control and 2 strained samples at 6 and 12 hours) were obtained and these were:1) control cell sample at zero hour2) strained cell sample harvested 6 hours following strain stimulus3) control sample at 6 hours4) strained cell sample harvested 12 hours following strain stimulus5) control cell sample at 12 hoursUsing a custom differential gene glass array service (CLONTECH LABS), the expression profile of 5000 genes in the 5 samples were compared. Differential expression 2.5 times that of control was used to determine up or down regulation of genes. Results: Our initial differential expression factor of 2.5, detected more than 200 genes at the 6 and 12 hour period. However, a more stringent differential expression filter factor of 5 produced 80 genes that were differentially up or down regulated at these time points. Certain genes hitherto unrecognised as mediators of mechanical strain were also differentially regulated and this finding is discussed. Discussion: The differential expression of early response genes such as c-fos, c-jun and signalling genes such as I-nos, e-nos are presented, as also the range of molecules important in extra and intra-cellular pathways. This study provides new information on the expression profile of currently accepted mechanotransducible genes and provides additional information on genes as yet un-identified as important in the transmission of mechanical strain. Knowledge from detailed gene profiling at further time points and strain levels, will be useful to plan strategies of therapy based on chronological gene expression in situations such as fracture healing and distraction osteogenesis.

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Dynamic Equibiaxial Strain Causes Intracellular Changes in Osteoblasts Cultured on RGD-Engineered Surfaces. E. A. Cavalcanti-Adam,*[1] I. M. Shapiro,[1] E. J. Macarak,*[2] R. J. Composto,*[3] C. S. Adams.*[1] [1]Biochemistry, University of Pennsylvania, School of Dental Medicine, Philadelphia, PA, USA, [2]Bioengineering, University of Pennsylvania, Philadelphia, PA, USA, [3]Chemical Engineering, University of Pennsylvania, School of Engineering and Applied Sciences, Philadelphia, PA, USA.

A primary goal of orthopedic research is to understand the mechanism by which mechanical deformation directs the activities of bone cells. In this report, we describe a method for covalently attaching RGD peptides to a deformable membrane. In addition, we show that application of dynamic equibiaxial strain modulates the responses of the ligand-adherent osteoblasts. To covalently attach the RGD peptide to an elastic deformable silicone surface, the membrane was functionalized by UV/ozone radiation and then aminated with 1 mM 3-aminopropyltriethoxysilane in hexane for 45 - 90 min. Purified RGD peptide (0.1mM) was then attached to the amino-group of the silane to form a covalent bond between the material surface and the peptide. MC3T3-El cells were deposited on the RGD-modified silicone surface and maintained in culture for 10-15 days. Three days after plating, cyclic dynamic equibiaxial strain was applied to the cells using a 13 station Flexercell apparatus. The strain was applied at 1.5% magnitude and at 15 cycles/minute for 2 hours. Controls for this study included non-stretched cells grown on RGD-treated membranes.Cells attached tightly to the RGD surface and spread rapidly. When subjected to dynamic strain, there was a profound change in osteoblast phenotype. The cells exhibited high alkaline phosphatase activity and an elevated level of mineral deposition. Stained with phalloidin, the osteoblast-like cells exhibited reorganization of actin filaments and generation of focal contacts. The cAMP levels of the cells were also determined. We noted a two-fold decrease in the cAMP concentration; moreover, disruption of cytoskeleton with cytochalasin D further modulated cyclic nucleotide levels. It is concluded that the covalent binding of RGD peptides to a silicone membrane provides a compatible biomimetic surface for the attachment and subsequent differentiation of osteoblast-like cells. Dynamic equibiaxial strain affects the cytoskeletal organization of the cell, probably through activation of integrin receptors. Finally, the fall in cAMP levels following application of physiological forces, indicates that the strain is transduced through the cyclic nucleotide system and is dependent on cytoskeletal integrity.

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Oscillatory Fluid Flow Induces Calcium Mobilization in Osteoblastic Cells via Extracellular ATP/UTP Interactions with P2Y Receptors. J. You,[1] C. R. Jacobs,[2] H. J. Donahue.[1] [1]Orthopaedics and Rehabilitation, The Pennsylvania State University College of Medicine, Hershey, PA, USA, [2]Biomechanical Engineering Division, Stanford University, Stanford, CA, USA.

Recently fluid flow has been demonstrated to be a potentially important physical signal for mechanical loading-induced changes in bone cell metabolism. We previously found that oscillatory fluid flow activated MC3T3-E1 osteoblastic cell intracellular calcium ($Ca^{2+}{}_i$) mobilization via the IP_3 pathway in the presence of 2% FBS. The aim of this study was to investigate the role of extracellular signaling molecules and their receptors in $Ca^{2+}{}_i$ mobilization induced by oscillatory fluid flow. First we demonstrated that oscillatory fluid flow (±20dynes/cm^2) in the absence of extracellular signaling molecules (i.e. no FBS in medium) failed to increase $[Ca^{2+}]_i$ in MC3T3-E1 cells. This suggests that extracellular signaling molecules in FBS are required to activate $Ca^{2+}{}_i$ mobilization. Apyrase (10U/ml), an enzyme that rapidly hydrolyses 5' nucleotide-triphosphates to monosphophates, prevented the fluid flow induced increases in $[Ca^{2+}]_i$ in the presence of FBS suggesting that ATP or UTP may be mediating the effect of fluid flow on $[Ca^{2+}]_i$. Furthermore, adding ATP or UTP to medium without FBS restored the ability of fluid flow to increase $[Ca^{2+}]_i$. However, adding ADP or UDP did not, suggesting that neither these molecules nor their receptors, P2Y1 and P2Y6, were playing a role in the fluid flow response. Adenosine, a P1 receptor agonist, and ATPγS, a P2X and P2Y11 receptor agonist, added to medium did not restore the ability of fluid flow to increase $[Ca^{2+}]_i$. PPADS, a P2X antagonist, did not have any effect on the $Ca^{2+}{}_i$ response in the presence of FBS. Thus, by process of elimination, our data suggest that P2Y receptors (P2Y2 or P2Y4) are involved in the $Ca^{2+}{}_i$ response to fluid flow. In addition, inhibiting the $G_{i/o}$ protein, to which P2Y receptors, but not other

purinoceptors, are coupled, inhibited fluid flow induced increases in $[Ca^{2+}]_i$ further suggesting that P2Y receptors are involved in the response. Taken together, these data suggest that oscillatory fluid flow acts through the interaction of extracellular nucleotides ATP/UTP with P2Y purinoceptors to induce $Ca^{2+}{}_i$ mobilization. Our finding is the first evidence showing that fluid flow and the extracellular signaling molecules ATP/UTP are essential regulators for bone cell mechanotransduction.

SU201

Up-Regulation of AP-1 Components in Mechanically-Stretched Human Osteoblastic Cells: Deciphering the Mechanotransduction Cascade. E. K. Basdra,*[1] D. Kletsas,*[2] A. G. Papavassiliou.[3] [1]Orthodontics, University of Heidelberg, Heidelberg, Germany, [2]Biology, N.C.S.R. "Demokritos", Athens, Greece, [3]Biochemistry, University of Patras, School of Medicine, Patras, Greece.

Osteoblastic cells transduce signals of mechanical loading that plays a key role in maintaining bone formation. In an attempt to elucidate the biochemical events associated with the conversion of mechanical stress to biological outcome, we examined cultured human periodontal ligament (hPDL) osteoblastic cells exposed to continuous stretch, in terms ofcellular parameters correlating known signaling cascades to the initialphase of osteoblast-specific transcriptional control. Time-courseexperiments revealed that mechanical stretch-loaded hPDL cells exhibit avery rapid and relatively sustained increase in the abundance of theimmediate-early gene products c-Fos and c-Jun, components of theactivator protein-1 (AP-1) transcription factor. Moreover, this increasein protein levels was paralleled by hyperphosphorylation and therebypotentiation of c-Jun, the principal modulator of AP-1 activity.Importantly, these inductive effects were partly or completely abolishedby pre-incubating the cells with SB 203580, PD 098059, and the novelcompound Y-27632, inhibitors of p38 mitogen-activated protein kinase(MAPK), MAPK kinase (MEK), and Rho kinase (RhoK), respectively. Theseresults consolidate AP-1 as the pivotal downstream effector in the earlyresponse of hPDL cells to continuous mechanical stretching, via thecoordinate stimulation of de novo synthesis and post-translationalregulation of AP-1 proteins. This "integrating" function of AP-1 ismediated through a mechanotransduction circuit that incorporates elements of well-defined upstream signaling protein kinase systems. REFERENCES:(1) Mechanical stress induces DNA synthesis in PDL fibroblasts by a mechanism unrelated to autocrine growth factor action.D. Kletsas, E.K. Basdra, A.G. PapavassiliouFEBS Letters, Vol. 430 No. 3, pp. 358-362 (1998).(2) Stretch-mediated activation of selective MAPK subtypes and potentiationof AP-1 binding in human osteoblastic cells.F.A. Peverali, E.K. Basdra and A.G. PapavassiliouMolecular Medicine, Vol. 7 No. 1, pp. 68-78 (2001).

SU202

Mechanosensitivity of Bone Cells to Oscillating Fluid Flow (OFF)Depends on Both Shear Stress and Flow Rate. T. R. Haut,*[1] C. E. Yellowley,[1] H. J. Donahue,[1] T. L. Haut-Donahue,*[1] C. R. Jacobs.[2] [1]Orthopaedics and Rehabilitation, Penn State College of Medicine, Hershey, PA, USA, [2]Mechanical Engineering, Stanford University, Stanford, CA, USA.

It has been demonstrated that bone adapts to its physical loading environment, however, the biophysical signals that regulate bone cells are not known. The aim of this study was to examine effects of shear stress and chemotransport, generated as a consequence of OFF, on cytosolic Ca2+ concentration ([Ca2+]i) and PGE2 production in mouse osteoblastic MC3T3-E1 cells. Ca2+ imaging: MC3T3-E1 cells cultured in monolayer were loaded with Fura-2 AM and placed in a parallel plate flow chamber. Following a 1min no flow period, cells experienced OFF at 1Hz for 3 min generated using a materials testing machine. 3 flow regimes were studied by altering flow rate and/or increasing fluid viscosity with neutral dextran: 1) constant peak shear stress (PSS), varied flow rate, 2) constant flow rate, varied PSS, 3) varied PSS with nutrient free HBSS. Image analysis software was used to capture and convert fluorescent signals into [Ca2+]i values. Ca2+i transients of 50 nM or greater were considered responses. PGE2: Cells were exposed to 1hr OFF using 3 flow regimes, all producing a PSS of 20 dynes/cm2 but with altered flow rates. Following flow, cells were incubated in fresh media for 1 hr, which was then collected for PGE2 analysis. PGE2 production was also measured in no flow, control cells. The following trends did not reach statistical significance, additional experiments are underway. At constant PSS (20 dynes/cm2), reducing flow rate from 18 ml/min to11.5 ml/min decreased the percent of cells responding with an increase in [Ca2+]i from 87.7±4.9% to 75.2±3.5%. At a constant flow rate of 4.5 ml/min, increasing PSS from 5 dynes/cm2 to 8.7 dynes/cm2 increased the percent of cells responding from 14.6±13% to 33.6±7.1%. In nutrient free HBSS only 10.8±7% of cells responded at 20 dynes/cm2 and 8.4±1.6% at 40 dynes/cm2. At a constant PSS of 20 dynes/cm2, decreasing flow rates from 43 to 28 to 18ml/min significantly decreased PGE2 production from 34±3.1 to 25.8±6.8 to 9.1±1.8 pg/µg of total protein, respectively ($p<0.05$). OFF elicited Ca2+i transients and increased PGE2 production in MC3T3-E1 cells. Decreasing flow rate while maintaining PSS decreased both Cai and PGE2 responses to OFF. The Cai response was increased when PSS was increased at a constant flow rate, and was significantly reduced in nutrient free HBSS. These data suggest that the cellular responses to OFF result from an interaction between shear stress and chemotransport. Further studies will determine whether this interaction occurs at a defined "mechanoreceptor" or is the result of convergence of two distinct signaling pathways.

SU203

Response of c-fos Promoter Activity to Brief, Low Amplitude Strains in Osteoblasts: A Role for Ets Transcription Factors? S. Pradhan,* S. Tsurel,* J. Berilla,* J. F. Welter.* Case Western Reserve University, Cleveland, OH, USA.

Osteoblastic cells respond to mechanical forces by activating signal transduction cascades and altering gene expression patterns. We examined the responses of MC3T3E1 mouse osteoblasts to short term, low level (1000 microstrain, 1Hz) loads applied by cyclic deformation of the growth surface. At these load levels, daily short-term loading significantly retards the ascorbate induced differentiation of the cells as measured by alkaline phosphatase and osteopontin expression. This effect peaked at 5 minutes of loading per day; loads of 1 or more hours per day accelerated the differentiation process slightly as measured by the same criteria. C-fos is known to respond to mechanical loading of bones in vivo, we therefore examined the effect of brief loading bouts on c-fos promoter activity.Stable lines of MC3T3E1 cells carrying the fos promoter driving a luciferase reporter gene were loaded for 0, 5 or 60 minutes. For these experiments cells were grown in MEM without ascorbate and were then either supplemented or not with 37.5mM ascorbate-2-phosphate at confluence. In cells which had not been pre-treated with ascorbate the c-fos promoter was essentially unresponsive to loads. Following 24 hours of ascorbate treatment, (placing these cells at the earliest stages differentiation) a 5 minute loading bout resulted in a marked (~50%) decrease in luciferase activity with a trough at 6-8 hours. Loading for 60 minutes caused a similar, but accelerated inhibition of luciferase activity with a trough at 2-4 hours after loading. 24 hours after loading, fos promoter activity had returned to baseline in cells loaded for 60 minutes but remained depressed at 75% of baseline in cells loaded for 5 minutes.Ets family transcription factors have been implicated in gene regulation in response to mechanical stimulation in several systems. The c-fos promoter contains a Serum Response Element which contains both a CArG motif responsible for binding the Serum Response Factor and an ets core motif CAGGAT which can bind ets factors. We therefore repeated these experiments using a mutant c-fos promoter in which the ets binding site is destroyed. The response of this mutant to loading for 60 minutes was indistinguishable from that of the wt-promoter. However, in contrast to the wt-promoter, the ets-mutated promoter responded to a 5 minute loading with a rapid increase in activity (~150%) which peaked at 10 hours before returning to baseline at 24 hours.These results suggest that although similar in magnitude, the inhibition of the c-fos promoter by 5 and 60 minute loading bouts are regulated by different mechanisms, and implicate the ets family of transcription factors in the response to the briefer loading events.

SU204

Osteoblast MAPK Signaling and AP-1 Activity Is Modulated by Changes in Gravity. C. S. Ontiveros,* A. Berzat,* L. R. McCabe.* Physiology, Michigan State University, East Lansing, MI, USA.

Previous studies demonstrate that changes in gravity affect the cellular and metabolic functions of osteoblasts. During spaceflight, microgravity conditions exist and bone formation is decreased. In contrast, hypergravity conditions have been shown to promote bone formation as seen with exercise. Histology illustrates that changes in osteoblast growth and mineralization are important contributors to these outcomes. Based on evidence demonstrating an important role for AP-1 and MAPK in regulating osteoblast growth and differentiation, we determined the influence of altered G force on AP-1 and MAPK activities. Using a rotating cell culture system (RCCS), we exposed mouse osteoblasts, MC3T3 E1, to simulated microgravity for 24 hours. At this time, levels of c-jun RNA and protein were increased by more than 2 fold compared to gravity controls. After 4 days under simulated microgravity, c-jun RNA levels returned to control levels. Modulation of AP-1 reporter assays correlated with increases in c-Jun. JNK was the predominant MAPK modulated under these conditions. To test the effects of the other extreme, hypergravity, osteoblasts were centrifuged at 13g for one hour and collected at various time points. An increase in c-Jun protein over controls was seen at 1 and 24 hours. ERK was also analyzed to determine a difference in activation under these conditions. An increase in phosphorylated ERK was seen at 1 and 24 hours after hypergravity exposure. In contrast, JNK and p38 activation were not influenced under these conditions. Preliminary studies using an ERK inhibitor suggest a potential role for ERK in hypergravity induced upregulation of c-Jun expression. Understanding the molecular events leading to the gravity associated changes in intracellular signaling and transcription factor activation can contribute to a better understanding of bone formation and bone loss pathologies.

SU205

High Intensity Sports Exert an Osteogenic Stimulus in Chinese Boys. A. Afghani,[1] B. Xie,*[1] J. Gong,*[2] Y. Li,*[2] C. A. Johnson.*[1] [1]Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA, USA, [2]Wuhan Public Health & Anti-Epidemic Institute, Wuhan, China.

Physical activity has been recognized to be an important determinant of bone gain or maintenance during adulthood. However, the role of mechanical loading on the pediatric skeletal system is less understood. The present epidemiological study was conducted to address the role of leisure physical activity, sports team participation, and intensity of sports on skeletal mass of Chinese adolescents. A total of 166 girls and 300 boys between the ages of 10 and 16 participated in this study. Bone mineral density (BMD) and content (BMC) of the forearm and the os calcis were measured using dual energy x-ray absorptiometry (DXA). Leisure physical activity and sports team participation were determined using questionnaire. The Compendium of Physical Activities was used to determine intensity of sports. High intensity sports included soccer, basketball, and running with an average MET value of 7.0; low intensity sports included volleyball, badminton, and table tennis with an average MET value of 4.0. After controlling for age, body mass index (BMI), and grip strength, there were no significant differences among groups of leisure activity in any of the bone mineral measures. In girls, there were also no significant differences among groups of sports teams. In boys, sports team participants however, had significantly greater BMD and BMC at the forearm and the os calcis after controlling for the confounders (age, BMI, grip strength). Adjusted means and SEM for forearm BMD were 0.34 ± 0.003 versus 0.36 ± 0.005 ($p<0.001$) for non-participants and participants, respectively. For forearm BMC, adjusted means and SEM were 3.11 ± 0.035 versus 3.36 ± 0.048 ($p<0.001$). Adjusted means and SEM for os calcis BMD were 0.52 ± 0.005 versus 0.54 ± 0.007 ($p=0.03$). For os calcis BMC, adjusted means and SEM were 2.33 ± 0.037 versus 2.48 ± 0.051 ($p=0.02$) for non-participants and participants of sports, respectively. High intensity sports participants had significantly greater forearm ($p<0.01$) and os calcis ($p<0.05$) BMD and BMC than the boys who reported no sports membership. The results of this study also suggest that low intensity sports do not exert an adequate osteogenic stimulus. This study supports the role of mechanical loading or exercise on the skeletal system of Chinese ado-

lescent boys. Interventions designed for the purpose of maximizing peak bone mass in children and reducing the risk for osteoporosis later in life should focus on sports team membership in high intensity type of exercises.

SU206

Bone Mineral Density in Active Prepubertal Females Compared to Sedentary Boys and Girls. D. Courteix, C. Jaffré,* L. Benhamou. Regional Hospital, IPROS, Inserm ERIT-M 0101, Orléans, France.

The gender difference in bone mass between boys and girls depends on bone sites and is maintained after adjustment for maturation and size differences. At the same bone age males have greater bone content and density than females. Moreover active boys and girls have greater BMC than their inactive peers (Bailey et al., 1999). The aim of this study was to compare the bone mass between active girls and sedentary boys. We measured anthropometrics characteristics in 35 active (10.2 yrs ± 1.4) and 47 non-active (9.7 yrs ± 1.2) girls and 20 sedentary boys (10.1 yrs ± 1). Active females were characterized by more than 4 hours of physical activity per week. Bone mineral content (BMC) and density (BMD) were measured at the total body, lumbar spine, hip and forearm using DXA. All subjects were prepubescent as regards the Tanner's criteria. Active girls did not differ from boys concerning anthropometrical characteristics while non-active girls had significantly lower lean mass and greater % fat mass than sedentary boys ($p<0.01$). Active girls had greater BMD than boys at the lumbar spine ($p<0.001$) and identical BMD at the other bone sites while non-active girls had significantly lower BMD at all bone sites compared to other groups (Table 1). Lumbar spine and trochanter BMC (adjusted for height) were found significantly greater in sporty girls ($p<0.001$). They had similar femoral neck BMC values than boys. At all other sites BMC values did not differ between the 3 groups. Our results suggest that physical exercise carried out before puberty could improve bone density in females, allowing them to compensate the natural gender difference observed in favor of boys. Table 1. BMD characteristics in each group (* *:p<0.01; * * *:p<0.001; ns: not significant)

BMD ($g.cm^{-2}$)	a Non Active Boys (n=20)	b Active Girls (n=35)	c Non Active Girls (n=47)	Statistical analysis
Total	0.898 (0.057)	0.885 (0.053)	0.851 (0.547)	* * a b vs. c
Lumbar spine	0.619 (0.077)	0.700 (0.085)	0.641 (0.074)	*** b vs. a c
Femoral neck	0.743 (0.078)	0.736 (0.088)	0.663 (0.072)	*** a b vs. c
Ward's	0.765 (0.108)	0.736 (0.102)	0.661 (0.095)	*** a b vs. c
Trochanter	0.599 (0.077)	0.590 (0.09)	0.537 (0.062)	*** a b vs. c
UD radius	0.310 (0.039)	0.327 (0.05)	0.305 (0.036)	ns
Mid radius	0.417 (0.037)	0.430 (0.046)	0.410 (0.033)	ns

SU207

Successful Adaptation of the In Vivo Non-invasive Ulna Loading Technique for the Mouse. K. C. L. Lee,* A. Maxwell,* L. E. Lanyon. Veterinary Basic Sciences, The Royal Veterinary College, London, United Kingdom.

The adaptive (re)modelling response of whole bones to mechanical stimuli can only be studied *in vivo*. Previous investigations applying artificial loads to bones *in vivo* have involved a number of species, but great benefit would be gained from extending this approach to the mouse, with its potential for genetic manipulation. This study aimed to adapt for the mouse, the non-invasive ulna loading model, which has been used in the rat.(1) This model avoids confounding effects of a periosteal response to local pressure.(2) Loading regimens were related to *in vivo* strains recorded from gauges bonded to the lateral and medial surfaces of the ulnar midshaft of 6 adult CD1 mice. Peak strains of 1680με and maximum strain rates of 0.03/s were recorded during locomotion and peak strains of 2620με and maximum strain rates of 0.10/s were recorded on landing from falls of 20cm. The left ulnae of 17 week old, female, CD1 mice were loaded for 10 mins, 5 days/wk, for 2 wks. Dynamic axial compression was applied via the flexed carpus and olecranon, using a 4Hz trapezoidal wave. Peak loads of 3.0N (n=10 mice) and 4.3N (n=7 mice) engendered 2000με and 3000με respectively at the lateral ulnar midshaft. Strain rate during loading and unloading was 0.1/s.Right ulnae served as non-loaded controls. All mice received calcein on the 3rd and final loading days and were sacrificed 3 days later. Serial transverse sections of each ulnar diaphysis (= 45% of the 15mm long ulnae) were analysed by histomorphometry. In control ulnae there was negligible modelling activity. By contrast, loading was associated with significant amounts of new bone formation, greatest 3-4mm distal to the midshaft. At this site, loading to 2000με resulted in lamellar periosteal new bone formation, causing a 5±0.4% ($p=0.005$) increase in cortical bone area, with no response endosteally. Moreover, loading to 3000με induced woven periosteal and lamellar endosteal new bone formation, expanding cortical bone area by 21±4% ($p=0.018$) and 1±0.3% ($p=0.028$) respectively. The endosteal new bone caused a 19±5% ($p=0.028$) reduction in marrow area. There was no evidence of damage, intracortical remodelling or resorption. The non-invasive axial ulna loading technique can be used in adult mice. As in the rat, short periods of cyclic loading engendering physiological strains, result in an osteogenic response, the size and character of which are related to peak strain magnitude. Important advantages of mice over rats include the absence of continued bone growth in adults and potential for genetic modification. References: 1) Torrance *et al*, 1994, Calcif. Tissue Int., 54, 241. 2) Akhter *et al*, 1998, Calcif. Tissue Int., 63, 442.

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SU208

Effects of Tower Climbing Exercise on Bone Mass and Turnover in Growing Mice. T. Mori,*[1] N. Okimoto,[1] H. Tsurukami,[1] A. Sakai,[1] Y. Okazaki,*[1] S. Uchida,[1] T. Notomi,*[2] T. Nakamura.[1] [1]Department of Orthopedic Surgery, University of Occupational and Environmental Health, Kitakyushu, Japan, [2]Division of Cerebral Structure, National Institute for Physiological Sciences, Okazaki, Japan.

To clarify the effects of voluntary tower climbing exercise on local turnover of trabecular bone and bone marrow cell development, 30 C57BL/6J mice, 8 weeks of age, were assigned to three body weight-matched groups: a baseline control, sedentary group, and exercising group. Mice voluntarily climbed a 100 cm tower to drink water from a bottle and to take food set at the top of it. The food intake for the sedentary group was adjusted to that of the exercising group of the previous day. After 4 weeks, the exercise group showed significantly increase in the bone volume (BV/TV), the mineralizing surface (MS/BS), the mineral apposition rate (MAR), and the bone formation rate (BFR/BS) in the trabecular bone of the proximal tibia compared to the sedentary group. The trabecular separation (Tb.Sp) was significantly decreased. In bone marrow cell culture experiment, the area of alkaline phosphatase positive - colony forming units - fibroblastic from the tibial bone marrow of the exercising mice increased significantly compared to that of the sedentary mice. These results show that the voluntary climbing exercise alters bone marrow cell development and increases the bone formation and bone volume in the trabecular bone.

SU209

The Effects of Cigarette Smoking and Physical Activity on the Bone Mineral Density of Collegiate Female Dancers. P. C. Fehling,*[1] Z. Aria,*[2] K. Sweet,*[2] M. Wolfson.*[2] [1]Exercise Science, Skidmore College, Saratoga Springs, NY, USA, [2]Skidmore College, Saratoga Springs, NY, USA.

The purpose of this study was to examine the effects of cigarette smoking on the bone mineral density (BMD) of collegiate dancers compared to a group of non-active controls. The subjects for this investigation were 45 college-aged females categorized as dancers (DAS) who smoke (n=10), dancers (DA) who do not smoke (n=12), non-active controls (CONS) who smoke (n=10), and non-active controls (CON) who do not smoke (n=13). Subjects had measurement of anthropometrics (height, weight, circumferences, skinfolds), current and past physical activity (by questionnaire), medical history, and smoking history. Dual energy x-ray absorptiometry (Lunar DXA-IQ, Waltham, MA) was used to determine BMD of the total body, lumbar spine (L2-L4), and proximal femur (neck), and to assess body composition. The DAS smoked an average of 10.3 ± 5.4 cigarettes per day for an average of 4.3 years (range: 1-8 years). The CONS smoked an average of 16.0 ± 5.9 cigarettes per day for an average of 6.2 years (range: 4-9). There was no significant difference among groups in age, height, weight or percent body fat (DXA). The DA had a significantly ($p<.05$) greater femoral neck BMD (1.15 ± .10 g/cm²) than the CONS (1.02 ± .12 g/cm²) and CON (1.01 ± .09 g/cm²). The DAS did not have a statistically different BMD than any of the groups at this site (1.08 ± .13 g/cm²). There were no significant differences among any of the groups for total body or lumbar BMD. The lack of significant effects of physical activity on the total body and lumbar region suggests that the physical activity of the dancers induced a site-specific response in the femoral neck, a weight-bearing area. In addition, these data suggest that smoking may have blunted the osteogenic effects of physical activity in the group of DAS.

SU210

Low Intensity, High Frequency Vibration Prevents the Decrease in Bone Bending Stress Induced by Ovariectomy of Adult Rats. B. S. Oxlund,* G. Ortoft,* T. T. Andreassen, H. Oxlund. Dept of Connective Tissue Biology, University of Aarhus, Aarhus, Denmark.

The effect of low intensity, high frequency vibration on bone mass, bone strength and skeletal muscle mass was studied in an adult ovariectomized (OVX) rat model. One-year-old female rats were allocated randomly to the following groups: start control, sham, OVX without vibration, OVX with vibration at 17 Hz (0.5 g), OVX with vibration at 30 Hz (1.5 g), OVX with vibration at 45 Hz (3.0 g). Vibrations were given 30 min/day for 90 days. The animals were labeled with calcein at day 67 and with tetracycline at day 84. The tibia mid-diaphysis was studied by mechanical testing and dynamic histomorphometry. OVX alone increased the periosteal bone formation rate and increased the medullary cross-sectional area i.e. increased endocortical resorption and outward antero-medial and lateral drifts of cortical bone at the tibia mid-diaphysis. OVX alone also resulted in a reduced bending stress of the tibia diaphysis. Vibration at 45 Hz of OVX rats induced a further increase in periosteal bone formation rate and inhibited the endocortical resorption seen in OVX rats. Vibration at the highest intensity (45 Hz) also inhibited the decline in bending stress induced by OVX. Neither OVX alone nor OVX with vibration influenced skeletal muscle mass. In conclusion, the results support the hypothesis that passive physical loading might have a beneficial effect on preservation of bone in OVX animals.

SU211

Bone Bending Strength, Skeletal Muscle Contraction Force and Body Weight are Highly Correlated in Growth Hormone Injected Adult Rats. H. Oxlund, N. B. Andersen,* G. Ortoft,* T. T. Andreassen. Dept of Connective Tissue Biology, University of Aarhus, Aarhus, Denmark.

The relationships between cortical bone bending strength, skeletal muscle contraction force and body weight were studied in 11-month-old female rats. Growth hormone (GH), 5 mg/kg, was injected daily sc for 3 months. The following groups were included: a start control, a saline injected and a GH-injected group, 14 rats in each group. The rats were anaesthetized with pentobarbital, and the in vivo maximal tetanic tension of the calf musculature (m. soleus, m. plantaris and m. gastrocnemius together) was analyzed in a materi-

als testing machine by stimulating the ischiadic nerve. The bending strength of the tibia mid-diaphyses was analyzed by 3-point bending. The bending strength was increased by 34%, the muscle contraction force by 55% and the body weight by 72% for GH-injected rats compared with saline (2p<0.00001 for all three parameters). Correlation coefficients: bone bending strength and muscle maximal tetanic tension r = 0.84 (2p< 0.00001); bone bending strength and body weight r = 0.85 (2p< 0.00001); muscle maximal tetanic tension and body weight r = 0.91 (2p< 0.00001). No significant correlation was found between bone bending strength and body weight for the control groups, while a significant correlation was found between bone bending strength and body weight for rats injected with GH (r = 0.66, 2p=0.01). Furthermore, a significant correlation was found between bone bending strength and maximal muscle tetanic tension for the control groups (r = 0.53, 2p<0.004). In conclusion, high correlation coefficients were found for cortical bone bending strength, skeletal muscle contraction force and body weight of adult rats injected with growth hormone. For the control groups no significant correlation was found between bone bending strength and body weight. These data support the hypothesis of a close interaction between skeletal muscle contraction force and bone strength.

SU212

Increased Muscle Mass Is Associated with Increased Bone Content, but not Density, in the Humeri of Adult Myostatin-deficient Mice. M. W. Hamrick,*[1] A. C. McPherron,*[2] O. Lovejoy.*[1] [1]Kent State University, Kent, OH, USA, [2]Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Myostatin (GDF-8) functions as a negative regulator of skeletal muscle growth and myostatin deficient animals show a marked increase in muscle mass compared to normals. We investigated the effects of increased muscle mass on bone morphology by examining cortical and trabecular bone content and density in the humeri of myostatin deficient mice. The myostatin gene was disrupted using homologous targeting in embryonic stem cells. The experimental group used in this study included 11 mixed-gender, adult mice homozygous for the disrupted myostatin sequence whereas the control group consisted of 11 mixed-gender, adult wild-type mice. Body mass, deltoid mass, and triceps mass were recorded immediately after sacrifice. Each humerus was then cleaned of all soft tissue and examined using pQCT densitometry. Cross-sectional slices 1 mm thick were scanned at three different positions along the humerus. These sections correspond to 15%, 40%, and 85% of total humeral length, respectively. Results show that the myostatin deficient mice weigh significantly more than controls (P=.01) and also have significantly larger triceps (P<.001) and deltoid (P=.001) muscles than controls. No significant differences were found in the content, density, and cross-sectional area of cortical and trabecular bone at the level of the distal humeral diaphysis (85% length). The deltoid crest of myostatin-deficient mice is, however, much larger than that of controls and as a result the experimental animals have significantly higher trabecular (P<.001) and cortical (P<.01) bone content, but not density (P>.05), in this region (40% length). Finally, the experimental animals have higher trabecular bone content and area (P<.05), but not density, in the region of the proximal humeral metaphysis (15% length). These results suggest that increased muscle mass produces a corresponding expansion of bone volume (but not density), and that these changes occur only in regions at or near muscle attachments.

SU213

Effect of Ibandronate on Bone Quality under 50% and 100% Weightbearing Conditions. M. Daphtary,*[1] C. Ruff,[2] J. Lee,*[1] J. Shapiro,[3] F. Bauss,*[4] L. Schultheis.*[1] [1]Medstar Research Institute, Washington, USA, [2]Johns Hopkins University, Baltimore, USA, [3]Uniformed Services University of the Health Sciences, Bethesda, USA, [4]Roche Pharmaceuticals, Mannheim, Germany.

The purpose of this study was to determine whether the efficacy of ibandronate in preserving bone quality was dependent on weightbearing and age. This study focused on evaluating peripheral Quantitative Computed Tomography (pQCT)-measured *in vivo* changes in bone properties over a period of 35 days in the humerus of ibandronate-treated rats subjected to either 100% (normal) or 50% (quantitatively reduced) weightbearing on their forelimbs. Virgin, female Sprague Dawley rats that were either 3 or 5 months old were injected 30 mg/kg of ibandronate as a single dose. Rats (n=23) were subjected to either 50% or 100% weightbearing on their forelimbs. To maintain 50% weightbearing, rats were hindquarter suspended and walked only on their forelimbs on a special platform. *In vivo* pQCT scans were performed on day 0 and 35 at the humerus to measure changes in trabecular bone density (TD), polar moment of inertia (PMI) and cortical area (CA). Polar moment of inertia is a measure of resistance to bending and torsion and cortical area is a predictor of axial strength. Cortical bone structural properties increased significantly under both 50% and 100% weightbearing conditions in 3-month-old rats. Statistically significant changes are indicated by "*" (Table 1). Changes in trabecular bone density under 50% weightbearing conditions were significantly less than the changes observed under 100% weightbearing conditions. Table 1: Changes in bone properties.

Age (months)	Weightbearing (%)	TD (mg/ccm)	CA (mm^2)	PMI (mm^4)
3	100	70.1 * (19%)	0.34 * (9%)	0.58 * (16%)
3	50	-2.3 (1%)	0.31 * (7%)	0.38 * (10%)
5	100	17.83 (5%)	0.19 (4%)	0.19 (4%)
5	50	-42.68 (-11%)	-0.06 (-1%)	0.08 (2%)

In 5-month-old rats there were no significant changes in cortical bone structural properties between 50% and 100% weightbearing conditions but changes in trabecular bone density were significantly less under conditions of 50% weightbearing. For each weightbearing condition, 3-month-old rats showed more benefit from ibandronate treatment on trabecular bone density and cortical area than 5-month-old rats; there was no significant difference in changes in polar moment of inertia. The effects of ibandronate on trabecular bone appear to be modulated by the magnitude of weightbearing. In contrast, the effects of ibandronate on cortical bone appear to be more sensitive to age-related phenomena. Thus, the efficacy of ibandronate in preserving bone quality is dependent on both the magnitude of skeletal weightbearing and phase of skeletal growth (age).

SU214

Acute Hormonal Responses to a Single High Intensity Exercise Session in Early Postmenopausal Women. W. Kemmler,*[1] K. Engelke,[1] L. Wildt,*[2] M. Pavel,*[3] W. Kalender.*[1] [1]Inst. of Medical Physics, Univ. of Erlangen, Erlangen, Germany, [2]Dept. of Endocr. Gynecology, Univ. of Erlangen, Erlangen, Germany, [3]Dept. of Internal Medicine, Univ. of Erlangen, Erlangen, Germany.

Appropriate exercise regimens have a positive influence on bone density that is mostly attributed to the bone muscle interface. However, the influence of physical exercise on hormones affecting bone metabolism is not well known. Here we investigated the effect of a single characteristic training session of the EFOPS study (Erlanger Fitness and Osteoporosis Prevention Study), a three year controlled trail in which 100 early (1-8 years) postmenopausal osteopenic women (-1>T-Score>-2.5 at L1-L4 or total hip) without medication or diseases affecting bone metabolism are exercised four times weekly. Two jointly held training sessions with 10-15 participants each are interleaved with 2 individual home sessions. 50 non-exercising women serve as control. Both groups are individually supplemented with Ca (max 1500 mg/day) and Vit D (max 500 iU/day) according to a nutritional analysis. 25 volunteers (57.9 ± 3.3 y; BMI: 25 ± 3.9) of the exercise group who were included in the EFOPS study for at least 18 month participated in the investigation. The training session consisted of low and high impact aerobics (20 minutes at 65-80% Hr_{max}), 4 x 15 multidirectional jumps (GRF: 3-4 x body weight), and resistance training (2-4 sets, 8 rep., 75% 1RM, 9 muscle groups with a 60-90 sec rest period separated each set). After a standardized breakfast (6:30) venous blood samples were taken at baseline just before the training (7:30), immediatly after (9:00), 2h after (11:00), and 22 h (8:00 next morning) after the training. Blood samples were kept on ice, serum was separated within 2 h and frozen at –20°. LH, FSF, E2, testosterone (T), free testosteron (fT), DHEA-S, SHBG, hGH, IGF-I, IGF-BP-3, and cortisol were analyzed using standardized RIA/EIA procedures. Parameters showing significant changes compared to baseline (grouped-t-test/wilcoxon test) are listed in the Table (*p<0.5, **p<0.1, ***p<0.01)..

	Baseline	< 5min post	2 h post	22 h post
DHEAS (ng/ml)	794 ± 330	852 ± 361 **	864 ± 395 **	
T (ng/ml)	0.17 ± 0.07	0.18 ± 0.10	0.13 ±0.05***	
fT (pg/ml)	0.83 ± 0.32	0.99 ± 0.51*	0.72±0.24*	
E2 (pg/ml)	13.8 ± 4.2	14.4 ± 4.7	16.4 ± 5.6 ***	
hGH (ng/ml)	1.9 ± 1.2	3.3 ± 2.5 *	1.2 ± 0.6 *	
IGF-I (ng/ml)	80.8 ± 39	78.5 ± 39	78.9 ±36	74.5 ± 38

In conclusion, a single intense exercise session typically used in osteoporosis prevention programs results in significant changes of hormones affecting bone metabolism in early postmenopausal women

SU215

Bone Metabolism and Stress Fractures among Female Recruits Undergoing Intensive Basic Training. A. J. Foldes,[1] M. Siderer,*[2] G. Mann,*[3] M. M. Popovtzer,*[1] N. Constantini.*[4] [1]Hadassah University Hospital, Jerusalem, Israel, [2]Border Police Headquarters, Jerusalem, Israel, [3]Me'ir Hospital, Kfar-Sava, Israel, [4]Wingate Institute, Netanya, Israel.

Stress fractures (SFx) constitute a major problem in military recruits, particularly in females. In order to investigate potential risk factors for SFx in this population, we prospectively studied 203 female recruits to combat units undergoing basic training. Bone mineral density (BMD) at the spine, hip, forearm and whole body, tibial speed of sound (SOS) and urinary deoxypyridinoline (DPD) were measured at baseline. SOS and DPD were determined also at the end of the 3-month training program. In cases with symptoms suggestive of SFx, bone scintigraphy was performed, and the severity of SFx was graded from 1 to 4. "Definite SFx" was defined as grade $\geq$ 2. Mean baseline BMDs and SOS values were within the normal range for age. Mean DPD (8.6 mM/mM creatinine) was slightly higher than the upper limit for adult premenopausal females (7.5 mM/mM creatinine), probably reflecting the tail of the bone accretion phase. By the end of the training course, a significant (P<0.01) increase was observed in mean weight (58.6 → 60.3 kg), SOS (3944 → 3959 m/sec) and DPD (8.6 → 9.6 mM/mM creatinine). Body fat (%) remained unchanged. Definite SFx were diagnosed in 17% of the recruits (n=34; average of 2.1 SFx per subject). These recruits were similar to those who did not sustain SFx (no or negative bone scan; n=148) with respect to height, weight, body fat, gynecological history, prior physical activity, night sleep duration, dairy consumption and smoking. Mean BMD at the spine, femoral neck and trochanter was 3 to 5% lower, while mean DPD was 8% higher in the SFx group. However, these differences, as well as between-group differences of other BMDs, SOS and DPD were not statistically significant. We conclude that SFx were frequent in female recruits undergoing basic training despite an apparent positive effect of the training on indices of bone metabolism. However, no clear association between the risk for SFx and various anthropometric, gynecological, lifestyle and bone variables could be demonstrated. Other factors, such as bone geometry, training program and the design of shoes and tacks (affecting the center of gravity) may contribute to the high incidence of SFx observed in female recruits.

SU216

The Effect of Skeletal Unloading on the Strength of the Femurs in Dahl Salt-Sensitive and Salt-Resistant Female Rats. S. B. Arnaud,[1] M. Thierry-Palmer,[2] T. Cleek,*[1] D. Cappiello,*[1] M. Dasalla,*[1] R. T. Whalen.[1] [1]Life Sciences Division, NASA Ames Research Center, Moffett Field, CA, USA, [2]Morehouse School of Medicine, Atlanta, GA, USA.

There are differences in the metabolism of calcium and vitamin D in Dahl salt-sensitive (SS) and –resistant (SR) rats. To determine whether these differences have an impact on the response of bone strength to skeletal unloading, we unloaded the hind limbs of female juveniles for 4 wks. Half the animals were fed low salt diets (0.3%) to avoid hypertension in SS and half were fed high salt (2%) diets to evaluate salt sensitivity. Femoral strength was estimated with our small animal torsional test machine. The ends of each bone were potted in a low meltng point metal. A gage length of 14 mm centered at the mid-diaphysis was internally rotated at 1deg/sec until failure. Wet weight s and bone lengths were also recorded. Muscle atrophy was estimated by the weight change of the ipsilateral soleus. Although the same age (11 wks), SS rats weighed 30 g more than SR rats at the start (220 and 182 g) and at the end (237 and 201 g) of skeletal unloading. BW in SS and SR controls were related to torque, Nmm (r=0.59, p=.01, n=24). There were no differences in the wet weights or lengths of the femurs in SS and SR. Ultimate strength was higher in SS controls fed high than low salt diets (382±42 vs 317±54 Nmm, p<.01), a salt effect not observed in SR. Strength was reduced 30% by unloading in SS fed high and 26% in SS fed low salt diets. Decreases were similar in SR (27 and 22%). Soleus muscle weights were similar in SS and SR controls fed low salt (90.6±.01 and 81.3±.01 mg), but not in SS fed high salt diets before (93.8±.01 and 80.9±.01 mg, p<.02).or after unloading (43.7±.01 and 30.8±.01 mg, p<.02). Soleus muscle weights could be related to femoral strength in SR (r=0.737, p<.01, n=26), but not in SS (r=0.132, n=23). SS and SR appear to be equally vulnerable to the skeletal effects of unloading, but there is a salt effect that enhances femoral strength and alters the prediction of bone strength based on muscle mass.

SU217

Effect of Natriuretic Peptides on the Bone Cells. D. Sohn,*[1] J. Lee,*[1] S. Ko,*[2] J. Kim,[3] S. Kim.*[1] [1]Department of Dental Pharmacology, Dankook University, Cheonan-si, Republic of Korea, [2]Department of Oral Biochemistry, Dankook University, Cheonan-si, Republic of Korea, [3]OCT Inc., Chenoan-si, Republic of Korea.

Bone is renewed by the remodeling cycle, in which bone resorption by osteoclasts is tightly coupled to new bone formation by osteoblasts. Although numerous factors participate in bone remodeling, the coupling of bone resorption to formation remains poorly understood. It has been suggested that bone and the vasculature system share many molecules in common. Natriuretic peptides are likely to be bone-active molecules also. This study was performed to clarify the possible roles of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) in the regulation of bone metabolism. ANP and CNP decreased the number of ROS17/2.8 and HOS cells after 48 hour treatment. Also, ANP and CNP decreased the cell viability of osteoblastic cells. In contrast, ANP and CNP increased the alkaline phosphatase activity of ROS17/2.8 and HOS cells. ANP did not affect the NBT reduction and nitrite production of osteoblastic cells. ROS17/2.8 and HOS cells produce and secrete gelatinase into culture medium, and this enzyme was thought to be the gelatinase A type according to the molecular weight determination. The gelatinase activity was not changed significantly by ANP and CNP treatment. After culture of mouse bone marrow cells with Vit D3 in the presence or absence of CNP, TRAP activity was increased dose-dependently by CNP. Mouse bone marrow cells were cultured on the OAAS plate (OCT Inc. Korea) with Vit D3 for 8 days and total resorption sites were captured by microscope and CCD camera. Resorption pits at total well surface were measured by image analysing program. Total numbers of pit were significantly increased by CNP treatment. Also, total resorption area and resorption area/total area were significantly increased by CNP. However, average resorption area (resorption area per pit) was not changed by CNP treatment. These results indicate that natriuretic peptides decrease the proliferation of osteoblast, while increase the activity of osteoblasts. Also, natriuretic peptides increase the osteoclastic bone resorptive activity. In conclusion, although the precise mechanism of natriuretic peptides remain to be elucidated, natriuretic peptides seem to be one of the important regulators of bone metabolism.

SU218

C-terminal Parathyroid Hormone-related Protein (107-139) Increases Osteoprotegerin Expression in Human Osteoblastic Cells. I. del Valle,*[1] A. Cortázar,*[2] P. Martínez,*[1] C. Guillén,*[2] M. Martínez,[3] P. Esbrit.[2] [1]Research Unit, La Paz Hospital, Madrid, Spain, [2]Bone and Mineral Metabolism Lab, Fundación Jiménez Díaz, Madrid, Spain, [3]Biochemistry Division, La Paz Hospital, Madrid, Spain.

Parathyroid hormone(PTH)-related protein (PTHrP) is present in bone, but its specific role in skeletal metabolism is ill-defined. The N-terminal region of PTHrP indirectly stimulates bone resorption by interacting with the common PTH/PTHrP type 1 receptor in osteoblasts. However, C-terminal PTHrP (107-139) appears to inhibit bone turnover by directly affecting both osteoblastic and osteoclastic function. Osteoprotegerin (OPG) is a decoy receptor for the osteoclast differentiation factor produced by osteoblasts, and it inhibits osteoclast formation. In the present study, we investigated the mechanism by which C-terminal PTHrP (107-139) might affect OPG expression in human osteoblastic cells. We also analyzed the interaction of this PTHrP domain with 1,25(OH)2D3 and TGF β1 in modulating OPG expression in these cells.Osteoblastic cells were isolated from human trabecular bone from subjects undergoing knee arthroplastia. These cells and human osteoblastic osteosarcoma cells MG-63 were grown in DMEM with 10-15 % fetal bovine serum. Confluent cells were serum-depleted before stimulation with the agonists followed by total RNA isolation. OPG and GAPDH (constitutive control) mRNA levels were analyzed by semiquantitaive RT-PCR using specific primers. We found that 10 nM PTHrP (107-139) maximally stimulated (2-fold over control) OPG expression within 2-6 h in both cell types. The effect of this peptide on OPG mRNA was similar to that induced by PTHrP (107-111), and it was dose dependent (10 fM -100 nM) in these cells. In contrast, PTHrP (1-36) induced an inconsistent effect on OPG mRNA, although generally inhibitory with 1-100 nM of this agonist, in both cell types. In addition, the effect of C-terminal PTHrP on OPG mRNA was inhibited by either 25 nM bisindolylmaleimide I or 100 nM calphostin, two protein kinase (PK) C inhibitors, but not by RpcAMPS, a PKA inhibitor, in these cells. OPG mRNA stimulation by this C-terminal domain of PTHrP was similar to, and not synergistic with, that induced by either 1,25(OH)2D3 (0.1-10 nM) or TGF β1 (0.5-5 ng/ml) in human osteoblastic cells.Our results indicate that N- and C-terminal PTHrP fragments interact in a different manner with OPG in human osteoblastic cells. The stimulation of OPG expression by C-terminal PTHrP in these cells provides further support to the putative role of this PTHrP domain as a bone resorption inhibitor.

SU219

Expression of Osteoclast Differentiating Factor (ODF, RANKL) and Osteoprotegerin (OPG) in Human Bone: Role of the Bone Remodeling Compartment in the Coupling Between Resorption and Formation. M. I. Holt,* A. Baattrup,* E. F. Eriksen.* University Department of Endocrinology, Aarhus Amtssygehus, Aarhus C., Denmark.

Bone resorption and formation sites are covered by a monolayer of cells forming a Bone Remodelling Compartment (BRC). This monolayer is alkaline phosphatase positive and expresses positive immunoreactivity for osteocalcin, osteonectin, insulin like growth factors 1,2; transforming growth factor alfa, and interleukin-1, displaying a protein expression pattern similar to that of neighbouring lining cells. Osteoclast formation depends on cell-cell contact between cells of the mononuclear lineage and osteoblastic cells secreting factors like macrophage colony stimulating factor (m-CSF) and osteoclast differentiating factor (ODF, RANKL). Due to the spatial and temporal separation of osteoclasts and osteoblasts during bone remodelling, other cells of osteoblast origin must be involved in this interaction. The lining cells covering the BRC are prime candidates for this function. Using immunohistochemical analysis of methacrylate embedded human bone we therefore investigated, whether these cells express ODF and OPG, and looked for coexpression with markers of osteoblast phenotype (Bone Morphogenetic Protein 2,7 (BMP 2,7) and Transforming Growth Factor-beta 1,2,3 (TGFb 1,2,3)).Positive staining for ODF was demonstrable in the outer layer of bone remodeling compartments (BRC's), lining cells, the osteoblast layer on bone forming surfaces, and osteocytes. Furthermore osteoclasts displayed strong immunoreactivity for ODF. No positive staining was detectable in mineralized bone matrix or osteoid. OPG also displayed positive staining in the outer layer of BRC's, lining cells, the osteoblast layer and osteocytes. However, contrary to what was seen for ODF, strong immunoreactivity was also detectable in mineralized bone matrix. TGFb1 displayed strong immunoreactivity in the outer layer of BRC's, the lining cell layer as well as the osteoblast layer, while TGFb2 and TGFb3 only exhibited positive staining in the outer layer of BRC's and lining cells. BMP2 and BMP7 also revealed strong immunoreactivity in the outer layer of the BRC, while the osteoblast layer revealed scattered positive stainingIn conclusion, lining cells as well as the outer layer of the BRC express both ODF, OPG, BMP 2,7 and TGFb 1,2,3, linking these osteoblastic cells to osteoclast differentiation through cell-cell contact. Furthermore, OPG is incorporated in bone matrix, making it plausible, that this inhibitor of osteoclast formation is involved in the feed back regulation of osteoclast activity during bone resorption.

SU220

Effect of Bisphosphonates on the Expression of OPG and RANKL in Mouse Calvarial Osteoblastic Cells. Y. H. Kim,* S. N. Kim,* G. S. Kim, J. H. Baek. Dept. of Pharmacology and Dental Therapeutics, College of Dentistry, Seoul National University, Seoul, Republic of Korea.

The mechanisms of inhibition of osteoclastic differentiation by bisphosphonates are not fully elucidated. Recently, factors that play important roles in controlling osteoclast differentiation have been identified; OPG is a potent inhibitor and RANKL is essential for osteoclast differentiation. To investigate whether bisphosphonates inhibit bone resorption through regulation of OPG-RANKL system, we performed semiquantitative RT-PCR of OPG and RANKL in mouse calvarial osteoblastic cells exposed for 48h to the following agents; alendronate (10^{-8} - 10^{-5} M), or pamidronate (10^{-8} - 10^{-5} M) alone, or combined with 1,25-$(OH)_2VitD_3$ (10^{-8} M)/dexamethasone (10^{-7} M). Alendronate alone decreased RANKL expression at low concentration but did not change OPG expression. Pamidronate did not change OPG and RANKL expression significantly. When bisphosphonates were exposed in the presence of 1,25-$(OH)_2VitD_3$/dexamethasone, alendronate recovered OPG expression reduced by 1,25-$(OH)_2VitD_3$/dexamethasone to the control level and decreased RANKL induced by 1,25-$(OH)_2$Vit D_3/dexamethasone slightly, while pamidronate decreased RANKL expression induced by 1,25-$(OH)_2VitD_3$/dexamethasone. Next, to confirm the inhibitory effect of bisphosphonates on the osteoclast formation, mouse bone marrow cells were cocultured with mouse calvarial osteoblastic cells in the presence of 1,25-$(OH)_2VitD_3$ (10^{-8} M) and alendronate (10^{-5} M) or pamidronate (10^{-5} M). After 6 days of coculture, multinucleated osteoclast-like cells were identified by TRAP staining and the number of osteoclastic cells were counted, and the total RNAs from coculture were subjected to semiquantitative RT-PCR. In this coculture, both alendronate and pamidronate decreased the osteoclast formation, but the expression of OPG and RANKL was not changed significantly. In this study, although alendronate or pamidronate increased OPG expression and decreased RANKL expression, the expression patterns of OPG and RANKL showed some discrepancies between experimental groups and we did not observe consistency between the change of the expression level of OPG and RANKL and the inhibitory effect of alendronate and pamidronate on the osteoclast formation. Based on these results, it could be suggested that OPG-RANKL system might not be the main target of bisphosphonates to inhibit bone resorption.

SU221

Bisphosphonates Differentially Regulate Gene Expression and Protein Secretion of the Anti-Resorptive Decoy Receptor Osteoprotegerin by Primary Human Osteoblasts. V. Viereck,[1] G. Emons,*[1] K. Froesch,*[1] L. C. Hofbauer.[2] [1]University of Goettingen, Goettingen, Germany, [2]University of Marburg, Marburg, Germany.

Bisphosphonates (BP) represent one of the most potent anti-resorptive drugs with potential benefits in the management of benign and malignant metabolic bone diseases characterized by enhanced bone resorption and bone loss. Despite their frequent use, the molecular mechanisms of BP on bone cells have largely remained unclear. Receptor activator of nuclear factor-kappaB ligand (RANKL) is the essential factor for osteoclast formation and activation, and enhances bone resorption. By contrast, osteoprotegerin (OPG) which is produced by osteoblastic lineage cells acts as a decoy receptor that neutralizes RANKL, and prevents bone loss. Various drugs commonly used in metabolic bone diseases have been demonstrated to modulate osteoblastic production of either RANKL, or OPG, or both. In this study, we assessed the effects of the BP pamidronate (PAM) and zoledronate (ZOL) on OPG mRNA steady state levels (by semiquantitative RT-PCR and Northern analysis) and protein production (by ELISA) in primary human trabecular osteoblasts (hOB) obtained from healthy donors. PAM increased OPG mRNA levels and protein secretion by hOB by up to two-fold in a dose-dependent fashion with a maximum effect at 10-6 M ($P < 0.0001$ by ANOVA) after 72 h. Similarly, ZOL enhanced OPG gene expression and protein secretion by hOB by two-fold in a dose-dependent fashion with a maximum effect at 10-8 M ($P < 0.0001$ by ANOVA) after 72 h, consistent with the higher biological potency of ZOL. Time course experiments indicated a stimulatory effect of PAM and ZOL on osteoblastic OPG protein secretion by 5.8- and 6.3-fold, respectively ($P < 0.0001$ by ANOVA). Both BP decreased OPG gene expression and protein production at a concentration of 10-4 M by 20 to 80% which, in part, was due to a cytotoxic effect. Analysis of cellular markers of osteoblastic differentiation revealed that PAM and ZOL induced a 2- to 3-fold induction of alkaline phosphatase mRNA levels and a 2- to 3-fold increase of type I collagen protein secretion. In conclusion, our data suggest that bisphosphonates are capable of modulating the production of OPG by human osteoblasts, identifying OPG as a potential osteoblastic BP target gene. Since, OPG production is a function of osteoblastic cell maturation, enhancement of OPG by BP may, at least in part, be related to the stimulatory effects of BP on osteoblastic differentiation.

SU222

Inhibition of Bone Formation by Extracellular ATP and UTP at Physiologic Levels: Nucleotide Effects on Bone Formation and Resorption Involve Different P2 Receptor Subtypes. A. Hoebertz, S. Mahendran,* G. Burnstock,* T. Arnett. Department of Anatomy and Developmental Biology, University College London, London, United Kingdom.

Extracellular nucleotides have been shown to act on bone cells via multiple P2 receptors, a receptor family divided into ionotropic P2X receptors and metabotropic P2Y receptors. The naturally occurring ligand ATP is a potent agonist at all receptor subtypes, whereas ADP (the first degradation product of ATP) and UTP act only at specific receptor subtypes. We recently reported that extracellular ADP, at nanomolar concentrations (FASEB J 15: 1139-1148, 2001), is a potent osteolytic agent, an effect likely to be mediated via the ADP-selective P2Y1 receptor subtype present on both osteoclasts and osteoblasts. In the present study we investigated the actions of extracellular nucleotides on osteoblastic function in cultures of primary rat calvarial osteoblasts maintained for 16-21 days in DMEM supplemented with ascorbic acid, B-glycerophosphate and dexamethasone. In contrast to their powerful effects on osteoclastic bone resorption, ADP and the selective P2Y1 agonist 2-methylthioADP were without effect on bone nodule formation at concentrations between 1 and 125 uM. However, both UTP, a P2Y2 and P2Y4 receptor agonist, and ATP strongly inhibited bone nodule formation at >=1 uM and >=10 uM, respectively, concentrations that reflect physiologic levels of nucleotides. Using in situ hybridization, we have shown that rat osteoclasts and rat osteoblastic cells express P2Y1 and P2Y2 receptors, respectively; however, in contrast to other studies we found no evidence for expression of the P2Y4 receptor. Interestingly, ADP and ATP at 0.2 to 2 uM also stimulated osteoclast formation in 10 day, stromal cell-free mouse marrow cultures in MEM supplemented with RANKL and M-CSF. Thus, the low-dose effects of extracellular nucleotides on bone resorption and formation appear to be mediated via different P2 receptor subtypes: UTP (which does not affect osteoclast function), signalling through the P2Y2 receptor, is a potent inhibitor of bone formation by osteoblasts, whereas ADP, signalling via the P2Y1 receptor is a major stimulator of osteoclast formation and activity. In conclusion, our results show that extracellular nucleotides, at physiologic levels, appear to play an important role as negative modulators of bone homeostasis.

SU223

Glucose Enhances Proton Efflux from Osteoblastic Cells and Lowers Extracellular pH: Possible Role in Diabetic Osteopenia. A. Santhanagopal, S. J. Dixon. Department of Physiology and Division of Oral Biology, The University of Western Ontario, London, ON, Canada.

Diabetes and hyperglycemia are associated with impaired bone formation and increased osteoclastic resorption, which can lead to osteopenia. Decreased extracellular pH (pHo) suppresses the formation and mineralization of bone. In addition to these effects on osteoblast activity, extracellular acidification markedly enhances the resorptive activity of osteoclasts. We hypothesized that hyperglycemia causes a decrease in the pHo of the local bone microenvironment by acting on cells of the osteoblast lineage. Thus, we used a Cytosensor microphysiometer to study the effects of glucose on proton efflux from osteoblastic cells and the resulting changes in pHo beneath the cell layer. UMR-106 cells and first passage rat calvarial cells were seeded on porous polycarbonate membranes. Solid-state sensors beneath the membranes were used to monitor pHo and proton efflux from cells. In subconfluent cultures, glucose acutely increased proton efflux in a concentration-dependent manner to maximum levels 3-4 fold above basal rates of 80-90 pmol proton equivalents/minute/sample. The dependence of proton efflux on glucose concentration displayed Michaelis-Menten kinetics with a *K*m of ~1.5 mM glucose. Moreover, within minutes, glucose decreased pHo in the compartment immediately beneath confluent UMR-106 cells by up to 0.18 ± 0.01 pH units, a change shown previously to be sufficient to activate osteoclastic resorption. Lactate efflux from UMR-106 cells was also dependent on extracellular glucose. In the presence of glucose, inhibitors of glycolysis (fluoride and iodoacetate), but not blockers of oxidative phosphorylation (azide and antimycin), markedly suppressed efflux of protons and lactate. In the absence of glucose, fluoride did not significantly alter proton efflux. Taken together, these findings indicate that glycolysis contributes predominantly to glucose-dependent proton efflux from osteoblastic cells. We conclude that glucose regulates proton efflux from osteoblastic cells, controlling local pHo in the extracellular compartment beneath the cell layer. In vivo, hyperglycemia may increase proton efflux from osteoblastic cells. The resulting decrease in pHo would suppress mineralization and stimulate osteoclastic resorption, thus contributing in two ways to the development of osteopenia in poorly controlled diabetes.

SU224

Normal Osteoclastogenesis Requires Connexin43 in both Stromal/ Osteoblast and Hematopoietic Compartments. F. Furlan,[1] J. Screen,*[1] Y. Abu-Amer,[2] L. Weitzmann,[1] F. Lecanda,[3] R. Civitelli.[1] [1]Bone and Mineral Diseases, Washington University, St Louis, MO, USA, [2]Orthopedic Surgery, Washington University, St Louis, MO, USA, [3]Pathology, University of Navarra, Pamplona, Spain.

We have demonstrated that osteoblasts are highly coupled by gap junctions formed primarily by connexin43 (Cx43), and that genetic deficiency of Cx43 leads to defects of skeletal development and dysfunctional osteoblasts. Since Cx43 is also present in cells of the monocyte-macrophage lineage and in osteoclasts, we have examined its functional role for osteoclast differentiation. Osteoblast enriched calvaria (clv) cells and, as a source of osteoclast precursors, spleen monocytes (mono) were isolated from Cx43-null and wild type (wt) newborn mice. Co-cultures of clv of different Cx43 genotypes with wt mono yielded TRAP-positive multinucleated cells in all co-cultures, although osteoclast number was significantly lower in co-cultures with Cx43-null than either heterozygous or wt clv. In this system, mono Cx43 gene dosage was uninfluential, as Cx43-null and heterozygous mono were able to undergo osteoclast differentiation as well as wt mono, when supported by wt clv. However, when osteoclast differentiation was induced in mono of different Cx43 genotypes by exposure to M-CSF and RANKL, significantly reduced formation of TRAP-positive cells was obtained in heterozygous mono, and Cx43-null mono were unable to undergo differentiation. Analysis of spleen cell content by flow cytometry did not reveal quantitative cellular defects in Cx43-null spleen cell preparations. When Cx43-null and wt clv were cultured in the same tissue culture dish but kept physically separated, TRAP-positive multinucleated cells formed from wt mono in contact with clv of either genotypes, although their number was lower in the areas populated by Cx43-null clv cells. Thus, wt clv can partially rescue the defective osteoclastogenic support of Cx43-null clv presumably via soluble factors. We also found that Cx43 deficient clv cells expressed lower RANKL and higher OPG mRNA relative to wt cells, whereas M-CSF abundance was very similar in different Cx43 genotypes. In summary, osteoblast support of osteoclastogenesis requires Cx43 in both osteoblast/stromal and hematopoietic compartments. Although loss of Cx43 leads to a reduced RANKL/OPG ratio, clv cells provide additional stimulatory mechanisms or survival factors that support monocyte differentiation in conditions of Cx43 deficiency. In conclusion, Cx43 is important in osteoclastogenesis.

SU225

The NP/NMP4/CIZ Transcription Factors Localize to the Osteoblast Nuclear Matrix, Mitochondria, and Cytoplasm. K. Torrungruang,*[1] J. Hock,[2] J. P. Bidwell.[2] [1]Periodontics, Indiana University School of Dentistry, Indianapolis, IN, USA, [2]Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA.

NP/NMP4/CIZ proteins are a family of transcription factors that reside in the nucleus and the cytoplasm. These proteins contribute to transcriptional activity of the type I collagen alpha1(I) polypeptide chain (COL1A1) and numerous matrix metalloproteinase genes (MMP). The NP/NMP4/CIZ proteins contain from five to eight Cys2His2 zinc fingers and a class II SH3 binding domain. Overlapping this latter domain is a putative AT-hook motif, which distinguishes the HMG-I(Y) subfamily of architectural transcription factors. As a first step toward determining the functional relationship between the cytoplasmic and nuclear NP/NMP4/CIZ compartments, we mapped their location in the osteoblast cytoplasm. Immunocytochemical analysis of primary osteoblasts and osteoblast-like cell lines demonstrated that NP/NMP4/CIZ co-localize with hsp60, a protein of the mitochondrial matrix, and with golgi protein 58K. Immunocytochemical analysis revealed no co-localization with vinculin, a component of the focal adhesions. Western analysis showed various NP/NMP4/CIZ isoforms in the osteoblast mitochondrial, cytosolic, and nuclear matrix compartments. This was consistent with multiple transcripts observed from RT-PCR analysis. Electrophoretic mobility analyses indicated that mitochondrial and cytoplasmic isoforms of NP/NMP4/CIZ do not bind to the COL1A1/MMP DNA consensus sequence recognized by some of the nuclear isoforms. From the present data, and previous studies, we hypothesize that NP/NMP4/CIZ are a family of "sorting isoforms", proteins derived from a single gene that mediate similar functions in different subcellular compartments.

SU226

Osteoblast and Osteoclast Activity in GATA-1 Deficient Mice. M. A. Kacena,[1] K. Wilson,*[1] Y. Xi,[1] C. M. Gundberg,[1] J. A. Lorenzo,[2] M. C. Horowitz.[1] [1]Yale University School of Medicine, New Haven, CT, USA, [2]University of Connecticut Health Center, Farmington, CT, USA.

The close juxtaposition of osteogenic, hematopoietic, and immune cells makes the bone

marrow the focus for many of the regulatory interactions required for the homeostatic development of bone. Mice deficient in the GATA-1 transcription factor experience a developmental block in megakaryocyte differentiation resulting in a phenotype characterized by greatly increased numbers of megakaryocytes in the spleen and bone marrow (10-100 fold increase), a drastic reduction of mature platelets (15% of control level), and increased bone mass (4 fold increase).To further understand the developmental progression of this bone phenotype we examined 6 week, 5 month, and 9 month-old male and female mice by histomorphometry and biochemistry. No differences were observed in 6 week-old mice compared to controls. However, 5 and 9 month-old animals had increased bone mass and increased serum osteocalcin levels, while urine cross-links levels varied dependent on sex. Increases in bone mass was observed in long bones and sternum, but not vertebrae or calvariae. Both 5 and 9 month-old GATA-1 deficient mice had increased osteoblast numbers and bone formation parameters while osteoclast and resorption parameters remained unaltered.To explore the potential mechanism(s) responsible for the increased bone mass in these animals, osteoblast and osteoclast cultures were examined in vitro. Osteoblasts were isolated from the calvaria of neonatal GATA-1 deficient (-/-) and C57BL/6 control (+/+) mice. There were no significant differences in proliferation, alkaline phosphatase expression, or osteocalcin secretion between the +/+ and -/- osteoblast cultures. Osteoclast-like cells were generated from co-cultures containing either +/+ or -/- osteoblasts cultured with either +/+ or -/- spleen cells in mix and match experiments or by culturing the spleen cells with M-CSF and RANKL. In all cases the number of osteoclasts generated from -/- spleen cell cultures was not different from +/+ cultures (defined as TRAP positive cells with >3 nuclei). The osteoclast-like cells resorbed bone equally well as assessed by pit resorption assays.In summary, -/- and +/+ osteoclast number and function are equivalent in vitro and in vivo indicating the increased bone mass in GATA-1 deficient mice (-/-) was not due to an osteoclast defect. Osteoblast function was normal in vitro, however, osteoblast number and bone formation parameters were significantly elevated in vivo. These observations suggest an interaction between megakaryocytes and osteoblasts in vivo which accounts for the increased bone mass.

SU227

Identification of Genes Specifically Regulated During Osteogenic Differentiation of Human Osteoprogenitor Cells by Microarray Analysis. F. Bassilana,* K. Seuwen,* H. J. Keller.* Bone Metabolism Research, Novartis Pharma AG, Basel, Switzerland.

Primary osteoprogenitor cells can be isolated from human trabecular bone and, upon specific stimuli, can be differentiated along distinct mesenchymal lineages such as osteoblasts, chondrocytes or adipocytes. To identify new genes linked to osteoblast differentiation, we have studied differential gene expression using high-density cDNA microarrays. Total RNA was isolated from control, early (4 days) and late (21 days) human trabecular bone cell cultures undergoing osteogenic differentiation, which was induced by 100 nM dexamethasone, 50 uM ascorbic acid and 10 mM b-glycerophosphate. Parallel expression profiling of almost 10'000 genes and ESTs was performed using UniGEM V cDNA microarrays from Incyte. In early differentiated cells, 16 genes were up- and 6 downregulated with > 2-fold difference compared to control cells. As expected, many more genes (195) were > 2-fold upregulated in 21 day cultures, representing mineralizing osteoblasts. Even more genes (496) were downregulated, when comparing to undifferentiated precursor cells at day 0. To assess the significance and specificity of differential expression of these genes during osteoblast differentiation, we have determined their tissue distribution by microarray analysis using RNA from brain, heart, kidney, liver, lung, trabecular bone, trachea, giant cell tumor, chondrocytes, MG63 osteosarcoma and skin fibroblasts. Microarray expression data from the different cells and tissues were normalized based on mean array fluorescence and were then entered into a Microsoft Access-based database also containing expression data from osteoblast differentiation. The database allows searches for "virtual" tissue expression of genes using search parameters such as gene accession number, description or sequence. Using the database, a query was established to select among the many regulated genes observed in late (21 days) differentiated osteoblasts only those with specific or preferential expression in the osteoblast lineage. By this way, we have identified 27 up- and 31 downregulated genes. Among the upregulated genes were known differentiation markers like IGF-II. In addition, we found several sequences that have not yet been associated with osteoblast differentiation. Differential expression was confirmed by ribonuclease protection assay for subsets of genes. In summary, we have identified several new genes that are preferentially expressed and regulated during osteoblast differentiation in vitro. These genes may play a role in osteoblast differentiation and bone formation.

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SU228

Characterization of Senescence-related Genes in MC3T3-E1 Pre-osteoblastic Cells Identified by Microarray Analysis. W. Huang, B. Carlsen, G. Rudkin,* N. Shah,* K. Ishida,* D. T. Yamaguchi, T. A. Miller.* VA Greater LA Healthecare System, Los Angeles, CA, USA.

The osteoblastic function of mouse pre-osteoblastic MC3T3-E1 cells, as measured by alkaline phosphatase activity and osteocalcin secretion, decreases during senescence resulting from serial passage. In order to uncover genes responsible for decreased osteoblastic function in senescent cells, we have studied passage-dependent change of gene expression in MC3T3-E1 cells. Changes in the expression pattern of 2,000 selected genes were examined simultaneously by comparing mRNA levels between MC3T3-E1 cells at passage 20 and passage 60 using the cDNA microarray analysis. Significant changes in the steady-state abundance of 27 mRNAs were observed in response to different passage numbers, including 17 known genes, 4 ESTs with homology to known genes, and 6 genes with no previously described function or homology. Northern blot analysis was used to verify and quantify the expression of selected genes, and revealed a significant higher level of up- and down-regulation as compared to microarray data. Among known genes, osteopontin (OPN) mRNA expression was up-regulated by as much as 15-fold in high passage cells. We found that the increase of OPN expression occurs gradually with the increase of passage numbers. In accordance with its mRNA expression, OPN protein level is highly elevated in high passage cells as revealed by immuno-blot on whole cell extracts. Treatment of high passage cells with OPN neutralizing antibodies stimulated proliferation of osteoblastic cells as measured by daily cell counts. Significance of OPN over-expression in in vitro aging of osteoblastic cells is currently under investigation. Effort is also being made to determine promoter elements responsible for OPN up-regulation. In summary, our results indicate the existence of a significant change in gene expression in osteoblastic cells undergoing in vitro aging. Such changes might be responsible for a reduction in bone regeneration in older osteoblasts.

SU229

The Bone Related Runx-2/Cbfa-1 Promoter Mediates Restricted Expression in Mesenchymal Condensations and Chondrocytes. C. J. Lengner,[1] H. Drissi,[1] J. Choi,[2] A. J. van Wijnen,[1] J. L. Stein,*[1] G. S. Stein,[1] J. B. Lian.[1] [1]Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA, [2]Medical Research Institute, Kyungpook National University Hospital, Republic of Korea.

The Runx-2/Cbfa-1 transcription factor is required for skeletal development and maturation of hypertrophic chondrocytes and osteoblasts. The gene is expressed as early as 9.5 days post coitum (dpc) during embryogenesis in a tissue-restricted manner. We have previously characterized several elements within 3 kb of the promoter that controls expression of the bone related Cbfa-1/til-1 (Type II/p57) isoform of Runx-2 in vitro. In these studies we addressed the spatio-temporal regulatory activity of the 3 kb promoter in vivo by generating transgenic mice in which this promoter drives expression of the lac Z reporter gene (3 kb-lac Z). The transgene construct was determined to be transcriptionally active in a variety of osseous and non-osseous cell lines in vitro. We selected three founder mice that highly expressed the transgene and bred them to form distinct transgenic lines. Analysis of the 3kb-lac Z transgenic mice after whole mount staining with X-gal reveals that promoter activity is localized to caudal somites as early as 8.5 dpc. Cryosectioning of embryos revealed that promoter activity progresses in a caudal to cranial manner in developing somites up to 13.5 dpc. At 13.5 dpc, when mesenchyme differentiates into pre-cartilage, expression of the transgene was observed only in chondrocyte condensations and early perichondrium. The intensity of X-gal staining in the cartilaginous region of the rib and its gradual decline towards the costochondral junction reflects expression of the 3 kb promoter in early differentiating chondrocytes. Chondrocyte-specific expression continues even after formation of bone (15.5-17.5 dpc), but no activity of the 3 kb promoter was observed in osteogenic lineage cells. Interestingly, the postnatal animals show X-gal staining only in the seminiferous tubules of the testes, where high levels of B-galactosidase expression are detected. Our findings indicate that the 3 kb promoter of the bone related isoform of Runx-2 is insufficient to support expression in osseous tissue, but does mediate expression in early mesenchymal condensations and the developing cartilage.

SU230

BMP Activity Is Required for Both Selective Growth and Differentiation of Osteoblasts to a Mineralized Matrix Through Differential BMP Receptor Utilization. S. E. Harris, M. A. Harris, D. Chen, G. R. Mundy. U of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

It is well established that BMPs, BMP-2, -4 , and -7 can accelarate osteoblast precursors to differentiate into a mineralized matrix both in vitro and in vivo. This phenomena requires an initial growth response or multilayering process that primarily functions through BMP receptor 1A. Dominant negative BMPR-1A transfected into osteoblasts retards growth of osteoblast precursors while dominant negative BMPR-1B constructs have little effect on growth; however dominant negative BMPR-1B completely blocks mineralized matrix formation. We hypothesize that BMP signaling through BMPR-1A instructs osteoblast to stay in a growth state while BMP signaling through the BMPR-1B instructs osteoblasts to decrease growth and begin the differentiation process. Endogenous BMP activity drives growth and differentiation in osteoblasts and added BMP 2 can accelerate both. To test this hypothesis we utilized 2T3 mouse osteoblasts in which BMP 2 accelerates both growth and differentiation over a 20 day period. Initial observations demonstrated that the ratio of BMPR-1A to BMPR-1B decreased as the osteoblasts slowed their growth rate and differentiated to the mineralizing phase. BMP 2 treatment accelerated this change in the BMPR-1A to BMPR1B ratio. 2T3 osteoblasts are also capable of forming a mineralized matrix, given sufficient time, in the absence of any added BMP ligand. Endogenous BMP activity was blocked by noggin and both growth and multilayering as well as differentiation to mineralized nodules was impaired. Addition of noggin at 0.5 ug/ml, in the absence of added BMPs, completely blocked the initial multilayering process and growth and stimulated a morphological change to a more dendritic state. As well, noggin blocked mineralized matrix formation at 15 days. Using gene expression profiling with microarrays, we analyzed a subset of genes whose expression was changed by either BMP 2 or noggin, in order to help define the role of endogenous BMP activity on osteoblast growth and differentiation and selective BMP receptor utilization. Many of the positive early growth response genes related to initial BMP action were decreased in noggin-treated osteoblasts, as well as a variety of other informative genes. These data demonstrate the necessary role of BMP activity in bone formation.

SU231

The Transcription Function of Dlx5 Is Regulated by a RING-finger Protein Praja-1 Through Ubiquitin-Dependent Degradation of Dlxin-1, a Dlx/Msx-Interacting Protein. A. Sasaki,* K. Ikeda, K. Watanabe. Dept of Geriatric Res, Natl Inst for Longevity Sci, Obu, Japan.

Msx2 and Dlx5 are homeodomain proteins which play an important role in osteoblast differentiation and whose expression is induced by BMP. Recently we have identified a novel protein, Dlxin-1, that associates with these homeodomain proteins and regulates Dlx5-dependent transcriptional function (J Biol Chem 2001). The expression of Dlxin-1 was detected in bone tissue including osteoblasts, suggesting that Dlxin-1 may contribute osteoblast differentiation through the regulation of not only Dlx5 but also Msx2. In order to

elucidate the molecular function of Dlxin-1, we employed yeast two-hybrid screening using the C-terminal necdin homology domain of Dlxin-1 as bait. Two closely related RING-finger proteins, praja-1 and mouse orthologue of rat Neurodap-1, were isolated as positive clones from mouse embryo cDNA library. Since proteins with RING-finger have been shown to bear E3 ubiquitin ligase activity, we hypothesized that praja-1 may act on Dlxin-1 as E3 enzyme to regulate its function through ubiquitination. GST pull-down and immunoprecipitation/western blotting assays following co-transfection of Dlxin-1 and praja-1 revealed that praja-1 binds to the C-terminal necdin-homology domain of Dlxin-1 in vitro and in vivo. Overexpression of praja-1 caused a decrease in Dlxin-1 protein level, which was reversed when a proteasome inhibitor was added. Overexpression of a praja-1 molecule with a mutation in the RING finger inhibited the decrease in Dlxin-1 protein, pointing to the importance of E3 activity associated with RING finger. Finally, expression of praja-1 downregulated Dlx5-dependent transcriptional activity in a GAL4-dependent assay. These results suggest that praja-1 regulates the transcription function of the homeodomain protein Dlx5 by controlling the stability of Dlxin-1 via a ubiquitin-dependent degradation pathway.

SU232

Identification and Differential Expression of TGF-β1, BMP-2 and Activin A Responsive Genes in Pre-osteoblastic Cells. D. S. de Jong,*[1] S. Bauerschmidt,*[2] E. J. J. van Zoelen,*[1] W. Olijve,*[1] W. T. Steegenga.*[1] [1]Cell Biology FNWI, University of Nijmegen, Nijmegen, The Netherlands, [2]Target Discovery Unit, N.V. Organon, Oss, The Netherlands.

Bone formation and remodeling are processes in which several members of the TGF-β family play various roles. BMP-2 treatment of pre-myeloid C2C12 cells causes the differentiation of these cells to osteoblastic cells, whereas TGF-β1 and activin A can not induce this differentiation process. Moreover, TGF-β1 is able to inhibit BMP-2-induced osteoblast differentiation in C2C12 cells, while no significant effect of activin A on this process was found. To identify BMP-2 responsive genes during early osteoblast differentiation, gene expression microarray experiments were performed. C2C12 cells were treated for 2 hr with BMP-2 and the expression patterns were analyzed. To determine the specificity of these genes for BMP-2, the expression patterns were compared to those of TGF-β1 or activin A treated cells. Using this approach, we have characterized 57 transcripts displaying significant expression by BMP-2, TGF-β1 and/or activin A. This set of regulated transcripts includes 31 ESTs and 26 genes of which 15 genes appear to be novel TGF-β family member target genes. Of the 26 genes, 14 have previously been associated with bone remodeling, supporting the idea that the novel target genes and the ESTs could be important modulators of bone differentiation. The 26 regulated genes included transcription factors, a neurohormone, a phosphate transporter, receptors, growth factors and a sulfotransferase. The 57 transcripts identified here could be categorized in BMP-2 specific (4) and TGF-β1 specific (6) genes, genes regulated by both BMP-2 and TGF-β1 or by all three growth factors (43) and genes inversely regulated by BMP-2 and TGF-β1 (4). The qualitative expression profiles of 2 inhibited and 6 stimulated transcripts were confirmed by Norhtern analysis or real time quantitative RT-PCR. Moreover, these latter two methods exhibited a profound quantitative difference in expression regulation (stronger) than was measured in the microarray analysis experiments.In summary, we used gene expression microarrays to identify 15 novel TGF-β family member target genes, of which 8 had never before been related to osteoblast differentiation. The potential role of these and the other genes in osteoblast differentiation is under current investigation.

Disclosures: University of Nijmegen,3; Netherlands Institute for Earth and Live Sciences (NWO-ALW),2; Genetics Institute (Cambridge, MA),5; N.V. Organon (Oss, The Netherlands),5.

SU233

Isolation and Characterization of Subpopulations of Cells Within the Osteoblast Lineage. I. Kalajzic,* A. C. Lichtler, D. W. Rowe. Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA.

To study osteoblast lineage differentiation our laboratory had previously developed a visible marker (GFP) under control of the Col1a1 promoter fragments, pOBCol3.6 and pOBCol2.3 (Kalajzic et al. JBMR 14, S165). A differential activation of these promoters was observed during osteoblast lineage progression in primary bone cell cultures. The pattern of GFP positive cells within bone was distinctly different. We considered that pOBCol3.6GFP marks a population of preosteoblastic cells and osteoblastic cells while pOBCol2.3 is active in mature stages of osteoblast differentiation. The aim of this study was to isolate and further characterize these subpopulations of cells.Freshly isolated calvarial cells were derived from 5-8 day old mice by a standard trypsin-collagenase digestion and analyzed by flow cytometry. The pOBCol3.6 transgene was expressed by 40-50% of total cell digest while pOBCol2.3 driven expression was observed in 15-20% of cells. Cultures initiated from a total cell digest were examined at days 7 and 15 of culture. The 3.6 promoter was active in 40% of the cells at both time points, while the 2.3 promoter showed activity only in 1-2% of cells by day 7 with an increase to 15% at day 15 and was localized to mineralizing nodules.Freshly isolated calvarial cells from pOBCol3.6 and pOBCol2.3 transgenic mice were separated into GFP+ and GFP- populations using fluorescent activated cell sorting. The purity of cell sorts was tested by FACS analysis and by GFP mRNA expression. Northern blot analysis showed that the 3.6 GFP positive population had 5 fold stronger Col1a1, BSP and OC expression than the 3.6 GFP negative population. When primary cells were grown under differentiating conditions and sorted, pOBCol3.6GFP positive cells showed enrichment in bone markers. These result correlate with the histological observation of inactivity of pOBCol3.6 expression in a number of osteocytes. The pOBCol2.3 positive cells were Col1a1, BSP and OC active while the GFP negative population expressed almost the same levels of Col1a1 with undetectable BSP and OC expression.Once isolated and replated pOBCol3.6GFP positive as well as GFP negative cells develop multilayered ALP positive and mineralized colonies that indicate the presence of an early uncommitted progenitor in the subpopulation of 3.6GFP negative cells.The studies using 2.3 driven transgene in replating experiments are ongoing. Cell activated sorting appears to be an appropriate technique to isolate different subpopulation of cells.

SU234

Primary Cultures of Human Osteoblasts Express a Calcium-Sensing Receptor (CaSR). H. K. Yoon,[1] K. H. W. Lau,[2] C. S. Hwang,*[1] Y. S. Kang,*[1] I. K. Moon,*[1] C. H. Yim,*[1] H. Y. Chung,*[1] K. O. Han,*[1] H. C. Jang,*[1] J. Y. Ahn,*[1] I. K. Han,*[1] Y. K. Choi.*[3] [1]Samsung Cheil Hospital & Women's Healthcare Center, Sungkyunkwan University, Seoul, Republic of Korea, [2]Pettis Mem. VAMC, Loma Linda, CA, USA, [3]Kyung Hee University, Seoul, Republic of Korea.

The CaSR is a G protein-coupled receptor that plays pivotal roles in extracellular Ca homeostasis. Because extracellular Ca is essential for mineralization, a major function of osteoblasts, it is of importance to know whether the CaSR is present in osteoblasts. While several studies reported that osteoblast-like cells express the CaSR, other studies failed to detect CaSR in human osteoblasts. This study sought to determine if primary cultures of normal human osteoblasts indeed express a CaSR. Primary human osteoblasts were derived from vertebra bone chips of 4 adult subjects (1 male and three females). To determine whether primary human osteoblasts express the CaSR, we first performed the immunoblot analysis, under reducing and non-reducing conditions, with a highly specific anti-CaSR monoclonal (ADD) antibody. In each primary osteoblast cell lysates, the ADD monoclonal antibody detected the anticipated CaSR immunoreactive doublet bands of ~175 and 190 kD, respectively, under both reducing and nonreducing conditions. This monoclonal antibody also detected the same doublet bands in parathyroid cell extract, but not in extracts of the HEK-293 cells. We next performed RT-PCR/nested PCR, under stringent conditions, using specific primers corresponding to unique regions of the human parathyroid CaSR cDNA sequence. In each primary human osteoblast, RT-PCR/nested PCR produced an amplified PCR fragment corresponding to the anticipated size of 369 bp. No such PCR product was obtained with RNA derived from HEK-293 cells. DNA sequencing of the PCR product from each primary human osteoblasts showed 100% sequence identity with the human kidney CaSR, but not with the human parathyroid CaSR, suggesting that the CaSR in human osteoblasts may be very similar, if not identical, to that in human kidney cells. We then used the CaSR PCR product as the probe in Northern analysis to identify CaSR mRNA species in each primary human osteoblast. In each case, a strong 5.2 kb transcript, which is similar in size to the major CaSR transcript in human kidney and parathyroid cells, was found. In addition to this 5.2 kb transcript, a weak 2.9 kb transcript was also found. Together, these findings suggest that primary human osteoblasts, under basal culture conditions, express a significant amount of CaSR mRNA. In summary, we conclude that normal human osteoblasts, under basal conditions, do express substantial levels of CaSR, which may play a role in the regulation of Ca homeostasis in osteoblasts.

SU235

CGI-135 Is a Chromatin Remodeling Protein Essential for Osteoblast Development. G. A. Candeliere, A. Li,* J. E. Aubin. Anatomy & Cell Biology, University of Toronto, Toronto, ON, Canada.

CGI-135 was identified by cDNA fingerprinting of osteoprogenitor cells to isolate differentiation stage-specific genes (EST 1.1 in G. A. Candeliere, Y. Rao, A. Floh, S. D. Sandler and J. E. Aubin, Nucleic Acids Research, 1999, Vol. 27, No. 4, 1079–1083). Bioinformatic analysis revealed it to be highly conserved across species, with putative interaction and regulatory motifs, but uncharacterized in function in any species to date. CGI-135 is broadly expressed during mouse embryogenesis, with intense expression in several tissues, including bone. Differential expression was observed in vivo and in vitro in osteoblastic cells with peak expression in newly formed post-proliferative osteoblasts, suggesting that CGI-135 has a role during the proliferation-terminal differentiation transition. Consistent with this, in the MC3T3-E1 cell line, CGI-135 expression levels and subcellular localization (nuclear-cytoplasmic translocation) change as cells shift between quiescence and lineage progression. A search of the Pathcalling database (Curagen Corporation) allowed us to deduce a putative interaction between CGI-135 and Ini1 (a known chromatin remodeling protein and tumor suppressor) and between CGI-135 and ASNA-I (an ATPase with an unknown cellular function). We confirmed that CGI-135 co-localizes and interacts, in vivo, with INI1 and ASNA-I in the MC3T3-E1 cell line. These results suggest that CGI-135 may function with Ini1 and ASNA-I to form a previously unknown higher order chromatin-remodeling complex. To assess a functional role for CGI-135, we used antisense compared to nonsense adenoviral constructs. Antisense constructs decreased CGI-135 expression which in turn resulted in a marked disorganization of chromatin structure, which we observed by Hoechst nuclear staining. Antisense-treated cultures had a decrease in cell number compared to nonsense-treated cultures; the lower cell number appeared to result not from changes in cell cycle, but from cells becoming non-adherent. Even with a low frequency of cell infection, the remaining adherent cell population showed delayed alkaline phosphatase and von kossa staining after infection of cultures at early, but not late, culture times . These data suggest that CGI-135 is a chromatin remodeling protein essential for osteoblast development.

SU236

Nicotine and Calcitonin Gene Related Peptide Receptor mRNA Expression in Osteoblast-like Cells. L. D. Crouch, A. Cha,* Y. K. Fung.* Departments of Oral Biology and Growth and Development, College of Dentistry, University of Nebraska Medical Center, Lincoln, NE, USA.

Smoking and nicotine negatively impact bone formation, maintenance, fracture healing and fusion. Indeed, one in every five to eight hip fractures may be attributable to smoking. One potential target for nicotine's suppressive actions on bone is calcitonin gene related peptide (CGRP), a neuropeptide that modulates local bone remodeling processes via osteoblast activation and osteoclast inactivation. To determine if nicotine suppresses elements of the CGRP signaling pathway, we preformed experiments to determine whether nicotine exposure impacts the mRNA expression of the CGRP receptor (CGRP-R) in osteoblast-like MG-63 cells. Nicotine exposure as brief as one hour, at concentrations similar to those observed in the plasma and saliva of smokers and smokeless tobacco users (0.4 to 10 μM), consistently suppressed CGRP-R mRNA expression, as assessed by semiquantitative RT-

PCR analysis. Four hour nicotine exposure at concentrations as high as 250 μM failed to alter β-actin expression or cell viability, suggesting a specific nicotine effect on CGRP-R expression. Experiments assessing nicotine's impact on CGRP-R protein expression and receptor-mediated signal transduction are continuing. These results suggest that nicotine decreases CGRP receptor mRNA expression in osteoblast-like cells at concentrations relevant to smoking and smokeless tobacco use, thus providing a potential mechanism for smoking's adverse consequences on bone.

SU237

Analysis of an Osteocalcin GFP Reporter Transgene in Transgenic Mice. Z. Kalajzic,*[1] P. Liu,[1] I. Kalajzic,[1] Z. Du,[2] A. Braut,*[3] E. Canalis,[2] R. Derynck,[4] M. Mina,[3] D. W. Rowe.[1] [1]Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA, [2]St. Francis Medical Center, Hartford, CT, USA, [3]Department of Pediatric Dentistry, University of Connecticut Health Center, Farmington, CT, USA, [4]University of California, San Francisco, CA, USA.

The osteocalcin (OC) promoter has been widely used to restrict transgenic expression of a variety of proteins to bone. To obtain a visual marker of the population of cells expressing this promoter, we generated transgenic mice carrying a green fluorescent protein (GFP) gene driven by the OC promoter. The construct containing 1.77 kb of the rat OC promoter linked to GFPemd was used to generate transgenic mice. Two of four founders proved to express the construct.When tissues from homozygous animals were analyzed by RT-PCR, GFP mRNA was present in long bones, calvaria, tail and brain, but was not detectable by Northern. Samples of femur and calvaria from 8 day and 2-3 month old transgenic animals were decalcified, paraffin embedded and examined by fluorescent microscopy using a FITC/DAPI/Texas red filter. Strong GFP expression was detected in a few osteoblastic cells lining the endosteal bone surface and in scattered osteocytes within the bone matrix. Similar findings were noted in the forming tooth in which only scattered odontoblasts expressed GFP and there was no detectable expression within the dental pulp. This limited pattern of OC-GFP positive cells contrasts with the uniform expression of a pOBCol2.3GFP, a collagen promoter fragment active in mature osteoblasts, in which most of the surface osteoblasts and odontoblasts and all of the matrix burried osteocytes are strongly positive. An additional difference between the pOBCol2.3 and OC-GFP expression pattern was absence of OC-GFP positive cells on the surface or within trabecular bone within the metaphysis. To assess the OC-GFP transgene expression during in vitro differentiation, marrow stromal cell and neonatal calvarial osteoblast cultures were established. The transgene was not expressed at earlier stages of culture (days 7 and 15), but by day 18-21 a few individual strongly positive cells developed within the mineralizing nodules. This pattern differs from pOBCol2.3GFP cultures which activate somewhat earlier (day 15-18) and in most of the cells within the mineralized nodule. Preliminary experiments examining GFP positive and negative cells from freshly digested calvaria after FAC sorting show that the OC-GFP negative cells still express OC mRNA. This analysis suggests that while this OC-GFP transgene does activate concomitant with the endogenous gene, the marked cells identify a subpopulation of mature OC expressing osteoblast/osteocyte cells.

SU238

Activity of a Dual Reporter Transgene Driven by 3.6Col1a1 and 2.3Col1a1 Promoters in Transgenic Mice. I. Marijanovic,* Z. Kalajzic,* M. S. Kronenberg, A. C. Lichtler, D. W. Rowe. Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA.

Quantitative and spatial analyses of promoter reporter constructs are not easily performed in intact bone. Quantitative assessment of b-galactosidase is unreliable and in situ hybridization for CAT or luciferase is technically demanding. To overcome this problem, we designed a construct for a dual reporter transgene containing CAT and GFP reporter genes under the control of 3.6 kb and 2.3 kb fragments of the rat Col1a1 promoter. Previously we have demonstrated that a 3.6 or 2.3 Col1a1 promoter fragment that includes 1.6 kb of the collagen first intron (pOBCol3.6 and pOBCol2.3) driving GFP produces fluorescent osteoblasts in intact bones of transgenic mice. We used the pIRESeGFP vector (Clontech) in which the CMV promoter drives a transcript containing a viral internal ribosome entry site (IRES) that is fused in frame with eGFP. A multicloning site is placed upstream of the IRES into which a second gene can be placed. After transfection, the resulting bicistronic mRNA is translated into two separate proteins. We modified the pIRESeGFP vector by inserting the CAT gene upstream of IRES and by replacing the CMV promoter with a 3.6Col1a1 or 2.3Col1a1 promoter (lacking the collagen first intron). eGFP was replaced by GFPsaphire (Packard) or GFPcyan (Clontech). The resulting constructs, Col3.6CATiresGFPsaph and Col2.3CATiresGFPcyan were initially shown to be functional transgenes in transient transfection experiments prior to pronuclear microinjection. Three founders with Col3.6CATiresGFPsaph and two founders with Col2.3CATiresGFPcyan were produced. Tail biopsies were GFP positive by direct microscopic examination and tail protein extracts showed CAT activity.Marrow stromal cell culture derived from 5 week old heterozygous F1 Col3.6CATiresGFPsaph mice was established under differentiating conditions. The CAT activity was at a very low level on day7 and increased with time, reaching the peak on day16. The GFP positive cells started to appear between day10 and day13 and their number increased throughout the culture period. GFP positive cells were predominantly localized within the mineralized nodules. Tissue sections of paraffin embedded femurs, tibiae and calvariae as well as calvariae cell cultures for Col3.6CATiresGFPsaph are in the process of analysis. The same studies will be performed for Col2.3CATiresGFPcyan mice when F1 mice become available. This work shows that the dual reporter concept appears to be a useful tool for the simultaneous visual and quantitative analysis of transgene activity in bone.

SU239

Beta-Catenin Signaling During Osteoblast Differentiation. S. S. Sheikh,* G. Mbalaviele, P. Warlow,* R. Civitelli. Washington University School of Medicine, Saint Louis, MO, USA.

b-catenin, the vertebrate homolog of the drosophila polarity gene product, armadillo, serves both as a bridge between cadherins and the actin-based cytoskeleton in adhaerens junctions and a transactivator, a critical component of the wnt pathway. Binding of extracellular wnt to their receptors (frizzled, fz) leads to stabilization and translocation of b-catenin from the cytoplasm to the nucleus where it associates with T-cell factor/lymphoid enhancer factor (Tcf-Lef) family members and modulate their transcriptional activity. Loss-of-function mutations of wnt-5a or wnt-7a, and Lef1 and Tcf1 in mouse lead to abnormal skeletal development and early embryonic lethality. We studied the role of b-catenin in osteoblast differentiation. Both the immature MC3T3-E1 osteoblastic cells and the pluripotent C3H10T1/2 mesenchymal cells express mRNA encoding several members of the wnt and fz families, including wnt-2b, 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 8d and fz-1, 4, 8, 9. While expression of fz was unchanged during BMP-2-induced MC3T3-E1 osteoblast differentiation, expression of some wnt members, and in particular, wnt-5a and wnt-7a, was increased up to 7 days of exposure to BMP-2. MC3T3-E1 cells also expressed b-catenin, and treatment with BMP-2 or LiCl, which mimics wnt signaling, induced b-catenin translocation from the membrane and cytoplasm compartments into the nucleus, consistent with an increase of transcriptionally active b-catenin pools. Nuclear translocation b-catenin occurred within 10 min, as confirmed by redistribution of a b-catenin-GFP fusion protein upon LiCl exposure. To further understand b-catenin function in osteoblast differentiation, a b-catenin mutant was created by deleting the armadillo repeats domain, which binds Tcfs/Lefs and cadherins. This mutant has been shown to accumulate in the nucleus and inhibit signaling. Transient expression in MC3T3-E1 cells by electroporation (>80 % transfection efficiency and >90% cell survival using GFP as readout) inhibited BMP-2-induced alkaline phosphatase activity by approximately 60 %. Another mutant was generated by deleting the N-terminal moiety of the molecule, thus creating a stable, transcriptionally active b-catenin. Interestingly, BMP-2-induced alkaline phosphatase activity in cells transfected with this mutant was approximately 125% of control cells transfected with an empty vector. In conclusion, undifferentiated osteoblastic cells express the components of the b-catenin signaling pathway, some of which are responsive to BMP-2. Modulation of b-catenin activity alters osteoblast responsiveness to BMP-2, suggesting a role of this molecule in osteoblast differentiation.

SU240

Dexamethasone Induces Glutamine Synthetase Expression in Human Osteosarcoma MG-63 Cells: A Novel Marker for Osteoblast Differentiation? A. Olkku,*[1] A. Linnala-Kankkunen,*[2] A. Mahonen.[1] [1]Department of Medical Biochemistry, University of Kuopio, Kuopio, Finland, [2]Department of Biochemistry, University of Kuopio, Kuopio, Finland.

Glucocorticoids are known to promote the development of the osteoblastic phenotype in osteoprogenitor cells and in this way contribute to osteogenic differentiation. Its effects on bone cell differentiation are complex, depending, e.g., on the maturation stage of the osteoblasts. It is known that glucocorticoids inhibit the proliferation of osteoblastic cells. Therefore, the high doses of glucocorticoids used in pharmacological purposes, decrease bone formation, which can cause for example glucocorticoid inducible osteoporosis.In the present study, we were interested in the potential effects of dexamethasone (Dx), a synthetic glucocorticoid, on protein expression in human osteoblastic cells. Using two-dimensional electrophoresis and mass spectrometry, we identified increased expression of glutamine synthetase (GS) in 100 nM Dx treated MG-63 cells, and the observed difference was confirmed by the Western blot analysis with anti-GS antibody. Dexamethasone increased the amount of GS in MG-63 cells in a time-dependent manner receiving a maximal, about 15-fold increase within 8 h compared to the control level. The elevated expression was maintained at least for 72 h. The increase in the GS expression was also dose-dependent with maximal effect at 100 nM Dx. Also 1,25-dihydroxyvitamin D3 (1,25D) inhibits the proliferation of MG-63 cells, but 1,25D did not affect or even slightly decreased the GS protein expression in our experiments. To further examine the combinatory effects of Dx and 1,25D on GS expression, the MG-63 cells were pretreated with 100 nM Dx for 24 h and then with Dx (100nM), 1,25D (10 nM), or both together. 1,25D alone or with Dx was able to decrease GS expression over 50% from the Dx-induced level. The effect of Dx on the GS expression was also determined using another human osteosarcoma cell line, SaOs-2. In those cells, however, the GS expression was not affected during the 72-h treatment. In fact, the proliferation of SaOs-2 was not significantly inhibited by 100 nM Dx. GS protein level is used as a differentiation marker in cells from mesenchymal origin, such as adipocytes and fibroblasts. In osteoblasts, which are derived from the same origin, the changes in glutamine synthetase expression levels resulting from Dx treatment may also be involved in differentiation process. Further studies are, however, necessary to clarify the role of glutamine synthetase in controlling proliferation and differentiation steps in osteoblastic cells.

SU241

Osteoblastic Differentiation of Murine Osteoblastic KS483 Cells Strictly Depends on Autocrine BMP Signalling. G. van der Horst,* R. van Bezooijen, M. Deckers,* J. Hoogendam,* H. Sips,* A. Visser,* C. Lowik, M. Karperien. Endocrinology, Leiden University Medical Center, Leiden, The Netherlands.

Bone morphogenetic proteins (BMPs) are members of the TGF-ß super-family, and regulate differentiation of osteoprogenitor cells. In this study, we investigated the effects of exogeneously added and endogenously produced BMPs on the differentiation of the murine pre-osteoblastic cell line KS483. In the presence of ascorbic acid and b-glycerolphosphate KS cells form alkaline phosphatase positive and mineralized nodules during an 18 days culture period. We first examined the expression of various BMPs, their receptors and intracellular signalling proteins (Smad proteins) using RT-PCR. Transcripts for BMP-2, -3, -4, -6 and -7 were constitutively expressed, while BMP-5 mRNA was not expressed

during differentiation of KS483 cells. mRNAs of the BMP type I receptors ALK-2 (ActR-I), ALK-3 (BMPR-IA) and ALK-6 (BMPR-IB), the BMP type II receptor as wells as Smad1 through 8 were all expressed and did not change during differentiation.Treatment of undifferentiated KS483 cells for 3 days with BMP-4 and BMP-6 strongly induced alkaline phosphatase (ALP) expression and activity dose dependently (50-100 ng/ml). Soluble truncated BMP receptors (strBMPR-IA and -IB) blocked BMP-4 (50 ng/ml) and BMP-6 (100 ng/ml) activity dose-dependently (complete inhibition at 250 ng/ml). Moreover, noggin blocked efficiently BMP-4- (complete at 250 ng/ml) but not BMP-6-induced ALP activity. Treatment with strBMPR-IA, -IB and noggin also inhibited endogeneously induced ALP activity dose-dependently. To test the role of endogeneous BMPs in osteoblast differentiation, KS483 cells were continuously treated with strBMPR-IA or noggin for 18 days. strBMPR-IA and noggin strongly reduced osteoblastic differentiation dose dependently, as assayed by ALP production, the number of bone nodules and the amount of mineral deposition. Inhibition of differentiation was also observed when cells were treated with the antagonists from day 4 to 11. Interestingly, addition of the antagonists during the mineralization phase (from day 11 to 18) blocked the progress of differentiation. Our results show that osteoblastic differentiation of KS483 strictly depends on autocrine BMP-signalling. Furthermore, the inhibitory effects of BMP-antagonists during the mineralization phase suggest that BMP activity is not only required for the initiation of osteoblast differentiation and the further development of early osteoblasts but is indispensable for late-stage osteoblast differentiation as well.

SU242

Mc2, a New cDNA, and Osteocalcin Are Inversely Regulated in Differentiating Bone Cells. M. Rumpler,* F. Varga,* K. Klaushofer. 4th Med. Dept., Hanusch-Hospital, Ludwig Boltzmann Institute of Osteology, Vienna, Austria.

During prolonged culture, the osteoblastic cell line MC3T3-E1 shows a differentiation process which is characterized by a temporal expression of marker genes of proliferation and differentiation, e.g. histones, collagen and osteocalcin (OCN). The expression of the latter protein is restricted to mature osteoblasts. In this process cell to cell interaction plays a critical role. To find genes regulated during the formation of confluent cell layers, we screened subconfluent and confluent MC3T3-E1 cell cultures by differential display. Two micrograms of RNA were reverse transcribed and amplified in a PCR reaction using an arbitrary and an oligo (dT) primer. The amplified PCR products were separated on a sequencing gel. One candidate was eluted, cloned and sequenced. To verify the differential expression northern blot hybridization was performed. For further expression studies MC3T3-E1, MDCK and ROS 17/2.8 cells were cultured in aMEM supplemented with 5% FCS. Total RNA was isolated from subconfluent or confluent cultures, and for long time experiments from cells cultured up to 21 days. The expression of both mRNAs Mc2 and OCN was normalized to 28S rRNA.A cDNA, named Mc2, which was upregulated in confluent MC3T3-E1 cells was isolated. We verified the existence of Mc2 mRNA in MC3T3-E1 cells and in primary osteoblasts isolated from calvariae by RT-PCR and sequencing. The Mc2 cDNA is represented by an open reading frame of 162 bps, exhibiting partial homologies (at the 3´ end) to various genes in the EMBL database. The predicted protein sequence contains a myristylation site and a phosphorylation site for protein kinase C. In MC3T3-E1 cells, the Mc2 expression increased in confluent cell layers at days 8 and 12, compared to the level in subconfluent cultures on day 4. In prolonged cultures up to 21 days, the expression of Mc2 mRNA decreased again to the level observed on day 4, while OCN mRNA strongly increased. Triiodothyronine, a potent stimulator of the OCN synthesis in MC3T3-E1 cells, promoted a downregulation of the Mc2 mRNA at days 8 and 12. Under these conditions the expression of OCN was upregulated. The more mature rat osteosarcoma cell line ROS 17/2.8 showed an increased expression of OCN mRNA already at confluence. Comparable to the results in MC3T3-E1 cells the expression of Mc2 decreased when OCN expression increased. In the epithelial MDCK cells, Mc2 mRNA expression increased in cultures containing cell cysts, which are described to characterize a differentiated phenotype in these cells. In conclusion our data show that the new cDNA Mc2 is regulated inversely to OCN expression in mouse- and rat osteoblasts. We therefore suggest that Mc2 plays a role in the differentiation process.

SU243

Rapid Hypertrophy of Secondary Chondrocytes Arising from Periosteal Precursors In Vivo. P. G. Buxton,[1] C. W. Archer,*[2] P. H. Francis-West.*[1] [1]Craniofacial Development, King's College London, London, United Kingdom, [2]School of Molecular and Biomedical Biosciences, University of Wales, Cardiff, United Kingdom.

Secondary chondrocytes arise from the periosteum of certain membrane bones in response to mechanical stimulation and have distinct properties to primary chondrocytes. Once formed secondary chondrocytes act as important growth centres in the face for pre- and post- natal growth. We have studied secondary chondrogenesis in the upper jaw of the chick (the quadrato-jugal) where it articulates with the proximally located quadrate. To determine the type of chondrocytes formed and their aetiology we have examined the expression of a number of chondrocyte markers by in situ hybridisation: type II collagen, type X collagen, Ihh, Cbfa-1, Sox-9 and Frzb. Secondary chondrocytes are evident at embryonic day11 in the quadrato-jugal and the in situ study revealed co-ordinate up-regulation of all of the above markers; Cbfa-1 was already expressed in the periosteum and continued to be so. This suggested a rapid pre-osteoblast to chondrocyte transition and so we assessed proliferation in this system. Two-hour pulse with BrdU followed by immediate fix and detection revealed a highly proliferative germinal region surrounding the secondary chondrocytes, which were themselves quiescent. We also examined the expression of several perichondrial markers to see whether changes in their domains presaged the shift to chondrogenesis: Bmp-4, Bmp-7 and Gdf-5. Bmp-4, -7 were found to be expressed throughout the periosteum of the membrane bone, and were unchanged by the initiation of secondary chondrogenesis. Gdf-5 was expressed only in the perichondrium of the cartilaginous quadrate and not around secondary chondrocytes. In summary both the expression data and the proliferation study indicate that the precursor population differentiates under mechanical stimulation into pre-hypertrophic chondrocytes by the expedient of Sox-9 up-regulation. Thus, unlike the zones evident in the maturation of primary chondrocytes, secondary chondrocytes undergo a very rapid differentiation into the hypertrophic state. Study of the distinct but juxtaposed populations of proliferative and differentiating cells that comprise this system will provide further insights into the compression-dependent mechanism underlying chondrogenesis.

SU244

Noggin Overexpression Precludes the Differentiation of Stromal Cells into Functional Osteoblasts, an Effect Enhanced by Cortisol. E. Gazzerro,[1] Z. Du,*[1] A. N. Economides,[2] A. M. Delany,[1] E. Canalis.[1] [1]Department of Research, Saint Francis Hospital and Medical Center, Hartford, CT, USA, [2]Regeneron Pharmaceuticals, Inc., Tarrytown, NY, USA.

Noggin, a glycoprotein secreted by osteoblasts, is known to bind bone morphogenetic proteins (BMPs) and antagonize their actions, although BMP independent activities have not been excluded. Transgenic mice overexpressing noggin under the control of the osteocalcin promoter develop marked osteopenia and spontaneous fractures. Histomorphometry reveals a normal number of dysfunctional osteoblasts, but despite these pronounced effects, the mechanisms of noggin action in bone remain unknown. To investigate the role of noggin in the early stages of osteoblastogenesis, murine ST-2 stromal cells were transduced with a retroviral construct (pLPCX) in which the CMV promoter drives noggin gene expression. Noggin expression was not detectable in ST-2 cells transduced with vector alone, whereas high levels of noggin transcript and immunoreactive protein were detected in cells transduced with pLPCX/noggin. Cells were cultured in the presence of 5 mM beta-glycerolphosphate and ascorbic acid for up to 28 days in the presence or absence of BMP-2 at 1 nM (Genetics Institute) or cortisol at 1 μM, both regulatory factors of osteoblastic cell differentiation. ST-2 cells overexpressing noggin displayed a modest decrease in proliferation but had impaired terminal differentiation when compared to control cells. In the initial phases of culture, noggin overexpressing cells were alkaline phosphatase positive, whereas after 28 days they did not form mineralized nodules, as assessed by alizarin red staining. Furthermore, noggin overexpression resulted in cellular detachment from the culture plate, suggesting impaired cell-cell or cell-matrix interactions. BMP-2 increased levels of alkaline phosphatase and the rate of mineralization of ST-2 cells transduced with vector alone, but these effects were blocked in cells overexpressing noggin. In contrast, cortisol decreased the differentiation of control ST-2 cells, and this effect was greatly enhanced in noggin overexpressing cells, indicating additive effects of the two agents. In conclusion, noggin precludes the differentiation of stromal cells into viable and functional osteoblasts, opposing the stimulatory effects of BMPs and enhancing the inhibitory effects of cortisol on this process.

SU245

Differentiation and Regulation of Dental Follicle Cells by Specific Factors. M. Zhao,* J. E. Berry,* A. J. Koh,* L. K. McCauley, B. L. Foster,* H. L. Viswanathan,* M. J. Somerman. University of Michigan, Ann Arbor, MI, USA.

The dental follicle, a loose connective tissue surrounding the tooth germ, is known to play a critical role in tooth eruption and root formation. Follicle cells are considered the stem cells for cementoblasts, periodontal ligament cells and alveolar osteoblast cells: the cells involved in formation of the periodontium. Further, follicle cells are considered to be responsible for recruiting and activating osteoclasts to the local site as required for tooth eruption. However, factors and mechanisms involved in regulating the behavior of follicle cells remain undefined. This study was performed to determine the effect of factors known to regulate mesenchymal tissues, BMP and PTHrP, on follicle cell activity. To obtain follicle cells in vitro, cells were isolated from the first molar region of 3-5 day old CD-1 mice and immortalized with SV40. Cultured cells were analyzed for the presence of BMP receptors IA/II and PTH/PTHrP receptor-1 by RT-PCR. Next, cells were treated with BMP-2 (10-100 ng/ml) or PTHrP (10-7 M) and were evaluated for gene expression by northern blot or RT-PCR. To examine mineralization in vitro, von Kossa stain was used. Further, to dissect the possible signaling pathways involved in BMP-2 action, inhibitors of cAMP/PKA, PKC and MAP kinase pathways were used. Results from RT-PCR indicated that follicle cells express transcripts for BMPR IA/II and PTH/PTHrP receptor-1. BMP-2, in a dose- and time-dependent manner, induced the gene expression of bone sialoprotein (BSP) and osteocalcin (OCN), but down-regulated osteopontin expression. Time course assay revealed that BMP-2 also increased the expression of cbfa-1, a master switch for osteoblastic differentiation, which was observed before the expression of BSP and OCN. BMP-2 also enhanced the rate of mineral formation in vitro in a dose-dependent manner. Inhibitors of cAMP/PKA and PKC had no effect on BMP-2-mediated gene expression of follicle cells, but U0126, a specific inhibitor of MEK-1/2 members of MAPK family, abolished BMP-2-mediated expression of BSP and OCN, indicating involvement of MAPK pathway. Further, results from RT-PCR revealed that PTHrP induced gene expression of RANKL, a factor known to regulate osteoclast activity. Results of these studies suggest that follicle cells play a prominent role in controlling critical events associated with later stages of tooth development, i.e., tooth eruption and formation of the periodontium. Defining the specific factors and mechanism involved will be important for therapies targeted at regulating both osteoclastic and osteoblastic activities, e.g., altered tooth eruption, periodontal diseases and osteoporosis.

SU246

MIP-1α and RANTES Inhibit Osteoblast Alkaline Phosphatase (ALP) Activity and Stimulate Matrix Metalloproteinase-2 (MMP-2) Activity as a Function of Upregulated Expression of Chemokine Receptor CCR1 During Osteoblast Development. X. Yu, P. Collin-Osdoby, Y. Huang,* P. Osdoby. Biology, Washington University, St. Louis, MO, USA.

Chemokines are key signals involved in the recruitment, development, activation, and survival of cells in normal physiological processes and numerous pathological disorders. Various chemokines including MIP-1 alpha and RANTES are locally elevated and contrib-

ute to persistent tissue damage at sites of inflammatory bone loss in such disorders as periodontal disease or rheumatoid arthritis. Chemokine production and receptor expression may therefore be important factors influencing bone cell function and remodeling processes. However, little is known about chemokine receptor expression or chemokine effects on bone cells. Here we report that chemokine receptor expression changes during murine osteoblast (OB) development and correlates with an altered responsiveness to corresponding chemokine ligands, thereby affecting OB differentiation and function. Pre-OB were outgrown from newborn mouse calvaria and cultured (27 days) with ascorbic acid, b-glycerophosphate, and dexamethasone to induce OB differentiation, high ALP activity, and mineralized nodule formation. A multiprobe RNase protection assay (RPA) was used to analyze murine chemokine receptor mRNA expression as a function of OB development. Whereas CCR3 and CCR5 expression were low or unchanged, CCR1 mRNA levels increased 3 to 4-fold, peaking by day 15 of culture. To learn whether such increased CCR1 expression could mediate corresponding chemokine ligand responses in developing OB, calvarial cells differentiated for 9, 15, or 21 days were treated (48h) with MIP-1alpha or RANTES (10-10 to 3´10-8 M) and effects on ALP and MMP-2 activities were measured. Neither chemokine altered ALP or MMP-2 activity in day 9 developing OB that expressed low levels of CCR1. In contrast, both MIP-1alpha and RANTES significantly ($p<0.05$) inhibited ALP (by 75%) and increased MMP-2 (3-fold) activities in the day 15 differentiating OB that expressed peak levels of CCR1. MIP-1a also reduced ALP activity (by 80%) in mineralizing OB formed by day 21 of culture, without affecting MMP-2 activity. ALP is a marker of OB differentiation that is associated with bone formation, while MMP-2 participates in the degradation of bone matrix proteins. Our results therefore suggest that MIP-1alpha and RANTES may exert catabolic effects on bone through inhibiting OB differentiation and bone formation, while stimulating OB MMP-2 activity and bone matrix breakdown. Elevated levels of such chemokines may therefore contribute to the osteopenia associated with various inflammatory disorders.

SU247

The Effects of *Pasteurella Multocida* Toxin (PMT) on Osteoblast Differentiation: A Role for Rho GTPases. D. Harmey,[1] G. Stenbeck,*[2] C. Nobes,*[3] A. J. Lax,*[4] A. E. Grigoriadis.[1] [1]Craniofacial Dev, KCL, London, United Kingdom, [2]Bone and Mineral Centre, UCL, London, United Kingdom, [3]LMCB, UCL, London, United Kingdom, [4]Oral Microbiology, KCL, London, United Kingdom.

Pasteurella multocida toxin (PMT) is a bacterial toxin that is the causative agent of the porcine bone-resorbing disease, atrophic rhinitis. However, neither its cellular target(s) in bone nor its mechanism of action is well understood. PMT is a potent mitogen, leading to activation of phospholipase C, increased production of inositol phosphates and calcium mobilisation, and also activation of the Ras/Raf/MAPK pathway. PMT also stimulates cytoskeletal rearrangements via activation of the small GTPase Rho. In this study, we have investigated the effects of PMT on osteoblast differentiation, and the relationship between its mitogenic effects and the activation of Rho.PMT treatment is mitogenic for primary mouse osteoblasts, and it markedly induced actin stress fibres in both undifferentiated osteoblasts as well as in cuboidal osteoblasts on the surface of developing nodules. PMT inhibited bone nodule formation in a dose-dependent manner, acting during the early stages of osteoprogenitor cell proliferation. Interestingly, this inhibition was seen at doses that did not stimulate proliferation, suggesting that the signalling pathways regulating these events may differ.To further investigate the effects of PMT on osteoblast differentiation and Rho-dependent signalling pathways, primary osteoblasts were treated with Rho inhibitors during differentiation. Treatment with C3 transferase or the Rho kinase inhibitor HA-1077 abrogated the PMT-mediated inhibition of nodule formation. In addition, treatment with Cytotoxic Necrotizing Factor (CNF), a bacterial toxin that activates Rho, produced a similar dose-dependent inhibition of bone nodule formation. As CNF did not stimulate osteoblast proliferation, these data further suggest that the mechanisms mediating the PMT effects on osteoblast differentiation and proliferation may not be identical. Finally, microinjection of neutralising Ras antibodies blocked PMT-induced BrdU incorporation in Swiss3T3 cells without affecting stress fibre formation, implicating the ERK/MAPK pathway in the mitogenic response. Moreover, preliminary evidence indicates that the MEK inhibitor PD98059 failed to block the PMT inhibition of nodule formation. Together, these results point to signalling pathways downstream of Rho GTPases as important negative regulators of osteoblast differentiation, and demonstrate that bacterial toxins such as PMT are useful tools for dissecting the signal transduction pathways involved in osteoblast differentiation and activity.

SU248

Raloxifene Stimulates Osteoblastic Differentiation and Mineralized Bone Nodule (MBN) Formation in Human SaOS-2 Cells and Opposes the Inhibitory Effect of PTH. Y. Lin,*[1] J. F. Liu,*[1] T. M. Murry,[1] J. Sodek,*[2] L. G. Rao.[1] [1]St. Michael's Hospital, Univ of Toronto, Toronto, Canada, [2]Faculty of Dentistry, Univ of Toronto, Toronto, Canada.

Raloxifene (Ral) therapy decreases bone loss in postmenopausal women. However,its mechanisms of action and interaction with other regulatory molecules are not fully understood.We have studied the interactive effects of Ral and parathyroid hormone 1-34 (PTH) on osteoblast differentiation and bone formation in mineralizing cultures of SaOS-2 cells. From day 8, Ral (provided by Eli Lilly Company, Indianapolis) was added intermittently for 24 hours of each 48hr cycle of medium changes until day 17. The number and area of MBN were then quantified in fixed, von Kossa-stained cells. Alkaline phosphatase activity (ALP) and ALP mRNA expression were determined in cell extracts after 24 hours of treatment with Ral with or without PTH. Our results demonstrate that intermittent treatment with Ral (10^{-8} and 10^{-6} M) increased the MBN number by 23.1 and 39.9%, respectively (both with $p<0.05$, compared to control) and MBN area by 86.8% and 154.0%, respectively (both with $p<0.01$). A single 24 hr treatment with PTH (10^{-8} M) at day 8 caused a reduction in MBN number by 46.8% and MBN area by 56.5%. When Ral (10^{-7} and 10^{-6} M) were added concomitantly with PTH, the MBN number increased by 108.9% and 122.6% (both $p<0.05$, compared to PTH alone) and MBN area by 203.1% and 321.2% (both $p<0.001$ compared with PTH alone). These effects of Ral more than restored the decrease in bone formation caused by 24 hr constant presence of PTH. Twenty-four hr treatment with PTH (10^{-8} M) at day 8 also reduced of ALP when analysed at day 9**,** but Ral (10^{-8} and 10^{-6} M) alone had no effect. Concomitant treatment with Ral (10^{-6} M) and PTH (10^{-8} M) only partially prevented PTH-induced reduction of ALP: control; 1700±68.9 nmol/min/mg protein, PTH; 1415±25.7, PTH+Ral; 1524±22.6, $p<0.05$ (compared with PTH alone). RT-PCR showed that ALP mRNA level also decreased after PTH treatment (ALP/GAPDH from 1.11±0.06 to 0.90±0.04), and Ral restored its mRNA level to 1.06±0.05 ($p<0.05$). Although both protein kinase (PK) A and PKC are thought to be involved in PTH-induced bone modulation, the PKA inhibitor 6-22 Amide (1.7 nM) and the PKC inhibitor-bisindolylmaleimide 1 (10 nM) did not influence Ral's effects on ALP activity, or MBN number and area. In preliminary studies, the regulation of the early response gene c-fos may be involved in PTH and Ral action. In conclusion, Ral stimulated MBN formation and effectively opposed the inhibitory effect of constant PTH exposure on bone formation in SaOS-2 cells. This is the first report showing an interaction of Ral with PTH on differentiation and mineralization in osteoblast cultures of human origin.

SU249

Reduced CpG Methylation Is Associated with Transcriptional Activation of the Bone-Specific Rat Osteocalcin Gene in Osteoblasts. M. A. Montecino,*[1] A. Villagra,*[1] J. Gutierrez,*[1] R. Paredes,*[1] J. Sierra,*[1] M. Puchi,*[1] M. Imschenetzky,*[1] A. van Wijnen,[2] J. Lian,[2] G. Stein,[2] J. Stein.*[2] [1]Biologia Molecular, Universidad de Concepcion, Concepcion, Chile, [2]Cell Biology, UMASS Medical School, Worcester, MA, USA.

Chromatin remodeling of the bone-specific rat osteocalcin (OC) gene accompanies the onset and increase in OC expression during osteoblast differentiation. In osseous cells expressing OC, the promoter region contains two nuclease hypersensitive sites that encompass the elements that regulate basal tissue-specific and vitamin D-enhanced OC transcription. Multiple lines of evidence indicate that DNA methylation is involved in maintaining a stable and condensed chromatin organization that represses eukaryotic transcription. Here, we report that DNA methylation at the OC gene locus is associated with the condensed chromatin structure found in cells not expressing OC. In addition, we find that reduced CpG methylation of the OC gene accompanies active transcription in ROS 17/2.8 rat osteosarcoma cells. Interestingly, during differentiation of primary diploid rat osteoblasts in culture, as the OC gene becomes increasingly expressed, CpG methylation of the OC promoter is significantly reduced. Inhibition of OC transcription does not occur by a direct mechanism because in vitro methylated OC promoter DNA is still recognized by the key regulators Runx/Cbfa and the vitamin D receptor complex. Furthermore, CpG methylation affects neither basal nor vitamin D-enhanced OC promoter activity in transient expression experiments. Together, our results indicate that DNA methylation may contribute indirectly to OC transcriptional control in proliferating osteoblasts and non-osseous cells by maintaining a highly condensed and repressed chromatin structure.

SU250

Conditioned Media Derived from Mechanically-Loaded Mouse Sutures Regulates Osteoblastic Cell Differentiation. W. Wang,[1] J. M. Taboas,*[2] J. L. Dreier,*[1] S. A. Goldstein,[2] M. A. Ignelzi, Jr..*[1] [1]Orthodontics/Ped Dentistry, Univ of Michigan Sch of Dentistry, Ann Arbor, MI, USA, [2]Orthopaedic Research Laboratory, Univ of Michigan, Ann Arbor, MI, USA.

Our previous studies have shown increased osteoid production, increased bone marker gene expression, increased alkaline phosphatase activity and fusion in mouse sagittal sutures that have been subjected to uniaxial compressive loads in vitro, but also in unloaded sutures that were co-cultured with these loaded sutures. These results suggest that soluble factors are produced as a consequence of mechanical loading and that these factors have the ability to trigger new bone formation in unloaded tissues. The purpose of this study was to study the effect of conditioned media (CM) derived from loaded, unloaded and torn sutures on the proliferation and differentiation of the osteoblastic cell line MC3T3-E1. MC3T3-E1 cells were treated for 7 days with CM harvested from loaded, unloaded and torn sutures. CM from loaded, unloaded or torn sutures had no effect on MC3T3-E1 cell proliferation. CM from loaded or torn sutures reduced alkaline phosphate (ALP) activity compared to unloaded sutures or media only controls ($p<0.001$). Many studies have shown that prostaglandin E2 (PGE2) is a potent molecule in bone formation. To determine the direct or indirect effect of PGE2 in our system, we used the cyclooxygenase inhibitor, indomethacin (10-6 M), to block PGE2 production in sutures and in MC3T3-E1 cells treated with CM. CM harvested from indomethacin-treated sutures had no effect on MC3T3-E1 cell proliferation. Treatment of sutures with indomethacin reversed the suppression of ALP activity by CM in MC3T3-E1 cells ($p<0.001$). To test the possibility that endogenous PGE2 was responsible for these results, indomethacin was also used to treat MC3T3-E1 cells. MC3T3-E1 cells pre-treated with indomethacin and then treated with CM from loaded, unloaded and torn sutures demonstrated increased ALP activity compared to MC3T3-E1 cells that had not been pre-treated with indomethacin ($p<0.001$). As revealed by Northern Blot analyses, Bone Sialoprotein (BSP), Osteocalcin (OCN), type I collagen and Osteopontin (OPN) expression increased in MC3T3-E1 cells following treatment with CM from loaded and torn sutures. When loaded sutures were treated with indomethacin, the resulting CM led to decreased expression of BSP, type I collagen and OPN in MC3T3-E1 cells. This study demonstrates that soluble factors produced by mechanically-loaded sutures regulate osteoblastic differentiation and gene expression in vitro and that PGE2 is a critical regulator of these events.

SU251

cDNA Microarray Analysis of Human Bone Marrow Stromal Cell mRNA Expression in Response to Dexamethasone. L. M. King,*[1] A. R. DeRubeis,*[1] L. Jia,*[2] C. Francomano,*[2] P. Robey.*[1] [1]CSDB, NIDCR, NIH, Bethesda, MD, USA, [2]MGB, NHGRI, NIH, Bethesda, MD, USA.

The bone marrow stromal cell (BMSC) population includes cells that have stem cell -

like properties, and under the appropriate experimental conditions, can give rise to bone, cartilage, adipose tissue, fibrous tissue and myelosupportive stroma. However, mechanisms that regulate the differentiation of the precursor cells into the osteoblast lineage are not well understood. Our previous studies showed that the glucocorticoid dexamethasone (Dex) increases the proliferation rate of BMSCs and their osteogenic potential, as demonstrated by in vivo transplantation. To further identify unique gene expression patterns underlying the proliferation and differentiation of BMSCs, we designed a filter microarray with representative cDNA clones of 2144 genes and ESTs, which were selected by their representation in NCBI public database dbEST libraries of skeletal-related tissues, and were arrayed by Research Genetics. Human BMSCs from two normal donors, one female (39 yr old) and one male (41 yr old) were cultured with and without Dex. Total RNA was prepared and hybridized to the microarray using standard techniques. The data were evaluated using P-SCAN software and the statistical package JMP (SAS, Inc).Our results showed that of the 1273 genes (59%) expressed above background, there was a 96% correlation between levels of gene expression between treatments. Only osteonectin showed a greater than two-fold difference in gene expression, with 2.2 fold down regulation by Dex. We evaluated mRNA expression of genes that showed a greater than 1.5 fold difference between treatments by real time semi – quantitative RT-PCR (LightCycler, Roche). Our PCR and microarray data were consistent for osteonectin, collagen I alpha 2 and an unknown gene, which were all downregulated by Dex. Only DNA polymerase POLD2 was consistently upregulated by Dex among samples and techniques. We also evaluated by PCR the expression of genes previously identified by differential display to be up or down regulated by Dex (big-h3, annexin VI). These results were consistent with our previous work indicating that our culture treatments were biologically equivalent. Our data suggest that small differences in gene expression regulated by Dex may have significant phenotypic consequences on BMSC growth and differentiation. Future studies will continue to optimize the use of microarrays to study patterns of gene expression as a function of BMSC maturation into different phenotypes, and as a function of disease. These studies will include the characterization of unknown genes for their roles in bone metabolism as they are revealed by this technology.

SU252

The HIV Protease Inhibitor Indinavir Uniquely Inhibits Bone Formation. M. W. H. Wang, S. Wei, S. L. Teitelbaum, F. P. Ross. Pathology, Washington University, St. Louis, MO, USA.

We find that HIV infected patients treated with protease inhibitors (PIs) have diminished bone mineral density t (p=0.02) and Z (p=0.04) scores relative to similarly-infected individuals not receiving these drugs. To identify the mechanisms by which PIs induce osteopenia we studied the effects of two commonly used drugs on osteoblast (OB) differentiation and function, in vitro, ex vivo and in vivo. We find that the PI Indinavir, at a pharmacologically relevant concentration (5μg/mL), virtually arrests the capacity of murine OBs to develop bone nodules, in vitro. This inhibitory effect of Indinavir does not reflect cell toxicity, as OB number is unaltered by the drug. In contrast to Indinavir, Ritonavir, which blocks osteoclast function (this meeting), does not inhibit bone nodule formation. The effect of Indinavir occurs only during the first half of the culture period, indicating it suppresses early osteoblastogenesis. Treatment of the murine osteoblastic line MC3T3-E1 with Indinavir prompts a dose dependent decrease in alkaline phosphatase activity, an early marker of OB differentiation. Similarly, when added to primary OBs as they differentiate in the presence of ascorbate and β-glycerophosphate, Indinavir blunts deposition of extracellular matrix, measured by Western blot analysis of both α1 and α2 chains of type I collagen. This result does not arise from a decrease in cell number, since levels of the cytoskeletal protein α-actinin are not altered by the drug. Reflecting its inhibitory effect on OB function, Indinavir also completely arrests bone formation in calvarial organ culture, both under basal conditions and when stimulated by statins. To test the effect of Indinavir on OB differentiation, ex vivo, we injected the drug, intra-peritoneally, into mice for two weeks, and assayed for the capacity of bone marrow cells to differentiate into OBs, in culture. We find a tenfold decrease (p<0.001) in OB colony forming unit formation by marrow derived from Indinavir-treated as compared to control animals. Finally these results are reflected in vivo, as mice administered Indinavir for two weeks develop a 12% decrease in spinal BMD. Thus, even though Indinavir is designed specifically to target a viral protease, it specifically dampens OB differentiation and function. These data suggest that Indinavir may promote the bone loss observed in HIV-infected patients.

SU253

Erythroid-Specific Expression of Human Growth Hormone in Marrow Stimulates Local Osteoblast Activity. N. W. Flay,[1] B. M. Vertel,*[1] D. King.[2] [1]Cell Biology and Anatomy, FUHS/CMS, North Chicago, IL, USA, [2]Biochemistry and Molecular Pathology, NEOUCOM, Rootstown, OH, USA.

Transgenic mice exhibiting low-level, erythroid-specific expression of human growth hormone (hGH) in bone marrow have denser and stronger bones than control counterparts, suggesting an anabolic influence upon bone surfaces. Studies are based on the hypothesis that erythroid-specific release of hGH in bone marrow of the transgenic mice imparts a localized effect upon neighboring cells, resulting in increased osteogenesis along marrow-lining bone surfaces. The hypothesis was tested using static and dynamic histomorphometric analyses of tibial and caudal vertebral bones of transgenic mouse lines at two stages of development: growth phase (seven weeks) and skeletal maturity (twelve weeks). Results revealed no differences between transgenic and control mice in osteoclast population sizes or in cell densities, but significant increases in osteoblast numbers in twelve-week transgenic tibial bones compared to control bones. Fluorescent images of calcein-injected mouse bones revealed increased amounts of cancellous bone at both ages in the transgenic compared to the control animals. Mineral apposition rate along endosteal surfaces of both bones significantly increased for both transgenic lines compared to control bones. In the transgenic line expressing higher hGH levels, the total mineralizing surface was increased in tibial bones at seven weeks and in both bones at twelve weeks. The conclusion is that local release of hGH into bone marrow cavities during erythropoiesis preferentially exerts anabolic influences along endosteal bone surfaces of transgenic mice in two ways: (1) by stimulating osteoblast activity, as reflected in the elevated mineral apposition rates, the amount of mineralizing surface and the percentage of mineralizing surface along transgenic bones as compared to control bones and (2) by increasing the osteoblast/osteoclast population ratio. Together, the effects allow for increased bone deposition during a single turnover cycle.

SU254

Effect of Endothelin-1 on Osteoprogenitor Proliferation and Differentiation and on Gene Expression in Cell Populations from Fetal Rat Calvaria. C. J. H. Veillette,* H. P. von Schroeder. University of Toronto, Toronto, ON, Canada.

Endothelin-1 (ET-1), a peptide produced by vascular endothelial cells (VECs), is implicated in the signaling between VECs and osteoblasts (OBs) in bone remodeling and repair. We investigated the effect of ET-1 on proliferation and differentiation of osteoblastic cells derived from fetal rat calvaria (FRC) using limiting dilution analysis (LDA). In addition, gene expression profiles in early FRC osteoblastic populations cultured in the presence and absence of ET-1 were determined with DNA microarrays. For LDA, osteoblastic cells isolated from FRC were plated in 96 well plates with cell densities from 6.25-800 cells/well. ET-1 (10^{-8}M) or vehicle alone was added with each medium change. β-glycerophosphate (10mM) was added during the final 4 days for osteoid mineralization. Microarray analysis of 1700 gene products was then performed on total RNA isolated from FRC cells cultured with ET-1 (10^{-8}M) or vehicle alone. RNA was collected at confluence (7 days of culture), and labelled with Cy3 or Cy5 fluorescent dyes. Array slides (Ontario Cancer Institute) were hybridized, and quantified using a Genepix scanner and analysis software (Axon Instruments). Data was normalized and processed using criteria and thresholds established from array data from control RNA. ET-1 increased CFU-F (fibroblastic colony forming units) by 1.5-fold, but increased CFU-AP (osteoblastic potential; alkaline phosphatase positive colonies) by 3-fold and CFU-O (osteoprogenitor cells; bone nodule forming cells) by 5.5-fold. ET-1 treatment resulted in a greater than two-fold upregulation of 18 gene products, including TGFβ 3 and 4, TNF receptor 4, corticosteroid-binding globulin, osteoblast cadherin, PDGF receptor, osteonectin, decorin, angiopoietin 1 receptor (Tie 1) and procollagen α-2(I) chain. Downregulated genes included TGF-β1 binding protein 1, insulin-like growth factor binding protein 4, osteopontin, IGF-II, and zinc finger protein A20. Our results show that ET-1 increases the frequency of cells with the capacity to form colonies with osteoblastic phenotype (AP^+) and bone nodules *in vitro*. In addition, several known key factors in osteoblastic differentiation, most notably the TGF-β and IGF signaling pathways, may be modulated by ET-1.

SU255

Expansion of Human CFU-O Progenitors in Stirred Suspension Bioreactors. D. Baksh,[1] P. W. Zandstra,*[2] J. E. Davies.[2] [1]Chemical Engineering, Biomaterials & Biomedical Engineering, Toronto, ON, Canada, [2]Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada.

The challenge of cell-based tissue engineering strategies is to expedite the delivery of large numbers of appropriate progenitors to the repair and/or regeneration site. Bone marrow stroma provides a source for such progenitor cells, referred to by some as mesenchymal stem cells (MSCs). Research has focussed on finding novel approaches for ex-vivo expansion of bone marrow derived stem cells, including MSCs for bone tissue engineering applications. All approaches to-date involve culturing whole bone marrow that contains low frequency of such cells that have been isolated based on their adherence to tissue culture treated substrates and passaging to achieve expansion. Such substrate dependent approaches have not yielded sufficient number of cells capable of elaborating morphologically identifiable bone matrix for clinical applications. Our previous work has shown that stromal cell precursors, detected as CFU-Fs (colony forming unit-fibroblast), proliferated in suspension bioreactor cultures containing interleukin 3 (IL-3) and Steel Factor (SF). These results have prompted our current investigation of cell culture conditions to optimize not only CFU-F but also osteoprogenitor cell expansion in suspension.Mononuclear human bone marrow-derived cells (hBMDC) were separated using a Ficoll-PaqueTM gradient. They were re-suspended at 5 x 106 cells/ml in flat-bottomed 100-ml spinner flasks. Cultures were supplemented with either no cytokines or combinations of recombinant human (rh) IL-3 (2 ng/ml), rh Steel Factor (rhSF, 10 ng/ml), or rh platelet derived growth factor (rhPDGF, 10 ng/ml). Cultures were maintained at 37oC in humidified atmosphere of 5% CO2 in air with constant stirring at 40 rpm. At days 5, 10, 15 and 20 cell counts and CFU-F and CFU-O assays were performed.Factorial experiments performed with hematopoietic cytokines and cytokines that are potent mitogens for proliferation demonstrated that cytokine addition influenced the output CFU-F and CFU-O colonies, where 5-10 fold expansion was observed when SCF and IL3 were added to the culture medium. Scanning and transmission electron microscopy revealed morphologically identifiable bone matrix, including cement line production and mineralized collagenous extracellular matrix. Our results demonstrate that it is possible to expand an osteoprogenitor population in suspension that maintains a capacity to form bone matrix.

SU256

N-Cadherin Abundance Modulates Osteoblast Function. L. Weitzmann,[1] S. Cheng,[1] L. Halstead,[1] R. Civitelli,[1] M. Amling.[2] [1]Division of Bone and Mineral Diseases, Dept of Internal Medicine, Washington University School of Medicine, St Louis, MO, USA, [2]Dept of Trauma Surgery, Hamburg University, Hamburg, Germany.

We have previously demonstrated that osteoblasts express a repertoire of cadherins, including N-cadherin (Ncad) and cadherin-11, and that a global inhibition of cadherin-mediated cell-cell interactions by dominant-negative constructs or inhibitory peptides disrupts osteoblast differentiation. To determine the selective role of Ncad in bone, we have used mice harboring a null mutation of the Ncad gene. Since homozygous loss of Ncad is embryonically lethal, we have focused on heterozygous Ncad+/- animals. Preliminary his-

tomorphometric analysis of 3-months-old male mice revealed a decreased bone volume/total volume in Ncad+/- mice compared with wild type Ncad+/+ mice (13.5 ± 2.3% versus 17.9 ± 3.5%, n = 4). Likewise, bone volume was tendentially lower in heterozygous females (15.2% versus 17.3%, n=2). To analyze the cellular basis of this phenotype, we analyzed several parameters of osteoblast function, including proliferation, expression of bone matrix proteins, and FGF2-induced chemotaxis, in osteoblasts obtained from newborn calvaria of Ncad+/- and Ncad+/+ newborn mice. Confirming previous observations of an Ncad gene dosage effect, calvarial cells derived from Ncad+/- mice express a lower abundance of Ncad mRNA and protein compared with Ncad+/+ cells. Analysis of [3H]thymidine incorporation indicated that the proliferation rate of Ncad+/- calvarial osteoblasts was only 75% relative to that of Ncad+/+ cells. Expression of osteoblast phenotypic markers, such as osteopontin, bone sialoprotein, type I collagen and alkaline phosphatase activity was not substantially different in Ncad+/- osteoblasts relative to Ncad+/+ cells. By contrast, Ncad+/- osteoblasts exhibited a higher FGF2-induced chemotactic migration on type I collagen and vitronectin than Ncad+/+ cells whereas chemotaxis on fibronectin was not altered. Furthermore, homotypic cell-cell adhesion was also decreased in Ncad+/- osteoblasts compared to wild type cells. In conclusion, Ncad is an essential phenotypic component of bone forming cells. Decreased abundance of Ncad does not critically alter the osteoblast differentiation program, but it decreases the proliferation rate of osteogenic cells and alters their ability to migrate on matrix substrata under chemotaxic stimulation. These abnormalities may lead to reduced bone mass in adult animals.

SU257

LIF Inhibits Osteoprogenitor Differentiation by Modulating Hyaluronic Acid Synthesis. D. Falconi,* J. Dreisziger,* J. E. Aubin. Department of Anatomy and Cell Biology, University of Toronto, Toronto, ON, Canada.

Hyaluronic acid (HA) is a high molecular weight extracellular glycosaminoglycan composed of repeating disaccharide units of β1-4GlcAβ1-3GlcNAc. Three integral plasma membrane glycosyltransferases, namely Hyaluronan Synthase 1, 2 and 3 (HAS1, HAS2 and HAS3) are responsible for the synthesis of HA. HAS1 and 2 are known to be expressed in the osteosarcoma cell line MG-63, with higher expression of HAS2 than HAS1. Moreover, HAS2 mRNA expression level is highly reduced by the synthetic glucocorticoid (GC), dexamethasone (DEX). Previously, we have found that LIF, given chronically or pulsed at 5 ng/ml at the end of the proliferation period, blocks osteoblast differentiation at the osteoprogenitor stage in rat calvaria (RC) cell cultures; this effect of LIF is antagonized by DEX. In order to determine the mechanism of this differentiation stage-specific effect of LIF, we performed differential display on RC cells treated with the cytokine during the sensitive period. Amongst LIF-regulated genes identified, HAS2 was up-regulated specifically in the sensitive time window, suggesting that the abundance of HA at this particular period could be responsible at least in part for the differentiation inhibitory effect of LIF. To address this possibility, we pulse (3-days) treated RC cells with different concentrations of HA (2, 1 and 0.5 mg/ml) during different proliferation and differentiation time windows; osteoblast differentiation was assessed by alkaline phosphatase and von Kossa staining. HA decreased alkaline phosphatase activity, nodule formation and mineral deposition, when it was present during a similar time window as sensitive to LIF; pulses during other time windows were ineffective. We therefore conclude that the increased concentration of HA, caused by LIF-induced up-regulation of HAS2, may contribute to the inhibitory effect of LIF on osteoprogenitor differentiation.

SU258

Connexin43-Mediated Gap-Junctional Communication Enhances PTH Responsiveness. G. D'Ippolito, P. Hernandez,* G. A. Howard, B. A. Roos, P. C. Schiller. GRECC and Research Service, Veterans Affairs Medical Center, and University of Miami School of Medicine, Miami, FL, USA.

Although PTH1R expression and receptor-mediated cAMP responsiveness are relatively constant during osteoblastic maturation, connexin43 expression and gap-junctional communication (Cx43/GJC) increases. The variable effect of PTH on in vitro ECM mineralization correlated with Cx43/GJC. PTH is inhibitory when Cx43/GJC is low (preosteoblastic) and stimulatory when Cx43/GJC is high (mature). This stimulation is blocked when GJC is inhibited. We hypothesize that Cx43/GJC modulates the responsiveness of osteoblastic cells to PTH. To test our hypothesis we transfected low-Cx43-expressing, poorly coupled rat UMR106.01 cells (representing a less mature phenotype) with a plasmid encoding a functional Cx43 (pCx43YFP, provided by Dr. DW Laird). Transient transfections of pCx43YFP increased GJC in UMR 106.01 osteoblastic cells. To evaluate the effect of modulation of Cx43/GJC on the responsiveness of the osteocalcin (OC) promoter (the prototype osteoblastic gene) to PTH stimulation, the pCx43YFP expression vector was cotransfected with pOClux, a luciferase reporter gene driven by cloned 1.1 Kb rat OC promoter sequences. The basal OC promoter activity was low in UMR106.01 cells, and PTH treatment had a 1.5-fold stimulatory effect, without modulating endogenous GJC. Transient cotransfection with pCx43YFP not only increased GJC and basal promoter activity but also enhanced the PTH responsiveness of the promoter up to 20 fold. The Cx43/GJC-modulated PTH responsiveness was dependent on treatment time and dose. The highest effect was detected at a concentration of 5-10 nM PTH after 4 hours of treatment. Chemical inhibition of GJC had similar effects on basal and PTH-stimulated OC promoter activity. In conclusion, Cx43/GJC enhances osteoblastic response to PTH. The molecular determinants involved in mediating this effect remain to be elucidated.

SU259

1-(5-oxohexyl)-3,7-dimethylxanthine, a Phosphodiesterase Inhibitor Activates MAPK Cascades and Promotes Osteoblast Differentiation by a Mechanism Independent of Protein Kinase A Activation. G. Rawadi,[*1] C. Ferrer,[*1] S. Spinella-Jaegle,[*1] B. Courtois,[*1] S. Roman-Roman,[1] Y. Bouali,[*1] R. Baron.[1 1]Aventis Pharma, Romainville, France.

Phosphodiesterase (PDE) inhibitors have been reported to affect osteoblastogenesis and osteoclastogenesis in vitro but little is known about the molecular mechanisms by which PDE inhibitors affect bone cells. In the present study we have investigated the effect of 1-(5- oxohexyl)-3,7-dimethylxanthine (pentoxifylline, PeTx), a non selective phosphodiesterase inhibitor, on osteoblastic differentiation in vitro by using two pluripotent mesenchymal cell lines, C3H10T1/2 and C2C12, able to acquire the osteoblastic phenotype in the presence of bone morphogenetic protein-2 (BMP-2). PeTx induces the osteoblastic markers, osteocalcin (OC) and Osf2/Cbfa1, in C3H10T1/2 and C2C12 cells. Moreover, PeTx enhances the BMP-2- induced expression of OC, Osf2/Cbfa1 and alkaline phosphatase (ALP). This activity can be at least partially attributed to the fact that PeTx is able to enhance BMP-2-induced Smad1 transcriptional activity. Although PeTx clearly stimulates PKA in the cells used here, pretreatment of cells with the PKA inhibitor H89 did not prevent the induction or enhancement of osteoblast markers by PeTx. In addition, transfection of C3H10T1/2 or C2C12 cells with a plasmid expressing PKI, a polypeptide inhibitor of PKA, had not significant effect on PeTx activity. These data clearly demonstrate that the effect of PeTx on osteoblast commitment is independent of PKA activation. Interestingly PeTx induces the activation of ERK1/2 and p38 kinase pathways independently of the activation of PKA. Selective inhibitors of these MAPK cascades prevented the acquisition of osteoblastic markers in cells treated with PeTx suggesting that activation of these two pathways plays a role in the effect of PeTx on osteoblastic differentiation. Previous studies have suggested that PDE4 may play a role in bone turnover. We have therefore examined the effect of PDE1, 2, 3, 4 and 5- specific inhibitors on ALP activity when combined to BMP-2. Surprisingly, when used at their respective IC50 concentrations, none of these inhibitors had a significant effect on ALP induction. However, at concentration 10 to 100 times higher than the IC50, only PDE4-specific inhibitors were able to enhance BMP-2-induced ALP activity. Based on these data, one can speculate that at that high concentration PDE4-specific inhibitors may target a distinct molecule than PDEs. In conclusion, PeTx has the potential to commit undifferentiated mesenchymal cells into the osteoblastic lineage by a mechanism involving MAPK but independent of the activation of PKA.

SU260

Identification of a Novel BMP-2 Inducible Kinase that Impairs Mineralization in vitro. A. E. Kearns,[1] M. M. Donohue,*[2] B. Sanyal,*[1] M. B. Demay.[2 1]Endocrinology, Mayo Clinic, Rochester, MN, USA, [2]Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

We have used the technique of differential display polymerase chain reaction (ddPCR) to identify genes that are regulated during endochondral bone formation. Template RNA was isolated from the prechondroblastic cell line, MLB13MYC clone 17, which acquires molecular markers of osteoblasts in response to BMP-2 treatment. A ddPCR product whose presence was dependent on treatment of these cells with BMP-2, was subcloned and used as a probe for northern analyses. The expression of the 7 Kb mRNA encoded by this ddPCR product was markedly increased (10-fold) by 72 hours of BMP-2 treatment (200 ng/ml) of the MLB13MYC clone 17 cell line. This transcript was also expressed in RNA isolated from spleen, kidney, lung, brain, heart and diaphragmatic muscle, but not liver. A Lambda zap II cDNA library was synthesized using mRNA isolated from MLB13MYC clone 17 cells treated with BMP-2. Using the ddPCR product as a radiolabeled probe, this library was screened to isolate the full-length cDNA represented by the ddPCR product. Four independent phage, spanning the entire cDNA were isolated and the inserts were sequenced. The deduced amino acid sequence identified this protein as a novel 126 kDa putative serine/threonine protein kinase, containing a nuclear localization signal (NLS). Studies were undertaken to confirm the functional properties of the kinase and NLS domains. The NLS was fused to green fluorescent protein (GFP) and was functional in directing GFP to the nucleus in transfected COS7 Cells. The kinase domain, expressed in E. coli, was shown to be capable of autophosphorylation as well as phosphorylation of myelin basic protein. Mutation of the kinase domain prevented autophosphorylation. This novel gene was named BIKe (BMP-2 Inducible Kinase).To identify a role for BIKe in endochondral bone formation, MC3T3E1 cells were stably transfected with vector containing the full-length coding region cloned into pcDNA 3.1 and with empty vector alone. Studies were performed on pools of 18 clones of stably transfected cells. BIKe significantly (>60%) decreased alkaline phosphatase activity relative to vector control (assessed at days 7 and 16). Delayed appearance of mineralization and magnitude of mineral deposition was decreased in the cells stably transfected with BIKe, relative to cells transfected with vector alone. Stable expression of BIKe did not delay the appearance of mRNA encoding selected markers of osteoblast differentiation, including Cbfa1, type I collagen and osteopontin. Studies directed at identifying the molecular substrates of this novel kinase are currently underway.

SU261

PKCdelta Mediates Activation of Erk Induced by Gq Protein-Coupled Receptors Via its Tyrosine Phosphorylation by Src or Fyn in MC3T3-E1 Osteoblast-like Cells. J. Caverzasio, J. Lemonnier,* G. Palmer, J. P. Bonjour. Division of Bone Diseases, University Hospital of Geneva, Geneva, Switzerland.

G protein-coupled receptors (GPCRs) are transducers of the anabolic effects of essential osteotropic factors such as parathyroid hormone and fluoride. Recent studies suggest the implication of both PKC and Src kinases in activation of Erk, a major mitogenic pathway, by Gq- or Gi-PCRs in MC3T3-E1 (E1) osteoblast-like cells. In the present study, we investigated which PKC and the signaling mechanism involved in Erk activation by PGF2alpha (PGF2a), a mitogenic GqPCR agonist, in E1 cells. In untreated E1 cells, Erk activity, measured by immunoprecipitation with a phospho-Erk antibody and an in vitro kinase assay, was undetectable. Exposure to 1 μM PGF2a induced a rapid and transient activation of Erk with a maximal effect at 5 min. The PKC inhibitor G06983 (10 μM) completely blocked and the selective PKCdelta (PKCd) inhibitor, rottlerin, dose-dependently (5-25 μM) blunted Erk activation induced by PGF2a suggesting an important role of PKCd in mediating this response. In the particulate fraction of untreated E1 cells, PKCd was already phosphorylated (phosph) on Ser-643 and Thr-505 (activated form) but it was not phosph on tyrosine residues. PGF2a induced a rapid and transient increase in the amount of phosph-Thr-505-PKCd (2.1 x) and of PKCd kinase activity (2.2 x) as well as a marked increase in

the phosph of PKCd on tyrosine residues (14.6 x). G06983 (10 μM) not only blocked Erk activation but also completely prevented the increase in activity and tyrosine phosph of PKCd induced by PGF2a. In addition, PP1, a selective Src kinases inhibitor, dose-dependently blunted the tyrosine phosph of PKCd and Erk activation induced by PGF2a. Immunoprecipitation and western blot analysis indicated that in untreated cells, PKCd is associated with Src and/or Fyn kinases in a signaling complex comprising at least 5 other proteins with MW of approx 125, 110, 40, 31 and 20 kD. Associated with the increase in PKCd tyrosine phosph, PGF2a enhanced the activity of Src and Fyn (3-4 x) and the amount of associated PKCd (4-5 x) with these kinases as well as the phosphorylation of other proteins in this signaling complex. In conclusion, data presented in this study describe a new signaling mechanism for Erk activation by GqPCRs. This transduction mechanism involves the transient association of activated PKCdelta with and its tyrosine phosphorylation by Src and/or Fyn kinases in the plasma membrane. Relocalisation and tyrosine phosphorylation of PKCdelta likely serves to recruit and activate downstream signaling effectors for activation of Erk by GqPCRs.

SU262

p38 Kinase and Its Downstream Target ATF-2 Are Involved in the Osteoblast Osmotic Response to Elevated Extracellular Glucose. M. Zayzafoon, L. R. McCabe. Physiology Department, Michigan State University, East Lansing, MI, USA.

Poorly controlled or untreated type I diabetes mellitus is characterized by hyperglycemia and is associated with decreased bone mass and increased fracture rates. Cells such as osteoblasts that lack the high Km glucose transporter (Glut2) have limited capability to absorb glucose and consequently are placed under hyperosmolar stress. Investigation of osmotic stress in mammalian tissues has focused principally on kidney and cellular responses to hyperosmolar conditions above 600 mOsm. Recently, we have shown that osteoblasts are sensitive to osmotic stress and respond to changes in extracellular glucose and mannitol as little as 5 to 10 mM (180-270 mg/dL). The osteoblast osmotic response is dependent upon protein kinase C activity and results in upregulation of c-jun and modulation of collagen 1 and osteocalcin expression. A role for osmotic stress was further demonstrated by 3-O-methyl-D-glucose uptake studies that show constant sugar uptake from 0-24 hours. To determine if diabetic hyperosmolar stress plays a role in activating MAPK in osteoblasts, we treated MC3T3-E1 osteoblasts (grown in 5.5 mM glucose) with 16mM glucose or mannitol for one hour. Western blotting demonstrates that p38 MAPK was significantly activated by both glucose and mannitol with no change in p38 levels. Increased activity of p38 MAPK was not inhibited by staurosporine, suggesting a PKC-independent pathway in this activation. This increase was time dependent peaking at 20 minutes and staying detectible after 24 hours. It was also concentration dependent, being detectable at concentration as low as 10mM and increasing gradually as total sugar concentration increased up to 22mM. ATF-2 activation followed the same pattern as p-38. EMSA studies showed an increase in AP-1 and CRE binding 1 hour after hypertonic treatment with glucose or mannitol. Supershift analysis demonstrated the involvement of c-Jun and ATF-2 in this binding. Corresponding to EMSA results, AP-1 and CRE transactivation increased 3 hours after sugar treatment. SB 203580 (p-38 MAPK inhibitor) was able to inhibit the phosphorylation of ATF-2 as well as the increase in c-Jun in response to hyperosmolality. Therefore, we propose that increased p38 MAPK activity and ATF-2 contribute to AP-1 and CRE activation, which ultimately leads to decreased osteoblast differentiation. These findings suggest that changes in cell volume maybe important in the modulation of cellular signaling pathways and elevated blood levels of glucose could contribute to at least one complication of diabetes, osteoporosis.

SU263

An Osteoclast Secreted Chemotactic Cytokine Stimulates Changes in Protein Phosphorylation and Activation of p42/p44 MAP Kinase in Human Mesenchymal Cells. K. I. Larsen,[1] W. Wang,*[2] X. Wu,*[1] J. P. Williams.[2] [1]Pathology, University of Alabama Birmingham, Birmingham, AL, USA, [2]Internal Medicine, University of Kentucky, Lexington, KY, USA.

Osteoclasts are terminally differentiated cells of hematopoietic origin. Mechanisms coordinating bone degradation by osteoclasts and new bone synthesis by osteoblasts are poorly defined. We have identified an osteoclast-secreted chemotactic cytokine that we have reported stimulates migration of human mesenchymal cells. The signaling pathways and receptors involved in mediating these effects have not been examined. We present data demonstrating that mim-1 stimulates rapid concentration and time dependent changes in protein phosphorylation in human mesenchymal cells. A GST-mim-1 fusion protein was constructed and used to purify mim-1 by GST-Sepharose affinity chromatography. Mim-1 was cleaved from the GST peptide with thrombin and purified. Western analysis, with a rabbit polyclonal antibody, confirmed the construct was cleaved. Human mesenchymal cells were incubated with increasing concentrations of purified mim-1 (0.1-2.0 μg/ml), washed and lysed in solubilization buffer containing a cocktail of protease and phosphatase inhibitors. Equivalent amounts of protein were resolved on 10% SDS PAGE and changes in protein phosphorylation were determined by Western analysis. Mim-1 stimulated a concentration dependent increase in tyrosine phosphorylation of five proteins with molecular weights of 42, 44, 48, 60 and 62 kDa. Changes in phosphorylation were maximal at 1 μg/ml (15 nM). Mim-1 (2 μg/ml) also stimulated time dependent changes in phosphorylation. Changes in phosphotyrosine were maximal by 3 minutes and declined toward control levels after 10 minutes, but remained elevated at 60 minutes. Western analysis with the phospho-specific p42/p44 MAP kinase antibody confirmed similar kinetics of activation of the MAP kinase-signaling pathway. In conclusion, expression and secretion of an osteoclast derived chemotactic cytokine that stimulates both time and concentration dependent changes in cellular phosphotyrosine levels and activates the MAP kinase pathway in osteoblastic precursor cells suggests a receptor mediated signaling pathway. Mim-1 dependent signaling in osteoblastic precursor cells is novel, and activation of MAP kinase suggests changes in transcription factor activity since MAP kinase is known to phosphorylate transcription factors upon activation and translocation to the nucleus.

SU264

The Rapid Decline in Immediate-Early Gene Expression After Stimulation by PTH Is Not Due to Decreased cAMP Levels or PTH Receptor Desensitization. X. Chen,* J. C. Dai, E. M. Greenfield. Case Western Reserve University, Cleveland, OH, USA.

In osteoblasts, PTH rapidly and transiently stimulates mRNA expression of many immediate-early genes, including IL-6 and *c-fos*. The goal of this study was to determine whether the decline in mRNA expression is due to the decreased cAMP levels that result from desensitization of the PTH receptor. We first confirmed that IL-6 and *c-fos* mRNA levels are rapidly and transiently stimulated by 100 nM PTH(1-34) in ROS 17/2.8 cells. Thus, mRNA levels are maximal 1 hour after PTH addition and return to baseline within 4 hours. This pattern is characteristic of immediate-early gene expression, and indistinguishable from that found in MC3T3-E1 cells, UMR106-01 cells, and primary cultures of rat osteoblasts. The increase in IL-6 and *c-fos* mRNA expression is due to the cAMP/PKA pathway, since it is also induced by forskolin but not by PTH(3-34). Consistent with this, PTH also induces rapid and transient elevation of intracellular cAMP levels, PKA activation, and phosphorylation of CREB, with maximal effects at 1, 15, and 30 minutes, respectively, after PTH addition. The rapid decline in mRNA levels is due to cellular desensitization, since preincubation with PTH(1-34) almost completely inhibits the IL-6 and *c-fos* mRNA response induced by a second challenge with PTH(1-34). Previous studies have shown that desensitization of the PTH receptor is primarily due to phosphorylation by G protein-coupled receptor kinase 2 (GRK2). We examined the role of GRK2 using antisense oligonucleotides and transfection with antisense plasmids under the control of the Tet-Off gene expression system. These approaches almost completely reduce the level of GRK2 mRNA and protein, and inhibit PTH-induced receptor desensitization by 62% and 81%, respectively, as assessed by measuring cAMP production induced by a second challenge with PTH(1-34). However, neither approach alters the rapid decline in IL-6 and *c-fos* mRNA levels. Negative controls included no treatment, sense oligonucleotides, sense transfectants, irrelevant transfectants, and antisense transfectants incubated with tetracycline. Addition of the phosphodiesterase inhibitor IBMX (100uM) completely blocks the decline in cAMP levels for at least 10 hours after stimulation with PTH(1-34). However, IBMX does not prevent the rapid declines in PKA activation, CREB phosphorylation, or expression of IL-6 and *c-fos* mRNA. Taken together, our results demonstrate that decreased cAMP levels and desensitization of PTH receptors are NOT responsible for the rapid declines in either activation of downstream signal transduction molecules or expression of immediate-early genes that occur following stimulation by PTH.

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The Specificity of Signal Transduction Responses to Osteoblast Perturbation. Y. M. Kim,* S. Teng,* R. Landesberg, R. W. Katz. Columbia University School of Dental and Oral Surgery, New York, NY, USA.

Extracellular signal regulated kinases (ERKs) are activated in osteoblast wound responses in vitro. ERK Phosphorylation results in kinase activation and subsequent phosphorylation of several transcription factors involved in cell proliferation. This can be demonstrated by increased rates of proliferation after perturbation of osteoblastic tissue culture monolayers. The purpose of the present study is to determe whether other mitogen activated protein kinases (MAPKs) may be activated in the wound response model. Stress activated protein kinases (SAPKs) [also known as C-jun NH2 kinases (JNKs)] are kinases of 46 and 55 kD. This second MAPK pathway is activated largely by inflammatory cytokines resulting from stress inducing stimuli. The p38 MAPK cascade constitutes a third MAPK pathway. Similar to the SAPK pathway, p38 MAPK is activated in response to a variety of cellular stresses.The osteoblast cell line, IRC 10/30 myc 1, was grown to confluence in 100 mm dishes, serum starved and then wounded. Cultures were harvested and cells lysed at various times after wounding. Anisomycin, a ribosomal poison which is known to activate p38 MAPK and SAPKs, was used to generate positive osteoblast responses in these specific cascades. Equal amounts of protein were electrophoresed in SDS polyacrylamide gels and the separated proteins were transferred to PDVF membranes. Antibodies to phospho-ERK,SAPK or p38 were used to probe the membranes for the presence of activated MAPKs using chemiluminescent immunoblotting techniques. Subsequent reprobing with antibodies specific for these enzymes independent of their phosphorylation state were used to confirm equal loading between lanes.Both p38 MAPK and SAPKs showed increased amounts of phosphorylation within 15 minutes when the cells were treated with anisomycin. The wounding protocol caused 4-6 fold increases in the amounts of phosphorylated ERK within ten minutes of wounding and returned to basal levels by three hours. There was no change in the levels of phosphorylated p38 MAPK or SAPKs in response to wounding. This indicates that the confluent cells respond to perturbation with the specific activation of the ERK cascade and do not show a significant cross activation of parallel but distinct signal transduction pathways.

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Insulin Induces Nuclear Translocation of IRS-1 with Activation of PI3-Kinase and p70 S6 Kinase in the Nucleus of UMR-106 cell. S. J. Kim, K. C. Seol.* Pharmacology, Kyung Hee University, School of Dentistry, Seoul, Republic of Korea.

Following the activation of insulin receptor tyrosine kinase in response to insulin, insulin signaling pathways are mediated by tyrosine phosphorylation of IRS-1 (insulin receptor substrate 1) and a series of serine/threonine kinases such as MAP kinase, PI_3-Kinase and p70 S6 Kinase. PI_3-Kinase activation leads to translocation of glucose transporter to plasma membrane and it is an upstream kinase responsible for the activation of p70 S6 Kinase. The PI_3-Kinase is an important mediator for the cell transformation, differentiation, mitogenesis and anti-apoptotic activity. PI_3-Kinase is mainly localized in the perinuclear region of many cells but it is also found in inner nuclear matrix components of osteosarcoma cells. In the present study, we explored to determine if insulin has any effect on the nuclear translocation of insulin receptor, IRS-1, PI_3-Kinase and p70 S6 Kinase and their potential interactions in the nucleus of UMR-106 cells. Significant amount of insulin

receptors and IRS-1 proteins were detected in the nucleus. IRS-1 and PI_3-Kinase appeared to translocate to the nucleus in a time dependent manner by insulin while insulin receptor levels were not changed by insulin treatment. Tyrosine phosphorylation of 85 KDa protein in the nucleus was significantly stimulated by insulin, suggesting PI_3-Kinase was activated in the nucleus by insulin treatment. p70 S6 Kinase, a downstream target of PI_3-Kinase was transiently appeared in the nucleus by insulin and its activity was stimulated by insulin. These results suggest that the insulin signaling system containing insulin receptor, IRS-1, PI_3-Kinase and p70 S6 Kinase operates in the nucleus of osteoblast cells. The nuclear insulin signaling may play an important role in the gene expression, differentiation and growth of osteoblast cells.

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Agonists for Peroxisome Proliferator Activated Receptor Increase Osteogenesis In Vitro and In Vivo. K. Still,* A. M. Scutt. Child Health, University of Sheffield, Sheffield, United Kingdom.

A number of substances, in particular prostaglandin E_2 (PGE_2), are known which stimulate bone formation when administered to adult animals. These anabolic agents are unsuitable for use in the clinic because of their profound side-effects. In vivo, PGE_2 is rapidly degraded, to PGA_2, and we have previously reported that some of the bone anabolic effects of PGE_2 may be caused by PGA_2 (Still and Scutt, Prostaglandins and other lipid mediators, In Press). In these studies, it was found that PGA_1 and A_2 both stimulated colony formation in a dose-dependent manner with a peak at 10^{-6} M and to a similar degree to PGE_2. PGA_1 and A_2 also dose-dependently stimulated collagen synthesis by neonatal rat calvaria, PGA_2 is known to bind to the family of PPAR nuclear receptors. Therefore, we investigated the effects of a number of PPAR agonists on colony formation using the CFU-f assay. Briefly, bone marrow cells were isolated from rat tibiae and femurae. 10^6 nucleated BMC were plated out in 55 cm^2 petri dishes in DMEM containing 10% FCS, 10^{-8} M dexamethasone and 50 µg/ml ascorbic acid. The medium was changed after 5 days and thereafter twice weekly. The cultures were maintained for 12 days after which the cells were washed with PBS and fixed by the addition of cold ethanol. After fixation, the cultures were sequentially stained for alkaline phosphatase (ALP), calcium and collagen-positive colonies. In these studies, agonists specific for the PPARs caused a dose-dependent increase in osteoblastic colony number and differentiation. In particular, Bezafibrate. Fenofibrate and linoleic acid, caused an anabolic response similar to, or greater than that of PGE_2. The effect of these drugs was then examined *in vivo*. Briefly, Wistar rats were injected daily with linoleic acid (1mg/kg or 0.3mg/kg), Bezafibrate (10mg/kg or 1 mg/kg) or Fenofibrate (10mg/kg or 1mg/kg) for 12 weeks. Metaphyseal bone mineral density was increased in all groups compared to the vehicle. In conclusion, PPARs do appear to play a role in bone formation.

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Interaction between BMP, Extracellular Matrix, and MAP Kinase Signaling Pathways in the Regulation of Osteoblast-Specific Gene Expression and Differentiation. G. Xiao,* R. Gopalakrishnan, D. Jiang,* E. Reith,* R. Franceschi. The University of Michigan, Ann Arbor, MI, USA.

Osteoblasts secret a complex extracellular matrix (ECM) containing collagenous and noncollagenous proteins, bone morphogenetic proteins (BMPs) and growth factors. Osteoblast-specific gene expression requires ascorbic acid (AA)-dependent assembly of a collagenous ECM. Matrix responsiveness requires an alpha2beta1 integrin-collagen interaction and mitogen-activated protein kinase (MAPK) activity which phosphorylates and activates the osteoblast-specific transcription factor, Cbfa1 (Xiao et al., 2000, J Biol Chem 275;4453). This study examines interactions between this integrin/MAPK-mediated pathway and signals initiated by BMPs contained in the osteoblast matrix. MC3T3-E1 cells were shown to constitutively express BMPs 2, 4, and 7. Noggin, a specific BMP inhibitor, reversibly blocked AA-induced gene expression indicating that autocrine BMP production was necessary for differentiation. Exogenously added BMPs 2, 4 or 7 only stimulated OCN and BSP mRNAs or OCN promoter activity in cells that were actively synthesizing an ECM (i.e. were grown in the presence of AA). A minimum of 4 d of AA pretreatment was required for cells that acquire the ability to respond to added BMP. Neither BMP7, AA or a combination of these two treatments stimulated Cbfa1 mRNA or protein levels as would be expected if regulation was mainly at the post-transcriptional level. U0126, a specific inhibitor of MAPK/extracellular signal-regulated kinase (MEK), blocked AA- or BMP7/AA-dependent gene expression in a time and dose-dependent manner that was closely correlated with inhibition extracellular signal-regulated kinase (ERK) phosphorylation. This work establishes that autocrine BMP production as well as integrin-mediated cell:collagen interactions are both required for osteoblast differentiation, and both these pathways require MAP kinase activity.

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PTHrP Prevents Osteoblast-like Cell Apoptosis Through the cAMP/PKA and AP-1 Signaling Pathway. A. Schneider, H. Chen, A. J. Koh,* C. Wang,* L. K. McCauley. School of Dentistry, University of Michigan, Ann Arbor, MI, USA.

Parathyroid hormone-related protein (PTHrP) is an autocrine, paracrine or intracrine cytokine that mediates anabolic and catabolic actions in skeletal tissues. It is known that low, intermittent administration of PTHrP results in anabolic effects on bone; however, the cellular and molecular control is not clearly understood. Recent studies indicate that anabolic actions of PTH are associated with an increased osteoblast lifespan due to decreased apoptosis. The purpose of the present investigation was to determine whether PTHrP had similar effects and elucidate the signaling pathway by which PTHrP exerts its protective role against osteoblast apoptosis. C3H10T1/2 (a pluripotential murine mesenchymal cell line) and MC3T3-E1 (a preosteoblastic murine cell line) cells were both utilized for these studies. Also, stable transfectants (PTH-1R WT) encoding the wildtype PTH/PTHrP receptor were generated in C3H10T1/2 cells with PerFect Lipid-2 and selected under G418. Cells were plated overnight at 30,000 cells/cm^2 and the following day, pretreated with PTHrP (1-34)(10^{-7}M; 1h) or vehicle. Dexamethasone (Dex)(10^{-7} M), a known stimulator of apoptosis, or vehicle was then added to cultures for 6h and cell viability determined by trypan blue exclusion. There was a significant reduction in viable cell number with Dex treatment ($p<0.05$), suggestive of an apoptotic event. Addition of PTHrP prior to Dex treatment resulted in numbers similar to vehicle only-treated cells and significantly higher than Dex-treated cultures ($p<0.05$), suggesting a survival-promoting effect. Likewise, treatment with forskolin (10^{-8} M) reversed the Dex-induced reduction in viable cell number, indicating that PTHrP exerts a protective effect through the cAMP pathway. PTH-1R WT cells had similar results under the same treatment protocol. In order to confirm the dependence of the PTHrP-induced anti-apoptotic effect on the cAMP/PKA pathway, downstream mediators CREB and the AP-1 complex, were altered in PTH1-R WT cells. Transient transfections with pCMV-CREB133 (a mutant preventing Ser133 phosphorylation) or TAM-67 (a c-jun dominant negative) were performed with Lipofectamine Plus. After a 3-hr incubation, cells were pretreated with PTHrP or vehicle prior to Dex treatment and cell viability assessed as described above. Both mutant vectors significantly blocked the PTHrP-mediated protective effect as compared to control transfectants ($p<0.05$). In summary, our findings suggest that PTHrP (1-34) mediates its survival effect by activating the PTH/PTHrP receptor, the cAMP/PKA signaling pathway and the AP-1 family of transcription factors.

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Function of Connexin 43 and Connexin 45 in the Reciprocal Interaction between Human Endothelial Cells and Human Bone Marrow Stromal Cells. B. Guillotin,[1] F. Villars,*[1] R. Bareille,[2] L. Bordenave,*[1] J. Amedee.[1] [1]U443-INSERM Université Victor Segalen Bordeaux 2, Bordeaux, France, [2]U443-INSERM, Bordeaux, France.

Bone development and remodelling depend on cellular interactions between bone-forming osteoblasts, bone-degrading osteoclasts, and other cells present within the bone microenvironment. Our previous data demonstrated that Human Umbilical Vein Endothelial Cells (HUVEC) and Human Bone marrow Stromal cells (HBMSC) are coupled and that one of the connexins identified in bone cells, connexin43 (Cx43) may be involved in this heterotypic interaction. The aim of our study is to investigate the function of the connexin45(Cx45) and to analyze their expression in the coculture HBMSC/ HUVEC in direct contact. HBMSC were cultured alone in IMDM+10% SVF or with direct contact with HUVEC for 6 days. Cell differentiation of HBMSC was evaluated by the measurement of alkaline phosphatase activity, osteocalcin synthesis by dot immunodetection or by RT-PCR analysis. HUVEC differentiation was assessed by the von Willebrand (vW) and CD31 expression identified also by dot-immunodetection or by RT-PCR analysis. Western blot was performed to measure the synthesis of both Cx43 and Cx45 in HBMSC or HUVEC cultured alone or in direct contact. Dot-immunodetection of bone specific markers and endothelial markers revealed that the direct contact between HUVEC and HBMSC both increases osteoblastic differentiation of HBMSC (ALP, OC) and HUVEC differentiation (vW, CD31). This “juxtacrine signalling” could involved a number of different heterotypic connexions which require adhesion molecules (cadherins, selectins, immunoglobulin superfamily, integrins) or gap junctions (connexins). Previous data revealed that both HBMSC and HUVEC expressed a gap junction protein, the connexin 43. Both RT-PCR, immunocytochemistry and Western Blot demonstrated now that HUVEC also expressed Cx45 mRNA and synthesized the protein, as well as HBMSC. Moreover, the synthesis of Cx43 and Cx45 are enhanced in the coculture HUVEC/HBMSC compared to the isolated cell cultures. Moreover, inhibition of connexin 43 synthesis using oligo-desoxyribonucleotides (ODN) antisens (10 mM for 6 days) decreases the effect of HUVEC co-cultures on HBMSC differentiation. In opposite, inhibition of connexin 45 synthesis using ODN antisens (10 mM for 6 days) appears to increase the ALP activity in HBMSC. In conclusion, this coculture of HUVEC and HBMSC appears to modulate both endothelial and osteoblastic phenotype. Moreover, these results confirm the function of Cx43 in osteoblastic differentiation and the opposite role of Cx45 in this differentiation process.

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The Capacity of Human Bone Marrow Stromal Cells to Form Bone in Vivo Is Maintained With Age. K. Stenderup,[1] C. Rosada,*[1] J. Justesen,[1] T. Al-Soubky,*[1] E. F. Eriksen,[1] F. Dagnaes-Hansen,*[2] M. Kassem.[1] [1]University Department of Endocrinology and Metabolism, Aarhus University Hospital, Aarhus, Denmark, [2]Department of Medical Microbiology and Immunology, University of Aarhus, Aarhus, Denmark.

Aging is associated with decreased bone formation that may result from an intrinsic senescence-associated decrease in osteoblastic cellular-functions or lack of signals (including growth factors and extracellular matrix components) necessary for the optimal osteoblast performance. We have previously shown that human bone marrow stromal cells (hMSCs) obtained from young and old donors exhibited similar *in vitro* proliferation rate and differentiation potential as evidenced by similar levels of osteoblastic markers (e.g. alkaline phosphatase and osteocalcin). However, it is not known whether these *in vitro* osteoblast characteristics can reflect osteoblast *in vivo* bone formation capacity. Thus, we studied the *in vivo* bone formation of hMSCs obtained from young and old donors. hMSCs were established from bone marrow aspirated from iliac crest from young donors (mean age 34 years) and old donors (mean age 70 years). hMSCs were cultured *in vitro* until confluence and 5 x 10^5 hMSCs were mixed with hydroxyapatite-tricalciumphosphate (HA/TCP) and implanted subcutaneously in immunodeficient mice (NOD/LtSz-*Prkdc*^{scid}). Based on a large number of experiments, we have found that 5 x 10^5 hMSCs per implant are optimal for inducing osteogenesis. We employed human breast fibroblasts (hBF) and HA/TCP alone as negative controls. Twelve implants containing hMSCs from young or from old donors were made. After 8 weeks, the implants were removed and embedded in methyl methacrylate. Sections were stained histochemically with Goldner-Trichrome and immunostaining was performed using human-specific antibodies against osteonectin and collagen type I. The amount of bone formed (bone volume/total volume, BV/TV) was quantified by point-counting. All implants containing hMSCs formed osteoid and lamellar bone of normal appearance and no bone was found in the implants containing HA/TCP alone or mixed with hBF. Donor origin of newly formed bone was demonstrated by immunostaining for osteonectin and collagen type I. We found that BV/TV formed in the

implants containing young cells (9% ± 6%, mean ± SD) was not significantly different from that formed in implants containing old cells (12% ± 6%, mean ± SD). Our study demonstrates that the capacity of hMSCs to form bone *in vivo* is maintained with age and suggests that the observed senescence-associated decrease in bone formation is due to a defect in the bone microenvironment, the nature of which remains to be determined.

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Genetic Labelling of Human Bone Marrow Osteogenic Stem Cells: Differentiation Potentials and Engraftment into NOD/SCID Mice. Z. Xia,*[1] K. Hollis,*[1] J. Rohll,*[2] J. T. Triffitt.[1] [1]Bone Research Laboratory, Nuffield Department of Orthopaedic Surgery, Oxford University, Oxford, United Kingdom, [2]Oxford Biomedica UK Ltd., Oxford, United Kingdom.

Osteogenic stem cells from bone marrow have great potential to form bone tissue when introduced to a host tissue site. The current study aimed to optimise the genetic labelling of human bone marrow osteogenic stem cells by using replication incompetent retroviral vectors encoding enhanced green fluorescent protein (eGFP) and neomycin phosphotransferase (neo) and to study the fate of these cells in vivo after implantation into immunocompromised animals. Murine leukemia virus (MuLV) vectors encoding eGFP and pseudotyped with the envelopes of either amphotropic murine leukemia virus (4070A), a mutant form of A-MuLV, which can utilise both Pit-1 and Pit-2 receptors (4070-3), gibbon ape leukemia virus (GaLV), feline endogenous virus (RD114) or with vesicular stomatitis virus G protein (VSV-G) were used to infect human bone marrow fibroblastic (HBMF) cells in vitro. Most efficient gene transfer was observed with the 4070A and 4070-3 pseudotypes. Transduced HBMF cells could also be selected by treatment with the antibiotic, G418. Cell counting, MTT assay, quantitative alkaline phosphatase (APase) and DNA assays were used to evaluate the proliferation and differentiation characteristics of transduced HBMF cells in vitro. eGFP-expressing cells were also implanted into a variety of tissue sites in severe combined immunodeficient/nonobese diabetic (NOD/SCID) mice. The results show that there is no significant difference in proliferation or differentiation characteristics between any groups of normal, eGFP-labelled and G418-selected cells ($p > 0.05$). The results of immunocytochemical staining for the presence of APase and osteocalcin suggest that eGFP-labelled cells have normal potential to differentiate s into osteoblast-like cells in vitro. eGFP expression in bone tissue was observed by fluorescence microscopy at both 1 and 2 weeks post-engraftment into NOD/SCID mice. In conclusion, the use of eGFP as a genetic-maker does not significantly affect the proliferation or differentiation of the HBMF cells over the time period studied. Optimisation of retroviral methods of gene delivery to HBMF will be of great value for study of the function and behaviour of osteogenic stem cells both in in vitro and in vivo and will have significant impact on the development of techniques for engineering of bone tissue.

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Evidence for Differentiation Ability of Human Preadipocytes into Osteoblasts. J. Justesen,* K. Stenderup,* S. B. Pedersen,* E. F. Eriksen, M. Kassem. University Department of Endocrinology and Metabolism, University Hospital of Aarhus, Aarhus, Denmark.

A close relationship between osteoblastic and adipocytic differentiation has been demonstrated. Human bone marrow stromal cells (MSCs) and trabecular osteoblasts are able to differentiate into mature adipocytes under appropriate culture conditions. However, it is not known whether cells from the adipocytic lineage are capable of differentiating into osteoblasts. Thus, we purified human preadipocytes and mature adipocytes from subcutaneous adipose tissue and tested their osteoblastic differentiation potential *in vitro*. Adipose tissue was obtained from human donors during liposuction. Preadipocytes were isolated by collegenase treatment, centrifugation, and plastic adherence. Mature adipocytes were treated with collegenase and washed before re-differentiation to a fibroblast-like morphology using 10% fetal calf serum (FCS). To promote osteoblast differentiation the cells were grown in 10% FCS alone or supplemented with calcitriol (10nM) or dexamethasone (100nM) and calcitriol (10nM). For *in vitro* mineralisation the preadipocytes were treated with 10% FCS and ascorbic acid (0.87mM). To promote adipogenesis the cells were grown in a medium containing insulin (100nM), T_3 (0.2nM), dexamethasone (100nM), and isobutylmethylxanthine (0.25mM). At the beginning of culture, the preadipocytes exhibited a fibroblast-like morphology similar to MSCs. However, in adipogenic medium the cells formed mature, lipid-filled adipocytes. Growing the cells in medium containing 10% FCS and calcitriol stimulated expression of osteoblast phenotype mRNA markers: Cbfa1, osteocalcin, alkaline phosphatase, and osteopontin. Dexamethasone together with calcitriol decreased the mRNA expression of the previously mentioned markers compared with calcitriol supplement alone. In the presence of ascorbic acid, a mineralised matrix could be visualised by alizarin red S staining. The ability of mature adipocytes to differentiate into osteoblasts was also studied. Mature adipocytes incubated with 10 % FCS lost their characteristic morphology and reverted to a fibroblast-like appearance. The expressions of osteoblastic markers are currently being investigated as well as the ability for *in vitro* and *in vivo* mineralisation. Preliminary studies show that the cells are positive for alkaline phosphatase staining and capable of differentiating into adipocytes. Our results demonstrate the existence of differentiation plasticity between adipocytic and osteoblastic cells and may suggest the presence of a population of stromal cells in different organs that can give rise to adipocytes and osteoblasts according to tissue needs.

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A Norvel Bone Resorbing Factor Gamma-glutamil Transpeptidase Stimulates Osteoclast Formation in Pathological Condition. S. Niida,[1] Y. Ishizuka,*[2] M. Kawahara,*[3] N. Maeda.*[1] [1]Anatomy, Hiroshima university School of Dentistry, Hiroshima, Japan, [2]Foundation and Advancement of International Science, Tsukuba, Japan, [3]Orthdontics, Hiroshima University, Hiroshima, Japan.

We have previously reported that gamma-glutamyl transpeptidase (GGT) was identified as a novel osteoclast forming factor involved in the bone metastasis of mouse T-lymphoma cells. The aim of this study was to characterize the biological activity of GGT in osteoclastgenesis. The anti-GGT antibody inhibited osteoclast-like cell (OCL) formation in mouse bone marrow culture in the presense of 10nM 1,25(OH)2D3 and GGT. Furthermore, OCL formation stimulated by higher concentration (100nM) 1,25(OH)2D3 or PTH alone was also blocked by the antibody. Then, we examined GGT expression in bone marrow cells using RT-PCR. Expression of GGT mRNA was detected in the samples, suggesting that GGT may play a role in OCL formation pathway involving 1,25(OH)2D3 or PTH. The antibody, however, did not neutralize the enzyme activity of GGT in these examinations. Previously, we confirmed GGT expression in arthritis of the CIA mouse. Culture of isolated cells from arthritic paws induced many OCLs without any exogenous stimulation of cytokines or hormones. This activity was also blocked by anti-GGT antibody. To further investigate the effect of GGT in the osteoclastgenesis, we examined the inhibition potential of the antibody in this culture system by varying the addition time of the antibody during a 5-day culture period. The OCL formation was inhibited by the antibody added within 24 hrs, whereas no effect was observed in examinations adding antibody on and after 48 hours. These results suggested that GGT serves as the OCL formation and thereby would participate in malignant bone resorption under the pathological conditions such as bone metastasis of tumor cells or RA. Moreover, our preliminary evidence indicated that GGT may play a role in the early stage of OCL formation. GGT is one of the most popular marker enzymes for diseases in clinical chemistry. The present study also suggests a novel clinical significance of GGT, which has not been considered so far, and would potentially provide a clue for mechanism of secondary osteoporosis induced by hepatic disease or RA of unknown etiology.

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Effects of Different Growth Factors on Human Osteoclast Differentiation and Resorption. T. A. Hentunen, H. K. Väänänen. Department of Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland.

The aim of this study was to develop human osteoclast differentiation and resorption assay for studying the regulation of human osteoclast differentiation and function. The combination of soluble forms of growth factors, receptor activator of NF-kB ligand (RANKL, also called TRANCE/ODF) and macrophage colony-stimulating factor (M-CSF) is powerful for inducing osteoclast formation and resorption activity. By culturing isolated human peripheral blood mononuclear cells (PBMC) for 8-21 days on bovine bone slices in the presence of RANKL (20 ng/ml) and M-CSF (10 ng/ml), we found that tartrate-resistant (TRAP)-positive multinucleated cells (MNC) started to be formed at day 14. Their resorption activity was rapidly increased after that. To find out if osteoclast precursors are adherent or non-adherent, isolated human PBMC were let to attach to a tissue culture dish overnight in the alpha-MEM- 10 % foetal calf serum. Non-adherent cells were collected and plated on bone slices (106/slice). Adherent cells were trypsinized from the dish and plated on bone slices (50000/slice). Cells were cultured for 21 days in the presence of RANKL and M-CSF. At the end of the culture, cells were stained for TRAP and TRAP-positive MNC were counted. Cells were then removed from bone slices by brushing and resorption pits were stained using peroxidase-conjugated WGA-lectin. In adherent and non-adherent cell cultures, there were 172.8 ± 62.9 and 240.5 ± 115.0 resorption pits/bone slice, respectively. Adherent cells had 14-fold higher pit formation capacity than non-adherent cells when pit numbers were compared to the number of cells plated. When we used the whole PBMC fraction, there were approximately 490.8 ± 126.8 pits and 88.5 ± 23.7 TRAP-positive MNC on bone slices after a 21-day culture. Using whole PBMC fraction, TNF-a (10 ng/ml) significantly enhanced RANKL/M-CSF-induced osteoclast formation and resorption 5-fold and 21-fold, respectively and dexamethasone (10-8 M) further stimulated this 5-fold and 12-fold, respectively. 17b-estradiol at 10-9 M inhibited RANKL/M-CSF/TNF-a/dexamethasone-induced osteoclast formation and resorption 55-61% as analyzed by counting the number of TRAP-positive MNC or by determining C-terminal telopeptide of type I collagen, CTx (CrossLaps, Osteometer) in the culture medium. This data suggest that the majority of osteoclast precursors reside in the adherent population of human PBMC and that human PBMC culture can be used to study the effects of estrogen and various substances on both human osteoclast differentiation and resorption activity.

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A Voltage Gated Proton Channel in Murine Osteoclasts During Development From Bone Marrow Cells. H. Mori,* H. Sakai, H. Morihata,* K. Sakuta,* M. Kuno.* Physiology, Osaka City University Graduate School of Medicine, Osaka, Japan.

Osteoclasts secrete the proton ion (H^+), mainly by an electrogenic H^+-ATPase, to resorb mineralized bone tissue. Acid-base balance is crucial in control of osteoclast functions, so that osteoclasts are equipped with various regulatory mechanisms of intracellular pH, such as H^+-ATPase, Na^+/H^+ exchanger, and Cl^-/HCO_3^- exchanger. It is postulated that a voltage-gated H^+ channel is involved in regulation of pH, but its existence and roles in cellular functions have not been identified in murine osteoclasts. In this study, we examined the H^+ currents in osteoclasts differentiated from murine bone marrow cells in the presence of a soluble receptor activator of NF-kappa B ligand (sRANKL) and a macrophage colony stimulating factor (M-CSF). Whole cell currents were recorded at a large pH gradient through the plasma membrane (δpH=1.8) in the absence of major monovalent ions (Na^+, K^+, Cl^-). Depolarizing voltage pulses activated currents characterized by outward rectification, slow activation, and reversible blockage by zinc ion. Either increasing extracellular pH or decreasing intracellular pH enhanced the current amplitude and accelerated the activation time course. As the reversal potential of the currents was linearly related to pH with a slope of 47.2 mV per δpH, H^+ was suggested to be the main charge carrier of the currents. The current density decreased greatly with an increase in number of nuclei, from 45 +- 12 pA / pF at +100 mV in cells with 3 to 5 nuclei (mean +- S.E.M., n = 9) to 9.6 +- 6.1 pA / pF in cells with more than 6 nuclei (n = 7). The activation time constant did not change between the two groups significantly (about 300 to 600 ms at +100 mV). The H^+ current was detected in only one of 16 osteoclasts generated from co-culture of bone marrow cells with ST2 cells, (0.6 +- 0.2 pA / pF at +100 mV, n = 16). These results suggest that murine osteoclasts generated in the presence of sRANKL express a voltage-gated proton

channel and that the current activity depends on the developmental status and the culture condition.

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Osteoclast Regulation by TNF-α Occurs Through Distinct Mechanisms In Vitro Versus In Vivo. K. Fuller, S. W. Fox,* B. Kirstein,* T. J. Chambers. Department of Cellular Pathology, St George's Hospital Medical School, London, United Kingdom.

Although TNF-α is crucial to the pathogenesis of inflammatory osteolysis, the mechanisms by which it induces bone destruction are unknown. Recently, it has been found to induce osteoclast differentiation in stroma-depleted bone marrow cell cultures, suggesting a direct effect of TNF-α on osteoclast-macrophage precursors. Yet there is strong evidence that TNF-α induces osteolysis in vivo indirectly, through induction of RANKL expression: TNF-α does not induce osteoclast formation in vivo if RANKL is absent or blocked; and TNF-α stimulates RANKL expression in osteoblastic cells. As an elegant resolution to this paradox, it has been suggested that TNF-α induces osteoclastic differentiation, in vitro and in vivo, through a direct effect on osteoclast precursors that have been 'primed' by prior exposure to RANKL, and that the preparations of bone marrow cells previously used in vitro have been contaminated with RANKL-expressing stromal cells. We have tested this hypothesis. We found that although TNF-α strongly synergised with RANKL for osteoclast-induction from stroma-depleted and non-depleted bone marrow cultures, TNF-α also induced osteoclastic cells in cultures in which stroma could not be detected. Moreover, osteoclast formation, even by low concentrations of TNF-α, was completely unaffected by the continuous presence of OPG, even at OPG concentrations that abolish osteoclast formation by supramaximal concentrations of RANKL. Further, TNF-α was able to induce osteoclastic differentiation in macrophage cell lines, and in macrophage colonies in methylcellulose. These experiments do not support a role for RANKL in priming precursors for osteoclast-induction by TNF-α. Nevertheless, they strongly suggest that osteoclastic cells can respond directly to TNF-α in vitro, unlike in vivo. We therefore compared the responsiveness to TNF-α of osteoclastic cells formed in vitro by incubation of bone marrow cells in RANKL plus M-CSF, with that of osteoclasts formed in vivo. We found that in vitro-generated cells were sensitive to activation by TNF-α, while osteoclasts ex vivo were unresponsive. There is thus a clear discrepancy between the responsiveness of osteoclasts and their precursors to TNF-α, in vitro vs in vivo. The behavior and responsiveness of osteoclasts and their precursors in vitro does not always predict the behavior of the resorptive system in vivo.

SU278

Expression of NADPH Oxidase 4 (NOX4) in Bone. S. Yang,[1] P. Madyashtha,*[1] S. Bingel,*[2] W. Ries,[1] L. Key.[1] [1]Pediatrics, Medical University of South Carolina, Charleston, SC, USA, [2]Comparative Medicine, Medical University of South Carolina, Charleston, SC, USA.

Superoxide generated by osteoclasts directly contributes to bone degradation. Inhibition of osteoclastic superoxide availability results in a reduction in bone resorption. NADPH oxidase, a common enzyme system that produces superoxide in white cell phagocytes, has been demostrated present and active in osteoclasts. Several studies suggest that NADPH oxidase is responsible for osteoclastic superoxide production. A membrane bound subunit, p91, is the catalytic domain of NADPH oxidase. Recently we have cloned a homology of p91, NOX4 (AF218723), from mouse. Nox 4 with 578 amino acids has 58% similarity in amino acids with the known p91 subunit of NADPH oxidase. Nox4 is present and active in bone, especially in osteoclasts. Expression of Nox4 in p91 knockout mutants is 2.5 times higher than that from wild type animal C57Bl/6. This result suggests that upregulation of Nox4 may be expanded in the absence of native p91 protein, enabling cells to generate adequate amount of superoxide to satisfy their biologic function and activity.

SU279

DNA Microarray Analysis of Fusing Macrophages Reveals Clues about the Functional Consequences of Multinucleation. J. Zhang,*[1] J. li,*[2] H. Sterling,*[1] G. Peet,*[2] R. Barton,*[2] A. Vignery.[1] [1]Orthopaedics, Yale School of Medicine, New Haven, CT, USA, [2]Genomics and Gene Therapy, Boehringer Ingelheim Pharmaceutical Inc, Ridgefield, CT, USA.

Osteoclasts are multinucleated cells that resorb bone, and differentiate by fusion of precursor cells that belong to the monocyte-macrophage lineage. While it is well established that cytokines, chemokines and growth factors that are produced by cells located in the osteoclast microenvironment control osteoclast differentiation and activation, the question as to whether osteoclasts themselves produce soluble molecules that control their differentiation and their activity has not been investigated. We have reported previously that freshly isolated rat alveolar macrophages cultured at high density spontaneously fuse to differentiate into multinucleated macrophages that express osteoclast functional markers, in the absence of exogenously added cytokine or factor (Int J Exp Pathol 81:291, 00). This suggested that macrophages control their multinucleation by means of factors that they produce. To investigate this possibility, we subjected fusing rat alveolar macrophages to DNA microarray analyses using Affymetrix technology which employs oligonucleotide hybridization, and includes mismatched control oligos. RNA was extracted from freshly isolated macrophages, and macrophages cultured at high density for one hour, one day or five days, when multinucleation reaches 99%. Experiments were repeated thrice. Our results indicate that macrophage multinucleation was accompanied by a differentially regulated expression of transcripts coding for the canonical osteoclast markers, for signaling molecules and for transcription factors. Multinucleation was also accompanied by a highly significant accumulation of transcripts coding for MMP proteases such as MMP-9 which increased over 100 fold in multinucleated cells when compared to freshly isolated cells. Most interesting was the strongly induced accumulation of transcripts coding for the cytokines IL-1a, IL-1β, IL-6 and TNFa , the chemokines MIP-1α and b, all of which took place on day five when transcripts coding for Macrophage Inhibiting Cytokine-1 mRNA had decreased. In addition to cytokines and chemokines, transcripts coding for extracellular matrix proteins and growth factors were highly induced, suggesting that multinucleated macrophages control their own microenvironment. This analysis revealed that macrophage multinucleation is accompanied by the regulated expression of a specific set of genes and suggested a sophisticated cross talk between osteoclasts and their microenvironment via a network of cytokines, chemokines and growth factors.

SU280

TNF-alpha and Other Osteoclast-stimulating Cytokines Inhibit Alendronate Activation of the Apoptosis-related MST1 Kinase, in Mouse Osteoclasts. H. Glantschnig,* G. A. Rodan, A. A. Reszka. Bone Biology & Osteoporosis Research, Merck & Co., Inc., West Point, PA, USA.

Nitrogen-containing bisphosphonates reduce protein geranylgeranylation, which leads to inhibition of osteoclast activity and ultimately osteoclast apoptosis, in vitro. We have identified the mammalian STE20-like (MST) 1 serine/threonine protein kinase as being activated by caspase cleavage during osteoclast apoptosis. However, it is not known where MST1 kinase activation fits into the osteoclast apoptotic pathway and how it is regulated. In this study we examined the effects of the osteoclast differentiation and survival factors RANKL, TNF-alpha, IL-1alpha, IL-6 and M-CSF on MST1 caspase cleavage and kinase activation, under basal conditions and during alendronate (ALN)-induced apoptosis.Osteoclast-like cells were differentiated from mouse bone marrow with MB1.8 osteoblasts. Osteoclast cultures were treated with or without the respective cytokine at 1 to 200 ng/ml for 18-20 hrs. Osteoclast protein lysates were collected and analyzed for prenylated proteins by immunoblotting and for kinase activity by in-gel-kinase assays, with myelin basic protein as substrate.We found that all cytokines tested, except IL-6, strongly suppressed the basal and ALN-induced 34 kDa MST1 kinase activity. We also observed that a separate 36 kDa protein kinase was subject to similar regulation as MST1. The suppression was dose dependent and the rank order potency was roughly M-CSF= TNFalpha= IL-1alpha>RANKL. Dose response studies revealed that maximum suppression of spontaneous apoptosis was achieved at lower concentrations than those required for maximum suppression of ALN-induced apoptosis, suggesting intersection of the cytokine and ALN pathways. The cytokines also suppressed apoptosis and MST1 activation induced by a specific inhibitor of geranylgeranylation. However, none influenced the prenylation of osteoclastic proteins, suggesting a downstream event. Whereas forskolin, an activator of PKA, was a potent inhibitor of apoptosis and MST1 kinase activation induced by ALN or CL2 (100 uM) after up to 24 hrs, the cytokine treatments did not influence the CL2 -induced effects when measured at 20 hrs.A time course showed that 3 hrs treatment with TNFalpha or IL-1alpha was sufficient to significantly reduce ALN-induced MST1 caspase cleavage and activity to basal levels. In parallel, a higher molecular weight kinase activity, identified as MST3, was increased.In conclusion we show that the kinases MST1 and MST3, are under the control of osteoclastogenic cytokines, most likely via regulation of caspase activities and through non-caspase intracellular signaling pathways, respectively.

SU281

Decreased Cancellous and Cortical Bone Mass in Female Mice Lacking the P2X7 Receptor. K. L. Chidsey-Frink,* H. Qi, D. T. Crawford,* H. A. Simmons, L. P. Audoly,* C. A. Gabel,* D. D. Thompson, H. Z. Ke. Pfizer Global Research and Development, Goton Labs., Groton, CT, USA.

The P2X7 receptor (P2X7R) is an ATP-gated, non-selective ion channel expressed by monocytes, macrophages, and osteoclasts. To understand the role of this receptor in regulating bone mass in mice, we characterized the bone phenotype of P2X7R-deficient (KO) mice as compared with genetically and age-matched wild-type controls (WT). The KO mouse line was generated by targeted gene disruption as previously published (Solle M et al., J. Biol. Chem., 2001;276:125-132). Female WT (n=13) and KO (n=13) mice were weighed and necropsied at 8 weeks of age. There was no significant difference in body weight in KO mice compared with WT controls. Similarly, there was no significant difference in femoral length between KO and WT controls, indicating that this receptor may not play a role in longitudinal bone growth. However, KO mice had significantly lower cortical content (-13%) and density (-5%) at the distal femoral metaphysis as determined by pQCT when compared with WT controls. In the femoral mid-shaft, KO mice had significantly lower total content (-12%) and density (-11%), trabecular content (-26%), cortical area (-8%), cortical content (-10%), periosteal circumference, and endocortical circumference when compared with WT controls. Distal femoral metaphyseal cancellous bone histomorphometric analysis showed that KO mice had significantly lower trabecular bone volume (-32%), thickness (-18%), number (-19%), and significant higher trabecular separation (+40%) as compared with WT controls. In addition, a non-significant increase in osteoclast surface (+46%, p=0.053) was found in KO mice as compared with WT controls. In summary, our results indicate that decreased cancellous and cortical bone mass and poor cancellous bone architecture was found in mice lacking the P2X7 receptor. We conclude that the P2X7 receptor plays an important role in both cortical and cancellous bone mass augmentations in rapidly growing female mice. The possible role of the P2X7 receptor in osteoclastic bone resorption is currently being investigated.

Disclosures: Pfizer Inc.,3.

SU282

Osteopontin is a Required Osteoclast Autocrine Regulating Rho Dependent Motility and Bone Resorption. M. Chellaiah,[1] N. Kizer,[2] U. Alvarez,*[2] J. Strauss-Schoenberger,*[2] L. Rifas,[3] S. R. Rittling,[4] D. T. Denhardt,[4] K. A. Hruska.[2] [1]Oral/Craniofacial Biological Services, University of Maryland, Baltimore, MD, USA, [2]Renal Division, Barnes-Jewish Hospital at Washington University, St. Louis, MO, USA, [3]Division of Bone & Mineral,

Barnes-Jewish Hospital at Washington University, St. Louis, MO, USA, [4]Cell Biology and Neuroscience, Rutgers University, Nelson Labs, Piscataway, NJ, USA.

Activation of the Rho GTPase stimulates podosome assembly in osteoclast and is required for motility and normal bone resorption rates (Chellaiah et al, JBC 2000). Since osteopontin (OP) stimulates podosome assembly and the pathways activated by Rho, we questioned whether there was dependency on OP in the activity of the downstream Rho targets, mDia1, Rho kinase (ROK), and CD44. In wild type osteoclasts, OP increased plasma membrane localization of CD44, association of CD44 with ROK, and mDia1 association with the podosome acting capping protein, gelsolin. In OP deficient osteoclasts, CD44 localization was disorganized and not ROK associated. Furthermore, mDia1-gelsolin association was markedly diminished. These biochemical and cell biologic abnormalities resulted in decreased osteoclast motility and bone resorption. Exogenous addition of OP to OP-/- OC's restored CD44 localization with ROK and increased mDia1-gelsolin association rescuing cell motility. Bone resorption was only partially restored by exogenous osteopontin which did not correct for the absence of OP in the resorption space beneath the ruffled border. A defect in osteoclast mediated bone resorption was demonstrated in vivo in osteopontin deficient mice. We conclude that osteopontin deficiency produces a decrease in cell motility and bone resorption through abnormal function of Rho effectors.

SU283

Direct Inhibition of Human Osteoclast Generation by 1,25 dihydroxy Vitamin D3. K. J. Splatt,[*1] C. J. Aitken,[*1] D. E. Myers,[1] J. M. Hodge,[*1] M. J. Constable,[*1] G. C. Nicholson.[1] [1]The University of Melbourne, Geelong, 3220, Australia.

The major physiological effect of $1,25(OH)_2D_3$ (VitD3) on bone is to mobilise calcium stores when dietary calcium is inadequate to maintain normal plasma calcium levels. This is achieved by stimulation of osteoclastic bone resorption. Paradoxically, when given systemically, VitD3 can increase bone density and reduce fractures and is used to treat osteoporosis. Current evidence indicates that VitD3 stimulates osteoclastogenesis indirectly, via stromal cells/osteoblasts, by up-regulating their expression of RANKL and down-regulating OPG expression. However, it has been reported that VitD3 acts directly on human osteoclast hematopoietic precursors to inhibit osteoclastogenesis (Itonaga *et al.*, 1999).In the current study we have investigated the effects of VitD3 on osteoclastogenesis in models employing human PBMC or $CD14^+$ cells treated with soluble RANKL (sRANKL) and human macrophage-colony stimulating factor (hM-CSF). PBMC were isolated from whole blood of normal donors using Ficoll-Paque. $CD14^+$ cells were separated from PBMC using MiniMACS $CD14^+$ magnetic beads and a VarioMAX column system. PBMC (10^6 cells per well) or $CD14^+$ cells (10^5 cells per well) were then cultured in 96-well plates on 4 x 4 x 0.5 mm slices of bovine cortical bone. Cells were cultured in alpha-MEM with 10% FBS for 21 days, in the presence of sRANKL (40 ng/ml) and hM-CSF (25 ng/ml), with or without VitD3 (10^{-11} M - 10^{-8} M). Multinucleated $TRAP^+$ cells were counted on the bone slices and resorption was quantified using scanning election microscopy.In both PBMC and $CD14^+$ cultures, VitD3 treatment consistently resulted in a concentration-dependent inhibition of osteoclast generation and bone resorption. These two effects where proportionate, indicating that VitD3 inhibited osteoclast differentiation rather than bone resorbing activity of the osteoclasts. The maximum effect (95% inhibition) occurred at 10^{-8} M VitD3 and the apparent EC_{50} was approximately 3×10^{-10} M. Dexamethasone (10^{-7} M) also markedly inhibited osteoclast generation and did not prevent the inhibitory effect of VitD3.These results confirm that VitD3 acts directly on human osteoclast precursors to inhibit their differentiation to osteoclasts. The possibility exists that VitD3 promotes differentiation of precursors to macrophages, thus preventing the development of the osteoclast phenotype. This direct effect of VitD3 contrasts sharply with its indirect, stromal cell/osteoblast-mediated effect to stimulate osteoclastogenesis and the physiological relevance of this response remains to be determined.(Itonaga *et al.*Biochem.Biophys.Res.Comm.264:590 1999)

SU284

Production of a Pure Population of Human Osteoclasts In Vitro as Identified by an Antibody to the Human Calcitonin Receptor. D. W. Dempster, K. Plavetic,* C. Hughes-Begos,* S. Neubort,* J. Nieves, F. Cosman, R. Lindsay. Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA.

A pure population of human osteoclasts would greatly facilitate studies on the molecular mechanisms underlying osteoclast formation and activity. One reason that this has not yet been achieved has been the lack of phenotypic markers that uniquely recognize osteoclasts in humans. We have, therefore, employed new polyclonal antibodies to the amino- and carboxy- terminals of the human calcitonin receptor (CTR) to characterize osteoclasts formed in vitro from peripheral monocytes. Peripheral blood mononuclear cells were obtained from healthy donors. The CD14- positive monocyte population (CD14+Mo) was isolated by immunomagnetic separation and cultured on bone slices in the presence of RANKL (10 ng/ml) and M-CSF (25 ng/ml) for 21 days. Multinucleated osteoclasts appeared at day 7 and their number and the amount of bone resorption increased steadily thereafter. At 21 days there was a mixed population of mono-and multi-nucleated cells present on the slice. The cells were stained with the CTR antibodies and counted. They were then counterstained with toluidine blue (to reveal any cells that were not stained by CTR antibodies) and re-counted. Cell numbers were the same before and after counterstaining, indicating that 100% of the cells stained positively for CTR. The multinucleated cells were associated with extensive resorption pits and the mononuclear cells were located in small, round resorption pits of similar size to the cell. This indicates that they too were capable of resorption. The cells also stained positively for less specific osteoclast markers, including the vitronectin receptor, and cathepsin K. In conclusion: 1.We have shown for the first time that it is possible to produce a pure population of human mono-and multinucleated osteoclasts in vitro. This will greatly facilitate studies on the molecular biology of human osteoclast formation and activity.2.Under appropriate conditions, all CD14+Mo are capable of forming osteoclasts in vitro.

SU285

Human Osteoclasts and Their Precursors Express Glucocorticoid Receptors Alpha and Beta. D. W. Dempster, C. Hughes-Begos,* K. Plavetic,* L. Stein,* S. Neubort,* J. Nieves, F. Cosman, R. Lindsay. Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA.

Glucocorticoid (GC) administration causes bone loss and osteoporosis in humans. The actions of GC on bone are complex and are thought involve effects on osteoclasts, osteoblasts and osteocytes. Furthermore, some individuals appear to be resistant to GC-induced osteoporosis. It has recently been shown in humans that, in addition to the functional GC receptor (GR alpha), a variety of cell types also express a dominant negative receptor (GR beta), and that over-expression of the GR beta may confer resistance to GC. The purpose of the present study was to determine which isoforms are expressed in human osteoclasts and their precursors in vitro. Peripheral blood mononuclear cells were obtained from healthy donors. The CD14- positive monocyte population (CD14+Mo) was isolated by immunomagnetic separation and cultured on bone slices or plastic in the presence of RANKL (10 ng/ml) and M-CSF (25 ng/ml) for 21 days. Multinucleated osteoclasts appeared at day 7 and their number and the amount of bone resorption increased steadily thereafter. Immunocytochemistry was performed on 21 day cultures using polyclonal antibodies that specifically recognize human GR alpha or beta. At 21 days, multinucleated osteoclasts were strongly positive for both GR alpha and beta. Immunoprecipitation was performed on total cellular protein extracted from these cultures using an antibody that recognized both GR alpha and beta. Western blot analysis of the immunoprecipitated proteins revealed a ≈94-kDa protein that specifically bound the GR alpha antibody and a ≈90-kDa protein that specifically bound the GR beta antibody. GR alpha and beta were also detected by Western blot analysis in CD14+Mo. This study marks the first time that both GR alpha and GR beta have been demonstrated at the protein level in human osteoclasts and their precursors. The existence of GR beta in these cells may provide a mechanism for modulation of the effects of GCs on bone resorption. For example, over-expression of GR beta in some individuals may confer resistance to the bone-resorbing effects of GC.

SU286

Combination of TNF-α and IL-1β Induced Osteoclast Formation and Bone Resorption Is Dependent on RANK Signal Transduction. J. Li,[1] S. Morony,[2] H. Tan,[2] W. Qiu,*[1] K. Warmington,*[2] V. Porkess,*[2] C. R. Dunstan,[3] P. J. Kostenuik,[2] D. L. Lacey,[2] W. J. Boyle.*[4] [1]Dept. of Inflammation, Amgen Inc., Thousand Oaks, CA, USA, [2]Dept. of Pharmacology and Pathology, Amgen Inc., Thousand Oaks, CA, USA, [3]Dept. of Product Development, Amgen Inc., Thousand Oaks, CA, USA, [4]Dept. of Exploratory Biology, Amgen Inc., Thousand Oaks, CA, USA.

Differentiation and activation of osteoclast is a process known to be controlled extracellularly by three TNF/TNFR family members RANKL/OPGL, RANK and OPG. Previously, we have determined that bone resorption induced by individual calcitropic hormones and proresorptive cytokines including TNF-α and IL-1β is dependent on RANK signaling in the osteoclast. However, several recent studies have suggested that the combination of TNF-α and IL-1β treatment in vitro may represent an alternative osteoclast differentiation and activation pathway independent of the RANKL-RANK interaction. In the current study, we investigate the effects of TNF-α and IL-1β on osteoclast formation and bone resorption in the RANK knockout mice. In vitro, in the presence of M-CSF, splenic hematopoietic precursor cells isolated from RANK knockout mice are unable to induce TRAP-positive osteoclast differentiation or resorption pits formation in response to either TNF-α alone or TNF-α plus IL-1β combination treatment. In contrast, splenic hematopoietic precursor cells isolated from wild type mice can differentiate into osteoclasts and form resorption pits on bone slices under the same conditions, although at a lower efficiency compared to their response to RANKL/OPGL. In vivo, the combination of TNF-α and IL-1β challenge in wild type mice induces profound hypercalcemia, osteoclast activation and bone resorption. Co-administration of OPG effectively inhibited these bone resorptive responses. Moreover, RANK knockout mice that received the combination challenge of TNF-α and IL-1β develop hypocalcemia and show no signs of osteoclast activation or bone resorption. Therefore, these results demonstrate that bone resorption induced by the combination of TNF-α and IL-1β is dependent on the presence of RANK signal transduction, and inhibition of the RANKL-RANK signaling pathway may be an effective therapeutic approach for various osteolytic bone and joint diseases.

Disclosures: Amgen Inc.,3.

SU287

Multiple Promoters Regulate Differential Expression of Murine Tartrate Resistant Acid Phosphatase (TRAP). N. C. Walsh,* D. A. Hume,* A. I. Cassady. Institute for Molecular Bioscience, University of Queensland, Brisbane QLD, Australia.

Tartrate resistant acid phosphatase (TRAP) activity is recognised as a terminal differentiation marker for osteoclasts and a subset of tissue macrophages. Low level expression is also detectable in some non-hematopoietic cells including the parenchymal cells of the liver and the mesangial cells of the kidney. This study characterises two novel TRAP mRNA transcripts that differ in their 5'UTRs. These alternatively spliced transcripts demonstrate unique expression patterns that provide insights into the regulation of TRAP gene expression in both hematopoietic and non-hematopoietic cells. The RIKEN Gene Encyclopaedia and UniGene databases were searched for ESTs homologous to the published TRAP mRNA sequence NM_007388. Alignment of both full length and partial cDNA sequences with NM_007388, identified two novel 5'UTRs which splice directly onto the first base of the translation initiation codon in Exon 2 (A+1TG). Open-reading frame analysis indicated that no in-frame translation initiation codons were present within the untranslated exons. Therefore the protein coding sequence of each transcript remains unchanged from that encoded by NM_007388.Genomic sequence analysis using BLAST searches of the NCBI HTGS and NR nucleotide databases identified both mouse and human genomic contigs

encoding the TRAP gene. The alternative 5' UTRs represented by the RIKEN sequences, AK011915 and BB561715, were placed relative to the A+1TG, at -5583 and -1714 bp respectively, in the mouse genomic sequence. Homologous sequences were located at -4147 bp and -1681 bp respectively, in the human genomic sequence. Differential expression of the novel TRAP 5' UTRs was observed by transcript specific semi-quantitative RT-PCR. Expression of the 5' UTR represented by AK011915 was limited to bone and spleen tissue suggesting that this transcript is specific to active osteoclasts and macrophages or alternatively their progenitor cells. As previously described the 5' UTR of NM_007388 was expressed strongly in bone and spleen with lower level expression evident in the kidney, liver and lung. The expression pattern for the 5' UTR of BB561715 was similar to NM_007388 with the exception that this transcript was not detected in bone. In addition the expression of the 5' UTR of BB561715 was elevated in liver and kidney compared to the other tissues analysed. This observation is important in delineating the function of this alternate 5' UTR and its promoter, as the cells expressing TRAP in the kidney and liver are predominantly non-hematopoietic cells.We conclude, that the regulation of murine TRAP gene expression is controlled by three distinct, tissue specific promoters.

SU288

Critical Roles of RANK Receptor Clusterinig for Stimulating the Osteoclast Development. T. Miyamoto, K. Iwamoto,* F. Arai, Y. Sawatani,* T. Suda.* Cell Differentiation, Kumamoto University, Kumamoto, Japan.

Osteoclasts are bone-absorbing multinuclear cells and their existence is strictly restricted on bone surface. RANK receptors are expressed on osteoclasts and transmembrane ligands, RANKL are expressed on osteoblasts and their interaction is required for osteoclast development. It is interesting to see the biological difference between the membrane associated RANKL and soluble RANKL (sRANKL) which is an extracellular portion of RANKL with myc tag. We have previously shown that the generation of TRAP-positive cells and multinuclear cells were severely decreased in methylcellulose culture (non-adherent condition) in the presence of M-CSF and sRANKL, compared with the liquid culture (adherent condition). However, expression of integrins on osteoclast precursors were not significantly decreased at the non-adhesion condition. (Blood, 2000). Here, we demonstrated that a dimer form of sRANKL linked with leucine-zipper (LZ-RANKL) or sRANKL with anti-myc-epitope antibodies increased the multinuclear TRAP-positive cells in non-adherent condition. This finding suggests that receptor clustering is critical for osteoclast development. We are now trying to overexpress RANK to induce the clustering to investigate whether it directly induce the full-maturation of osteoclasts or not.

SU289

Inflammatory Acute Phase Reactants in Rheumatoid Arthritis, alpha 1-Antitrypsin and C-Reactive Protein, Inhibit Osteoclastogenesis. S. Kotake,*[1] N. Udagawa,[2] T. Okamoto,*[3] Y. Nanke,*[1] K. J. Kim,*[1] S. Momohara,*[1] S. Saito,*[1] N. Takahashi,[2] H. Oda,*[1] T. Tomatsu,*[1] N. Kamatani.*[1] [1]Tokyo Women's Medical University, Tokyo, Japan, [2]Showa University, Tokyo, Japan, [3]Toray Industries, Inc., Tokyo, Japan.

To identify a novel factor that inhibits osteoclastogenesis in the synovial tissue of patients with rheumatoid arthritis (RA), we purified a factor from RA synovial tissues by hydrophobic column chromatography followed by ion-exchange column chromatography, and chromatofocusing. A factor with the inhibitory effect of osteoclast formation in mouse co-culture system was further purified to apparent homogeneity by polyacrylamide gel electrophoresis. The factor was finally identified as human α1-antitrypsin by amino acid sequencing. Human α1-antitrypsin (0.5 – 5 μg/ml) dose-dependently inhibited osteoclastogenesis in a co-culture of osteoblasts and bone marrow cells induced by 1,25-dihydroxyvitamine D3. α1-antitrypsin positive cells were immunohistologically detected in synovial tissue from patients with RA. Next, we investigated the effect of another inflammatory acute phase reactant, C-reactive protein (CRP), on osteoclastogenesis. CRP (10 – 100 μg/ml) dose-dependently inhibited osteoclastogenesis from human monocytes induced by soluble RANKL and M-CSF. Similarly, CRP strongly inhibited osteoclast differentiation in mouse co-culture system. The inhibitory effect was detected at a low concentration of CRP (10 μg/ml = 1 mg/dl), which indicates very mild inflammation. In summary, these findings suggest that inflammatory acute phase reactants in RA, α1-antitrypsin and CRP, inhibit osteoclastogenesis in human and mouse culture system. The production of these reactants is induced by proinflammatory cytokines such as IL-6, IL-1, or TNFα, which induce osteoclastogenesis. Thus, these acute phase reactants may play an important role in feedback inhibition in systemic and/or local osteoclastogenesis in inflammatory diseases such as RA.

SU290

Negative Regulation of Osteoclast Differentiation by c-Fos-Dependent Transcriptional Activation of Interferon-Inducible Genes. K. Matsuo,[1] M. Radolf,*[2] H. Auer,*[2] P. Steinlein,*[2] E. F. Wagner.*[2] [1]Deparment of Geriatric Research, National Institute for Longevity Sciences (NILS), Aichi, Japan, [2]Research Institute of Molecular Pathology (IMP), Vienna, Austria.

c-Fos is a transcription factor which is essential for osteoclast formation. Mice lacking c-Fos develop osteopetrosis since osteoclast progenitors fail to differentiate. We performed genome-wide screening of c-Fos dependent transcripts in the macrophage-osteoclast lineage using cDNA microarray hybridization. M-CSF dependent macrophages were prepared from bone marrow or spleens of wild-type mice and from spleens of *Fos*-/- mice, which lack bone marrow cavity. These cells were cultured under osteoclastogenic conditions in the presence of M-CSF and RANKL for 1, 2 and 6 days. At day 6, approximately 10% of wild-type cells stained positively for tartrate-resistant acid phosphatase (TRAP) activity, while *Fos*-/-cells remain TRAP-negative. Unexpectedly, in addition to osteoclast marker genes encoding TRAP and calcitonin receptor, a set of genes which are known to be inducible by type I interferons (α and β), including *Ifit1, Ifit2, Ifit3, Scyb10* and *Irf7*, were preferentially transcribed in the wild-type culture. Induction of interferon-inducible genes such as *Mx1* and *Ifi27* were detected as early as day 1 in wild-type cells. Northern blot analysis using independent cultures confirmed higher expression of interferon-inducible genes in wild-type cells than *Fos*-/- cells. Furthermore, type I interferons as well as poly I-C, which induces interferon signaling, inhibited osteoclastogenesis in culture. These observations suggest that c-Fos is involved in inhibitory signaling of osteclastogenesis through activation of interferon-inducible genes in progenitor cells. The functions of interferon-inducible genes as well as the potential roles of Fra1 and Fra2 in the suppression of inhibitory signaling are currently being investigated. In conclusion, c-Fos seems to be required not only for positive but also for negative regulation of osteoclast differentiation.

SU291

Inhibitory Effect of Cyclosporin A on Fusion of Osteoclast Precursors. Y. Jin,*[1] S. Li,*[1] E. Lee,*[1] Y. Won,[2] S. Lim.[1] [1]Internal Medicine, Medical School of Yonsei University, Seoul, Republic of Korea, [2]Internal Medicine, Medical School of Kwandong University, Kangwondo, Republic of Korea.

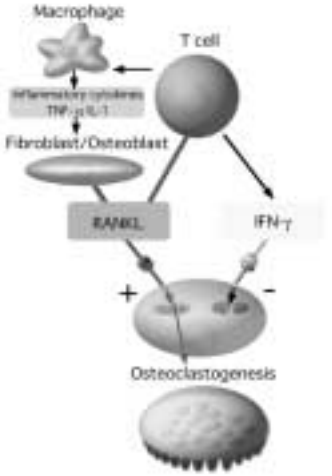
In this study, we attempted to investigate the in vitro effect of Cyclosporin A (CsA) on osteoclastogenesis using a mouse bone marrow culture system. Firstly, the long bone marrow cells were isolated from femur and tibiae, and then were incubated in complete α-MEM media overnight. Next day, the non-adherent cells were removed, and the left cells were further cultured in the presence of ODF and M-CSF, with or without CsA. After 3 days, the cells were subjected to TRAP (Tatrate resistant acid phosphatase) assay and acid phosphatase activity assay. TRAP-positive multinucleated cells (MNC) containing at least three nuclei were counted as osteoclasts. As a result, co-treatment with CsA at 10-6M and CsA 10-7M significantly inhibit induction of osteoclast by M-CSF and ODF. The TRAP-positive MNC numbers (No./well) of positive control (treatment with M-CSF and ODF only), CsA (10-6M), and CsA (10-7M), were 602±22, 9±1 and 125±19, respectively. In the acid phosphatase activity assays, CsA did not show significant effects on their activities. Their acid phosphatase activities (Sigma Units/well) were 0.074±0.008 (Negative control), 6.192±0.085 (Positive control), 6.224±0.040 (CsA 10-6M) and 6.321±0.227 (CsA 10-7M), respectively. In addition, we also detected if CsA could influence the induced MNCs as to evaluate its effect on osteoclast apoptosis. After induction of MNCs, they were treated with CsA for 9 hours, and then, their TRAP-positive MNC numbers were counted. The result showed that treatment of CsA at 10-6M and 10-7M did not result in the significant difference compared to untreated group. These results suggest that CsA had significantly inhibitory effect on fusion of these precursors rather than inhibiting the formation of osteoclast precursors. And CsA might inhibits multinucleated osteoclast formation through inhibitory effect on fusion of osteoclast precursors.

SU292

T-cell Regulation of Osteoclastogenesis by RANKL and IFN-gamma. H. Takayanagi,[1] H. Oda,[2] K. Nakamura,[2] T. Taniguchi.*[3] [1]Dep of Immunology and Orthopaedic Surgery, Univ of Tokyo, Tokyo, Japan, [2]Dep of Orthopaedic Surgery, Univ of Tokyo, Tokyo, Japan, [3]Dep of Immunology, Univ of Tokyo, Tokyo, Japan.

Bone resorption is regulated by the immune system, where T cell expression of RANKL may contribute to pathological conditions such as autoimmune arthritis. However, it is unknown whether activated T cells maintain bone homeostasis by counterbalancing the action of RANKL. We suspected that T cells also have a negative regulatory pathway of osteoclastogenesis: 1) Normal activation of immune system does not always lead to excessive osteoclastogenesis. 2) Osteoclast formation is limited to skeletal tissues, while T cells can infiltrate anywhere in the body. 3) T cell cytokines such as IFN-gamma and IL-4 have inhibitory effects on osteoclastogenesis. Therefore, we investigated the effect of activated T cells on osteoclastogenesis by coculture of mouse splenic T cells and RANKL/M-CSF-stimulated bone marrow cells. Using IFN-gamma receptor deficient mice, we show that T-cell production of IFN-gamma essentially contribute to the suppression of osteoclastogenesis. We further investigated the molecular mechanism by which IFN-gamma inhibits RANKL signaling by EMSA and immunoblotting. IFN-gamma induces rapid degradation of the RANK adapter protein, TRAF6, resulting in strong inhibition of the RANKL-induced activation of NF-kB and JNK. This inhibition of osteoclastogenesis is rescued by overexpressing TRAF6 in precursor cells, indicating TRAF6 is indeed the target critical for the IFN-gamma action. Furthermore, we provide evidence that the accelerated degradation of TRAF6 requires its ubiquitination, which is initiated by RANKL, and IFN-gamma-induced activation of the ubiquitin-proteasome system. Our results suggest that TRAF6 is essential for osteoclast differentiation, and a good therapeutic target in the treatment of osteopenic diseases. We conclude that the effect of T cells on osteoclastogenesis depends on the balance between RANKL and IFN-gamma, which protects against bone loss upon T- cell activation. Inflammation-induced bone destruction may be caused by a scarcity of IFN-gamma and the enhanced expression of RANKL (on both fibroblasts/osteoblasts and

T cells), as summarized in the figure.



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Calvarial and Long Bone Marrow Respond Differently to Osteoclast Differentiation Factor RANKL. T. J. de Vries,*[1] A. M. Schoenmaker,*[1] W. Beertsen,[2] V. Everts.[1] [1]Periodontology (ACTA) and Cell Biology and Histology (AMC), AMC, Amsterdam, The Netherlands, [2]Periodontology (ACTA), ACTA, Amsterdam, The Netherlands.

Until recently, it was believed that multinucleated osteoclasts constitute a homogeneous group of cells which degrade bone similarly at all bone sites in our body. We recently showed that calvarial and long bone osteoclasts differ substantially in the way they resorb bone matrix. Whereas long bone osteoclasts primarily use cathepsins, calvarial osteoclasts use both cathepsins and matrix metalloproteases (V. Everts et al., FASEB J.13, p. 1219-1230, 1999). Based on these findings we hypothesized that different populations of osteoclasts arise from fusion of different site-specific monocytic precursor cells.This hypothesis was tested by generating osteoclasts from two bone marrow sources and comparing their properties. To this end we cultured bone marrow isolated from either calvariae or long bones in the presence of macrophage colony stimulating factor (M-CSF) and receptor activator of NF-kappaB ligand (RANKL). Cultures were stained for TRAP activity and the bone-resorbing capacity of the osteoclast-like cells was tested by seeding them on bone slices. A striking difference between the two populations concerned the number of nuclei per cell. The osteoclasts generated from calvarial marrow developed more rapidly and contained more nuclei per cell after a standardized culture period of 5 days. However, after seeding the cells from the two different marrow sources on bone slices no differences were observed in their bone resorbing capacity. Another remarkable difference between the two types of bone marrow cells was their response to RANKL after a preculture period with M-CSF. Bone marrow was cultured for 3 days with M-CSF upon which RANKL was added for another 5 days. After 3 days of M-CSF pretreatment and upon addition of RANKL, bone marrow cells from calvariae had developed into TRAP positive cells which also expressed calcitonin receptor, carbonic anhydrase II and the integrin unit beta-3 . Under these conditions, however, hardly any TRAP positive cells were observed in long bone marrow cultures. In addition we found that these long bone marrow cultures did not express calcitonin receptor and showed diminished carbonic anhydrase II and integrin unit beta-3 expression when pretreated with M-CSF. These observations support the view that site-specific precursor cells exist which can react differently to in situ stimuli such as RANKL.

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Teprenone Inhibits Human Osteoclastogenesis. Y. Nanke,* S. Kotake,* N. Ichikawa,* T. Furuya,* N. Kamatani.* Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

It has been reported that the inhibitory effect of Vitamin K2 (Menatetrenone)on bone resorption may be related to its side chain. (K. Hara et al. 16:179-184, Bone 1995). Teprenone, an antiulcer drug, has almost the same chemical structure as that of the side chain of Menatetrenone. Therefore, we investigated whether Teprenone also has an inhibitory effect in osteoclastogenesis. Human peripheral blood was collected from healthy normal volunteers. Peripheral blood mononuclear cells (PBMC) were then cultured for 3 days in 48-well plate in the presence of M-CSF (100 ng/ml). Non-adherent cells were then removed. The adherent peripheral mononuclear cells, as described above, were cultured in the presence of various concentrations of Menatetrenone or Teprenone with human soluble RANKL (100 ng/ml) and M-CSF (100 ng/ml). We used the adherent peripheral mononuclear cells as monocytes in the culture system. Osteoclast-like cell formation was evaluated by immunohistological staining for vitronectin receptors after culture for 7 or 8 days. Menatetrenone(10^{-6} M) inhibited the formation of osteoclast-like cells induced in the presence of soluble RANKL. Teprenone (1 μg/ml) also inhibited the formation of osteoclast-like cells. Since it has been reported that the serum concentration of Teprenone reaches 2.5 μg/ml after the administration 150 mg of Teprenone, we speculate that Teprenone may inhibit osteoclast formation physiologically. Thus, Teprenone may be effective in not only protecting the gastric mucosa but also in preventing bone loss.

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The Effect of Periostin on Osteoclast Differentiation and Activation. J. Wilde,[1] K. Hiura,[1] M. Yokozeki,[1] S. Kido,[1] D. Inoue,[1] T. Matsumoto,[1] A. Kudo,[2] K. Moriyama.[1] [1]University of Tokushima, Tokushima, Japan, [2]Tokyo Institute of Technology, Yokohama, Japan.

The periodontal ligament (PDL) plays an important role in the remodeling of the alveolar bone during orthodontic tooth movement. Many factors are involved in the mechanisms of tooth movement in response to an orthodontic force. Periostin is a 90 kDa secreted protein preferentially expressed in the periodontal ligament (PDL) and the periostium, of which mRNA is increased in the compression sites of the PDL during experimental tooth movement in vivo, and is induced by uni-axial mechanical stress in PDL cells in vitro(ASBMR 22nd Annual Meeting). The aim of this study was to analyze the effect of periostin in osteoclasts (OCL) differentiation and activation. OCL derived from human peripheral blood mononuclear cells, unfractionated mouse bone cells (BC) and unfractionated rabbit BC were cultured on bone slices. The OCL differentiation was observed by TRAP staining of the multinucleated cells and their activation was evaluated by pit assay of the bone slices. We found that human OCL differentiation and activation were partially inhibited, after two weeks of culture in the presence of conditioned medium (CM) from 24-hours-uni-axial mechanically stressed human periodontal ligament fibroblastic cells (hPDLF). These results were confirmed when unfractionated mouse BC cultures showed an inhibitory effect in the OCL differentiation and activation after five days of culture with 600 ng/ml recombinant mouse periostin. Furthermore, when unfractionated rabbit BC were co-cultured for four days with hPDLF transfected with a vector carrying periostin gene or CM from these cells, OCL differentiation and activation were also inhibited even in the presence of anti-human osteoprotegerin antibody. Levels of osteoprotegerin (OPG) were confirmed by western blotting and no difference of this protein was found among all the groups and controls. These results suggest that periostin might be a putative negative regulator of OCL differentiation and activation induced by mechanical stress in a pathway different from that of OPG.

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Human Osteoclast Formation and Bone Resorption by A Mechanism Independent of RANK/RANKL: Role of IL-6 and IL-11. O. Kudo,*[1] A. Sabokbar,*[1] I. Itonaga,*[1] Y. Fujikawa,*[2] T. Torisu,*[2] N. A. Athanasou.[1] [1]University of Oxford, Oxford, United Kingdom, [2]Oita Medical School, Oita, Japan.

Osteoclast progenitors can differentiate into mature bone resorbing osteoclasts in the presence of RANK ligand (RANKL), which is expressed on bone stromal/osteoblastic cells and macrophage colony stimulating factor (M-CSF). Osteoprotegerin (OPG) inhibits RANKL-induced osteoclast formation and bone resorption. It has been reported that tumour necrosis factor-α (TNF-α), a potent cytokine involved in regulation of osteoclast activity via a primary effect on osteoblasts, can directly (in the presence of M-CSF) induce the differentiation of osteoclast progenitors into mature osteoclasts. These studies showed that TNF-α-induced osteoclast formation is independent of RANK/RANKL interaction. In the present study we sought to determine whether IL-6 and IL-11, two other potent stimulator of bone resorption, can similarly induce osteoclast formation and bone resorption in vitro. Mononuclear cells were isolated from peripheral blood of healthy volunteers and cultured for up to 3 weeks on glass coverslips and dentine slices in the presence of: (i) RANKL and M-CSF; (ii) IL-6 (plus soluble IL-6 receptor), M-CSF ± OPG; (iii) IL-11, M-CSF ± OPG; (iv) IL-6 (plus soluble IL-6 receptor), M-CSF ± anti gp130; (v) IL-11, M-CSF ± anti gp130. The extent of osteoclast formation and bone resorption was determined by generation of TRAP-positive multinucleated cells on glass coverslips and lacunar resorption on dentine slices. We noted that addition of IL-6 (plus soluble IL-6 receptor), and IL-11 in the presence of M-CSF, but in the absence of RANKL, was sufficient to induce the formation of TRAP-positive multinucleated osteoclast-like cells which were capable of lacunar resorption in vitro. The addition of OPG to the cultures containing IL-6 or IL-11 did not inhibit osteoclast formation and bone resorption, indicating that IL-6 and IL-11 induce osteoclast formation by a mechanism independent of RANK/RANKL. Both IL-6- and IL-11-induced osteoclast formation was significantly inhibited in cultures containing anti-gp130 antibodies. Our results indicate that IL-6 and IL-11, factors which have been associated with inflammation and bone disorders could directly induce osteoclast precursors to differentiate into active bone resorbing osteoclasts.

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Gender Differences in 1,25-Dihydroxyvitamin D3 Effects on Osteoclast Formation From Circulating and Bone Marrow Precursors. M. Jevon,*[1] A. Sabokbar,*[1] D. G. Roodman,[2] S. D. Neale,*[1] N. A. Athanasou.[1] [1]University of Oxford, Oxford, United Kingdom, [2]University of Texas Health Sciences, San Antonia, TX, USA.

A number of bone diseases characterised by excessive osteolysis (e.g. osteoporosis and Paget's disease) exhibit a marked gender difference in prevalence. Bone resorption is carried out by osteoclasts, which are formed by fusion of circulating mononuclear precursor cells of haematopoietic origin. A number of humoral factors, including 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], are known to influence osteoclast formation from circulating and marrow precursors. In this study, we have determined whether there are sex-related differences in the sensitivity to the effect of $1,25(OH)_2D_3$ on osteoclast formation from circulating and marrow precursors. Human peripheral blood mononuclear cells (PBMCs) were co-cultured with UMR 106 osteoblast-like cells in the presence of M-CSF and $1,25(OH)_2D_3$ [10^{-7}-10^{-10}M]. In addition, the effect of variable concentrations of $1,25(OH)_2D_3$ on long-term human marrow cultures was also determined. As assessed by the formation of TRAP-positive and VNR-positive multinucleated cells (MNC) and lacunar bone resorption, long-term human marrow cultures and co-cultures of PBMCs and UMR 106 cells in males and females showed no evidence of osteoclast formation when 10^{-10}M $1,25(OH)_2D_3$ was added. An increase in the number of TRAP-positive and VNR-positive MNC was noted in males when 10^{-9}M and 10^{-8}M $1,25(OH)_2D_3$ was added to PBMC-UMR 106 co-cultures

and marrow cultures ($p>0.005$ and $p>0.05$ respectively). At all concentrations of added $1,25(OH)_2D_3$, an increase in the extent of lacunar resorption was noted in male UMR 106-PBMC co-cultures compared with females. These results indicate that there are gender-related differences in osteoclast formation and bone resorption in terms of sensitivity to the effect of $1,25(OH)_2D_3$. These findings have implications for the pathogenesis of osteolytic conditions such as Pagets disease, where there is increased osteoclastogenesis and evidence of increased $1,25(OH)_2D_3$ sensitivity has been noted.

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Characterization and Cloning of the Annexin II Receptor. H. Maeda,[1] S. V. Reddy,[1] N. Kurihara,[1] G. D. Roodman.[2] [1]Medicine/Hematology, Univ of TX Health Science Ctr @ SA, San Antonio, TX, USA, [2]Medicine, UTHSCSA/VA Medical Center, San Antonio, TX, USA.

We have previously identified Annexin II (AXII) as a novel factor produced by osteoclasts (OCLs) that stimulates OCL formation through induction of GM-CSF production by marrow stromal cells (JCI 103:1605, 1999). Annexin II occurs predominantly as a heterotetramer (AXIIt) formed by two p11-kDa and two 36-kDa subunits. To further characterize the mechanism responsible for AXIIt's effects on OCL formation, we examined the role of the p11 and p36 subunits in this process. Immunocytochemical analysis showed that human OCLs express AXIIt using either a monoclonal antibody specific for the p11-kDa or 36-kDa subunits. Consistent with our previous finding that AXIIt indirectly stimulated OCL formation, AXIIt induced CFU-GM colony formation by unfractionated human bone marrow, but not by highly purified hematopoietic precursors lacking stromal cells. Furthermore, biotinylated AXIIt bound to normal human marrow stromal cells and the human marrow-derived PSV10 stromal cell line. Scatchard analysis of ^{125}I-AXIIt binding to PSV10 cells demonstrated a single class of receptors with a Kd = 5.79 nM and Bmax = 2.13 X 10^5 receptors/cell. Binding of ^{125}I-AXIIt to PSV10 cells or normal marrow stromal cells was not blocked by annexin III or annexin V. To further characterize the AXIIt receptor, cross-linking experiments were performed in the presence of DTME with biologically active biotinylated AXIIt and intact PSV10 cells. SDS-PAGE analysis identified a 55-kDa complex on PSV10 cells that was specifically cross-linked with AXIIt. Western blot analysis of the AXIIt receptor complex with monoclonal antibodies specific for the AXII p11-kDa or 36-kDa subunit demonstrated that the p11 but not the 36-kDa subunit was cross-linked to the putative receptor. We then screened a human bone marrow cell mammalian expression library for the AXIIt receptor (AXIIR) cDNA by transiently expressing the library in NIH3T3 cells and testing for binding of ^{125}I-AXIIt to the cells. We identified a 1.2-kb AXIIR cDNA. Transient expression of the AXIIR cDNA in NIH3T3 cells resulted in significantly increased levels of ^{125}I-AXIIt binding to the cells that was blocked by 100X unlabeled AXIIt but not by annexin V. A search of the human genome database and hybrid cell analysis revealed that the AXIIR cDNA was a novel human-specific clone that mapped to chromosome 5. These data demonstrate that a previously unknown and novel AXIIR is present on human marrow stromal cells that may mediate the effects of AXIIt on OCL formation.

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Lycopene Inhibits Osteoclastic Bone Resorption Mediated by Reactive Oxygen Sp. L. G. Rao,[1] N. Krishnadev,*[1] L. J. F. Liu,*[1] T. M. Murray,[1] A. V. Rao.*[2] [1]Department of Medicine, St. Michael's Hospital and University of Toronto, Toronto, ON, Canada, [2]Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada.

Osteoclasts have been shown to produce reactive oxygen species (ROS) that can stimulate bone resorption. In this study, we tested whether dietary lycopene, a potent antioxidant carotenoid naturally present in tomatoes, can inhibit osteoclastic bone resorption mediated by ROS. We determined the effects of a water-dispersable lycopene on the differentiation of mononucleated cells to tartrate-resistant acid phosphatase-positive (TRAP+) multinucleated osteoclasts and on the activity of these cells to resorb bone. Cells from bone marrow prepared from rat femur were plated onto 16-well calcium phosphate coated osteologic discs and cultured in alpha-MEM medium supplemented with β-glycerophosphate and ascorbic acid. The cells were treated with vehicle or PTH-(1-34) and varying doses of water-dispersible lycopene at each medium change every 48 hours. At day 8, the cells were removed from the discs with detergent and the resorption pits quantified in a Microst Reader. In a parallel experiment, the cells were cultured in 12-well plastic dishes and at day 8, the cells were stained for TRAP. Results showed that PTH stimulated resorption pits from 2% to 4.5%. Both the basal (one-way ANOVA, $p<0.05$) and PTH-stimulated resorption ($p<0.005$) were significantly inhibited in a dose-dependent manner by lycopene at concentrations from 10^{-7}M to10^{-5} M. When the fixed, stained cells were examined under a microscope, we observed a nearly complete inhibition of TRAP+ multinucleated cell formation in cultures treated with lycopene at a concentration of 10^{-5}M compared to vehicle- or PTH-treated cells. Osteoclasts reduced the nitroblue tetrazolium (NBT) to purple-colored formazan, indicating the presence of ROS in these cells. The formazan-staining cells were decreased by treatment with 10^{-5} M lycopene, indicating that lycopene inhibited the formation of ROS-secreting osteoclasts. In conclusion, we have shown that lycopene inhibited basal and PTH-stimulated osteoclastic bone resorption, as well as the formation of TRAP+ multinucleaneated osteoclasts. These effects were exerted by inhibition of the activity of ROS produced by osteoclasts. These findings are novel and may be important in the pathogenesis, treatment and prevention of osteoporosis.

SU300

Involvement of Vacuolar H^+-ATPase (V-ATPase) in Specific Incorporation of Risedronate into Osteoclasts. M. Takami,[1] K. Suda,*[2] N. Udagawa,[1] J. Woo,[2] K. Nagai,*[2] T. Sasaki,*[3] N. Takahashi.[1] [1]Biochemistry, School of Dentistry, Showa University, Tokyo, Japan, [2]Bioengineering, Tokyo Institute of Technology, Yokohama, Japan, [3]Histology, School of Dentistry, Showa University, Tokyo, Japan.

Although it is believed that bone-resorbing osteoclasts (OCLs) specifically incorporate bisphosphonates (BPs), the precise mechanism of the BP incorporation by osteoclasts is not known. We previously showed that BPs disrupted actin rings (clear zones) formed in normal OCLs, but failed to destroy actin rings in OCLs derived from osteopetrotic *oc/oc* mice. In this study, we found that the activity of vacuolar H^+-ATPase (V-ATPase), is essentially involved in the incorporation of risedronate into OCLs. OCLs prepared from mouse co-cultures were placed on dentine slices in the presence or absence of risedronate. The configuration of actin rings in OCLs and resorption pits on dentine slices were observed. Actin rings in OCLs were also observed in the presence of specific inhibitors of V-ATPase, bafilomycin A1 or concanamycin A, and a proteinase inhibitor, E64. Disruption of actin rings was evaluated as an index of risedronate incorporation into OCLs. (1) Treatment of OCLs cultured on dentine slices with risedronate completely disrupted actin rings formed in OCLs, and inhibited pit formation by OCLs on the slices. (2) Bafilomycin A1 and concanamycin A strongly inhibited pit-forming activity of OCLs but failed to disrupt actin rings. (3) When OCLs were treated with with E64, decalcified spots that might be formed by osteoclastic acidification were observed on dentine slices. However, neither digestion of dentine matrix at the decalcified spots nor disruption of actin rings was induced by E64. (4) Treatment of OCLs with risedronate failed to disrupt actin rings in the presence of Bafilomycin A1 and concanamycin A. In contrast, risedronate disrupted actin rings of OCLs in the presence of E64. (5) Calcitonin disrupted actin rings in OCLs in the presence and absence of bafilomycin A1, concanamycin A or E64. Thus, risedronate failed to disrupt actin rings formed in OCLs in the presence of inhibitors of V-ATPase. These results suggest that acidification by V-ATPase is involved in specific incorporation of BPs into OCLs.

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Effects of Flavonoids, Naturally Antioxidant Substances, on Osteoclastic Activities. A. Wattel,* R. Mentaverri,* F. Lorget,* C. Mullié,* C. Dupont,* S. Kamel, M. Brazier. Laboratoire de Pharmacie Clinique, Faculté de Pharmacie, Amiens, France.

Nutrition is considered as an important factor in the prevention of osteoporosis, a common disease in the western world. Among the various item which are reported to impact on bone health, flavonoids have been suggested to have benefit effects. Flavonoids, considered as micronutrients, are divided into different subfamilies characterised by their chemical structure. In this study, we have been interested in flavonols (quercetin, kaempferol, myricetin, fisetin, rutin), isoflavonoids (genistein, daidzein), flavanones (hesperetin, taxifolin) and catechin. In order to research a potential role of flavonoids on osteoclastic activities, we have tested several flavonoids on osteoclasts obtained from ten-day-old rabbit long bones. Bone resorption has been evaluated in vitro after a 48-hours culture on bovine bone slices by pit area measurement. We have shown that all flavonoids, tested at concentrations between 0.1 and 50 µM, inhibit bone resorption in a dose dependant manner. Only rutin, a glycoside of quercetin, doesn't inhibit bone resorption. We have listed them according to their IC_{50} or concentration inhibitory 50% of basal resorption. Kaempferol (IC_{50} = 0.8 µM) seems to be the most potent bone resorption inhibitory flavonoid, followed by genistein. However, daidzein looks like being less efficient. Hesperetin and taxifolin, both belonging to flavanones, have the greatest index : respectively 19.6 and 60.1 µM (see following table).

	Kaempférol	Genistein	Fisetin	Catechin	Myricetin	Quercetin	Daidzein	Hesperetin	Taxifolin
IC_{50} (µM)	0.8	1.3	2.3	3.4	4.7	5.7	7.4	19.6	60.1

One of the most important mechanisms of inhibition of bone resorption is osteoclast apoptosis induction. By using Hoechst staining, we have evaluated effects of flavonoids at the concentration of 50 µM on osteoclast programmed cell death in a high purified population of osteoclasts. All the flavonoids, except rutin and at a least degree the flavanones, dramatically induce osteoclast apoptosis, particularly flavonols. Proapoptotic effects of those flavonoids could explain, at least in part, the inhibition of bone resorption observed, but there are probably others mechanisms involved, like antioxidant properties, which have been described for flavonoids. Our results suggest that flavonoids, which are commonly present in human diet, could influence bone remodelling and might explain the relative protection against postmenopausal osteoporosis, observed in Asian women, who are great consumers of foodstuffs rich in flavonoids like soja and tea.

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Human Osteoclasts Release CTX Collagen Fragments from Bone Slices Allowing Quantification of Resorption by the CrossLaps Assay. M. A. Karsdal,[1] J. Nielsen,*[1] H. Lou,*[1] J. Jacobsen,[2] N. Foged,*[1] J. M. Delaissé,[1] P. Qvist.[2] [1]OSTEOPRO A/S, Herlev, Denmark, [2]Osteometer Biotech, Herlev, Denmark.

The quantitative pit formation assay is a central method for studies of osteoclasts function in vitro and has been widely used to screen new chemical entities for their anti-resorptive potency. However, these screenings have until recently been limited to the use of either osteoclasts of animal origin or human osteoclastoma-derived osteoclast-like cells, and therefore may not have been optimal for identification of new drug candidates. This study presents a new method for using human monocyte-derived osteoclasts in the pit formation assay. This method includes a simple quantification of bone resorption through ELISA measurement of CTX collagen fragments released into the conditioned medium by the human osteoclasts. Monocytes were isolated from peripheral blood and cultured for 14 days in the presence of both RANKL and M-CSF. At day 14, the newly formed pre-osteoclasts were detached by trypsin treatment and cell scraping followed by replating on bovine cortical bone slices in the presence of both RANKL and M-CSF. After a 7 day culture period, cells stained positive for TRAP and most were multinucleated. Their osteoclastic phenotype was further confirmed by immunohistochemistry and zymography showing their expression of calcitonin receptor, αvβ3, MMP-9 and actin ring formation.

The CTX release into the culture medium was measured by the CrossLaps for Culture ELISA and compared to the total plan area of resorption pits. According to end point measurements at various culture times, the amount of CTX released by the replated human osteoclasts correlated strictly with pit area. Dynamic measurements in the Crosslaps assay performed by multiple sampling of conditioned medium over time from the same osteoclast culture, confirmed the continuous resorption by the osteoclasts. Furthermore, the present method of replating osteoclasts assured a much more homogeneous resorption when compared to the classical 3 week human monocyte culture on bone substratum. When replated osteoclasts were cultured in the presence of the bisphosphonate, pamidronate, or the carbonic anhydrase inhibitor, ethoxyzolamide, both pit formation and CTX release was inhibited to the same degree, with IC50 values of approx. 1 µM for pamidronate and approx. 10 µM for ethoxyzolamide. We propose that the continuing search for novel anti-resorptive drug candidates should preferably be done in cultures of human rather than animal osteoclasts, and that the selection of compounds according to potency should be based on dynamic and/or end point quantification of the release of collagen fragments such as CTX into the culture medium.

SU303

Distribution of the Osteoclast-Selective 116KDa (a3 isoform) V-ATPase Subunit in Human Tissues. I. E. James,*[1] B. R. Bradley,*[1] S. M. Blake,[1] C. O. McMonigal,*[1] J. G. Emery,[1] C. N. Bender,*[1] K. M. Steplewski,*[2] M. Gowen,[1] M. W. Lark.[1] [1]Musculoskeletal Diseases, GlaxoSmithKline, King of Prussia, PA, USA, [2]Gene Expression Sciences, GlaxoSmithKline, King of Prussia, PA, USA.

Osteoclasts dissolve bone mineral and expose the protein matrix for subsequent proteolysis by acidifying the subcellular compartment. Acidification is achieved by means of a vacuolar-type ATPase (vATPase) that is polarized to the ruffled membrane of the resorbing osteoclasts. The vATPases are found on other organelles where they also function to pump protons across a variety of membranes. The vATPases are multi-unit enzymes consisting of a catalytic cytoplasmic V1 domain that is attached to a membrane anchored Vo complex. The 116KDa subunit is part of the membrane anchored complex and contains a number of membrane-spanning regions. There are 3 reported isoforms of the 116KDa complex and knockout of the mouse a3 isoform and mutations in the human a3 isoform result in severe osteopetrosis. Although the a3 isoform was originally reported to be osteoclast-specific, subsequent Northern blot and PCR data suggest that it may be ubiquitously expressed. In this study, we have used quantitative PCR (TaqMan) and Northern blot analysis to determine the 116KDa mRNA distribution in a large panel of human tissues. In addition, immunocytochemistry has been used to determine the tissue distribution of the OC-116KDa protein, using a rabbit polyclonal anti-peptide antibody. The anti-peptide antibody (anti-81PPKGRLPAPPRDL94) and the primers used for the mRNA analyses were designed so that they would not detect the TIRC7 alternative splice form of the 116KDa subunit that is detected on activated T-cells. The TaqMan and Northern blot analyses confirmed that the mRNA for the a3 isoform is not only present in osteoclasts but is also present in other tissues, in particular pancreas, spleen, peripheral blood mononuclear cells and macrophages. Preliminary immunostaining with the anti-peptide antibody demonstrated strong and selective reactivity against osteoclasts in human osteoclastoma tissue. In addition, weaker staining was also observed in pancreas and kidney. Taken together these data suggest that the 116KDa a3 isoform is not specific for osteoclasts at either the mRNA or protein level. Furthermore, the data suggest that the 116KDa a3 isoform is critical for osteoclast function, but other 116KDa isoforms may substitute for the a3 isoform in other unaffected cells and tissues.

SU304

Reveromycin A Induces Apoptosis in Activated Osteoclasts, not in Inactivated. J. Woo,[1] M. Kato,*[1] H. Osada,*[2] P. H. Stern,[3] K. Nagai.*[1] [1]Chubu University, Kasugai, Japan, [2]REKEN, Wako, Japan, [3]Northwestern University, Chicago, IL, USA.

Reveromycin A(RMA), a natural low molecular weight compound isolated from Streptomyces sp., was initially identified to inhibit the mitogenic activity of epidermal growth factor. We have found that RMA inhibits bone resorption through inducing apoptosis in osteoclasts, and that the effect of RMA is accompanied by cytochrome c release from mitochondria into cytoplasm, caspase activation and nuclear fragmentation in osteoclasts. The aim of the present study is to investigate the specific effect of RMA on activated osteoclasts. RMA inhibited the survival of osteoclast-like cells (OCLs) purified from cocultures of osteoblastic calvarial cells and bone marrow cells. The inhibitory effect of RMA on the survival of other several human and mouse cell lines such as monocytes, macrophage and immune cell lines was 100 times less than that on OCLs. RMA induced apoptosis in activated OCLs having actin ring and resorption capability, which are formed by the fusion of mononuclear preosteoclasts(pOCs) in the presence of RANKL. However, it did not induce apoptosis in inactivated OCLs without actin rings, which are formed by the fusion of pOCs in the presence of MCSF. RMA had no effect on OCLs in which actin rings were disrupted by calcitonin, and on OCLs without actin rings which were formed on collagen gel . The effect of RMA on activated OCLs was blocked by concanamycin B, a specific V-ATPase inhibitor or by acetazolamide, a carbonic anhydrase inhibitor, which can prevent the acidification around membrane of osteoclasts. The release of cytochrome c, caspase activation and nuclear fragmentation induced by RMA were also blocked by these inhibitors. Taken together, our results indicate that specific effect of RMA on activated osteoclasts may result from acidic condition around membrane of osteoclasts that increase cell permeability of RMA by suppressing dissociation of protons from carboxyl groups. Therefore, R M A would be an anti-resorptive agent with high specificity against activated osteoclasts as well as an indicator to identify activated osteoclasts.

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Reduced Trabecular Resorption in OSVDR Transgenic Mice Associated with Lower RANKL/OPG Ratio is Evident In Vivo but not In Vitro. G. P. Thomas,* P. A. Baldock,* S. U. K. Baker,* R. F. Enriquez,* J. A. Eisman, E. M. Gardiner. Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW, Australia.

Actions of vitamin D on bone are pleiotropic with 1,25-(OH)2D3, the active metabolite of vitamin D, stimulating both formation and resorption. Osteoclastic resorption is mediated through the RANKL/OPG regulatory pathway via immature osteoblasts or stromal cells. 1,25-(OH)2D3 increases the RANKL/OPG expression ratio, consistent with its pro-resorptive activity in osteoclastic regulation. However, OSVDR transgenic mice, overexpressing the vitamin D receptor specifically in mature cells of the osteoblastic lineage, have wider, stronger long bones than wildtype FVB/N mice, associated with elevated periosteal osteoblast activity. Also, their trabecular bone volume and thickness is increased in caudal vertebrae associated with a decrease in osteoclast surface rather than an increase in osteoblast activity. The present study was designed to investigate the potential role of changes in the RANKL/OPG pathway in the inhibition of osteoclastic resorption via mature osteoblasts.In the current study the analyses of trabecular bone were extended to 17-day and 4-month old OSV3 transgenic and FVB/N wildtype mice. RANKL/OPG expression levels were measured by semi-quantitative RT-PCR from RNA of whole bones and in vitro primary osteoblastic cell cultures. Resorption was also measured in organ cultures of 45Ca-labelled long bones.Trabecular osteoclast surface was lower in the femora of 17-day and 4-month old OSV3 mice (OSV3 vs FVB: 17-day = 10±2% vs 23±5%, $p<0.01$; 4-month = 13±1% vs 16±1%, $p<0.01$). The lower resorption in 4-month old OSV3 mice was associated with a decreased RANKL/OPG ratio in both treated and untreated transgenic bones (OSV3 vs FVB: cont = 2±0.2 vs 3±0.6; treat = 3±0.2 vs 5±1, $p<0.01$). This relationship was maintained in 1,25-(OH)2D3 treated mice, as the ratio increased similarly in both mouse lines in response to the hormone (1.5-fold, $p<0.01$). In contrast to the in vivo result, basal and 1,25-(OH)2D3-treated resorption in bone organ cultures from OSV3 mice were comparable to FVB/N (both lines 1.8-fold hormonal increase in 45Ca release, $p<0.01$). Similar results were obtained in primary osteoblastic cell cultures, with comparable basal expression and 1,25-(OH)2D3-induced increases in the RANKL/OPG ratio (both lines 2-fold, $p<0.01$).Thus, RANKL/OPG expression appears to mediate the reduced OSV3 resorption in vivo but these differences are much reduced in vitro. This dichotomy suggests that physiological regulatory mechanism(s) acting upstream of RANKL/OPG in these transgenic mice are important components of the modulation of bone mass in vivo.

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Daidzein and Genistein Effects on Osteoclastic Activity From Differentiated Human Blood Monocytes. S. Gnidehou,* M. E. Cohen-Solal, M. C. de Vernejoul, J. M. Garel. INSERM U349, Paris, France.

Nutrition may be an important factor in the prevention of postmenopausal osteoporosis. Soy isoflavones (daidzein and genistein) were shown to prevent bone loss associated with ovariectomy in rats. Thus, we have investigated the direct effects of Daidzein (D) and Genistein (G) on human osteoclast differentiation and activity. Human peripheral blood monocytes were differentiated under CSF-1 (25ng/ml) and RANKL (30ng/ml) to mature osteoclasts after 18 days of culture. The bone resorbing activity of multinucleated TRAP+ cells was evaluated by the pit area measured on dentine slices. Total RNA of the cells were used for semi-quantitative RT-PCR.In one set of experiments, 17βestradiol (E2), D and G were added on day 12 of culture in doses ranging from 0.1 nM to 1 µM. A decrease of pit area was observed under 10 nM of E2, D, and G treatments. However, the number of TRAP+ multinucleated cells was unchanged after E2, D, and G administration. Since others (Chikazu et al., 2000) have shown that FGF-2 directly activates mature rabbit osteoclasts, we have treated our culture system by FGF-2 (1nM) addition on day 12. In our conditions, the markers of osteoclastic activity cathepsin K and MMP9 were unchanged after FGF2 treatment. FGF receptors mRNAs were detected on day 18; only FGFR1 was markedly expressed in human osteoclasts. FGFR4 was not expressed, but FGFR2 and FGFR3 were slightly expresssed. However, E2 at 10 nM added on day 2 increased the FGFR1 mRNA at the end of the culture. Conclusions: Our results suggest that D and G like E2 inhibit bone resorption by a direct effect on osteoclast activity. Moreover, in contrast to the data obtained in rabbits, the markers of osteoclast differentiation were unchanged after FGF2 treatment of the human cells. Since E2 inhibits bone resorption,the increase of FGFR1 mRNA level after E2 treatment was unexpected.

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Physiological Significance of NMDA Receptors Expression in Osteoclast. R. Mentaverri,* A. Wattel,* C. Mullie,* C. Dupont,* F. Lorget,* S. Kamel, M. Brazier. Faculty of Pharmacy, AMIENS, France.

It has been recently demonstrated that osteoclasts express functional N-Methyl-D-Aspartate receptor (NMDAR). However, their physiological significance is presently unknown. We have previously described that MK801 (dizocilpine maleate), a specific NMDAR opened channel blocker, decreases in a dose dependant manner in vitro bone resorption at least in part by inducing osteoclast apoptosis(1). In this paper we investigated the molecular mechanism involved in the MK801-induced osteoclast apoptosis. Mature osteoclasts were purified from ten-day-old rabbit. To confirm that the effects of MK801 on osteoclast viability was related to the NMDAR we examined the effect of D-AP5 (2-amino-5-phophonovalerate) which bind to the NMDAR ligand site, and of the (-) isomer of MK801. D-AP5 although less effective than MK801 induced a significant increase in osteoclast apoptosis in a dose range (10-1000µM)which correlated well with it's effects on bone resorption. The (-) isomer of MK801 (100 µM) also induced a significant increase of osteoclast apoptosis but in a lesser extent than the (+) MK801 (100 µM). These results suggest that the effects of MK801 on osteoclast viability depend probably on the NMDAR activity. Z-DEVD-FMK, a specific caspase 3 inhibitory peptide, reversed significantly the effects of MK801 on osteoclast in a dose dependant manner (10µM-100µM) suggesting that the MK801-induced osteoclast apoptosis is dependant of caspase 3 activity, the main

effector of apoptotic process. Cycloheximide (0.1μg/ml), a protein synthesis inhibitor, also completely blocked osteoclastic cell death induction signifying that new protein synthesis is necessary to induce apoptosis.We have shown that increasing extracellular calcium concentration from 1.8 mM to 3 mM partially reversed both MK801-induced bone resorption inhibition and osteoclast apoptosis. In central nervous system, it has been reported that NMDAR is permeable to calcium allowing a calcium entry which in turn activate NO Synthase (NOS). Therefore we tested the hypothesis that in osteoclast NMDAR has the same function. Low doses of SNAP (0.01-1μM), a specific NO donor, also reversed partially the MK801-induced apoptotic cell death whereas no effects were observed when SNAP alone was added in the culture medium.From these results, it can be concluded that activation of NMDAR may be required for osteoclast survival. As in neuronal cells, signal transduction of NMDAR may imply calcium influx and NO biosynthesis. Further research are necessary to understand the link between NO production depending on calcium influx through NMDAR and osteoclast survival. (1) Mentaverri et al. (2000) J. Bone Miner. Res. 15, SU268

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Inhibition of p38 MAP Kinase Induces Apoptosis in Mature Primary Osteoclasts but Not in Mononucleated Precursors. M. A. Karsdal, N. T. Foged,* J. M. Delaissé, A. Lochter.* OSTEOPRO A/S, Herlev, Denmark.

In bone tissue, survival of cells plays an important role in the maintenance of tissue homeostasis. Recent findings demonstrate that survival and activity of osteoclasts are precisely regulated through interactions with supporting cells and by the action of several cytokines and growth factors. It has previously been demonstrated that osteoclastogenesis is severely impaired in the presence of a p38 MAP kinase inhibitor. However, primary mature osteoclasts have not been used in these studies, and the role of MAP kinases in the function of mature osteoclasts still remains to be investigated. In the present study, the survival of primary mononucleated pre-osteoclasts and multinucleated osteoclasts isolated from rabbit long bones was assessed by using TUNEL assay and nuclear fragmentation as indicators of apoptosis. Inhibitors of MAP kinases were added after cells were allowed to adhere, at which time about 10% of multinucleated osteoclasts displayed apoptotic nuclei. After 20 hours, osteoclast apoptosis in the presence of 10 μM of the p38 MAP kinase inhibitors PD169316, SB203580 and SB202190 was observed in 65% of the osteoclasts, whereas apoptosis in control cultures was still at 10%. Osteoclast apoptosis in the presence of 50 μM of PD98059, an inhibitor of the p44/42 activation, increased to 35% at this time. Most interestingly, TRAP positive mononuceated pre-osteoclasts did not undergo apoptosis in the presence of the p38 MAP kinase inhibitors, which corresponds well to previously reported data on the role of p38 MAP kinases in osteoclastogenesis. Furthermore, apoptosis levels comparable to control were observed when osteoblasts, fibroblasts or epithelial cells were treated with the p38 MAP kinase inhibitors for 2 days. When osteoclasts were plated on bovine bone slices to assay resorption, the p38 MAP kinase inhibitors totally abrogated resorption at 10 μM (100%) while the p44/42 MAP kinase inhibitor at 50 μM affected resorption less severely (<20% reduction). Likewise, when p38 MAP kinase inhibitors were used in organ cultures of either tibiae or calvariae, they completely blocked resorption, while the p44/42 inhibitor had only a slight effect on resorption (<20% reduction). These data present for the first time evidence that p38 MAP kinase is critical for survival of mature osteoclasts, and that p38 MAP kinase plays different roles during osteoclastogenesis and in mature resorbing osteoclasts. Thus, the p38 MAP kinase might constitute a possible target for the treatment of diseases with augmented osteoclast function.

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p38 MAP Kinase Is Involved in Osteoclast Differentiation but Not in Osteoclast Activation. X. Li,*[1] N. Udagawa,[1] K. Itoh,*[1] T. Suzawa,[1] K. Suda,*[1] Y. Murase,*[2] T. Nishihara,*[2] T. Suda,[1] N. Takahashi.[1] [1]School of Dentistry, Showa University, Tokyo, Japan, [2]Kyushu Dental College, Fukuoka, Japan.

The RANKL-RANK interaction plays essential roles in osteoclast differentiation and activation through MAPK (mitogen-activated protein kinase) signaling pathways such as JNK, ERK and p38. Although p38 MAPK is shown to be involved in many cellular processes including proliferation, differentiation and apoptosis, it is still unclear how p38 MAPK regulates osteoclast differentiation and function. We have shown here that p38 MAPK-mediated signal is essential for RANKL-induced osteoclast differentiation, but this signal is not necessary for osteoclast function induced by not only RANKL but also IL-1 and LPS. SB208530, a special inhibitor of p38 MAPK, blocked osteoclast formation in the co-culture of mouse bone marrow cells and primary osteoblasts treated with $1,25(OH)_2D_3$. SB208530 completely inhibited osteoclast formation in bone marrow cultures treated with RANKL and M-CSF. SB208530 showed no effect on the expression of RANKL mRNA induced by $1,25(OH)_2D_3$ in primary osteoblasts. SB208530 showed no inhibitory effect on the survival of osteoclasts supported by RANKL or M-CSF. The pit-forming activity of osteoclasts placed on dentin slices was not inhibited by SB208530. This compound showed no inhibitory effects on bone resorption in organ cultures of long bones obtained from newborn mice. Mature osteoclasts prepared from the co-culture as well as bone marrow macrophages (osteoclast precursors) expressed similar amounts of p38 MAPK. Phosphorylation of p38 MAPK was induced in bone marrow macrophages in response to RANKL but not in mature osteoclasts. LPS, TNFα and IL-1 similarly stimulated phosphorylation of p38 MAPK in bone marrow macrophages. But, LPS, TNFα and IL-1 failed to induce phosphorylation of p38 MAPK in mature osteoclasts, though those factors stimulated the survival of osteoclasts. These results suggest that p38 MAPK plays important roles in osteoclast differentiation, but not in osteoclast function. TNF and IL-1 family members could not activate p38 MAPK in osteoclasts. This further suggests that p38 MAPK signaling pathway is not involved in osteoclast function induced by these cytokines.

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rDRAK1, a Novel Kinase Related to Apoptosis, Is Expressed in Osteoclasts and Induces Apoptosis. H. Kojima,[1] T. Uemura,[2] A. Nemoto,*[2] R. Honma,*[3] M. Ogura,*[4] Y. Liu.*[5] [1]Tissue Engineering Research Center(TERC), National Inst. of Advanced Industrial Science and Technology(AIST), Domestic Research Fellow(JST), Tsukuba, Ibaraki, Japan, [2]TERC, AIST, CREST JST, Tsukuba Ibaraki, Japan, [3]TERC, AIST, Tsukuba Ibaraki, Japan, [4]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba Ibaraki, Japan, [5]Liver Cancer Institute, Shanghai Medical University, Shanghai, China.

In this paper, we report a novel serine/threonine kinase, rabbit death-associated protein (DAP) kinase-related apoptosis-inducing protein kinase 1 (rDRAK1) involved in osteoclast apoptosis. For several years, we have tried to clone osteoclast-specific genes involved in osteoclast resorption (cycle) including apoptosis. For this purpose, it was necessary to isolate primary osteoclasts of sufficient number and purity for biochemical or molecular biological study. Recently, an efficient method for the isolation of rabbit osteoclasts was established by Kakudo et al. Using a cDNA library of highly enriched rabbit osteoclasts, we obtained several fragments of genes which might be related to osteoclast resorption cycle. One of them has high homology with hDRAK1 a member of the DAP kinase subfamily, and it was termed as rDRAK1. DAP kinase is a calcium/calmodulin (CaM)-regulated serine/threonine protein kinase, and an effector of ganma-interferon-mediated apoptosis in HeLa cells. By the screening of a rabbit osteoclast cDNA library and 5'-RACE, we obtained a full-length of this cDNA. The sequencing data indicated that rDRAK1 has 88.0%, 44.6%, 38.7% and 42.3% identity with hDRAK1, DAP kinase, DRP-1 and ZIP-kinase, respectively. To demonstrate the role of rDRAK1 in rabbit osteoclast apoptosis, we verified the effect of typical osteoclast survival factors (IL-1, M-CSF and ODF) on DRAK1 mRNA expression by a quantitative reverse transcription polymerase chain reaction (RT-PCR) method. The results suggested that these three survival factors were proved to inhibit rDRAK1 expression in rabbit osteoclasts. After transfection of a rDRAK1 expression vector into cultured osteoclasts, overexpressed rDRAK1 was exclusively localized to the nuclei and induced apoptosis. Hence, rDRAK1 might play an important role in the core apoptosis program in osteoclast.

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Regulation of Osteoclast Survival by Tumor Necrosis Factor-α Inhibition of Caspases. H. Kwak,*[1] K. Kwack,*[2] Z. Lee,[3] H. Kim.*[4] [1]Microbiol.&Immunol., Chosun University School of Dentistry, Gwangju, Republic of Korea, [2]Immunomodulation Research Center, University of Ulsan, Ulsan, Republic of Korea, [3]National Research Lab. of Bone Metabolism, Chosun University School of Dentistry, Gwangju, Republic of Korea, [4]Research Center for Proteineous Materials, Chosun University, Gwangju, Republic of Korea.

Terminally differentiated mature osteoclasts have a short life span. The survival of osteoclasts appears to be dependent on osteotrophic factors presented by osteoblast/stromal cells as in the absence of the supporting cells, osteoclasts easily undergo apoptosis *in vitro*. A few studies have reported that the survival of osteoclasts is regulated by certain cytokines and hormones including estrogen, tumor growth factor-β, macrophage colony stimulating factor, interleukin 1, and receptor activator of NF-κB ligand. In the course of searching for new factors that regulate the survival of mature osteoclasts, we found that TNF-α induces the survival of differentiated mature osteoclasts. Mouse bone marrow cells were cocultured with calvarial osteoblasts on collagen gel for 6 days in the presence of 1α,25-dihydroxyvitamin D_3 and prostaglandin E_2. Osteoclasts were isolated from the coculture to ~95% purity of TRAP-positive cells. When TRAP-positive multinuclear cells were counted, 19.7 ± 2.0% of purified osteoclasts remained attached after 24-h culture in the absence of any ectopic factor. In the presence of 20 nM TNF-α, the survival rate was increased to 70.8 ± 6.9%. Addition of neutralizing antibodies against TNF receptor type 1 and type 2 supressed the survival effect of TNF-α. hHGF (80 ng/ml), mSCF (20 ng/ml), hKGF (50 ng/ml), hEGF (0.8 ng/ml), hNGF (3 ng/ml), mIL-6 (0.12 ng/ml) plus hIL-6R (30 ng/ml), mIL-3 (0.2 ng/ml), mIL-11 (0.3 ng/ml), mIL-17 (6 ng/ml), mMDC (40 ng/ml), mSDF-1α (18 ng/ml), hBMP-5 (2 μg/ml), and human pleiotrophin (16 μg/ml) did not increase osteoclast survival at concentrations more than two-fold of ED_{50}. The dying osteoclasts showed apoptotic characteristics in 4-6-diamidino-2-phenylindole (DAPI) staining. The TNF-α treatment reduced the percentage of cells with fragmented chromosome by 49.8% in DAPI staining. Caspase-9 and -3 activities increased in osteoclasts incubated in the absence of TNF-α by 2.35 ± 0.57 and 2.02 ± 0.16 folds, respectively. Addition of TNF-α reduced the caspase-9 and -3 activity increases to 1.35 ± 0.12 and 1.45 ± 0.05 folds, respectively. These results demonstrate that TNF-α stimulates the survival of mature osteoclasts by supressing caspase activities.

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The Akt and ERK Signaling Pathways are Involved in Tumor Necrosis Factor-α Stimulation of Osteoclast Survival. S. Lee,[1] H. Kim,*[2] Z. Lee.[3] [1]Microbiol. & Immunol., Chosun University School of Dentistry, Gwangju, Republic of Korea, [2]Research Center for Proteineous Materials, Chosun University, Gwangju, Republic of Korea, [3]National Research Lab. of Bone Metabolism, Chosun University School of Dentistry, Gwangju, Republic of Korea.

A few cytokines such as macrophage colony stimulating factor, interleukin 1, and receptor activator of NF-κB ligand are known to stimulate the survival of osteoclasts. However current understanding on the molecular mechanism by which these survival factors prevent osteoclasts from death is preliminary. Several signaling pathways have been implicated in the regulation of cell death. The phosphatidylinositol 3-kinase (PI-3K)/Akt pathway has been demonstrated to transduce antiapoptotic signals from various stimuli including growth factors, cytokines, and neuronal cell differentiation. Mitogen-activated protein kinase (MAPK)/ERK is activated upon phosphorylation by MEK which can be

activated by Raf-1. Raf-1 activation can be achieved by GTP-bound activated form of Ras. The Ras/ERK pathway has also been shown to be an important component for survival of neuronal and hematopoietic cells. However the roles played by Akt and MAPK/ERK for osteoclast survival have not been studied in detail. In this study, we report that tumor necrosis facor (TNF)-α induces the survival of mature osteoclasts by engaging the PI-3K/Akt and ERK signaling pathways. In the absence of added survival factors, purified mouse osteoclasts showed 10-20% suvival after 24-h culture whereas the survival rate was greatly increased in the presence of TNF-α to 65-75%. The effect of TNF-α was blocked by LY294002 known to specifically inhibit PI 3-K. The phosphorylation extent of Akt, which reflects the activation of this PI 3-K downstream target was increased by the treatment of TNF-α. Furthermore treatment with LY294002 blocked the increase in Akt phosphorylation by TNF-α. In addition the treatment of PD98059 that inhibits the ERK upstream kinase MEK1 abolished the effect of TNF-α on survival of purified osteoclasts. The involvement of ERK was further supported by the observation that TNF-α caused an increase in phosphorylation of ERK1 and ERK2 that was inhibited by treatment with PD98059. We also tested whether the antiapoptotic mechanism of PI 3-K/Akt pathway in the TNF-α-induced osteoclast survival would involve NF-κB activation. TNF-α stimulated the DNA binding activity of NF-κB in mature osteoclasts. Treatment of the cells with LY294002 did not affect the TNF-α-stimulated NF-κB activation. Therefore TNF-α may increase osteoclast survival by stimulating the PI 3-K/Akt and MEK/ERK pathways independent on NF-κB activation.

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RANKL and VEGF Induce Osteoclast Recruitment, Mediated Through the p42/44 MAPK-Pathway. K. Henriksen, M. A. Karsdal, N. T. Foged,* J. M. Delaissé, M. T. Engsig. OsteoPro A/S, Herlev, Denmark.

The ability of various growth factors such as RANKL, VEGF and M-CSF to promote osteoclast formation in vitro as well as in vivo is well established. However, additional regulatory functions, e.g. stimulation of resorptive activity, have been observed for these growth factors. In this study we address the role of RANKL and VEGF in osteoclast recruitment to the future site of resorption. Both RANKL and VEGF dose dependently stimulated invasion of murine bone marrow derived osteoclasts into type I collagen matrix in modified Boyden invasion chambers, reaching maximal effect at 100 ng/ml and 1 ng/ml respectively. By adding growth factors to the upper and/or the lower compartment of the invasion chamber, we observed that these stimulations were in part caused by chemotactic effects and in part by a general stimulation of osteoclast motility. The natural antagonists of RANKL and VEGF, OPG and endostatin respectively, specifically abrogated the corresponding growth factor induced invasion. Furthermore, in cultures of embryonic metatarsals isolated at Day 17 after gestation, 1 day before marrow cavity formation, OPG and endostatin prevented osteoclast invasion into the developing marrow cavity indicating physiological roles of RANKL and VEGF in osteoclast recruitment. To investigate the signal transduction pathways of the growth factor-induced osteoclast recruitment, osteoclasts in cultures were stimulated with RANKL or VEGF, and the activation of the MAP Kinases (MAPK) was followed by immunoblotting. Stimulation by RANKL or VEGF induced a transient activation of the p42/44 MAPK, but not the p38 MAPK. In addition, 10 μM PD98059, a synthetic inhibitor of p42/44 activation, abrogated the RANKL and VEGF induced stimulations of osteoclast invasion in the invasion chamber assays, without significantly affecting basal invasion level or osteoclast viability. Correspondingly, in cultures of embryonic metatarsals, osteoclast recruitment to the developing marrow cavity was prevented by 10 μM PD98059. In contrast, in cultures of 45Ca pre-labeled embryonic tibiae, where mature osteoclasts are already present and actively resorbing at the bone surface, PD98059 showed only partial inhibition of bone resorption as assessed by monitoring 45Ca release. We conclude that RANKL and VEGF induce osteoclast recruitment, mediated through p42/44 MAPK. Furthermore, we propose that this function plays a relevant role during development and in pathological situations.

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Inhibition of RANKL/RANK Signaling as a Mechanism of the Bone-sparing Effects of p38 MAPK Inhibitors in Rodent Arthritis. G. Mbalaviele, S. Settle, G. Anderson,* J. Portanova.* Arthritis and Inflammation, Pharmacia Corporation, St. Louis, MO, USA.

Rheumatoid arthritis (RA) is a chronic inflammatory disease that causes progressive destruction of the articular cartilage and subchondral bone. While the etiology of RA is unclear, it is known that cytokines such as tumor necrosis factor (TNF)-α and interleukin-1 play critical roles in the inflammatory process which leads to joint destruction. Since mitogen-activated protein kinase (MAPK) pathways are important in the production of cytokines and their downstream signaling functions, p38 MAPK (p38) was studied in RA. To evaluate potential beneficial effects of p38-based therapy on bone in RA, the effect of p38 inhibitors in rodent models of this disease were tested. Radiologically and histologically, p38 inhibitors prevented up to 100% joint swelling and bone and cartilage destruction in mouse collagen- and rat streptococcal cell wall-induced models of human RA. The cellular and molecular mechanisms underlying the bone-sparing effects of p38 inhibitors were investigated by using rodent culture systems, in which osteoclast formation from progenitor cells are RANKL-dependent events. First, the expression of p38 in mouse RAW 264.7 osteoclast precursors was determined by Western analysis. These cells expressed p38α, but not p38β, p38δ and p38γ isoforms. Treatment of RAW cells with soluble (s)-RANKL induced a rapid phosphorylation of p38, reaching a peak at 15 min. This phenomenon was not associated with a change in p38 protein level. RANKL also activated extracellular signal-regulated protein kinase (ERK) and NF-κB pathways in these cells and the p38 inhibitors had no effect on these pathways. To understand the role of p38 in osteoclastogenesis, mouse bone marrow cells or RAW cells were treated with 50 ng/ml s-RANKL and 10 ng/ml macrophage-colony stimulating factor (M-CSF) in the absence or presence of p38 inhibitors. Osteoclastogenesis was inhibited up to 100% by p38 inhibitors without evidence of cell toxicity, in a dose-dependent manner (0-10 μM), but not by an inactive analog agent. p38 inhibitors blocked osteoclastogenesis in rodent as well as in human purified bone marrow $CD34^+$ hematopoietic stem cell cultures. The inhibitors had no effect on cell proliferation and adherence. In conclusion, the data show that osteoclast precursors are targets of p38 inhibitors, and provide a mechanism of the bone-sparing effect of these inhibitors. Together with our own and other studies showing that TNF-α and RANKL synergistically induce osteoclastogenesis, p38-based therapies may be beneficial in preventing bone loss associated with inflammatory diseases such as RA.

Disclosures: Pharmacia Corporation,3.

SU315

Mechanisms Controlling Osteoclast Apoptosis. A. K. Shaw,[1] D. A. Pascoe,*[2] C. Giulivi,*[3] M. J. Oursler.[2] [1]Biochemistry, University of Minnesota Duluth, Duluth, MN, USA, [2]Biology, University of Minnesota Duluth, Duluth, MN, USA, [3]Chemistry, University of Minnesota Duluth, Duluth, MN, USA.

The rate at which bone is resorbed during both normal bone metabolism and pathological bone loss is directly influenced by the number of osteoclasts present. Regulating the rates of differentiation and death controls the number of osteoclasts present. Recent advances have greatly increased our understanding of osteoclast differentiation, but knowledge of the process of osteoclast apoptosis has lagged. In vitro studies have shown that many osteoclasts, once removed from the support of stromal cells, undergo apoptosis. It is the purpose of the studies reported here to resolve the molecular events that determine the fate of purified osteoclasts. To accomplish this goal, we have differentiated mouse osteoclast-like cells in the presence of stromal support cells. Once differentiated, the osteoclast-like cells were isolated from the support cells and the apoptotic response assessed by examining chromatin condensation. Within 90 minutes, there was evidence of apoptotic death in nearly half of the cells. Blocking initiator caspase 8 or effector caspases 3 or 6 greatly reduced the apoptotic response. Inhibition of protein synthesis resulted in apoptotic death of nearly all of the osteoclast-like cells whereas Actinomycin D had no effect on survival, suggesting that survival of osteoclasts requires protein synthesis in the absence of translational changes. We next investigated potential pathways likely to be involved in osteoclast survival in this model. Blocking the MEK/ERK pathway had no effect on survival and there was no increase in phosphorylation of either of these second messengers, suggesting that this pathway is not involved in osteoclast survival in the absence of support cells. Moreover, there was no evidence of changes in activation of survival-promoting NFκB. Interestingly, blocking PI3K dramatically increased osteoclast apoptosis. Several lines of evidence suggested that mitochondria are not involved in the process of apoptosis: there was no detectable change in mitochondrial membrane potential or increase in release of hydrogen peroxide during or preceding apoptosis. Addition of FCCP, a mitochondrial uncoupler that promotes mitochondrial depolarization, decreased hydrogen peroxide production. In addition, loss of ATP production by disrupting the electrochemical potential had no influence on osteoclast survival. Taken together, these data support that osteoclast apoptosis is similar to other hematopoietic cells in that it is caspase 8 and 3 driven yet differs in that apoptosis is carried out in the absence of a mitochondrial involvement.

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The Role of Trp Channels in Osteoclast Calcium Sensing. B. D. Bennett,[1] M. X. Zhu,*[2] K. Tustison,*[1] U. Alvarez,*[1] T. Sugatani,*[1] K. A. Hruska.[1] [1]Internal Medicine, Renal Division, Washington University School of Medicine, St. Louis, MO, USA, [2]Pharmacology and Neurobiotechnology, Ohio State University, Columbus, OH, USA.

Handling of extracellular calcium, $[Ca^{2+}]_e$, by cells in bone is important due to the large amount of Ca^{2+} liberated by osteoclasts during bone resorption. Influx of Ca^{2+} into osteoclasts may impact bone resorption by interacting with important signaling pathways mediating apoptosis and cell motility. $[Ca^{2+}]_e$ is putatively "sensed" by OCs, and the associated rise in intracellular calcium is linked to PLC (1) and may involve PLC-linked transient receptor potential (trp) calcium channels (2). Since, trp channels supply Ca^{2+} to endoplasmic reticulum calcium pumps (SERCA) filling intracellular calcium stores, trp may influence intracellular metabolism of calcium generated by the extracellular calcium load brought about by resorption. Osteoclasts express trp3 and 6 with trp3 being the dominantly expressed protein. Trp6 is localized in vesicular compartments associated with the ER. Trp6 overexpression potentiated Ca-sensing and increased ER Ca stores. In contrast, trp3 was localized in the plasma membrane and expression of a trp3 dominant negative construct revealed inhibition of store-operated calcium influx, but only minor inhibition of Ca-sensing. Since Ca-sensing is PLC dependent and does not require filled stores (1) and trp6 is PLC-linked and can be activated even after store-depletion, we conclude that osteoclast Ca-sensing may occur through trp6 and that trp3 is linked to Ca store-operated influx. These data together with the known trp physical interaction with ER bound receptors and plasma membrane bound PLC, suggest a model where a trp-PLC-receptor complex is part of the vast ER region in multinucleated giant cell OCs which operates as the OC calcium store fed by trp3 and trp6. (1) Bennett, BD et al. Endocrinology (2001) 142(5). (2) Bennett, BD et al JBMR (2000) 15: S517.

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Caffeine Consumption, Rates of Hip Bone Loss and Risk of Hip Fracture. M. C. Homan,*[1] P. G. McGovern,*[1] P. J. Bowman,*[1] K. L. Stone,[2] M. C. Nevitt,[2] D. C. Bauer,[2] K. E. Ensrud.[3] [1]University of Minnesota, Minneapolis, MN, USA, [2]University of California, San Francisco, CA, USA, [3]VA Med Center & Univ of Minnesota, Minneapolis, MN, USA.

Research has been inconclusive regarding the relationship of caffeine consumption to both bone density and risk for fracture. The association between caffeine consumption and rates of bone loss in very elderly women is uncertain. We examined the associations between caffeine consumption (current and lifetime), and rates of hip bone loss and risk of hip fracture in 9704 women aged 65 years or older at baseline enrolled in the Study of Osteoporotic Fractures. Current and lifetime measures of caffeine consumption were obtained by questionnaire at the baseline visit (1986-1988). Participants were followed an average of 10.6 years for hip fractures; all 632 fractures were confirmed by review of films or x-ray reports. Bone mineral density (BMD) at the total hip and its four subregions was

measured in 6020 participants at the second and fourth exams (average 3.7 years between exams). We calculated the mean annualized percent change in hip BMD by quartile of caffeine consumption using the least squared means procedure. Cox regression was used to analyze the association between caffeine consumption and risk of first incident hip fracture. There was no evidence of an association between age-adjusted caffeine consumption (current or lifetime) and rates of hip bone loss; further adjustment for potential confounders (clinic, smoking, alcohol use and estrogen use) did not alter our findings. Women with low caffeine consumption (second quartile) compared with those with no consumption had an increased risk of hip fracture that persisted despite adjustment for covariates. However, the test for trend across quartiles of caffeine consumption did not reach significance. We found no evidence that lifetime caffeine consumption was related to risk for hip fracture.

Quartiles of current caffeine intake	Mean percent change in BMD/year*		Relative Hazards for hip fracture (95% CI)*
	Femoral neck	Total hip	
1 (0 mg/d)	-0.45	-0.57	1.0 (Referent)
2 (1-139 mg/d)	-0.54	-0.59	1.29 (1.04,1.61)
3 (140-239 mg/d)	-0.50	-0.64	1.19 (0.96,1.47)
4 (240-630 mg/d)	-0.55	-0.51	1.23 (0.97.1.55)
Test for trend	p=0.20	p=0.40	p=0.10

*Age-adjusted.We conclude that increasing caffeine consumption is not associated with rates of bone loss or risk for hip fracture in elderly women

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Seasonal Variations in 25-Hydroxy Vitamin D, Parathyroid Hormone and 1,25 Dihydroxy Vitamin D in a Healthy Population of Western Canadians. D. Rucker, J. A. Allan,* G. H. Fick,* D. A. Hanley. Medicine, University of Calgary, Calgary, AB, Canada.

Individuals living in countries at higher latitudes are more prone to seasonal vitamin D insufficiency that can lead to secondary hyperparathyroidism and subsequent bone loss. In this study we evaluated the seasonal prevalence of vitamin D insufficiency (defined as 25OHD <50nmol) and seasonal variations in 25-hydroxy vitamin D (25OHD), parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D (1,25OH2D) in a healthy, community dwelling population of Western Canadians at 51.4° North. A total of 188 randomly selected subjects (60 men and 128 women, aged 27-89 yrs) from the Calgary cohort of the Canadian Multicentre Osteoporosis Study participated. Individuals were excluded only if baseline 25OHD were 200 IU vitamin D/day. Fasting overnight blood samples were collected in three-month intervals: February, May, August and November. Commercial RIA assay kits (DiaSorin Inc.) were used to measure serum 25OHD, PTH, and 1,25-OH2D and regression models for longitudinal data were built separately for each hormone to determine the effects of age, BMI, season, sex and other potential predictors. Non-significant variables were removed from the final model. Serum levels of 25OHD, PTH and 1,25OH2D and (geometric mean ± SD) are shown in the table below. The predicted rise of 25OHD during the spring and summer months and subsequent decrease during fall and winter months was seen. Vitamin D insufficiency was found in 61% of subjects at least once during this 12-month study. Though 1,25OH2D and PTH declined throughout the year, these changes were well within the reference range, and had no relationship to 25OHD levels. PTH was not a significant predictor for 25OHD (P=0.104), indicating that serum PTH should not be used to predict the need for vitamin D supplementation. Though we documented a high prevalence of vitamin D insufficiency, the implications of seasonal 25OHD insufficiency on bone health need further investigation.

	FEB	MAY	AUG	NOV
25OHD (nmol/L)	53.4±1.5	57.6±1.5ac	67.8±1.4bc	49.9±1.4ac
PTH (ng/L)	35.5±1.6	35.6±1.6	32.4±1.6ad	30.4±1.7bd
1,25OH2D (pg/ml)	63.5±1.5	55.4±1.7be	53.8±1.6be	49.3±1.7be

Values designated by a or b differ significantly from FEB (aP<0.05; bP<0.001); cAdjusted for age, BMI (kg/m2) and holiday travel; dAdjusted for age, sex BMI and serum calcium; eAdjusted for age, BMI, PTH, 25OHD and inorganic phosphate

SU319

Fruit Intake Is Associated with Better Bone Mass Among Hong Kong Chinese Early Postmenopausal Women. Y. Chen, S. C. Ho, R. Lee,* S. Lam,* J. Woo.* Department of Community & Family Medicine, The Chinese University of Hong Kong, New Territories, Hong Kong Special Administrative Region of China.

The effects of nutritional factors on bone mass is still largely uncertain, most of the previous studies focused primarily on the role of nutrients, especially calcium, in bone health. Little attention was paid to different food groups. This study examined the association of habitual fruit and vegetable intakes with bone mass in a community-based cross-sectional study. A total of 668 Chinese early postmenopausal women with an age range of 48-62 y and within 10 y natural menopause were recruited into the study between July 1999 to December 2000. Inclusion in the study required that subjects be ambulatory with no medical conditions known to affect bone density or calcium metabolism. Habitual food intake in the past 12 months was assessed by using purpose-designed semi-quantitative food-frequency questionnaire. Bone mineral density (BMD) and bone mineral content (BMC) were measured with dual-energy X-ray absorptiometry (DEXA) at whole body (WB), lumbar spine (LS) and left hip (LH). Physical activity, menses history, demographic data were assessed by standardized questions. Partial correlation analysis showed that higher fruit intake was significantly associated with better BMD and BMC at WB, LS and LH after controlling for age, years since menopause and body mass index. The partial correlation coefficients of fruit intake with BMD and BMC were 0.119 (p<0.01) & 0.142 (p<0.001) at WB, 0.131 (p<0.01) & 0.168 (p<0.001) at LS, and 0.087 (p<0.05) & 0.106 (p<0.01) at left femoral neck, respectively. The results remained significant after further adjustment for dietary calcium, protein, phosphorus intakes, and physical activity index in stepwise multivariate regression models. Fruit intake accounted for 1.7% & 1.4% (p<0.001), 2.0% & 2.8% (p<0.001) and 0.6% (p=0.033) & 1.0% (p=0.008) of changes in BMD and BMC at whole body, lumbar spine and left femoral neck, respectively. Bone mass at lumbar spine had a closer correlation with fruit intake than that at left hip. No significant correlation was observed between vegetable intake and BMD and BMC among the three sites. The results suggested that higher fruit intake might be related to a better bone mass in early postmenopausal women.

SU320

A Longitudinal Study of Calcium Intake and Peak Bone Mass in Chinese Women. S. S. G. Chan,[1] S. C. Ho,[1] P. C. Leung.*[2] [1]Department of Community & Family Medicine, The Chinese University of Hong Kong, New Territories, Hong Kong Special Administrative Region of China, [2]Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, New Territories, Hong Kong Special Administrative Region of China.

Our study on premenopausal women has shown that peak bone mass (PBM) is attained in early thirties and small loss of spinal bone mineral density (SBMD) occurs after PBM attainment. Previous cross-sectional analysis has shown an association between energy-adjusted calcium intake (Ca-D) and SBMD in young premenopausal women, but longitudinal data in Asian women are lacking. This study aims to report the effect of Ca-D in the maintenance of PBM in Chinese women aged 21-40. 145 women aged 21 to 40 had been followed up for two years (mean 2.17 years, SD=0.17). We had obtained baseline measurements of SBMD (L2-L4) with the use of dual energy x-ray densitometre (Norland XR26). Dietary intake of Ca was assessed through quantitative food frequency method. Information on body measurement, physical activity, particularly weight bearing activity were also obtained at baseline. Repeated measurements of SBMD were obtained at 2-year followup and the outcome variable was the percentage change of SBMD over the followup time.A mean 1.3% decline of SBMD was observed. Univarate regression analysis shows a positive association of Ca-D with % change of SBMD. Ca-D accounted for 4.5% of variance of bone change. Multivariate regression analysis shows the beneficial effect of Ca-D still remains after adjusting for bone area, followup time, lean body mass and weight bearing activity. The coefficient of % change of SBMD of the second Ca-D quartile comparing with the lowest quartile was 3.41 (SE=1.38) (p=.015) in the adjusted model which accounted for 32% variance of bone change. This study shows that adequate calcium intake is a significant dietary factor for PBM maintenance in Chinese women.

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Bone Turnover with In vivo Smoke and Ethanol Exposure. D. M. Cullen,[1] A. C. Peyton,*[1] M. J. Gentry-Nielsen,*[2] M. P. Akhter.[1] [1]Medicine, Creighton University, Omaha, NE, USA, [2]Medical Microbiology, Creighton University, Omaha, NE, USA.

Smoking has been associated with low bone mass in humans, but is also associated with heavy drinking behavior. In this study we hypothesized that the deleterious effects of smoking and ethanol exposure on bone would be additive. Young male Sprague-Dawley rats (100-120g) were exposed to mainstream and sidestream smoke twice/day weekdays and once/day weekends for 55 minutes (5 cigarettes) for 7.5 weeks. For the last 8 days, the control and smoked groups were each randomized to alcohol or chow food. Half received liquid diets with 35% of the calories from EtOH, the other half were maintained on the standard rat chow. In smoke exposed rats, carboxyhemoglobin levels ranged from 18.2-25.2% and nicotine averaged 67 (8)ng/ml. Blood EtOH averaged 65 (9) mg/dl. Calcein was injected 8 and 2 days before collection. Difference between groups (Smoke-EtOH, Smoke-Chow, Sham-EtOH, Sham-Chow, n=6/gr) were compared by two factor ANOVA with P<0.05 and reported as mean (SEM). Histomorphometry was used to measure tibial cortical periosteal and endocortical surfaces and cancellous endosteal surfaces. Endpoints for analysis included bone area (BV/TV); mineralizing surface (MS); and mineral apposition rate (MAR). With EtOH consumption, periosteal and cancellous mineralizing surface were lower while endocortical and endosteal MAR were greater compared to Chow fed rats. Cancellous bone volume was lower in EtOH than Chow rats. Smoke effects were greater in chow than EtOH fed rats for periosteal and cancellous mineralizing surfaces. In conclusion, acute alcohol exposure results in rapid bone loss and suppressed bone formation. There was a pattern of deleterious smoke effects in chow fed rats, but no combined effects for EtOH and smoke. Acute, heavy alcohol exposure appears to be more detrimental to bone than smoking.

	Chow-Sham	Chow-Smoke	EtOH-Sham	EtOH-Smoke
Peri MS [ab]	80 (2)	73 (3)	64 (3)	69 (2)
Peri MAR	2.8 (0.1)	2.6 (0.1)	2.6 (0.1)	2.4 (0.1)
Endo MS	46 (4)	60 (5)	45 (4)	45 (4)
Endo MAR [a]	2.1 (0.3)	2.4 (0.2)	2.8 (0.3)	2.8 (0.1)
Canc. BVTV [a]	9.2 (1.1)	8.6 (0.7)	5.7 (0.8)	6.1 (0.7)
Canc. MS [ab]	26.4 (1.2)	19.0 (2.2)	13.3 (1.6)	16.5 (1.8)
Canc. MAR [a]	1.4 (0.1)	1.3 (0.1)	1.7 (0.1)	1.6 (0.1)

[a]difference Chow vs EtOH; [b] interaction of chow and smoke

SU322

Body Composition Reference Ranges for American Men and Women. T. L. Kelly. Hologic, Inc., Bedford, MA, USA.

Whole body DXA measurements of body composition were employed to develop %Fat reference ranges for American men (n=254) and women (n=1723). Obese and overweight thresholds for DXA %Fat were defined based on the gender-specific young adult prevalence of these conditions in the United States (NHANES, 1997.) In the 1997 NHANES survey, subjects with a BMI above 25 (41% of men and 33% of women) were considered overweight and subjects with a BMI greater than or equal to 30 (14% of men and 12% of women) were considered obese. DXA %Fat reference ranges were developed based upon these percentages.

	Normal (% Fat)	**Overweight** (% Fat)	**Obese** (% Fat)
Male	4.0%- 25.2%	25.3%- 30.3%	≥30.4%
Female	12.0%- 29.2%	29.3%- 35.2%	≥ 35.3%

The lower limit of the Normal %Fat range represents fat which is essential for normal physiological functions. Essential fat is found in the bone marrow, nerve linings, heart, lungs, liver, muscle, and in a host of other tissues. It constitutes approximately 12% of body mass in women and 4% in men. When the relationship between BMI and %Fat was examined more closely, the two indices of obesity were only moderately correlated (r^2= 0.65 for women and 0.34 for men) with high prediction errors between BMI and %Fat (SEE 4.6% Fat for women and 5.8% for men). The relatively high prediction error and poor correlation in male subjects may indicate a deficiency in the ability of BMI to capture obesity, particularly in men. For example, a short but muscular male subject may have a BMI in the overweight range and a relatively low %Fat value. As a consequence, BMI may overstate the prevalence of obesity in this subgroup. It is postulated that DXA %Fat measurements are superior to BMI for determining the prevalence of obesity. Further, the reference ranges presented here will facilitate the interpretation of DXA %Fat measurements in clinical medicine and in obesity research.

SU323

Bone Strength Properties with Tobacco Smoke and Ethanol Exposure in Rats. M. P. Akhter,[1] T. W. Hull,*[1] M. J. Gentry-Nielsen,*[2] D. M. Cullen,*[1] R. R. Recker.*[1] [1]Medicine, Creighton University, Omaha, NE, USA, [2]Medical Microbiology, Creighton University, Omaha, NE, USA.

Low bone mass has been reported to be associated with smoking and drinking behavior. In this study we hypothesized that the deleterious effects of smoking and ethanol (EtOH) exposure on bone biomechanical properties will be additive. This study evaluated the effects of both tobacco smoke and ethanol exposure on bone biomechanical properties in rats. Young male rats (100-120g) were exposed to main-stream and side-stream cigarette smoke in a whole-body exposure chamber, set at 70 to 100 mg smoke particulates/m^3 for a 55 minutes session (12 session/wk) for 7.5 weeks. Smoking chamber was developed at Creighton University Biomechanics Lab. For the last 8 days, the control and smoke-exposed groups were each randomized to EtOH or standard chow food. Half received liquid diets with 35% of the calories from EtOH, the other half were maintained on the standard rat chow. In the smoke exposed rats, carboxyhemoglobin levels ranged from 18.2-25.2% and nicotine averaged 67(8) ng/ml. Blood EtOH averaged 65(9) mg/dl. Vertebral bodies (L4) were collected, prepared, subjected to compression testing (stroke rate of 3mm/min, using Instron 5543 testing system), and analyzed for structural [ultimate(ULT) & yield(YLD) load, and Stiffness(STIF)], material [ultimate (ULTRS) & yield (YLDRS) stress and flexural modulus (MOD)] strength, and bone density (BMD) parameters. Difference between groups (Chow-Sham, Chow-Smoke, EtOH-Sham, EtOH-Smoke, n=6rats/group) were compared by two-factor ANOVA with P<0.05 and reported as mean (SEM). Structural strength parameters (ULT, YLD), material strength parameters (ULTRS, YLDRS), and BMD were lower in the EtOH group as compared to the CHOW fed group (Table). There is greater EtOH effect on rat bone biomechanical properties as compared to smoke exposure. In conclusion, heavy alcohol (ethanol) exposure seems to deteriorate vertebral bone strength and density more than smoking alone.

Bone Strength mean(SEM)	**CHOW-SHAM**	**CHOW-SMOKE**	**EtOH-SHAM**	**EtOH - SMOKE**
Ultimate load (N) [a]	171(10)	166(11)	144(16)	133(7)
Yield load (N) [a]	142(10)	145(9)	115(15)	111(13)
Stiffness (N/mm)	746(57)	720(66)	715(56)	664(49)
Ultimate stress (N/mm^2) [a]	24(2)	26(2)	20(2)	20(1)
Yield stress (N/mm^2) [a]	20(1)	22(2)	16(2)	17(2)
Flexural Modulus (N/mm^2)	562(49)	555(55)	533(37)	521(27)
BMD (mg/mm^2) [a]	1.99(0.09)	2.1(0.09)	1.93(0.04)	1.80(0.07)

[a] difference Chow vs EtOH

SU324

Oral Calcium Intake, Vitamin D Concentrations and Renal Calcium Excretion in Women with and without Osteoporosis. I. M. Frieling,* S. Hachfeld,* M. Dickmann,* H. P. Kruse.* Nephrology/Osteology, Medical Clinic, University Hospital Hamburg, Hamburg, Germany.

Current laboratory diagnostic guides of osteoporosis imply the examination of renal calcium excretion in 24 hour urine to find out possible pathogenic important hypercalciuria and to estimate oral calcium needs. Renal calcium excretion reflects intestinal calcium absorption, which depends on oral calcium intake and vitamin D levels. Our primary endpoint is to confirm this assumption even in a large cohort of patients.We examined 109 pre- and postmenopausal women from 22 to 89 years, mean 59,7 years. All patients were introduced in our special out-patient clinic with a suspected or diagnosed osteoporosis, however part of the subjects already received oral calcium and/or vitamin D supplementation. All women received an established calcium diet questionnaire and another one to check their knowledge about the calcium content of different food. Simultaneously they were questioned whether they were informed about osteoporosis by their GP or were organized in any osteoporosis selfaidgroup. Laboratory routine tests were performed, also the examination of calcium, anorganic phosphate, creatinine, albumine, alkaline phosphatase, bone specific alkaline phosphatase, 25-OH-vitamin D, 1,25(OH)2-vitamin D and PTH in serum and deoxypyridin cross-links in second morning urine and calcium excretion in 24 hour urine.The average daily oral calcium intake including possible supplementation was 1032 mg, minimum 113 mg and maximum 3078 mg. The medium renal calcium excretion was 5,06 mmol/d (0,30 - 14,70). The average of laboratory tests of bone turnover and calcium regulating hormones were normal, vitamin D 27,2 ng/ml (4,9 - 78,0). Between oral calcium intake and renal calcium excretion a weak, but significant positive correlation was found (r=0,26; p=0,013), but no correlation between renal calcium excretion and 25- OH vitamin D and 1,25(OH)2 vitamin D levels. Bone specific alkaline phosphatase and deoxypyridin cross-links had a positive correlation (r=0,69; p<0,0001). PTH had a negative correlation to 25-OH vitamin D, but not to 1,25 (OH)2 vitamin D.The results confirm the assumption, that renal calcium excretion depends on oral calcium intake. The poor correlation does not allow any conclusions in the special case. The so called secondary hyperparathyroidism depends on vitamin D levels; there is no direct correlation to oral calcium intake. Bone specific alkaline phosphatase increases with increasing PTH. In this case vitamin D levels have no diagnostic relevance.

SU325

The Influence of Dietary Calcium and Vitamin D Intake on Osteoporotic Fracture Risk. K. Michaëlsson,[1] A. Wolk.[2] [1]Department of Orthopaedics, University hospital, Uppsala, Sweden, [2]Department of Medical Epidemiology, Karolinska Institutet, Stockholm, Sweden.

The etiologic role of dietary calcium and vitamin D intake in primary prevention of osteoporotic fractures is uncertain. To further explore these associations we used data from a large population-based prospective cohort study with a setting in a high incidence area of osteoporotic fractures in central Sweden. We estimated nutrient intake using data obtained from a comprehensive validated food-frequency questionnaire in a cohort comprising 61,463 women, aged 38-76 years at baseline in 1987-90. Women with first fractures of the hip (n=1535), pelvis (n=524), spine (n=405), distal forearm (n=1972) and proximal upper arm (n=633) were identified through December 31, 2000, by linkage to out- and in-patient registers and by scrutinizing x-ray records. Those with fractures prior to the entry to the cohort (n=774) were excluded from analysis. During an average 11.1 years of follow-up, we observed 3,986 women with a fracture at any site. Rate ratios for fractures (RR) and 95% CI were estimated using Cox proportional hazards models. We found no overall association between calcium intake and fracture risk. RR for fracture of the hip per 300 mg/day increase in dietary calcium intake was 1.04 (95% CI 0.97-1.12). Corresponding RR for pelvic fractures was 1.03 (95% CI 0.91-1.17), for vertebral fractures 1.07 (95% CI 0.92-1.23), for distal forearm fractures 0.98 (95% CI 0.92-1.05), for upper arm fractures 1.01 (95% CI 0.90-1.13) and for any of the sites RR 1.01 (95% CI 0.96-1.06). Highest compared to lowest decile of calcium intake conferred a RR of 0.97 (95% CI 0.84-1.12). Furthermore, no association between dietary vitamin D intake and fracture risk was observed; RR per µg increase in daily vitamin D intake was 0.99 (95% CI 0.96-1.02) for any fracture. No interaction between calcium and vitamin D intake was observed. Highest deciles of both calcium and vitamin D intake compared to the lowest deciles of both nutrients revealed a RR of 1.07 for all fractures (95% CI 0.81-1.41). No significant association was found between calcium or vitamin D intake and fracture risk even after exploring subgroups such as different categories of age or body mass index. Although our study is potentially limited by the observational design, we conclude that individuals who have a low or a high dietary calcium or vitamin D intake have the same osteoporotic fracture risk as those with average intakes of these nutrients.

SU326

Dairy Products and Radial Bone Density among Elderly Women - The HUNT Study, Norway. S. Forsmo,*[1] A. Langhammar,*[1] L. Forsén,*[2] B. Schei.*[1] [1]Norwegian University of Science and Technology, Trondheim, Norway, [2]National Institute of Public Health, Oslo, Norway.

The fracture incidence in Norway is one of the highest in the world. Traditionally, dairy products constitute the main calcium source, and their consumption, especially of milk is also among the highest in the world. This remains a paradox. The purpose of this study was to analyse the association between dairy products (milk, cheese) and bone mass density (BMD) in elderly Norwegian women. As part of a population-based health study during the years 1995-97 in the Norwegian county of Nord-Trøndelag (HUNT study), distal and ultradistal radial bone mass density was measured with single X-ray absorptiometry (SXA-technology) in 18265 men and women above 19 years. All women 65 years or elder, resident in the county, were invited for densitometry and 5057 women aged 65-79 at invitation were eligible for this study. The non-dominant arm was measured, provided no previous fracture in distal radius. A comprehensive questionnaire included several alimentary questions. The milk question involved four levels: none, less than one, 1-2 and 3 or more glasses of milk. Cheese intake was measured as slices of bread with cheese (similar four levels). Milk and cheese intake was reported by 94.7% and 95.4%, respectively, of the 4510 women (89%) with valid answers. A total of 82.2% reported at least one glass of milk daily. There was no variation by age. Women reporting no or less than one daily glass of milk had significantly increased risk of low BMD (Z-score<-1) at both distal and ultradistal radius than women with daily milk intake, OR=1.4 (1.1-1.7). No or scarce cheese intake was not found to be associated with low BMD. In a multivariate model controlling for age, weight, age since menopause and hormone replacement therapy, milk and cheese were sig-

nificantly positively associated with distal and ultradistal radial BMD. The overall contribution to the BMD variance was, however, small, R2=0,4%. In this model, weight (positive) and age (negative) were the strongest predictors of BMD. According to the model, a woman of 70 years, 70 kg, 20 years since menopause and drinking at least 3 glasses of milk a day would have about 5% higher radial BMD than a non-milk drinking woman with otherwise same characteristics. In summary, milk consumption in Norway is frequent, even in older age. There is a statistically significant positive association between the consumption of dairy products and BMD, stronger for milk than for cheese. Its overall contribution to BMD seems, however, small.

SU327

Tobacco Smoke Exposure and Bone Biomechanical Properties. M. P. Akhter,[1] G. R. Haynatzki,[1] T. W. Hull,*[1] D. M. Cullen,[1] R. R. Recker,[1] G. C. Gairola.*[2] [1]Medicine, Creighton University, Omaha, NE, USA, [2]Toxicology, University of Kentucky, Lexington, KY, USA.

Epidemiological evidence suggests that tobacco smokers are at increased risk of bone fractures (Am J Med 106(3):311-4,1999). However, a recent meta-analysis of several studies failed to show an effect of smoking on bone mass in pre-menopausal women (BMJ 315:841-846, 1997). In the present study, using mouse as an animal model, we have determined if biomechanical strength characterstics of femurs are influenced by experimental exposure to cigarette (tobacco) smoke. Female C57BL mice (11-12 wks old) were exposed to side-stream cigarette smoke in a whole-body exposure chamber, set at 30mg smoke particulates/m^3 for 4hrs/d and 5 d/wk for 12 weeks. Femurs were collected, subjected to 3-point bending test (stroke rate of 3mm/min, using Instron 5543 testing system), and analyzed for structural [ultimate(ULT) & yield(YLD) load, and Stiffness(STIF)] and material [ultimate (ULTRS) & yield (YLDRS) stress and flexural modulus (MOD)] strength variables. Elevated levels of blood carboxyhemoglobin and pulmonary CYP1A1 protein confirmed the exposure of mice to cigarette smoke. Two-group t-test was used to analyze the differences (P<0.05) between smoke and non-smoke-exposed groups for all the biomechanical strength variables. All structural strength variables (ULT, YLD, STIF) and material strength variables (ULTRS, YLDRS) were significantly (P<0.05) lower in the smoke-exposed group as compared to the control (non-smoke group). The flexural modulus was marginally lower in the smoke-exposed group (P<0.1) (Table).

Bone Strength mean (SEM)	Smoke (n=18)	Non-Smoke (n=6)
Ultimate load (N)	17.5 (0.3) [a]	21.2 (0.6)
Yield load (N)	14 (0.4) [a]	17.0 (0.8)
Stiffness (N/mm)	116 (3.8) [a]	140 (7.8)
Ultimate stress (N/mm^2)	104 (3.8) [a]	122 (3.6)
Yield stress (N/mm^2)	83 (3.7) [a]	100 (7.0)
Flexural Modulus (N/mm^2)	2247 (125) [b]	2604 (134)

[a] P <0.05, [b] P <0.1

The results suggest a deleterious effect of tobacco smoke exposure on biomechanical properties (both structural and material strength) of femurs in mice.

SU328

The Relationship Between Diet and Bone Mineral Density in Older Men. K. L. Stone,[1] T. Blackwell,[1] E. S. Orwoll,[2] J. C. Cauley,[3] E. Barrett-Connor,[4] R. Marcus,[5] M. C. Nevitt,[1] S. R. Cummings.[1] [1]University of California, San Francisco, CA, USA, [2]Oregon Health Sciences University, Portland, OR, USA, [3]University of Pittsburgh, Pittsburgh, PA, USA, [4]University of California, San Diego, CA, USA, [5]Stanford University, Palo Alto, CA, USA.

Osteoporosis is a concern among both men and women, although little is known about the correlates of bone mineral density (BMD) and fracture risk in older men. To test whether dietary intake of selected nutrients is related to BMD in elderly men, we analyzed preliminary data from the Osteoporotic Fractures in Men ("MrOS") study.During the baseline examination, a 70+ item food frequency questionnaire (Block Dietary Data Systems) was administered in 1705 men aged 65 and older. BMD of the whole body (WBMD), total hip (HBMD) and total spine (SBMD) were assessed by DXA (Hologic QDR 4500W). Food questionnaires were analyzed to obtain estimated daily dietary intake of selected nutrients.Using linear regression, we tested the association of dietary intakes with WBMD, HBMD and SBMD. Intakes were categorized as low (lowest quintile), medium (2nd through 4th quintile) and high (upper quintile) for the analyses. All results were adjusted for age, body weight, physical activity, and total energy intake. Further adjustment for race, smoking, alcohol, caffeine and health status did not change the results.In separate models, dietary calcium, potassium, protein and lutein (a carotenoid abundant in dark green vegetables) were related to both WBMD and HBMD (results for HBMD shown in table). However, in combination only calcium, potassium and lutein remained significant.

Adjusted mean total hip BMD (g/cm2) by levels of dietary intake.

	Low	Medium	High	p(trend)
Calcium	0.94	0.96	0.97	0.001
Potassium	0.94	0.96	0.97	0.001
Protein	0.95	0.96	0.97	0.03
Lutein	0.94	0.96	0.96	0.03

Lutein shares many dietary sources with vitamin K and has been shown to correlate with rates of bone loss in older women. Higher dietary intakes of calcium, potassium and lutein may contribute to bone health in older men, however further research is needed to confirm these findings, and to determine whether these relatively modest differences in BMD will translate into significant differences in fracture risk among older men

SU329

Prediction of Fracture Risk with Age, Years Since Menopause, Bone Mineral Density and Pre-existing Fracture. H. Watanabe,*[1] M. Fukunaga,[2] M. Shiraki,[3] Y. Ohashi.*[1] [1]Department of Biostatistics, University of Tokyo, Tokyo, Japan, [2]Department of Nuclear Medicine, Kawasaki Medical School, Okayama, Japan, [3]Research Institute and Practice for Involutional Diseases, Nagano, Japan.

Prediction of fracture risk is useful for determining treatment policy, and the formula was estimated using age, years since menopause, bone mineral density and pre-existing fracture based on a prospective cohort study. The subjects were 524 healthy women after natural menopause.The subjects' age, years since menopause (YSM), height (BH), weight (BW) and bone mineral density (BMD) were determined at the beginning of the study with seven years follow up. The fracture risk was analyzed by Cox regression analysis. The optimum model was chosen from the combination of all explanatory variables (the score method). Since age and YSM have a high correlation, transformation was done for YSM for avoiding multicollinearity. The mean (±s.d.) of each baseline variable was : age 63.4±10.4 years, height 150.5±7.7 cm, weight 51.6±8.7 kg, YSM 14.6±10.3 years, BMD (DPX) 0.966±0.185 g/cm^2, and pre-existing fracture was found in 18.5% (97/524) of subjects. As a result, the incidence of fracture was 15.6% (82/524). As for the sites of the fracture, they were 67 of vertebrae, five of femur neck, nine of forearm, two of rib, and four of the other (five subjects had two fractures at a different site). In prediction formula, age, height, weight, BMD, previous fracture, and YSM (continuous variable) were significant respectively and the selected model was P=1-exp(-exp(.0971(age)-1.94(BMD)+1.12(fr)-.631(YSM_1)-1.00(YSM_2)-6.90)x year), where P is the cumulative probability of fracture, (fr) is pre-existing fracture (yes:1, no:0), YSM_1 and YSM_2 are dummy variables (YSM_1=1 when YSM>=5 and YSM_1=0 when YSM<5; YSM_2=1 when YSM>=12 and YSM_2=0 when YSM<12).

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Influence of Serum 25-OHD on Hip Fracture in the Elderly. G. Martini, R. Valenti,* S. Giovani,* S. Salvadori,* G. Silvestri,* R. Nuti. Metabolic Disease Unit, University of Siena, Siena, Italy.

Vitamin D is required for efficient absorption of dietary calcium and for mineralization of bone. Reduced vitamin D (25-OH) levels are associated with increase of parathyroid hormone (PTH) which stimulates bone resorption and bone loss. It has been shown a decrease in serum 25-OHD with advancing age and it has been postulated that hip fracture can be due to hypovitaminosis D but only limited data are available on vitamin D status in patients admitted for acute osteoporotic fracture. The aim of the study was to evaluate serum 25-OHD concentrations, serum PTH levels and serum markers of bone turnover in women with acute hip fracture.The study was conducted in 74 patients with hip fracture and 73 ambulatory control subjects (mean ± SD: 80.4 ± 9.3 years and 71.2 ± 6.1 years respectively). All patients had normal renal function and none were taking vitamin D supplementation. Fractured patients were selfsufficiency before accident.Bone metabolism was evaluated by: serum calcium and phosphate (standard methods), serum bone alkaline phosphatase (Metrabiosystem), serum crosslaps (Osteometer), serum 25OHD (Biomedica), iPTH 1-84 (DRG Int.). No differences among groups were appreciated as regards serum calcium, serum phosphate and bone alkaline phosphatase. Significantly higher values of serum crosslaps were found in hip fracture patients (2483 ± 1911 pmol/L vs 1915 ± 1068 pmol/L; p<0.05). Serum 25OHD and PTH did not differ significantly between the groups. Only 4 patients with hip fractures had low levels of serum 25OHD (<12 ng/ml). Our data indicate that fractured patients included into the study have normal vitamin D status. The condition of increased bone resorption may be due to a condition of increased calcium malabsorption related to a reduced 1,25(OH)2D synthesis.

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Bioelectrical Impedance Analysis and Body Composition in Hip Fracture Patients. I. Weller, N. Payne, J. Schatzker. M.E. Muller Program, Sunnybrook & Women's College Health Sciences Centre, Toronto, ON, Canada.

Purpose: To determine the feasibility of using bioelectrical impedance analysis (BIA) and to obtain preliminary data on body composition in hospitalized hip fracture patients. Methods: Hip fracture patients (n=18) who met the inclusion (³ 80 yr, female) and exclusion criteria (unable to obtain informed consent, fracture as a result of severe trauma or metastatic cancer, implanted defibrillator, renal disease) were recruited. BIA was used to assess fat and fat free mass. BIA is an inexpensive, non-invasive technique to measure body composition in epidemiologic studies and is an important improvement over BMI . Measurements were carried out with a RJL Systems analyser under standardized conditions, using the standard tetrapolar technique according to the manufacturer's instructions for distal placement on the hand and foot of the unaffected side. An average of measures taken on two consecutive days was used in the analysis. Height and weight were recorded. Results: We experienced little difficulty in recruitment and even the cognitively impaired patients did not find the procedure unpleasant or intrusive. The following table summarizes the body composition data obtained from our study and compares it to data in the literature on healthy elderly women.

	Pilot Study (n=18)	Baumgartner (n=56)
	Mean (SD)	Mean (SD)
Age (yr)	84.9 (3.6)	> 80 yr

Weight (kg)	54.9 (8.5)	59.0 (8.5)
Height (cm)	157.9 (8.0)	156.3 (6.1)
BMI (kg/m^2)	22.0 (3.0)	24.2 (3.3)
Fat Free Mass (kg)	35.7 (11.5)	37.3 (3.2)
Fat Mass (kg)	19.2 (17.0)	21.4 (7.3)

The patients in the two studies are comparable with respect to age and height. Body weight, body mass index (BMI), fat mass and fat free mass are lower in the hip fracture patients compared to healthy elderly. Conclusions: BIA is a feasible measure to use on elderly hip fracture patients. These preliminary results suggest that the lower weight and BMI in elderly female hip fracture patients is due to both low fat mass and fat free mass.

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Prevalence of Vertebral Fracture Among Patients with Chronic Obstructive Pulmonary Disease (COPD) in Canada. A. Papaioannou,[1] J. D. Adachi,[1] W. Parkinson,*[2] N. C. Ferko,*[3] G. Ioannidis,[1] E. Jurriaans,*[1] L. Probyn,*[1] G. Cox,*[1] G. Stephenson,*[4] N. Hannigan.*[1] [1]Medicine, McMaster University, Hamilton, ON, Canada, [2]School, Rehabilitation Sciences, McMaster University, Hamilton, ON, Canada, [3]Clinical Health Sciences, McMaster University, Hamilton, ON, Canada, [4]Procter & Gamble Pharmaceuticals, Toronto, ON, Canada.

Research in osteoporosis has suggested that vertebral fractures may coexist with COPD. Vertebral fractures secondary to osteoporosis reduce quality of life and are a risk factor for hip fracture. Because these fractures can be prevented with appropriate medications, recognition and treatment of high-risk patients is warranted. The purpose of this study is to determine 1) the prevalence of vertebral fractures among patients with COPD admitted to acute care compared to age-matched controls and 2) the extent to which COPD patients with vertebral fractures were discharged on osteoporosis therapy. The design is a case-controlled study of 149 COPD patients randomly identified by ICD-9 codes and 145 sex-and age matched controls 50 years of age and over who were randomly identified by ICD-9 codes, and who had a lateral-anterior/posterior chest radiograph, admitted to an acute care hospital in Hamilton, Ontario in 1999. Outcomes were established using a chart review to identify diagnoses, medication use, and chest radiograph interpretations. The sample included 154 women and 140 men with a mean age of 72.2 years (SD 12.2) and 71.9 years (SD 8.2), respectively. The prevalence of any type of vertebral fracture reported by radiograph was found to be 15/149 (10.1%) in the COPD patients and 3/145 (2.1%) in the controls. The prevalence of either vertebral fracture or osteopenia was 26/149 (17.4%) in the cases and 13/145 (8.9%) in the controls. The proportion of patients exposed to either oral or inhaled corticosteroids at the time of admission was 73.2% in the cases and 4.1% in the controls. The proportion of patients with fractures who were discharged on osteoporosis therapy (bisphosphonates or hormone replacement therapy) was 4/18 (16.6%). Of all patients with confirmed vertebral fractures, 7/18 (38.8%) had a diagnosis of osteoporosis indicated in their medical records. This study demonstrates an increased prevalence of vertebral fractures in COPD patients. Osteoporosis and vertebral fractures are often overlooked in discharge summaries. This high-risk group should be targeted for the prevention and treatment of osteoporosis related fractures.

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Hip Fracture Prediction Ability of Hip Axis Length and a Proposed Indicator of Femoral Neck Strength. A. S. Karlamangla,*[1] E. Barrett-Connor,[2] G. A. Greendale.[1] [1]Division of Geriatrics, UCLA School of Medicine, Los Angeles, CA, USA, [2]Department of Community and Family Medicine, UCSD School of Medicine, La Jolla, CA, USA.

Previous studies have found that longer hip axis length (HAL) is associated with higher hip fracture risk in women. It is postulated that this association exists because HAL is a surrogate measure of the moment arm and is hence proportional to the bending moment at the femoral neck. While the bending moment determines the magnitude of the forces within the bone, the ability of the bone to resist breakage depends also on its cross sectional area and density. An integrated measure that incorporates femoral neck axis length (FNAL), femoral neck width (FNW), and bone mineral density (NeckBMD) in the femoral neck: (NeckBMD * FNW) / FNAL might therefore be a better predictor of femoral neck strength and fracture risk. We conducted a pilot study in a cohort of 134 women (mean age 72 years) from the Rancho Bernardo Study who had DEXA bone density scans of the hip between 1988 and 1991, and had not had a hip fracture prior to the scan. FNAL and FNW were measured manually on hard copy printouts of the hip DEXA scans. 13 of the women had hip fractures by 1998. Logistic regression was used to examine the prediction ability of HAL and the proposed integrated femoral neck strength measure for incident hip fractures, after adjusting for age, height, weight, and body mass index. We found no significant association between HAL and the risk of incident hip fracture (Adjusted OR=0.73; 95% CI: [0.30, 1.8]). After adjustment, each standard deviation increase in our proposed femoral neck strength measure reduced the risk of incident hip fractures by 60% (Adjusted OR=0.38; 95% CI: [0.16, 0.92]). Including 21 additional women in the study, who had DEXA scans of the hip between 1988 and 1991 and had fractures in the contralateral hip prior to DEXA acquisition, did not alter the observed lack of association between HAL and fractures. The adjusted odds ratio of HAL for hip fractures (prevalent or incident) was 0.97 (95% CI: [0.50, 1.8]). We conclude that the proposed integrated strength measure may provide better prediction of hip fracture risk than any single geometric measurement. This finding warrants further investigation in a larger sample.

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Femoral Neck Bone Mineral Density and Femoral Neck Axis Length of Chinese Females and Males: Differences Between Patients With Osteoporotic Fracture in the Femoral Neck and Age-Matched Controls. T. Hai,[1,2] J. Yebin,[1] L. Xian-Zheng,[2] S. Ren,[2] H. K. Genant.[1] [1]Radiology, Osteoporosis & Arthritis Research Group, San Francisco, CA, USA, [2]Dept. of Orthopedic Surgery, Beijing Friendship Hospital, Beijing, China.

PURPOSE: Fracture resistance or bone biomechanic strength depends mainly on bone mineral density and bone geometric properties. The higher prevalence of osteoporotic hip fracture in Caucasians than in Asians may be due to differences in the hip geometry. We investigated in a Chinese population the differences in the femoral neck bone mineral density (BMD) and the femoral neck axis length (FNAL) between patients with osteoporotic hip fracture and age-matched controls. METHOD/MATERIALS: We evaluated 106 patients with femoral hip osteoporotic fracture, including 70 females with age ranging from 60 to 83 years (mean 69±9 years) and 36 males with age ranging from 60 to 93 years (mean 71±9 years). In addition, age-matched subjects without hip fracture, including 70 females and 40 males, were served as controls. They were examined by dual x-ray absorptiometry (DPX-L, Lunar Corporation, Wisconsin), and their femoral neck BMD (g/cm2) and FNAL (mm) were determined. RESULTS: The femoral neck BMD in the fractured females (0.642±0.09 g/cm2) and males (0.683±0.13 g/cm2) was significantly lower (p0.05) in FNAL between the fractured females (86±4 mm) and the female controls (86±5 mm), and between the fractured males (97±6 mm) and the male controls (96±5 mm). There was no difference (p>0.05) in BMD between the fractured females and the fractured male controls, while BMD was greater in the non-fractured males than in the non-fractured females (p<0.001). FNAL was longer in males than in female of both fractured groups and the controls (p<0.001). CONCLUSIONS: Loss of the femoral neck BMD was associated with femoral neck fracture in both Chinese females and males. FNAL was not associated with femoral neck fracture and could not be used to predict femoral neck fracture. The Chinese males had longer FNAL than Chinese females. In patients with femoral neck fracture, loss of BMD was greater in males than in females.

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Effect of Estrogen on Fracture Callus Quality in an Ovariectomized Rat Model. H. Ouyang,*[1] R. Mendelsohn,*[1] A. Tomin,[2] A. L. Boskey,[2] P. J. Sherman,*[2] E. P. Paschalis.[2] [1]Chemistry, Rutgers University, Newark, NJ, USA, [2]Mineralized Tissues, Hospital for Special Surgery, New York, NY, USA.

Epidemiological studies and recent animal experimentation in ovariectomized rats support the concept that estrogen deficient osteoporosis is associated with delayed fracture healing. It is further uncertain whether it is the state of decreased bone mass by which osteoporosis is defined, or the deficiency of estrogen that is responsible for abnormal fracture repair. In the present study, the hypothesis that estrogen deficient osteoporosis impairs fracture healing due to altered fracture callus quality, was tested using Fourier transform infrared microscopic imaging (FTIRI). FTIRI allows the analysis of non-demineralized thin tissue sections. Each image analyzes a 400 x 400 um2 area with a spatial resolution of ~7 um. The outcomes of the analyses were: a) mineral/matrix ratio (corresponding to ash-weight, b) mineral crystallinity (crystallite size/perfection in the c-crystallographic axis), and c) relative ratio of collagen cross-links (pyr/DHLNL). Measurements were made both in the fracture callus and in bone away from the fracture site in each animal (three images/site/animal). The results were reported both as color-coded images and pixel population distribution histograms (employed for statistical comparisons; Kruskal-Wallis test). A standard transverse, closed fracture of the right femur was created at 12 weeks in 16 female Sprague-Dawley rats ovariectomized at six weeks of age. Half these animals received subcutaneous, continuous release estrogen throughout the study, while the other half received only the carrier. The animals were sacrificed at 4, 6, 8, and 12 weeks after fracture. Methacrylate-embedded thin (~ 3um) sections were analyzed by FTIRI. When the fracture callus area was compared to the adjacent bone, the mineral/matrix, mineral crystallinity and collagen cross-link ratio (Pyr/DHLNL) had lower values in the callus area for the 4, 6, and 8 week time points. By the 12th week, all three parameters were statistically similar, except mineral crystallinity, which remained lower in animals not treated with estrogen. When the fracture callus area quality was compared among the two groups, the mineral/matrix ratios were statistically similar, whereas both the mineral crystallinity and collagen cross-link ratio (Pyr/DHLNL) exhibited statistically significant higher values in estrogen treated animals at all time-points. The results are consistent with an osteoclast-suppression mechanism of action for estrogen, and are in excellent agreement with previously reported mechanical testing of the fracture callus results (4-point bending test) in the same groups of animals.

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Volumetric Bone Density and Fracture Risk in Men and Women in the Dubbo Osteoporosis Epidemiology Study (DOES). J. R. Center,*[1] T. V. Nguyen,*[1] N. K. Henderson,[2] N. A. Pocock,[3] J. A. Eisman.[1] [1]Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW, Australia, [2]Curtin University of Technology, Sheraton Park, WA, Australia, [3]Dept of Nuclear Medicine, St Vincent's Hospital, Sydney, NSW, Australia.

Bone mineral density (BMD), a non-invasive predictor of hip fracture risk, is a 2 dimensional (areal) and not a true volumetric measure, thus incompletely accounts for size. The two-fold greater hip fracture rate in women versus men has been attributed to this lower areal BMD. However, differences in bone size and geometry may also be factors and true volumetric BMD may provide additional information. The aim of this study was to examine gender differences in volumetric BMD, areal BMD and bone size in relation to hip fracture. DOES has examined 3000 men and women aged 60+ years since 1989. All subjects with a hip fracture that occurred prior to or within 3 months of a DXA scan (42 women and 11 men) were compared with 932 women and 680 men without any fractures. Femoral neck volumetric BMD was calculated (LUNAR DPX-L) assuming it to be cylindrical. Femoral neck axis length (FNAL) had previously been measured on a subset of sub-

jects (n = 126 for men and n = 109 for women).Areal BMD was lower in women than men in both the non-fracture and hip fracture subjects. However, volumetric BMD was the same in both sexes for non-fracture (0.31 ± 0.06 vs 0.31 ± 0.05, p = 0.90, women and men, respectively) and hip fracture subjects (0.25 ± 0.04 in both sexes). Both areal and volumetric BMD were significantly lower in hip fracture subjects. Estimated cross-sectional area (CSA) of the femoral neck was higher in men than women for both non-fracture (11.3 ± 1.8 vs 8.6 ± 1.5, p = 0.001) and hip fracture subjects (11.6 ± 1.9 vs 8.1 ± 1.8, p = 0.0001) but did not differ according to fracture status. Areal BMD, CSA and FNAL were positively correlated with height while volumetric BMD was largely independent of height. In men, but not women, there was a significant correlation between FNAL and CSA (r = 0.39, p = 0.0001 and r = 0.14, p = 0.15, men and women respectively). Thus volumetric BMD, which is the same in men and women, is lower in hip fracture groups. The differential fracture rate between the sexes is therefore not solely related to "true" bone density. The larger bone size and CSA in men may off-set the mechanical disadvantage of a longer femoral neck explaining the lower fracture rate in men. The interrelationships between volumetric bone density, size and geometry at the hip warrant further evaluation in relation to hip fracture risk.

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Risk Factors of Falls and its Consequences in Postmenopausal Women with Osteoporosis. M. Glueer,[1] H. Minne,[2] M. Pfeifer,[2] B. Begerow,*[2] A. Lazarescu,[2] W. Pollähne.*[2] [1]Bundesanstalt Milchforschung, Kiel, Germany, [2]Institut Klinische Osteologie, Bad Pyrmont, Germany.

Falls play a crucial role in the development of appendicular fractures - most importantly of the hip. Their effects on health and Quality of Life need further investigation. Also the reasons for falls are not completely known though various risk factors have been identified yet (e.g. Sway, Pfeifer et al.). We investigated these issues in 283 women (age 63.5±7.9) with postmenopausal osteoporosis who attended a rehabilitation program at baseline. Subsequently we followed 124 of these women (64.9±7.6) over an one year period. Fall characteristics and Quality of Life were assessed using standardised questionnaires and functional tests. Vertebral fractures were assessed on radiographs by an experienced radiologist. At baseline 127 (43.5%) women reported about at least one severe fall in the past 5 years. Of those 61.4% recalled at least one fall, 19.7% reported about 2 falls and 18.9% suffered from 3-10 falls. Slipping (n=65, 51%) and tripping (n=40, 31%) were the most frequent reasons for falling. Only three patients reported dizziness as a reason for falling. 17.3% could not recall the reasons of the fall. The 'fallers' were older (p< .037), had more multimorbidities (p<.002), had a slightly elevated BMI (p<.056), had a somewhat poorer balance (walking backwards on a line, p<0.079) and had more bone related surgeries (.021). They suffered more frequently from coronary heart disease (p<.029) and tended to be hypertonic (p<.10). Fallers had a signficantly higher number of wrist fractures (p<.001) and rib fractures (p<.005) but not more vertebral fractures (p<.89). Five out of six hip fractures in the total sample were reported by the fallers (p<0.05). Fallers had more pain in general (p<.041), tended to have suffered longer from back pain (p<.055), reported an elevated disease-related anxiety level (p<.002), but not more functional limitations (p<.25). Within the group of fallers, pain increased with increasing number of falls (p<.033) and a trend to higher functional limitations (p<.15) was reported as directly fall-related consequences.After one year, 19 (15%) of the 124 women experienced a new severe fall. Among the factors listed above only age (p<.11), coronary heart disease in general (p<.16), hypertension (p<.15), and social extroversion (p<.09) showed trends for predicting higher risk of falling. Effects are probably weak due too small number of incident falls.These data underline the multifactorial pattern of determinants for risk of falling. Results stress the necessity for preventing falls in order to reduce the risk of appendicular fractures, fall-related pain, and disease-related anxiety.

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Defining Incident Vertebral Deformities: Another Approach. J. Fechtenbaum,[1] B. Giraudeau,[2] P. Ravaud,[3] S. Kolta,*[1] C. Roux.*[1] [1]Cochin Hospital, Paris, France, [2]Clinical Research Center, Tours, France, [3]Epidemiology and Biostatistics, Bichat Hospital, Paris, France.

Semi quantitative (SQ) and quantitative (Q) methods are used to diagnose vertebral deformities in clinical studies. In Q method, a fixed percentage (- 15 % or - 20 %) of decrease in any vertebral height is used. This percentage has been established in order to obtain the highest sensitivity and specificity as compared to SQ results. There are discrepancies between the 2 methods, and an adjudication is mandatory. Thus these 2 assessments are not independent.From a large population of osteoporotic postmenopausal patients, 100 women were randomly selected among those women without any incident vertebral deformity according to SQ performed at baseline and 1 year later. Thoracic and lumbar spine X-rays were performed using standardized method of acquisition. Morphometry was performed from T4 to L4. For any vertebral height a threshold was calculated at - 1.96 SD, where SD is the standard deviation of the difference between the 2 measurements. Thresholds on the height ratios have also been determined with the same method. An incident fracture was defined as a decrease of more (in absolute value) than the associated threshold. In a second step, we applied these thresholds to a database of 16 089 vertebrae obtained from a similar population with a 3 year follow-up. In this population 414 incident fractures have been diagnosed using adjudication between SQ and Q (- 20 %) results.The diagnostic threshold was highly dependent on the considered vertebra and height, ranging from 1.5mm (anterior height T7 and T8) to up to 3.7mm (middle height L1). We diagnosed 369/414 fractures as compared to 332/414 using a fixed threshold of - 20 %. Considering the number of patients having at least one incident fracture, sensitivities and specificities were 89.1 %, 98.2 % for our method and 80.2 %, 99.7 % for the fixed threshold.This method determines a quantitative threshold for each height, ratio and vertebra. It is based on the long term reproducibility. It can be used independently of semi quantitative assessment and may be crossed with SQ results to determine deformities diagnosed by both methods, without adjudication.

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Vertebral Deformities and Underlying Occult Pathology; An Explanation for the Increased Mortality of Patients with Prevalent Vertebral Deformities? R. G. J. Versluis,*[1] S. E. Papapoulos.[2] [1]General Practice, Leiden University Medical Center, Leiden, The Netherlands, [2]Endocrinology, Leiden University Medical Center, Leiden, The Netherlands.

Vertebral deformities are usually asymptomatic but are associated with increased mortality. No causal relation to mortality has been identified in epidemiological studies. In an investigation of simple methods to identify osteoporosis in women in general practice lateral spine x-rays were obtained in a random cohort of 449 women aged between 55 and 84 years. Vertebral morphometry was performed with the method of Eastell et al. In 44 women (10%) grade II (severe) vertebral deformities were identified. These women were invited to the practice and 42 attended. Medical history was obtained, and examination and a laboratory screening were performed. The most common abnormality was vitamin D deficiency/insufficiency which was present in 24% of the women. 22 women were referred back to their GP with management instructions while the rest were invited to a specialized bone clinic for detailed investigations. 19 women attended. In 5 of these women (12% of those with grade II deformities investigated) serious pathology was identified (multiple myeloma, chronic lymphatic leukemia, coeliac disease, Paget's disease and primary hyperparathyroidism). Thus, severe vertebral deformities were associated with serious, previously unknown pathology, in this randomly selected cohort. These results suggest that occult underlying pathology may contribute to the association of vertebral deformities with mortality documented in epidemiological studies. Moreover, the frequency and the seriousness of these deformities strongly suggest that lateral spine x-rays should be part of case-finding strategies in osteoporosis.

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The Influence of Anthropometric Factors on the Risk of Incident Vertebral Fracture. D. K. Roy,[1] J. D. Finn,*[1] D. Felsenberg,[2] O. Johnell,[3] M. Lunt,*[1] T. W. O'Neill,[1] J. Reeve,[4] A. J. Silman,*[1] EPOS Study Group.[1] [1]ARC Epidemiology Unit, University of Manchester, Manchester, United Kingdom, [2]Freie University, Berlin, Germany, [3]Malmo General Hospital, Malmo, Sweden, [4]Institute of Public Health, Cambridge, United Kingdom.

The aim of this study was to investigate the role of anthropometric variables on the incidence of vertebral fracture in European men and women.Men and women aged 50 to 79 years were recruited from population registers in 28 centres for participation in a prospective study of osteoporotic fractures - the European Prospective Osteoporosis Study (EPOS). Those who took part in the baseline survey had lateral thoracolumbar radiographs performed. Height and weight were measured in a standard fashion. Duplicate spinal radiographs were performed a mean of 3.8 years following the baseline survey. Incident vertebral fractures were defined using a combination of the point prevalence and 20% change in vertebral height criteria. Poisson regression was used to determine the influence of baseline weight, height and body mass index on the occurrence of incident vertebral fracture.3228 men (mean age 63.2 years) and 3456 women (mean age 62.2 years) were included in this analysis. During the follow-up period 197 subjects experienced an incident vertebral fracture. After adjusting for age and gender, those in the lowest quintile of weight (relative risk [RR]=1.4; 95% confidence interval [CI] 1.0, 2.1) and body mass index (RR=1.5; CI 1.1, 2.1) had an increased risk of incident vertebral fracture compared to those in higher quintiles. Height did not appear to predict incident vertebral fracture.These data suggest that low body weight is associated with an increased risk of incident vertebral fracture.

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Between-Centre Variation in Limb Fracture Rates Is Due in Part to Variation in Rates of Falling. D. K. Roy,[1] S. R. Pye,*[1] M. Lunt,*[1] T. W. O'Neill,[1] J. Reeve,[2] A. J. Silman,*[1] EPOS Study Group.[1] [1]ARC Epidemiology Unit, University of Manchester, Manchester, United Kingdom, [2]Institute of Public Health, Cambridge, United Kingdom.

There is important geographic variation in the occurrence of the major osteoporotic fractures. The aim of this study was to determine the extent to which between-centre variation in limb fracture rates across Europe is explained by variation in rates of falling.Men and women aged 50-79 years were recruited from population based registers in 31 European centres. Subjects were followed by postal questionnaire to ascertain the occurrence of incident fractures and were also asked about the occurrence and number of recent falls. Self reported fractures were confirmed, where possible by review of the radiographs, medical record or subject interview. In the analysis subjects contributed follow-up (person-years) from the date of the baseline survey until either the occurrence of limb fracture, death or the end of the study. Poisson regression was used to assess how much of the observed between-centre differences in limb fracture rates could be explained by between-centre differences in fall rates.6320 men (mean age 63.8 years) and 6803 women (mean age 63.1 years) completed at least one questionnaire. During a median follow up time of 3 years, 3659 falls were reported by men and 4829 by women. There were statistically significant between-centre differences in the occurrence of both upper and lower limb fractures. After age adjustment, the centre specific fall rates were significant determinants of upper and lower limb fractures. Between-centre variation in fall rate explained 22% and 6% of the between-centre variation in the incidence of these fractures respectively.A significant proportion of the observed between-centre differences in limb fracture rates may be explained by differences in fall rates in those centres.

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Multiple Prevalent Vertebral Fractures are Associated with Greater Physical Impairment than Single Fractures. K. Aoyagi,[1] P. D. Ross,[2] H. Jinbayashi,[3] M. Ito.[4] [1]Dept of Public Health, Nagasaki University, Nagasaki,

Japan, [2]Merck & Co., Inc, Rahway, NJ, USA, [3]Dept of Orthopedic Surgery, Nagasaki University, Nagasaki, Japan, [4]Dept of Radiology, Nagasaki University, Nagasaki, Japan.

Patients with multiple existing vertebral fractures have greater pain and physical impairment, and greater risk of subsequent fractures, compared to patients with a single vertebral fracture, who in turn have greater impairment and fracture risk than patients without an existing vertebral fracture. However, most data comes from Caucasian populations, and there is relatively little data on the prevalence and consequences of multiple vertebral fractures in Japanese women. We examined the associations of the number of prevalent vertebral fractures and other characteristics with physical functioning among 584 Japanese women ages 40 to 90 years. Lateral spine radiographs were obtained and radiographic vertebral fractures were assessed by quantitative morphometry, defined as vertebral heights more than 3 SD below the normal mean. A self-administered questionnaire was used to survey participants about difficulty in performing selected basic and instrumental activities of daily living (ADL). The prevalence of vertebral fractures increased significantly with age. The prevalence of one or more vertebral fractures was 15% overall, and 50% among women ages 80 and over. The prevalence of women with 2 or more vertebral fractures was 8% overall, and 37% among women ages 80 and over. Impaired function was defined as difficulty performing 3 or more ADLs. In an age-adjusted logistic regression model, the odds of impaired function was 1.4 (95% CI: 0.7-2.9) times higher for women with a single vertebral fracture, and 3.1 (95% CI: 1.4-6.8) times higher for women with 2 or more fractures, compared to women with no fractures. In multiple variable logistic regression models, 2 or more vertebral fractures remained a significant predictor of impaired function (odds ratio, OR: 4.1, 95% CI: 1.8-9.5), independent of age, number of painful joints, and BMI, but one fracture was not (OR: 1.5, 95% CI: 0.7-3.2). Additional adjustment for back pain did not alter these findings. In conclusion, Japanese women with 2 or more vertebral fractures have greater impairment than those with only one fracture.

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Association of Health Status and Physical Performance with Self-Reported History of Fracture. H. Fink,*[1] M. Kuskowski,*[1] M. Nevitt,[2] J. Cauley,[3] K. Stone,[2] K. Ensrud.[4] [1]GRECC, VA Medical Center, Minneapolis, USA, [2]U CA, San Francisco, USA, [3]U Pittsburgh, Pittsburgh, USA, [4]U MN, Minneapolis, USA.

Little is known about the impact of clinical fractures (fx) on health status and physical function in older men. We addressed this issue with data from MrOS, an ongoing, prospective cohort study of men aged ≥ 65 yrs. At baseline, data were collected regarding previous fx, current health status (SF-12 quality of life physical component summary scale [PCS-12] and mental component summary scale [MCS-12]), physical function (impairment in IADLs), and common chronic conditions. Measures of physical performance were maximal bilateral grip strength (kg, Jamar dynamometer) and leg extensor power (watts, Nottingham Power Rig), chair stands (ability and time to complete 5 stands without use of arms) and 6-meter walk speed (m/sec). The odds ratio of being in the most impaired quintile (vs. other quintiles combined) for each health measure was determined as a function of self-reported history of hip, spine or wrist/forearm fx. For each skeletal site, men with 1 or more fx of that type at age ≥50 yrs were compared to men without any fx at age ≥ 50 yrs. Results were adjusted for baseline age, physical activity and number of comorbid conditions. Of 2006 men enrolled in MrOS, 28 reported a history of hip fx(s) (median time since fx 5.5 yrs), 45 had a spine fx(s) (median 6.0 yrs) and 81 had a wrist/forearm fx(s) (median 10.0 yrs).

	Hip fx	**Spine fx**	**Wrist/forearm fx**
	OR (95%CI)	OR (95%CI)	OR (95%CI)
PCS-12	2.5 (1.1-6.0)*	1.9 (0.97-3.9)	1.0 (0.6-1.9)
MCS-12	1.2 (0.5-3.0)	0.8 (0.3-1.7)	0.7 (0.4-1.3)
IADL impairments	0.9 (0.3-2.4)	2.6 (1.3-5.1)*	1.1 (0.6-1.9)
Grip strength	1.1 (0.4-2.7)	2.1 (1.1-4.1)*	1.2 (0.7-2.1)
Leg extensor power	0.5 (0.2-1.7)	2.2 (1.05-4.5)*	0.7 (0.4-1.4)
Chair stands	2.7 (1.2-6.1)*	1.1 (0.5-2.2)	0.9 (0.5-1.6)
Walk speed	1.4 (0.6-3.4)	3.5 (1.8-6.9)*	0.9 (0.5-1.6)

*p<0.05

Though numbers of fx were small, among older, community-dwelling men, history of hip or spine fx, but not wrist/forearm fx, was associated with significantly increased odds of impairment in multiple measures of health status or physical performance. While men with hip fx appear more likely to have trouble arising from a chair, those with spine fx appear more likely to have IADL impairments, slow walk speed, and low grip strength and leg power. Prospective data are needed to confirm a causal relationship between fx and poor health outcomes in men.

SU344

Lengthy Hospitalizations Associated With Vertebral Fractures Despite Control for Comorbid Conditions. N. Ferko,*[1] A. Papaioannou,[2] J. D. Adachi,[2] W. Parkinson,*[3] G. Stephenson,*[4] M. Bedard.*[5] [1]Clinical Health Sciences, McMaster University, Hamilton, ON, Canada, [2]Medicine, McMaster University, Hamilton, ON, Canada, [3]School, Rehabilitation Sciences, McMaster University, Hamilton, ON, Canada, [4]Procter & Gamble Pharmaceuticals, Toronto, ON, Canada, [5]Lakehead Psychiatric Hospital, Thunder Bay, ON, Canada.

This study established whether length of hospital stay (L.O.S.) in Canadians 50 and older is attributable to their vertebral fractures versus comorbid conditions. The study used a case control design and data in the Canadian Institute for Health Information (CIHI) database on hospital discharges in Ontario, Alberta, and British Columbia between April 1,1996 to March 31, 1997. Patients with vertebral fractures were identified by International Classification of Diseases (ICD-9) codes. L.O.S. constituted the dependent measure in a multivariate linear regression that calculated the independent contributions to L.O.S. by vertebral fractures while controlling for: age, gender, province, discharged deceased, hip fractures, all other fractures, motor vehicle accidents, all other injuries, and the major disorder classifications in ICD-9. There were 846,651 unique patients in the database. Their overall mean age was 70.0 years. Women comprised 50.2% of the sample. Mean L.O.S. for the entire data base sample was 6.54 days (95%CI=6.53, 6.55). Mean L.O.S. for all patients admitted for vertebral fractures was 10.1 days (95%CI=9.8, 10.4). L.O.S. attributed solely to vertebral fractures was 4.8 days based on a 50 year old woman with no comorbid conditions, and 6.1 days based on a 75 year old woman. Of 18 health conditions, vertebral fractures were among the top 3 in accounting for L.O.S. along with hip fractures and mental disorders which accounted for 5.9 days and 6.1 days in a 50 year old woman. Among patients admitted for other problems, comorbid vertebral fractures added 2.1 days. These findings indicate that hospital stays are lengthy even after controlling for comorbid conditions.

SU345

Significance of Vertebral Deformity as Indicated by the SF-36 Quality of Life Measures. S. A. Jackson,*[1] L. Robertson,*[1] J. D. Adachi,[2] A. Tenenhouse.[3] [1]University of Alberta, Edmonton, AB, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]McGill University, Montreal, PQ, Canada.

The basic definition of vertebral deformity has in the past been somewhat controversial, leading to a wide variation in prevalence and incidence data reported by different studies. Current opinion favors the use of population-based norms and a statistical definition of abnormality such as 3 standard deviations or 4 standard deviations below a reference normal. The 3 standard deviation criteria is usually termed a Grade 1 deformity and the 4 standard deviation a Grade 2. Because of this statistical definition, it is uncertain if a Grade 1 deformity is sub-clinical and therefore represents an insignificant clinical impact. We have attempted to answer this question using the SF-36 health related quality of life instrument and vertebral deformity data of the Canadian Multicenter Osteoporosis Study. CaMos is a prospective cohort study, currently in year five, involving 9423 randomly selected, community based men and women aged 25 years or more. Information collected included the Medical Outcomes Study 36-item Short Form (SF-36). This instrument produces eight quality of life metrics, which relate to physical and mental health. Two summary scores are also produced. Vertebral shape is measured by digital morphometry of lateral spine radiographs and deformity categorized using the CaMos reference norms, which are population derived.There are significant differences (P<0.01) in the SF-36 Physical Functioning metric between groups with no vertebral deformity and those with only one Grade 1 or Grade 2 deformity. There is no difference in the metric between the Grade 1 and Grade 2 groups.Lumbar deformities tend to produce more degradation in physical functioning than do thoracic deformities in both male and female groups. The degradation in physical functioning as total vertebral deformity increases is mirrored by falling spine BMD.The mental health metric is not affected by the extent of vertebral deformity.In conclusion, the SF-36 quality of life instrument indicates that a Grade 1 vertebral deformity is clinically significant and validates the choice of 3 standard deviations as a critical normal limit.

SU346

Survival and Functional Outcome after Intertrochanteric and Femoral Neck Hip Fractures: A One-Year Prospective Cohort Study. S. Boonen,[1] P. Autier,*[2] M. Barette,*[3] D. Vanderschueren,[1] P. Haentjens.*[4] [1]K.U.Leuven, Leuven, Belgium, [2]Center for Research in Epidemiology, Luxembourg, Luxembourg, [3]Institut Bordet, Brussels, Belgium, [4]Academisch Ziekenhuis V.U.B., Brussels, Belgium.

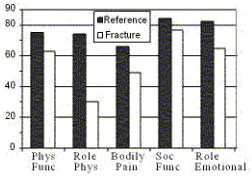
We conducted a prospective study among elderly women with a first hip fracture to document survival and functional outcome, and to determine whether outcomes differ by fracture type.The design was a one-year prospective inception cohort study reflecting standard day-to-day clinical practice. The main outcome measures were survival and functional outcome, both at hospital discharge and one year later. Functional outcome was assessed using the Rapid Disability Rating Scale version-2. Of the 170 women originally enrolled, 84 (49%) had an intertrochanteric and 86 (51%) a femoral neck fracture. There were no significant differences between the two groups with respect to median age (80 and 78 years, respectively) or type and number of comorbidities at the time of injury (data not shown). At hospital discharge, intertrochanteric hip-fracture patients had a higher mortality (8.5% versus 1.2%; $p < 0.05$) and a worse walking ability (0.4 units difference; $p < 0.05$). One year later, mortality was still higher after intertrochanteric fracture (24% versus 11%; $p < 0.05$), but functional results among surviving patients were similar in both groups. During the one-year period after hospital discharge the functional status improved by 3.9 units ($p < 0.01$) for intertrochanteric and by 2.6 units ($p < 0.01$) for femoral neck fracture patients. In both groups, this improvement was related to a significant improvement in walking ability ($p < 0.01$).Our results confirm that trochanteric fracture occurrence is associated with higher mortality than femoral neck fracture. However, among those who survive, functional outcome at one year is similar.

SU347

Quality of Life After Different Types of Osteoporotic Fractures. I. Hallberg,* O. Löfman, O. Wahlström, G. Toss. Osteoporosis Unit, University Hospital, Linkoping, Sweden.

General prevention against osteoporotic fractures is often advocated, but many local authorities still hesitate. More knowledge about the impact of fractures on Health Related Quality Of Life (HRQOL) is needed. 600 consecutive women 55-75 years old with a recent

suspected fragility fracture were invited for osteoporosis investigation including BMD measurement and health-related quality-of-life questionnaire (SF-36). SF-36 was chosen as it is widely used for various diseases and thus allows for comparison. Age and sex matched reference values for SF-36 were obtained from a local randomised reference population (n= 1059). The present study constitute baseline in a follow-up study. Preset exclusion criteria were high-energy trauma (31) and on-going osteoporosis treatment (62). Active and passive refusers were 45 and 155 respectively. Of the 303 included (mean age 67.5) 55 had a vertebral fracture, proximal humerus 37, distal forearm 171 and 40 a hip fracture. 148 (49%)of the women had one or more previous fracture. 45.5% had osteoporosis, 46.2% osteopenia in hip and/or spine while 8.3% had a normal BMD according to the WHO definitions and the machine specific reference (NHANES III and Favus, Hologic 4500). The lowest HRQOL scores of physical as well mental functions were seen in after vertebral and hip fractures (see figure) but scores were reduced also after humerus and forearm fractures, even after 3-6 months. HRQOL was correlated to BMD and inversely correlated to number of previous fractures but not to age. Figure: SF-36 Life Quality Score (selected) in total



fracture group in relation to reference population.Conclusions: Vertebral and hip fractures but also fractures of the forearm and proximal humerus fractures impair HRQOL. Impairment was correlated to number of previous fractures and to BMD. Impairment was significant even after 3-6 months and futher follow-up is needed.

SU348

The Impact of Osteoporotic Fractures on Health Related Quality of Life (HRQL) As Measured by the Health Utilities Index. E. A. Papadimitropoulos,[1] J. D. Adachi,*[2] G. Ioannidis,*[2] C. Berger,*[3] L. Pickard,*[2] J. Prior,*[4] D. A. Hanley,*[5] W. P. Olszynski,*[6] T. Murray,*[7] T. Anastassiades,*[8] J. P. Brown,*[9] S. Kirkland,*[10] C. Joyce,*[11] L. Joseph,*[3] A. Papaioannou,*[2] S. Poliquin,*[3] A. Tenenhouse.*[3] [1]Research and Development, Eli Lilly Canada Inc., Scarborough, ON, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]McGill University, Montreal, PQ, Canada, [4]University of British Columbia, Vancouver, BC, Canada, [5]Calgary University, Calgary, AB, Canada, [6]University of Saskatechewan, Saskatoon, SK, Canada, [7]University of Toronto, Toronto, ON, Canada, [8]Queen's University, Kingston, ON, Canada, [9]Laval University, Ste Foy, PQ, Canada, [10]Dalhousie University, Halifax, NS, Canada, [11]Memorial University, St.John's, CA, Canada.

HRQL was examined in relationship to prevalent fractures in individuals (n=3630) 50 years of age and older participating in the Canadian Multicentre Osteoporosis Study. Participants were sub-divided into three groups according to their fracture status: clinically recognized main fractures (at the hip, spine, wrist/forearm, pelvis and ribs), subclinical vertebral deformities (deformities detected by x-ray), and no prevalent fractures. Extensive baseline data were collected on all participants. The health utilities index *HUI), Mark II and III, instrument was used for HRQL as an outcome measure. The Mark II questionnaire assesses current HRQL acorss six attributes (sensation, mobility, emotion, cognition, self-care and pain), the Mark III assesses eight attributes (vision, hearing, speech, ambulation, dexterity, emotion, cognition and pain). Multi-attribute utility scores were calculated and may vary from 0 (death) to 1 (perfect health). We performed multivariate regression analyses modeled for Mark II and Mark III multi-attribute utility scores. Regression coefficient parameter estimates and 95% confidence intervals (CI) were determined. A total of 887 (24.4%) and 608 (16.7%) participants experienced main fractures and subclinical vertebral deformities, respectively. Participants with main fractures (-0.027;95% CI: -0.052, -0.001) and subclinical vertebral deformities (-0.015; 95% CI: -0.027, -0.004) had lower HRQL (as measured by Mark II) compared with those without fractures. Furthermore, moderate/severe disability in the main fracture, subclinical deformities and no fracture groups was 12.5%, 10.5% and 6.5% for sensation; 11.5%, 5.1% and 2.4% for mobility; 4.5%, 3.1% and 3.7% for emotion, 0.2%, 0.3% and 0.3% for cognition; 2.3%, 0.3% and 0.3% for self care; and 22.4%, 20.7% and 14.3% for the pain attribute. In conclusion, both main fractures and subclinical vertebral deformities have a negative impact on HRQL.

SU349

Bone Mineral Density and Its Lifestyle Correlates in Middle-Aged Men. M. R. Sowers. Epidemiology, University of Michigan, Ann Arbor, USA.

More men are living longer, extending the duration of time during which they experience age-related bone loss. The National Center for Health Statistics projects that the number of men older than 70 will double between 1993 and 2050. Thus, the prevalence of osteoporosis in men promises to become an issue as a greater number of men will live past the age of 70, the age at which the frequency of hip fractures increases exponentially. This study examines modifiable risk factors for bone in men using a cross-sectional design and a population-based sample. The risk factors include measures of current weight, current obesity (amount of lean and fat mass), past weight, current physical activity level, past and current smoking behavior, diet (including calcium and fluoride from drinking water) and current alcohol consumption. Data were collected from 171 Caucasian men between the ages of 35 and 65years (average age of 51 years), living in three communities in Iowa whose mineral content of the drinking water provided for great variation in both calcium and fluoride intake. Bone mineral density(BMD) was measured using dual-energy X-ray absorptiometry (DXA; Lunar Corporation, Madison, WI) at three sites: proximal femur, lumbar spine, and whole body. Body composition (fat and lean mass) was measured with the total body scan. Historical weight, physical activity, smoking, alcohol use and diet came from interviews. Men, on average, weighed 90 kg, while average weight at age 20 was 76 kg. 35% of men were nonsmokers and 40% were ex-smokers. At the femoral neck, age ($p<0.001$), weight at age 20 ($p<0.0009$), current smoking ($p<0.07$) and an interaction term between smoking status and weight explained 28% of the total BMD variability. Current or ex-smokers with greater weight had greater BMD. Similar relationships were seen in the total body BMD measure. At the lumbar spine, smoking status was negatively associated with BMD ($p < 0.01$) while weight at age 20 ($p <0.01$), and moderate alcohol consumption ($p <0.05$) were positively associated with BMD, explaining 11% of the variation. This study of BMD in middle-aged men suggests the importance of factors that may contribute to "peak" bone mass in men and indicates that potentially modifiable factors amenable to intervention may be more pronounced in men than in women.

SU350

The Association Between Leptin and Gonadal and Adrenal Steroids, Insulin-like Growth Factor I, Insulin-like Growth Factor Binding Protein 3 and Bone Mass Density in Greek Healthy Males. F. G. Papadopoulou,*[1] T. Konstandinidis,*[2] G. Koliakos,*[3] G. E. Krassas.[2] [1]Endocrine Clinic, Panagia Hospital, Thessaloniki, Greece, [2]Endocrine Dept, Panagia Hospital, Thessaloniki, Greece, [3]Dept. of Biological Chemistry, Aristotle University, Thessaloniki, Greece.

Pathogenesis of osteoporosis in men remains obscure. The aim of this study was to evaluate the correlation between leptin and gonadal and adrenal steroids, insulin like growth factor I (IGF-I), insulin like growth factor binding protein 3 (IGFBP-3) and bone mass density (BMD) in Greek healthy males. Three hundred and sixty three healthy male volunteers were investigated. The mean age was 51.3±8.7 yrs and body mass index (BMI) was 27.5±3.7 kg/m2. In all subjects the levels of leptin, total testosterone (TTe), free testosterone (FTe), dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), estradiol (E2) and sex hormone binding globulin (SHBG) were measured. Bioavailable testosterone was determined using a method modified from Tremblay and Dube. The ratio of (E2/SHBG, nmol/nmol) was used as an index of free estradiol (FE2I) while the ratio of total testosterone to SHBG (TT/SHBG, nmol/nmol) was used as an index of free androgen (FAI). In all subjects BMD at four skeletal sites, lumbar spine (LS), femoral neck (FN), Ward's triangle (WT), and finally trochanter (Tr), was measured using dual-energy x-ray absorptiometry (DEXA). T-score, Z-score and g/cm2 values were estimated.In the group of the 363 healthy males the mean hormone levels were as follows: Leptin 15.8±17.8 ng/ml, TT 19.4±9.6 ng/ml, FTe 16.0±5.0 pg/ml, BTe 40.3±25.1, FAI 91.5±77.4 nmol/nmol, DHT 338.9±328.5 pg/ml, mean DHEA 8.8±11.7 ng/ml, E2 40.6±28.2 pmo/lt, FE2I 3.0±8.1 pmol/nmol, and finally SHBG levels were 30.9±22.1 nmol/lt. BMD in gr/cm2 were as follows: LS 1.01±0.14, at FN 0.85±0.85, at Tr 0.75±0.11 and finally at WT was 0.68±0.17 while the T-score was at LS -0.78±1.38, at FN -1.03±4.64, at Tr -0.27±0.97 and at WT -0.93±1.78. The Spearman and Kendall correlation coefficients revealed that serum leptin levels correlated negatively with IGFI ($r= -0.15$, $p= 0.003$) and with FAI ($r= -0.11$, $p= 0.050$), while it was positively correlated with BMI ($r= 0.25$, $p= 0.01$), BMD in Tr ($r= 0.20$, $p= 0.010$), and BMD in FN ($r= 0.18$, $p= 0.022$). Linear multiple regression was used to assess the independent effect of several variables on circulating levels of leptin. Serum levels of both FAI and IGF-I correlated negatively with serum leptin independently from influences of BMI and leptin but the influence of FAI is dependent on the influence of IGF-I. Finally, a stepwise forward method was also used.It is concluded that serum leptin correlates negatively with FAI and IGF-I and positively with BMD in FN and Tr. It could be postulated that IGF-I and androgens play an important role on the regulation of circulating leptin and also that the latter has an impact on BMD in males.

SU351

Low 17-ß-estradiol and Free Estriol - A Risk Factor in Idiopathic Osteoporosis in Men. B. E. Gerbert,*[1] H. Heinze,*[2] J. Schulze.*[2] [1]Clinic of Internal Medicine III, University of Dresden, Dresden, Germany, [2]University of Dresden, Dresden, Germany.

In recent publications a low serum level of 17ß-estradiol in osteoporotic men was observed. The aim of this study was to investigate the influence of 17ß-estradiol and free estriol on bone mineral density in men.We investigated 108 men (51,70 ± 13,38 years) with idiopathic osteoporosis. Men with a T-Score below -2,5 at spine or proximal femoral were excluded. Measurement of bone mineral density was taken by dual x-ray absorptiometry (Lunar 400). We excluded hypovitaminosis D, hyperthyreoidism, hypogonadism and other specific diseases of the bone in the patients.The patients were subdivided into two age-groups, men younger than 35 years (29,44 ± 4,12 years, n = 16) and men older than 35 years (55,58 ± 10,29 years, n = 92). We investigated the serum level of 17ß-estradiol (E2), free estriol (E3) and other bone-specific parameters.Results: No difference in bone mineral density at spine and femoral neck was seen between the two groups. We found a significant difference in the bone mineral density at femoral neck and a lower BMD at femoral trochanter in the older men.Frequently we found an extremely low serum level of 17ß-estradiol and free estriol. We measured a significant lower serum level of 17ß-estradiol in the younger men than in the older men (21,46 ± 34,66 pmol/l, resp. 51,71 ± 63,26 pmol/l,p=0.02). In 69% of the younger men and 43% of the older men we could describe a serum level of 17ß-estradiol below 37 pmol/l. Serum level of free estriol was significant lower in the younger men (0,02 ± 0,05 nmol/l, resp. 0,13 ± 0,20 nmol/l). In 85% of the younger men and 53% of the older men we measured a serum level of free estriol <0,01 nmol/l.In 55 percent of the men both the serum level of 17ß-estradiol and the serum level of free estriol were undetectable with our laboratory kit (17ß-estradiol < 37 pmol/l, LIA, Bayer Diagnostics; free estriol < 0,11 nmol/l, ultrasensitive RIA, DSL-Diagnostics).Conclusion: The defect in 17ß-estradiol and free estriol can cause the lost of bone foundation in the young.

SU352

Applying a Simple Clinical Tool Based on Age and Body Weight which Identifies Osteoporosis in Asian Women to a Cohort of Singapore Men. L. K. H. Koh,[1] F. L. Thoo.*[2] [1]Endocrinology, Singapore General Hospital, Singapore, Singapore, [2]Diagnostic Radiology, Changi General Hospital, Singapore, Singapore.

The objective of this study was to determine if a simple scoring index for predicting osteoporosis among Asian women would apply to a cohort of Singapore men. A simple scoring index had been developed previously, based on multiple variable regression modeling using data from postmenopausal women in 8 East and Southeast Asian countries. The final index contained only 2 variables (age and body weight) and achieved 91% sensitivity and 45% specificity, with an area under the curve (AUC) of 0.79. We applied this model to a cohort of 98 normal Singapore men aged 61 ± 8.8 years (range 50 - 88 years) who had hip DXA measurements in 1999. The index based on age and weight was calculated for each subject, and ranged from -7 to 8. Based on the original scoring index cut-offs of -4 and -1, three osteoporosis risk categories were defined: 4% were categorized as high risk, 24% as moderate risk, and 72% as low risk. Using the male value for femoral neck BMD T ≤ -2.5 SD (0.655 g/cm^2), 75% of men with high risk, 26% with moderate risk, and 13% with low risk had osteoporosis. At a cut-off of ≤ -1, a sensitivity of 50%, specificity of 78%, and an AUC of 0.71 was achieved. Using the female value for femoral neck BMD T ≤ -2.5 SD (0.570 g/cm^2), the proportion having osteoporosis was 75%, 9% and 4% respectively for high, moderate and low risk categories. A sensitivity of 63%, specificity of 76%, and an AUC of 0.84 was achieved using the same cut-off of ≤ -1. Using a cut-off of ≤ 0, the sensitivity increased to 72% with the male osteoporosis threshold value and 100% with the female osteoporosis threshold value. The scoring index developed for women, based on age and body weight, had some predictive ability for detecting osteoporosis in men in our population. The index performed somewhat better if the female threshold for osteoporosis, and a higher scoring index cut-off was used. This simple risk assessment tool could help clinicians select high risk male patients for BMD measurements and intervention before fractures occur.

SU353

Secondary Causes of Osteoporosis in Men. T. W. O'Neill, S. R. Pye,* K. R. Adams,* J. P. Halsey,* P. Klimiuk,* S. M. Knight,* B. Pal,* A. Potter,* P. L. Selby, I. M. Stewart,* D. R. Swinson.* ARC Epidemiology Unit, University of Manchester, Manchester, United Kingdom.

Osteoporosis is an important health problem in men. The aim of this study was to characterise the cause(s) of osteoporosis in men with vertebral fractures.A descriptive survey method was used. The setting was medical out-patient clinics in nine hospitals in the North West of England. Men attending these clinics with radiographically confirmed vertebral fractures were included in the survey. For each subject the attending clinician characterised what they considered to be the main cause of the fracture. Fractures due to osteoporosis were categorised as either primary or secondary osteoporosis and, if secondary the underlying cause(s) recorded.159 men with vertebral fracture (mean age 60.8 years, SD=13.5) were recruited. In 154 the fracture was considered to be due to osteoporosis. In the other 5 the fracture was due to trauma (2), malignancy (2) and Paget's disease (1). Of the 154 with osteoporosis 81 (53%) were considered to be due to primary or idiopathic osteoporosis and 73 (47%) due to secondary causes. Of these 73, the secondary causes were : cortico-steroids 34 (46%), alcohol excess 10 (14%), hypogonadism 7 (10%), gastro-intestinal disease 1 (1%), other causes 7 (10%), more than one underlying cause 14 (19%).In this hospital based survey, approximately half of all men with an osteoporotic vertebral fracture were considered to have secondary osteoporosis with corticosteroids, alcohol excess and hypogonadism being the most frequent underlying causes.

SU354

BMD in Calcaneus in 3200 18 Year Old Men Is Related to Physical Activity and Lifestyle Factors. C. Nyman,*[1] L. Hulthén,*[2] O. Johnell,*[3] R. Kullenberg,*[4] K. Landin-Wilhelmsson,*[2] R. Lorentzon,*[5] E. Norjavaara,*[6] E. Orwall,*[7] U. Pettersson,*[5] L. Samuelsson,*[8] D. Mellström.*[1] [1]Dept. of Geriatric Medicine, Goteborg University, Goteborg, Sweden, [2]Dept. of Internal Medicine, Goteborg University, Goteborg, Sweden, [3]Dept. of Orthopaedics, Malmo University, Malmo, Sweden, [4]Dept. of Radiophysics, Goteborg University, Goteborg, Sweden, [5]Dept. of Orthopaedics, Umea University, Umea, Sweden, [6]AstraZeneca, Goteborg, Sweden, [7]Oregon Health Services, Portland University, USA, [8]The National Service Administration, Regional Office, Goteborg, Sweden.

BMD is a predictor of fractures and survival in men. The question is if BMD in men is an expression of general health and physical capacity. Most of (96%) 18 year old men in Sweden participate in a two days test of functional capacity for selection to the compulsory military service. BMD in calcaneus was measured with DXA, CalScan, and a questionare included nutritional habits, physical activity, medications etc. Blood is sampled for genetic studies. Isometric muscle strength was measured in Nm (Newton-meters) using Isokai. Physical capacity, VO2max, was estimated from a maximal stress test. This was performed on a bicycle-ergometer and maximal oxygen uptake was measured in L O2/min. BMD significantly correlated to height r=0.04, weight r=0.18, muscle strength r=0.26, oxygen uptake r=0.12 and endurance test r=0.20. Smoking and inhalation of corticosteroids were significant risk factors. Calcium intake (calculated from milk and cheese intake), vegetable intake and years of training were protective factors. Calcaneus AREA significantly correlated to height r=0.55, weight r=0.42, muscle strength r=0.14, years of training r=0.14. A multivariate analysis showed that height, weight, muscle strength and years of training also were independent predictors of calcaneus BMD.

SU355

Free of Bioavailable Estradiol is a Determinant of Bone Loss in Community-Dwelling Elderly Men: A Longitudinal Study. S. J. A. Goemaere,[1] H. Zmierczak,*[2] I. Van Pottelbergh,[2] K. Toye,[2] M. Daems,*[2] J. M. Kaufman.[2] [1]Unit for Osteoporosis and Metabolic Bone Disease, Ghent University Hospital, Ghent, Belgium, [2]Ghent University Hospital, Ghent, Belgium.

Serum Levels of free (F) and bioavailable (Bio) testosterone (T) and estradiol (E2) in men decline with age. Whether these hormonal changes contribute to senile bone loss remains unclear. Cross-sectional studies have shown weak and inconsistent associations of sex-steroid levels with bone mineral density (BMD), recent studies suggesting a stronger impact of (F or Bio) E2 than T.The present observational study assessed bone loss in ambulatory elderly men (age 71-86y at baseline) recruited from the population register of a semi-rural community. From the 352 men willing to participate (participation rate 47%) 49 subjects were excluded because of factors affecting bone metabolism or sex steroid levels and 108 men dropped out for a variety of reasons. The remaining 195 subjects (mean age: 75.5 +/-3.9y; mean BMI: 26.4 +/- 3.4 kg/m2) were followed at yearly intervals for a median period of 4 years. Data collection included DXA (Hologic QDR1000+), fasting serum samples before 10 a.m. for determination T, E2, SHBG and albumine; FT, BioT, FE2 and BioE2 were calculated using a validated 2nd degree equation. The present analysis is based on baseline hormone levels and first and last DXA measurement. The annualized change in bone density (mean +/- sd) at proximal femur and distal forearm was -0.29 +/- 0.93% and -0.31+/- 0.54%, respectively. The BMD loss was significantly related to age (r= 0.26,p<0.001; r= -0.18,p=0.01;respectively) and to BMI (r= -0.14,p=0.06; r = -16,p=0.03;respectively). Neither FT nor BioT were associated with bone loss at the hip or the forearm. However, FE2 and BioE2 were significantly negatively associated with bone loss at the forearm (r=-0.18;p=0.02), the lowest FE2 quartile (Q1) being associated with higher bone loss (Q1vsQ4: -0.45 +/-0.68% vs -0.21 +/- 0.35%; p = 0.03). Similar trends for association of FE2 and BioE2 with bone loss at the hip were significant for the trochanter subregion only (p< 0.05).In conclusion, this longitudinal study does not support the view that interindividual variations in serum (F of Bio) T play a direct role in the determination of senile bone loss in ambulatory men, but does indicate a role for serum levels of (F or Bio) E2, its aromatization product.

SU356

Association of Bone Turnover with Longitudinally Assessed Bone Loss in Community-Dwelling Elderly Men. S. J. A. Goemaere,[1] H. Zmierczak,*[2] I. Van Pottelbergh,*[2] R. Demuynck,*[2] H. Myny,*[2] J. M. Kaufman.[2] [1]Unit for Osteoporosis and Metabolic Bone Disease, Ghent University Hospital, Ghent, Belgium, [2]Ghent University Hospital, Ghent, Belgium.

Bone turnover indices can predict bone loss in postmenopausal women. Little is known about the association of biochemical markers of turnover and prospectively assessed bone loss in elderly men.In the present longitudinal study bone loss in ambulatory elderly males (age 71-86y), recruited from the population register in a semi-rural community, was assessed. From the 352 men willing to participate (participation rate 47%) in the longitudinal study, 49 subject were excluded because of factors interfering with bone metabolism or sex steroid levels and 108 men dropped out for a variety of reasons. The remaining 195 subjects (mean age: 75.5 +/- 3.9y; mean BMI: 26.4 +/- 3.4 kg/m2) were followed at yearly intervals for a median period of 4 years. Baseline data collection included fasting serum samples (before <10:00 a.m.) for determination of levels of sex steroids, osteocalcin (OC), bone specific alkaline phosphatase (BsAP) and serum (s) crosslinks by immunoassay and second void urine samples for creatinine, urinary (u) crosslinks and deoxypyridinoline (DPD). Longitudinal change in bone mineral density was evaluated by DXA (Hologic QDR1000+) . Data analysis was done by Pearson correlation, linear regression and analysis of variance after logarithmic transformation of bone turnover parameters and is based on the baseline values for bone turnover indices and the first and last DXA measurement. In the present group of elderly male, the annualized percent loss of bone density (mean+/- sd) at total hip (HIP) and distal forearm (ARM) was 0.29 +/-0.93% and 0.31 +/- 0.54% respectively. The baseline bone turnover as determined by biochemical markers was not related to age, BMI or sex steroid levels. Consistant positive associations (Pearson correlations) were found between all bone turnover indices and bone loss rate (r= 0.16 to 0.22, p= 0.03 to 0.002).

Linear Regression of %BMD loss by bone turnover markers. Entries are coefficients (p-value)

	OC	BsAP	uCrosslinks	sCrosslinks	DPD
ARM	0.24 (0.01)	0.36 (0.002)	0.15 (0.04)	0.23 (0.001)	0.30 (0.05)
HIP	0.26 (0.13)	0.34 (0.09)	0.25 (0.03)	0.24 (0.05)	0.65 (0.007)

Quartile analysis of bone turnover markers (by ANOVA) showed consistent trends towards increase of bone loss at either hip or forearm associated with the highest quartiles for bone turnover markers, approaching statistical significance for bone resorption markers (F= 2.2 to 3.2, p=0.07 to 0.01). In conclusion, in elderly ambulatory men, higher bone turnover as assessed by biochemical markers is associated with higher rates of bone loss

SU357

Dietary Mineral Intake and Low Bone Mass in Men: The VALOR Study. D. R. Miller,*[1] E. A. Krall,*[2] J. J. Anderson,*[2] S. E. Rich,*[2] A. Rourke,*[3] J. Chan.*[4] [1]CHQOER / MAVERIC, Veterans Administration, Bedford, MA, USA, [2]Boston University, Boston, MA, USA, [3]CHQOER, Veterans Adminstration, Bedford, MA, USA, [4]MAVERIC, Veterans Adminstration, Boston, MA, USA.

Low dietary intake of calcium has been associated with low bone mineral density

(BMD) and increased risk of fractures in women. Reports from recent studies have suggested that this association also applies to men and that dietary intake of magnesium and other minerals also may influence BMD. We studied relationships of dietary mineral intake with low bone mass in the VA Longitudinal Osteoporosis Research (VALOR) study, a cohort of men aged 50 to 91 years (mean ±SD=70±8). BMD of the femoral neck and radius were measured with dual energy x-ray absorptiometry (model DPX-IQ, Lunar Corp., Madison, WI); for the femur, the averages of right and left sides were computed. Low bone mass (osteoporosis and osteopenia) was defined as femoral neck BMD <-1 SD below the mean value of males age 20-29 years. Dietary mineral intake was estimated using a semi-quantitative food frequency questionnaire developed for use in the elderly, with nutrient values computed by Channing Labs (Boston, MA). General linear modeling was used to test differences in BMD and logistic regression modeling was used to estimate odds of low bone mass across quintiles and other categories of nutrient intake; all models included adjustment for age, body mass index, and total calorie intake. Men with calcium intake of less than 600 mg/day (50% of the RDA) had lower BMD and higher odds of low bone mass (3.5, 95% confidence interval (CI) =1.8-6.7); above 600 mg/day, there was no apparent increase in BMD with increasing calcium intake. Similarly, men with magnesium intake of less than 300 mg/day (71% of the RDA) had lower BMD and higher odds of low bone mass (3.7, CI =1.8-7.5), with no increase in BMD with intakes above 300 mg/day. These associations were independent of each other and the odds of low bone mass in men with both low calcium and magnesium intake, relative to those with higher intakes of both, was 6.0 (2.7-13). We also observed a direct association between BMD and potassium intake (femoral neck BMD across quintiles of intake: 0.89, 0.92, 0.95, 0.97.1.00, p=0.01) and the odds of low bone mass in men with intake below 2.5 gms/day was 2.5 (CI =1.3-4.9). These findings were not substantially altered with adjustment for other factors, including family history, exercise, tobacco use, alcohol use, and other dietary factors. Many older men consume relatively low levels of calcium, magnesium, and potassium, and our results suggest that increasing intakes of these minerals in those men may slow bone loss and reduce risk of fracture.

SU358

Osteoporosis in Young Adults: The Result of a Changing Life Style? M. A. R. Lissens,*[1] G. Akyuz.*[2] [1]Physical Medicine and Rehabilitation, University Hospital Gent, Gent, Belgium, [2]Physical Medicine and Rehabilitation, Marmara University, Istanbul, Turkey.

The incidence of osteoporosis is known to increase exponentially after the age of 50 years. In women bone loss accelerates around the menopause. In recent years however, low bone mineral density (BMD) is seen more and more in young adults, as well males as females. In this study, BMD was determined in 50 healthy young adults (age 20 to 45), 28 females and 22 males (mean age 31.18 ± 8.51 years) using dual-energy X-ray absorptiometry (DXA) of the lumbar spine (L2-L4). The mean BMD was found to be 1.013 ± 0.199 g/cm2..The mean T-score was 87.56 ± 13.46 % (ranging from 59 up to 127%) or -1.21 SD (ranging from -4.04 to +1.2 SD). Twenty eight or 56% of these young adults showed a T-score below -1 SD. Eight of them (16%) fulfilled the WHO-criteria of osteoporosis and 20 (40%) of osteopenia. The changing life style of young adults, including a lack of physical activity, a lack of exposure to sunshine, low calcium and high caffein dietary intake, alcoholic beverages, soft drinks, smoking and drugs might at least partially explain these findings. In an attempt to test this hypothesis, BMD values were correlated with physical activity (walking, sports and exercise) and with daily calcium intake. A nearly significant positive correlation with calcium intake (p=0.058) and with physical activity (p=0.073) was found. However, calcium dietary intake was estimated only semi-quantitatively and retrospectively, and physical activity (type and duration) was very difficult to quantify. Moreover, other influencing factors that might be important were not taken into account.

SU359

Weight Cycling—A Population Risk Factor for Past Fragility Fracture: Data From the Canadian Multicentre Osteoporosis Study. C. L. Hitchcock,*[1] L. Kramer,*[2] M. A. Petit,[1] L. Joseph,*[3] S. I. Barr,*[1] H. A. McKay,[1] Y. M. Vigna,*[1] C. Berger,*[3] B. Lentle,*[1] J. C. Prior,[1] CaMOS Research Group.[3] [1]U of British Columbia, Vancouver, BC, Canada, [2]Simon Fraser U., Burnaby, BC, Canada, [3]McGill U, Montreal, PQ, Canada.

This analysis explores weight cycling (loss and regain of 10 or more pounds) as a risk factor for fragility fracture and low bone mineral density (BMD) in a population-based sample of Canadian men and women (CaMOS). Weight cycling was related to fragility fracture and BMD in the British Columbia cohort (ASBMR 2000). Weight cycling information was available for 9357 participants (2866 men and 6481 women) averaging 60 and 63 years of age, respectively (25-103 years), at 9 Canadian urban centres.Participants were interviewed using a questionnaire for demographics, medical history, lifestyle factors, quality of life, and history of fractures, including the degree of trauma involved. Fragility fractures are those that occur with a fall from a standing height or less. Femoral neck (FN) and lumbar spine (LS) BMD were measured by Dual Energy X-ray Absorptiometry, and standardized across centres. Linear models examined weight cycling, fragility fracture and BMD as related to: centre, age, weight, body mass index (BMI), current alcohol, caffeine, vitamin D and calcium use, lifetime tobacco use, eating attitudes, happiness, worry, and exercise. One third of men (33%) and 41% of women reported 1 or more weight cycling episodes (mean and 95% CI: 1.1 (0.9,1.2) and 1.6 (1.5, 1.7) per person respectively). Weight cycling differed across the centres (range of centre averages for women: 34.7%-46.1% and men: 24.4%-41.3%). Weight cycling was correlated with higher BMI (Spearman correlation=0.35 in women and 0.29 in men). Twenty-four percent of men and 27% of women reported past fragility fractures. BMD averages (CI) for women were: LS: 0.93 (0.928, 0.937); FN: 0.71 (0.707, 0.713) and for men were: LS: 1.04 (1.033, 1.046); FN: 0.81 (0.806, 0.816) g/cm^2. Past fragility fracture was related to weight cycling episodes for men (odds ratio (OR) = 3.93 [CI 1.690, 9.023] for ≥ 4 relative to 0), but our results are inconclusive for women (OR = 1.11 [CI 0.651, 1.852]). In a linear model with all factors for women, BMD (g/cm²) was lower for those with ≥ 4 weight cycling episodes than for those reporting none: FN: -0.013 (-0.035, 0.008) and LS:-0.022 (-0.054, 0.01). For men the BMD differences were: FN: -0.048 (-0.091, 0.005) and LS:-0.033 (-0.061, -0.004). There are a number of interactions with other variables, including centre-to-centre variation. These national, population-based data suggest that weight cycling is related to past fragility fracture and is a risk factor for low bone mineral density.

SU360

Public Disease Osteoporosis - Risky-Trends in Germany. C. A. E. Günther, H. Kießling, O. Guenther, A. Kapner, L. Wiske, D. Arnold. Deutsches Zentrum für Osteoporose, Johannesbad Reha-Kliniken AG, Bad Füssing, Germany.

Introduction: Risk factors are important in the pathogenesis of Osteoporosis. Therefore we carried out a special designed survey. It was meant to give conclusions - especially in the second year of "Bone and Joint Decade" and in the "Year of Osteoporosis" - about the risk behavior of the German population. Methods: We used a standardized questionnaire to investigate the Osteoporosis-risk-behavior. In total 560 persons (aged: 43,4 ± 18,6 years, 343 w, 217 m) throughout Germany were asked. To prove significance we used c2-test and u-test. Results: The daily milk consumption (ml per day) amounted to 330 (highest: age group < 20 years 450ml, lowest: age group 40 to 49 y. 218ml, p < 0,05). The consumption of cola beverages (ml per week) came up to 544ml with the highest consumption in the age group under 20 years drinking 1640ml (w 572, m 2290, p < 0,01). 16,9% declared to be abstinent from alcohol, 45,9% (w 55,7, m 31,5, p < 0,001) drank little, 21,6% (w 16,8, m 28,7, p < 0,001) consumed alcohol moderately and 15,7% (w 9,2, m 25,3, p < 0,001) higher amounts. An average of 3,8 hours per week is being spend on sporting activities (w 3,4, m 4,6, p < 0,05). 1,7 hours per day are spent in front of the television (w/m n.s.) and there is no age-related change in habits. 8,7% are abstinent. Women and men spent 2,4 hours per day in a sitting position with computer works (w/m n.s.). The maximum amount occurred between the ages 20 to 29 with 4,6 hours per day.(p< 0,001). Visiting a fast-food-restaurant is practiced about 1,5 times per month (w/m n.s.). Only the age group 20 to 29 years goes there more often (3,5 times per month, p< o,o5 to p< 0,001). Conclusion: The average amount of calcium intake - calculated from the amount of about 450ml milk per day - is too low. The high cola intake of young people - and therefore high intake of phosphate - should let us rise our forefinger. The alcohol consumption shows that women seem to have more likely "osteoporosis-protective" (less than 14g alcohol per day) and men "osteoporosis-destructive" drinking habits (more than 14g alcohol). Comparison of hours spend in front of the television plus computer and the hours spent exercising shows that - depending on the age group - 6 to 10 times more time is wasted inactive than used for physical activities. The frequency visiting fast-food restaurants should not harm our bones. In summary we must come to a more bone friendly behavior.

SU361

Risk Factors for Development of Osteoporosis and Cardiovascular Disease in Postmenopausal Danish Women: The PERF Study. Y. Z. Bagger, B. J. Riis,* P. Alexandersen,* L. B. Tankó, C. Christiansen. Center for Clinical and Basic Research, Ballerup, Denmark.

In order to evaluate a number of possible risk factors for osteoporosis and cardiovascular disease in postmenopausal women, we have followed a group of 8000 postmenopausal Danish women who had previously participated in various clinical studies of osteoporosis since 1977. Follow-up times varied between 5 to 22 years. The aim of the present Prospective Epidemiological Risk Factors (the PERF study) is to answer the following questions:1. How bone mass and bone metabolism at various stages of life predict osteoporosis and cardiovascular disease?2. How body fat mass at various stages of life influence the pathogenesis of osteoporosis and cardiovascular disease?3. How serum lipid profile at various stages of life predict the development of osteoporosis and cardiovascular disease?4. How previously administered HRT or bisphosphonate therapy influences the development or prevention of osteoporosis and cardiovascular disease?5. How previously administered HRT influence the cognitive function in postmenopausal women?6. Are there any genes or combination of genes that can predict osteoporosis, cardiovascular disease and frequent coexistence of these?From 2000 to 2001, 8000 postmenopausal women were invited to the PERF study. At present the age of the participants is between 60 and 80 years. Two subsets of the study population participated in clinical trials involving therapy with either HRT (~1000 women) or bisphosphonates (~1000 women). About 70% of the original women have been re-examined during 2000 to 2001. Fasting blood samples including DNA analysis are at disposal for analysis. Measurement of BMD, X-ray of columna, mammography, cognitive test and ECG are performed. The questionnaires including history of hip/wrist fractures as well as of diagnosed cardiovascular diseases (angina, myocardial infarct, claudicatio, stroke) are also obtained. About 15% of women, who did not wish to participate in the PERF study or who were lost to follow-up, were excluded. For the ill and immobile women, a phone interview is performed in order to obtain medical histories. In case of death, reports of death and autopsy are obtained for verification of the cause of death. Serial BMD measurements, bone markers, X-ray of columna and blood samples at baseline and different times during the follow-up period are available.About 600 women died during the follow-up period. Of the followed study population, 25% have vertebral fractures and 20% non-vertebral fractures. About 30% of women have hypertension and 10% atherosclerotic diseases (angina pectoris, myocardial infarct, claudicatio, or stroke). First reports by the various research groups are expected in 2002.

SU362

Successful Strategy for Using a Mailed, Self-administered Questionnaire in Women Age 55 or Older to Determine Their Risk of Osteoporosis. J. T. Schousboe,*[1] C. R. DeBold,*[1] L. S. Kuno,*[2] K. Delaney-Mroz,*[2] T. A. Abbott.[3] [1]Park Nicollet Osteoporosis Center, Park Nicollet Health Services, Minneapolis, MN, USA, [2]Health Research Center, Park Nicollet Institute, Minneapolis, MN, USA, [3]Merck & Co., Inc., West Point, PA, USA.

We used the Simple Calculated Osteoporosis Risk Estimation (SCORE), a six item questionnaire that assesses risk of low bone density, to recruit women age ≥55 yr for an osteoporosis study. Women 5 or more yr past menopause, not on hormone replacement

therapy (HRT), and with a SCORE ≥8 were eligible for study entry. In the original study design, patients were to be provided questionnaires at clinic visits. Because of the low return rate, we employed two sequential strategies of mailing SCORE to female patients (≥55 yr) from two clinics. Strategy I asked women to score themselves and to call back if their SCORE was ≥8. Strategy II asked a separate cohort of female patients from both clinics to fill out SCORE and mail it back to be scored. Those that had a clinic visit during the time that SCORE was being handed out by clinic staff or had SCORE mailed to them were exposed women. Those that completed it in the office (office use of SCORE), called us (Strategy I) or mailed it back to us (Strategy II) were responders. Those with a SCORE ≥8, not on HRT or anti-resorptive therapy, and who had not had a prior BMD test were qualifiers. The following table shows for each strategy the number of exposed women age ≥55 exposed, and the number and percentages of responders and qualifiers.

Strategy	# Exposed Women	# Responders (%)	# Qualifiers (%)
Office Use of SCORE	8,646	1,328 (15.4%)	299 (3.5%)
Strategy I	1,473	93 (6.3%)	36 (2.4%)
Strategy II	1,474	650 (44.1%)	140 (9.5%)

Response to the questionnaire handed out in the offices was poor, because the clinic staff felt too busy to hand it out and answer questions about it. Response to the mailed questionnaire was also poor if recipients were asked to score themselves (Strategy I), but much greater if they mailed it back for scoring (Strategy II). The low percentage of qualifiers was due to the restrictive study inclusion/exclusion criteria and underestimates the number of woman at risk for osteoporosis. In conclusion, mailed questionnaires returned for scoring may be a very effective, efficient strategy for identifying many women at risk for osteoporosis, who could be targeted for education, bone density testing, or study recruitment.

Disclosures: Merck & Co., Inc.,2; Proctor & Gamble, Inc.,5.

SU363

Applying a Simple Clinical Tool to Identify Osteoporosis in a Cohort of Singapore Women. L. K. H. Koh,[1] D. C. E. Ng,*[2] F. X. Sundram,*[2] A. P. A. Tan,*[3] C. L. Ong.*[3] [1]Endocrinology, Singapore General Hospital, Singapore, Singapore, [2]Nuclear Medicine, Singapore General Hospital, Singapore, Singapore, [3]Diagnostic Radiology, KK Women's & Children's Hospital, Singapore, Singapore.

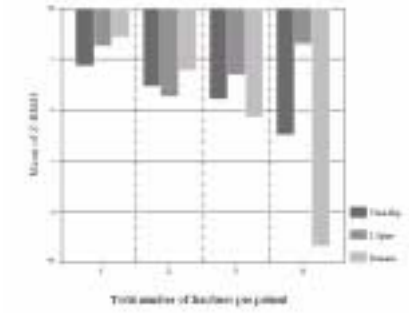
The objective of this study was to determine if a simple scoring index for predicting osteoporosis among Asian women would apply to a cohort of Singapore women. A simple scoring index had been developed previously, based on multiple variable regression modeling using data from postmenopausal women in 8 East and Southeast Asian countries. The final index contained only 2 variables (age and body weight) and achieved 91% sensitivity and 45% specificity, with an area under the curve (AUC) of 0.79. We applied this model to a cohort of 125 normal Singapore women who had DXA hip measurements in 1996. The women were aged 60 ± 7.5 years (range 50 - 89 years) and met the same inclusion criteria as the scoring index. The index based on age and weight was calculated for each subject, and ranged from -10 to 7. Based on the original scoring index cut-offs of -4 and -1, three osteoporosis risk categories were defined: 4% were categorized as high risk, 43% as moderate risk, and 53% as low risk. Eighty percent of women with high risk, 24% with moderate risk, and only 2% with low risk had osteoporosis (femoral neck BMD T ≤ -2.5 SD). Using scoring index values of -3 and 0, the proportion of women was 12%, 54% and 34% and proportion having osteoporosis was 47%, 15% and 2% respectively for high, moderate and low risk categories. In our population, the risk index achieved a sensitivity of 94%, a specificity of 64%, and an AUC of 0.83. The scoring index had acceptable predictive ability for detecting osteoporosis. Different cut-offs on the index could be chosen for our population depending on whether testing more women, or having a higher yield for detecting osteoporosis was desired. This simple risk assessment tool could help clinicians select patients for BMD measurements and intervention before fractures occur.

SU364

Women With Osteoporotic Fracture. Case for Investigation? O. C. Lofman, I. Hallberg, G. Toss. Center for Public Health Sciences, University Hospital of Linkoping, Linkoping, Sweden.

600 consecutive women 55-75 years old with a recent suspected fragility fracture (FF) were invited for osteoporosis investigation including BMD measurement, a questionnaire about risk factors and physical and laboratory investigations for causative diseases. The present study constitute baseline data in a follow-up study. Preset exclusion criteria were high energy trauma (31) and on-going osteoporosis treatment (62). Active and passive refusers were 45 and 155 respectively Of the 303 included 55 had a vertebral fracture, 37 in proximal humerus, 171 had a fracture in distal forearm and 40 in the hip. Mean age at fracture of distal forearm was 66.6 , prox. humerus 67.3, spine 68.9 and hip 69.3 years. 148 of the women had one or more previous fracture (49%). 45.5% had osteoporosis and 46.2% had osteopenia in hip and/or spine while 8.3% had a normal BMD according to WHO definitions and the machine specific reference (NHANES III and Favus, Hologic 4500). Age-adjusted BMD of total hip and forearm (but not spine) was inversely correlated to the number of previous fractures. BMI was 26 (16-42) kg/m^2. 37 (12%) were current smokers. 38 (12.5%) had on-going treatment with oral corticosteroids. Coeliac disease was found in seven patients

. Fig: BMD (Z-score) in different sites related to number of fractures



Conclusion: Fractures predicted reduced BMD independent of site; fractures of the spine and hip was a better predictor of low BMD than proximal humerus or distal forearm. A fracture at these sites in 55-75 year old women may therefor warrant an osteoporosis investigation.

SU365

The Multifunctional Role of Vitamin D in the Elderly Population of the PRO.V.A. Study. L. Sartori,[1] E. Musacchio,*[1] G. Baggio,*[2] E. Manzato,*[1] G. Crepaldi.[1] [1]Department of Medical and Surgical Sciences, University of Padova, Padova, Italy, [2]Padova General Hospital, Padova, Italy.

Vitamin D is recognized as an important cofactor in the pathogenesis of osteoporosis and osteomalacia in the elderly population. Beside direct effects on bone metabolism, vitamin D can also interfere with muscle strength and physical performance. Physical disability is highly prevalent among older persons and represents both a significant marker of illness and an important predictor of further adverse outcomes. Low levels of vitamin D could then constitute a critical issue in evaluating the risk for osteoporosis-related fractures in the elderly population.Pro.V.A. is an ongoing, observational, population-based study designed to assess the health and physical function as well as to identify the causes and predictors of physical disability in 3000 men and women 65+ years of age, randomly selected from the two separate local health registries of the Veneto region in Italy. All subjects underwent physical, biological, clinical and instrumental evaluation to measure the health status together with an extensive physical performance testing to assess the presence of functional impairment.In the Pro.V.A. study population, 25-OH vit. D3 (vit. D) levels progressively decreased with age, showing a 48% reduction between the youngest (65-69 yrs) and the oldest (90+ yrs) age groups. There were highly significant correlations of vit. D with PTH ($p<0.005$), DHEA ($p<0.0005$) and calcaneous QUS ($p<0.0005$), as well as negative correlations with serum bALP ($p<0.05$) and urinary Xlaps ($p<0.0005$). As a whole, 12% of our population (7% of the males and 16% of the females) had vit. D concentrations below 25 nmol/L. Twenty-seven percent of the subjects showed functional disability in ADL, with a substantial prevalence for the female group (F:M=3:2). Vit. D was also significantly reduced in many different disability-related settings such as walking impairment ($p<0.0002$), history of falls ($p<0.0002$), and previous femoral fractures ($p<0.0002$).Our results suggest that, in the elderly subjects, vitamin D has indeed a multifunctional role in bone health. Its function in bone metabolism, together with a likely role in musculoskeletal disabilities and falls, makes it a key player and a powerful marker for bone-related functional disabilities. These features also underline the importance of maintaining adequate vitamin D levels through dietary or pharmacological supplements, especially in the oldest age groups.

SU366

Decreased Bone Mineral Density in Postmenopausal Women With Self Reported Wrist Fractures Before Menopause. C. Fiorano-Charlier,*[1] A. Ostertag,*[1] J. P. Aquino,*[2] M. C. de Vernejoul,[1] C. Baudoin.*[1] [1]INSERM U349, Paris, France, [2]Observatoire de l'âge, Paris, France.

Post menopausal fractures are associated with low bone mass, however the role of a low peak bone mass in young adults to determine latter osteoporosis questions the value of pre menopausal fracture. We investigated if the self-reported past history of pre menopausal wrist and non-wrist fractures could be similarly associated to the actual bone density and could predict osteoporosis**Patients and methods.** We recruited, in a recipients fund, 453 volunteers women with a median age of 64 years (range 50 - 83 years), without any metabolic bone diseases, previous femoral neck fracture or prevalent vertebral fracture. The actual bone density was measured at femoral neck (FN) and lumbar spine (LS) using a Lunar DPX-L. Osteoporotic patients were defined by a T-score < 2.5 in at least one bone site. Measurements and interviewed were performed in our center by trained technicians. We analysed T-score using analysis of variance and multiple comparison procedure, with significant level fixed at 0.05. Estimate of osteoporotic risk ratio (RR) was based on logistic regression (S-PLUS software).**Results.** As expected, the 319 women who did not report any fracture had a higher T-score at LS (- 0.93 ± 1.44) than women who reported a previous fracture. Among the women who reported a first fracture before menopause, compared to the non fractured women, only those who reported a wrist fracture had an actual decreased LS T-score (-1.77 ± 1.20, n=15) while the women who reported only a non-wrist fracture before menopause had no significant decrease LS T-score (-1.26 ± 1.00, n=36).The T-score of 34 women with a postmenopausal wrist fracture (-1.51 ± 1.28) and of the 49 women who reported only a non-wrist fracture (-1.85 ± 1.27), both differed significantly from non-fractured women. Identical results were observed at the FN. These 5 groups of women had comparable demographic characteristics and life style.The osteoporotic risk differed in the 5 groups described above, after adjustment on weight, age, hormonal replacement therapy and hip fracture in the family. There was 20% of osteoporotic patients among non fractured women. RR for pre-menopausal wrist fractures was 2.4 (95%CI:1.4-4.3) whereas it was 1.2 (0.7-2.4) for pre-menopausal non wrist fractures.**Conclusion.** We conclude that self reported wrist fractures but no other fractures

occurring before menopause are likely to be associated to osteoporosis when they are 65 years old and are therefore a strong indication for screening

SU367

The Effect of Cigarette Smoking on Bone Density in 1485 Early Postmenopausal Women. Five Years Follow Up on the Danish Osteoporosis Prevention Study. A. P. Hermann,[1] L. S. Stilgren,[1] N. U. Kolthoff,*[2] C. L. Tofteng,[3] L. Mosekilde.[4] [1]Department of Endocrinology, Odense University Hospital, Odense, Denmark, [2]Hilleroed Hospital, Hilleroed, Denmark, [3]Osteoporosis Research Center, Hvidovre Hospital, Copenhagen, Denmark, [4]Department of Endocrinology, Aarhus Amtssygehus, Aarhus, Denmark.

The aim of our present study was to evaluate the effect of tobacco smoking on changes in regional and total BMD in recently menopausal women, allocated to HRT or no treatment. We analysed 5-year follow-up data on BMD in a national comprehensive cohort study including 2016 early postmenopausal women. Initially the women were allocated to a randomised or a non-randomised arm by their own choice. In both arms treatment was given as open labelled, cyclic combined estrogen and gestagen. Treatment was initiated with oral medication, but in case of side effects a change to transdermal therapy was possible. Smoking habits were recorded at study start and after 1, 2, and 5 years. BMD in the whole body, spine, and hip were measured during the same visits, using Hologic 1000W and 2000 equipment. In the HRT group 508 women (72.3 %) completed 5 years treatment and in the control group 977 (74.4 %) were still untreated after 5 years. In the control group we found a decline in BMD of 0.03, 0.07, and 0.05 g/cm2 in the whole body, spine, and femoral neck respectively. In the HRT-treated group an increase in BMD of 0.02 g/cm2 was found in whole body and spine, while BMD of the femoral neck was unchanged. In the untreated group no difference in bone loss was found between the 352 (36%) current smokers and the 625 non-smokers. In the HRT group the 214 (42%) smokers gained significantly less BMD in the whole body ($p<0.001$), and had a significant decline in bone density in the femoral neck in contrast to the non smokers ($p=0.02$). No difference was seen in the spine. There was no significant difference in weight gain between smokers and non-smokers. Furthermore the differences between smokers and non-smokers were independent of initial body weight or weight change during the follow-up periode. In conclusion we could not demonstrate any negative effect of cigarette smoking on BMD in 977 early postmenopausal women during 5 years of follow-up. Concomitant cigarette smoking significantly attenuated the positive effect of HRT on BMD in 508 women starting treatment shortly after menopause.

SU368

Bone Mineral Density is Not Decreased in Multiparous Amish Women. E. A. Streeten, D. McBride, T. I. Pollin,* A. L. Lodge,* B. D. Mitchell, A. R. Shuldiner.* Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD, USA.

Pregnancy is associated with a reduction in trabecular bone mineral density (BMD), in spite of maternal compensatory changes in calcium homeostasis. The changes in BMD in pregnancy are interesting and important, but perhaps a more clinically relevant question is: What is the effect of parity on later BMD and fracture risk? Some data suggests that multiparity is not a risk factor for osteoporosis. The purpose of this study was to evaluate the relationship between parity and BMD in Old Order Amish women,a population noted for its large family sizes.Methods: Women for this study were participants in the Amish Family Osteoporosis Study (AFOS), enrolled between 1997 and Jan. 2001. Probands for the AFOS were identified by word of mouth on the basis of diagnosed osteoporosis or family history of osteoporosis. First degree family members of the probands, spouses of probands and the spouses' first-degree family members were also invited to participate. BMD was measured by DXA at the spine(L1-4), hip and forearm in 406 Amish women (mean age=49.4 yrs).Results: BMD for spouses of probands, a more random sampling of the Amish than the probands, were similar to BMD in NHANES Caucasians. Mean age at menarche was 13.5; menopause 48.8 years. The mean daily calcium intake was 930 mg (n=100).The mean number of offspring per woman was 5.8 (range: 0-16), with 227 having at least 6 children. Overall, there was no correlation between parity and BMD at any site. Because parity and BMD are both strongly influenced by age, we computed age-adjusted BMD levels and evaluated the relationship with parity, this time restricting our analysis to the 170 women who were enrolled because they were spouses of the proband or probands' relatives. There was no significant difference in age-adjusted BMD between women with 0 children (n=24) and women with 8 children or more (n=24)at either the spine (0.989 vs 0.947 g/cm2), total hip (0.862 vs 0.877 g/cm2) or ultradistal radius (0.425 vs 0.440 g/cm2), nor was there any evidence for a linear trend between age-adjusted BMD and parity. Similar results were obtained comparing Z score and parity and when restricting this analysis to women aged 60 years and older.Conclusion: There is no correlation between increasing parity and BMD in Amish women. Although Amish women appear to be similar to the general population of Caucasian women with respect to potential risk factors for osteoporosis, we cannot rule out the existence of other characteristics unique to Amish women that could be protecting them from parity-related bone loss.

Disclosures: Osteoporosis Advisory Board for Proctor and Gamble,5.

SU369

Contribution of Age and Clinical Risk Factors to DEXA-Based Hip Fracture Risk Stratification Using a Multidimensional Model. W. D. Leslie,[1] C. Metge,[2] L. Ward.*[3] [1]Department of Medicine, University of Manitoba, Winnipeg, MB, Canada, [2]Dept of Community Health Sciences, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada, [3]Department of Nuclear Medicine, St. Boniface General Hospital, Winnipeg, MB, Canada.

Hip fractures are independently associated with advancing age, specific clinical risk factors (CRF), and low BMD. The use of T-scores for reporting BMD ignores the contribution of age and CRFs. We previously developed a multidimensional model of hip fracture risk that incorporates patient age, bone mineral density (BMD), and the results of 11 specific CRFs: poor general health, inactivity, immobility, current smoking, greater height, height loss, low body weight, hyperthyroidsim, fractures after age 50, falls, and family history of osteoporotic fracture (JBMR 2000;15:S416). The purpose of this study was to compare the contribution of the multidimensional mode (M-DM) with a unidimensional model (1-MD) based on BMD alone.We selected 213 consecutive postmenopausal females (mean age 65.3, range 50-87.9) with CRF data referred for BMD assessment of fracture risk. Absolute hip fracture risk (over the next 5 years and remaining lifetime) was estimated using both the M-DM and 1-DM (latter assumes constant patient age of 65 and three common CRFs - family history of osteoporosis, fall in last 12 months and ambulatory less than 4 hours/day). The ratio of absolute hip fracture risks (1-DM/M-DM) was derived for each patient and displayed in a modified Bland-Altman plot (geometric mean vs ratio with upper and lower 95% CI).The fracture risk ratio was 0.8±2.2 for hip fracture in the next 5 years and 1.1±1.9 for remaining lifetime, with respective 95% CIs of 0.16-3.8 and 0.3-3.9. This indicates a large contribution of M-DM over 1-DM in fracture risk stratification. The ratio was not constant across the risk range (P1) while M-DM exceeded 1-DM at higher levels of risk (ratio<1).In conclusion, we have shown that a multidimensional approach to hip fracture risk stratification is feasible and greatly modifies risk stratification based on BMD alone.

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A Score-Based Index For The Assessment of Vertebral Fracture Risk. F. Bertoldo,*[1] G. Franchina,*[1] A. Fracalossi,*[2] S. Zenari,*[1] V. Lo Cascio.[2] [1]Dipartimento di Scienze Biomediche e Chirurgiche, Medicina D, Verona, Italy, [2]Dipartimento di Scienze Biomediche e Chirurgiche, Medicina Interna D, Verona, Italy.

From a clinical perspective, the major goal for intervention in patients with osteoporosis is to reduce fracture risk. The challenge for the physician is to identify those individuals who are candidates for pharmacological treatment. The prediction of fracture has proved to be unsatisfactory if based only on BMD T-score and the intervention threshold depends upon a set of other independent well known risk factors. The aim of our study was to test a score-based index (SI) to assess the risk of osteoporotic fractures. The SI was calculated giving an arbitrary score to each of the following independent risk factors: age, familiar history of fractures, prevalent osteoporotic fractures, BMD T-score, bone turnover expressed as NTX urinary levels, falls in the last 2 years. We examined in a 2-year retrospective study the incidence of new V-FX performing a lateral spine radiograph in 316 postmenopausal women aged 63.7+ 3.4 yrs whose medical history, complete physical examination, femoral BMD (Lunar Expert XL), NTX urinary levels, lateral spine radiographs were collected at the baseline. We excluded patients with secondary osteoporosis or treated in the last 2 years with bisphosphonates, estrogens or SERM for more than 6 months continuously. V-FX was defined as a difference of at least 15% between posterior and central and/or anterior vertebral heights. At baseline 84 out of 316 patients had at least a V-FX (26.6%). One or more new V-FX were observed in 40 patients (12.6%); 25 out of 40 (65.5%) were previously fractured. Patients with incident fractures had a baseline BMD T-score not different from patients without new V-FX (-2.1+1.2 and -2.1+0.9 respectively; $p= 0.13$) but SI was significantly higher in the former (21.7+9.3 vs 12.8+0.5 respectively; $p= 0.0001$). Dividing the patients with new V-FX by tertiles of BMD T-score, 37.5% fell in the lowest (T-score -1.7) (chi square 4.05,d.f.2; $p = 0.13$, compared with patients without new V-FX). When the patients with new V-FX were stratified on the basis of SI tertiles, 85% fell in the highest (score >20) and 2.5% in the lowest (score <12.8) tertile (chi square 71.2, d.f. 2; $p = 0.001$ compared with patients without new V-FX). Our preliminary data suggest that SI, taking in account a set of independent risk factors, is more predictive than BMD T-score alone in identifying patients at risk for new fractures although larger and prospective evaluation should be performed.

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Determinants of Hip Fracture Type—Effects of Bone Mineral Density (BMD), BMD Distribution, and Load Condition. M. Mizuno,[1] A. Harada,*[1] M. Takemura,*[1] H. Okuizumi,[2] E. Tanaka,*[3] S. Yamamoto.*[3] [1]Orthopaedic Surgery, Chubu National Hospital, Obu, Japan, [2]Biomechanics Research Laboratory, University of Michigan, Ann Arbor, MI, USA, [3]Mechano-Informatics and Systems, Nagoya University, Nagoya, Japan.

This study elucidates the determinants in two types of hip fracture: trochanteric fracture and cervical fracture.Twenty-two embalmed, cadaveric femora (6 males and 10 females; mean age of 77.4±6.4 years (±SD), ranging from 61 to 89 years old at time of death) were examined for the effects of load condition on the femoral head, bone mineral density (BMD), and BMD distribution of the proximal femur on hip fracture type. Dual energy X-ray absorptiometry (DXA) and a hip strength analysis (HSA) program (Lunar Corp.) were used to measure BMD in the femoral neck, Ward's triangle, trochanter, and upper and lower femoral neck regions. A 60x10 mm range of interest (ROI) was specified in the subcapital area, and BMD was measured in the upper and lower subcapital regions. The upper subcapital BMD versus lower subcapital BMD ratio (subcapital-U/L) was calculated as the density distribution ratio. Mechanical fracture tests were performed with the femoral head facing upward and the angle of the neck axis and load to the femoral head set in the axial plane 15~20 degree anterior (anterior load: 7 cases), 0 degree (neck axis load; 8 cases), and 15~20 degree posterior (posterior load; 7 cases). In the coronal plane the angle between the neck axis and the femoral head load was 40~50 degree viewed from the femoral anterior side. An 8.4 kg weight was dropped on the femoral head. Statistical analysis included Student's t-test, and chi square test for independence.Results of the mechanical fracture tests disclosed trochanteric fracture in 15 cases, and cervical fracture in 7 cases. No difference

was observed in the absolute value of the densities of the examined areas in both fracture types. Excluding 3 cases which could not be measured due to corrupted digital data, the subcapital-U/L density ratio in 19 of the 22 cases was lower in 7 cases of cervical fracture (0.55±0.08) than in 12 cases of trochanteric fracture (0.64±0.06) ($p<0.01$). The mean CV value of subcapital-U/L was 4.97%. Load condition was not associated with hip fracture type ($p=0.2$).We concluded that hip fracture type was associated with the BMD distribution in the subcapital region.

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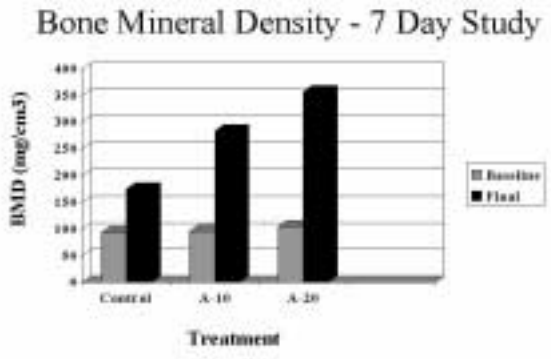
In Vivo Rat Assay: Detection of Significant and Dose-Responsive Bone Resorption by pQCT Quantification in 7 days. N. A. McHugh, H. Vercesi,* R. W. Egan,* J. A. Hey.* Allergy, Schering-Plough Research Institute, Kenilworth, NJ, USA.

Animal models which simulate osteoporosis such as ovariectomized animals (post-menopausal), glucocorticoid-induced and senescence related osteopenia are typically conducted in aged animals over long periods of time. In the case of senescence-induced osteoporosis, effects may not be observed for 3 to 6 months. The cost of boarding and large amounts of drugs that are needed to sustain these chronic models are often prohibitive. Moreover, this protracted study time is rate-limiting to evaluation and development of novel therapeutic agents. The purpose of this study was to determine if the Schenck 21 day old weanling rat model can be used to quantify bone resorption or growth by pQCT scanning techniques. Using the resorption inhibitor alendronate sodium (bisphosphonate), we not only observed significant changes to bone growth but a dose-response effect as well. Because the 21 day old rat is growing at a fast rate, all groups showed a significant increase in bone mineral density compared to their baseline values; however, we only observed an average increase of 86% in the control rats versus the 195% and 241% increases measured in the low dose (10ug/kg) and high dose (20ug/kg) of alendronate sodium, respectively. Although the relevance and appropriateness of this model to adult human disease may be limited, it provides a useful model for mechanistic pre-screening of therapeutic modalities or efficacy assessment to help guide dose selection for chronic long term osteoporosis models.



Disclosures: Schering-Plough Research Institute,3.

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Normal Bone Volumetric Density in Adults with Life-long, Untreated, Severe Growth Hormone Deficiency Due to Genetic GHRH Receptor Deficiency. H. G. Maheshwari,*[1] R. Bouillon,[2] G. Baumann.*[1] [1]Center for Endocrinology, Metabolism and Molecular Medicine, Northwestern University, Chicago, IL, USA, [2]Division of Endocrinology, Catholic University, Leuven, Belgium.

Growth hormone (GH) and insulin-like growth factor I (IGF-I) are important hormones for bone elongation and accretion during childhood. While the role of GH and IGF-I in bone growth is well established, their importance for bone mineralization is less well understood. Adults with GH deficiency (GHD) frequently have osteopenia, as assessed by Dexa scanning, and bone mineral density (BMD) improves with prolonged GH replacement treatment. However, the full impact of life-long GHD on bone structure and mineralization is only incompletely known because in Western societies children with GHD are invariably treated with GH, and because in adults GHD is frequently associated with other pituitary hormone deficits leading to hypogonadism, hypothyroidism and/or hypoadrenalism. The recent discovery of a genetic syndrome of severe isolated GHD due to an inactivating mutation in the GH releasing hormone receptor (GHRH-R) in Pakistan, where affected patients were not treated, affords a unique opportunity to assess the impact of life-long, isolated GHD commencing at conception on bone mineralization. To this end, we studied 4 affected adult male patients (age 23-30 years) at Northwestern University by Dexa scanning, using a Hologic QDR-4500A scanner. The patients' social, nutritional, general health, endocrine, and genetic characteristics have been previously reported (JCEM 83:4065, 1998). They had not been treated with GH or any other medical therapy. Dexa results were compared to normative values derived from the Hologic database. Areal BMD at L2-L4 was 0.144±0.021 g/cm2, corresponding to a Z score of -3.18±1.24 (mean±SD). Total body BMD was 0.995±0.056 g/cm2 (Z score of -1.68 ± 0.64). Because of the small size of these patients (height Z score -6.3 to -8.0), we calculated apparent volumetric BMD (BMAD) as a more accurate index of true or volumetric density. BMAD at L2-L4 was 0.144±0.021 g/cm3, corresponding to a Z score of -0.87± 1.21; total body BMAD was 0.0076±0.0006 g/cm3 (Z score 1.79±1.2). Only one patient had a BMAD score more than 2 SD below normal in the lumbar spine; all other values were within the normal range. We conclude that bone mineralization in these GHRH-R deficient patients with life-long, untreated, severe, isolated GHD is relatively normal, as assessed by volumetric BMD measurement. This finding raises questions about the role of GHD as a cause of osteopenia when areal BMD is used as the sole criterion. Additional studies of this issue in larger, clearly defined GHD cohorts are required to reach a firm conclusion.

Disclosures: Pharmacia Corporation,6.

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Increase in Weight and BMI Does Not Lead to Bone Mass Recovery in Adolescents with Anorexia Nervosa. M. B. Oliveri,[1] M. S. Parisi,*[1] L. Del Río.[2] [1]División Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Cetir Centre Médic, Barcelona, Spain.

Osteopenia and osteoporosis are established complications of anorexia nervosa. However, whether the diminution in the bone mass of adolescent patients (before they reach their peak bone mass) can be recovered partly or completely and the factors that determine recovery have not been clearly established to date. A total of 113 young female patients diagnosed with anorexia nervosa were studied. Bone mineral density of the lumbar spine (LS BMD) was determined by DEXA (LUNAR DPX-L) at baseline. Follow-up determinations were performed at one year in 38 patients, and at 3 years in 21. The Z score of LS BMD (ZLS) was calculated by comparing to a control population of 393 women aged 11 to 19 years. Baseline evaluation: age (Mean ± SD): 15.9 ± 2.1 years (range: 11-19); BMI: 16.1 ± 1.8; diminution in body weight according to age (dw/a): 21.0%, LS BMD 0.999 ± 0.153 g/cm2; ZLS: -1.2. ZLS and LS BMD correlated positively with BMI: r= 0.21and 0.29 ($p<0.05$) and with weight: r= 0.32 and 0.44 ($p<0.01$) respectively (Spearman rank coefficients). The population was divided in groups according to age (table 1).Results at 12 months showed an average 12% increase in weight from 40 to 44.8 kg ($p<0.001$), an increase in BMI from 16.6 to 17.9 ($p<0.001$); dw/a improved from -19.3 to -13.3% ($p<0.01$), whereas LS BMD (g/cm2) remained unchanged: 1.004 ± 0.143 to 1.004 ± 0.118 g/cm2. At 36 months follow-up, results showed an average increase in weight from 38.2 to 44.5 kg (16%) ($p<0.01$) and in BMI from 15.8 to 17.9 ($p<0.001$); dw/a improved from -23.6 at baseline to -15.7 ($p<0.01$) but no improvement LS BMD was observed: 0.951 ± 0.145 to 0.957 ± 0.132 g/cm2 (Wilcoxon signed ranks test). Conclusions: 1) Baseline evaluation revealed decreased LS BMD values, which were lowest in the 11-13 years group, associated with a significant diminution in BMI and weight; 2) In spite of the significant improvement in weight and BMI at 1 and 3 years follow-up, LS BMD did not recover. This finding coupled with the weak correlation between BMI, weight, and LS BMD observed at baseline would seem to indicate that weight loss and recovery only partly influences bone mass. Table 1: ** $p<0.001$compared to B and C * $p<0.02$ compared to B (Mann-Whitney Test)

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Change in Housing Condition Modifies Bone Turnover in Old Control Cynomolgus Monkeys for Six Months. J. Legrand,[1] C. Fisch,[2] P. Guillaumat,[2] R. Forster,[2] S. de Jouffrey,*[2] J. Claude.*[3] [1]Beaufour -Ipsen, Paris, France, [2]CIT, EVREUX, France, [3]Faculty of Pharmacy, Paris, France.

Mature female cynomolgus monkeys from Mauritius, maintained as breeding gangs and approximately weighing 4 kg, were allocated to two groups. The first group of 10 was sent immediately to the experimental facility (CIT, Evreux, France), where the animals were housed in individual cages and sham-operated one month after arrival. The second group of 12 was kept at Mauritius for 12 months, the animals being acclimated to individual cages, before being sent to the experimental facility where they were similarly housed and sham-operated one month after arrival. Bone mineral density (BMD) of lumbar vertebrae was measured by DXA and blood and urine samples were taken for quantification of osteocalcin (OSTEO) and deoxypyridinoline urinary clearance (DPyr/creat) before surgery and every 3 months thereafter. Five animals from the first group and two from the second experienced chronic diarrhea and severe depression symptoms during the first 3 months after arrival at the experimental facility. They were excluded from the study. In addition, a third female of the second group developed a malabsorption syndrome and was removed from analysis. The BMD of the remaining animals of the first group decreased to 95% of the pre-surgery value at three months and then increased back to 98% at 6 months, being then relatively constant. OSTEO and Dpyr/creat were higher and more dispersed in animals from the first group before surgery, and both markers increased in this group at 3 months after surgery. The BMD of all animals of the second group and the biochemical markers of bone turnover remained stable during the study and very close to 100 % of the value measured before surgery. Bone turnover was clearly induced in the animals from the first group during the first months after arrival at the experimental facility. Such a change was not observed in the animals of the second group and can be inferred to the change of housing conditions. Any other factor like age, breeding history, food composition being similar between both groups can be excluded as a causal factor. Old females, used to living in large gangs, seem to experience a high level of stress when caged individually as reflected by the observed depression symptoms and diarrhea. Such symptoms have been reported to be associated with increased secretion of corticosteroids, that may trigger BMD decrease. In conclusion, six months appear to be needed after housing change for a recovery and stabilization of the bone metabolism status of cynomolgus monkeys. Transportation or sham-operation does not appear to affect BMD or bone turnover biochemical markers of animals acclimated to single housing condition.

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Early Cortical Bone Loss in Rheumatoid Arthritis Is Detectable by Digital X-Ray Radiogrammetry but Not by DXA. T. Jensen,*[1] M. Klarlund,*[1] K. Jensen,*[2] H. Skjødt,*[1] L. Hyldstrup.[3] [1]Clinic of Rheumatology, Hvidovre Hospital, Hvidovre, Denmark, [2]Research Center of Magnetic Resonance, Hvidovre Hospital, Hvidovre, Denmark, [3]Endocrine Unit, Hvidovre Hospital, Hvidovre, Denmark.

Periarticular and generalised bone loss is an early feature of rheumatoid arthritis and measurements of regional bone mineral density (BMD) in forearm and hands have been

used as a marker of regional osteopenia and development of irreversible joint damage. The aim of the present study is to evaluate whether Digital X-ray Radiogrammetry (DXR, Pronosco X-posure System) applied to the standard hand x-rays taken with the purpose of disease evaluation also can be used for detection of cortical bone loss. Further, to compare the changes in cortical bone of the hand detected by DXA and DXR. DXR provides a composite bone mass estimate using a weighted average of cortical thickness and bone width measurements at the 3 metacarpals (2-4).Twenty-one patients with unclassified polyarthritis and 51 patients with early rheumatoid arthritis (median disease duration 3/3 months, range 1-24/1-24, respectively) with active joint disease were followed with monthly intervals up to 2 years. Bone erosions in the hands were determined by x-ray (posterior/anterior position) and MRI at entry (n=71) and after 1 and 2 years (RA, n=45). BMD in hands and forearm were evaluated every 6 months with DXA and DXR. In patients with RA, DXR-BMD in left and right metacarpals demonstrated significant reductions after 6 month and throughout the study period (p0.05). Both in patients who developed erosive disease (n=22) as well as in patients without erosions (n=20) DXR-BMD decreased significantly. No significant changes in BMD measured by the DXA-technique were observed in any patient group. Comparing changes in DXR-BMD in the groups of patients with erosive and non-erosive disease, a significantly greater bone loss was seen in the first group after 24 months, but only at the left hand ($p<0.01$, Mann Whitney test). In conclusion, peripheral bone loss in early rheumatoid arthritis can be detected by digital x-ray radiogrammetry, using the same hand x-rays taken for evaluation of disease progression. The degree of bone loss seems to be related to severity of the disease.

Disclosures: Pronosco AS,2.

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Growth-Hormone Effects on Muscle/Bone Interactions in Hypophysectomized Rats. J. L. Ferretti,[1] G. R. Cointry,[1] S. Feldman,*[1] R. Gordon.*[2] [1]Center for P-Ca Metabolism Studies (CEMFoC), School of Medicine, Natl. University of Rosario, Rosario, Argentina, [2]Dept. of Immunology, School of Medicine, Natl. University of Rosario, Rosario, Argentina.

The GH effects on muscle / bone interactions are still poorly understood. This study analyzes the effects of 0, 30 or 150 mUI/d of sc GH for 45 days on bone mass, material properties, architecture (pQCT) and mechanical properties (3-point bending tests) of femur diaphyses, and the gastrocnemius wet mass in adult, hypophysectomized (Hx) Sprague-Dawley rats (n = 5,8,8).The hormone improved the architectural indicators (periosteal -not endosteal- perimeter, cortical thickness, cross-sectional area and moments of inertia, CSMI's), the "mass" and calcification indicators (BMC, volumetric BMD) of cortical bone, and the mechanical indicators (diaphyseal stiffness and strength -fracture load-). The elastic modulus (intrinsic stiffness) of cortical bone did not change but correlated linearly with the diaphyseal stiffness and strength, with similar slopes but different intercepts for each group of animals. This suggests the participation of other (geometric) variables in the pathogenesis of GH-induced changes. The cortical area and CSMI's correlated with the improvements in mechanical properties showing a single slope for all groups, suggesting that GH effects depended more upon changes in bone architrecture than in material quality. Treatment also induced an "anabolic" shift of the "distribution/quality" curves (CSMI/volumetric BMD or elastic modulus) of the total bone to the upper-right side of the graphs, following a dose-response fashion. Gastrocnemius mass (y) was increased parallelly to the improvements in CSMI's and mechanical properties (x) but treated rats showed a shift to the right in the graphs, suggesting a decreasing impact of muscular hypertrophy on the diaphyseal design as the GH dose increased.No negative effects on the bone and muscle variables studied were detected. Treatment seems to have added new, normal or slightly overmineralized material of normal intrinsic stiffness, chiefly on the periosteum, thus improving bone design and strength showing a dose-related pattern. The significant but decreasing influence of the dose-dependent, progressive gastrocnemius hypertrophy on bone changes may be explained because GH increased more muscle mass than muscle strength. If so, then, at progressively higher doses, the induced improvement in bonre mass and design would have depended more upon metabolic effects (balance between bone formation and resorption) than on biomechanical interactions.

SU378

Reversibility of Corticosteroid-induced Osteoporosis. N. Galofré,* A. Díez-Perez, S. Serrano,* L. Mariñoso,* X. Nogués, M. J. Peña,* L. Mellibovsky,* J. Blanch, J. Carbonell,* J. Aubía.* Hospital del Mar, Barcelona, Spain.

Background. Deleterious effect of corticosteroids on bone is well recognized. However the reversibility of those effects are not fully understood. In a rat model we analyze the recovery of the effects of a single dose of corticosteroids. Methods. Seven groups of male Sprague-Dawley rats (7 per group, body weight 250 g) were studied. One group (Basal) was sacrificed at baseline. Two groups (S1 and S5) received saline solution. Groups LD1 and LD5 received prednisolone 0.5 mg/kg (low dose) every day. Groups HD1 and HD5 (high dose) received daily 2 mg/kg of prednisolone. All treatments were given subcutaneously for one week. Groups S1, LD1 and HD1 were sacrificed at the end of the first week. The remaining were sacrificed at the end of the 5th week, after 28 days without treatment. All animals received tetracycline the days 7 and 2 before sacrifice. The 4th vertebral body was removed for hystomorphometric analysis.Results: In the LD groups, OS/BS decreased at the weeks 1 and 5, MS/BS at the week 5 and WTh at the weeks 1 and 5. In the HD groups there was a decreased MAR and WTh at the week 1 and decrease in N.Oc/T.Ar, Oc.S/BS, MS/BS, WTh and Ac.f at the week 5 ($p<0.05$ for all the comparisons, ANOVA, Scheffe method). Conclusion: Corticosteroids at low dose induce an early depression on bone formation that persists after their cessation. Bone resorption is increased, at high corticosteroid doses, after treatment is discontinued.

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Glucocorticoid Administration Decreased Bone Strength Attended With Deterioration of Cortex and Cortico-trabecular Junction in the Growing Minipig. S. Ikeda,*[1] Y. Morisita,*[2] H. Tsutsumi,*[3] M. Ito,[4] A. Shiraishi,*[5] S. Arita,*[1] T. Nakamura.[1] [1]Department of Orthopaedic Surgery, University of Occupational and Environmental Health, Kitakyusyu, Japan, [2]Chugai Pharmaceutical Co.Ltd., Tokyo, Japan, [3]CSK Research Park,INC., Suwa, Japan, [4]Department of Radiology, Nagasaki University school of Medicine, Nagasaki, Japan, [5]Product Research Laboratory,Chugai Pharmaceutical Co.Ltd., Tokyo, Japan.

To examine the effects of glucocorticoid administration in minipig as a model of drug-induced osteoporosis, sixteen female minipigs, 8 months of age, were assigned to 3 groups. Four animals were sacrificed at start for baseline control. Six were subcutaneously injected at a daily dose of prednisolone 0.5 mg/kg body weight (BW) dose in five days a week for 26 weeks (steroid group), and six received vehicle (control). BW and biochemical markers in serum and urine were measured at 0, 13, and 26 weeks. At sacrifice, the 2nd and 3rd lumbar vertebral body (L2, 3) and the left femur were dissected out. After measuring sizes, bone minerals were measured by pQCT at the mid portions of L3 and femur. The images of trabecular microarchitecture in lumbar bone were obtained and trabecular parameters were analyzed by Micro-CT. Then, mechanical tests for failure, compression on L2 and three-point bending on femur, were performed. Increases in BW did not significantly differ between steroid and control groups. Serum osteocalcin, urinary NTx and pyridinoline levels in steroid group significantly reduced at 13 and 26 weeks. The length values of L2 and femur in steroid group were significantly smaller than those of controls. The bone width, however, did not significantly differ from the controls. The length of L2 and femur were significantly reduced in the steroid group. While BMD values of trabecular area of L3 in steroid group did not significantly reduced, BMD values of cortex and cortico-trabecular junction area were significantly reduced. BMD values of femur were significantly reduced. In the images of trabecular microarchitecture of L3 in the steroid group, growth plate was still remained and deterioration of cortex and cortico-trabecular junction was visible. In steroid group, BS/BV, Tb.Th, structure model index (SMI), and trabecular bone pattern factor (TBPf) were significantly differ. Both ultimate load and maximum energy absorption values in steroid group on L2 and femur were significantly reduced, respectively. These data clearly demonstrated that glucocorticoid administration indicated the reduction of bone turnover and decreased bone strength attended with deterioration of cortex and cortico-trabecular junction in the growing minipig.

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Clinical Factors Do Not Identify Patients at Risk of Corticosteroid Osteoporosis. P. L. Selby,[1] L. Garcia,*[2] J. E. Adams,[3] M. Davies.[1] [1]Musculoskeletal Research Group, University of Manchester, Manchester, United Kingdom, [2]Medicine for the Elderly, Manchester Royal Infirmary, Manchester, United Kingdom, [3]Imaging Science and Biomedical Engineering, University of Manchester, Manchester, United Kingdom.

Corticosteroid use is one of the major secondary causes of osteoporosis and osteoporotic fracture. Not all patients receiving long-term corticosteroids will develop osteoporosis and treatment should be targeted at those patients at risk. The National Osteoporosis Society (NOS) in the United Kingdom has developed clinical guidelines which identify patients as being at risk of corticosteroid induced osteoporosis on the basis of clinical risk factors including: presence of pre-existing fracture; dose of corticosteroid administered; and presence of other risk factors for postmenopausal osteoporosis. The utility of these factors in identifying at risk patients has not been tested and we have examined their effectiveness at identifying osteoporosis in patients receiving corticosteroids. 88 consecutive patients referred for bone densitometry on the basis of corticosteroid use were studied. Information on the dose of corticosteroids, presence of fractures, and other risk factors for osteoporosis was extracted from the patients' hospital records. The mean age of the patients was 55 (SD 14) years, 68% were female and 21% had osteoporosis as judged by WHO criteria (T score < -2.5); using the less stringent NOS criterion (T score < -1.5), 42% were osteoporotic. Bone mineral density was negatively related to age ($r=0.56$, $p<0.001$) and positively related to body mass index ($r=0.32$, $p=0.019$). There was no relationship between the dose of corticosteroid on either bone mineral density ($F=1.02$, $p=0.36$) or the presence of osteoporosis using either of the above criteria($\chi^2=2.3$, $p=0.32$ NOS and $\chi^2=1.8$, $p=0.41$ WHO). Patients with pre-existing fractures had lower bone mineral density than those without ($\delta=1.39$SDU $p=0.003$) furthermore they were at higher risk of osteoporosis ($\chi^2=7.8$, $p=0.01$ NOS; $\chi^2=4.8$, $p=0.04$ WHO). None of the other risk factors for osteoporosis was associated with decreased bone density or increased osteoporosis. These results indicate that clinical risk factors other than fracture are a poor means of identifying patients on corticosteroids who are at risk of osteoporosis. There is no substitute for the use of bone mineral density measurements in such patients.

Disclosures: Various pharmaceutical companies,5.

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Reduced Bone Size and Volumetric Density in Men Receiving Corticosteroid Therapy. Y. Duan,* R. MacIsaac,* C. McDonald,* X. F. Wang,* A. Lee,* E. Seeman. Department of Medicine, Austin & Repatriation Medical Centre, Melbourne, Australia.

Deficits in bone mineral content (BMC) in patients receiving corticosteroid therapy (CST) is attributed to bone loss due to reduced bone formation. We asked whether reduced bone formation in the subperiosteal region of bone may produced reduced bone size as well as reduced volumetric BMD (vBMD). We studied 142 men aged 21 to 88 years (mean 62.6 yrs) treated with prednisolone for chronic obstructive pulmonary disease (COPD, n = 68), rheumatoid arthritis (RA, n = 23), inflammatory bowel disease (IBD, n = 19), and vasculitic disease (VAS, n = 32). The patients received an average of 14.1 mg CST daily (range, 1-75) for 7.4 yrs (range, 0.08 to 40). Controls compromised 395 healthy men aged

17 to 91 years. Bone size and vBMD at the third lumbar vertebra (L3) and femoral neck were measured by postero-anterior (PA) scanning using dual-energy x-ray absorptiometry. Vertebral body width was obtained from PA scan. Femoral neck width was measured at the middle point of femoral neck axis length. Vertebral volume (V) was estimated as: V = (scan area of L3)3/2, femoral neck volume = 3.14 * (width/2)2 * height of scan region. vBMD was estimated as BMC/V. The data were expressed in absolute terms and as the number of standardized deviation (SD) scores above or below the age-matched normal mean (Z score, Mean ± SEM).Patients received CST has reduced vertebral width (– 0.38 ± 0.08 SD, p < 0.001), BMC (– 0.75 ± 0.09 SD, p < 0.001) and vBMD (– 0.64 ± 0.08, p < 0.001). There was no difference in vertebral height. Reductions were seen in the femoral neck (width, – 0.21 ± 0.07 SD; BMC, – 0.72 ± 0.10 SD; and vBMD, – 0.61 ± 0.08 SD; all p < 0.05 to < 0.001). Reduced bone size was responsible for about 50% of the deficit in vertebral BMC and 28% of the deficit in femoral neck BMC. After adjustment for age, there were no relationships between bone width or vBMD and cumulative dose of CST (r = 0.02 to 0.13, all NS). In summary, men receiving CST have smaller bones and less bone in the smaller bones. Smaller bone size accounted for about 30-50% of the deficit in BMC and may be due to reduced periosteal bone formation during growth or ageing. The reduced periosteal bone formation may have its origins in growth, ageing or both, and may be due to selection bias, the chronic illness or CST. Men received CST are at increased risk for vertebral and hip fractures because of the smaller bone size and reduced vBMD.

SU382

Effects of Oral Glucocorticoids on Regional Bone Mineral Density and Soft Tissue Composition in Japanese Women. S. Takata,[1] H. Yonezu,*[2] N. Yasui.*[1] [1]Orthopedic Surgery, The University of Tokushima, Tokushima, Japan, [2]Orthopedic Surgery, Oe Kyodo Hospital, Oe, Japan.

We studied the effects of oral glucocorticoids (OGCs) on bone mineral density (BMD) and soft tissue composition in Japanese Women. Fifty-seven women, 29 to 74 years of age, were divided into two groups: women receiving at least 7.5 mg of oral prednisone daily (OGCs group, n=27) and healthy women (control group, n=30). The BMD of the 2nd to 4th lumbar vertebrae (L2-4BMD), head, arms, legs, ribs, thoracic vertebrae, lumbar vertebrae and pelvis as well as the lean mass and fat mass of the head, arms, legs, and trunk were measured by dual energy X-ray absorptiometry. L2-4BMD and BMDs of the lumbar spine, thoracic spine and pelvis of the OGCs group were significantly lower compared to the control group (P <0.001). The fat mass of the trunk and head was significantly higher in the OGCs group than in the control group (P <0.001); whereas, there was no significant difference regarding lean mass between the two groups. The results showed that prolonged treatmen with OGCs was associated with a decrease of the BMDs of spine and pelvis and an increase of the fat mass of the trunk and head. We conclude that OGCs affects weight-bearing and axial bone which is rich in cancellous bone, and that OGCs facilitates the proliferation of adipose cells in the head and trunk, changing thereby the distribution of adipose tissue in women under prolonged daily treatment with OGCs.

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The Effects of Corticosteroid on Trabecular Bone Architecture, Growth Plate and Bone Marrow are only Partially Reversible: An MRI/MRS in the Rabbit. M. Takahashi,*[1] F. W. Wehrli,[1] L. Hilaire,*[1] B. Zemel.[2] [1]Radiology, University of Pennsylvania Medical Center, Philadelphia, PA, USA, [2]Division of G.I. and Nutrition, Children's Hospital of Philadelphia, Philadelphia, PA, USA.

We have previously shown that two-week treatment with dexamethasone (Dex) induced a significant reduction in trabecular bone volume that occurred at the expense of uniform trabecular thinning without affecting network architecture. Paralleling the loss in bone volume was a conversion of hematopoietic to yellow marrow and atrophy of the epiphyseal growth plate. The purpose of the continuation of this study was to investigate whether there is reversal and full re-establishment of skeletal defects upon cessation of steroid treatment.Twelve male rabbits (6 month old) were either subcutaneously implanted a pellet for slow-release Dex at a dose of 0.6mg/kg/day or sham operated, at baseline. Animals were anesthetized and imaged at two time points (0, 2 wks) and, additionally, at three time points (4, 6, 10 wks) after removal of the pellets (sham operation for the controls). In-vivo µ-MRI was performed on a 1.5T whole-body imager for quantifying trabecular morphology in the distal femur. A 3D data set of 4.5mm thickness was processed using bone volume fraction (TB/TV) mapping. Bone marrow composition was measured spectroscopically in axial sections in the epiphysis and metaphysis using a fast chemical shift imaging technique.In the control group, TB/TV remained constant during the protocol duration in these relatively mature animals. However, in the treatment group TB/TV rapidly decreased within 2 wks of Dex treatment as observed previously, but recovering rapidly within 2 wks upon cessation of treatment (at 4 wks), and returning to normal levels at 6 wks. The controls showed approximately linear thinning of the growth plate thickness as would be expected during maturation. In contrast, all treated animals showed more rapid thinning during the 2 wks of treatment, with decelerated thinning after removal of pellets, and there was no difference between the groups at 10 wks. Fat fraction in the metaphyseal marrow was greater at 2 wks in the treatment group. The treatment group remained at this level throughout the protocol even after ending the treatment. Although fat fraction in the control group increased gradually, it was still lower than in the treatment group at 10 wks, suggesting irreversible reduction of hematopoesis. In summery, these results suggest in vivo the partial reversibility of CS exposure in bone volume. However, the CS-induced inhibition of chondrocyte proliferation and/or cartilage matrix production and conversion of hematopoietic to adipocytic marrow do not appear to be fully reversible.

SU384

Inhaled Glucocorticoids Are Associated With Decreased Bone Mineral Density. J. A. Pasco,[1] M. A. Kotowicz,[1] M. J. Henry,*[1] E. Seeman,[2] G. C. Nicholson.[1] [1]Clinical & Biomedical Sciences, Barwon Health, The University of Melbourne, Geelong, Australia, [2]Medicine, The University of Melbourne, Heidelberg, Australia.

Whereas oral glucocorticoid therapy is an established cause of osteoporosis, the effects of inhaled glucocorticoids remain unclear. We examined the association between current use of inhaled glucocorticoids and BMD in 1,171 women aged 20-94 yr (median 51.8 yr) from an age-stratified, population-based random sample recruited for the Geelong Osteoporosis Study. BMD (Lunar DPX-L) was measured at the spine (PA and lateral, L2-4), proximal femur, whole body and distal forearm. Self-reported medication use and lifestyle factors were documented by questionnaire. Women with a history of using HRT, oral or topical glucocorticoids, or non-current use of inhaled glucocorticoids, were excluded. Univariate analysis indicated that the 60 inhaled glucocorticoid users were heavier than non-users (mean±SD, 75.8±21.0 vs 67.4±14.0 kg, p=0.003), but did not differ in age (median (range), 49.1 (20-87) vs 51.9 (20-94) yr, p=0.98), height (161.4±8.1 vs 160.2±6.9 cm, p=0.3), dietary calcium intake (623±315 vs 658±370 mg/day, p=0.4), or BMD at any site (all p>0.05). There was no difference in the proportion of glucocorticoid users who smoked cigarettes (38% (n=23) vs 39% (n=428), p=0.98) or who were very active (8.3% (n=5) vs 10.4% (n=116), p=0.6). Multivariate analysis (adjusting for age, weight, ±height) indicated a negative association between use of inhaled glucocorticoids and BMD at the lateral spine (mean±SE, 0.576±0.023 vs 0.618±0.006 g/cm^2, p=0.07), femoral neck (0.875±0.016 vs 0.900±0.004 g/cm^2, p=0.1), whole body (1.086±0.010 vs 1.108±0.002 g/cm^2, p=0.03) and ultradistal forearm (0.285±0.007 vs 0.298±0.002 g/cm^2, p=0.07), representing lower BMD by 6.8%, 2.8%, 2.0%, and 4.2%, respectively. Adjusting for physical activity, smoking and calcium intake did not alter the pattern of associations. Since unspecified doses, frequency of use and different inhaler devices would all influence systemic bioavailablity, different inhaled glucocorticoids were grouped together in this study. Despite this limitation, our cross-sectional, epidemiological study suggests that inhaled glucocorticoids are associated with reduced BMD. It is unclear whether this is attributable to the action of glucocorticoids or to the underlying disease.

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Hypocalcemia Increases Serum Osteocalcin in Control but Not Warfarin-Treated Monkeys. D. C. Krueger, J. A. Engelke,* T. N. Kawahara,* N. C. Binkley. Institute on Aging, University of Wisconsin, Madison, WI, USA.

Moment to moment serum calcium regulation likely involves soluble hydroxyapatite precursors. Bone matrix proteins, including osteocalcin (Oc), a vitamin K (K) dependent protein may stabilize these precursors. To assess this possibility, we evaluated the effect of induced hypocalcemia on serum Oc concentration in rhesus monkeys. Initially, 20 skeletally mature male monkeys (mean age 11.9 years) were randomly assigned to receive intravenous saline (8.6 ml/kg/hr) or citrate (48 mg/kg/hr). The control group received saline for 40 minutes; following 10 minutes of saline, the treatment group received sodium citrate to induce hypocalcemia. Blood specimens were obtained at 0, 2, 5, 10, 12, 15, 25, and 40 minutes via central venous catheter. Whole blood ionized calcium, serum Oc, bone specific alkaline phosphatase (BSAP) and parathyroid hormone (PTH) were measured at all timepoints. Ionized calcium was reduced (p < .001) after five minutes of citrate infusion and continued to decline for study duration. Compared to saline, citrate induced elevation (p < .0001) of PTH at two and Oc (p < .05) at 15 minutes; BSAP concentration was not altered. We hypothesized that Oc may be involved in moment-to-moment maintenance of normocalcemia by contributing to hypocalemia correction and speculated this would be impaired in a K insufficient state. As such, we subsequently studied 18 adult male monkeys (mean age 14.2 years) that had been randomly assigned to control or warfarin (W) therapy for 30 months. All animals received saline as above for 10 minutes at which time the infusion was switched to citrate as above for 30 minutes, then restarted saline for an additional 30 minutes. Blood specimens were obtained at 0, 10, 12, 15, 25, 40, 50, 60 and 70 minutes via central venous catheter. Whole blood ionized calcium and serum Oc were measured at all timepoints. Ionized calcium was reduced (p < .001) after two minutes of citrate and continued to decline, then returned toward normal within 10 minutes after citrate discontinuation (p < .001) without difference between groups. Serum Oc was elevated in the control group by 15 minutes of citrate (p < .05); this increase was not observed in the W group.In conclusion, citrate infusion produced hypocalcemia equally in control and W groups. Hypocalcemia induction produced a rapid Oc elevation in control but not W treated animals which may reflect lower bone Oc content following long term anticoagulation. However, in spite of a blunted Oc response, hypocalcemia was corrected equally in the W treated group. This suggests that if Oc plays a role in moment-to-moment calcium homeostasis, PTH may compensate for its deficiency. Evaluation of PTH concentration in this model is being performed.

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Changes in Parathyroid Hormone and Phosphate Circadian Rhythms Following Growth Hormone Replacement in Adult Growth Hormone Deficient Patients. A. M. Ahmad,* J. Thomas, A. Clewes, M. Hopkins, R. Guzder, K. Imtiaz, H. Ibrahim, B. H. Durham, M. J. Diver, J. P. Vora, W. D. Fraser. Endocrinology & Clinical Chemistry, Royal Liverpool University Hospital, Liverpool, United Kingdom.

Adult growth hormone deficiency (AGHD) predisposes to osteoporosis. The underlying mechanism remains unclear. Parathyroid hormone (PTH) and phosphate circadian rhythms, well-established in healthy subjects, are altered in AGHD and postmenopausal women with osteoporosis, suggesting that dynamics of PTH secretion may be important for bone metabolism in these patients. Growth hormone replacement (GHR) increases bone mineral density (BMD) in AGHD that may be regulated by changes in PTH secretion. We studied the effects of GHR on PTH and phosphate circadian rhythms in AGHD patients. 14 AGHD patients (mean age[SEM]: 49.5[2.6]yrs), receiving appropriate pituitary hormone

replacement, except GHR, were recruited. Half-hourly blood samples were collected over 24-h, prior to and after 12 months on GHR. GH was commenced at 0.5 IU/d and titrated at 4 weekly intervals to achieve insulin-like growth factor I standard deviation score (IGF-I SDS) between median and the upper reference limit. PTH, phosphate, calcium and albumin were measured on all samples. Circadian rhythms were analysed using Cosinor analysis. Significant circadian rhythms for PTH and phosphate in all individuals ($p<0.001$), but not for adjusted calcium, were observed prior to and following GHR. PTH mesor decreased (4.6[0.4] vs 4.0[0.3] pmol/l, $p<0.05$) and phosphate mesor increased (1.08[0.02] vs 1.21[0.03] mmol/l, $p<0.01$) following GHR with an associated decrease in PTH amplitude ($p<0.01$) and increase in PO4 amplitude ($p=0.004$). 24-h mean adjusted calcium did not change significantly. No significant shift in PTH and phosphate acrophase was observed. PTH and phosphate circadian rhythms were maintained following GHR, but there were significant changes in PTH and phosphate mesor and amplitude in the absence of significant changes in serum calcium. GHR in AGHD may, therefore, have a regulatory role on PTH and phosphate secretion and changes in PTH secretory pattern may play an important role in bone metabolism in AGHD.

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Bone Evaluation in Adults With Cystic Fibrosis. C. Cormier,[1] I. Hadda,[1] J. Souberbielle,[2] J. Ruiz,*[1] C. Kindermans,[2] D. Hubert.[3] [1]Rheumatology A, APHP Cochin Hospital, Paris, France, [2]Physiology, APHP Necker Hospital, Paris, France, [3]Pneumology, APHP Cochin Hospital, Paris, France.

Bone Evaluation in Adults with Cystic FibrosisOsteoporosis is frequent in adults with chronic cystic fibrosis (CF). The pathogenesis of bone disease is not well understood. We measured bone mineral density (BMD) in 54 patients with dual energy x-ray absorptiometry (HOLOGIC QDR 4500) in lumbar spine, non dominant femoral neck and we measured 25 hydroxyxyvitamin D (25 OHD), osteocalcin (OC) in 31 patients and IGF1 in 21 patients.The mean age was: 29.3 + 8.6 years in 28 women and 26 men. CF began early in life (8.8 + 14 years), 83% of patients had exocrine pancreatic insufficiency. These cases of CF were very severe : 81% had chronic bronchial colonisation with pseudomonas, Forced Expiratory Volume (FEV) was 38.3 + 17.4% and BMI was 18.6 + 3 kg/m2. Only 11 patients used corticosteroïds and the mean dietary calcium intake in the whole population was moderate: 846 + 417mg/day. The mean T-score for the spine (-1.89 + 1.1) and for the total femur(-1.61 + 1.11) was lower in men ($p<0.01$ at spine,$p<0.001$ at hip). In the whole population, 28% were osteoporotic (18% in women, 38% in men), 49% were osteopenic and only 23% had normal spine BMD. 27% patients had fractures with lower T-scores than patients without fracture. The mean 25 OHD (in 31 patients) was 14.7 + 8.8ng/ml and 48% (n = 15) were 800 IU/d in 40% (n = 6) of the latter. There was osteoblastic depletion with low mean OC Z-score (-1.19 + 2.1) independently of corticosteroids (only in 16%) and gender. The mean IGF1Z-score was –0.52 + 1.51, lower in men (-1 + 1.3) than in women(-0.1 + 1.6). Eight patients had IGF1 Z-score <-1 (4 F, 4 M) and 3 <-2 (2F, 1M). Spine BMD correlated positively with FEV and IGF1. In conclusion, osteoporosis is common in CF and more severe in men. FEV and IGF1 Z-score are significant determinant of BMD.

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Reduction in Cortical Bone Mass and Enhancement in Trabecular Bone Mass in Klotho-mutant Mice Were Rescued by Adenovirus Mediated Transfer of Soluble Klotho Gene. K. Kashimada,[1] T. Yamashita,[1] T. Shiraki-Iida,*[2] N. Kawaguchi,[1] Y. Nabeshima,*[3] M. Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Imperial College, London, United Kingdom, [3]Kyoto University, Kyoto, Japan.

Klotho mutant mice exhibit multiple aging-related phenotypes including decrease in cortical bone mass in diaphyses and increase in trabecular bone mass in metaphyses. However, the function of klotho protein is not clear yet. There are two isoforms of klotho protein, membrane-bound form and soluble form. Membrane type is a full length isoform and composed of KL1 and KL2 domains and a single transmembrane domain. Soluble type contains only KL1 domain, and lacks the transmembrane domain. Because of this structure, soluble klotho has been suggested to exist. Klotho gene is expressed highly in kidney and brain, however not in bone where phenotypes could be seen suggesting the role of soluble form in bone metabolism. To investigate the role of soluble klotho gene in bone metabolism, we injected adenovirus vector carrying soluble klotho gene. Adenovirus containing either soluble klotho gene or LacZ gene was prepared by COS-TPC method and 5×108 pfu of virus were injected into the tail vein at 4-5 weeks old klotho mutant mice. The femoral bones were analyzed after ten or twenty weeks of injection. Morphological analysis of bone was performed by micro CT and trabecular bone volume in epiphysis and cortical bone thickness in diaphyses were evaluated. Ten weeks after the injection, trabecular bone volume in the femur of soluble klotho gene transfeced klotho mice was significantly decreased compared to LacZ adenovirus-injected mice($p<0.05$). Thus osteopetrotic change was improved by the adenovirus injection. In contrast to the male mice, female klotho mutant mice, soluble klotho gene did not show any difference in the cancellous bones compared to LacZ injected group. Such sexual difference was also observed in terms of cortical bone mass. In male klotho mutant mice, significant increase in the cortical bone volume and cortical bone thickness was observed by soluble form transfection ($p<0.05$). Twenty weeks after the injection, cortical bone thickness in male klotho mice was resumed to the levels similar to the wild type mice. However in female klotho mice, cortical bone volume, and cortical bone thickness were not altered by the klotho gene transfection indicating the gender dependent responses to exogenous soluble klotho gene expression. In conclusion, adenovirus mediated soluble klotho gene expression rescued bone phenotypes in male klotho mutant mice, while female klotho mutant mice were resistant to such soluble klotho gene transfection.

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Altered Relationships Between Mineral and Lean Masses in Obese, Eulgycemic, Hypernisulinemic Women. M. M. R. Ulla,[1] M. Stivala,*[2] F. Ghiglione,*[2] R. Noriega,*[2] G. Cointry,*[3] J. L. Ferretti.[3] [1]Endocrinology and Osteology, C.E.O.M., Córdoba, Argentina, [2]CEOM, Córdoba, Argentina, [3]CEMFoC, Rosario, Argentina.

The aim of this study was to describe and correlate the changes in fat, lean and mineral masses (FM, LM, BMC) in euglycemic women showing both, higher than normal basal serum insulin levels and the typical distribution of the abdominal fat.We measured the whole-body BMC, FM and LM (DEXA, Norland XR-36) and the serum glucose and insulin levels both in basal conditions and after a stimulation test by glucose 75 gr. administration in 24 women aged 19 to 60 years (pre and post-menopausal). The BMC was expressed either in crude form or statistically adjusted to a common, 18-kg FM (FA-BMC) according to the natural logarithmic association between those variables. Both the BMC/LM and the FA-BMC/LM ratios were calculated. For comparison purposes, patients were classified into 3 groups according to their basal insulin levels (I:26 µIU/ml).Positive correlations between basal serum insulin and body weight, fat mass or lean mass were observed. No correlation was found between the BMC and the basal serum insulin. The BMC or FA-BMC/LBM ratio decreased exponentially with the basal serum insulin level or the body weight. Correlations between the BMC or FA-BMC and the LBM were lineal for all groups I, II and III, and parallel to those shown by 400 age and sex-matched, normal controls. However, significant differences (ANCOVA, always $p<0.001$) were observed between the intercepts for the different groups, showing the decreasing order I> II>III. The intercept difference with respect to the control subjects was slightly positive significance of these results was unaffected by the pre or post-menopausal condition of the women. No associations were observed between any of the variables studied and the magnitude of the difference between the basal and glucose-stimulated serum insulin levels.Results show that, despite to show no apparent effect on serum glucose in these patients, the increased basal insulin activity enhanced their body weight and fat and lean masses. In addition, should the LM be proportional to the muscle mass, these patients should have a disproportionate enhancement of the muscle mass with respect to the relatively unaffected bone mass. If so, then the excess of insulin would have reduced the natural, biomechanical influence of muscles on the skeleton, proposed changing the setpoint of the biomechanical control of bone mass and structure according to the bone "mechanostat" theory (CTI 62:1, 1998).

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Attitude and Knowledge of Israeli Physicians Regarding the Diagnosis of Osteoporosis. I. Vered,[1] P. Werner.[2] [1]Endocrinology, Sheba Medical Center, Tel Hashomer, Tel Aviv University, Israel, [2]Gerontology, University of Haifa, Haifa, Israel.

Physicians' attitude, knowledge and practice regarding the diagnosis of osteoporosis were examined through a mail survey of 323 Israeli physicians. The survey was sent to a stratified sample of 1900 Israeli physicians based on their medical speciality and geographic location (overall response rate 19%). The structured questionnaire addressed attitudes and knowledge regarding the definition of osteoporosis, its prevalence, risk factors, diagnostic methods and perceptions regarding their cost-effectiveness. The majority of the respondents (89%) supported screening for osteoporosis in postmenopausal women, especially for high risk women. Overall, the participants had good knowledge about the definition of osteoporosis and its risk factors, but poor knowledge about its prevalence (<50% reported the correct prevalence). Female physicians had better knowledge and stronger attitudes than male physicians, 95.7% of female physicians supported massive screening vs. 87.3% of male physicians ($p<0.05$). Better knowledge of osteoporosis was associated with having more than 50% female patients, physician's female gender, younger age (<45 years) and less seniority (<15 years of practice). The majority of the participants reported being familiar with and using all types of clinical methods. They also rated these methods as highly cost-effective. The knowledge and use of clinical assessment was associated with physicians' female gender, family practice, working for an HMO, and having >50% female patients. Among the densitometry and imaging methods, the participants were familiar and used mainly the DXA and spinal X-ray. A clear association was found between the practice of the respondents and their perception of cost-effectiveness.In sum, although the responding physicians showed good knowledge of the topic, opportunities for improvement still exist, especially in the older, male and senior sector of the prfession.The use of diagnostic methods depends to a great extent on the knowledge and attitudes of the physicians. Documenting the level of knowledge and attitudes of physicians regarding osteoporosis may affect their practice and guide in the development of educational programs aimed at expanding the knowledge of the disease.

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Effect of Stress on the Bone Mineral Content of Rhesus Monkeys Susceptible and Resistant to Dietary Induced Obesity. B. Weekley,*[1] I. Rogers,*[1] J. Szumiloski,*[1] L. Van der Ploeg,*[2] D. MacNeil,*[2] P. Mathers,*[1] D. Pisacreta,*[1] H. Klein.*[1] [1]Merck and Co., Inc., West Point, PA, USA, [2]Merck and Co., Inc., Rahway, NJ, USA.

Obesity is known to protect against development of osteoporosis through poorly understood mechanisms. However, recent studies suggest that leptin inhibits bone formation possibly through a central mechanism . Furthermore, leptin may act as a stress mediator by suppressing the activity of the hypothalamic-pituitary-adrenal axis, an effect which may differ in individual animals depending on the degree of adiposity. Previous work in this laboratory has characterized a model of Dietary Induced Obesity (DIO) in the rhesus monkey. In that model it was apparent that some animals were susceptible to DIO and were termed Dietary Responders(DR) whereas some animals were resistant to the obesity producing effects of a high fat high calorie dietary supplement and were considered dietary non responders (DNR). This study was conducted to evaluate the effect of stressors on the physiologic and phenotypic characteristics of DR (n=6) and DNR (n=4). Dual Energy X-

ray absorpiometry (DEXA; GE Lunar Corp.) scans were conducted to assess changes in Bone Mineral Content (BMC) and body fat in response to chronic stress. Endocrine and clinical pathology parameters were also assessed to evaluate the response to stress. Stressors consisted of 14 weeks of chair restraint training (2-3 times per week), sham dose administration (2-3 times per week) followed by a surgical procedure to implant temperature telemetry transmitters.Stress induced changes include body weight (-5.9±1.1% in DR vs.-6.7±2.6% in DNR), per cent body fat (-7.5±1.6% in DR vs -6.4±2.5% in DNR), and Bone Mineral Content (0.43±0.09% in DR vs 0.14±0.54% in DNR).Endocrine changes in response to stress include leptin (-34.4±9.9% in DR vs -10.8±27.3% in DNR), insulin (-34.6±3.6% in DR vs 45.2±35.9% in DNR) and glucose (-13.7±5.5% in DR vs 6.6±6.4% in DNR). Correlation of changes in body weight and BMC in response to stress (r=-0.74 in DR vs r=-0.97 in DNR) and correlation of changes in leptin and BMC in response to stress (r=-0.01 in DR vs r=-0.97 in DNR) suggest that stress may differentially affect metabolic regulation of bone mineral content in DR and DNR. Such differences may serve as an animal model for the study of the mechanisms by which obesity and body weight contributes to regulation of bone mineral content.

Disclosures: Merck and Co., Inc.,3.

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Acute Hypoglycemia suppresses Parathyroid Hormone and Bone Turnover Markers: An Insulin Clamp Study. J. A. Clowes, R. T. Robinson,* A. Eagleton, S. R. Heller,* R. Eastell, A. Blumsohn. Clinical Sciences (North), University of Sheffield, Sheffield, United Kingdom.

Bone turnover markers (BTM) decreases acutely following both oral glucose and feeding. The mechanism for this is unclear. We examined the acute effect of a hyperinsulinemic euglycemic clamp (E) and stepwise hyperinsulinemic hypoglycemic clamp (H) on bone turnover and parathyroid hormone (PTH). Sixteen healthy male volunteers (mean age 22) attended on two occasions at 0800 after an overnight fast. They were randomised in a double blind crossover study to H or E clamp protocols. At each visit subjects received an intravenous insulin infusion at a constant rate (1.5 mUnits/kg/min). 20% dextrose was infused at a variable rate to maintain plasma glucose at 5 mmol/l (E clamp). For the hypoglycaemic clamp, glucose was lowered in a stepwise manner to reach 2.5 mmol/l (H clamp) at time 60 minutes, and maintained at that level until 105 minutes. Samples were collected at 0, 45, 75 and 105 minutes for measurement of PTH (Roche Elecsys), serum C-terminal telopeptide of type I collagen - beta CTX (βCrosslaps; Roche Elecsys; a marker of bone resorption), serum procollagen type I N-terminal propeptide - PINP and osteocalcin - OC (Roche Elecsys; markers of bone formation). Results were compared at 0 and 105 min using paired t tests. The H clamp resulted in decreases in βCTX by 37% (Figure), OC by 5%, PINP by 15% (all P<0.001) and PTH by 12% (P<0.05). E Clamp resulted only in increases in PINP by 4% and PTH by 23% (P<0.01). There was no change in plasma insulin between 0 and 105 minutes during either the H or E clamps (P>0.05). We conclude that hyperinsulinemic hypoglycemia is associated with acute suppression of bone turnover. This may be related to acute changes in PTH, hypoglycemia or a hormone released in response to hypoglycemia. Hyperinsulinemia itself does not appear to be the driving mechanism.

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Effects of GH on the Mineral, Lean, and Fat Masses in Pan-Hypopituitary Men and Women. H. C. Hermberg,*[1] H. Fideleff,*[1] A. Chervin,*[1] G. Stalldecker,*[1] I. Sinay,*[1] P. Sobrado,*[1] G. R. Cointry,*[2] J. L. Ferretti.[2] [1]KIMS Group, Hospital Alemán, Buenos Aires, Argentina, [2]Centro de Estudios de Metabolismo Fosfocálcico (CEMFoC), Facultad de Medicina, Univ. Nacional de Rosario, Rosario, Argentina.

The GH effects on the muscle/bone relationships have been scarcely investigated. This study analyzes the relationships between mineral, lean, and fat masses (BMC, LM, FM) in adult patients with growth hormone deficiency (AGHD) (age 23-60 years, 14 men and 15 females), both before and after treatment with replacement doses of GH during 12 to 18 months. Whole-body measurements were made by DEXA and compared with data from 600 age-matched, normal men and post-menopausal women. The BMC data were analyzed both in crude form and statistically adjusted to a common, 18-kg FM (FA-BMC) according to the natural, logarithmic association involved [Bone 22:683,1998].Concerning the whole-body measurements, the slopes of the correlations between BMC or FA-BMC (y) and LM (x) were similar to those shown by controls in men and women, both before and after treatment. The intercepts were similar to those of controls for the crude BMC in both pre- and post-treated men, and lower than that in all other instances. Treatment enhanced all, BMC or FA-BMC and LM correlatively in men and women, but failed to improve the intercepts of any of the impaired correlations between BMC and LM. However, the slopes of the significant, linear correlations between the changes induced in BMC or FA-BMC (y) and in LM (x) were slightly higher for men than women. The number of patients was insufficient to detect any effect of HRT on those differences. Assuming a direct proportionality between LM and muscle masses, results suggest that the affected men tended to maintain the normal muscle-bone relationships but had a disproportionately high FM, while women seemed to show a low bone mass independently of the FM. Nevertheless, the anthropometrical correlation between BMC and LM remained positive and parallel to normal in all patients, both before and after treatment, and was also respected by the treatment effects on both kinds of variables. Therefore, GH treatment would have been generally useful to enhance both bone and muscle masses following the physiological proportions, but either unable or much less effective than that to improve the bone/muscle proportion. The slightly more positive effects shown by men, if confirmed in further studies, would indicate 1. a more effective stimulation of bones by the men's more massive muscles, and/or 2. a gender-related effect of GH perhaps in connection with the particular, negative influence of the lack of estrogen in the affected women.

SU394

Body Weight, Calcium Intake, and the Acute PTH Response to an Oral Calcium Load. J. Krakauer,[1] M. Breitenbach,*[1] B. Mikulec,*[1] D. Ferry,*[1] R. Karcher,*[1] J. Levine,*[2] M. Kleerekoper.[3] [1]Beaumont Hospital, Royal Oak, MI, USA, [2]Mayo Clinic, Rochester, MN, USA, [3]Wayne State University School of Medicine, Detroit, MI, USA.

To further evaluate a reported association between body weight and calcium intake (JCEM 85:4635-8; 2000) we studied the effects of an oral calcium load on serum PTH in 19 postmenopausal women. Body mass was measured at baseline and 1 year later and the one-year change in BMC was measured by DEXA. Calcium citrate 600 mg was given orally at the one-year follow-up and PTH measured at 0, 30, 60, and 120 minutes.

The Table shows results of either bivariate correlations or linear regression models:

Dependent Variable		Variables in model	Significance P =
Δ Body weight	Adjusted R^2 = 0.23	(-)$PTHs_{120"}$, [(Ca x PO_4)/Cr] $s_{30"}$	.04
Δ Lumbar Spine BMC	Adjusted R^2 = 0.45	Δ Body weight, Nondairy calcium intake	.003
Δ Femoral Neck BMC	r = 0.47	$PTHs_{30"}$	.04
Lumbar Spine BMC	Adjusted R^2 = 0.32	$PTHs_{120"}$, Leptin*	.02
Femoral Trochanter BMC	Adjusted R^2 = 0.44	Weight, (-)$PTHs_{30"}$,Leptin*	.008
Radius 33% BMC	Adjusted R^2 = 0.45	(-)PTH_0, (-)$PTHs_{30"}$, (-) $PTHAC_{60-120"}$	.007
Ultradistal radius BMC	r = -0.50	$PTHs_{30"}$	.03

* Mean serum leptin (4 samples per subject) , PTHsx", is the fractional change in PTH at time x" relative to time 0 (PTH_0). $PTHAC_{60-120}$ is the area under the PTH curve from 60" to 120".

One-year increase in body weight was associated with the less suppression of PTH at 120" and with greater suppression of the Ca x PO_4 filtered load at 30" (adjusted r^2 0.23, p=0.04). An increase in body weight and in non-dairy calcium intake was associated with an increase in spine BMC (adjusted r^2 0.45, p=0.003). There was no association between change in body weight and change in BMC at the hip or radius. The increase in femoral neck BMD was associated with increased PTH suppression at 30 minutes while the reverse was observed at the femoral trochanter and the radius (proximal and distal sites). There was no association between changes in lumbar spine BMC and PTH suppression by calcium citrate. These data suggest a complex relationship between the PTH axis, changes in body weight, and changes in BMC.

SU395

Risedronate (RIS) Improves Vertebral Bone Architecture and Strength in Ovariectomized Minipigs, as Measured by 3-Dimensional Micro-Computed Tomography. T. E. Dufresne,* P. A. Chmielewski,* B. Borah,* G. J. Gross,* M. C. Prenger,* R. Phipps. Procter & Gamble Pharmaceuticals, Mason, OH, USA.

The role of changes in trabecular architecture to loss of bone biomechanical strength in osteoporosis and the effects on bone architecture of osteoporosis therapies are currently of great interest. The effects of RIS on trabecular architecture in the vertebra of calcium-restricted ovariectomized minipigs, a model of postmenopausal osteoporosis, were investigated using 3-dimensional micro-computed tomography (3D μCT). Eighteen-month-old Sinclair S-1 minipigs (n=11/group) were ovariectomized (OVX) and treated daily with either vehicle or RIS (0.5 mg/kg or 2.5 mg/kg) for 18 month. The L4 vertebral cores were imaged using a Scanco Medical scanner at 35 micron isotropic voxel resolution. For the highest Risedronate dose group (2.5 mg/kg), there was a 76% decrease in activation frequency compared to the vehicle group. Several standard and new architectural parameters were significantly altered (p<0.05) in the high-dose RIS group compared with vehicle-treated animals. Bone Volume/Tissue Volume (BV/TV), Trabecular Thickness (Tb.Th.), Trabecular Number (Tb.N), and connectivity density increased significantly, while marrow star volume and trabecular separation decreased significantly in the treatment group. A more uniform variation of marrow space thickness, measured as the standard deviation of trabecular separation (Tb.Sp-SD), was observed in the RIS group. Treatment also resulted in significant decreases in the % Bone in the load direction and in the degree of anisotropy, indicating that there was a significant preservation of trabeculae in the plane orthogonal to the load direction. Both maximum stress and apparent modulus were significantly increased in the high dose RIS group compared to the vehicle group. Multiple linear regression of the maximum stress with architectural parameters revealed that BV/TV% predicted 76% of the maximum stress, while Tb.Th. and TB.Sp-SD together predicted 84%, and the combination of BV/TV%, Tb.Sp-SD, and Tb.N predicted 91% of the maximum stress. When compared with BV/TV alone, the addition of architectural parameters to the model added substantially to the predictive ability with respect to strength. The 3D mCT data show that RIS improves trabecular architecture in the minipig vertebra. This architectural improvement contributed to stronger bone, consistent with the significant fracture prevention benefits observed with RIS in clinical trials.

SU396

Alendronate 70 mg Once Weekly and Alendronate 10 mg Once Daily Preference Study in Postmenopausal Women with Osteoporosis. J. Palmisano,*[1] E. M. Lewiecki,[2] C. Rosen,[3] R. S. Bockman,[4] L. K. Vanaman,*[1] M. Smith,*[1] L. Wang,*[1] J. Yates.[1] [1]Merck & Co., Inc., West Point, PA, USA, [2]New Mexico Clinical Research & Osteoporosis Center, Albuquerque, NM, USA, [3]Maine Center for Osteoporosis, Bangor, ME, USA, [4]Hospital for Special Surgery, New York, NY, USA.

Objectives: Primary: To examine patient preference of Once Weekly (OW) 70 mg alendronate** (ALN) to Once Daily (OD) 10 mg alendronate**. Secondary: To examine which treatment regimen, OW ALN or OD ALN is considered more convenient and would provide the greatest overall compliance. Design: This open-label, crossover study is being conducted at 40 U.S. sites with 287 women. Patients were randomized (1:1) to receive either ALN 70 mg OW or ALN 10 mg OD for 4 weeks and then crossover to the opposite treatment for 4 weeks after a 1-week washout period. Preference, convenience, and compliance will be assessed by a patient administered Questionnaire. Results: Analysis of the Questionnaire completed by 273 women to evaluate preference, convenience and compliance for Once Weekly ALN and Once Daily ALN therapy will be available Summer of 2001. Conclusions: This is the first study to provide a preference comparison between Once Weekly 70 mg ALN and Once Daily 10 mg ALN.**Trademark: Fosamax®, Merck & Co., Inc., West Point, PA.

SU397

Risedronate Significantly Reduces the Risk of Non-Vertebral Fractures in Postmenopausal Osteoporotic Women in Just One Year. M. Bolognese,[1] I. Fogelman,[2] P. Guesens,*[3] D. A. Hanley,[4] I. Barton,*[5] P. Bettica.[5] [1]Bone Health Center, Bethesda, MD, USA, [2]Guy's Hospital, London, United Kingdom, [3]University of Maastricht, Maastricht, The Netherlands, [4]University of Calgary, Calgary, Canada, [5]Procter & Gamble Pharmaceuticals, Staines, United Kingdom.

To evaluate the one-year treatment effect of risedronate on reducing non-vertebral fractures, 4 clinical studies conducted in Europe and North America were analyzed (n=4845). In 2 of these (BMD-NA and BMD-MN), ranging in length from 18 to 24 months, women ≤ 80 years old, were enrolled based on low lumbar spine BMD (T-score < -2.0). In the other two (VERT-NA and VERT-MN), with a study duration of 3 years, women £ 85 years old and at least 5 years postmenopausal were enrolled based on low lumbar spine BMD (T-score ≤ -2) and one prevalent vertebral fracture (VERT-NA) or two or more prevalent vertebral fractures (VERT-NA and VERT-MN). All patients received either placebo or risedronate 2.5 or 5 mg daily. Patients also received a calcium supplement (1 g/d). Vitamin D supplements were provided (500 IU) if baseline serum 25-OH Vitamin D levels were low. Because of the known relationship between low BMD and fracture prevention benefit of anti-resoprtiveagents, we analyzed data for subjects with a lumbar spine T-score of < -2.5 (W.H.O. panel definition of osteoporosis). Non-vertebral fractures were collected as adverse events. Kaplan-Meier estimates were used to derive the percent of patients who had an incident non-vertebral fracture. Cox regression analysis was performed to test differences in treatment groups. In women, with a lumbar spine BMD T-score < -2.5, risedronate was associated with significant 56% (p=0.002) non-vertebral fracture risk reduction in 1-year when all studies were pooled. In the BMD-NA and BMD-MN studies, the one-year risk reduction was 71% (p=0.01). In conclusion, in postmenopausal women with low baseline lumbar spine BMD, risedronate significantly reduced non-vertebral fracture risk up to 71% in just 1 year. These data are consistent with the previously documented prevention of vertebral fractures in the first year of treatment and point to a rapid onset of risedronate's skeletal benefits in osteoporosis.

SU398

Effectiveness of Intravenous Pamidronate in the Treatment of Osteoporosis. H. Cohen,* C. Rhys-Dillon,* W. D. Evans, J. C. Martin,* J. Morgan,* K. T. Rajan. Royal Glamorgan Hospital, Llantrisant, United Kingdom.

The use of bisphosphonates has become established for the treatment of osteoporsosis. In the present study, intravenous pamidronate was used in patients who were unable to tolerate oral bisphosphonates.Intravenous pamidronate was administered to 49 patients, of whom 41 were women and 9 were men. For both sexes, mean (SEM) age was 67 (1.5) years. Of the women, 37 were postmenopausal and 4 premenopausal. Four patients had peptic ulcer, 4 carcinoma of the breast, 6 deep vein thrombosis, 2 inflammatory bowel disease, 15 asthma, 8 rheumatoid arthritis and 1 polymyalgia rheumatica. Eleven subjects were taking oral steroids 6 (1) mg daily for a period of 8.8 (2.8) years. Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (Hologic QDR 4500 Acclaim Elite). Baseline BMD T-score was -3.78 (0.20) in the lumbar spine and -3.64 (0.18) in the femoral neck.Of the total, 42 patients have been treated for at least 6 months and 23 for 12 months. At 6 and 12 months respectively, lumbar spine BMD increased by 3.7 (0.8)% ($p<0.0001$ vs baseline) and 2.5 (1.5)% ($p<0.1$ vs baseline). The corresponding values for the femoral neck were 2.1 (2.1)% and 0.3 (2.6)% (p NS in both cases). There were tolerable side-effects in 16 cases: flu-like symptoms (10), headache (3), nausea (2) and bone pain (1).Intravenous pamidronate is well-tolerated in this group of patients. At 6 and 12 months, this treatment produces significant BMD increases in the lumbar spine but not in the femoral neck.

SU399

Efficacy of Once a Week Risedronate in Comparison with Once-Weekly Alendronate Therapy for Postmenopausal Osteoporosis: One-year Data

S. J. Wimalawansa. Medicine, Endocrinology, Robert Wood Johnson Medical School, New Brunswicck, NJ, USA.

The efficacy of bisphosphonate therapy in prevention and treatment of postmenopausal osteoporosis is now well established. However, the compliance with oral bisphosphonates is often inadequate due to the difficulty in the routine of taking these agents and the associated upper gastrointestinal adverse effects. The aim of this study was to evaluate the efficacy of once-weekly administered risedronate (i.e, infrequent dosing of 30 mg once a week) on BMD in comparison with a group of patents who had once-weekly administered alendronate (60 mg, 6 x 10 mg) on bone mineral density (BMD) and biomarkers over a one year period. The secondary aims were to monitor the differences in adverse events and the compliance between the two medications. Risedronate was administered once a week, and 46 patients who completed one-year follow up were included in the analysis. These data were compared with an age-matched comparable group of patients who were treated with once-weekly administered alendronate. Patients in both groups had BMD <2.5 SD in the lumbar spine. BMD was measured by DXA in the lumbar spine and in the total hip at the beginning, and after one year. Biochemical markers of bone turnover; osteocalcin and N-telopeptides were also measured in both treatment groups. BMD and biomarker data were compared between the two treatment groups. Results: BMD increased significantly in both groups. The increments of BMD were similar in both groups of patients; 5.2% vs. 5.0% in the lumbar spine, and 3.8% vs. 3.5% in the total hip in risedronate and alendronate groups, respectively. Patients on both regimens had significant and equal suppression of biochemical markers. Risedronate and alendronate therapies suppressed serum osteocalcin by 28% and 27%, and urinary N-telopeptides by 39% and 41%, respectively. Patient compliance (measured by taking medications for 42 weeks or more during the year; i.e., 80% adherence to therapy) was 89% in the risedronate-treated group and 87% in the alendronate-treated group. There were no statistical differences in BMD or biomarkers. The incidences of adverse event profiles or adherence to therapy were not different between the groups. Conclusion: Once-weekly administered risedronate (30 mg) and alendronate (60 mg) are highly effective and equally in enhancing BMD and suppressing the markers of bone turnover. Both drugs were well tolerated with high rate of adherence to therapy. This is the first study to compare the effects of infrequent administration (i.e., once a week therapy) of the two FDA approved oral bisphosphonates (i.e., alendronate and risedronate) in patients with established postmenopausal osteoporosis.

Disclosures: Proctor & Gamble Pharmaceuticles,5.

SU400

Intermittent, Intravenous Pamidronate Therapy: Highly Effective Treatment for Postmenopausal Osteoporosis. S. J. Wimalawansa. Medicine, Endocrinology & Metabolism, Robert Wood Johnson Medical School, New Brunswicck, NJ, USA.

It is now well established that oral bisphosphonate therapy is highly effective in prevention and treatment of osteoporosis. However, adherence to therapy is poor due to the inconvenience of taking this medications and associated upper gastrointestinal adverse effects. Both these could be overcome by administering bisphosphonate agent intravenously, on intermittent basis. A total of 580 postmenopausal women with lumbar spine bone mineral density (BMD) <2.5 SD (mean BMD 3.6 S.D) were treated with intravenously administered pamidronate once in 6-month, over a period of 6 years (average follow up of 4.6 years). Over 90% of these patients were unable to tolerate oral bisphosphonate. Two separate infusions of pamidronate, 90 mg of each were administered a week apart. In all patients, pamidronate was given in one liter of normal saline administered over 5-hour period in the outpatient setting. Repeat administration of pamidronate was done on 6-monthly basis for the duration of the study (i.e, single infusion of 90 mg, twice a year for 4 years). Four-year treatment data was compared with a comparable group of patients who received once daily alendronate for a 4-years (n=91). BMD was measured by DXA in the lumbar spine and in the hip at the beginning, and then annually using the same DXA scanner. Serum osteocalcin and N-telopeptides were also measured at the beginning, and then annually. Changes of BMD and biochemical markers were similar in pamidronate and alendronate-treated groups over the four-year follow-up period. Although biomarkers remain suppressed to a similar degree in both groups, the BMD responses in the long run were higher in the pamidronate treated group: 13.5 ± 0.1 vs. 8.7 ± 0.2 in the lumbar vertebrae ($p<0.01$), and 9.7 ± 0.1 vs. 6.5 ± 0.3 in the hip ($p<0.01$). Unlike with the oral therapy, once the drug is administered intravenously in the office setting, there is no compliance problem with the medication. It is likely that if the compliance is optimal, alendronate-treated patients also may have achieved a further increment of BMD, in comparable to that observed with pamidronate-treated patients. Yearly basis, the cost of these two medication regimens was comparable. Furthermore, since intravenous therapy bypasses the stomach and esophagus, there are no associated upper gastrointestinal problems, and so the associated medical costs. Therefore, taken together, intravenous pamidronate therapy is a practical and highly effective way of administering a bisphosphonate to improve BMD in patients with postmenopausal osteoporosis. This is particularly true for patients who can not adhere or to tolerate oral bisphosphonates.

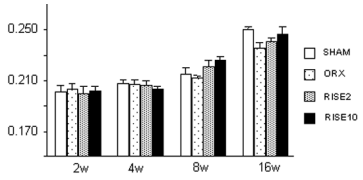
Disclosures: Proctor & Gamble Pharmaceuticles,5.

SU401

Preventive Effects of a Bisphosphonate (Risedronate) on Mandibular Bone Loss in an Animal Model of Male Osteoporosis (the Orchidectomized Rat). E. Lerouxel,* H. Libouban,* M. F. Moreau,* M. F. Basle,* M. Audran, D. Chappard.* LHEA, Fac Medicine, Angers, France.

In man, hypogonadism is associated with osteoporosis and can be appreciated by dual

energy X-ray absorptiometry (DXA) on axial and peripheral skeletal pieces. However, there are some controversies about the relationships between bone loss in these sites and at the mandible. Osteoporosis has been suggested as a risk factor for implant failure, alveolar bone loss and tooth loss, but data supporting this hypothesis are limited.The effects of ORX on bone mass and the presumed preventive activity of an antiresorptive compound (risedronate) were studied in the orchidectomized (ORX) rat using DXA. We have studied four groups of rats (6 animals per group) : sham-operated control rats (SHAM), ORX control rats (ORX), ORX treated with risedronate 2 or 10 µg/kg/day (RISE2 and RISE10). Risedronate was injected subcutaneously 5 days per week. Rats were sacrified at 2, 4, 8 or 16 weeks post-ORX. DXA was performed on an Hologic QDR 4500 (with small animal software) on each left hemimandible. BMC measurements on the whole hemimandible had a high CV (~ 4%) due to the very low density of the ramus mandibulae. Analysis was also restricted on a rectangular customized region of interest centred on the molar region (R1), which included the alveolar bone, the 3 molars and the horizontal part of the incisor. In this ROI, the CV was ~ 2%.A significant bone loss became evident at 16 weeks in the ORX group ($p<0.02$ versus SHAM). At 16 weeks, BMC-R1 in the RISE2 and RISE10 groups was increased versus the ORX group, this increase being more important in the RISE10 group than in the RISE2 group (respectively 4.5% and 2%). In the treated groups, BMC-R1 was not significantly different from SHAM animals but did not reached significance versus ORX. The mandibular area, that does not grow by an endochondral process, represents a most suitable region for the study of bone loss due to sex hormone deprivation. Risedronate appears to be a preventive treatment for orchidectomy-induced bone loss.



SU402

Efficiency of Intravenous Bisphosphonate Therapy in Patients with Osteoporosis. J. Steindorf,* B. Relke,* I. Boesel,* H. J. Heberling.* City Hospital Leipzig, Leipzig, Germany.

The medical therapy of osteoporosis with oral bisphosphonates represents an established procedure. Recently, various prospective studies have described a successful treatment of osteoporosis using this medication. However, oral bisphosphonates have limitations in a part of patients with side effects or preexisting diseases concerning gastrointestinal tract. The intravenous application of bisphosphonates appear to be an alternative therapeutic tool for this subgroup of patients. In the present study, we assessed the effect of intravenous bisphosphonates in relation to the bone mineral density (BMD).34 patients (29 women, 5 men, age 63 +- 9 years) with different causes of osteoporosis were analyzed, retrospectively. These patients were treated with 30 mg pamidronate (Aredia) or 2 mg ibandronate (Bondronat) intravenously every 3 months. The BMD were determined at the lumbar spine and at the neck of the femur before treatment and following one year using the DXA-measurement.We have detected a significant increase of the BMD at the lumbar spine by 5.2% ($p<0.02$). In 20 patients (62.5%) we found an increase of BMD at this point by more than 2%. Four patients (12.5%) have shown an impairement of the BMD. That might be associated with the malabsorption syndrom in all of these cases. The BMD at the neck of the femur was not related to any significant differences as compared before and after one year bisphosphonate infusion in our patients group. Additionally, we found no differences in the outcome concerning the age of the patients, the cause of osteoporosis or the kind of bisphosphonate.Our data are comparable with the results of recent studies using oral bisphosphonates or the selective estrogen receptor modulator raloxifene in the treatment of osteoporosis. Thus, the intravenous application of bisphosphonates appear to be an acceptable alternative method for a subgroup of patients with gastrointestinal suffering.

SU403

Alendronate 70 mg Once Weekly vs. Placebo: Tolerability Study in Patients with Osteoporosis. S. L. Greenspan,[1] E. M. Field-Munves,*[2] R. P. Tonino,*[3] B. Mako,*[4] M. E. Smith,*[4] J. Yates,*[4] J. Palmisano.*[4] [1]Osteoporosis Prevention & Treatment Center, Pittsburgh, PA, USA, [2]Springhouse Professional Center, Allentown, PA, USA, [3]Good Health, Burlington, VT, USA, [4]Merck & Co., Inc., West Point, PA, USA.

In order to examine the percentage of patients reporting upper gastrointestinal (UGI) adverse experiences (AE) while on alendronate** (ALN) 70mg once weekly as compared to placebo (PBO), we enrolled 450 men and women with osteoporosis from 48 centers in the USA in a double-blind, randomized, PBO-controlled trial for 12 weeks. Patients were randomized to ALN 70 mg once weekly or ALN PBO once weekly in a 1:1 ratio. We also examined the percentage of patients that discontinued due to UGI AEs, changes in a marker of bone resorption (urinary N-telopeptide), and overall safety and tolerability.Of the 450 enrolled patients, 422 (94%) completed the study and 28 (5%) patients discontinued for any reason. The blind has not been broken so additional results will not be available until summer 2001. We conclude that this will be the first clinical trial to provide a comparison of the overall safety, tolerability and change in resorption of ALN 70 mg once weekly vs PBO.**Trademark: Fosamax®, Merck & Co., Inc., West Point, PA

Disclosures: Susan Greenspan, MD,2,8; Richard Tonino, MD,2,5,8.

SU404

Lack of Gastric Adaptation to Weekly Versus Daily Alendronate Administration in Rats. J. L. Wallace,*[1] W. McKnight,*[1] M. Dicay,*[1] M. A. Blank.*[2] [1]University of Calgary, Calgary, Canada, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Aminobisphosphonates, such as alendronate, have well characterized irritant effects in the upper GI tract. In animal studies, acute administration of alendronate exacerbated gastric damage induced by indomethacin and, when given daily, delayed healing of pre-existing gastric ulcers. It has been proposed that administration of alendronate on a weekly, rather than daily basis, may have a lower potential for inducing upper GI irritation. However, daily administration of other gastric irritants (to animals or humans) leads to a progressive increase in gastric resistance to injury (gastric adaptation). In the present study, we examined gastric injury induced by 21 days of daily (20 mg) versus weekly (140 mg) alendronate administration to rats. The drugs were given orally in a vehicle of saline. Subgroups rats were euthanized at the end of 1, 2 or 3 wk of treatment for blind assessment of the extent of hemorrhagic gastric damage (corpus and antrum). In rats given alendronate daily, the extent of damage to the stomach was greatest at the end of wk 1 (mean damage area of 3.5 ± 1.4 mm^2; $p<0.05$ vs. 0.3 ± 0.3 mm^2 in vehicle group), and by wk 3 had decreased to 1.7 ± 0.9 (not significantly different vs. vehicle). In contrast, no such adaptation of the stomach was observed in rats given alendronate once per week (mean damage area of 3.6 ± 1.1 at wk 1 and 3.7 ± 1.2 at wk 3). Antral damage is often considered to be the most clinically significant. A small amount of antral damage was observed at wk 1 in the daily alendronate group (25% incidence; mean ulcer area of 0.3 ± 0.3 mm^2), but no antral damage was observed at wk 2 or 3 in this group. In contrast, the incidence of antral damage did not change with time in the group treated weekly with alendronate (25-30%), but the severity of damage increased considerably (mean ulcer size of 3.0 ± 1.0 mm^2 at wk 1 and 5.6 ± 1.2 mm^2 at wk 3; $p<0.05$ vs. vehicle). Daily alendronate administration results in an adaptive response manifest by reduced gastric (particularly antral) damage with time. In contrast, gastric adaptation does not occur with weekly administration of alendronate, with antral damage becoming progressively more severe.

SU405

Effectiveness of Bisphosphonates Is Related to Compliance and Adherence. H. Schober,*[1] A. Döring,*[2] R. Andresen,*[3] G. Kundt,*[4] H. Schmidt-Gayck.[5] [1]Internal Medicine, Community Hospital, Wolgast, Germany, [2]Community Hospital, Wolgast, Germany, [3]Municipal Hospital, Güstrow, Germany, [4]Biostatistics Univ., Rostock, Germany, [5]Dept. Science, Lab.Soc., Heidelberg, Germany.

Bisphosphonates (BP's) are an effective antiresorptive drug treatment in osteoporotic patients. Well defined study groups allow an outcome prediction. However, in clinical practice with unselected patients, under less strict control, the effect has yet to be evaluated. The effectiveness of the BP's may be influenced by compliance and adherence. I.V. treatment with BP's ensures a defined dosage. The objective of this observational study was to compare the effect of 4 different BP's (oral and I.V.) on BMD and bone turnover changes in unselected patients.Between 1996 and 1999 161 female patients underwent spinal QCT and were thus diagnosed suffering osteoporosis according to the WHO-criteria. All patients were treated with BP's, they were divided in four groups: 2 groups with oral BP treatment (group I: Etidronate (ETD); 14 days 400mg/d, 76 days 500mg Calcium/d), (group II: Alendronate (ALN); 10mg/d) and 2 groups with I.V. BP-treatment (group III: Pamidronate (APD); 30 mg every 3 months) and group IV: Ibandronate (IBD); 2 mg every 3 months). The I.V. treatment was chosen in patients with severe comorbidity and/ or on multiple drug therapy (>3 drugs). All patients received Vitamin D 3 and 500 mg Calcium/ d. There were no significant differences between the four groups concerning estrogen exposure , estrogen use and number of pregnancies. All participants underwent QCT at baseline and after one and two years. Urinary crosslinks were collected at noon at baseline after 3 and 12 months.Characteristics of the patients: Group I: n: 39, age: 63,3 y, BMD: 73,2 mg/ml, Comorbidity: 36%; Group II: n: 21, age: 58,9 y, BMD: 75,2 mg/ml, Comorbidity: 20%; Group III: n: 61, age: 66,0 y, BMD: 68,3 mg/ml, Comorbidity: 59%; Group IV: n: 40, age: 65,7 y, BMD: 64,7 mg/ml, Comorbidity: 52%. Bone turnover was significantly decreased after 3 and 12 months in group III and IV using I.V. BP'S: APD ($p<0,01$) and IBD ($p<0,07$). In the orally treated patients no significant changes in bone turnover could be observed, even though the BMD increased in group I (ETD) and decreased in group II (ALN).

BMD changes

	BMD (mg/ ml)baseline	BMD (mg/ml) after one year	BMD (mg/ml) after two years
Group I	73,2	81,0	90,2
Group II	75,2	73,3	59,8
Group III	68,3	67,3	69,1
Group IV	64,7	58,7	63,7

though the I.V. treatments with BP's decreased the bone turnover significantly, no increase in BMD was seen, but the BMD was stabilized. The difference between the orally treated patients- BMD increase in group I and BMD decrease in group II may be to due compliance and adherence problems

SU406

Tri-Monthly Intravenous Injections of Ibandronate (2mg) for Treatment of Postmenopausal Osteoporosis. M. Pecherstorfer,[1] N. Loho,[1] S. Mirzai,[2] K. Brenner,[1] N. Hoyle,*[3] D. Felsenberg.[4] [1]Department of Medicine and Medical

Oncology, Wilhelminenspital, Vienna, Austria, [2]Department of Nuclear Medicine, Wilhelminenspital, Vienna, Austria, [3]Roche Diagnostica, Penzberg, Germany, [4]Freie Universität Berlin, Berlin, Germany.

The aim of the study was to evaluate the efficacy of treating postmenopausal osteoporosis with i.v. ibandronate administered trimonthly. Twenty females (median age 71.5 a, median time since last menstruation 25 a) with primary osteoporosis according to WHO criteria were included in the study. Nine patients had prevalent vertebral fractures. None of the 20 patients had been treated with bisphosphonates, calcitonin, fluorides or hormones within 3 months prior to study entry. All patients took calcium and vitamin D supplements during the study. Every three months the patients received an i.v. injection of 2 mg ibandronate dissolved in 20 ml of saline solution. Bone mineral density (BMD) was determined not more than 2 weeks before the first injection and after 12 and 24 months by dual energy x-ray absorptio-metry (Hologic 2000). We measured BMD only in vertebral bodies that appeared normal on radiographs obtained within 1 month prior to measurement of BMD. Serum indicators of bone resorption (Elecsys ß-CrossLaps/serum) and of bone formation (Elecsys P1NP, pre-launch assay) were measured every 6 months. After two years of treatment, BMD increased in the lumbar spine by 5.5% (Friedman test p= 0.007), in the trochanter maior by 5.4% (p= 0.013), and in the total hip by 3.4% (p= 0.008). There were no significant changes in BMD in the femoral neck, in the regio intertrochanterica or in Ward´s triangle. Serum levels of CrossLaps and P1NP decreased significantly (p= 0.014 and p= 0.010, respectively). During the observation period, one of the patients suffered a new impression fracture of vertebral body L4. The most frequent adverse event was transient generalized muscle pain experienced by 7 patients following the first injection of ibandronate. Fever and/or impairment of renal function did not occur in any of the patients. In conclusion, after 2 years of treatment, the increase in BMD demonstrates that interval therapy with 2 mg ibandronate injected intravenously every 3 months is effective in inhibiting bone loss due to osteoporosis. To improve antiresorptive efficacy, shorter treatment intervals or higher doses might be considered.

SU407

Can Alendronate Be Taken Before Lunch or Dinner? A Randomized Trial in Osteoporotic Women. P. D. Delmas,[1] E. Confavreux,*[1] P. Garnero,*[2] G. Genolet,*[1] B. Gibelin,*[3] J. Yates.*[4] [1]Inserm Research Unit 403 and Claude Bernard University, Lyon, France, [2]Synarc, Lyon, France, [3]M.S.D., Paris, France, [4]MRL, Rahway, USA.

The current mode of administration of alendronate (FOSAMAX) is experienced as constraining by some patients. In order to test the efficacy of a more flexible dosing schedule, we randomized 139 postmenopausal women (67 ± 5 yr. old) with osteoporosis (mean spine T score -2.8) to 3 dosing regimens of alendronate, 10 mg/day, in an open label design : ½ hour before breakfast, 1 hour before lunch, or 1 hour before dinner. All patients had been fasting for at least 4 hr before taking alendronate. The following markers were measured at baseline, 3, 6 and 12 months: serum osteocalcin (OC) (Elsa OsteoTM, Cis), serum bone alkaline phosphatase (BAP AlkphaseTM, Metra), serum and urinary type I collagen C telopeptide (CTX, crosslapsTM, Osteometer). The decrease in bone turnover at each time point was highly significant (p< 0.001 for all markers in each group) and did not differ significantly (ANOVA) between the 3 dosing groups at 6 months (Table).

		Median% decrease (IQ) at 6 months of			
Patients groups	**n**	**OC**	**BAP**	**sCTX**	**uCTX**
1- Before breakfast	47	-39.9 (21)	-38.3 (20)	-71.2 (28)	-86.5 (25)
2- Before lunch	39	-38 (20)	-42 (27)	-74.4 (21)	-82 (18)
3- Before dinner	39	- 36.6 (17)	-33.5 (25)	-60 (40)	-72.2 (29)

The percentage of responders was not different between groups. For example, the % of patients of groups 1,2 and 3 who showed a superio equal 45% decrease of urinary CTX at 12 months was 90%, 95% and 89% respectively. The mean change (and % of patients with an increase) of spine BMD at 1 year in groups 1, 2, and 3 were at 4.95% (89), +5.54% (100) and +5.9% (100) respectively. At the total hip, the BMD changes were +2.87%, +3.49% and +2.98%, with 96%, 100% and 97% of responders respectively. In summary, our study suggests that alendronate 10 mg daily, given either 1 hour before lunch or dinner in patients fasting for 4 hours, has the same efficacy in decreasing bone turnover and increasing BMD as alendronate given fasting ½ hour before breakfast. We conclude that a more flexible dosing can be offered to osteoporotic patients on alendronate therapy

Disclosures: MSD,2.

SU408

Concentration of Gonadotrophin and Testosterone in Men with Osteoporosis Following Bisphosphoniates Treatment. J. Glogowska-Szelag,*[1] M. Nowak,*[1] B. Kos-Kudla,*[1] M. Dabrowska,*[2] Z. Brodzinski,*[2] J. Szelag.*[2] [1]Department of Pathophysiology and Endocrinology, Silesian Medical University, Zabrze, Poland, [2]Silesian Medical Centre of Osteoporosis, Katowice, Poland.

Osteoporosis is a disease in which low bone mass and microarchitectural deterioration of bone tissue lead to increase in fracture risk. Osteoporosis is one of the major worldwide problems facing postmenopausal women and also men above 40 years old. Primary osteoporosis is caused by genetic factors (androgen insufficiency in men) and life style. Among variety of risk factors of secondary osteoporosis the most important are: gastroenterological, endocrine, renal and haematological disorders. Treatment of osteoporosis should consider of good general nutrition, physical exercise and antiresorptive drugs.The present work was aimed at demonstrating whether in men a protracted disease such as osteoporosis and used modes of pharmacological treatment -bisphosphoniates, calcium and vitamin D preparates have an effect on basal testosterone and gonadotrophin (FSH, LH) levels in blood serum. The study involved 15 men aged 45-65 suffering from osteoporosis treated with bisphosphoniates 10mg/24h, preparations calcium 500mg/24h and vitamin D 420 I.U/24h and a control comprising 10 healthy men age-matched with the study groups.The study was driven before the onset of treatment and after six months farmacological treatment. Gonadotrophins and testosterone levels in blood serum were assessed using RIA method. Bone mineral density was mesaured before treatment and six months after. Statistical significance is determined based on Student`s test for p<0,05. Because study is in progress results and other data will be presented and discussed at the Congress.

SU409

Risedronate Reduces the Risk of Clinical Vertebral Fractures in Just 6 Months. N. B. Watts,[1] S. Adami,[2] C. H. Chesnut.[3] [1]Emory University, Atlanta, GA, USA, [2]Clinicizzati di Valeggio, Valeggio, Italy, [3]University of Washington, Seattle, WA, USA.

A number of clinical studies have shown that risedronate rapidly and consistently reduces morphometric vertebral fracture risk up to 70% in just one year. Data from two of these trials (VERT-NA and VERT-MN), with fracture efficacy as primary endpoint, were analyzed to determine the efficacy of risedronate in reducing the risk of clinical vertebral fractures. In the North American trial (VERT-NA), 2458 women were enrolled based on low lumbar spine BMD (T-score ≤ -2) and one prevalent vertebral fracture or two or more prevalent vertebral fractures. In VERT-MN, 1226 women were enrolled based on two or more prevalent vertebral fractures. All patients received either placebo or risedronate (2.5 or 5 mg) daily. In addition calcium supplementation was provided at 1000 mg/day and 500 IU of Vitamin D was provided if baseline levels were low. Clinical vertebral fractures were reported as adverse events and diagnosed by a physician. All clinical fractures included in this analysis were radiographically confirmed during the study. Twenty six per cent of the morphometric fractures were also reported as clinical fractures in line with previous literature (Nevitt, et. al., Arch. Int. Med. 160:77,2000). The incidence of clinical vertebral fractures was calculated using Kaplan-Meier estimates of the survival function. There was a statistically significant 69% reduction in clinical vertebral fracture risk in 1-year (pooled studies, p=0.009) in the risedronate 5 mg/day group. At 6 months, clinical vertebral fracture incidence was 1% in the placebo group and 0.1% in the risedronate 5 mg group. At 9 months, the incidence rates were 1.6% and 0.3% for placebo and risedronate groups respectively. At 12 months, these rates were 1.7% and 0.6%. From 6 months on, risedronate 5 mg/day statistically significantly reduced the risk of clinical vertebral fracture compared to placebo (p<0.01).In conclusion, risedronate 5 mg/day therapy significantly reduces the risk of clinical vertebral fractures from as early as 6 months.

SU410

Tiludronate in Horses: Tolerance and Effects on Bone Resorption. A. M. Varela,*[1] O. M. Lepage,[2] P. Garnero,[3] M. Marcoux.*[1] [1]Département de Sciences Cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, PQ, Canada, [2]Département Hippique, Ecole Nationale Vétérinaire de Lyon, Marcy l'Etoile, France, [3]INSERM research unit 403, and Synarc, Lyon, France.

The purpose of this study was to evaluate intravenous tolerance of tiludronate in horses and its short-term effects on bone resorption.Five healthy adult horses (French Trotters) 6 to 12 years of aged received 1 mg/kg of tiludronate in 1L saline intravenously during 30 min. Horses were kept in stocks under continuous medical observation for the first 3 hours after treatment. Complete clinical examination was realized before, 15 min., 30 min., 2h, 6h, and 24h after treatment. Blood calcium (total and ionized) was monitored immediately before, 30 min., 2h, 6h, and 24h post-treatment. Biochemical parameters reflecting renal (plasma creatinine) and hepatic functions (AST and GGT) were measured before and 24 hours after treatment. Bone resorption was evaluated before and 24 hours after tiludronate administration by measuring serum C-terminal crosslinking telopeptide of type I collagen (CTX) using automated immunoassay (Bêta Crosslaps, Elecsys, Roche Diagnostics).No adverse drug reactions were observed. A slight transient tachycardia (a mean increase of 8 bpm above the baseline level) without dysrhythmias was noted in two horses at +30 min., in two other horses at +2h, and in one horse at both times. Tachycardia was present as well as a transient slight hypocalcemia in these horses except one horse in which tachycardia was noted without hypocalcemia at +2h. Nevertheless hypocalcemia was present with or without tachycardia, in all horses at + 30 min. However, 6h-post treatment, all values had reverted back to normal. There were no changes in creatininemia and hepatic parameters during the study.Serum CTX concentrations before treatment were found to be higher in younger horses (0.260 ng/ml in two 6-year-old horses vs. 0.076 ng/ml in three 10-12 year-old horses). Twenty-four hours post tiludronate I.V. administration, serum CTX levels markedly decreased by an average 67.9% (p<0.01 vs baseline).In conclusion, slow intravenous administration of tiludronate is well tolerated in horses without any clinically relevant adverse effect. It induces a rapid and marked decrease in serum CTX, indicating antiresorptive efficacy.

Disclosures: CEVA Animal Health,2.

SU411

Short-Term Compliance to Daily Alendronate Treatment in 1877 Patients with Osteoporosis. The ECMO Study. E. J. A. Roldán,[1] A. L. Negri.*[2] [1]Gador SA, Buenos Aires, Argentina, [2]Casasco SA, Buenos Aires, Argentina.

Fracture prevention efficacy of most anti-osteoporotic drugs is actuallyhampered due to low rates of adherence, early withdrawals, or frequent mistakes with the administration procedures. Among other reasons, health education appears as a relevant feature. The ECMO study is an educational program aimed to improve adherence to prescriptions and compliance to daily administration of alendronate 10 mg (MARVIL 10), in patients with primary osteoporosis diagnosed either by DXA (WHO criteria) or the existence of a low energy fracture (mainly spine crush). One hundred and ninety physicians and 1877 patients from all over the country (1843 post menopausal women and 34 men) are participating in the program. The sample includes a great variety of sanitary, economic, and technical con-

ditions that will allow the further clustering of data and the observation of particular factors affecting compliance. Adherence was monitored through an independent telephone network. Of the patients included in the study, 5.49% never bought the medication; 2.98% abandoned during the first month; 8.2% during the second month; 5.69 during the third month and 3.79 the fourth month of therapy. The program is still ongoing. Causes of withdrawal were: patient's personal reasons 42%, attending physician's advise 17%, adverse effects 19%, economic problems 20%; another physician's advise 2%. Lack of adherence to an osteoporosis treatment is associated to factors other than tolerability and costs. Personal disconfort with treatment challenge adherence. Hence, health education inosteoporosis should be still enhanced.

SU412

Suppressed Bone Turn-over by 3 Years Incadronate disodium Treatment Increases Microdamage Accumulation, but Does Not Reduced Bone Mechanical Strength in Dog's Rib. S. Mori, Y. P. Cao,* T. Mashiba,* S. Komatsubara,* H. Norimatsu. Kagawa Medical University, Kagawa, Japan.

It has been reported that microdamage is accumulated by bisphosphonates(BPs) treatment, while increases bone mass and decreases fracture incidence. But cause-effect relationships of these findings are still unknown. This study aimed to evaluate the change of bone turnover, microdamage accumulation and mechanical strength of the dog's rib following 3 years BPs treatment.Materials and Methods: 29 dogs, 1 year old, were divided into three groups: control group (n=10) was given lactose, low--dose group(0.3mg/kg/day, n=10) and high--dose group(0.6mg/kg/day, n=9) were treated with incadronate disodium (YM-175, Yamanouchi Pharm. Japan). All dogs were treated for three years and double-labeled with calcein before sacrifice. pQCT measurement and 4 point bending test were performed on 9th rib. The samples were stained with either basic fuchsin or Villanueva bone stain, then embedded in MMA and cut cross-sectionally. Histomorphometrical measurements were performed using a semiautomatic digitizing system to get total area, cortical area, activation frequency and microdamage density. Results: Total cross-sectional area and cortical area were not significantly different among three groups. YM-175 suppressed intracortical remodeling (Ac.f) dose dependently. This increased microdamage accumulation in both low-dose and high-dose groups. However, YM-175 significantly increased cortical BMD in a dose-dependent manner, which resulted in significant increasing mechanical strength in both low-dose and high-dose groups. Conclusion: This study showed that suppressed bone remodeling by YM-175 increased microdamage accumulation although the sectional area was unchanged, however, bone strength was increased with YM-175 because of the increased mineralization.

SU413

Short-Term Cost-Effectiveness of Osteoporosis Treatment: Actonel Versus Fosamax. A. N. A. Tosteson,[1] D. Grima,[2] D. Becker.[2] [1]Dartmouth College, Lebanon, NH, USA, [2]Innovus Research, Inc., Burlington, ON, Canada.

The purpose of this study was to evaluate the cost-effectiveness and budget impact of Actonel therapy vs. Fosamax therapy in the treatment of US osteoporosis patients. A fracture incidence-based model of the natural history of osteoporosis was used to estimate outcomes for patients treated with Actonel and Fosamax. The model's conceptual underpinning is a Markov process whereby patients transition across outcome states over time. The model includes the most common types of osteoporotic fractures (hip, vertebra, wrist, "other"). The base case analysis was conducted on a hypothetical cohort of women aged 65 with low bone mineral density (BMD) and prevalent vertebral fracture, with 3 years of treatment and observation with Actonel or Fosamax. Published fracture unit costs and AWP prices were used (Actonel $1.95/day; Fosamax $2.21/day). The model used published efficacy rates in terms of relative risk reductions for hip fracture (60% Actonel; 51% Fosamax) and vertebral fracture (49% Actonel; 47% Fosamax). The analysis excluded wrist and other fractures due to lack of published efficacy in both therapies. A discount rate of 3% was applied to costs and outcomes. Model outputs included fractures, costs, and quality-adjusted life years (QALYs). Analytic methods included incremental cost/any fracture avoided, cost/QALY gained, and overall budget impacts. Sensitivity analyses were conducted by varying efficacy rates, unit costs, discontinuation rates, follow-up duration, and patient age. In the base case, Actonel produced greater reductions in fractures compared to Fosamax per 1,000 patients (all fractures: 134 vs. 144; hip fractures: 23.1 vs. 28.3) and higher QALYs (2,247 vs. 2,245). The total cost per patient was lower for Actonel ($3,184) compared to Fosamax ($3,617) due to better efficacy and lower acquisition costs. Lower costs and higher efficacy for Actonel translated into strong cost-effectiveness outcomes. When comparing Actonel to no therapy, incremental cost/hip fracture avoided was $13,376, while incremental cost per QALY gained was $17,886. The budget impact analysis revealed actual cost savings of $488 per patient (discounted) under Actonel vs. Fosamax. The sensitivity analyses suggest possible cost-savings under Actonel compared to no therapy when using a lifetime follow-up, or the upper confidence limit for hip fracture efficacy (80%). Actonel is cost saving vs. Fosamax due to lower costs and better efficacy. Compared to no therapy, Actonel is highly cost-effective producing a cost/QALY ratio within accepted ranges for therapy adoption. Over a lifetime horizon Actonel may be cost saving compared to no therapy.

SU414

Single Intravenous Administration of Zoledronic Acid Exerts a Long-Term Protective Effect Against Cancellous and Cortical Bone Loss in Ovariectomized Rats. J. A. Gasser, J. R. Green. Arthritis & Bone Metabolism, Novartis Pharma AG, Basel, Switzerland.

Oral bisphosphonate therapy requires frequent (usually daily) administration using a relatively cumbersome procedure to avoid interference with food and drinks. The aim of our study was to investigate the duration of bone protective effects in ovariectomized rats of a single intravenous dose of zoledronic acid, a highly potent, new generation bisphosphonate compound. Skeletally mature, 7 month-old virgin Wistar rats (10 / group) were sham-operated or ovariectomized (OVX) and treated with a single intravenous injection of zoledronic acid at doses of 0.8, 4, 20, 100 or 500 µg/kg. Changes in the mass and density of cancellous and cortical bone, as well as in structural cortical parameters, were measured in the proximal tibial metaphysis at 4-weekly intervals by peripheral quantitative computed tomography. NTx-levels (Osteomark®, Ostex, Seattle) were measured at the same time points in serum prepared from blood drawn from the retro-orbital venous plexus.Vehicle treated OVX-rats showed rapid cortical thinning through endocortical resorption with a loss of up to 20% at 12 weeks ($p<0.01$, Dunnett's test). Cancellous bone mineral density steadily decreased to 50% ($p<0.01$) at week 12. A single i.v. administration of 0.8 µg/kg zoledronic acid led to partial protection against cortical and cancellous bone loss at 4 and 8 weeks but no longer at 12 weeks. At a five times higher dose (4 µg/kg), zoledronic acid prevented cancellous bone loss for up to 8 weeks before a slow decrease became apparent. However, cortical thinning was still reduced significantly for up to 12 weeks at a dose of 4 µg/kg. Full protection of all cancellous and cortical bone parameters was achieved at 20 µg/kg, while higher doses even resulted in a 4% increase in cortical thickness at 12 weeks.These results suggest that a single intravenous dose of zoledronic acid is well tolerated and exerts a long-term, dose-dependent suppression of bone turnover, and significant protection against cancellous and cortical bone loss in this rat model of osteopenia. Based on the different length of the bone remodeling cycle in rats and humans, these data support the use of an infrequent dosing regimen for zoledronic acid of only 1 or 2 intravenous injections per year for the treatment of osteoporosis.

SU415

Long Term Predictions of Less than Daily Alendronate Treatment. C. J. Hernandez,* G. S. Beaupré, R. Marcus, D. R. Carter. VA Palo Alto HCS, Palo Alto, CA, Stanford University, Stanford, CA, USA.

Less than daily alendronate administration is an attractive way to simplify the treatment process. A recent year long study demonstrated that twice weekly and weekly alendronate treatment regimen are therapeutically equivalent to daily (Schnitzer et al. Aging, 2000 12(1):1-12). Most osteoporosis patients will maintain an alendronate regimen for much longer than one year. It is unclear if the difference between daily and less than daily treatment will be the same after ten years as after one. We use a computer simulation of bone remodeling that is based on clinical measurements to predict the long term changes in BMD caused by daily and less than daily treatment regimen. The computer simulation used in this analysis calculates the activity of basic multicellular units (BMUs) and has been shown to predict BMD changes caused by daily alendronate treatment (Hernandez et al. ORS 2001, 475). BMU response to oral doses averaging 10mg per day (70 mg weekly, 35 mg twice weekly, etc.) is represented by reducing the activation frequency by the same amount as measured in a clinical study. The difference between daily and less than daily regimen of the same cumulative dose is that during less than daily treatment more alendronate is eliminated from the body before the next dose. The rate of elimination and the corresponding reduction in BMU response are calculated based on published findings (Khan et al. JBMR 12(10):1700-7).

Treatment Frequency	Predicted BMD increase after 1 year	Predicted BMD increase after 10 years
Daily	5.37%	11.98%
Twice Weekly	5.12%	11.38%
Weekly	4.94%	10.96%
Twice Monthly	4.73%	10.47%
Monthly	4.49%	9.90%

Two alendronate regimen are considered therapeutically equivalent if the difference between BMD changes is within 1.5%. By this definition our results suggest that all treatment regimen considered may be therapeutically equivalent to daily administration after the first year of treatment. After ten years of treatment only twice weekly and weekly regimen are predicted to be equivalent to daily. We suggest that, for antiresorptive drugs, the length of an equivalence study may have a significant influence on the conclusions. Our predictions of long term treatment suggest that twice weekly and weekly regimen may be equivalent to daily administration after long term treatment.

SU416

Heavy Housework along with Calcium Is Beneficial for Hip and Forearm BMD in Elderly Women. R. A. Brownbill, C. Lindsey, J. Z. Ilich. University of Connecticut, Storrs, CT, USA.

It is widely accepted that activities of moderate or vigorous intensity are beneficial to bone, but it is not clear whether less intense activities such as housework, gardening or stair climbing benefit bone mass in postmenopausal women, since few studies have addressed these activities. The purpose of this study was to determine if lower intensity activities such as heavy housework, gardening or stair climbing, as well as calcium (Ca) intake are associated with bone mass. Participants were 136 Caucasian women, mean±SD age 68.6±7.1 y (range 57.4-88.6) all healthy and not taking medications known to affect bone. BMD at multiple skeletal sites was measured by DPX-MD (Lunar Corp, Madison, WI), with specialized software for the forearm, femur, lumbar spine and whole body. Ca intake was assessed by a shortened food frequency questionnaire (NOF, 1998) which quantifies calcium intake based on intake from milk, cheese and yogurt along with a correction factor. Physical activity was assessed with the Allied Dunbar National Fitness Survey (PH Fentem et al, Sports Council and Health Education Authority, London, England, 1994). Activities assessed included hours of participation per week in heavy housework (such as vacuuming and scrubbing floors), heavy and light gardening, home improvement activities (such as painting) and stair climbing. 89% of women engaged in regular heavy housework (2.1±4.3hr/week) and stair climbing (86.8±85.8 stairs/d) and 54% in gardening (1.0±2.2 hr/week).In multiple regression models corrected for number of years in menopause and body weight, heavy housework and Ca intake (834±365 mg/d) were significant predictors of the neck, trochanter, and total hip BMD with R2adjusted 0.30, 0.26, and 0.27, respectively, as

well as of the forearm (ulna) BMD at 1/3 distance, R2adjusted 0.16. Gardening, home improvement activities and stair climbing were not related to any of the bone variables. This could be due to the fact that only half of the women engaged in gardening and that the stairs climbed per day might have been miscounted.These findings indicate that lower intensity activities such as housework, may positively influence bone mass in postmenopausal women and therefore should be recommended along with adequate Ca diet to elderly women in which moderate or vigorous exercise may not be appropriate. Future studies looking at the physical activity and bone mass relationship in elderly women need to address the affects of lower intensity activities, since these are common forms of activity engaged in by this segment of population.

SU417

Body Weight is a Key Determinant of Bone Mass in Elderly Women, While the Effect of Physical Activity, Muscle Strength and Muscle Mass Is Limited. P. Gerdhem,* K. Ringsberg,* K. Åkesson, K. Obrant. Department of Orthopaedics, Malmö University Hospital, Malmö, Sweden.

In the Malmö Osteoporosis Prospective Risk Assessment (OPRA) study 1044 women, all 75 years of age, were recruited from the population files. In 995 of the women, the effect of current body weight, weight-change from age 50 to age 75, height, physical activity, muscle strength, and muscle mass on bone mass was investigated. Isometric muscle strength (knee extension and flexion) was tested by a computerized dynamometer and the present activity level was assessed as an activity score. The women were examined with DXA (total body, hip- trochanter and neck and lumbar spine- LII- LIV). From the total body scan mode, total fat mass and lean body mass were assessed. In a forward, stepwise regression model we found body weight to be the only variable having any substantial influence, on the total variability (16-34% depending on skeletal site) in BMD. Given weight, none of the other variables contributed to more than 1% of the variability in BMD in any of the skeletal regions. For example:

Determinants of Total Body BMD	**r^2**	**p**
Body weight	0,32	<0,0000
Physical activity	0,008	=0,001
Muscle strength	0,007	=0,002
Lean mass	0,005	=0,012

When current body weight (including fat and lean mass) was replaced by weight-change, weight-change was more determining for BMD (r^2=0,04-0,13, p<0,0000) than physical activity and muscular strength. Each 10 kg weight-change predicted a change in T-score between 0.40- 0.65 SD. Conclusion: Our findings suggest that, contrary to common belief, physical activity, muscle strength, and muscle mass, are of minor importance for bone mass in elderly women. In the elderly, high body weight or weight-gain, irrespective if caused by increased muscle or fat mass, overrule the effect of increased muscle strength and activity level on bone mass.

SU418

Effects of Oral Alendronate and Exercise on Tibia and Physical Performance in Early Postmenopausal Women. K. Uusi-Rasi,*[1] H. Sievänen,*[1] P. Kannus,*[1] A. Heinonen,*[1] M. Pasanen,*[1] S. Cheng,[2] I. Vuori.*[1] [1]UKK Institute for Health Promotion Research, Tampere, Finland, [2]University of Jyväskylä, Jyväskylä, Finland.

The purpose of this one-year randomised (concerning alendronate double blind) placebo-controlled intervention trial was to evaluate the effects of jumping exercise and alendronate treatment on tibia and physical performance in early postmenopausal women. 164 women were randomly assigned into four groups: 1) 5 mg of alendronate + exercise (Al^+Ex^+), 2) 5 mg alendronate (Al^+Ex^-), 3) placebo + exercise (Al^-Ex^+), and 4) placebo (Al^-Ex^-). The inclusion criteria were no regular exercise or previous bone fractures, 1-5 years after menopause, no current or previous use of drugs or illness affecting bone metabolism, no contraindication to exercise and alendronate, femoral neck t-score $\geq$ -2.5, and the FSH level > 30 IU/l. Measurements were done at baseline and end of the study. The final data were obtained from 153 women. Bone mineral content (BMC), total area (TotA), cortical area (CoA) and polar section modulus (SSI) of the midshaft and distal part of the tibia were measured with peripheral quantitative computed tomography (Stratec XCT 3000). Supervised high-impact jump training was given 3 times a week for 12 months. Between-group differences were estimated by analysis of covariance using baseline values as covariates. Compliance defined as percentage of attendance at all available training sessions was 56% and for taking alendronate/placebo pills 93%. The mean increase in the leg-extensor power was 8.5% more in the exercise group compared to the non-exercisers. The respective group differences were 2.5% in the dynamic body balance, and 3.1% in the estimated oxygen uptake in favour of the exercise group. Table 1. The mean percentage changes in bone variables.

Variable	**Al^+ Ex^+ N=37**	**Al^+ Ex^- N=38**	**Al^- Ex^+ N=37**	**Al^- Ex^- N=39**
Distal tibia BMC	0.4	0.4	0.2	-1.7
CoA : TotA ratio	6.0	2.6	5.4	1.4
SSI	5.9	2.0	3.8	0.3
Tibial shaft BMC	-0.2	-0.5	-0.1	-1.0
CoA	0.0	-0.7	0.5	-0.6
SSI	-0.4	0.0	0.0	-0.6

The results suggest that exercise improves the mechanical competence of the distal tibia by strengthening its cortex. The mean CoA:TotA ratio was 3.7% (95% CI 0.1 to 3.7%) and SSI 3.6% (0.3 to 7.1%) greater in the exercisers than in the non-exercisers. In addition, both alendronate treatment and exercise maintained bone mass at the distal tibia compared to the Al^-Ex^- group (P=0.051). At the tibial shaft, neither alendronate nor exercise had statistically significant effects on bone, except a 1% (0.1 to 1.9%) greater CoA in the exercisers than in the non-exercisers.

SU419

High Strain Group Exercise Over 6 Months Increases Lean Muscle Mass and Bone Mass and Density in Postmenopausal Osteopenic Women. A. J. Kapsabelis,[1] G. Skarantavos,[2] G. Prevena,*[1] S. Mitakidis,[3] E. Gioni,[4] A. Galanos,[4] G. Lyritis.[4] [1]Perfecture of Keratsini, Keratsini, Greece, [2]Rheumatology Clinic, Kat Hosptial, Athens, Greece, [3]Ika Amfiali, Keratsini, Greece, [4]Laboratory for the Research of Musculoskeletal Diseases, University of Athens, Athens, Greece.

The purpose of this research was to study the effect of high strain, group exercise in lean muscle mass, bone mass and geometry of postmenopausal osteopenic women. For this reason 56 postmenopausal women were randomly allocated into 2 groups: A (N: 34, age: 60±11) and B (N: 22,age: 62±10) and were subjected in laboratory tests of bone metabolism. Group A was subjected for 6 months in 3 times a week, 1-hour program of aerobic dancing, stepping, rope jumping and resistance training. Group B (control) had had no exercise. All women at the beginning and at the end of the research were subjected in measurement of their left tibia by pQCT (Stratec 2000) with slices at 4%, 14%, 38% and 66% of the tibia length from the distal end of the tibia. No statistical differences were noted between groups at the beginning of the study. At the end the exercise group presented significant augmentation of lean muscle area (p=0.002), significant augmentation of total bone mass (p=0.029), total bone density (p=0.018) and subcortical bone mass (p=0.004) at the 4% slice, whereas the control group showed no differences. The control group experienced a significant loss of total bone mass at the 38% slice (p=0.029), whereas the exercise group maintained total bone mass (p>0.05) According to our results, a 6 months high strain, group exercise program in postmenopausal, osteopenic women results in: (1) Augmentation of lean muscle mass of the calf. (2) Augmentation of the total bone mass and bone density of distal tibia, mainly attributed to the subcortical bone. (3) Maintenance of cortical bone mass of mid-tibia.

SU420

"Osteofit" – A Community-Based Exercise Program Reduces Fall Risk by Improving Dynamic Balance in 65-75 Yr Old Women Who Have Osteoporosis. K. M. Khan,[1] C. Waterman,[2] M. Donaldson,[3] N. Carter,[3] T. Liu-Ambrose,*[3] M. Petit,[2] A. Mallinson,[2] K. Kruse,[2] P. Janssen,[2] L. Riddell,[2] J. C. Prior,[2] A. Heinonen,[3] H. A. McKay.[3] [1]BC Women's Hospital & Health Centre & Dept Family Practice, UBC, Vancouver, BC, Canada, [2]BC Women's Hospital & Health Centre, Vancouver, BC, Canada, [3]Human Kinetics, UBC, Vancouver, BC, Canada.

We conducted a single-blind randomized controlled trial to assess the effect of "Osteofit" on balance and muscle strength in women who have osteoporosis (t-score < -2.5). Osteofit is a community-based exercise program of BC Women's Hospital and Health Centre for women with osteoporosis. It has been adopted by over 50 community centres in British Columbia, Canada. We recruited 97 community-dwelling women who had osteoporosis or established osteoporosis aged 65-75 yrs (mean 69.3 (3.3)) and randomized them to intervention (Osteofit) or control social visits. We report results in 80 women who completed 20 week followup measurement [n = 40 in control group, 40 in Osteofit exercise group]. We assessed health history, current medication and quality of life by questionnaire. Static balance was measured by computerized dynamic posturography (Equitest), dynamic balance by timed figure of 8 run, and quadriceps strength by dynamometry at baseline and at 20 weeks. Un-paired t-tests were used to compare baseline characteristics between groups. Exercise and control groups were well matched at baseline for age, height (157.7 (5.6); 156.6 (7.5) and weight 59.1 (11.7); 62.9 (13.3). The control group, as compared to exercisers, reported fewer years of estrogen use (2.0 (5.1) vs 3.6 (5.5) yrs , fewer medications (2.0 (1.6) vs 2.6 (1.6)), and higher activity levels (14.7 (18.1); 9.2 (9.0) hours per week) at baseline (all NS). ANCOVA was used to examine differences between groups after controlling for age, medications, baseline activity, and years of estrogen use. Percent change over 20 weeks (means adjusted for age, medications, physical activity, and years of estrogen use) for control and exercise group are presented in the table (Mean (95% CIs)).

Percent change (Mean & 95% CI)over 20 weeks in control group and exercise ('Osteofit') groups

	Control	**Exercise (Osteofit)**	**p**
N	40	40	
Knee extension strength / ht (kg/m)	0.6% (-8.5 to 9.7)	8.8% (-0.1 to 17.8)	0.22
Figure of 8 velocity (m/sec)	3.1% (-0.1 to 6.4)	7.9% (4.7 to 11.1)	0.04
Composite Balance Score (Equitest)	-0.5% (-5.0 to 3.9)	2.9% (-1.6 to 7.3)	0.29

n average, performance for the control group did not change or declined over time, while the exercise group improved or maintained static and dynamic balance and knee extensor strength. A community-based exercise program is an effective means to improve risk factors for falling, and thus fracture, in women who are particularly at risk because of osteoporosis.

SU421

Twelve Month Efficacy of Home-Based Exercise for Improving the Quality of Life of Elderly Women with Symptomatic Osteoporosis Related Vertebral Fractures. K. Winegard,*[1] A. Papaioannou,[2] W. Parkinson,*[3] N. Ferko,*[4] J. D. Adachi,[2] N. McCartney,*[1] C. Webber.[5] [1]Kinesiology, McMaster University, Hamilton, ON, Canada, [2]Department of Medicine, McMaster University, Hamilton, ON, Canada, [3]School, Rehabilitation Sciences, McMaster University, Hamilton, ON, Canada, [4]Clinical Health Sciences, McMaster University, Hamilton, ON, Canada, [5]Radiology, McMaster University, Hamilton, ON, Canada.

The purpose of this study is to establish whether a 12 month home exercise program can improve the quality of life of elderly women with symptomatic osteoporosis-related vertebral fractures. Previous research has implicated poor physical function as a risk factor for subsequent fractures in the elderly who have osteoporosis related vertebral fractures. This ongoing study involves 78 women. Data are available on 48 women with an age range of 61 to 88 years (mean age=74.4 years). Radiographic examinations revealed that 49 entered with one vertebral fracture and 29 with fractures of multiple vertebrae. Participants are assigned to exercise or control groups using randomized stratification to control for age (over, under 70 years) and number of vertebrae fractures (single, multiple). A 12 month exercise program that incorporates tailored range of motion, strengthening, and aerobic conditioning is performed for at least 60 minutes 3 times weekly. Following 2 training visits, a kinesiologist meets with participants once per month for six months. Exercise continues on a self-directed basis for 6 additional months. Controls provide outcome measures only. The primary outcome measure included a disease specific osteoporosis quality of life (QOL) questionnaire. The QOL questionnaire assessed symptoms, emotional and physical function, activities of daily living and leisure/social aspects. Results for 12 month outcome on the first 48 subjects show a trend ($p=0.12$) towards improvement on overall disease specific QOL in the exercise group, that is accounted for largely by improved symptoms ($p<0.05$) and physical functioning ($p<0.01$). These data suggest that minimal contact home exercise may improve the quality of life of women with vertebral fractures which may be of clinical significance given the minimal therapeutic intervention required to achieve this.

SU422

Effects of Endurance Exercise and Chronic Alcohol Consumption on Musculoskeletal Components in Skeletally Mature Male Rats. A. H. Reed,*[1] H. L. McCarty,*[1] G. L. Evans,*[2] R. T. Turner,[2] K. C. Westerlind.[1] [1]AMC Cancer Research Center, Denver, CO, USA, [2]The Mayo Clinic, Rochester, MN, USA.

Lifestyle factors are known to affect skeletal development and integrity. Specifically, endurance exercise has been reported to increase bone volume whereas chronic alcohol consumption has been shown to result in a decrease in bone formation and an increased incidence of osteopenia. In certain physically active populations (e.g. military recruits, college athletes), there is a high prevalence of alcohol consumption, yet the combined effect of exercise and alcohol consumption on the skeleton has not been evaluated. To investigate the independent and interactive effects, 6 mos male SD rats were stratified by wt and randomized to 1 of 5 groups (n=10/grp): Baseline, Exercise+Alcohol diet (Ex-EtOH), Exercise+Normal diet (Ex), Sham-exercise+EtOH diet (Sham-EtOH), and Sham-exercise+Normal diet (Sham). Alcohol-fed rats received Bio-Serv Liquid Rat Diet LD 82 ad lib (35% caloric intake). Non-alcohol fed rats were pair fed the same diet with a Maltose Dextrin caloric substitute. Exercise was conducted on a motorized treadmill 5 d/wk for 16 wk with duration and speed increasing to a maximum of 25 m/min, 30 min, 15% grade. Sham-exercise rats were placed on a stationary treadmill at 15% grade for matching time periods. Fluorochrome labels were administered 3 d prior to baseline (tetracycline; 20mg/kgBW), and 2 and 10 d prior to sacrifice (calcein; 20 mg/kg BW). Muscle weights (per 100g BW), were significantly larger in the exercised rats vs. the sham rats [0.310 vs 0.279mg (heart), 0.041 vs 0.034mg (soleus), 0.297 vs 0.255mg (rectus)], respectively. Alcohol had no effect on skeletal muscle weight, but resulted in significantly larger heart weights in both alcohol-fed groups. BFR/BV was significantly decreased in the alcohol-fed rats (0.240 %/d) vs. rats on the normal diet (0.354 %/d) and was associated with a significant decrease in LS/BS (Alcohol 9.55% vs. Non-alcohol 18.15%). Alcohol consumption also negatively affected cortical bone. Mean cortical and cross-sectional areas were significantly lower in the alcohol-fed groups (4.90 um2 and 6.44 um2, respectively) compared to non-alcohol fed (5.24 um2 and 6.77 um2, respectively). Exercise resulted in no significant change in cancellous or cortical bone measurements and did not attenuate any of the reductions in bone formation associated with alcohol consumption. It is speculated that an increase in muscle mass with a concomitant decrease in bone formation, such as that observed in the Ex-EtOH group, may place the skeleton at risk for fracture. This may be most applicable in physically active populations, where stress fractures are suspiciously common.

SU423

Bone Density Changes With a Progressive Strength Training Program in Postmenopausal Women: Dose-Response Hypotheses. T. G. Lohman, E. C. Cussler,* S. B. Going, L. B. Houtkooper,* L. M. Metcalfe.* University of Arizona, Tucson, AZ, USA.

The relationship between the amount of weight lifted in one year and the amount of bone mineral density (BMD) change was examined in 140 post-menopausal women. Half of the sample had been taking hormone replacement therapy (HRT) for the past 1 to 6 years and half had not been taking HRT. The women performed 8 different individual exercises including the weighted squats, military press, seated row, lat pull down, back extension, rotary torso, weighted march and seated leg press at three weekly sessions with 6 to 8 repetitions of two sets. The progressive strength training program was increased every two months to maintain exercise intensity at 70% of 1 RM. Attendance averaged 71.5% ± 19.8% for the total sample over one year. Dual energy x-ray absorptiometry (DXA) was used to measure total body and regional BMD at the hip, spine, and arm at baseline and one year. Increases in femur trochanter (1.7 ± 3.4%), femur neck (1.1 ± 3.8%), lumbar spine 2-4 (0.4 ± 2.3%) and total BMD (0.2 ± 1.1%) were found after one year. Total weight lifted in relation to change in BMD was slightly quadratic, with greater BMD changes associated with larger amounts of weight lifted than predicted from a linear relationship. Furthermore, those on HRT showed a more significant dose-response relationship with exercise than those not on HRT. Total weight lifted was positively and significantly associated ($p<.05$) with trochanter BMD change, but not with femur neck, lumbar spine, or total BMD. Weight lifted in individual exercises also significantly related to trochanter BMD change (lat pull down, row, military press, squats and rotary torso) but not to lumbar spine or femur neck. Total BMD change was related to only one specific exercise (weighted march). Changes in association with standard deviations of weight lifted indicate that a dose response relationship is apparent in the femur trochanter only. The progressive strength training program had impact dose-response effects on femur trochanter BMD but not other sites.Supported by: NIH AR39559 and Mission Pharmacal

SU424

Quantitative Ultrasound and Dual X-Ray Absorptiometry Parameters: Interaction of Leisure Physical Activity and Estrogen Receptor Genotype. S. Dodin,[1] C. Blanchet,*[1] Y. Giguere,*[2] M. Dumont,*[3] M. Al-Akoum,*[1] S. Côté,*[1] N. Laflamme,*[4] F. Rousseau.*[4] [1]Quebec Menopause Center, Québec, Canada, [2]Research Unit in Human and Molecular Genetics, Quebec, Canada, [3]Nuclear Medecine Department, HSFA, Québec, Canada, [4]Signalgène Inc., Montreal, Canada.

The purpose of the study was to assess the magnitude of the interactions between leisure physical activity (LPA), estrogen receptor PvuII polymorphism (ESR1) and bone parameters measured by quantitative ultrasound (QUS) or dual x-ray absorptiometry (DXA) in 848 healthy French Canadian menopausal women (age : 57.8 ± 7.2 years, weight : 66.2 ± 12.4 kg, height : 158.3 ± 5.8 cm and body mass index : 26.4 ± 4.9). They answered a questionnaire that included leisure physical activity frequencies over the last three months. According to the questionnaire, three groups of leisure physical activity were formed (sedentary, moderately active and active). Bone mineral density (BMD) was measured at the lumbar spine (L2-L4) and at the femoral neck (FN) by DXA. QUS parameters of the right calcaneus, broadband ultrason attenuation (BUA), speed of sound (SOS), and stiffness index (SI), were measured by a AchillesTM ultrasound bone densitometry. The ESRI genotype analysis was done by PCR allele-specific oligonucleotide amplification. According to leisure physical activity, and after adjustment, significant difference was observed at the femoral neck (active vs sedentary; $p=0.009$) (active vs moderately active; $p=0.019$) and at the calcaneus (active vs sedentary; $p=0.0002$). No such association was found at the lumbar spine. In this sample, no significant difference was observed between ESRI genotypes and adjusted bone parameters at the lumbar spine, femoral neck and stiffness index. However, analyses of adjusted bone parameters according to ESRI and leisure physical activity revealed a significant difference in active women only for stiffness index. Active women, who exercised three times or more a week, carrying the «PP» and «Pp» genotypes had a higher stiffness index than active women carrying the «pp» genotype (PP vs pp; $p=0.004$) (Pp vs pp; $p=0.004$). In conclusion, these results suggest that gene-environment interactions such as leisure physical activity and ESRI genotype may play a role in maintaining the bone architecture in active menopausal women. However, those results should be verified in a prospective study.

SU425

Initial Bone Mineral Density Does Not Predict One-Year Bone Density Change in Weight-Lifting Postmenopausal Women. E. C. Cussler,* R. M. Blew,* S. B. Going, T. G. Lohman. University of Arizona, Tucson, AZ, USA.

The American College of Sports Medicine position stand on osteoporosis and exercise presented the "principle of initial values" which proposes that individuals with the lowest levels of bone mineral density (BMD) have a greater capacity for percent improvement in training studies; those with average or above average bone mass gain the least BMD. Although often accepted by investigators, the principle is based on previously documented responses of other physiological systems. Therefore, the aim of this study was to assess the validity of this presupposition in postmenopausal women by examining the relationship between initial BMD and BMD changes following a one-year exercise intervention program. One hundred forty healthy, postmenopausal women participated 3 d/wk in progressive weight lifting exercises targeting major muscle groups and attended an average of 71.5 ± 19.8% of the total sessions. BMD of the femoral neck, trochanter, AP spine, and total body were measured at baseline and one year using dual energy x-ray absorptiometry. For each bone site, multiple linear regression models including baseline BMD, lean soft tissue, percent body fat, fat free mass, and body weight were developed to predict change in BMD. Models were also adjusted for age, hormone replacement use, and time of study entry (cohort). Initial BMD ranged from -3.9 to +4.3 standard deviations of young adult female norms (T-score). The percent BMD change at one-year varied from -6.8% to +12.7%. Age and hormone use were significantly associated with bone change at several bone sites; however, initial BMD failed to show a statistically significant relationship to BMD change at any site. For every g/cm2 increase in initial neck BMD, a decrease in change of 0.0164 g/cm2 was predicted ($p<.40$). A one g/cm2 increase in baseline trochanter was associated with an increase of 0.0003 g/cm2 ($p<.98$). Similar results were found for the lumbar spine and total body BMD change. Inclusion of lean soft tissue, percent body fat, fat free mass, and body weight in separate prediction models did not alter the results. These results suggest that the initial level of BMD does not predict the magnitude of change in BMD for postmenopausal women as previously proposed. Supported by: NIH AR39559 and Mission Pharmacal

SU426

Correlation Between Maximal Effect (Emax) Delivered During Fitness Test and Bone Mineral Density (BMD) in Children and Adolescents. K. Kramme,*[1] L. Mortensen,[2] K. Froberg,*[3] H. Beck-Nielsen,*[1] K. Brixen,[1] P. Charles.[2] [1]Department of Endocrinology, Odense University Hospital, Odense, Denmark, [2]Department of Endocrinology, Aarhus University Hospital, Aarhus, Denmark, [3]Institute of Physical Education and Biomechanics, University of Southern Denmark, Odense, Denmark.

Previous studies have suggested that exercise may be one of the most important modifyable factors for accretion of bone mass in childhood and adolescence. Thus, some studies in adults have reported a positive correlation between VO2max and BMD. VO2max, however, depends on body weight which in itself is closely related with BMD. To circumvent this interdependence we evaluated the association between the maximal effect delivered during fitness test and lumbar spine and whole-body BMD in children and adolescents.This cross-sectional study comprised 157 females and 149 males aged 8 to 21 years (median 14 years). Participants were tested on an ergometer bicycle, and BMD subsequently measured using a Norland DXA-scanner. The relationship between BMD and Emax, body weight, height, age, and sex was evaluated using multiple backwards regression analysis.Results are shown in the table. Data are shown as partial correlation coefficients. (* p<0.05, **p<0.01, ***p<0.001. n.i=not included in the final model)

	BMD Lumbar Spine	BMD Whole Body
Sex	0.33***	n.i
Age	0.46***	0.44***
Body weight	0.23***	0.55***
Height	0.17**	n.i.
Emax	0.15*	0.18**
Combined model	0.87***	0.89***

In conclusion, the maximal effect delivered during exercise test correlates significantly with BMD in the lumbar spine and whole body in children and adolescents. Our study supports the idea that exercise to some agree affects accretion of bone mass during childhood and adolesence

SU427

Reductions in Bone Turnover Predict Exercise-Induced Increases in Bone Mineral Density in Mature Premenopausal Women. K. M. Winters,*[1] C. Loughleed,*[1] C. M. Snow.[2] [1]Exercise Science, Northern Arizona University, Flagstaff, AZ, USA, [2]Bone Research Laboratory, Oregon State University, Corvallis, OR, USA.

Reductions in biochemical markers of bone turnover are associated with favorable BMD responses to anti-resorptive drug therapy. However, their utility in assessing the effect of exercise on BMD is poorly understood. Therefore, we sought to determine if changes in biochemical markers of bone turnover could predict changes in BMD in mature premenopausal women (n=24; age: 40 ± 4yrs) who participated in 12 months of jump and resistance training. To assess changes in non-exercising women, an age-matched control group (n=13; age: 40 ± 4yrs) was also followed over the same time period. The training program consisted of 100 jumps and 100 repetitions of lower body resistance exercise, performed 3 days per week for 12 months. Both BMD and biochemical markers were measured at baseline and post exercise (12 mos.). BMD of the proximal femur (greater trochanter (GT); femoral neck (FN); total hip (Thip)) and of the lumbar spine (LS) was assessed by DXA (Hologic 4500A). Serum osteocalcin and urinary deoxypyridinoline cross-links, adjusted for variations in urine volume (creatinine), were measured by ELISA. In response to training, GT and Thip BMD increased significantly (+2.8% and +1.5%, respectively), nearly so for FN BMD (+1.3%), but not for LS BMD (+0.5%). BMD did not change at any site in controls (GT: +0.8%; FN: -0.4%; Thip: +0.6%; LS: -0.8%). In response to training, both biomarkers decreased (-11.2% and -16.8% for osteocalcin and deoxypyridinoline, respectively), but decreases were only significant for osteocalcin. Neither biomarker changed significantly in controls (+2.1% and +1.0% for osteocalcin and deoxypyridinoline, respectively). Regression analyses sought to determine the ability of biochemical markers to predict exercise-induced changes at each bone site. Predictor variables entered into the analyses included, baseline osteocalcin and deoxypyridinoline and 12-month changes in each biomarker. Reductions in deoxypyridinoline significantly predicted increases in GT BMD (R-squared=0.2; p=0.04), while reductions in osteocalcin significantly predicted increases in Thip BMD (R-squared=0.2; p=0.03). Biochemical markers could not predict changes in FN nor LS spine BMD, however neither of these sites significantly increased following the training program. Thus, reductions in biochemical markers of bone turnover significantly predicted successful responses to osteogenic exercise in adult premenopausal women. These findings suggest that biochemical markers may be useful for evaluating the osteogenic response to increased mechanical loading during the premenopausal years.

SU428

Does Follow-up of Women Receiving Antiresorptive Therapies Using Bone Markers Increase Effectiveness of Treatment? R. D. Chapurlat, S. R. Cummings. Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA, USA.

The use of biochemical markers of bone turnover has been advocated to improve follow-up of women receiving antiresorptive therapies for osteoporosis. They may detect women who do not respond to treatment - or who do not adhere - early, and thus would allow rapid adaptation of treatment. This strategy, however, is not yet supported by trials showing it improves effectiveness of treatments.To explore the value of markers of bone turnover to monitor antiresorptive treatments of osteoporosis, we conducted a decision analysis using a decision tree and Markov modeling. The base case is the treatment of a 60 years old osteoporotic woman with a total hip T score of -3, using a second generation bisphosphonate during 5 years. We have compared two strategies: treatment of this women without specific monitoring; and treatment of this women with measurement of a serum marker of bone resorption after 3 months of treatment, with the possibility to change the treatment if response to treatment assessed by this marker was not satisfactory. The second treatment could be PTH. Sensitivity analyses have been done on adherence to treatment and probability of response to treatment. Most probabilities and utilities we used were drawn from the recent NOF effectiveness analysis on osteoporosis. We assumed that follow-up did not influence adherence to treatment, as this has never been demonstrated in a randomized trial. So, in the base case, the proportion of women who adhere to treatment in the long-term was set at 50% for both the follow-up and non-follow-up branches. We found that the expected value of the follow-up branch (8.1560 QALYs) was slightly greater than that of the no follow-up branch (8.1532 QALYs). This result was confirmed in sensitivity analyses. Specifically, in a two-way sensitivity analysis, the follow-up option was always better than the no follow-up option when the proportion of women who adhered with follow-up was equal or superior to the proportion of women who adhered without follow-up. In cases where follow-up increased adherence, there were increments in the expected value of the follow-up branch. For example, if the proportion of women adherent to treatment was increased from 50% to 60% by follow-up, then the expected value of the follow-up branch was raised from 8.1560 QALYs to 8.1800 QALYs. The probability of non response to treatment did not influence results. In conclusion, our decision analysis model suggests that follow-up of osteoporotic women treated with a second generation bisphosphonate during a 5 years period using an early measurement of a serum marker of bone resorption slightly increases effectiveness of the treatment.

SU429

Effects of Bone Anti-Resorptive Agents on the Anabolic Actions of Basic Fibroblast Growth Factor in Aged Ovariectomized Rats. U. T. Iwaniec, N. G. Mitova-Caneva,* T. J. Wronski. Department of Physiological Sciences, University of Florida, Gainesville, FL, USA.

The purpose of this study was to determine whether prior and concurrent administration of the anti-resorptive agents estrogen and risedronate suppresses the bone anabolic response to treatment with basic fibroblast growth factor (bFGF). Three month old female Sprague Dawley rats were ovariectomized (OVX) or sham-operated and left untreated for 12 months to establish cancellous osteopenia in the OVX group. The rats were then injected sc with estrogen (10 μg/kg, 4 days/week), risedronate (5 μg/kg, 2 days/week), or vehicle for 4 weeks. At the end of the second week of anti-resorptive treatment, catheters were inserted in the jugular veins of all rats and vehicle or bFGF (Chiron Corp., CA) at a dose of 250 μg/kg was injected daily for the remainder of study. Lumbar vertebrae were collected from each rat and processed undecalcified for bone histomorphometry. Data were analyzed with the Kruskal-Wallis test followed by a nonparametric posthoc test. Treatment of OVX rats for 14 days with bFGF resulted in markedly increased osteoblast surface (Ob.S/BS), osteoid surface (OS/BS), and osteoid volume (OV/TV) compared to vehicle treatment of sham-operated and OVX controls. Prior and concurrent treatment with anti-resorptive agents did not suppress the effects of bFGF on these indices of bone formation. The lack of fluorochrome labeling in cancellous bone of bFGF-treated rats (data not shown), despite increased osteoblast surface, was indicative of an inhibitory effect of bFGF on bone mineralization.

Cancellous Bone Histomorphometry (mean ± SD)

Group	n	Ob.S/BS (%)	OS/BS (%)	OV/TV (%)
Con+Veh+Veh	4	1.2±1.1	1.3±1.0	0.0±0.0
Ovx+Veh+Veh	6	8.7±5.1	7.9±4.7	0.1±0.1
Ovx+Veh+bFGF	7	28.2±15.8[a,b]	39.0±18.7[a,b]	3.5±2.2[a,b]
Ovx+Est+bFGF	10	47.0±19.4[a,b,c]	54.6±24.8[a,b]	5.6±3.7[a,b]
Ovx+Ris+bFGF	9	26.4±17.2[a,b]	29.1±19.9[a,b]	3.8±3.8[a,b]

[a]different from Con+Veh+Veh, [b]different from Ovx+Veh+Veh, [c]different from Ovx+Veh+bFGF, P<0.05

These findings indicate that prior and concurrent treatment with the anti-resorptive agents estrogen and risedronate does not inhibit the bone anabolic response to treatment with bFGF.

SU430

Risk Factors for Osteoporosis-related Fractures in an Academic Practice: Medical and Biochemical Interventions. B. J. Edwards,[1] K. Halvey,*[2] A. Arseven,*[1] J. Clarke,*[1] J. Kelly,*[1] C. Wilson.*[2] [1]Medicine, Northwestern University, Chicago, IL, USA, [2]Nursing, Northwestern Memorial Hospital, Chicago, IL, USA.

Osteoporosis is a common disease in older Americans, with estimated prevalence of over 50%, however diagnosis rates are still low (<15%). Physicians who care for older patients may greatly impact clinical outcomes by preventing disabling vertebral and hip fractures. The goal was to identify risk factors and evaluate the tolerability of medications and mechanical intervention in this group of older patients during 1998- 2000. Patient education was emphasized as a means for adequate medication administration. Tolerability of bisphosphonates (weekly alendronate and risedronate) and receptivity of hip protectors and physical therapy was evaluated.Methods: physicians and nurse practitioners reviewed fracture trials and literature on hip protectors and gait training. Patient education materials, and

algorithms were developed. Patients seen in the older adult services at Northwestern Memorial Hospital had an osteoporosis evaluation as part of their medical care. Physician reminders initially used. Individualized education was performed by the nurse practitioner and written instructions were used. In cases of memory loss (dementia) instructions were given to family or caregiver. Compliance was assessed by nurse practitioner. Elderly patients seen in an academic older adult practice have a high number of risk factors for hip fractures, namely low bone density, history of fractures and gait disorders (falls). Osteoporosis awareness increased in all physicians. Alendronate on a weekly basis and risedronate were well tolerated by patients. Compliance with the use of hip protectors and physical therapy was noted in the majority of cases.

Results

Sex	**Female n=76**	**Male n=12**
Age Height (inches) Weight (lbs)	80 +/-7 yr; 60 +/-3 in.; 129 +/- 25 lb	77 +/-7 yr; 70 +/-3 in.; 159 +/-28 lb
BMI (Kg/m2)	26 +/-5	27 +/-4
Spine BMD gm/cm2;	0.80 +/-0.15; T = -2.2 +/- 1	0.85 +/-0.2; T = -2.3+/- 1
Femoral neck BMD (gm/cm2); score	0.68 +/- 0.1; T = -2.4 +/-1	0.68 +/-0.1; T = -2.1 +/-0.6
History of fracture	42%	50%
Gait Disorder (%	63%	73%
Folstein MMSE	26 +/- 5	28 +/- 2
Months of treatment	10 +/-2	9 +/-3
Side effects	3/76 (CP, heartburn	1/12 (CP/heartburn)

Conclusion: Older adults have a number of risk factors for osteoporosis-related fractures. Comprehensive recommendations including medication use, activity and safety issues may be made in an effort to reduce hip fracture risk. These measures are well received and tolerated by older patients.

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SU431

Practice Variations Among US Physicians in the Diagnosis, Prevention, and Treatment of Osteoporosis in Postmenopausal Women: A Systematic Literature Review. T. Y. Kuo,[1] T. C. Gallagher,*[2] L. Gelberg,*[1 1]Department of Family Medicine, UCLA, Los Angeles, CA, USA, [2]Department of Community Health, University of Illinois, Champaign, IL, USA.

Osteoporosis is a major US public health problem. Despite recent advances in pharmacological treatments and bone density screening, and the establishment of national guidelines for the diagnosis, prevention, and treatment of this disease in postmenopausal women, there is little evidence to suggest that US physicians are following these standards. One potential explanation for this may be that differences in provider characteristics could contribute to large practice variations in care. We conducted a systematic literature review to examine this possibility. This review was conducted based on a search of the MEDLINE database (period: January 1980 to April 2001) using the MESH terms OSTEOPOROSIS, PHYSICIANS, and PRACTICES, and the bibliographies of pertinent articles identified from the search. The consistency and reliability of the search was established using two different electronic search engines that produced similar results. The initial search yielded 78 articles. Of these, only studies that met the following criteria were selected for detailed review: 1) studies published as observational surveys or randomized-controlled trials in peer-reviewed English-language journals; 2) studies that included comparison groups of physicians from different disciplines; 3) studies including only US physicians; and 4) studies that were considered clinically relevant by experienced clinicians who manage osteoporosis in women. We rated the methodology of each study using a method quality screen and considered only those that met more than 60% of the criteria. This review is not a meta-analysis. Of the initial 78 articles, only nine studies met the review criteria. The response rates for the various survey studies range from 45% to 75% and the sample sizes range from 50 to 664. Results of the review suggest that large practice variations exist among US physicians. For example, adherence with recommended standards for prescribing therapies for osteoporosis (range 5% to 80%) and ordering bone density screening (range 12% to 40%) was demonstrated to be highly variable among US physicians. Additionally, provider characteristics such as gender, years in practice, and specialty were noted to greatly affect prescribing rates of various therapies. These findings suggest that provider characteristics are important contributing factors to large practice variations among US physicians. Thus, in order to improve care of osteoporosis in postmenopausal women, more research is needed to elucidate how these provider characteristics affect medical decision-making.

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The Therapeutic Effects of the Bisphosphonate Evaluated by Quantitative Ulstrasonography in Postmenopausal Osteoporosis. Y. Rhee,*[1] H. Park,[2] K. Park,[3] S. Lim.[1] [1]Internal Medicine, Medical School of Yonsei University, Seoul, Republic of Korea, [2]Internal Medicine, Samchuk Hospital, Samchuk, Republic of Korea, [3]Department of Obstetrics and Gynecology, Medical School of Yonsei University, Seoul, Republic of Korea.

There have been many therapeutic modalities against the postmenopausal osteoporosis with variable results reported. Especially classic hormone replacement therapy and recent application of the bisphosphonates showed not only the increase in the bone mineral density but also significant decreased incidence of the fractures. However, those therapeutic effects were mainly evaluated by the dual energy X-ray absorptiometry(DEXA) which is usually known to reflect the changes in the trabecular bones. As the fractures involving cortical bones such as Colles' fracture is not uncommon, we measured the bone mineral density by quantitative ultrasound to see the effects of the bisphosphonates against the osteoporotic bones comparing with the DEXA method. Eighty female patients who were diagnosed to have postmenopausal osteoporosis showing more than -2.5 standard deviation of BMD by the DEXA were enrolled. They were treated with 10 mg of alendronate daily. The BMD were measured on the initial visit and after 12 months by DEXA, and on the 3rd, 6th, and 9th months by QUS. The changes of BMD or SOS was calculated as the last BMD or SOS minus initial BMD or SOS divided by initial BMD or SOS.The changes of BMD by QUS using T-score of the speed of sound(SOS) were -0.09%, -0.40%, 4.85% on the radius and -0.76%, 2.59%, 1.79% on the tibia measured on the 3rd, 6th, and 12th months respectively. The changes of BMD measured by DEXA between the 12 months of treatment showed 10.8% increase in the lumbar spine, -0.1% in the femoral neck, 9.64% in the Ward's triangle, and 5.59% in the trochanter of the femur. In conclusion, the increase rate of BMD were much slower in the value of BMD by QUS meaning DEXA might be better in evaluating the effects of the bisphosphonates rather than QUS.

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Development and Evaluation of a Decision Aid for Women with Established Osteoporosis. A. Cranney,[1] A. M. O'Connor,*[1] M. J. Jacobsen,*[1] P. Tugwell,*[1] J. D. Adachi,[2] D. S. Ooi,[3] R. Goldstein,*[1] L. Waldegger,*[4] V. Hum,*[4] G. Wells.*[1 1]Ottawa Health Research Institute; University of Ottawa, Ottawa, ON, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]University of Ottawa, Ottawa, ON, Canada, [4]Ottawa Health Research Institute, Ottawa, ON, Canada.

The purpose of this study was to develop and evaluate the impact and acceptability of a decision aid for postmenopausal women with osteoporosis who are considering options to prevent bone loss and fractures. Patients find it difficult to assimilate the vast amount of knowledge available on osteoporosis treatment options, and make a decision during a clinic visit. We have developed an evidence-based decision aid based on the Ottawa Decision Support Framework, and incorporated results derived from our Cochrane systematic reviews. The decision aid is designed to assist women with decisions about multiple therapeutic options and it is to be reviewed prior to their visit with their practitioner. The decision aid consists of an audio-guided workbook outlining information on lifestyle and therapeutic options for osteoporosis and their outcomes, steps in decision making, and a personal worksheet summarizing womens' personal perception of risk; values; and decision predisposition. Eighteen postmenopausal women with osteoporosis (T score ≤ -2.5 SD) participated in a before/after pilot test of the decision aid. Outcomes used to evaluate the decision aid included: knowledge of osteoporosis, expectations of osteoporosis outcomes and benefits of therapies; decisional conflict; and acceptability. Outcomes were analysed using Wilcoxon Rank sum test for non-parametric data and paired t-tests for parametric data. The mean age of the women was 61 years and the mean bone density T score was -3.0 SD. Most women found the decision aid balanced, useful in making a decision and acceptable in length. After using the decision aid, women had significantly reduced decisional conflict scores ($p < 0.009$), significant increases in knowledge from 47% to 83% ($p<0.0001$), and increases in realistic expectations of osteoporosis outcomes and benefits of therapies from 17% to 56% ($p<0.0001$). Our decision aid shows promise as an adjunct to counseling women who are deciding about osteoporosis therapy. Evaluation of long-term adherence to chosen therapy will be conducted in a randomized control trial.

SU434

Prevalence of Low Bone Mass Among Postmenopausal Chinese Canadian Women Attending Primary Care Clinics. A. M. Cheung, F. Chan,* R. Chaudhry,* A. Shik,* N. Forde.* Women's Health and Osteoporosis Programs, University Health Network and, Dept of Medicine and Public Health Sciences, University of Toronto, Toronto, ON, Canada.

The purpose of our study is to determine the prevalence of low bone mass among postmenopausal Chinese Canadian women seen in primary care settings. We recruited through 7 primary care practices serving large populations of Chinese immigrants. These practices were distributed across the Greater Toronto Area, in areas with high concentration of Chinese immigrants -- Scarborough, Richmond Hill, Markham, North York, and downtown Toronto. On randomly selected days, our interviewer would go to the clinic, and recruit and interview consecutive eligible patients. We collected data on demographics, risk factors for osteoporosis, calcium intake, physical activity, medication use, comorbid conditions, and fall and fracture history. We also collected data on height and weight and measured calcaneal bone density using the Sahara ultrasound device (Hologic, Inc).A total of 148 postmenopausal women (mean age = 64.6; range = 44 to 86) were recruited. On average, these women have been in Canada for 11.3 years (range = 2 to 57 years). Twenty percent reported being diabetic; 63% reported having cardiovascular disease; 6% reported having either kidney or liver disease; and just under 10% reported having osteoporosis. Mean age at menarche was 14.2 years. Mean age at menopause was 48.2 years. 61.9% of these women breastfed their children. Their mean height and weight were 60 inches and 121 lbs. Using Sahara ultrasound, the mean BUA was 70.3, mean SOS was1532.5, and mean calculated BMD was 0.46813 g/cm, with a mean T-score of -1.006. One in twelve women has T score of -2.5 or below and 48% have T-scores between -1 and -2.5. Physical activity score, obtained using a validated physical activity questionnaire for the elderly, was 3.5, 3.1 and 2.0 for women in the normal, osteopenic and osteoporotic ranges. Overall, 29% are on HRT, 14% are on bisphosphonates, 12% are taking calcium supplements and 3% are taking vitamin D. Those who self-reported being osteoporotic were more likely to be taking medication (64%) than those whose measured BMD was in the osteoporotic range (25%). The same was true of calcium supplements (21% vs. 16%) and vitamin D (7% vs. 0%). In summary, the prevalence of osteoporosis, as measured by calcaneal bone density, among our sample of Chinese Canadian women is lower than that found among postmenopausal Canadian women generally. Of those who self-reported being osteoporotic, 29% had T-scores in the osteoporotic range and 57% in the osteopenic range. Also, very few women in the sample were taking calcium or vitamin D supplementation regardless of BMD.

Disclosures: Eli Lilly and Co.,2; Novartis,2; Procter and Gamble Pharmaceuticals,2.

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Measurement of Urinary Helical Peptide (alpha-1(I) 620-633) to Predict Long-Term Response to Alendronate Therapy in Elderly Women. P. He,*[1] M. J. Cerelli,*[1] D. K. Jenkins,[1] S. L. Greenspan.[2] [1]Quidel Corporation, Santa Clara, CA, USA, [2]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

Measurement of urinary Helical Peptide (uHelPep), a new marker of bone resorption was evaluated for early prediction of changes in bone mineral density (BMD) in response to alendronate therapy in elderly women. Measurements of bone markers for early detection of effectiveness and monitoring of alendronate therapy for postmenopausal osteoporotic women have been previously reported. The pyridinium crosslinks and related peptides that reflect degradation of the ends (or telopeptide regions) of the collagen molecule are the most widely reported markers. The helical peptide, a novel marker derived from the central triple helix region of collagen type I alpha-1 chain residues 620-633, was isolated and identified from the urine of a Pagetic patient. We have developed a competitive ELISA to measure HelPep in urine.In a community-based study spanning 2.5 yr, 120 ambulatory elderly women were randomized to receive either placebo or alendronate in a double-blind trial of the efficacy of alendronate for preserving or increasing bone mass. The initial 5 mg/day dosage of alendronate increased to 10 mg/day at 18 months. Urine samples and BMD data were collected every 6 months. UHelPep/Creatinine levels were determined in a randomly selected subset of the study (19 placebo- and 19 alendronate-treated) and compared to BMD at 6-month intervals. Following alendronate treatment, the mean uHelPep/Cr change from baseline was -60% (SEM 6.9%) by the first 6-month timepoint and -86% (SEM 2.5%) by the 30-month timepoint. At 6 months, the decreases in uHelPep/Cr were highly correlated with long-term increases in AP spine BMD (r=-0.73, p=002) at 2.5 yr in patients receiving alendronate therapy. For combined placebo- and alendronate-treated subjects, changes in uHelPep/Cr levels at 6 months were strongly correlated with changes in both total hip BMD and AP spine BMD at 24 and 30 months (TH BMD: 24 mo: r=-0.61, p=0.0002; 30 mo: r=-0.64, p=0.0001: AP spine BMD: 24 mo: r=-0.40, p=0.02, 30 mo: r=-0.65, p<0.0001). In conclusion, short-term changes in uHelPep/Cr levels can predict subsequent long-term changes in BMD in elderly women receiving alendronate therapy. UHelPep, the first peptide marker derived from the central triple helix of collagen I, has proven to be a dynamic marker of collagen degradation and has demonstrated utility to assess skeletal health.

SU436

Effects of Two Herbal Extracts on the Bone Metabolism. K. Oh,*[1] S. Kim,*[1] J. Kim,*[2] S. Ko,*[3] H. Kim,[4] J. Baek,[5] H. Ryoo,[6] J. Kim.[3] [1]OCT Inc., Cheonan-si, Republic of Korea, [2]Dept. of Oral Histology, Dankook University, Cheonan-si, Republic of Korea, [3]Dept. of Oral Biochemistry, Dankook University, Cheonan-si, Republic of Korea, [4]Dept. of Oral Anatomy, Seoul National University, Seoul, Republic of Korea, [5]Dept. of Pharmacology and Dental Therapeutics, Seoul National University, Seoul, Republic of Korea, [6]Dept. of Oral Biochemistry, Kyungpook National University, Taegu, Republic of Korea.

The present study was performed to investigate whether two herbal extracts play roles in the bone metabolism. We examined cellular activities of osteoblasts by measurement of cell proliferation rate, alkaline phosphatase(ALP) activity, mRNA expression of osteopontin(OPN), ALP and collagen(COL), Cbfa1 expression, and osteoprotegerin(OPG) secretion. Osteoclast activity was assayed by measuring TRAP activity, and the number and area of resorption pits after culture of osteoclast precursor cells on the calcium-phosphate coated culture dish (OAAS, OCT Inc.). There was an maximum 300% increase in proliferation rate of osteoblastic cells (MG63) after treatment with fraction RCb1M, and 15% increase with fraction RGa1W, when compared to the control. RCb1M- and RGa1W-treated osteoblastic cells (HOS, human osteosarcoma cell) showed a statistically significant increase in ALP activity. RCb1M at day 3, and RGa1W at day 14 increased the COL, ALP and OPN mRNA expression from HOS cells. Also both of fractions increased the expression of the Cbfa1 gene, which was detected by p6xOSE2-Luc-transient transfected ROS and C2C12 cells. Secretion of OPG, which was detected from MG63 cell supernatant by western blot analysis, showed marked increases after treatment of RCb1M as well as RGa1W. RGa1W showed significant decreases in the number of TRAP (+) multinucleated cells and area of resorption pits. Taken together, RCb1M and RGa1W stimulate the proliferation and bioactivities of bone-forming osteoblasts, and inhibit activities of bone-resorbing osteoclasts. Also, in vivo studies using ovariectomy-induced osteoporotic rat experimental models revealed that these fractions increased the thickness of cortical bone and trabecules of tibia. We believe that these fractions contain effective substances that have the potential to overcome osteoporosis.

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TSE-424, a Novel Tissue Selective Estrogen, Reduces Biochemical Indices of Bone Metabolism in a Dose Related Fashion. S. Ronkin,* L. Clarke,* P. Boudes,* G. Constantine.* Wyeth Ayerst Research, Radnor, PA, USA.

Purpose: TSE-424 has demonstrated estrogen agonist effect on the skeleton and on lipid metabolism without estrogen antagonist effect on breast cancer cells or endometrium in preclinical models. This trial explores the effects of TSE-424 on biochemical indices of bone metabolism. Methods: A prospective, randomized, double-blind placebo-controlled clinical trial enrolled a total of 494 healthy postmenopausal women and evaluated 3 doses of TSE-424, administered daily for 3 months. Raloxifene (60 mg) served as an active control. All treatment groups received calcium (600 mg/day). Markers of bone resorption and formation were measured at 3 months. Results: TSE-424 produced a dose-related decrease in markers of bone remodeling after 3 months of treatment. Doses as low as 5 mg daily resulted in statistically significant reductions in markers as compared to placebo. Treatment with raloxifene (60 mg) also resulted in statistically significant reductions in markers as compared to placebo. TSE-424 and raloxifene were well tolerated.Conclusion: This controlled clinical trial demonstrates dose-related reductions in markers of bone remodeling with low doses of TSE-424. Higher doses of TSE-424 are currently under study.

Disclosures: Wyeth Ayerst Research,3.

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Effect of Estrogen Injection on Bone Mineral Contents in the Ovariectomized Rats Fed Normal or Low Calcium Diet. Y. S. Lee,*[1] M. S. Kim.*[2] [1]Food and Nutrition, Seoul National University, Seoul, Republic of Korea, [2]Seoul National University, Seoul, Republic of Korea.

Estrogen deficiency in post menopause results in a marked loss of bone, that can be prevented by estrogen replacement and ameliorated by increased dietary calcium. This study was conducted to investigate how estrogen injection could reduce bone loss in reference to estrogen deficiency with different dietary calcium levels. Nine week-old female rats (Sprague-Dawely) were ovariectomized and then injected estrogen subcutaneous three times a week as dose 10μg/kg BW. The rats were fed diets containing of normal (0.5%) or low (0.1%) Ca. Serum Ca and P level were not changed by ovariectomy and estrogen injection. Serum alkaline phosphatase activity and urinary hydroxyproline level increased in ovariectomized rats and decreased only in the rats fed normal Ca diet after estrogen injection. Serum tartrate-resistance acid phosphatase activity has not shown any charge. Breaking force of femur has shown a tendency to decrease in the rats fed low Ca diet, but it does not seem to hold any statistical significance. Bone minerals (Ca and P) contents decreased in ovariectomized and increased significantly in the rats injected estrogen and fed normal Ca diet. This result suggests sufficient Ca intake and estrogen injection may protect bone loss due to post menopause.

SU439

Effect of Isoflavone and/or Estrogen Addition on Bone Mineral Contents in the Ovariectomized Rats. M. S. Kim,*[1] Y. S. Lee.*[2] [1]Food and Nutrition, Seoul National University, Seoul, Republic of Korea, [2]Seoul National University, Seoul, Republic of Korea.

Estrogen replacement is a widely accepted therapy for postmenopausal bone loss and associated increased incidence of bone fracture. Due to a possible risk of estrogen dependent reproductive cancer, it is advisable for public health to search for an alternative to estogen replacement therapy, such as dietary isoflavone. This study was conducted to investigate whether the soy isoflavone intake and/or estrogen injection could reduce postmenopausal bone loss and replace estrogen therapy by the use of ovariectomizd rat model fed on low-Ca diet. Nine week-old female rats (Sprague-Dawely) were ovariectomized and then fed low (0.1%)-Ca diet with isoflavone supplementation (80 or 160ppm). Some ovariectomized rats were also fed the same diet and injected estrogen subcutaneous. Serum Ca and P levels were normal in all the rats. Serum alkaline phosphatase activity increased by ovariectomy operation and decreased only in the rats fed isoflavone 160ppm diet and injected estrogen. Serum tartrate-resistance acid phosphatase activity and urinary hydroxyproline level diet not show any difference between control group and experimental groups. Bone minerals (Ca and P) contents decreased in ovariectomized and increased in the rats fed isoflavone 80ppm supplementation diet or the rats fed the diet without isoflavone and injected estrogen. Therefore, the effect of isoflavone 80ppm supplementation was similar to that of estrogen injection, but any beneficial effect of isoflavone intake and concurrent estrogen injection has not been observed. This result suggests that appropriate isoflavone supplementation (80ppm in this study) can prevent postmenopausal bone loss without estrogen injection and may serve as an alternative to estogen therapy.

SU440

Raloxifene Attenuates Bone Loss in Mechanically Unloaded, Ovariectomized Female Rats. M. R. Allen,[1] J. L. Stafinsky,*[1] C. Nolan,*[2] S. A. Bloomfield,[1] C. L. Smith.[2] [1]Health and Kinesiology, Texas A&M University, College Station, TX, USA, [2]Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA.

Mechanical unloading results in loss of bone mineral density (BMD) and is associated with an increased risk of fracture. Raloxifene is a selective estrogen receptor modulator (SERM) which inhibits bone loss associated with reductions in circulating sex steroids. To determine if raloxifene could alleviate unloading-induced bone loss, we examined the ability of raloxifene, in comparison to estradiol or placebo, to maintain BMD in 5-month old, ovariectomized (OVX) virgin female Sprague Dawley rats subjected to 28 days of hindlimb suspension (HLS). After OVX, animals were allowed to recover for ~four weeks and then randomized into raloxifene (R; 535 μg/day; n=10), 17beta-estradiol (E2; 12 μg/day; n=9) or placebo (n=8) treatment groups. Hormones were administered via slow release pellets implanted immediately prior to the initiation of HLS. The tibia proximal metaphyses (total slice BMD, and cortical and cancellous compartment BMD) were assessed *in vivo* by peripheral quantitative computed tomography (pQCT; Stratec Research-M) prior to OVX, prior to HLS and post-HLS. At the conclusion of the unloading period, serum, urine and tissues were collected for analyses. Uterine wet weights in E2-treated rats were 4-fold greater than in placebo controls confirming appropriate hormone administration; raloxifene treatment was without effect. Urinary pyridinium cross-links and serum osteocalcin values were 50.4% and 39.3% lower in R and 31.0% and 30.0% lower in E2 treatment groups, respectively, in comparison to placebo indicating a reduction in bone turnover. Animals lost an average of 13.0% of total BMD at the proximal tibia as a result of OVX prior to HLS. Subsequent treatment with either R or E2 maintained total BMD over the period of HLS, while total BMD loss of 12% occurred in control animals. Interestingly, trabecular BMD loss was detected for all groups (placebo -30.4%, R -24.3% and E2 -19.6%), while cortical BMD was increased in R (+3.2%) and E2 (+3.5%) treated, but not placebo (-0.7%) groups. *Ex vivo* pQCT measurements of excised femur distal metaphyses also indicated that total BMD was greater in R (+16.5%) and E2 (+31.0%) groups than in placebo

controls although this was due largely to an increase in trabecular and not cortical BMD. These results indicate that treatment of ovariectomized, unloaded female rats with raloxifene or estradiol alleviates loss of BMD associated with mechanical unloading, and suggests that estrogen receptor based therapies may have site-specific effects on the cortical or trabecular compartments depending on the skeletal site examined.

SU442

Serum Placental Protein-14 (PP14): A Novel Marker of SERM Action in Postmenopausal Women. L. B. Tanko, L. Warming,* Y. Z. Bagger, I. Byrjalsen,* C. Christiansen. Center for Clinical and Basic Research, Ballerup, Denmark.

Selective estrogen receptor modulators (SERMs) may have a great future as a widely prescribed medication for postmenopausal women based on their protective effects against the development of osteoporosis, breast cancer and cardiovascular diseases. However, at present no easy-to-do laboratory test is available that could assist clinical trials or the general clinical practice in determining the optimal dosing of SERMs. Therefore, the aim of the present study was to investigate whether serum PP14, a well-known endometrial secretory protein, could serve as a marker of SERM action in postmenopausal women. Serum samples obtained from two prospective, double-blinded, placebo-controlled SERM trials were used for the analysis. Participants of trial I received treatment with various doses of raloxifen (30,60,150 mg/day), whereas those of trial II received treatment with various doses of levormeloxifen (1.25,5,10,20 mg/day). Serum PP14 and endometrial thickness at baseline and after 6, 12 and 24 months were measured by radioimmunoassay and transvaginal ultrasound, respectively. During the treatment period, serum PP14 and endometrial thickness showed no significant changes in the placebo groups. After 6 months of the treatment, 150 mg, but not 30 and 60 mg, raloxifene induced a significant increase in serum PP14. Levormeloxifen, in all tested doses, induced significant increases. These increases were all of similar degree and significantly more pronounced than that induced by 150 mg raloxifen ($p<0.001$). After 6 month, raloxifen induced dose-dependent, but clinically non-significant increases in endometrial thickness (max. increase to 150 mg raloxifen ~50%). Levormeloxifen, however, frequently induced clinically significant increases in endometrial thickness independently of the dose used (max. increase ~260%). In both clinical trials, the drug-induced changes in serum PP14 showed significant correlation with the respective changes in endometrial thickness. The correlation between SERM-induced changes in endometrial thickness and serum PP14 were characterized by an r value of 0.62 ($n=195$, $p<0.0001$). These observations with serum PP14 are in line with previous studies reporting on other biological effects (e.g. bone and lipid metabolism, adverse effects) of these doses of raloxifen and levormeloxifen. Based on these results, we propose the PP14 assay as a useful tool for the monitoring of SERM action that could thereby assist both the drug industry to plan clinical trials with upcoming SERMs as well as the clinical practice engaged with treatment of postmenopausal women with currently available SERMs.

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Relationships Between Age and Prior Use of HRT on Vertebral Fracture Risk Reduction with Raloxifene in Postmenopausal Women: Results from the MORE Trial. O. Johnell,[1] J. A. Kanis,[2] S. Sarkar,*[3] W. Wu,*[3] C. de Laet,*[4] A. Oden,*[5] I. Pavo.*[6] [1]Dept. of Orthopedics, Universitetssjukhuset MAS, Malmo, Sweden, [2]Dept. of Human Metabolism and Clinical Biochemistry, University of Sheffield, Sheffield, United Kingdom, [3]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA, [4]Dept. of Orthopedics, Institute for Medical Technology Assessment, Rotterdam, The Netherlands, [5]Statistical Consultant, Gothenberg, Sweden, [6]Lilly Area Medical Center, Vienna, Austria.

The aim of this study was to determine whether age or prior use of hormone replacement therapy (HRT) influenced the effectiveness of raloxifene (RLX) on vertebral fracture (VF) risk. The effects of RLX 60 mg/daily were studied in the MORE (Multiple Outcomes of Raloxifene Evaluation) trial in postmenopausal women ≤80 years old, mean age 66.5 years) with osteoporosis, randomized to placebo (N=2576) or RLX 60 (N= 2557). Separate logistic regression models analysed the relationships between VF risk and age, or VF risk and prior use of HRT. The models estimated the risk of having at least 1 new VF at 3 years for RLX- or placebo-treated patients, with age as a continuous variable or prior use of HRT as a categorical (yes/no) variable. RLX significantly decreased VF risk in women of all ages. Younger women at the lower third percentile of age had a somewhat greater reduction in VF risk (43%) with RLX than older women. The interaction effect for age and treatment was significant ($p=0.095$).

Percentile	Age (yr)	Relative Risk (95% Confidence Interval)
33rd	63.6	0.57 (0.41, 0.73)
50th	66.7	0.62 (0.48, 0.76)
67th	70.0	0.68 (0.53, 0.83)

Prior use of HRT was associated with a greater effect on VF reduction. The interaction effect for age and treatment was significant ($p=0.066$). The RR was 0.47 (95% CI 0.31, 0.70) in women with prior HRT use, and 0.72 (95% CI 0.58, 0.91) in women with no prior HRT use. In women in the lowest tertile for age, the RR for ≥1 new VF at 3 years was 0.23 (95% CI 0.08, 0.66) in women with prior HRT use and 0.58 (95% CI 0.35, 0.95) in women with no prior HRT use (interaction p= 0.096). We conclude that raloxifene 60 mg/day decreases the risk of new vertebral fractures at 3 years, and may have a greater effect in younger postmenopausal women, particularly in women younger than 63.6 years who had previously taken HRT.

Disclosures: Eli Lilly andCompany,1,2,3,5,8.

SU444

Effects of Tibolone and Combined 17 Beta Estradiol and Norethisterone Acetate (NETA) on Serum C-Reactive Protein (CRP) in Healthy Postmenopausal Women. P. Garnero,[1] C. Roux,[2] C. Benhamou,*[3] C. Pelissier,*[4] C. Jamin.*[5] [1]Inserm Unit 403, Synarc, Lyon, France, [2]Hôpital Cochin, Paris, France, [3]Hôpital Porte Madeleine, Orléans, France, [4]Hôpital Hôtel Dieu, Paris, France, [5]Hôpital Bichat, Paris, France.

Serum CRP is an independent risk factor for the development of cardiovascular diseases. Recently it has been shown that conjugated equine estrogen given to postmenopausal women markedly increases serum CRP levels (Walsh et al., 2000). The aim of this study was to compare the effects of tibolone, a tissue specific steroid with combination of estrogenic, androgenic and progestogenic properties and a combination of 17 beta estradiol and NETA (E2+NETA) on serum CRP in healthy postmenopausal women. One hundred and thirty nine postmenopausal women (mean age: 55 yr: 44-68 yr) were randomly assigned to receive tibolone (1.25 or 2.5 mg/day) or 17 beta estradiol (2mg/day) plus NETA (1 mg/day) for 2 years. Serum CRP, assessed with an ultrasensitive assay (detection limit, 0.2 mg/L), was measured at baseline, 6 months, 12 months and 24 months. The table represents the median (interquartile) changes from baseline expressed in percentage (%) and absolute levels (Abs.) after 6, 12 and 24 months of treatment.

*p<0.0001, p<0.05 vs baseline

Treatment group	6 months change		12 months change		24 months change	
	%	Abs.(mg/L)	%	Abs.(mg/L)	%	Abs.(mg/L)
Tibolone 1.25 (n=52)	+106* (223)	+0.65* (1.00)	+70* (202)	+0.40* (0.95)	+129* (320)	+0.75* (2.80)
Tibolone 2.5(n=39)	+89** (217)	+0.75** (1.20)	+104** (261)	+0.90** (1.80)	+73** (199)	+0.65** (1.55)
E2+NETA (n=48)	+139* (344)	+0.90* (1.90)	+129* (203)	+0.90* (2.00)	+133* (198)	+0.80* (1.38)

No significant differences in the increase of serum CRP levels between groups was observed at any time points. The median increase at 6 months for tibolone and E2+NETA was comparable to the 84% increase reported previously for 0.625 mg/day conjugated estrogen. Exclusion of patients with infection, women undergoing surgery and women with a CRP level at baseline above 10 mg/L (n=10) did not alter the results. In conclusion, we found that tibolone and E2+NETA significantly increased serum CRP levels to a comparable extent. Relationship between elevated CRP levels with tibolone and E2+NETA and cardiovascular disease events require further studies

SU445

Hormone Replacement Therapy in Osteopenic Postmenopausal Women with Systemic Lupus Erythematosus - Bone Mineral Density and Biochemical Markers of Bone Turnover at Baseline. H. P. Bhattoa,[1] E. Kiss,*[2] P. Bettembuk,[1] A. Balogh.[1] [1]Regional Osteoporosis Center, Dept. of Obstetrics and Gynecology, University of Debrecen, Debrecen, Hungary, [2]3rd Dept. of Internal Medicine, University of Debrecen, Debrecen, Hungary.

Although there are valid concerns regarding the use of exogenous estrogens in women with SLE, there are also potential health benefits to be considered. Several salutary effects of postmenopausal estrogens assume particular importance in SLE where the risks of osteoporosis, exaggerated by menopause (natural or cyclophosphamide-induced) and glucocorticoids, are substantial. Moreover, hormone replacement therapy (HRT) is associated with a 40% reduction in the risk of coronary artery disease, higher levels of high-density lipoprotein-cholesterol, and decreased levels of low-density lipoprotein-cholesterol and plasminogen-activator inhibitor type 1, benefits that should be especially applicable to postmenopausal women with SLE. We have planned a prospective examination of BMD and biochemical markers of bone turnover for a year in a placebo controlled double blind study. Postmenopausal SLE women have been randomly allocated to placebo or treatment group (50 µgram transdermal estradiol). Both groups receive 5 mg continuos oral medroxyprogesterone acetate, 500 mg calcium and 400 IU vitamin D_3.The patients confirmed to the ACR SLE criteria, T score < -1.0 and had no other disease influencing bone metabolism. Exclusion criteria were: risk factors for thrombo-embolism, serious liver or kidney disease, vaginal bleeding of unknown origin, breast tumor, endometrial carcinoma, endometriosis. Follow-up visits are planned at 3, 6, 9 and 12 months. A total of 35 postmenopausal SLE women (mean age (years) 54 ± 8.9; BMI (kg/m^2) 28 ± 4.1; SLEDAI 3.5 ± 3.1; SLICC 3.28 ± 2.04; daily corticosteroid dose (mg/day) 6 ± 4.5) have been enrolled in the study. The baseline results ($n = 35$) are as follows: L_1-L_4 BMD (gm/cm^2) 0.815 ± 0.130; femur neck BMD (gm/cm^2) 0.680 ± 0.119; serum crosslaps (ng/ml) 0.56 ± 0.13; serum osteocalcin (ng/ml) 22.3 ± 14.2.Hormone replacement therapy has been initiated in patients with SLE applying strict inclusion and safety criteria.

SU446

Effect of Raloxifene Hydrochloride (RLX) on Bone Mineral Density (BMD) and Bone Turnover Markers in Diabetic Women: The Multiple Outcomes of Raloxifene Evaluation (MORE) Trial. K. E. Ensrud,[1] J. A. Cauley,[2] L. Zhou,*[3] T. M. Mason,*[3] P. J. Bowman,*[1] K. D. Harper.[3] [1]VA Medical Center & Univ of MN, Minneapolis, MN, USA, [2]Univ of Pittsburgh, Pittsburgh, PA, USA, [3]Lilly Research Laboratories, Indianapolis, IN, USA.

Findings from a number of longitudinal studies suggest that older women with diabetes mellitus (DM) have higher rates of bone loss in spite of having higher BMD than non-diabetic women. However, the effect of antiresorptive treatment on bone loss and bone turn-

over in diabetic women is uncertain. To test whether RLX has similar effects on bone in women with and without DM, we analyzed data from MORE, a randomized trial that enrolled 7705 postmenopausal women with osteoporosis aged ≤80 years. Women were randomized to RLX 60 mg/d, RLX 120 mg/d, or placebo (PL). DM was defined at baseline by ≥1 of the following criteria: self-report, use of hypoglycemic agents or insulin, and fasting plasma glucose ≥ 7.0 mmol/L. We compared the effect of RLX (combined doses) vs. PL on mean % changes in BMD and median % changes in bone turnover markers from baseline to 36 months within subgroups defined at baseline by DM; 7253 women (94%) had a baseline and ≥1 follow-up measurement. At 36 months, among diabetics, RLX increased mean BMD at the spine by 2.3% and hip by 1.5% compared to PL. Similarly, among non-diabetics, RLX increased mean BMD at the spine by 2.6% and hip by 2.2% compared to PL. There was no evidence of an interaction between DM status and treatment (p>0.53 at spine, p>0.62 at hip). RLX reduced serum osteocalcin (OC) to a similar degree among diabetics and non-diabetics. Median reductions in urinary C-telopeptide excretion (Ntx) were also greater in the RLX group compared to PL, but the comparison was significant only among non-diabetics. There was no evidence of an interaction between DM status and treatment (p>0.85 for OC, p>0.68 for Ntx). Our findings were similar when the analyses were performed for each RLX dose group vs. PL.

	DM (n=270)		No DM (n=6983)	
	PL (n=90)	RLX (n=180)	PL (n=2344)	RLX (n=4639)
Mean % change in BMD				
Lumbar spine	0.17	2.49 *	0.26	2.86 *
Femoral neck	-0.85	0.67 ^	-0.96	1.29 *
Median % change in turnover				
OC	1.0	-22.9 #	-8.7	-28.7 *
Ntx	12.2	-19.8	-8.3	-33.8 *

*p<0.001 vs. placebo; # p<0.01 vs. placebo; ^ p< 0.05 vs. placebo

We conclude that the effects of raloxifene on BMD and bone turnover are similar among diabetic and non-diabetic women.

Disclosures: Eli Lilly & Co., Inc.,2; Merck, Inc.,2; Roche Global Development- Palo Alto,2; Berlex Laboratories, Inc.,2.

SU447

Combined Ginsenosides and Low Dose of Estrogen Administration Has a Synergetic Effect on Osteopenia of Rats Induced by Ovariectomy. L. Cui,[1] T. Wu,*[1] X. Q. Liu,*[1] Y. Y. Liu,[1] Q. N. Li.[2] [1]Department of Pharmacology, Guangdong Medical College, Zhanjiang City, Guangdong, China, [2]Bone Biology Laboratory, Guangdong Medical College, Zhanjiang City, Guangdong, China.

Ginsenosides as anti-aging drugs are well known through oriental countries. Based on their anabolic pharmacological actions and sex hormones like effects, we had conducted experiments to evaluate the anti-osteoporosis effect of ginsenosides in animal models. Our previous study had first demonstrated that a proper dose of ginsenosides could partially prevent bone loss of estrogen deficiency rats by inhibiting osteoclasts bone resorption but only had a mild depression of bone turnover. The objective of this study was to determine whether low dose of estrogen in combining with ginsenosides can completely prevent bone loss in ovariectomized rats. Methods: Four-month-old ovariectomized rats were treated either with 100 and 300mg/kg/BW of ginsenosides and 30 and 100μg/kg/BW of 17alpha-ethynylestradiol alone, respectively, or combined 100mg/kg/BW of ginsenosides and 30μg/kg/BW of 17alpha-ethynylestradiol administration for 10 weeks. Double in vivo fluorochrome labeling was given. The undicalcified longitudinal proximal tibial metaphyseal sections were cut and stained with Goldner's Trichrome for the bone histomorphometric analysis. Results: The rats lost 74% of bone volume and induced high bone turnover after OVX when compared with the sham group. Bone volume was increased by 205%(15.6% vs 5.1% in OVX) in higher dose estrogen treatment group while it was increased by 105%(10.4% vs 5.1% in OVX) in lower dose, the two doses of estrogen inhibited osteoclasts surface (by -65% and -55%, P<0.01) and decreased bone turnover rate (by -85% and -83%, P<0.01). Higher dose of ginsenosides increased bone volume by 84% (9.4% vs 5.1% in OVX) and decreased bone turnover rate (by -64%, P<0.05) while lower dose of ginsenosides did not effect significantly on the bone histomorphometric indices. However, combined each low dose of ginsenosides and estrogen achieved well preventive effects: increase of 202% in bone volume, decrease of -66% in bone turnover rate and -72% in osteoclasts surface. The combined effect in preventing bone loss equals to that the high dose of estrogen alone did. This results indicate that use of low dose of estrogen plus ginsenosides as a strategy in prevention of osteoporosis not only reduce side effect of estrogen but maintain its power for the treatment effect as well. Ginsenosides do not strongly inhibit bone formation as estrogen dose and this may be benefits to bone qualify. The mechanism of ginsenosides on bone metablism needs further study.

SU448

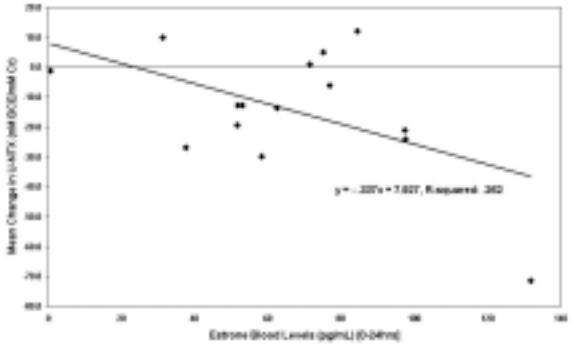
Nongenotropic Activation of MAP Kinases and Prevention of Apoptosis by SERMs in Osteoblasts/Osteocytes: A Property Shared by Hydroxytamoxifene and Idoxifene, but not Raloxifene. S. Kousteni, T. Bellido, L. I. Plotkin, L. Han, R. S. Weinstein, R. L. Jilka, S. C. Manolagas. Division of Endocrinology & Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Estrogens prevent osteoblast and osteocyte apoptosis in vivo and in vitro, most likely by activating a Src/Shc/ERK signaling pathway via an estrogen (ER) or androgen (AR) receptor-mediated nongenotropic action. A shortened working life span of the bone-forming cells, in combination with a prolongation of the life span of osteoclasts, may account for the focal imbalance between bone resorption and formation that follows loss of sex steroids. Based on evidence that selective estrogen receptor modulators (SERMs) are estrogen agonists on bone, we have investigated whether representatives of these compounds have similar or different effects to those of estrogen on Src/Shc/ERK activation and prevention of apoptosis of calvaria-derived murine osteoblastic cells and MLO-Y4 osteocytic cells. Both hydroxytamoxifene (Tam) and idoxifene (Idox) prevented etoposide-induced apoptosis in a dose dependent fashion, at concentrations ranging from 10^{-10} to 10^{-7} M. The same results were reproduced in HeLa cells transiently transfected with the ERα. The effects of the two SERMs on apoptosis were mediated via activation of the Src/Shc/ERK signaling pathway, as the specific inhibitors of the Src and ERK kinases, PP1 and U0126, abrogated both kinase activation and anti-apoptosis. Further, as published before for E_2, the effect of Tam and Idox could be shown in HeLa cells carrying a truncated version of the ERα consisting of the ligand binding domain fused to a membrane localization sequence. In addition, as shown elsewhere in this meeting for E_2, Tam and Idox activated the serum response element in HeLa cells through a MAP kinase-dependent stimulation of Elk-1 binding to this *cis* element. In sharp contrast to Tam and Idox, raloxifene (Ral) at concentrations as high as 10^{-6} M was completely ineffective in all these assays. Nonetheless, as shown by others, at 10^{-8} M, Ral like estradiol suppressed TNFα plus IL-1β-induced IL-6 activity in HeLa cells transiently transfected with the ERα. These results demonstrate that some but not all SERMs mimic the nongenotropic effects of estrogens. Whether the lack of nongenotropic actions of Ral, in the face of estrogen-like genotropic activity, plays a role in its weaker anti-osteoporotic efficacy compared to classical HRT or bisphosphonates, owing to an inability to prolong the life span of osteoblasts and osteocytes, will require further studies.

SU449

Association Between Steady-State Cenestin® (Synthetic Conjugated Estrogens, A) Plasma Estrone Concentrations and U-NTX Biochemical Marker of Bone Turnover in Postmenopausal Women. R. E. Stevens, S. A. Ayres, K. V. Phelps.* Clinical Affairs, Duramed Pharmaceuticals, Inc., Cincinnati, OH, USA.

Biochemical markers of bone metabolism have been shown to correlate to changes in BMD. A reduction in bone turnover markers of about 30% below baseline values has been shown to be associated with maintenance of bone mass in menopausal women treated with anti-resorptive therapy. In menopausal women the primary circulating estrogen is estrone. Limited data is available correlating plasma estrogen concentrations and biochemical markers in menopausal women after ERT. The present study was designed to evaluate the effect of Cenestin, a synthetic conjugated estrogens product on bone turnover in menopausal women. A post-hoc comparison examined the association between biochemical bone marker response and plasma unconjugated estrone (the biological active moiety) concentrations after 3 months of Cenestin. Fifty healthy, menopausal women, 1-3 years after cessation of menses, 40-65 years of age, were randomly assigned to receive either 0.625 mg/day Cenestin (n=35) or placebo (n=15). Fifteen of the 35 women on Cenestin had blood samples taken at Day 90. Blood samples were collected on Day 90 at -24, 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 14, 16, 24 and 36 hrs post dosing. Capillary GC/MS analysis was performed using negative ion chemical ionization with selective ion monitoring to quantitate estrone. The biochemical marker, urinary N-telopeptide of type I collagen (U-NTX) was analyzed by automated ELISA (Vitros Eci, Ortho-Clinical Diagnostics). Overall, Cenestin in contrast to the placebo group, normalized bone turnover to the premenopausal level. The reduction in U-NTX was approximately -34.1% below baseline values. At Day 90, steady-state estrone concentrations for the 15 women on Cenestin averaged 65 pg/mL. Analysis of the data demonstrated a significant negative correlation between steady-state estrone concentrations (Day 90) and U-NTX, Day 90 (r = -0.51, p=0.05). Overall Cenestin was effective in reducing bone turnover in menopausal women. This study reaffirms the clinical importance of monitoring biochemical markers of bone metabolism in the assessment of the efficacy of therapy in the prevention of bone loss in menopausal women. In addition, monitoring estrone concentrations is a supportive adjunct to monitoring the response to ERT.



SU450

Fibroblast Growth Factor-2 Induces a Dual Control of Apoptosis in Human Calvaria Osteoblasts. F. Debiais, E. Hay,* P. J. Marie. INSERM U349, Paris Cedex 10, France.

Recent data indicate that Fibroblast Growth Factor (FGF)/FGF Receptor (FGFR) interactions activate apoptosis in mouse and human calvaria osteoblasts. However, nothing is known on the mechanisms that are involved in the control of apoptosis by FGFs in osteoblasts. In this study, we have determined the effect of FGF-2 on apoptosis and the underlying mechanisms in normal human calvaria osteoblasts and in immortalized human neonatal calvaria (IHNC) cells. Apoptosis was determined by terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) analysis or trypan blue staining. Treatment with rhFGF-2 (50 ng/ml) significantly increased by 22 % the number of TUNEL-positive osteoblastic cells cultured in serum-deprived primary human calvaria cells, at 96 h of treatment ($p<0.02$). However, this late pro-apoptotic effect of FGF-2 was not found at earlier time-points. Indeed, rhFGF-2 had no effect on apoptosis at 72 h. Moreover, rhFGF-2 decreased by 48 % ($p<0.05$) the number of apoptotic cells from 6 to 24 h of treatment, showing that FGF-2 induced a biphasic effect on apoptosis in human osteoblasts. A similar dual effect was found in IHNC cells cultured in serum-deprived conditions. The analysis of mechanisms involved in this dual effect showed that rhFGF-2 (50 ng/ml; 6-96h) did not affect caspase-8 and caspase-9 activities in serum-deprived IHNC cells. In contrast, rhFGF-2 decreased caspase-2 activity by about 20 % ($p<0.05$) at 15-48 h but increased this activity by 35 % ($p<0.05$) at 96 h in IHNC cells. Consequently, rhFGF-2 significantly reduced effector caspase (-3,-6,-7) activity by 18 % ($p<0.05$) at 15-48 h and increased this activity by 25 % at 96 h ($p<0.05$). Further RT-PCR and western blot analyses showed that rhFGF-2 rapidly increased by 2-fold mRNA levels for the anti-apoptotic factor NF-kB at 16-39 h which was followed by a 3-fold increase in NF-kB protein levels at 24-48 h. In contrast, rhFGF-2 decreased the expression of the anti-apoptotic protein Bcl-2 by 2.5 fold at 48 h, whereas Bax levels were unchanged. This study shows that FGF-2 induces a dual effect on apoptosis in human osteoblasts through selective modulation of NF-kB and Bcl-2 levels, leading to biphasic effects on caspase-2 activity, effector caspases and DNA fragmentation. This identifies a novel time-related dual effect of FGF-2 on apoptosis in human calvaria osteoblasts.

SU451

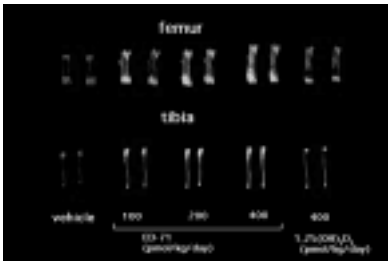
Prevalence of Gsalpha Gene Mutations in Fibrous Dysplasia and Fibrous Dysplasia-like Low-Grade Central Osteosarcoma. K. Pollandt,*[1] C. Engels,[2] E. Kaiser,[1] M. Werner,*[2] G. Delling.[2] [1]Center for Biomechanics, University Hospital Hamburg-Eppendorf, Hamburg, Germany, [2]Bone Pathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany.

Activating point mutations of the Gsalpha gene resulting in the substitution of arginine at position 201 mainly by either cysteine or histidine have been identified as the underlying defect of fibrous dysplasia (FD). Because consecutive studies have indicated that the presence of one of these mutations is a constant finding in FD, evaluation of mutational analysis as a possible tool in the diagnostic process of FD and its differential diagnoses is of interest. Therefore we analysed the presence of the described mutations in the specimen of nine patients with monostotic and one patient with polyostotic FD and in the specimen of five cases of fibrous dysplasia-like low-grade central osteosarcoma (fd-like lgcOSA). Fd-like lgcOSA is one of the most important differential diagnoses of FD because of its low-grade malignant behaviour. Recognition of fd-like lgcOSA is important, because incorrect diagnosis will lead to inadequate therapy, initial surgery will be followed by local recurrence with consecutive increase of the risk of transformation on a tumor of higher grade malignancy with the potential of lung metastases.The paraffin-embedded specimen were obtained from the files of the Hamburg Bone Tumor Registry. The samples were applied in restriction digestion analysis with Nla III, single stranded conformational polymorphism (SSCP) analysis and sequencing. We could demonstrate a R201H mutation in six cases with monostotic FD and a R201C mutation in the remaining three cases with monostotic FD and in the case with polyostotic disease. These results demonstrate that the presence of Gsalpha gene mutations is a constant finding in FD.In fd-like lgcOSA we could demonstrate a lack of mutations in in eight different samples of four cases. In one patient we identified a R201C mtation. This patient did not have a history of proceeding FD at the same or a different location. As our resluts demonstrate a low prevalence of Gsalpha gene mutations in this tumor in contrast to FD, mutational analysis may be an additional helpful parameter in individual cases for the differential diagnosis of FD and fd-like lgcOSA.

SU452

Synthetic Vitamin D Analog, ED-71, Can Cure Rachitic Bone of Hypophosphatemic Mice without Hypercalcemia. H. Tanaka,[1] M. Inoue,[1] Y. Seino.[2] [1]Department of Pediatrics, Okayama University, Okayama, Japan, [2]Okayama University, Okayama, Japan.

X-linked hypophosphatemic vitamin D resistant rickets(XLH) is a most common form of hereditary rickets, which is characterized by profound hypophosphatemia, normocalcemia, hyperphosphaturia. Recent findings indicate that the disorder is due to defective Phex gene product. However, the pathogenetic mechanism has not been fully disclosed. The treatment regimen consists of mainly relatively high dose of active vitamin D and phosphate supplement. This treatment may sometimes be complicated with hypercalcemia, nephrocalcinosis and secondary hyperparathyroidism. ED-71,2b-(3-hydroxypropoxy)1a,25-dihydroxyvitamin D_3, is a synthetic vitamin D analog and has a unique character such as long serum half life. This led us the idea that ED-71 may be a candidate for more effective and less harmful agent for the treatment of XLH. To determine the effects on bone mineral metabolism, Hypophosphatemic mice (Hyp), 4 weeks of age, were assigned to six groups. Group 1 was the vehicle control. Group 2-5 were given ED-71 at 40, 100, 200, 400 pmol/kg of body weight (BW) everyday for 3 weeks. Group 6 was treated with 1,25-dihydroxyvitamin D3 at 400 pmol/kg of body weight. After 3 weeks treatment, bones were retrieved and bone morphometric analysis was performed. Group 2-5 did not show significant hypercalcemia after 3 weeks treatment of ED-71 whereas Group 6 demonstrated hypercalcemia (12.8 mg/dl). Similarly serum phosphorus level did not show significant differences among group2-4. Group 5 and 6 demonstrated significant high levels of serum phosphorus (8.7, 15.4 respectively). As shown in figure, the bones from Group3-5 and 6 showed increased BMD. Moreover, Group 6 showed lower BMD than Group 3-5 while Group6 showed highest serum Calcium and phosphorus levels. Histomorphometrical analysis indicated dose dependent increase in trabecular bone volume (BV/TV), trabecular number (Tb.N) and decrease in osteoid surface in ED-71 treated mice (Group 3-5). On the other hand, the bone from Group6 showed minimal changes in BV/TV, Tb.N and osteoid surface. From these results, we conclude that ED-71 is a promising candidate for the treatment of XLH.



SU453

Hypophosphatasia: Molecular Diagnosis of Rathbun's Original Case. S. R. Mumm,[1] J. Jones,*[1] P. Finnegan,*[1] M. P. Whyte.[2] [1]Division of Bone and Mineral Diseases, Washington University School of Medicine and Barnes-Jewish Hospital Research Institute, St. Louis, MO, USA, [2]Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children, St. Louis, MO, USA.

In 1948, Dr. J.C. Rathbun characterized the disorder *hypophosphatasia* when he reported paradoxically low levels of *alkaline phosphatase* (ALP) activity in blood and in several tissues from an infant who died with rickets and epilepsy (Am J Dis Child 75: 822, '48). Hypophosphatasia is now recognized to be an inborn error of metabolism featuring deficient activity of the tissue nonspecific isoenzyme of ALP (TNSALP) due to deactivating mutations in *TNSALP.* In 1996, 50 years after the death of their son, the parents of Rathbun's patient provided to us their blood for *TNSALP* analysis. Denaturing gradient gel electrophoresis (DGGE) is established in our laboratory to investigate the molecular pathology of hypophosphatasia. Analysis of genomic DNA included PCR amplification of all 11 coding exons of TNSALP and DGGE was used to identify abnormalities. PCR products for exons containing mutations/polymorphisms were re-amplified and sequenced. We find that compound heterozygosity involving two missense mutations in *TNSALP* caused the death of Rathbun's patient. In the mother, the missense mutation was identified in exon 5, a G to A change at nucleotide number 340 (G340A) causing an amino acid change (alanine to threonine) at position 97 (Ala97Thr). This mutation has not been reported. We looked for the G340A change in 84 individuals without hypophosphatasia, using an allele-specific oligonucleotide assay. The G340A change was not detected in the 168 alleles and, therefore, was not a polymorphism. In fact, alanine at position 97 is conserved in *E. coli* ALP protein, suggesting that this amino acid has a critical role in ALP function. In the father, a missense mutation was detected in exon 9, an A to C change at nucleotide number 881 (A881C) causing an amino acid change (aspartic acid to alanine) at position 277 (Asp277Ala). This alteration has been reported in several cases of hypophosphatasia and may represent one of the most common mutations. He also showed a common polymorphism in exon 12 (T1565C; Val505Ala) that causes an amino acid change but is not considered detrimental to ALP activity or contributory to disease. Hence, the molecular basis for severe forms of hypophosphatasia can be deduced by studying the TNSALP genes in parents or other family members. Our work is now focused on a molecular nosology for hypophosphatasia to improve both our understanding of the physiological role of TNSALP and prognostication for this disorder that manifests with striking variable severity and sometimes unpredictable lethality.

SU454

A Highly Sensitive PCR Method Detects Activating Mutations of the *GNAS1* Gene in Peripheral Blood Cells of Patients with McCune-Albright Syndrome or Isolated Fibrous Dysplasia. C. Ding,* Z. Deng,* M. A. Levine. Pediatrics, Johns Hopkins University, Baltimore, MD, USA.

McCune-Albright syndrome (MAS)is characterized by the triad of fibrous dysplasia (FD), skin pigmentation, and autonomous endocrine disorders. Somatic mutations in the *GNAS1* gene that replace Arginine 201 in Gsα have been identified in affected tissues from patients with complete MAS or isolated components of the triad. These missense mutations inhibit the GTPase activity of Gsα and lead to constitutive activation of adenylyl cyclase. Conventional methods to detect mosaic mutations of *GNAS1* have required PCR analysis of genomic DNA from affected tissues or mulitiple rounds of tandem PCR and endonuclease digestion to enrich for mutant alleles in genomic DNA from other tissues. Recently, a novel PCR-based method that uses a protein nucleic acid (PNA) primer to inhibit amplification of the wild type allele was shown to detect the *GNAS1* R201 mutation in bone lesions from 8/8 FD patients (Bianco *et al,* J Bone Miner Res 2000;15:120-8). In the present study we applied this method to the analysis of genomic DNA from peripheral blood cells of 10 patients with MAS and 3 patients with isolated FD. PCR was performed in a 100-µl reaction using 2.5 U of Taq polymerase, 500 ng of genomic DNA, 1 µg each of forward and reverse primers, in the absence or presence of 2 µg of PNA. After initial denaturation to 94C for 15 min, samples were cycled 40 times (94C for 30s, 68C for 60s, 55C for 30s, 72C for 60s), with final extension for 7 min at 72C. In the absence of PNA, a strong 325 bp PCR band was generated from all samples; in the presence of PNA there was an approximately 50-90% reduction in the intensity of this PCR product. Direct sequencing of the PCR products demonstrated R201 mutations in 3/10 MAS patients and 0/3 FD patients in the absence of PNA. By contrast, in the presence of PNA an R201 mutation was

easily detected in all 10 MAS patients (5-R201H; 4-R201C; 1-R201L) and all 3 FD patients (3-R201C). In mixing experiments using wild type and mutant DNA samples, we were able to determine the presence of a mutant *GNAS1* allele in the equivalent of 1 cell in 500 to 1000. DNA samples from normal subjects showed wild type *GNAS1* sequence in the absence of PNA, and no mutation in the presence of PNA. We conclude that inclusion of a specific PNA primer in the PCR for the R201 mutation allows the selective amplification of low numbers of mutant alleles, and permits detection of activating mutations in genomic DNA from peripheral blood cells in patients with MAS and FD. The presence of cells bearing the *GNAS1* activating mutation in the circulation of patients with FD suggests that somatic mosacism is more widespread than clinically suspected, or that cells from FD lesions are shed into the circulation.

SU455

Positional Dissociation Between the Genetic Mutation Responsible for Pseudohypoparathyroidism Type Ib and the Associated Methylation Defect at Exon A/B: Evidence for a Long-Range Regulatory Element Within the Imprinted GNAS1 Locus. M. Bastepe, J. E. Pincus,* H. Jüppner. Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.

Pseudohypoparathyroidism type Ib (PHP-Ib) is a paternally imprinted disorder, which maps to chromosome 20q13.3 with GNAS1 at its telomeric boundary. GNAS1 encodes the stimulatory G protein (Gs-alpha), XL, and NESP55, as well as A/B and a presumably non-coding antisense (AS) transcript. Several of the GNAS1-specific exons and their respective promoters undergo parent-specific methylation. NESP55 is therefore derived only from the maternal allele, while XL, AS, and A/B are derived only from the paternal allele; in contrast, Gs-alpha appears to be transcribed from both parental alleles. In PHP-Ib, exon A/B lacks methylation of the maternal allele. More extensive methylation abnormalities were observed in pseudohypoparathyroidism associated with paternal uniparental isodisomy of chromosome 20q (patUPD20q) and in a small PHP-Ib kindred. Yet unidentified chromosome 20q13.3 mutations and patUPD20q thus appear to lead to abnormal methylation of exon A/B and sometimes other GNAS1 exons, and these epigenetic changes are associated with PTH-resistant hypocalcemia. Patient F-V/51, and other affected members of the previously reported PHP-Ib kindred F, were now shown to a display the disease-specific methylation defect at exon A/B. Since F-V/51 was recombinant between GNAS1 intron 3 and the telomeric end of chromosome 20q, most of the 13 coding Gs-alpha exons were excluded as being involved in the pathogenesis of the disease. To further delineate the telomeric boundary of the PHP-Ib locus, we investigated F-V/51 with several new intragenic microsatellite markers. These studies revealed that F-V/51 remains recombinant at an informative single nucleotide polymorphism (SNP) located 1.2 kb upstream of XL. These findings suggest that the genetic defect responsible for abnormal methylation of exon A/B and presumably PTH-resistant hypocalcemia is located in kindred F, and probably in other PHP-Ib patients, at least 30 kb centromeric of the abnormally methylated exon A/B. A GNAS1 region further upstream is therefore predicted to exert, presumably through imprinting of exon A/B, long-range effects on Gs-alpha expression.

SU456

Osteopathia Striata with Cranial Sclerosis: Iliac and Cranial Bone Histology in an Adolescent Girl. L. M. Ward,[1] F. Rauch,[1] R. Travers,[1] M. Roy,*[2] J. Montes,*[3] G. Chabot,[2] F. H. Glorieux.[1] [1]Genetics Unit, Shriners Hospital, McGill University, Montreal, PQ, Canada, [2]Département de Pédiatrie, Hôpital Ste-Justine, Montreal, PQ, Canada, [3]Departments of Surgery and Pediatrics, McGill University, Montreal, PQ, Canada.

Osteopathia striata with cranial sclerosis (OS-CS) is a rare skeletal dysplasia characterized by linear striations of the long bones and osteosclerosis of the cranium. Few descriptions of the bone histology in this condition exist, and cranial histology has not previously been reported. Here we describe an adolescent girl with severe OS-CS, where the long bone striata were of little clinical significance but the cranial sclerosis resulted in considerable disfigurement and disability. The results of bone histology at the iliac crest are also reported. The patient is a French-Canadian girl born in February 1986. The diagnosis of OS-CS was made at 4 years of age in the context of multiple medical problems including the Pierre-Robin sequence (laryngotracheal stenosis, cleft palate, bilateral conductive hearing loss) and anal stenosis. At 14 years of age, she was fully mobile without fractures or limb pain, but had unilateral facial paralysis due to facial nerve entrapment and persistent conductive hearing loss. Due to progressive headaches, she underwent intracranial pressure monitoring, at which time a full-thickness cranial biopsy was obtained. A biopsy at the iliac crest was also performed following dual tetracycline labeling. Qualitative evaluation of the iliac specimen revealed preservation of the lamellation under polarized light. Fluorescent studies showed distinct dual tetracycline labels. Quantitative histomorphometric parameters compared to fourteen age-matched controls revealed thick trabeculae and an increased cancellous bone volume, with no evident alteration in bone remodeling activity.

	OS-CS	Controls(± 1SD)
Core Wi (mm)	4.6	7.1± 1.8
Ct. Wi (mcm)	1002	897± 331
BV/TV (%)	40.0	24.4± 4.3
Tb. Th. (mcm)	225	148± 23
O. Th. (mcm)	7.2	6.7± 1.8
OS/BS (%)	6.8	6.7± 4.5
MS/BS (%)	15.8	11.7± 5.0
MAR (mcm/d)	0.81	0.87± 0.09
BFR/BS ($mcm^3/mcm^2/y$)	46.9	37.3± 16.7
Oc.S/BS (%)	1.29	0.94± 0.38

Qualitative evaluation of the cranial biopsy showed successive layers of periosteal bone covering a compact cortical compartment with tightly packed haversian canals. There was no evidence of woven bone presence.

SU457

The Use of Protein Nucleic Acid (PNA) in the Quantification of Arg201 Mutations in FD/MAS and Cancer. A. Karadag,*[1] M. Riminucci,[2] P. Bianco,[3] N. Cherman,*[1] P. Gehron Robey,[1] L. W. Fisher.[1] [1]CSDB, NIDCR, NIH, Bethesda, MD, USA, [2]Universita dell L'Aquila, L'Aquila, Italy, [3]University of Rome, Rome, Italy.

Activating missense mutations of the GNAS1 gene encoding the alpha subunit of the stimulatory G protein (Gs) have been shown to be the cause of fibrous dysplasia and McCune Albright syndrome (FD/MAS) and to occur in a number of endocrine cancers. Because these mutations are always somatic, only a portion of the cells contains the activating mutation. Last year we reported a method of identifying the mutation by DNA sequencing of the mutant allele after blocking the PCR amplification of the wildtype allele with PNA. Recently we have also been successful using the PNA/PCR/sequencing approach with genomic DNA extracted from sections of archival paraffin blocks thus permitting the determination of mutations in patients who are either no longer available or for whom fresh tissue is difficult to obtain. Furthermore, we have developed frequency resonance energy transfer (FRET) hybridization probe (HP) sets that permit us to use a LightCycler to determine the mutation without sequencing the PCR products. The usefulness of these techniques is limited to identifying the mutation and can not be used for determining the relative abundance of the mutant cells in the sample tested. After the report from our laboratory that the development of the FD/MAS lesion may require both normal and mutant cells, we set out to develop methods that would permit the determination of the ratio of normal to mutant cells within cell populations either in vivo or in vitro. First, standard DNA-based hybridization FRET probes (DNA-DNA HP) were designed to quantify the relative abundance of normal and mutant alleles in the PCR product. Melting curves of the DNA-DNA HP were useful for quantifying some mutations but for others the difference in melting temperatures of the perfect match vs. the single basepair mismatch were too similar to permit accurate quantification. To overcome this, we have developed DNA-PNA FRET hybridization pairs for which the melting temperatures of the relevant allelic sequences differ by 10-15 C. Using these techniques, we have found that different areas contain different proportions of mutant and normal cells. In conclusion the use of PNA, particularly in conjunction with the LightCycler, has proven to be a useful tool in identifying and quantifying known mutations in cell cultures, fresh tissue and in archival paraffin sections.

SU458

Participation of Fos and Src in Cadmium-Induced Bone Changes in Mice. A. Regunathan,* M. H. Bhattacharyya. Biosciences Division, Argonne National Laboratory, Argonne, IL, USA.

Mice deficient in either fos or src develop osteopetrotic bones and fail to erupt teeth. Osteopetrosis in fos-/- mice is attributed to a demonstrated lack of osteoclasts, indicating that osteoclast generation from bone marrow stem cells requires fos. Osteopetrosis in src-/- mice is attributed to the demonstrated presence of osteoclasts that fail to become activated to resorb bone, indicating that osteoclast cell activation requires src. Osteoblasts from both transgenic mouse strains are normal and fully capable of supporting generation and activation of normal osteoclasts in cell culture systems. We conducted studies to determine whether cadmium could cause decreased bone mass in the absence of either the fos or the src gene products. Male and female mice each heterozygous for fos-deficiency were mated to produce fos-/- and fos+/o offspring (+/o = +/+ or +/-). Fos-/- pups were identified by lack of teeth on Day 15 (Day 0 = date of birth). Pups were divided into four groups: no Cd, fos+/o; +Cd, fos+/o; no Cd, fos-/-; and +Cd, fos-/-. Pups administered with cadmium received daily subcutaneous injections (50 mg Cd/kg body wt.) on Days 18-20. From weaning (Day 21), Cd was administered through drinking water at 10 ppm for the first two weeks and 20 ppm for the second two weeks prior to sacrifice (Day 48). An analogous protocol was followed starting with mating male and female mice each heterozygous for src-deficiency. Results demonstrate that src-/- pups were 24% lower in body weight on Day 45 than their src+/o littermates; however, cadmium had no effect on body weight independent of src gene status and did not stimulate tooth eruption in src-/- pups. In contrast, cadmium decreased growth in the fos-/- offspring by 17%. And 40% of the +Cd, fos-/- pups showed eruption of a single upper tooth, noting that all fos-/- pups were toothless in the absence of cadmium administration. Histomorph studies on the bone of both strains of transgenic mouse were performed and show distinct differences between the control and deficient mice. Through Van Kassa/toluidine blue staining, it is clear that the growth plate of the deficient animal is much larger in size than that of the wild type. In addition, the marrow cavity of the deficient tibae appears to be filled with primary spongeosa-like bone that has not been remodeled. Both these results point to the osteopetrotic nature of the bones. The question needs to be answered as to whether there are marked differences between the deficient animal treated with cadmium and the one which was not. Results indicate that cadmium can cause a decrease in bone mass through a pathway independent of the fos gene products. However, src appears to be required for cadmium to stimulate changes involved in bone remodeling and tooth eruption.

SU459

Reversal of Hypocalcemia in Young Vitamin D Depleted Rats by Oral Calcium, D_3 or Calcitriol. I. Bone Densitometry and Survival Rate During the Repletion and Redepletion Phases. G. Mailhot,* N. Dion,* L. G. Ste-Marie, M. Gascon-Barré. Hôpital Saint-Luc, Centre de recherche du Centre hospitalier de l'Université de Montréal, Montréal, PQ, Canada.

Chronic hypocalcemia secondary to vitamin D_3 (D_3) depletion (Ca-D-) perturbs not only extra- but also intracellular calcium homeostasis (Mailhot et al., Endocrinology, 2000). The purpose of the studies was to investigate the lasting effects of oral calcium alone (Ca+) , D_3 or calcitriol (CT) on calcium and bone metabolism using a repletion-redepletion protocol as experimental paradigm. Male Ca-D- rats received calcium alone (3% Ca gluconate in drinking water), D_3 (6.5 nmol/d), or CT (28 pmol/d) for 14 d, and were then switched back to the original Ca-D-diet. Normocalcemic rats paired for age or weigth served as Controls. All repletion protocols normalized serum (Se) calcium, parathyroid hormone (PTH) and alkaline phosphatase, increased urinary (U) calcium and decreased U phosphorus ($p<0.05$) but Ca+ rats showed a lesser weight gain, hypophosphatemia and very low Se D_3 metabolites compared to the D_3, CT, and Controls groups ($p<0.05$). Animals of all groups survived the repletion protocol. Upon redepletion, Se and U calcium rapidly decreased in Ca+ while Se phosphorus and PTH increased but all animals died within five days. No death was observed in the D_3 or CT groups over a 7 wk period despite a progressive declined in Se and U calcium to the original Ca-D- values and a return of the secondary hyperparathyroidism state. Investigation of whole body, femur and lumbar spine bone mineral density and content revealed normalization by D_3 and CT ($p<0.05$) but not by oral calcium alone where no accretion over Ca-D- was observed. Our data thus indicate that oral calcium alone does not improve bone mineral density in Ca-D- rats despite normalization of Se calcium and PTH suggesting an impairment in bone calcium accretion possibly secondary to hypophosphatemia. It is postulated that the incapacity of the bone calcium reservoir to provide sufficient calcium to sustain Se calcium during calcium deprivation may be the cause of death in these animals clearly underlying the necessity to add D_3, or CT to calcium repletion in the young growing Ca-D- rat.

SU460

Reversal of Hypocalcemia in Young Vitamin D Depleted Rats by Oral Calcium or Calcitriol. II. Histomorphometry of the Proximal Tibia Following Repletion and Redepletion Phases. N. Dion,* G. Mailhot,* C. Deschênes,* M. Gascon-Barré, L. G. Ste-Marie. Hôpital St-Luc, Centre de recherche du CHUM, Montreal, PQ, Canada.

In the growing rat, bone metabolism is sensitive to the calcium/phosphorus (Ca/P) ratio, rickets being induced by vitamin D (vit. D) deficiency diets with high calcium and low phosphorus content. To study the bone metabolism response to oral calcium (Ca+) or calcitriol (CT), animals with severe hypocalcemia secondary to vit. D depletion were repleted with diets containing either a high calcium content or CT for 14 d, and were subjected to a 3 d redepletion protocol using a non-rachitogenic Ca-D- diet. Unsupplemented Ca-D- rats matched for age and weight were also studied. All proximal tibiae were recovered at sacrifice and histomorphometric measurements were performed on trabecular bone at the secondary spongiosa. Vit. D replete normocalcemic rats paired for weight served as controls (C). Structural (BV/TV, Tb.Th, Tb.Sp, Tb.N) and resorption parameters (ES/BS and Oc.S/BS) were found to be similar in all groups. Compared to C, Ca-D- rats showed hyperosteoïdosis (OV/BV $p<0,01$; OS/BS $p<0,01$; O.Th $p<0,001$) with lower active bone cell covered surfaces (Ob.S/OS $p<0,001$ and N.Oc/E.Pm $p<0,05$). Undetectable tetracycline labeling (TL) confirmed the mineralization defect induced in this group. Repletion with CT induced normalization of static formation parameters to a level similar to that of C. The presence of linear TL confirmed ongoing mineralization but, at a slower rate than C as indicated by the absence of double labeling. Compared to Ca-D-, the significant increase of N.Oc/E.Pm ($p<0,01$) could reflect bone response to 3 d redepletion. In the Ca+ group, static formation parameters tended to be lower than in Ca-D- but higher than in CT. The absence of well defined TL confirmed the persistence of mineralization defect albeit normalization of serum Ca^{2+}. Growth plate in this group was significantly thicker ($p<0,001$) mainly due to higher hypertrophic chondrocyte zone (HC). Our data show that chronic calcium and vit. D depletion in the rat induces osteomalacia which is corrected by calcitriol repletion. During repletion with calcium alone, growth retardation was partly corrected but rickets developed probably due to hypophosphatemia. The high mortality rate observed in this group during the redepletion phase may be due to severe hypocalcemia resulting from failure to mobilize calcium from bone and/or an increase demand for calcium from the large HC at a time when serum phosphorus was rising concomitantly with a decrease in the serum Ca^{2+} thus creating a favorable Ca/P ratio for mineralization despite the calcium deprivation.

SU461

Urinary Osteopontin Concentration Is Correlated with Intratrochanteric but not Femoral Neck Bone Mineral Density for Male Kidney Stone Formers. K. M. Kellum,*[1] J. S. Lindberg,[1] L. L. Hamm,*[2] A. Burshell,*[3] F. E. Husserl,*[4] F. E. Cole.*[5] [1]Nephrology Research, Alton Ochsner Medical Foundation, New Orleans, LA, USA, [2]Nephrology, Tulane University Medical Center, New Orleans, LA, USA, [3]Endocrinology, Alton Ochsner Medical Foundation, New Orleans, LA, USA, [4]Nephrology, Alton Ochsner Medical Foundation, New Orleans, LA, USA, [5]Research, Alton Ochsner Medical Foundation, New, LA, USA.

Osteopontin is a protein produced by kidney cells and bone cells, both osteoblasts and osteoclasts. Urinary osteopontin concentration [OPN] is related to femoral neck bone mineral density (fnbmd) in postmenopausal stone forming women (PMSF) on estrogen replacement therapy. Femoral neck bone and itratrochanteric bone have different compositions of cortical bone and trabecular bone. For male stone formers (MSF), fnbmd and intratrochanteric bone mineral density (itbmd) may have different relationships to [OPN] due to the effect of testosterone.14 MSF submitted 24-hour urine collections, which were analyzed for [OPN] by ELISA. Fnbmd and itbmd were determined by DEXA scan within 1 year of OPN collection. [OPN] was not correlated to fnbmd ($p=0.3$). However, [OPN] was positively, linearly correlated to itbmd ($p<0.05$).In MSF [OPN] was related to itbmd; in contrast, for MSF, like PMSF -ert, [OPN] as not related to fnbmd. Urinary osteopontin may be produced by bone, as well as kidney, to provide CaOXSF inhibition in the urine. In the presence of testosterone, osteoblasts may exceed osteoclasts in the production of OPN, since testosterone stimulates osteoblasts. With osteoblast stimulation, bone mineral density is increased. The intratrochanteric area contains more trabecular bone, which may be more sensitive to testosterone, than the femoral neck, and as a result, itbmd may be more influenced by the presence of testosterone and be more responsive to changes in OPN production.

SU462

Study of Bone Mass in Chronic Hepatitis C Male Patients Treated with Alpha-Interferon and Ribavirin. G. Martinez,*[1] E. Jodar,*[1] F. Hawkins,[1] J. Solis.*[2] [1]Endocrinology Service, Hospital 12 de Octubre, Madrid, Spain, [2]Gastroenterology Service, Hospital 12 de Octubre, Madrid, Spain.

The effects of antiviral therapy on bone mass and mineral metabolism are being investigated. In this cross-sectional study we have evaluated 30 chronic hepatitis C caucasian male patients (mean age 41±5,2 years, range 31-48) randomly selected from our outpatient clinic, treated with alfa-interferon (IFN) alone or in combination with ribavirin (RBV). Patients and Methods: Thirteen patients (group 1) were treated with IFN(3 MU tiw) for 12 months and 17 patients (group 2) were treated with IFN(3 MU tiw) plus RBV (1000-1200 mg/day). Lumbar (L2-L4) BMD was measured with DXA (Hologic QDR 4500, Waltham, MA, USA) at the end of treatment. As control group 218 healthy caucasian Spanish males were used. T and Z-scores of lumbar BMD were obtained for each patient. Bone formation markers (serum osteocalcin & bone-specific alkaline phosphatase), bone resorption (urinary pyridinoline & 24 hrs. calcium excretion) and calcitropic hormones (serum iPTH and 25-OH vitamin D) were measured using well standarized methods. Results: We haven´t found differences between groups in clinical characteristics (age, known duration of disease, route of transmision of HCV, serum ALT levels, HCV viral load or Knodell 's histologic activity index score). Bone mass was significantly lower in group 2 than in group 1 patients (BMD 0.889±0.066 vs 1.108±0.076; T-score -1.25±0.55 vs 0.57±0.63; Z score -0.89±0.43 vs 0.67±0.54, $p<0.001$). 9 patients(52.9%) in group 2 had osteopenia ($-2.5<$ T-score<-1.0), but none had T-score<-2.5. Mean values of bone markers were in the normal range in both groups. No significant differences were found between groups in bone alkaline phosphatase, osteocalcin, pyridinoline or calcitropic hormones. Twenty-four hour urinary calcium excretion was significantly disminished in group 2 (79±36 mg/day vs 218±97 mg/day, $p<0.001$). Conclusion: These preliminary results show a decreased bone mass and low bone turnover after combination therapy of chronic hepatitis C that includes RBV. This could suggest a deletereous effect of RBV on bone metabolism. Longitudinal studies are needed to ascertain the effect of RBV on bone mass.

SU463

Urinary Osteopontin Concentration Is Correlated with Both Femoral Neck and Intratrochanteric Z-Scores in Postmenopausal Kidney Stone Forming Women on Estrogen Replacement Therapy. K. M. Kellum,*[1] J. S. Lindberg,*[1] L. L. Hamm,*[2] A. L. Burshell,*[3] F. E. Husserl,*[4] F. E. Cole.*[5] [1]Nephrology Research, Alton Ochsner Medical Foundation, New Orleans, LA, USA, [2]Nephrology, Tulane University Medical Center, New Orleans, LA, USA, [3]Endocrinology, Alton Ochsner Medical Foundation, New Orleans, LA, USA, [4]Nephrology, Alton Ochsner Medical Foundation, New Orleans, LA, USA, [5]Research, Alton Ochsner Medical Foundation, New Orleans, LA, USA.

Osteopontin (OPN) is a protein produced by kidney and bone cells and is found in the urine of normal and stone forming humans. Urinary OPN concentration ([OPN]) is positively, linearly correlated to femoral neck bone mineral density (fnbmd) in postmenopausal kidney stone forming women (PMSF) on estrogen replacement therapy (ERT). Femoral neck is mainly cortical, while intratrochanteric has more trabecular bone. Since these bone types have different responses to ERT, intratrochanteric bone mineral density (itbmd) under the influence of ERT may not be related to [OPN]. PMSF 9 on ERT and 12 not on ERT were studied. Each submitted a 24hour urine collection and [OPN] was measured using ELISA. Fnbmd and itbmd were measured by DEXA scan taken within 1 year of OPN measurement. The bone mineral density values are expressed as z-scores to correct for age differences. Analysis by linear regression was performed and p-values less than 0.05 were considered significant. For the PMSF on ERT, the femoral neck z-score (fnzs) and the intratrochanteric z-score (itzs) were positively, linearly correlated to [OPN] ($p<0.05$ for each). In contrast, for PMSF not on ERT, neither fnzs nor itzs were correlated to [OPN] ($p=0.2$ and 0.4 respectively). ERT has a similar effect on the relationship between [OPN] and both fnbmd and itbmd despite these areas having different compositions of cortical and trabecular bone. This correlation may represent a positive effect of ERT on osteoblast activity, and as osteoblasts are stimulated to produce OPN, bone mineral density is increased.

SU464

The Concentration of Marrow Stromal Precursor Cells in Skeletal and Metabolic Disorders. S. A. Kuznetsov, N. Cherman,* J. S. Lee, M. T. Collins, P. Gehron Robey. CSDB, NIDCR/NIH, Bethesda, MD, USA.

Bone marrow stromal precursor cells, also referred to as colony forming units - fibroblastic (CFU-Fs), form discrete colonies (clones) of fibroblast-like cells *in vitro*. CFU-F populations include multipotential precursor cells able to differentiate along osteogenic, chondrogenic, adipogenic, and stromal pathways. The CFU-F concentration among marrow cells is relatively stable under physiological conditions but can be significantly altered by acute hemorrhage, irradiation, or curettage, and in humans, in the course of several

hematological disorders. We have applied this parameter to a variety of skeletal and metabolic conditions. Marrow single cell suspensions were prepared from surgical specimens and plated into 25 cm^2 flasks. Colonies containing at least 50 cells were counted between day 10 and 14 and CFU-F numbers per $1x10^5$ nucleated cells were calculated.

Patient Age	Diagnosis	Age, years	CFU-F number ± SEM	P *<0,05 **<0.01
<20 yrs of age	Corrective surgery (N=18)	1 to 19	47.5 ± 3.7	
	Achondroplasia (SADDAN)	15	1.1 ± 0.5	**
	Marfan syndrome	14	25.5 ± 1.9	ns
	Sprengel deformity	5	44.8 ± 4.2	ns
	Craniosynostosis	8 mo.	56.0 ± 3.5	ns
	Craniosynostosis	3 mo.	65.3 ± 6.0	ns
	Craniosynostosis	4 mo.	306.7 ± 18.6	**
	Sickle cell anemia	4	630.0 ± 17.3	**
>20 yrs of age	Normal; trauma (N=4)	42 to 57	34.2 ± 6.0	
	Total lipodystrophy	31	0.03 ± 0.03	**
	Total lipodystrophy	29	0.47 ± 0.27	**
	Pseudoachondroplasia	36	1.5 ± 0.6	**
	Paget's disease	75	6.2 ± 1.1	**
	Job's disease	42	11.7 ± 3.5	*
	Job's disease	31	29.7 ± 0.9	ns
	Partial lipodystrophy	48	18.5 ± 0.9	ns
	Partial lipodystrophy	27	25.0 + 2.7	ns
	Lupus erythromatosis	48	24.8 ± 0.8	ns
	Alcaptinuria/ochrinosis	58	120.5 ± 6.1	**
	Stickler syndrome	64	223.3 ± 3.3	**

We conclude that, in a number of skeletal and metabolic disorders, CFU-F concentration can be substantially, or even drastically, affected. Both the direction and the magnitude of the change depend on the nature and severity of the pathological process. Thus, CFU-F concentration may be useful for differential diagnosis, in particular, for classification of vaguely delineated disorders, including osteoporosis. In addition, our data point to a role of CFU-Fs in pathophysiology of bone/bone marrow. Knowledge of CFU-F concentration and activity will likely provide valuable insight into mechanisms of a wide variety of skeletal and metabolic conditions.

SU465

Growth Hormone (GH) Does Not Alter Cortical Bone Porosity in Adult GH-Deficiency - A 12 Month Double-Blind Placebo Controlled Study. K. E. Koroma,[*1] E. Hauge,[2] H. Brockstedt,[*3] J. S. Christiansen,[*3] T. B. Hansen,[*1] F. Melsen,[2] C. Hagen,[*1] K. Brixen.[1] [1]Endocrinology, Odense University Hospital, Odense, Denmark, [2]Pathology, Aarhus University Hospital, Aarhus, Denmark, [3]Endocrinology, Aarhus University Hospital, Aarhus, Denmark.

Fracture incidence is increased in patients with adult-onset GH-deficiency (GHD). Moreover, these patients have reduced bone mineral density (BMD). Short-term GH-treatment decreases whereas long-term treatment increases BMD. It has been suggested that this initial decrease in BMD is explained by an increase in the remodeling space in the cancellous bone (1). In this study we examined the changes in cortical porosity in order to evaluate the remodeling space in the cortical bone.Twenty-nine patients with adult-onset GH-deficiency, aged 21-61 (mean 45.5) years (19 men and 10 women) were randomized to treatment with GH (Norditropin, Novo-Nordic, Denmark) 2.0IU/m2/day or placebo. None of the patients had previous acromegaly. Substitution with other hormones than GH were continued unchanged throughout the study. GH-deficiency was defined as a maximal GH-peak of <10mg/l during insulin-tolerance test (blood glucose <2.0mmol/l). Contra-lateral bone biopsies were obtained from the iliac crest before and after 12 months of treatment. Biopsies were embedded undecalcified in methylmethacrylate and sections of 7-8 µm were cut on a heavy-duty microtome parallel to the longitudinal axis of the randomly rotated specimen. Sections were stained with Goldner-trichrome for light microscopy or left unstained for fluorescence microscopy. Twenty-four paired biopsies were available for determination of cortical porosity (Ct.Po), absolute cortical width (A.Ct.Wi), quiescent (QSi), formative (FSi), and eroded cortical osteons (ESi) as previously decribed (2). Data on osteon diameter, wall width, and Haversian canal width will be presented.Results are shown in table 1.

Table 1. Differences between groups are indicated by * p<0.05 and (*) p<0.10

	Placebo (n=12)		GH-treatment (n=12)	
	Before	After	Before	After
Ct.Po(%)	5.8 (0.6)	8.0(0.6)	5.6(0.6)	7.3(0.9)
ESi(%)	2.2(0.6)	2.6(0.8)	1.8(0.5)	3.3(1.0)
FSi(%)	8.9(2.7)	7.6(1.8)	6.3(2.5)	11.0(1.4)(*)
QSi(%)	88.9(2.6)	89.7(1.8)	91.9(2.7)	85.7(2.0)*
A.Ct.Wi(mm)	1.5(0.1)	1.3(1.0)	1.1(0.1)	1.1(0.1)

No significant changes in Ct.Po or A.Ct.Wi were seen. However, QSi decreased (p=0.04) and FSi tended to increase (p=0.07) while ESi was unchanged.In conclusion, GH treatment does not alter cortical bone porosity (i.e. the remodeling space). The decrease in BMD seen during short-term GH treatment in GH-deficiency is still not fully explained.1) Brixen K et. al. J.Bone Miner.Res.2000;15:293.-300. 2) Brockstedt H et al.: Bone, 1996;18:67-7

SU466

Over-production and Secretion of MIP-1alpha and beta by Multiple Myeloma Cells Clinically Correlates with Enhanced Bone Resorption and Development of Destructive Bone Lesions. T. Hashimoto,[1] M. Abe,[1] T. Ohshima,[*1] H. Shibata,[*1] S. Ozaki,[*1] D. Inoue,[1] T. Matsumoto.[1] [1]University of Tokushima, Tokushima, Japan.

Multiple myeloma (MM) develops and expands in the bone marrow, and exhibits a devastating bone destruction by enhanced bone resorption. We have demonstrated that macrophage inflammatory protein (MIP)-1alpha and beta are secreted from most of MM cells and stimulate osteoclast formation and function. Because enhanced osteoclastogenesis in co-cultures of bone marrow with MM cells is almost completely abrogated by neutralizing antibodies against MIP-1alpha and beta, these chemokines appear to be predominant mediators of MM-induced osteolysis. In order to further clarify the role of these chemokines, we investigated the clinical correlation between secretion of MIP-1alpha and beta by MM cells and the number of radiographycally determined destructive bone lesions as well as the levels of metabolic bone markers: urinary excretion of C-telopeptide (CTx) and deoxypyridinoline (Dpd) for resorption and serum levels of bone-specific alkaline phosphatase (BALP) and osteocalcin (OC) for formation. For MIP-1 measurements, MM cells were purified from bone marrow mononuclear cells by a positive selection using a human MM cell-specific anti-HM1.24 monoclonal antibody raised in our laboratory, and cultured for 2 days. MIP-1 concentrations in the culture supernatants were determined by ELISA. MIP-1alpha and beta secretion was detectable in 20 and 21 out of 28 patients, respectively. MM cells from patients with multiple bone lesions secreted a significantly higher amount of MIP-1alpha and beta than those from patients with less than one bone lesion (2417+/-822 vs 14+/-7 for MIP-1alpha, p<0.01, and 1462+/-845 vs 24+/-13 for MIP-1beta, p<0.01, respectively: pg/million cells). Furthermore, the amount of MIP-1alpha and beta secreted by MM cells showed a significant correlation with the bone resorption markers, and MIP-1alpha secretion higher than 100 pg/million cells was associated with multiple bone lesions and elevated resorption markers without exception. In contrast, the formation markers were relatively suppressed and showed no significant correlations with MIP-1 secretion, although some patients with increased resorption and MIP-1 secretion also exhibited comparable elevation of BALP values. These results demonstrate correlation between the severity of bone destruction and MIP-1 production by MM cells and further provide a clinical evidence for a causal role of MIP-1 in the development of MM bone lesions. Suppression of MIP-1 production and/or actions may thus be a rational therapeutic strategy for the MM bone disease.

SU467

Raloxifene Lowers Serum Calcium and Markers of Bone Turnover in Primary Hyperparathyroidism. M. R. Rubin,* K. Lee,* S. J. Silverberg. College of Physicians & Surgeons, Columbia University, New York, NY, USA.

Most patients with primary hyperparathyroidism (PHPT) are postmenopausal women. Estrogen replacement therapy (ERT) decreases total serum calcium by about 0.5 mg/dl in PHPT. Raloxifene HCl (Rlx), a selective estrogen receptor modulator, has skeletal antiresorptive properties similar to ERT. We therefore investigated the ability of Rlx to decrease serum calcium and markers of bone turnover in PHPT. 16 postmenopausal women with asymptomatic PHPT were randomized to receive 8 weeks of Rlx 60 mg/d or placebo, followed by a 4-week washout period. Measures were obtained at 0,1,4, 8 &12 weeks, with triplicate assays for calcium at 0 and 8 weeks to increase statistical power. At baseline, the groups were well matched (mean ± SEM age: Rlx: [n=8]: 64 ± 2; Control: [n=8]: 63 ± 4 years). There were no differences between groups in baseline serum calcium (Rlx: 10.8 ± 0.2; Control: 10.6 ± 0.1 mg/dl), PTH (Rlx: 107± 25; Control: 85 ± 18 pg/ml), phosphorus (Rlx: 2.4 ± 0.1; Control: 2.7 ± 0.1 mg/dl), total alkaline phosphatase (Rlx: 99 ± 9; Control: 95 ± 8 U/L), 1,25-$(OH)_2$vitamin D (Rlx: 59 ± 7; Control: 48 ± 4 pg/ml), osteocalcin (Rlx: 11.4 ± 1.6; Control: 11.0 ± 1.4 nmol/L), serum N-telopeptide (Rlx: 21.2±3.4; Control: 21.1 ± 3.1 nmol BCE/L) or urinary calcium excretion (Rlx: 243 ± 40; Control: 237 ± 45 mg/G creatinine). The groups also had similar bone mineral density at the lumbar spine, femoral neck and distal radius. During administration of Rlx, serum calcium decreased significantly by 8 weeks (10.8 ± 0.2 to 10.4 ± 0.2 mg/dl, p<0.05; Control: 10.6 ± 0.1 to 10.4± 0.1, p= NS), and returned to baseline by 4 weeks without Rlx (10.8 ± 0.2 mg/dl). No decrease was seen at week 1 or 4. In association with the changes in serum calcium, bone markers decreased at week 8 in the Rlx group (Osteocalcin: 11.4 ± 1.6 to 9.9± 1.6 nmol/L, p <0.05; Control: 11.0 ± 1.4 to 11.5 ± 1.7; NS. Serum Ntx: 21.2±3.4 to 17.3±2.8 nmol BCE/L, p<0.05; Control: 21.1 ± 3.1 to 20.4 ± 3.0; NS). Both returned to baseline 4 weeks off Rlx (Osteocalcin: 11.1± 2.1 nmol/L; Ntx: 18.2±2.3 nmol BCE/L). The 8 weeks of Rlx administration did not affect serum PTH (107± 25 to 122 ± 31 pg/ml), 1,25-$(OH)_2$vitamin D (59 ± 7 to 55 ± 4 pg/ml), total alkaline phosphatase (99 ± 9 to 101± 10 IU/L) or urinary calcium excretion (243 ± 40 to 242± 37 mg/G creatinine). Thus, by 8 weeks of treatment with Rlx, postmenopausal women with mild PHPT experienced a modest decline in serum calcium and markers of bone turnover, with no alteration in PTH levels. Calcium and bone marker changes were similar to those observed with ERT in PHPT, and returned to baseline with discontinuation of Rlx. We conclude that the decrease in serum calcium observed in patients with PHPT is due to the skeletal anti-resorptive effects of Rlx.

Disclosures: Eli Lilly,2.

SU468

Serum OPG in Patients with Primary Hyperparathyroidism (PHP) Before and Following Surgery. L. S. Stilgren,* B. Abrahamsen, K. Brixen,* A. R. Madsen,* L. Hegedüs,* H. Beck-Nielsen.* Endocrinology, Odense University Hospital, Odense, Denmark.

Osteoprotegerin (OPG) is a recently described member of the tumor necrosis factor family and is known to induce osteclastic differentiation from hemopoietic precursors. It is well known that the bone metabolism is increased in patients with PHP it is,however, unknown whether this is mediated by cytokines or primarily a direct effect of PTH. In the present study we examined serum OPG in patients with PHP before and after surgery. The study comprised 12 patients (females/males=10/2), aged 53 to 76 years,(median 62.3) with PHP confirmed by elevation of plasma PTH (range 7.7- 43.2 pmol/l; median 13.7) and elevated serum ionized calcium(range 1.33-1.78 mmol/l; median 1.44). None of the patients had renal disease or other conditions known to affect bone metabolism, or received bisphosphonates, glucocorticoids, or fluoride. Two postmenopausal women received estrogens and continued this troughout the observation period. The follow-up in these women was done at the same time in their menstrual cycle. Patients were investigated before and one year after successful parathyroidectomy. Serum was collected after an overnight fast and OPG measured using enzyme-linked immunosorbent assay (ELISA). BMD of the spine and femur were measured using a Hologic 2000 DXA-scanner. As expected PTH level was significantly decreased (p=0.002). There was, however, no significant difference between serum OPG level before and after surgery (P=0.26).

	Mean ± SEM
Serum OPG before surgery	44.3 ng/ml ± 20.4
Serum OPG after surgery	41.8 ng/ml ± 24.9

There was a significant increase in BMD_{spine} (p=0.0001) and $BMD_{total\ hip}$ (p=0.0004), but no correlation with serum OPG (r=-0.04; r=-0.05). After surgery there was no significant correlation between serum OPG and serum PTH (Spearman r=-0.16,n.s.) alkaline phosphatase (r=0.15,n.s.) or age (r=-0.20,n.s.) These findings indicate that PTH does not significantly alter serum OPG. Our results, however, do not rule out that PTH may change local (e.g. osteoblastic) OPG production.

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Recovery of Bone Mass in Primary Hyperparathyroidism After Parathyroidectomy Is Predicted by Baseline Bone Turnover and Bone Density. L. Cianferotti,[1] E. Vignali,[1] F. Cetani,[1] E. Ambrogini,*[1] F. Golia,*[1] A. Picone,*[1] P. Miccoli,*[2] C. Marcocci.[1] [1]Dipartimento di Endocrinologia, Università di Pisa, PISA, Italy, [2]Dipartimento di Chirurgia, Università di Pisa, PISA, Italy.

Primary hyperparathyroidism (PHP) is associated with an increased bone turnover and various degrees of bone loss. Successful parathyroidectomy (PTX) is usually followed by a decrese of markers of bone turnover within the normal range and an increase in bone mineral content. Aim of this study was to identify preoperative parameters which might predict the recovery of bone mass after removal of affected glands. The study group was composed of 49 hyperparathyroid patients (mean age 54.6 yrs): 15 men and 34 women (9 premenopausal and 25 postmenopausal). The patients were evaluated before and one year after successful PTX. Basal evaluation included measurement of bone mineral density (BMD, g/cm2) at lumbar spine and femur, and assessment of several parameters of bone turnover, such as total and ionized serum calcium, PTH, osteocalcin (BGP), bone alkaline phosphatase (BALP) and urinary deoxypyridinoline. A direct correlation was observed between basal levels of PTH and BGP (p<0.005, r=0.4) and PTH and BALP (p<0.0005, r=0.6). A moderate degree of bone loss was present at all sites (mean lumbar Z score=-1.28; femoral neck Z score=-0.92). Serial measurements of BMD were obtained in each patients over a period of 1 year after PTX. BMD variably increased in most patients one year post surgery; the mean increase was about 10% either at lumbar spine or femur. The percent recovery of BMD was directly correlated with basal PTH, BALP, BGP levels and inversely correlated with preoperative Z-scores (Table 1). In conclusion, basal measurement of PTH, BALP, BGP and BMD may predict the recovery of bone mass in patients with PHP following PTX; these parameters might be considered as additional criteria for the selection of patients to be submitted to surgery.

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Higher Serum PTH Levels After Parathyroidectomy (PTX) in Patients with Primary Hyperparathyroidism (PHPT): Implications for Pathogenesis of the Disease and Relevance to PTH Treatment in Osteoporosis. D. S. Rao,[1] N. Parikh,*[1] E. R. Phillips,*[1] M. Honasoge,[1] G. B. Talpos.*[2] [1]Bone & Mineral Research Laboratory, Henry Ford Hospital, Detroit, MI, USA, [2]Surgery, Henry Ford Hospital, Detroit, MI, USA.

It has generally been assumed that PTH secretion returns to normal after PTX in patients with PHPT, but little data exists to support this belief. We previously reported that bone loss after PTX is attenuated. We also reported that modest elevations in serum PTH levels, as in patients with mild PHPT, blunt the skeletal effects of estrogen depletion (ASBMR 1997 & 1998). Others have reported on continued bone gain after PTX. These data imply that an anabolic stimulus is present in such patients. We now hypothesize that PTH hypersecretion continues even after PTX because of the altered set-point of the remaining parathyroid cells. Accordingly we studied 82 women after PTX. We excluded 110 patients since post-PTX serum PTH level was not measured. However, there were no systematic differences between the 82 included and the 110 excluded patients in age, pre-PTX serum calcium, PTH, or 25-OHD, or parathyroid adenoma weight. In comparison to healthy age matched or young normal women the 82 patients higher serum PTH levels. The relevant data are in the Table:

Variable	**Patients**	**Controls**	**p value**
Age (years)	63 ± 12	65 ± 5	0.017
Serum Ca (mg/dl)	9.27 ± 0.54	9.40 ± 0.31	0.055 (young normal)
Serum PTH (pg/ml)	50 ± 22	36 ± 12	<0.001 (age-matched)
Serum PTH (pg/ml)	50 ± 22	29 ± 18	<0.001 (young normal)

There was no difference in serum creatinine levels between the two groups. The pre- and post-PTX 25-OHD levels in the 82 patients were similar. CONCLUSIONS: PHPT patients continue to secrete higher than normal PTH even after "curative" PTX possibly due to altered set-point. Since cell proliferation is very low in the parathyroid cells a second adenoma due to monoclonal expansion is unlikely in the remaining life expectancy of most patients. This continued higher PTH secretion may explain the observed anabolic effects on bone both in PTX and no-PTX mild PHPT patients. Thus, our new data reconciles the seeming paradox of osteoporosis in PHPT and PTH treatment for osteoporosis. We propose that a PTH level of <100 pg/ml is anabolic whereas a level >100 pg/ml is catabolic to bone. Further studies of steady state and dynamic PTH testing are needed either to confirm or refute this hypothesis.

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Bone Gain After Surgical Cure of Primary Hyperparathyroidism Is Demonstrated by Quantitative Ultrasound. E. Segal,* B. Raz,* S. Ish Shalom. Rambam Medical Center, Haifa, Israel.

Primary hyperparathyroidism (PHPT) is often associated with increase in bone turnover and fragility. Whereas most patients with PHPT are asymptomatic, approximately 50% meet one or more criteria for parathyroidectomy.The aim of this study was to evaluate the magnitude of change in bone strength as measured by the Sunlight Omnisense ™ (Omnisense, Sunlight Medical Ltd. Tel-Aviv, Israel), after surgical cure of PHPT. Speed of sound (SOS) was measured in all patients by the Omnisense few days prior to surgery and at 4, 8 and 12 months after surgery. Measured sites included: distal 1/3 radius and midshaft tibia. Serum levels of PTH, calcium, albumin, phosphate, creatinine, alkaline phosphatase and 25(OH)D3 were assessed before and after surgery. Thirty three patients, 27 women and 6 men aged 35-83 completed baseline assessment, 11 patients (all women, aged 45-83) completed 8 month of post surgical follow-up. Prior to surgery on average the group had negative Z-score at the radius and the Tibia (Radius -1.52±0.38; Tibia -1.50±0.39). One group t-test had a p=0.002 indicating that this negative Z-sore is statisticaly significant below zero. In all patients the PTH level returned to normal after surgery. In 11 patients, that were assessed eight months after surgery was demonstrated an increase of 0.42±0.94 at the radius and 0.39±0.53 at the tibia. (Radius, -2.05±0.76 to -1.64±0.60; Tibia -1.89±0.59 to -1.50±0.57), this increase had P<0.05 with one-tail paired t-test. The precision of SOS measurement was 0.44% at the radius and 0.59% at the Tibia.Z-score increase was seen in 67% of the radius and 82% at the Tibia. The significant positive change (positive change greater than 1.8*measurement-error) was seen in 67% and 45% of the group. Conclusion: Our data confirm that PHPT patients suffer from osteopenia at the appendicular sites prior to surgery. Most of these patients demonstrated significant bone gain in the first year post surgery. Bone gain post parathyroidectomy can be detected with the Omnisense as early as eight months post surgery.

Disclosures: Sunlight Medical Ltd.,2.

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A Comparison of Volumetric Densitometry Techniques in Pediatric-Onset Systemic Lupus Erythematosus. E. von Scheven,*[1] J. A. Shepherd,[2] X. G. Cheng,[2] H. K. Genant.[2] [1]Pediatric Rheumatology, University of California, San Francisco, San Francisco, CA, USA, [2]Radiology, University of California, San Francisco, San Francisco, CA, USA.

Variation in body size and skeletal maturation among pediatric cohorts limits the usefulness of areal densitometric assessments by Dual X-ray Absortiometry (DXA). Utilizing Quantitative Computed Tomography (QCT) as the "gold standard" volumetric measurement, we evaluated areal and volumetric DXA determinations for correlation with QCT among healthy children, and for discrimination of abnormal BMD among children with Systemic Lupus Erythematosus (SLE). 77 healthy female children (age 7.0-21.9 years [mean 14.9], 55% Caucasian, 18% Asian, 25% Hispanic and 2% mixed) and 42 female subjects with pediatric-onset SLE (age 9.5-21.9 years [mean 16.1], 24% Caucasian, 35% Asian, 31% Hispanic and 10% mixed) were evaluated. We performed DXA (L1-L4, Hologic QDR 4500) to determine APBMD (areal BMD), APBMAD (volumetric BMD, BMC/AP^1.5), Lat BMD (lateral spine L1-3) and Hologic Lat VolBMD (Lat BMC/vol determined by dimensions obtained on AP and Lat scans); and QCT (L1-3, GE 9800 Scanner, UCSF QUIET Algorithm) to determine the volumetric BMD. Linear regression was used to identify the contribution of age and weight and to determine the correlation (r^2) between age-adjusted and age- and weight- adjusted DXA determinations and QCT. Among healthy controls, comparison of DXA determinations to QCT revealed slightly greater correlation (r^2) for volumetric determinations (APBMAD, r^2=0.52 and Lat VolBMD, r^2=0.46) compared to areal determinations (APBMD, r^2=0.41 and latBMD, r^2=0.42). After adjusting for age and weight all correlations improved, with areal determinations (APBMD, r^2=0.83 and latBMD, r^2=0.80) demonstrating similar correlation to QCT as volumetric determinations (APBMAD r^2=0.70 and lat VolBMD r^2=0.61). This suggested that DXA may serve as a surrogate for QCT in children. However, evaluation of these age- and weight- adjusted determinations (z-scores) for disease discrimination revealed the greatest separation of SLE subjects utilizing QCT, and better approximation by DXA utilizing areal determinations (APBMD and latBMD) than volumetric determina-

tions (APBMAD and Lat VolBMD) (see table below).

Variable	SLE z-score (mean)	P -value
QCT	- 0.86	< 0.0001
APBMD	- 0.78 *	< 0.0001
LatBMD	- 0.77 *	0.005
APBMAD	- 0.42 *	0.02
Lat VolBMD	- 0.49	0.03

* both weight- and age- adjusted z-score In summary, children with SLE demonstrate diminished BMD compared to healthy controls. QCT achieves the greatest disease separation; and after adjusting for age and weight, although all are statistically significant, APBMD and Lat BMD demonstrate the best approximation of QCT.

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Continuous Increase of Spine BMD with Biphosphonates: Cyclical Intravenous APD Following Oral APD Therapy in Children with Osteogenesis Imperfecta. C. Tau,[1] C. Mautalen,[2] O. Brunetto,*[3] V. Alvarez,*[1] M. Rubinstein.*[4] [1]Endocrinologia, Hospital de Pediatria J.P.Garrahan, Buenos Aires, Argentina, [2]Osteopatias Medicas, Hospital de Clinicas, Buenos Aires, Argentina, [3]Endocrinologia, Hospital de Pediatria P. Elizalde, Buenos Aires, Argentina, [4]Central Laboratory, Hospital de Pediatria J.P.Garrahan, Buenos Aires, Argentina.

Osteogenesis Imperfecta (OI) is an hereditary disease characterized by severe bone fragility with frequent fractures and progressive skeletal deformities. Biphosphonates administration improves the quality of life and decreases fractures in children with OI. The aim of this study was to analyze the effect of 1 year of cyclical intravenous pamidronate (IV-APD) in 14 children (5F,9M) with OI type I,n=3; OI type III,n=4 and OI type IV, n=7, age(X±SEM): 8.1±1.4 (range 1.6 to 19.7 years old). Every child received IV-APD (provided kindly by Gador) at 3-4 month intervals for 1 year, in cycles of 3 consecutive days, (dose 7.3 ± 0.62 mg/kg/year (range 3.9 to 11.1 mg/kg/year). Patients also received calcium supplement 0.5 to 1 gr/day and vitamin D 400-800 IU/day during therapy. Prior to intravenous therapy, these patients have received 1 year of oral pamidronate. Serum calcium(SCa) was analysed the 3rd day of the first cycle.Spine bone mineral density (BMD) L1-L4 was measured by DEXA. Knees X-rays were taken every 6 months. Basal height Z-score (range 0.93 to -9.75) increased, but not significantly after therapy: -4.0±0.8 vs -3.6±0.8. BMD increased significantly after 1 year of treatment (0.35 ± 0.03 to 0.45 ±0.03 gr/cm2, p<0.02) and Z-Score BMD (-4.1± 0.2 to -2.8 ± 0.3, p <0.005). There was an average annual BMD increase of 31.5% (range 4 to 82%). The patients had an annual BMD increase of 22.5% during previous oral APD therapy. Fractures, oscilating between 0.7 to 21 per year before therapy, decreased sharply with oral APD (3.8±1.4 vs 1.3±0.4 by year, p<0.05) and continued low with IV-APD (1.1±0.3 by year, p <0.02 vs baseline). As in normals,the Z-score BMD was positively correlated with height before and after 1 year of treatment (r=0.52, p <0.05 and 0.73, p <0.003 respectively). SCa decreased significantly (9.6±0.2 to 8.6±0.2 mg/dl, p<0.001) at the end of the first cycle. 25OHD increased significantly after 1 year of IV therapy: 21±1 to 28±2 ng/ml(p<0.001) probably due to a greater outdoor activities. X-rays showed dense lines under growth plates in most patients. Conclusion: One year of intravenous cyclical pamidronate improved bone mass in children with Osteogenesis Imperfecta with a diminution of the number of fractures and better quality of life. The BMD had a continuous increase even in patients who had previously augmented the BMD after oral APD.

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A Collaborative Group for the Identification of Mutations in Infantile Malignant Osteopetrosis. P. Vezzoni,*[1] W. Van Hul,*[2] A. Teti,*[3] G. F. Carle.[4] [1]Istituto di Tecnologie Biomediche Avanzate, Consiglio Nazionale delle Ricerche, Segrate, Italy, [2]Department of Medical Genetics, University of Antwerp, Antwerp, Belgium, [3]Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy, [4]IFR50 Génétique et Signalisation-UMR6549 CNRS, Université de Nice-Sophia Antipolis, Nice, France.

Infantile Malignant Osteopetrosis (arOP) is a recessive inherited disease characterized by a defect of osteoclast resorptive function. Osteoclast resorption failure leads to a general increase in bone density, causing bone marrow fibrosis, severe anemia, massive hepatosplenomegaly, progressive blindness and deafness. This disease has a fatal outcome within the first decade of life and the only treatment to date is bone marrow transplantation. The severity and progressiveness of the disease underline the importance of early diagnosis, ideally even in prenatal life. Recent studies by several groups have shown that the majority of the patients affected with arOP present mutations in the gene encoding the osteoclast-specific 116 kDa subunit of the vacuolar proton pump (ATP6i). The Atp6i gene is being studied as well in mutant mice. Another gene involved in the pathogenesis of arOP, CLCN7, has been recently characterized both in mice and humans. So far more than 70 patients have been collected and analysed for mutations in the ATP6i gene. The CLCN7 gene is being investigated in the subset of patients lacking detected ATP6i mutations. As some patients do not seem to bear mutations in either ATP6i or CLCN7 genes, the search for new genes that may interfere with normal osteoclast function is currently being carried out. In order to facilitate the studies on this rare disease and in particular to define genotype/phenotype correlations, we have created a network gathering clinicians and researchers interested in autosomal recessive osteopetrosis and the associated animal models (contact: carle@unice.fr)

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Comparison of Mineral Characteristics in *oim/oim* Mice and Human Osteogenesis Imperfecta. N. P. Camacho,[1] W. Tivol,*[2] K. Buttle,*[2] C. L. Raggio,*[1] S. B. Doty,*[1] W. J. Landis.[3] [1]Research Division, Hospital for Special Surgery, New York, NY, USA, [2]Wadsworth Center for Laboratories and Research, Albany, NY, USA, [3]Northeastern Ohio Universities College of Medicine, Rootstown, OH, USA.

Osteogenesis imperfecta (OI) is a heritable disease caused by molecular defects in type I collagen. It is characterized by skeletal deformities, fragile bones and multiple fractures. The homozygous OIM mouse (*oim/oim*) produces type I collagen composed of α1 homotrimers rather than $(\alpha 1)_2(\alpha 2)$ heterotrimers, and phenotypically models moderate-to-severe human OI (Type III). Currently, *oim/oim* mice are used in research to evaluate drug therapies in OI, but questions remain concerning their validity as an animal model of human OI. The purpose of this study was to confirm that their bone mineral ultrastructure is similar to that in human OI. Tibial cortical bone from two children with Type III OI was obtained under an IRB-approved protocol during surgery. Tibial bone was also obtained from *oim/oim* and wildtype (+/+) control mice under an IACUC-approved study. All bone samples were prepared anhydrously, embedded in Spurr resin and thin-sectioned for examination without staining by high voltage electron microscopy. Results: Both human OI tissues appeared heterogeneous, having adjacent tissue regions that were variable in electron density and texture of mineral and matrix. Selected area electron diffraction of human bone crystals confirmed an apatitic mineral phase with some crystal orientation, but that was also variable among tissue regions. The *oim/oim* bone was heterogeneous in appearance as well, but the mineral was less crystalline and/or oriented than that of human OI bone. Further, unlike human OI bone, the *oim/oim* tissue was densely mineralized and obscured matrix structural features. This observation is in agreement with other studies where the bone from these mice has been shown to be highly mineralized. The +/+ bone was not as mineralized as *oim/oim* bone, and the crystals were more clearly visualized. By electron diffraction, this mineral phase was the most highly oriented compared to human OI and *oim/oim* bone. In summary, the ultrastructural heterogeneity present in human OI is also apparent in *oim/oim* mice, as is reduced mineral crystallinity. However, the presence of dense mineral in the *oim/oim* mice is different from the human OI phenotype, and may contribute to bone fragility in this animal model.

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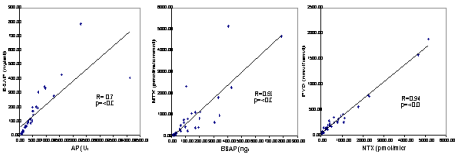
Markers of Bone Metabolism in Fibrous Dysplasia. S. Akintoye,*[1] J. Lee,*[1] C. Chebli,*[1] S. Booher,*[1] S. Wientroub,*[2] P. Bianco,*[3] P. G. Robey,*[1] M. T. Collins.*[1] [1]NIH, Bethesda, MD, USA, [2]Dana Childrens Hosp., Tel Aviv, Israel, [3]University of Rome, Rome, Italy.

Fibrous dysplasia of bone (FD) is characterized by replacement of bone marrow and bone with a benign fibroosseous tissue composed primarily of an expanded population of bone marrow stromal cells. Serum and urine markers of bone metabolism are sometimes used to monitor the extent of disease, its progression and response to therapy. In this study we evaluated multiple markers in a population of patients with a spectrum of disease severity. Thirty-six patients with FD (13 males, 23 females, age range 7-57) were evaluated. Disease severity ranged from mild (monostotic) to severe disease with virtually all bones involved (panostotic). We examined bone "formation" markers alkaline phosphatase (AP), osteocalcin (OC), and bone-specific alkaline phosphatase (BSAP), and bone "resorption markers", pyridinoline/creatinine (PYD) and deoxypyridinoline/creatinine (DPYD) cross-links and collagen N-telopeptide cross links/creatinine (NTX). The ranges and normal ranges ( ) were as follows: AP 55-4115 U/L (37-116), OC 7-610 mg/L (2-15), BSAP 10-787 ng/ml (<2-24), PYD 31-2000 nmol/nmol (18-40), DPYD 5-2000 mmol/mol (5-14), NTX 31-5137 pmol/μmol (0-64). There was a high positive correlation between all markers of formation and resorption.



The degree of elevation of the markers parallels the severity of disease, such that those patients with monostotic FD had the lowest and those with severe disease had higher values. These data indicate that the range of values for markers of bone metabolism in FD is wide; that the degree of elevation reflects the degree of FD severity and that any marker is useful in evaluating disease severity.

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Suppression of Arthritic Bone Destruction by Adenovirus-mediated Dominant Negative Ras Gene Transfer to Synoviocytes and Osteoclasts. A. Yamamoto, Y. Kadono, T. Miyazaki, T. Akiyama, T. Ogata,* I. Nakamura, H. Oda,* K. Nakamura, S. Tanaka. Department of Orthopaedic Surgery, The University of Tokyo, Tokyo, Japan.

Rheumatoid arthritis (RA) is a chronic systemic disorder of unknown etiology, characterized by inflammation, pathological proliferation of activated synovial cells and destruction of affected joints. Small GTPase Ras, protein product of protooncogene *ras*, plays a well-recognized role in relaying mitogenic intracellular signals and mediates cell growth, and we previously reported that signaling pathways mediated by Ras is essential for the survival of osteoclasts. In malignant tumor cells, mutations in the *ras* gene are frequently observed, which result in progression of the tumor growth. Mutations of *ras* gene in synovial specimens are also observed in some cases of RA patients, indicating that Ras signaling is involved in the pathogenesis of RA. The purpose of this study was to clarify the role of Ras-mediated signaling pathways in synoviocyte activation and bone destruction in arthritic joints. Primary synovial fibroblast-like cells (SFC) were obtained from RA

patients at the time of joint replacement surgeries. Replication-deficient adenovirus vector carrying the dominant negative mutant of *ras* gene ($AxRas^{DN}$) was constructed by homologous recombination in 293 cells. $AxRas^{DN}$ markedly reduced proliferation of SFCs in an MOI (multiplicity of infection)-dependent manner, as determined by bromodeoxyuridine incorporation and cell proliferation assay. Interleukin (IL)-1 is an important proinflammatory cytokine involved in RA pathology and a potent inducer of IL-6. IL-1 rapidly induced activation of MAP kinases (ERK, p38, and JNK) in SFCs, and their activation was inhibited by Ras^{DN} overexpression. IL-1-induced IL-6 production was also suppressed by Ax Ras^{DN} in both transcriptional and protein levels. The *in vivo* effect of $AxRas^{DN}$ on arthritic bone destructon was investigated using rat adjuvant arthritis as an animal model of RA. Direct injection of $AxRas^{DN}$ into ankle joints of adjuvant arthritis rats markedly ameliorated inflammation and suppressed bone destruction in the affected joints. In conclusion, our data indicate that Ras-mediated signaling pathways are involved in activation of RA synoviocytes as well as osteoclast activation in arthritic joints, and that adenovirus-mediated inhibition of Ras signaling can be a novel approach for RA treatment targeting both synoviocyte activation and osteoclastic bone destruction.

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Profile of the Risk Factors for Unilateral and Bilateral Knee Osteoarthritis: A Case-Control Study. J. Brantus,*[1] I. Reading,*[1] P. Croft,*[2] D. Barrett,*[3] D. Coggon,*[1] C. Cooper.*[1] [1]Environmental Epidemiology Unit, Southampton General Hospital, Southampton, United Kingdom, [2]North Staffordshire Medical Institute, Stoke-on-Trent, United Kingdom, [3]Dept of Orthopaedic Surgery, Southampton General Hospital, Southampton, United Kingdom.

Objectives: To examine the association of body mass index (BMI), Heberden's nodes and previous knee injury with unilateral or bilateral osteoarthritis (OA) of the knee in a case-control study.Methods: We performed a population-based case-control study in three health districts of England (Southampton, Portsmouth and North Staffordshire). 540 men and women aged 45 years and over, consecutively listed for surgical treatment of primary knee OA, were compared with 540 controls matched by age, sex and family practitioner. Results: 256 (47.4%) of cases had unilateral knee OA, and 274 (50.7%) of cases had bilateral involvement. Independent risk factors for knee OA included obesity (odds ratio [OR] 7.3; 95% CI 4.8-11.2), previous knee injury (OR 4.9; 95% CI 3.2-7.4), and hand joint involvement (OR 2.3; 95% CI 1.5-3.5). All three risk factors were significantly associated with the risk of unilateral and bilateral knee OA, but previous knee injury was most strongly associated with unilateral disease, while obesity was more strongly associated with bilateral involvement.Conclusion: The results suggest that the relative contribution of mechanical and constitutional factors in the pathogenesis of unilateral or bilateral knee OA might vary. They confirm the importance of significant knee injury and obesity as risk factors for unilateral and bilateral involvement respectively.

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The Inhibitory Effects of Incadronate on Bone and Joint Destruction in Adjuvant Arthritis. Comparison of Daily and Weekly Administration. T. Shuto, H. Zhao,* G. Hirata, A. Matsuo,* Y. Iwamoto.* Department of Orthopaedic Surgery, Kyushu University, Fukuoka, Japan.

We recently demonstrated that daily administration of incadronate inhibits bone and joint destruction in rat adjuvant arthritis (AA) when we injected the incadronate prior to the onset of arthritis (J Orthop Sci 5: 397-403, 2000). In the present study, we examined if incadronate given after the onset of arthritis prevents bone and joint destruction in AA. We also compared the inhibitory effects of daily and weekly injection of incadronate in established arthritis rats. Forty-eight female Lewis rats were randomly allocated into 6 groups (8 rats/group). Five groups were given intradermal injection of heat-killed *Mycobacterium butyricum* for induction of AA. In the four incadronate-treated AA groups (2 groups for 0.1 and 1 mg/kg/day, the other 2 groups for 0.1 and 1 mg/kg/week), incadronate was injected subcutaneously from day 14 (the day of immunization with the adjuvant was termed day 0) to day 42 (the end of the experiment). The effects of incadronate in AA rats were evaluated according to arthritis score, hind paw volume, radiological score and histological findings. This experimental protocol was approved by the Committee of the Ethics on Animal Experiment in Graduate School of Medical Sciences, Kyushu University. Both daily and weekly administration of incadronate suppressed not only arthritis score and hind paw volume but also radiological score in established AA rats. The histopathological destructive changes in AA were also inhibited by incadronate. The number of TRAP-positive cells in bone marrow spaces in the incadronate-treated groups was also decreased. Weekly as well as daily injection of incadronate was effective in prevention of inflammation and bone and joint destruction in established AA rats. We propose that the ability of incadronate to ameliorate the pathological changes of AA in rats warrants further evaluation with regard to the treatment of various arthritic conditions, including human rheumatoid arthritis.

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Pregnancy-Associated Osteoporosis: A Series of 5 Cases. S. Venkatachalam,*[1] A. K. Bhalla.*[2] [1]Rheumatology, Royal National Hospital for Rheumatic Diseases, Bath, United Kingdom, [2]Royal National Hospital for Rheumatic Diseases, Bath, United Kingdom.

Pregnancy-associated osteoporosis is not well recognised. Back pain in pregnancy or postpartum is not always related to posture. We communicate a short series of 5 cases with Pregnancy-associated osteoporosis seen in our unit in the last decade.Illustrative case: A 33 year-old previously healthy primipara presented with severe back pain and impaired mobility 3 days after delivery. The initial treating physicians excluded neural compression with MRI of the thoracolumbar spine and treated her with opiates and physiotherapy. The back pain was unrelenting when we saw her 4 months after onset. She had adequate calcium intake and had no anorexia or malabsorption. She had never received steroids, heparin or anticonvulsants. Her grandmother had a hip fracture. She had lost 5cm of height and had dorsal kyphosis and exaggerated lumbar lordosis. Radiographs showed multiple vertebral fractures from T1 to L5. Bone mineral density (BMD) was low at both the lumbar spine (Z=-2.46) and the hip (Z=-1.95). Full blood count, plasma viscosity, liver, renal and thyroid function, protein electrophoresis, serum calcium, phosphorus, magnesium, alkaline phosphatase, vitamin D and PTH and 24 hour urine calcium excretion were all normal. Bone markers showed increased resorption (Dpd/Cr). Bone biopsy was normal. Her back pain improved with analgesics and physiotherapy. She also received calcium, vitamin D and Alendronate.Two other women aged 29 and 37 years respectively, also presented with back pain and vertebral fractures after their first delivery. They had no other risk factor for osteoporosis. All investigations were normal except low lumbar spine BMD. One of them had an uncomplicated second pregnancy.Another 33 year-old woman presented with back pain and vertebral fractures of T6,T8 and L1, six weeks after her second delivery. She had low lumbar spine BMD (Z=-3.56) which improved after a year (7%) and has remained stable 7 years later.A 29 year-old woman was diagnosed incidentally as the lumbar spine BMD was reduced significantly (8%) after her third delivery. Her mother suffered from systemic mastocytosis and osteoporosis. The BMD recovered in 6 months. Conclusion: Pregnancy-associated osteoporosis is not uncommon. It should be suspected when a woman presents with unrelenting back pain in pregnancy or postpartum. The back pain improves gradually but the patients are left with the vertebral deformity and poor self-image. Calcium or bisphosphonates may be useful in treatment though spontaneous recovery is the norm.

SU481

Expression of PTHrP and Its Cognate Receptor in the Microcirculation of the Human Rheumatoid Synovium. **J. Funk,[1] H. Wei,*[1] J. Chen,*[1] W. Carley.*[2] [1]Medicine, University of Arizona, Tucson, AZ, USA, [2]Bayer Corporation, New Haven, CT, USA.**

The rheumatoid synovium is an extremely vascular tissue. Indeed, angiogenesis is thought to play an important role in the tumor-like growth of the cytokine-rich rheumatoid synovium. Parathyroid hormone-related protein (PTHrP), a vasoactive peptide that has recently also been shown to play a role in tumor angiogenesis, is produced in increased amounts by the rheumatoid synovium where its expression has been localized to synoviocytes and the synovial vasculature. Studies were therefore undertaken, using both human synovial tissue and primary cultures of human rheumatoid synovial microvascular endothelial cells, to identify the type of vascular cells expressing PTHrP or its cognate receptor (PTHR1) in the rheumatoid synovium. In synovial tissue specimens obtained from 5 patients with rheumatoid arthritis undergoing joint replacement surgery, endothelial cells were the primary site of PTHrP expression in the synovial vasculature; immunoreactive PTHrP was located in both the nucleus (speckled pattern) and cytoplasm of these cells. In contrast, smooth muscle actin-positive pericytes surrounding the endothelial cells were the primary site of vascular PTH/PTHrP receptor (PTHR1) expression. Immunoreactive PTHR1 was present in pericyte nuclei, while the cytoplasm appeared PTHR1-negative. Faint PTHR1 immunoreactivity was also occasionally seen in synovial endothelial cells. Expression of PTHrP by human synovial endothelial cells was confirmed by *in vitro* studies using primary cultures of microvascular endothelial cells isolated from the human rheumatoid synovium. Cultured synovial endothelial cells expressed PTHrP mRNA, as assessed by RT-PCR; were immunopositive for PTHrP, displaying the same intracellular distribution of PTHrP staining as *in situ* cells; and secreted PTHrP(1-86) protein, as assayed by Nichols PTHrP IRMA. These results suggest a possible paracrine loop for PTHrP activity in the synovial microcirculation, wherein N-terminal-containing PTHrP peptides secreted by the synovial endothelium act on surrounding PTHR1-positive pericytes. While previous studies have also reported nuclear PTHR1 localization in non-vascular cells, the physiologic role of nuclear PTH/PTHrP receptors and putative mechanisms of nuclear receptor localization have not been elucidated.

SU482

PTHrP Neutralizing Antibody Inhibits Streptococcal Cell Wall (SCW)-Induced Bone Resorption and Granuloma Formation, but Not SCW-Induced Joint Inflammation in Lewis Rats. J. Chen,* S. Davie,* G. Stafford,* J. Funk. Medicine, University of Arizona, Tucson, AZ, USA.

Recent reports documenting increased expression of parathyroid hormone-related protein (PTHrP) in the synovium and synovial fluid of patients with rheumatoid arthritis suggest that this bone-resorbing peptide may play a role in the pathophysiology of joint destruction in rheumatoid arthritis. To test this hypothesis, the effect of PTHrP neutralizing antibody on disease progression was tested in streptococcal cell wall (SCW)-induced arthritis, a classic animal model of rheumatoid arthritis. In response to SCW treatment (25-30 µg SCW/g body weight ip), 100 g female Lewis rats developed an acute phase of joint swelling. Subsequent to a brief nadir in disease activity, animals then developed a chronic phase of joint swelling which is known to be T cell-dependent. During this chronic phase of arthritis, serum pyridinoline (Metra Biosystems Serum Pyd), a biochemical marker of bone resorption, increased 2-fold in SCW-treated animals ($p<0.01$). As has been reported for rheumatoid arthritis in humans, while serum levels of PTHrP, assayed by Nichols PTHrP IRMA, did not increase during the acute or chronic phase of SCW-induced arthritis, PTHrP expression increased locally in the arthritic joints of these animals, as determined by immunohistochemical staining. The granulomas that develop in the spleens and livers of SCW-treated rats in response to local SCW deposition were also immunopositive for PTHrP. Treatment with PTHrP neutralizing antibody (1.5-3 µl/g ip twice weekly) had no effect on joint swelling, but prevented the increase in serum pyridinoline that occurred during the chronic phase of arthritis. PTHrP antibody treatment also inhibited the formation of splenic and hepatic granulomas, as assessed by histopathologic evaluation of excised tissues. These findings suggest that endogenous PTHrP is an important meditator of inflammation-associated bone resorption. Additionally, the unanticipated finding of a protective effect of PTHrP antibody in preventing granuloma formation suggests a potentially important role for PTHrP in modulating the immune response in granulomatous disorders.

SU483

Validity of Patient-Reported Diagnoses of Osteoarthritis and Rheumatoid Arthritis: Results from the Canadian Multicenter Osteoporosis Study (CaMOS). A. Berard,*[1] J. Adachi,[2] W. Olszynski,[3] L. Pickard,*[4] J. Kedra,*[3] G. Ioannidis.*[4] [1]Epidemiology, Albert Einstein College of Medicine, Bronx, NY, USA, [2]Rheumatology, McMaster University, Hamilton, Canada, [3]Osteoporosis Center, Saskatoon, Canada, [4]McMaster University, Hamilton, Canada.

Background: Due in part to patient confusion regarding the clinical presentations of OA and RA, it is necessary to evaluate the validity of self-reported OA and RA at the same time. As of now, no studies have looked at the validity of patients' self-reports of OA along with self-reports of RA. Objective: Validate patients' self-reports of OA and RA using rheumatologist diagnosis as a gold standard, in a population of patients living in the community.Method: A sample of patients included in CaMos was selected for this validation study. To be eligible patients had to be 1) in CaMos, 2) from the Hamilton, ON or the Saskatoon, SA sites, 3) 45 years or older, 4) and still living in the community. At each site, a list of all CaMos participants who reported having OA or RA at any time between inclusion and August, 31, 1999, was prepared by the site coordinator. 10% of patients who reported having OA and 50% of patients who reported having RA were randomly selected; of the remaining CaMos participants who did not report having OA or RA, a 5% random sample was also selected for inclusion in this study. One of the two participating rheumatologists made the diagnoses of OA or RA in a blinded manner according to the American College of Rheumatology criteria. Sensitivity, specificity and positive and negative predictive values were calculated using the rheumatologists' diagnosis as a gold standard. Results: 140 patients were included in this validation study: 41 who reported OA, 68 RA, and 43 no OA/RA. The mean age (standard deviation) of the study group was 67 (8) years. Using the rheumatologists' diagnosis as a gold standard, self-reported OA was 32% sensitive and 84% specific with positive predictive value of 90% and negative predictive value of 21%; self-reported RA was 100% sensitive and 54% specific with positive predictive value of 10% and negative predictive value of 100%. Patients who reported having no OA and no RA were 100% accurate for the diagnosis of RA and 29% accurate for the diagnosis of OA.Conclusion: Although self-report of OA has higher specificity than self-report of RA, this study suggests that they are not sufficient measures to identify patients in healthy living individuals: OA/RA medication history or rheumatologist diagnosis could be used in addition to patient's self-report to increase sensitivity and specificity in epidemiologic studies.

SU484

Bone Loss in HIV: Effects of Treatment Type, Insulin Resistance, and Fat Redistribution. T. T. Brown,* M. Ruppe,* R. Kassner,* T. Kehoe, P. Kumar,* J. Pezzullo,* J. Timpone.* Georgetown University, Washington, DC, USA.

Highly active anti-retroviral therapy (HAART) for HIV has been associated with multiple metabolic abnormalities, including insulin resistance, dyslipidemia, central fat accumulation and peripheral fat wasting. Reduced bone mineral density has also been described in this population and has been attributed to protease inhibitor (PI) use. Associations with the other metabolic abnormalities remain unclear. We performed a cross-sectional analysis of 46 HIV patients in our clinic population on HAART: 24 on PIs and 22 on non-nucleoside reverse transcriptase inhibitors (NNRTIs) (85% men, mean age 40 yrs, mean CD4 675 cells/mm3, 72% with viral load < 400 copies). Bone density at the spine, hip, and forearm was determined, in addition to a lipid profile and a glucose tolerance test (OGTT). Body fat composition was done using DXA. A laboratory evaluation for secondary causes of bone loss was also completed.Patient characteristics between the PI and NNRTI groups were similar except for duration of HAART therapy [40 mo. (PI) vs. 24 mo. (NNRTI), p<0.0001]. Bone loss at any site using WHO criteria:

T score	< - 2.5	-1.0 to - 2.5	< - 1.0
All (N=46)	8.7%	54.3%	63.0%
PI (N=24)	16.7%	54.1%	70.8%
NNRTI (N=22)	0.0%	54.5%	54.5%

One mineral density was significantly reduced in the PI group at the femoral neck (P=0.019) and total hip (P=0.008) compared to the NNRTI group. These differences were maintained after controlling for duration of therapy. There was no laboratory evidence of secondary etiologies. Dyslipidemia was more prevalent among the PI group (54% v. 13.6%, chi square 8.31, P=0.004), but was not associated with bone loss at any site. Insulin sensitivity (as estimated from OGTT) did not differ between the groups, but was correlated with increased spine, forearm, and ultradistal radius BMD in the PI group (r=0.603, P=0.002; r=0.439, P=0.032; r=0.436, P=0.033), but not in the NNRTI group. These associations remained significant after controlling for duration of therapy. Measures of central fat accumulation and peripheral fat wasting were not significantly different between groups after controlling for duration of therapy. However, central adiposity (trunk fat mass: total fat mass) was significantly associated with reduced BMD at the ultradistal radius (r=0.547, P=0.013) in the PI group, but not in the NNRTI group and trends were seen at the total forearm (P=0.071) and hip (P=0.063) in patients on protease inhibitors. Bone loss is prevalent among HIV patients on HAART and is more pronounced in patients on protease inhibitors. Insulin resistance and central adiposity may be associated with decreased BMD in PI patients, but not NNRTI patients.

SU485

The Relationship of Serum Leptin With Body Composition and Bone Mineral Status in Children With Cystic Fibrosis. M. Sood,*[1] L. Patel,*[2] N. Zaman,*[2] M. Super,*[1] G. Hambleton,*[1] J. Adams,[3] P. Clayton,*[2] Z. Mughal.[4] [1]The Cystic Fibrosis Clinic, Manchester Children's Hospitals, Manchester, United Kingdom, [2]Department of Child Health, The University of Manchester, Manchester, United Kingdom, [3]Clinical Radiology, Imaging Science & Biomedical Engineering, The University of Manchester, Manchester, United Kingdom, [4]Department of Paediatric Medicine, Saint Mary's Hospital for Women & Children, Manchester, United Kingdom.

Deficits in weight, height and bone mineralization have been observed in cystic fibrosis (CF) despite improvements in management. In mouse models, leptin not only affects satiety and thus the body weight, but it is also proposed to inhibit bone formation by a central hypothalamic action. We investigated the pattern of serum leptin by sex and pubertal status, and its relationship with body mass index (BMI), fat mass (FM), lean mass (LM) and total & regional bone mineral content & density in children with CF.Twenty-nine patients (median age 10.0; 17 pubertal; 12 girls) with CF on an unrestricted diet and pancreatic enzyme supplements were studied. BMI was expressed as SD scores using 1990 UK standards. Dual-energy X-ray absorptiometry (Hologic QDR 4500 scanner) was used to measure FM,LM, bone mineral content (BMC) and areal bone mineral density (BMD) of the whole body (TBBMC&D), lumbar spine (LS BMC&D) and total hip (TH BMC&D). Serum leptin concentrations were determined by radioimmunoassay. Values were log-transformed and also expressed as SD scores using local reference values.Serum leptin (median; SDS) was lower than normal references in prepubertal (2.7 µg/l; -0.5 SDS, P=0.007) and pubertal boys (2.3 µg/l; -0.6 SDS, P=0.0001), and prepubertal girls (2.4 µg/l; -1.0 SDS, P=0.01) but not in pubertal girls (7.8 µg/l; 0 SDS). The relationship between log leptin and BMI SDS (r=0.39, P=0.04) was weaker (P=0.001) than in healthy children (r=0.55, P=0.0001). Log leptin was associated with FM (r=0.86, P=0.0001) and age (r=0.45, P=0.01) but not LM (r=0.31, P=0.1). TBBMC (r= - 0.49, P=0.001), TBBMD (r= - 0.54, P=0.004), LS BMC & BMD (r = - 0.43, p=0.03), TH BMC (r= - 0.57, p=0.002) and TH BMD (r= - 0.58, p=0.001) were all inversely related to log leptin per unit fat mass. From these data we conclude that serum leptin in CF was principally determined by fat mass as in normal children. Unlike healthy children, the relationship between serum leptin and BMI SDS in CF was weaker and the inverse relationship with LM in normal children was not seen. In CF children, leptin adjusted to FM appears to be inversely related to bone mineral content & density.

SU486

Regulation of Apolipoprotein E Expression in Bone by Parathyroid Hormone. T. E. Hefferan, M. Zhang,* A. Maran, R. T. Turner. Department of Orthopedics, Mayo Clinic, Rochester, MN, USA.

The mechanisms by which parathyroid hormone (PTH) acts on bone are complex and are not fully understood. In order to examine the molecular regulation in bone by PTH, our laboratory utilized microarray analysis to determine which genes PTH regulates. Initially microarray analysis was done on RNA extracted from the tibial metaphysis of intact, 3-month old female Sprague Dawley rats. These rats were injected subcutaneously with vehicle or human PTH (1-34) at a dose of 80 ug/kg and sacrificed 12 hours later. The analysis of the RNA by microarray revealed a regulation of numerous genes including upregulation of apolipoprotein E with 12 h PTH treatment compared to control treated animals. We were interested in PTH regulation of apolipoprotein E since polymorphism of the gene has been associated with low bone mineral density. To confirm the microarray data, next we conducted a 24-h time course study using intact, 6-month old female Sprague Dawley rats. The animals were injected subcutaneously with vehicle or 80 ug/kg human PTH (1-34) and sacrificed at 0, 2, 4, 8, 12, 16 and 24 h after treatment. Northern blot analysis of RNA extracted from tibial metaphysis showed apolipoprotein E expression to be upregulated at the 16 h time point compared to control animals. A third study was conducted to determine the effect of longer-term pulsatile PTH treatment on apolipoprotein E expression in bone. In this study, 6-month old intact female Sprague Dawley rats were injected subcutaneously with vehicle or human PTH (1-34), 80ug/kg, daily for 7 days and sacrificed within 24 h of the last treatment. Northern blot analysis of RNA extracted from the femur metaphysis showed apolipoprotein E expression to be upregulated in the PTH treated animals compared to the control animals. We have shown apolipoprotein E expression to be upregulated in PTH treated animals, with a peak expression occurring 16 h after treatment and this upregulation was maintained through a 7-day course of treatment. Our data indicates that systemic PTH regulates apolipoprotein E expression in bone.

SU487

Treatment with Parathyroid Hormone Fragments Increases Fracture Strength and Callus Amount, and Strength and Callus Amount Normalize after Treatment Withdrawal. T. T. Andreassen,[1] G. E. Willick,*[2] P. Morley,*[2] J. F. Whitfield.[2] [1]Department of Connective Tissue Biology, University of Aarhus, Aarhus, Denmark, [2]Institute of Biological Science, National Research Council of Canada, Ottawa, Canada.

The effects of parathyroid hormone (PTH) fragments [(1-34)NH2, (1-31)NH2, and [Leu27]cyclo(Glu22-Lys26)(1-31)NH2], on mechanical strength and callus amount in rat tibial fractures were measured after 56 and 112 days of healing. The PTH fragments were injected at a dose of 15 nmol/kg once each day only during the first 56 days of the 112-day healing period. Control animals with fractures were given vehicle. There were 18-20 animals in each subgroup. No differences were found between the three PTH fragments. The combined results are given below (PTH fragments versus control; Mean with (SEM); N=newton).By the 56th day of healing, the PTHs had increased the ultimate load (N) to 51 (3) from 32 (4) in the control rats (p=0.001); the ultimate stiffness (N/mm) to 359 (20) from 227 (32) in the controls (p=0.001); the energy absorption at ultimate load (Nmm) to 5.7 (0.4) from 3.9 (0.3) in the controls (p=0.016); and the external callus volume (mm3) to 67 (4) from 54 (5) in the controls (p=0.047).By day 112 (56 days after stopping the PTH injections) there were no differences between the PTH-treated and controls animals. The ultimate load (N) was 63 (4) versus 56 (5) in the controls (p=0.26). The ultimate stiffness (N/mm) was 384 (18) versus 378 (39) in the controls (p=0.87). The energy absorption at ultimate load (Nmm) was 8.2 (0.8) versus 6.1 (0.7) in the controls (p=0.09). The external callus volume (mm3) was 50 (4) versus 49 (4) in the controls (p=0.95).Changes from day 56 to day 112 of healing: ultimate load continued to rise in both groups (p<0.001), with no difference in the rise between the groups (p=0.17). Ultimate stiffness continued to rise in

both groups (p=0.005), and the rise was higher in the control group compared with the PTHs (p=0.03). Energy absorption at ultimate load continued to rise in both groups (p<0.001), with no difference in the rise between the groups (p=0.63). External callus volume declined in both groups (p =0.009), with no difference in the decline between the groups (p=0.23).In conclusion, treatment with PTH fragments for 56 days increased mechanical strength and callus volume. After withdrawal of the PTH treatment at day 56 of healing, the strength continued to increase and the external callus volume declined, but by day 112 no differences in fracture strength and callus volume were found between control rats and PTH-treated rats.

SU488

Aluminium Inhibits the Ex Vivo Expression of Parathyroid Hormone. J. L. Fernández-Martín,*[1] A. Rodríguez-Rodríguez,*[1] C. Díaz-Corte,*[1] M. Naves,*[1] M. T. Fernández-Coto,*[2] J. B. Cannata-Andía.[1] [1]Bone and Mineral Research Unit. Instituto Reina Sofía de Investigación, Hospital Central de Asturias, Oviedo, Spain, [2]Department of Biochemistry, Hospital Central de Asturias, Oviedo, Spain.

In previous studies we found that aluminium inhibited PTH mRNA in rats with chronic renal failure. However, it is not yet known whether the mechanism is pre or post-transcriptional. The aim of this study was to find out an "ex vivo" model able to reproduce the "in vivo" findings in order to perform future studies at transcriptional level (run-on).The study was carried out in parathyroid glands extracted from 30 rats with normal renal function cultured "ex vivo". The 2 parathyroid glands from each rat were removed and placed in two different dishes (each one in one dish). Each experiment was performed pooling the parathyroid glands from the 10 rats (10 parathyroid glands in each dish). The parathyroid glands were washed for 8 hours using a lab-made medium (Hepes 25 mM, glucose 12 mM, L-glutamine 4 mM, sodium piruvate 1 mM, bovine serum albumin 0.1%, insulin 0.1 U/mL, penicillin 100 U/mL, streptomycin 100 U/mL, NaCl 125 mM, KCl 5.9 mM, $MgCl_2$ 0.5 mM, $CaCl_2$ 1.4 mM and phosphate 1 mM at pH=7.4). During the washing period the medium was changed every 2 hours. After 8 hours of washing the parathyroid glands were incubated at 37ºC for 24 hours in a medium containing either citrate (control) or aluminium citrate (10 and 100 µM). The ionic Ca, P and intact PTH in the medium and PTH mRNA in the parathyroid glands were measured. Intact PTH was measured by RIA (Nichols Institute®) and PTH mRNA was measured by Northern blot using a specific cDNA probe.Both concentrations of aluminium (10 and 100 µM) reduced the amount of PTH released by the parathyroid glands to the medium (47.2% with 10 µM and 34.2% with 100 µM). The mRNA of PTH was also lower in the glands cultured with aluminium (57.1% for 10 µM and 41.6% for 100 µM). There were no differences in Ca and P levels.Aluminium inhibited not only the PTH release but also the PTH synthesis measured by Northern blot in parathyroid glands cultured "ex vivo" as it has been previously demonstrated "in vivo". Therefore, the "ex vivo" model used in this study reproduces the "in vivo" results making it suitable to perform further studies at transcriptional level.

SU489

Immunolocalization of PTH Receptors in Osteoclasts of Rat Metaphysis. C. V. Gay, B. Z. Zheng,* V. R. Gilman,* A. M. Mastro.* Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA.

Many studies show that PTH and PTHrP activate osteoclasts indirectly through action on osteoblasts. However, a small body of literature where a variety of approaches have been used, indicates that direct action also occurs. Here we report immunoreactivity of osteoclasts to antibodies to PTH receptor. Metaphyses from 4 and 15 mo. old rats were fixed in 4% paraformaldehyde, demineralized, dehydrated and embedded in paraffin. Sections mounted on glass slides were prepared for immunostaining by removing the paraffin, rehydration and treatment with proteinase K (10 µg/ml in Tris buffered EDTA, 10 min). Polyclonal rabbit anti-PTH receptor (PRB-635P; BAbCO, Berkeley, CA) at 1/50 and 1/100 dilutions was applied for 4 hrs. Cy3-conjugated donkey anti-rabbit secondary antibody (Chemicon, Temecula, CA) was used at dilutions of 1/100 and 1/200. All antibody solutions and rinses contained 1% donkey serum, 0.5% Tween 20 in PBS. Digital confocal microscopy was used to obtain fluorescent images. Alternate sections from each tissue block were stained with TRAP to identify osteoclasts. All sections were digitized so that identical cells could be identified in the TRAP stained section and its partner section, which was immunostained. Large, multinucleate osteoclasts on the surfaces of trabecular bone were stained in sections of both young and old rat metaphysis. Rounded osteoclasts were stained diffusely throughout the cytoplasm. Osteoclasts which were flattened onto bone surfaces, contained intensely stained internal punctate regions. The internal staining of active osteoclasts suggests that occupied receptors are internalized. This is consistent with an earlier report that internalization of PTH by isolated osteoclasts occurs within 20 min (Agarwala and Gay, J. Bone and Mineral Res. 7:531-539, 1992). Osteoblasts and some bone marrow cells were also stained. Isotype controls (rabbit IgG) were negative. While the cell types that were immunopositive were the same in both young and old rat metaphysis, osteoclasts were larger and more plentiful in the old rat samples and osteoblasts were present in greater numbers in the young bone samples. Several factors may have contributed to the detection of PTH receptor in osteoclasts in this study. These include selection of metaphysis from rat as the model, where numerous, active osteoclasts were found; use of proteinase K and detergents to foster penetration of antibodies into the sections; use of laser scanning confocal microscopy to enhance the sensitivity of detection; and digitizing images so that identical regions of bone in both TRAP and immunostained sections could be identified.

SU490

Evaluating the Role of Bone ECM Proteins in PTH-Dependent Inhibition of Mineralization in MC3T3-E1 Cells: Evidence for Involvement of MGP. R. Gopalakrishnan,*[1] M. T. Chan,*[1] H. Ouyang,*[2] M. J. Somerman,[1] L. K. McCauley,[1] R. T. Franceschi.[1] [1]Periodontics/Prevention/Geriatrics, University of Michigan, Ann Arbor, MI, USA, [2]Cariology, Restorative Sciences and Endodontics, University of Michigan, Ann Arbor, MI, USA.

As part of its catabolic activity in bone, parathyroid hormone (PTH) is known to rapidly inhibit osteoblast-mediated mineralization in vivo. However, the mechanism of this inhibition is not well understood. To determine the basis for this effect, we used a strongly mineralizing subclone of MC3T3-E1 cells and changes in mineralization and gene expression were examined after PTH treatment. Using a 45Ca uptake assay, we found that cells began losing the ability to mineralize after as little as 3h of PTH treatment. While evaluating the mRNA expression of both positive and negative regulators of mineralization, we found that the time course for the inhibtion of mineralization best correlated with the induction of matrix Gla protein (MGP). MGP was rapidly induced one hour after PTH treatment and was increased by 5 to 10-fold after 3-6h, a response requiring protein synthesis. Furthermore, warfarin, an inhibitor of MGP g-carboxylation, reversed effects of PTH on mineralization. In contrast, osteocalcin, the other principle Gla protein of bone and a putative inhibitor of mineralization, was inhibited after prolonged (>12 h) PTH treatment. Treatment with forskolin, a cAMP synthesis activator, also increased MGP levels comparable to that following PTH treatment. Taken together, these results indicate that PTH-dependent inhibition of mineralization in osteoblasts is best explained by induction of MGP. We are currently evaluating the use of other PKA and PKC pathway activators and inhibitors to elucidate the signaling pathway/s involved in the PTH induction of MGP.

SU491

HPLC Fractionation and Detection of PTH Fragments in Patient Samples Using Two New Automated Assays Measuring PTH 1-84 and PTH 1-38. C. W. Cody,* J. S. Hutchison,* E. Guthrie,* J. Lu,* P. Miller,* M. Caulfield, R. E. Reitz. Nichols Institute Diagnostics, Quest Diagnostics, San Juan Capistrano, CA, USA.

This study was designed to investigate the different types of non (1-84) PTH fragments present in patient samples. Individual chronic renal failure patient samples, patient pools containing high or normal levels of PTH, and chemically synthesized PTH peptide standards were fractionated by reverse phase HPLC. HPLC fractions were assayed using 3 different PTH assays: the current Nichols Advantage Intact PTH assay and 2 newly developed automated PTH assays to PTH 1-84 and PTH 1-38.

PTH is considered to be bioactive if it is intact at the N-terminal; both of the new PTH assays incorporate an antibody that will only bind to PTH if the N-terminal region is intact. The bioactive assays differ with respect to their specificity towards the C-terminal region of PTH. The PTH 1-84 assay has the same specificity towards the C-terminal region as the current Nichols Intact PTH assay. The PTH 1-38 assay will also detect smaller N-terminal intact PTH fragments (chemically synthesized PTH 1-34 and PTH 1-38).

HPLC results showed that patient samples contained variable amounts of the two major forms of PTH as detected using the current Nichols Advantage Intact PTH Assay. The first peak to elute (non 1-84 PTH) had a retention time similar to that of chemically synthesized PTH 7-84. The second peak had a retention time identical to that of chemically synthesized PTH 1-84. The two HPLC peaks were also assayed using the new bioactive PTH 1-84 and PTH 1-38 assays. Both of the assays detected only the second HPLC peak (PTH 1-84) in all patient samples investigated. The PTH 1-38 assay also has the ability to detect smaller PTH fragments that have an intact N-terminus. Results indicate that small amounts of such fragments are present in some patient samples. The amount observed in different patient samples was highly variable, even in the presence of protease inhibitors.

We have demonstrated that two new Nichols assays (PTH 1-84 and PTH 1-38) do not detect non 1-84 PTH, which is similar to PTH 7-84, in HPLC fractionated patient samples. These assays have different specificities towards the C-terminal region of PTH, which distinguish small from large bioactive fragments.

SU492

Hypercalcemia and Calcified Brain Metastases Due to Ectopic Production of Intact Parathyroid Hormone by Small Cell Cancer. V. Botea,* R. Munasinghe,* G. Edelson.* Internal Medicine, Sinai Grace Hospital, Detroit, MI, USA.

Introduction: Humoral Hypercalcemia of Malignancy due to PTH-related protein (PTH-rP) is well recognized. However, only a few cases of hypercalcemia due to ectopic production of intact parathyroid hormone (iPTH) from non-parathyroid cancer have been reported. Very rarely brain metastases can be detected as calcified lesions but the association with hypercalcemia is not well established. Case report: A previously healthy 50 year old man presented with a two month history of back and hip pain. He also reported recent weight loss, nausea, vomiting and increasing constipation. Physical examination revealed a confused, dehydrated, cachectic patient with clubbing of fingers and tenderness over the hips and neck. Laboratory studies revealed a total serum calcium of 19.1mg/dl and a phosphorus of 5.2mg/dl. A CT scan of the head showed multiple bilateral calcified metastases. Chest CT scan showed a right lung cavitary mass. A bone survey revealed osteolytic lesions of the left femur. Biopsy of a femoral lesion yielded histology consistent with small cell carcinoma. The serum iPTH level was high (107pg/ml, N=10-65). An immunohistochemistry stain was strongly positive for iPTH in the biopsy tissue. Discussion: To date only seven cases of true ectopic production of iPTH from non-parathyroid malignancies have been reported of which only two have been from small cell carcinoma. None of these cases were associated with calcified brain metastases. The commonest reported cause of calcified brain metastases is lung cancer. There does not appear to be a recognized association between the presence of hypercalcemia and the tendency for metastases to calcify.

SU493

Evaluation of PTH Molecular Forms and of PTH-Fragments/PTH(1-84) Ratio in a Transversal Study Covering 30 Years of Renal Transplantation. J. H. Brossard,[1] L. Rousseau,*[1] G. St-Louis,*[2] M. Paquêt,*[2] M. Sadouk,*[1] P.

D'Amour.[1] [1]Medicine, Centre de recherche du CHUM, Hôpital Saint-Luc, Montréal, PQ, Canada, [2]Medicine, Hôpital Notre-Dame, Montréal, PQ, Canada.

To better understand the regulation of calcium (Ca) and bone metabolism in renal transplanted patients (RTP), we measured Intact (I-), Cyclase Activating (CA-) and carboxyterminal (C-) PTH as well as total corrected Ca (Ca_{tc}), phosphate (PO_4), alkaline phosphatase (AP) and creatinine (Cr) in over 400 patients transplanted over 30 years. To eliminate confounding factors, we decided to retain only the 106 patients with Cr level < 100 pmol/L. These patients were compared to 111 normal individuals (NI) of similar age and sex. Mean serum Ca_{tc} concentration was higher in RTP (2.47±0.12 vs 2.40±0.08 mmol/L, $p < 0.0001$) , and 23.6% of these patients had values above 2.55 mmol/l. Mean serum PTH concentrations were also increased in RTP (I-PTH :8.3±4.2 vs 2.5±1.1 pmol/L, $p< 0.0001$; CA-PTH :5.3±3.1 vs 1.9±0.8 pmol/L, $p< 0.0001$; C-PTH :17.5±8.9 vs 8.6±1.9 pmol/L, $p< 0.0001$) and 59.4, 69.8 and 82.1% of patients had elevated values respectively. Values of the C-PTH/I-PTH ratio (2.26±0.77 vs 3.81±1.19, $p< 0.0001$) and of the C-PTH/CA-PTH ratio (3.37±1.51 vs 5.11±1.80, $p< 0.0001$) were also decreased in RTP compared to NI. In NI, PTH values correlated negatively with Ca_{tc} concentrations ($r =-0.196$, $p< 0.05$) while they did not correlated (I- and CA-PTH) or correlated positively (C-PTH) with Ca_{tc} concentrations ($r = 0.264$, $p< 0.01$) in RTP. PTH values tended to decrease with time since transplantation, correlated negatively with PO_4 values ($r = -0.411$, $p < 0.0001$) and positively with AP values ($r = 0.211$, $p < 0.05$) in RTP. These results indicate elevated PTH values in a majority of RTP, related to hypophosphatemia and elevated bone turnover. Low C-PTH/I-PTH or C-PTH/CA-PTH ratio do not favor an adaptation to an increased parathyroid function but suggests different degrees of clinical or subclinical primary hyperparathyroidism in these patients, with little improvement with time.

SU494

Effects of Parathyroid Hormone (PTH) on Gene Expression of RANK Ligand (RANKL), Osteoprotegerin (OPG) and the Cognate Receptor for PTH in Mice In Vivo. S. S. Lu,[1] M. Ducayen-Knowles,*[1] D. W. Demster,[1] R. Lindsay,[2] A. Iida-Klein.[1] [1]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Clinical Research Center, Helen Hayes Hospital, West Haverstraw, NY, USA.

We have previously demonstrated that intermittent administration of PTH increases bone mass and reverses ovariectomized (Ovx)-induced bone loss in mice. However, the molecular mechanisms underlying the PTH effects are not well understood. Accordingly, we examined the effects of PTH on gene expression of a regulator of osteoclast recruitment, RANK-ligand (RANKL), its decoy receptor osteoprotegerin (OPG), as well as effects on the cognate receptor for PTH (PTH1R), during both Ovx-induced bone loss and treatment with PTH in intact and Ovx mice (C57BL/J6).

Twelve-week-old mice were ovariectomized or sham-operated, and followed for 2 and 4 weeks. At 4 weeks post-Ovx, mice were treated with PTH (hPTH 1-34, 40mcg/kg, 5 days/ week, s.c.) for 3 and 7 weeks. Following sacrifice total RNA was extracted from the entire right tibia, pooled, and mRNA levels measured by RT-PCR and Northern Analysis. As control, intact adult mice (age 10 weeks) were treated with PTH at the same dose for 3 and 7 weeks also.

Ovx increased mRNA levels for RANL over the first 4 weeks (55 and 104% at 2 and 4 weeks, respectively). By 7 and 11 weeks RANKL mRNA had fallen below baseline by 33 and 67%, respectively. OPG mRNA was reduced by Ovx at all time points falling 80% by 11 weeks. Ovx had no effect on PTH1R mRNA. In intact animals, however, PTH administration stimulated gene expression of RANKL and PTH1R by 4 and 3 fold, respectively, at all time points, without effect on OPG. Following Ovx, PTH suppressed expression of RANKL below intact levels, by 67% after 7 weeks of treatment, but stimulated OPG and PTH-1R expression by 2-3 fold. Thus, independently of estrogen status PTH given intermittently stimulates expression of its own receptor in mice in vivo. However, the response of RANKL is dependent upon estrogen status, increasing in intact, but decreasing in estrogen-deficient animals in response to PTH. Similarly the effect of PTH on OPG expression is determined by estrogen status, mRNA increasing only in Ovx animal. These modulations in expression of osteoclastogenesis regulators may help explain differences in response to PTH in estrogen replete and deplete animals.

SU495

Concentration-dependent Effects of Parathyroid Hormone on Calcium Signaling Pathways in Endothelial Cells. D. Throckmorton,*[1] D. Kurscheid-Reich,*[2] Q. Zhong,*[1] K. Ding,*[1] R. McCarthy,*[2] P. Barrett,*[3] C. M. Isales.[1] [1]Medical College of Georgia, Augusta, GA, USA, [2]Yale University, New Haven, CT, USA, [3]Pharmacology, University of Virginia, Charlottesville, VA, USA.

We have previously reported that parathyroid hormone (PTH) has specific effects on a human umbilical vein endothelial cell line(ECV-304) including stimulating 3H thymidine incorporation and endothelin-1 release. Further studies were performed to characterize the signaling cascades initiated by PTH. We report that PTH induced the appearance of voltage sensitive calcium channels. While treatment of ECV-304 cells with extracellular potassium did not increase intracellular calcium, as measured with aequorin, if the cells were pretreated with PTH (10nM) then subsequent exposure to increasing extracellular potassium, raised intracellular calcium. Furthermore, treatment of ECV304 cells with PTH (10nM) induced the appearance of calcium channels at hyperpolarized potentials, as measured by patch clamp. Increases of intracellular calcium can be associated with activation of phospholipase C. We found that PTH increased the lipid second messenger, ceramide but not diacylglycerol content. Since elevations in [Ca2+]i and phospholipid turnover are signals for the activation of protein kinase C (PKC), the cells were screened for PKC isoforms. PTH induced a redistribution of the PKCe to the particulate fractions of cell homogenates. In summary, PTH induced PKC translocation through a calcium-phospholipid pathway in an endothelial cell line. Since ceramide is known to cause smooth muscle relaxation we speculate that PTH-induced smooth muscle relaxation may be mediated by ceramide.

SU496

Familial Hypocalciuric Hypercalcemia (FHH) Caused by an R648stop Mutation in the Calcium-Sensing Receptor (CaR) Gene: A Case Report of an 84-year-old Female Proband with Parathyroid Enlargement and Elevated Serum Parathyroid Hormone (PTH) level. M. Yamauchi,*[1] T. Sugimoto,[1] T. Yamaguchi,[1] S. Yano,*[1] J. Wang,*[2] E. M. Brown,[2] M. Bai,*[2] K. Chihara.*[1] [1]Third Division, Department of Medicine, Kobe University School of Medicine, Kobe, Japan, [2]Endocrine-Hypertension Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

In this study, we report an 84-year-old female proband in a Japanese family with FHH caused by a R648stop mutation in the CaR gene. She presented with hypercalcemia (11.4 mg/dl), hypocalciuria (Cca/Ccr=0.003), hypermagnesemia (2.9 mg/dl), high serum intact PTH level (292 pg/ml), and parathyroid enlargement by image diagnoses (ultrasonography, CT and subtraction scintigraphy) at age 71. Family screening revealed a total of 9 hypercalcemic persons (all intact PTH values less than 62 pg/dl) among 17 family members tested, and thus a diagnosis of FHH was established. At age 74, she underwent resection of three clearly enlarged parathyroid glands due to concern that coexistent primary hyperparathyroidism was the cause of her persistently high PTH levels, but hypercalcemia persisted postoperatively. Histological examination of the resected parathyroid glands was characteristic of lipohyperplasia, with normal levels of expressions of Ki67, vitamin D receptor, and the CaR as assessed by immunohistochemistry. Both the proband and all affected family members had a heterozygous nonsense (R648stop) mutation in the CaR gene by sequence analysis. This mutation is located in the first intracellular loop and would be predicted to produce a truncated CaR having only one transmembrane domain (TMD) and lacking its remaining TMDs, intracellular loops and C-terminal tail. Western analysis of HEK293 cell membranes transiently transfected with this mutant receptor showed cell surface expression of the truncated protein at a level comparable to that of the wild type CaR. The mutant receptor, however, exhibited no intracellular Ca responses when exposed to high extracellular Ca. The proband's clinical course was complicated by renal tubular acidosis and nephrotic syndrome, which may have contributed to her elevated serum PTH and parathyroid gland enlargement. This report is the first to show that a R648stop CaR mutation yields a truncated receptor that is expressed on the cell surface but devoid of biological activity.

SU497

Extracellular Calcium Transcriptionally Induces Cyclooxygenase-2 in Osteoblasts via an ERK Signaling Pathway. S. Choudhary,*[1] S. Godwin,*[2] S. Mellas,*[3] S. Wadhwa,[2] B. Jacobs,*[3] C. Alander,[1] L. G. Raisz,[1] C. Pilbeam.[1] [1]Medicine, Univ. of Conn. Health Ctr., Farmington, CT, USA, [2]Oral Biology, Univ. of Conn. Health Ctr., Farmington, CT, USA, [3]Dept. of Orthodontics, Univ. of Conn. Health Ctr., Farmington, CT, USA.

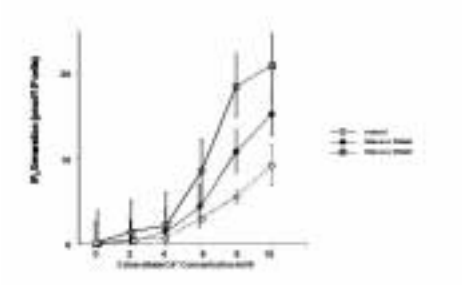
Elevation of extracellular calcium has been shown to inhibit osteoclastic bone resorption and stimulate osteoblastic proliferation and chemotaxis and may act to couple resorption to formation. Prostaglandins produced by cyclooxygenase-2 (COX-2) are potent regulators of bone resorption and formation, and might mediate some effects of extracellular calcium. We examined the ability of extracellular calcium to induce COX-2 gene expression and its promoter activity in cultures of primary osteoblasts sequentially digested from neonatal calvaria of mice transgenic for -371/+70 bp of the COX-2 5'-flanking DNA fused to a luciferase reporter gene. Extracellular calcium (7.5 mM) induced COX-2 mRNA expression in these cells, measured by Northern blot and increased PGE_2 production, measured by RIA. COX-2 mRNA was induced within 30 min and levels peaked at 6 h. A similar time course was observed for the stimulation of luciferase activity, with increases of up to 15-fold at 6 h. Extracellular calcium dose-dependently stimulated luciferase activity over the range 1.8-10 mM with peak effect at 10 mM. Treatment of the cultures with various calcium receptor agonists, such as, Gd (10-500 microM) and Mg (3-50 mM), which can mimic the effects of extracellular calcium on parathyroid cells, did not stimulate COX-2 promoter activity. Hence, the receptor mediating effects of extracellular calcium on COX-2 expression in osteoblasts may differ from the calcium receptor on parathyroid cells. On Western analysis, extracellular calcium (5 mM) stimulated phosphorylation of ERK1/2 within 3 min; levels peaked at 10 min and decreased to basal at 30 min. Treatment with a specific inhibitor of the ERK1/2 pathway, PD-98059 (50 microM), reduced the extracellular calcium stimulation of luciferase activity at 5 hours by 72-75%. We conclude that extracellular calcium induces COX-2 gene transcription largely via an ERK1/2 pathway. We speculate that increased extracellular calcium resulting from osteoclastic resorption might induce osteoblastogenesis via induction of COX-2 and associated prostaglandins.

SU498

High Glucose Condition Enhances the Sensitivity of Calcium Sensing Receptor. H. Kanazawa, H. Tanaka, Y. Seino. Department of Pediatrics, Okayama University, Okayama, Japan.

Human calcium-sensing receptor (CASR) is expressed in many organs, senses the extracellular calcium concentration, and plays a central role of calcium homeostasis. Although the CASR is also able to change the sensitivity by several factors, such as ion strength, its regulatory mechanism has not been well documented. It has been reported that the secondary hyperparathyroidism in end-stage diabetic nephropathy progress more slowly than in the other end-stage renal diseases. To clarify the regulatory mechanisms, we examined the effect of high glucose condition on the function of the CASR. HEK.293 cell transfected with parathyroid CASR cDNA was used for the experiments. Under high (50mM), moderately high (30mM), and normal (4.5mM) glucose conditions in growing medium, cells were cultured. As an osmotic control, D-mannitol was also added to the growing medium. Increasing extracellular calcium concentration caused intracellular calcium elevation and IP_3 accumulation, and the response was paralleled by glucose concen-

tration. Extracellular calcium dose response curve was shifted to the left side compared to normal glucose condition by both IP_3 measurement and Fluo3 (ED50 were about 2.8mM, 3.8mM, and 4.5mM by Fluo3 respectively). D-mannitol did not augmented IP_3 response, and slightly shifted the curve left compared to that of control. Moreover, under high glucose condition, more dimerized CASR was detected than under the regular glucose condition by Western blotting. Similarly MAPK activation was enhanced under the high glucose condition. These findings suggest that high glucose condition enhance the sensitivity of CASR and dimerization of CASR. Underling mechanisms may involve MAPK pathway. Our result may be able to explain the abnormality of calcium homeostasis in high glucose condition, especially in end stage renal failure of diabetic nephropathy.



SU499

Expression of Ca^{2+}-Sensing Receptors (CaRs) in Chinese Hamster Ovary (CHO) Cells Mediates Coupling to Phospholipase C Activation and Inhibition of Adenylate Cyclase Activity. W. Chang,[1] S. A. Pratt,*[1] T. H. Chen,*[1] K. Krapcho,[2] E. Nemeth,[2] D. Shoback.[1] [1]Endocrine Unit, VAMC, University of California San Francisco, San Francisco, CA, USA, [2]NPS Pharmaceuticals, Inc, Salt Lake City, UT, USA.

High extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$) and other divalent cations inhibit parathyroid hormone (PTH) secretion by activating Ca^{2+}-sensing receptors (CaRs). CaRs stimulate phospholipase C (PLC) and couple to the opening of ion channels. Both mechanisms are thought to contribute to intracellular Ca^{2+} mobilization, a key pathway involved in regulating PTH secretion. High $[Ca^{2+}]_o$ and divalent cations also inhibit cyclic AMP (cAMP) accumulation in parathyroid and renal cells. The role of this pathway in mediating the ionic control of secretion and other functions has been difficult to test due to lack of appropriate model systems. We tested whether known CaRs couple to inhibition of cAMP by transiently transfecting bovine parathyroid CaRs into HeLa, HEK-293, COS-7, and CHO cells. None of these systems exhibited detectable inhibition of forskolin (FORSK)-stimulated cAMP by high $[Ca^{2+}]_o$. Clones of CHO cells stably expressing high levels of CaRs, selected on the basis of immunoblotting, however, demonstrated marked, dose-dependent inhibition of FORSK-stimulated cAMP accumulation by high $[Ca^{2+}]_o$ and Sr^{2+} and PLC activation as assessed by total inositol phosphates (InsPs). The ability of the calcimimetic NPS-1377 to modulate both signaling pathways was also tested at 0.5 mM Ca^{2+} (see TABLE; N=2-4 experiments). InsPs and cAMP levels were not significantly changed by these CaR agonists in CHO cells expressing control vector. Our studies confirm that CaRs couple to inhibition of adenylate cyclase activity in CHO cell clones expressing high levels of CaRs with a sensitivity to $[Ca^{2+}]_o$ similar to previous work in parathyroid and renal cells. Studies are in progress to identify whether inhibition of cAMP is mediated via inhibitory GTP binding proteins or indirectly via Ca^{2+} mobilization. This system should prove useful in determining the molecular basis for differential coupling of CaRs to multiple downstream effector pathways involved in the control of secretion, proliferation, and other actions of the CaR in target cells.

SU500

Stimulatory Effects of Strontium Ranelate (S12911) on the Rat and Mouse Cation-Sensing Receptor. J. Coulombe,*[1] H. Faure,*[1] B. Robin,*[2] Y. Tsouderos,[2] M. Ruat.*[1] [1]Laboratoire de Neurobiologie Cellulaire et Moléculaire, UPR 9040, Gif Sur Yvette, France, [2]Institut de Recherches Internationales Servier, Courbevoie, France.

Strontium ranelate (S12911 - PROTOS®) has been shown to increase proliferation of preosteoblastic cells and to decrease osteoclasts activity, leading to an increase of bone formation and a decrease of bone resorption. The extracellular cation-sensing receptor (CaSR), a G protein coupled receptor expressed by various cell types, is stimulated by divalent cations and could play a key role in strontium ranelate mechanism of action. The pharmacological properties of strontium ranelate toward cloned rat CaSR, expressed in Chinese hamster ovary (CHO) cells and the mouse CaSR constitutively expressed in AtT20 cells (Ferry et al., 1997), have been evaluated. Due to the low solubility of strontium ranelate in the culture medium, strontium chloride ($SrCl_2$) was used to test high Sr^{2+} concentrations. The tested Sr^{2+} concentrations ranged from 0.1 to 15 mM of Sr^{2+} in the presence of 0.3 or 2 mM of Ca^{2+} in the culture medium. The activity of ranelic acid, the organic part of strontium ranelate, on the CaSR was tested using sodium ranelate. The activation of the CaSR was evaluated by the production of tritiated inositol phosphate (^{3}H-IP). Results were obtained from 3 separate experiments performed in triplicate, and are expressed as mean ± SD. Strontium ranelate stimulated the ^{3}H-IP response both in CHO(CaSR) and AtT20 cells and its effects were more pronounced in the presence of high calcium concentrations (2 mM). In AtT20 cells and in the presence of 2 mM Ca^{2+}, the effects of strontium ranelate were already evident at 0.4 mM Sr^{2+} and strontium ranelate induced a 15 fold increase in the ^{3}H-IP response at 5.4 mM Sr^{2+}. These data indicate that Sr^{2+} is an agonist of the rat and mouse CaSR. Sodium ranelate had limited or no effect on the ^{3}H-IP response induced by calcium in CHO(CaSR), suggesting that ranelic acid does not intervene in the strontium ranelate ^{3}H-IP response observed through the CaSR. Higher Sr^{2+} concentrations (up to 15 mM) were obtained with $SrCl_2$. In CHO(CaSR) and in the presence of 2 mM Ca^{2+}, $SrCl_2$ stimulated the ^{3}H-IP response with an EC50 of 5.3 ± 0.8 mM with a maximal effect representing 80% of the maximal effect of calcium chloride. The effects of $SrCl_2$ were more modest at low calcium concentrations (0.3 mM). These data further characterize the effects of Sr^{2+} toward the rat and mouse CaSR and suggests that strontium ranelate is an agonist of this receptor with a lower affinity than Ca^{2+}.

Disclosures: IRI Servier,2.

SU501

Heterozygous Truncations of the Ca2+-Sensing Receptor Predict a Functional Deletion of the Receptor in an Infant with Neonatal Severe Hyperparathyroidism. T. Ratajczak,*[1] B. K. Ward,*[1] A. Magno,*[1] B. G. A. Stuckey,*[1] M. Burrows,*[2] T. W. Jones,*[2] E. A. Davis,*[2] D. Minchin.*[3] [1]Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Perth, Australia, [2]Endocrinology, Princess Margaret Hospital, Perth, Australia, [3]General Surgery, Sir Charles Gairdner Hospital, Perth, Australia.

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant disorder of high penetrance characterised by lifelong moderate, but generally asymptomatic hypercalcemia. In contrast, neonatal severe hyperparathyroidism (NSHPT) corresponds to a more acute form of hypercalcemia that can be life threatening without parathyroidectomy. Both disorders result from inactivating mutations of the Ca2+-sensing receptor (CaR) gene. The human CaR, consisting of 1078 amino acids, contains a seven membrane-spanning motif characteristic of G protein-coupled receptors and is abundantly expressed in the parathyroid glands and kidney. We report a case of NSHPT in a five-month old male infant with a history of failure to thrive, hypotonia and developmental delay. Biochemical investigation revealed hypercalcemia (serum calcium concentration of 4.48 mmol/L; normal range 2.15-2.75 mmol/L), hypocalciuria and hypersecretion of PTH. Skeletal X-rays showed changes consistent with hyperparathyroidism. After initial management to reduce hypercalcemia, total parathyoidectomy was performed. Post-operatively the patient continues to make excellent developmental progress.Family history revealed a large number of maternal relatives with known hypercalcemia consistent with FHH. There was no history of consanguinity in the parents of the proband. For the proband, automated direct sequence analysis of PCR fragments amplified from genomic DNA, incorporating the entire CaR open reading frame, revealed heterozygous nonsense mutations in the CaR at codons 94 (GGA to TGA/G94Stop) and 648 (CGA to TGA/R648Stop). The mutations abolished and introduced restriction enzyme recognition sites for XcmI and DdeI, respectively. Digestion with these enzymes confirmed both mutations in the infant, but only the R648Stop mutation was present in the proband's hypercalcemic mother and other affected maternal relatives. Analysis of 50 unrelated normal subjects revealed the absence of both mutations.The R648Stop mutation, located immediately before the second transmembrane domain, would produce a truncated CaR lacking domains critical for G protein-coupled signalling. The G94Stop mutation, in the extracellular domain, would yield a severely truncated receptor that would not be anchored in the cell membrane. Together these mutations potentially correspond to a functional deletion of the CaR in the NSHPT patient.

SU502

Calcium Receptor Active Compounds Modulate cAMP Levels in Bovine Parathyroid Cells. W. L. Heaton,* P. S. Jacobson,* N. E. Lloyd,* E. F. Nemeth, K. J. Krapcho.* NPS Pharmaceuticals, Salt Lake City, USA.

In bovine parathyroid cells (bPTc) elevation of cAMP by either dopamine (DA) or β-adrenergic receptors is inhibited by increasing concentrations of extracellular Ca^{2+} ($[Ca^{2+}]_{ex}$). These inhibitory effects of $[Ca^{2+}]_{ex}$ on cAMP levels are believed to be mediated by calcium receptor (CaR) activation of a pertussis toxin-sensitive inhibitory G protein (G_i). To determine if the effect of $[Ca^{2+}]_{ex}$ is specifically due to CaR activation, and not other Ca^{2+} -dependent processes, we tested the ability of potent and selective small molecule activators (calcimimetic: NPS 1377) or inhibitors (calcilytics: NPS 2143 and NPS 89636) to affect isoproterenol (ISO)- and DA- stimulated cAMP accumulation in dispersed bPTc. Cells were incubated in buffer containing 0.5 mM $[Ca^{2+}]_{ex}$ and 0.5 mM isobutyl methlyxanthine with or without test compounds for 15 minutes at 37ºC in 96-well plates. Following incubation, cells were lysed and total cAMP content was measured. Increasing $[Ca^{2+}]_{ex}$ or adding NPS 1377 completely inhibited cAMP stimulation by ISO or DA. Both CaR agonists were slightly less potent at inhibiting DA- versus ISO-stimulated responses (Table 1). The calcilytics NPS 2143 or NPS 89636 completely reversed the inhibitory effects of $[Ca^{2+}]_{ex}$ or NPS 1377, but failed to affect the inhibition of ISO-stimulated cAMP by prostaglandin F_{2a}.

Table 1	**DA-stimulated cAMP**	**ISO-stimulated cAMP**
$[Ca^{2+}]_{ex}$	IC_{50} = 1.24 ± 0.09 mM (n=4)	IC_{50} = 1.08 ± 0.04 mM (n=5)
NPS 1377	IC_{50} = 44.5 ± 9.5 nM (n=3)	IC_{50} = 11.3 ± 2.5 nM (n=5)

When we evaluated the pertussis toxin (PTX) sensitivity of the $[Ca^{2+}]_{ex}$ mediated effect on ISO- and DA-stimulated cAMP responses, significant differences were noted. While PTX treatment alone (18 h with 0.5 μg/ml), blocked $[Ca^{2+}]_{ex}$ inhibition of DA-stimulated cAMP, $[Ca^{2+}]_{ex}$ still inhibited ISO-stimulated cAMP accumulation by 60%. Because calcilytics and calcimimetics modulate cAMP levels in bPTc in opposite directions, the results confirm that the effects of $[Ca^{2+}]_{ex}$ on cAMP production are mediated by the CaR. The differential effects of PTX however, reveal differences in the regulatory influences of the CaR on dopaminergic or β-adrenergic receptor-mediated increases in cAMP levels.

SU503

Insights into the Biochemical Life Cycle of the Vitamin D Receptor. C. Encinas Dominguez,* P. W. Jurutka, P. D. Thompson, J. C. Hsieh, M. L. Thatcher,* C. A. Haussler,* G. K. Whitfield, M. R. Haussler. Biochemistry & Molecular Biophysics, College of Medicine, University of Arizona, Tucson, AZ, USA.

The biological actions of 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) are mediated by the nuclear vitamin D receptor (VDR) that functions as a ligand-dependent transcriptional regulator. We have developed a 4-stage model that succinctly summarizes the VDR-life cycle, and have tested this model using a variety of experimental approaches, including pull-down assays with GST-fusion proteins, as well as examination of the functional activity of VDR and its putative coregulators in transiently-transfected mammalian cells. The 4 stages of the VDR life cycle are: 1) unoccupied VDR acts as a transcriptional repressor; 2) VDR, upon binding $1,25(OH)_2D_3$, initially recruits retinoid X receptor (RXR), followed by coregulators with histone acetyl transferase (HAT) activity; 3) this first group of coactivators dissociates, and is replaced by a second complement of coactivators that promotes formation of the transcription preinitiation complex; and 4) VDR is ubiquitinated, then eventually degraded. Phosphorylation of VDR may be involved in all four stages. Stage 1: pull-down experiments with GST-fusion proteins indicate that unliganded VDR can associate with both its DNA responsive element and a corepressor such as the silencing mediator of retinoid and thyroid receptors (SMRT). Stage 2: pull-down assays reveal that liganded VDR has less affinity for SMRT, but becomes available to interact with RXR plus coregulators like steroid receptor coactivator-1 (SRC-1) and CREB-binding protein (CBP), the latter two possessing HAT activity. We have further observed in transactivation assays that CBP is a powerful enhancer of VDR activity. Thus, we propose that CBP-VDR, as well as SRC-1-VDR association, is a significant prerequisite for transactivation by liganded VDR. Stage 3: VDR interacts with the mediator protein $DRIP_{205}$, which in turn attracts the preinitiation complex (PIC) containing RNA polymerase II. The interaction with $DRIP_{205}$ likely follows dissociation of SRC-1 and CBP, which may lose their affinity for VDR after self-acetylation. Stage 4: in cotransfection experiments, we observe that Trip1 attenuates VDR activity, likely due to the ability of Trip1 to promote ubiquitination, followed by proteolysis, of VDR. Thus, the VDR life cycle can be summarized by the progressive action of four sets of factors: SMRT, RXR/SRC-1/CBP, $DRIP_{205}$/PIC, and Trip1. A testable conclusion from this model is that the role of $1,25(OH)_2D_3$ is primarily in the transition from Stage 1 to Stage 2, but the continued presence of $1,25(OH)_2D_3$ is likely necessary also for the progression to Stages 3 and 4.

SU504

An Osteoblast-specific Activation of the Vitamin D Receptor by a Non-calcemic Analog of Vitamin D_3. A. Ismail,*[1] M. R. Uskokovic,[2] S. Peleg.[1] [1]Endocrine Neoplasia and Hormonal Disorders, The University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA, [2]Roche Bioscience, Palo Alto, CA, USA.

The vitamin D analog Ro-26-9228 restores bone mineral density without inducing hypercalcemia in osteopenic rats. Ex-vivo experiments have shown that the analog upregulated, preferentially, gene expression in trabecular bone but not in the duodenum of female rats. In culture, the potency of Ro-26-9228 to induce vitamin D receptor (VDR)-mediated gene transcription had an ED_{50} of 0.5-1 nM in the osteoblast-like cells, ROS 17/2.8, but in the intestinal-like cells, Caco-2, the analog had an ED_{50} of 100-400 nM. The affinity of Ro-26-9228 for the in vitro-synthesized VDR (ivtVDR) had a Kd of 6.5 nM. In contrast, the potencies of Ro-26-9228 to induce a protease-resistant conformation and interaction of ivtVDR with the coactivator GRIP had ED_{50}s of 400 and 700 nM, respectively. We hypothesized that osteoblasts may possess a factor or a mechanism that potentiates the assembly of the analog-bound VDR in a transcriptionally active conformation, whereas the intestinal cells and the reticulocyte lysate do not have this factor or mechanism. To test this hypothesis, we incubated ROS 17/2.8 and Caco-2 cells with 1,25-dihydroxyvitamin D_3 ($1,25D_3$) or Ro-26-9228 (0.01 –1000 nM) for 1 hour. The ability of the extracted cellular VDR-ligand complexes to interact with GST-GRIP was examined by pull-down assays, using western blot analysis to quantify the activated VDR. We found that the ED_{50}s for $1,25D_3$ to induce interaction of VDR from either osteoblasts or from intestinal cells with GST-GRIP were 0.05 nM and 0.1 nM, respectively. In contrast, the ED_{50}s for Ro-26-9228 to induce interaction of VDR from osteoblasts or from intestinal cells with GST-GRIP were 5 nM and >1000 nM, respectively. Competition assays in intact cells indicated that the greater potency of Ro-26-9228 to activate VDR from osteoblasts was not due to a better uptake or greater affinity of the analog for VDR in these cells. The potency of Ro-26-9228 to increase the amounts of immunoreactive VDR was similar in osteoblasts and in intestinal cells (ED_{50}=5 nM). Furthermore, the incubation of intact intestinal cells or intestinal cell extract with the proteasome inhibitor MG132 increased the amounts of immunoreactive VDR but did not increase the potency of Ro-26-9228 to induce interaction of intestinal VDR with GST-GRIP, suggesting the mechanisms for VDR stabilization and VDR activation were distinct. We speculate that the differential activation of cellular VDR by Ro-26-9228 involves osteoblast-specific posttranslational modifications that stabilize the VDR-analog complexes in a conformation that favors interaction with transcription coactivators.

SU505

The Vitamin D Receptor is Required for Normal Epidermal Differentiation. Z. Xie,[1] L. Komuves,*[1] Q. Yu,*[1] D. C. Ng,*[1] C. Leary,*[1] T. Yoshizawa,*[2] S. Kato,[2] D. Bikle.[1] [1]Endocrine Unit, VA Medical Center, University of California, San Francisco, CA, USA, [2]Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan.

The active form of vitamin D, 1α,25-dihydroxyvitmin D ($1\alpha,25(OH)_2D$), binds to specific vitamin D receptors (VDR) located in the nucleus of target cells to activate vitamin D-responsive genes. While classic vitamin D target tissues include intestine, kidney and bone, where $1\alpha,25(OH)_2D$ acts to maintain serum calcium levels and to build and preserve bone, compelling evidence has emerged in recent years that vitamin D is also critical for the differentiation of a large number of normal and malignant cells including keratinocytes. Keratinocytes synthesize $1\alpha,25(OH)_2D_3$, contain $1\alpha,25(OH)_2D_3$ receptors, and are induced by $1\alpha,25(OH)_2D_3$ to differentiate in vitro. However, the importance of $1\alpha,25(OH)_2D_3$ on epidermal differentiation in vivo is unclear. To investigate the physiologic role of the VDR in mediating epidermal differentiation in vivo, we examined differentiation markers in the epidermis of VDR knockout mice (VDRKO). Involucrin, a component of the cornified envelope, is a suprabasal marker of keratinocyte differentiation that is expressed in the late spinous layer and throughout the granular layer in the epidermis. Profilaggrin and loricrin are expressed the granular layer of the epidermis. All three differentiation markers as detected by immunostaining showed decreased expression in VDRKO mice from one to three weeks after birth. However, these differences in expression became less apparent between VDRKO mice and wild type littermate controls after three weeks. In situ hybridization was then carried out using specific complementary ribonucleic acid probes for these three differentiation markers. The results showed reduced mRNAs for all of these three differentiation markers at birth in VDRKO mice, but these differences from wild type littermates were no longer apparent after three weeks. Our data indicate that the VDR is required for normal differentiation of the epidermis during the early post natal period, a time at which no alteration in the hair follicle is apparent.

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Bending Analysis of VDR Binding to Positive and Negative DNA Response Elements. A. Alimov,*[1] X. Peng,*[2] H. Malluche,[1] N. Koszewski.[1] [1]Division of Nephrology, Bone & Mineral Metabolism, University of Kentucky, Lexington, KY, USA, [2]Biostatistics Consulting Unit, University of Kentucky, Lexington, KY, USA.

The effects of VDR/RXR heterodimer binding on DNA structure were investigated by circular permutation analysis. The VDR complex binds most avidly to direct repeat +3 (DR+3) DNA elements, termed vitamin D response elements (VDREs), to regulate both positive and negative gene transcriptional events. Circularly permutated DNA probes containing either the avian PTH (aPTH) or a sequence comprised of a double mutation of the aPTH sequence (dmPTH) were prepared. The aPTH VDRE has been shown to confer vitamin D-dependent repression of gene transcription while the dmPTH sequence is associated with enhanced transcriptional activity in response to hormone. The two sequences were identical save for two base-pairs in their respective 3' half-sites, GGGT***GT*** vs. GGGT***CA***, and previous work had demonstrated altered DNA contact points as observed from interference footprints from heterodimer binding in addition to the opposing transcriptional activities. All probes were generated by the insertion of synthetic oligonucleotides of identical length encompassing the binding sites and confirmed by sequence analysis. VDR/RXR-containing extracts were prepared from baculovirus-infected Sf9 insect cells and all binding reactions were performed in the presence of 1,25-dihydroxyvitamin D3 and buffered 120 mM potassium chloride. In all cases, VDR/RXR heterodimer complexes bound to probes in which the binding site was located closer to the center of the permutated fragment migrated more slowly than when the site was nearer to the ends. The relative mobilities from 3 independent experiments were plotted against the distance between the center of the binding site and the ends of the DNA probe. DNA distortion angles were calculated from the amplitude of the bestfit cosine function for each set of data. Based on this analysis heterodimer binding to the aPTH VDRE yielded a calculated distortion angle of 38^o, while the dmPTH site produced a significantly larger distortion angle in excess of 51^o. In addition, the observed center of distortion was displaced from ca. +1 for the aPTH VDRE to ca. -3 for the dmPTH sequence. Based on these data it can be concluded that small changes in the sequence of a VDRE can have a profound impact on DNA structure, and provides additional insight into explaining differences in transcriptional outcomes in response to hormone from nearly identical DR+3 response elements.

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Analysis of the Immune System in Vitamin D Receptor Knockout Mice Reveals No Major Abnormalities. L. Cao,* B. Wang,* Y. Lee,* Y. Fu, Y. C. Li. The University of Chicago, Chicago, IL, USA.

In addition to its central role in the regulation of calcium homeostasis, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] is known to regulate the immune system, and impaired immune functions have been observed in vitamin D-deficiency. We, therefore, examined the effect of VDR ablation on the immune system in VDR null mice. In the spleen, a 36% increase in spleen size and cell number was observed in VDR null mice, with a moderate alteration in the T and B cell compartments, but the populations of splenic monocytes/macrophages and NK cells were not changed. Neither were the $CD4^+$, $CD8^+$ and $CD4^+8^+$ cell populations in the thymus. Despite the changes in the spleen, no differences were observed between VDR null and wildtype mice in splenocyte proliferation and cytokine production, as well as in the production of immunoglobulins. Bone marrow-derived macrophages from VDR null mice were normal in the expression of TNF-α and nitric oxide synthase (NOS) as well as in NO production, and the phagocytic activity of VDR(-/-) peritoneal macrophages was normal as well. Furthermore, no significant differences were observed between VDR null and wildtype mice in response to *Listeria monocytogenes* infection or in tumor rejection. These data indicate that, in contrast to vitamin D deficiency, VDR inactivation does not significantly compromise the immune system.

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Changes in Tyrosin Phosphorylation of Cytoplasmic Proteins Induced by 1,25(OH)2D3: Rol of VDR. C. Buitrago, R.Boland and A.R. De Boland.* Dept. Biologia, Bioquimica & Farmacia, Universidad Nacional del Sur, Bahia Blanca, Argentina.

In skeletal muscle cells tyrosine phosphorylation of cellular proteins plays a key role in 1,25(OH)2 -vitaminD3 [1,25(OH)2D3] dependent modulation of non-genomic responses, such as activation of the extra cellular signal-regulated mitogen-activated protein (MAP)

kinase isoforms ERK 1/2 and the ubiquitous citoplasmatic kinase Src, as well as the phosphorylation of the oncoprotein c-myc. (JBC 275: 36021-36028, 2000). In the present study we evaluated the involvement of the vitamin D nuclear receptor (VDR) in tyrosine phosphorylation of Src, c-myc, of ERK 1/2 induced by 1,25(OH)2D3 . Muscle cells were transfected (4 h) with ODN against VDR mRNA [anti-VDR1, 5'-TPGTCCTTGGTGATTTTGCAPG-3' (antisense against the poli A region from Gallus gallus VDR mRNA); anti-VDR2, 5'-TPCGATGACTTTCTGCTGCTPC-3' and anti-VDR3 5'-TPCCTTCATCATCCCAATGTPC-3' (antisenses against internals nucleotide sequences of VDR mRNA]. Muscle cells were then treated with 1 nM 1,25(OH)2D3 (1-5 min), followed by cell lysis, inmunoprecipitation with anti-Src and anti c-myc antibodies and inmunoblot analysis using anti-phosphotyrosine antibody. Cell lysates were also subjected to western blott assays with anti-active (phospho) MAP kinase antibody.A direct immunobloting of total proteins with a monoclonal anti VDR antibody revelaed a 80% suppression of the VDR expression. The treatments with ODNs significantly reduced (-94%) c-myc tyrosine phosphorylation induced by the hormone. An increase in Src phosphorylation (+70%) was observed in transfected cells, reflecting a decrease in Src activation induced by 1,25(OH)2D3 . However, hormone-dependent MAPK tyrosine phosphorylation was not affected by ODNs transfection. Taken together, these results suggest that VDR participates in the tyrosine phosphorylation of the oncoprotein c-myc and in the dephosphorylation of the cytosolic tyrosine kinase Src triggered by 1,25(OH)2D3 and that hormone activation of MAP kinase is independent of VDR, and may involve interactions with other signalling mechanisms.

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1α,25(OH)$_2$Regulates Matrix Vesicle PKC Directly via G-proteins and Indirectly During Organelle Biogenesis. B. D. Boyan,[1] V. L. Sylvia,[1] D. D. Dean,[1] D. Casasola,*[1] Z. Schwartz.[2] [1]Orthopaedics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [2]Periodontics, Hebrew University, Jerusalem, Israel.

Matrix vesicles (MVs) are extracellular organelles that modulate mineral formation, enzymatic modification of the matrix, and activation of local growth factors in the growth plate, all of which are sensitive to 1α,25(OH)$_2$D$_3$ [1,25]. Whereas 1,25 stimulates PKC activity in growth zone (GC) chondrocyte cultures through direct action on the plasma membrane (PM), PKC activity in MVs produced by the cells is inhibited by 1,25. Here, we examined the regulation of MV PKC by 1,25 during organelle biogenesis and via nongenomic mechanisms in the matrix. 1,25 caused a decrease in MV PKCζ specific activity within 9 min at 10^{-9} and 10^{-8}M, but stimulated PM PKCα activity in confluent GC cultures. At 12 or 24 h after treatment with 1,25, PKCζ activity in MVs was increased, while PM PKCα was unchanged. This suggests that the rapid response was due to a direct action of 1,25 on the MVs and PMs, and the effect at 12 and 24 h was due to new MV biosynthesis and altered PKC content. The effect of 1,25 was stereospecific and metabolite-specific; 1β,25(OH)$_2$D$_3$ and 24R,25(OH)$_2$D$_3$ had no effect. Ab99, a specific antibody against the 1,25-mVDR, blocked the PKC increase at 24 h, indicating the 1,25-mVDR was involved. Increased MV PKC at 24 h was due in part to phospholipase C (PLC)-dependent production of diacylglycerol, which is also mediated by the 1,25-mVDR. Monensin blocked the effect of 1,25 on PKC at 24 h, indicating that packaging of PKC was blocked. To investigate the signaling pathway responsible for the direct regulation of PKC, MVs were isolated from GC cultures and treated for 9 min with 1,25 in the presence and absence of specific inhibitors of various signaling pathways. Inhibition of PI-PLC (U73122), PLD (wortmannin), G_i (pertussis toxin), or G_s (cholera toxin) had no effect. In contrast, GDPβS blocked the inhibitory effect of 1,25 on MV PKC, indicating that it was mediated by G_q protein. The effect of 1,25 on MV PKC was also receptor-mediated; Ab99 blocked the 1,25-induced inhibition of MV PKC. Arachidonic acid, which is released by growth zone chondrocytes in response to 1,25, stimulated PKC when directly added to MVs. These results indicate that MVs are regulated by 1,25 at three levels: (1) during their production, (2) through direct action of the seco-steroid on the membrane, and (3) through production of additional regulatory factors, such as arachidonic acid. Moreover, the opposing effects of 1,25 and arachidonic acid on PKC may act to modulate nongenomic, autocrine signal transduction at sites distant from the cell.

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Activation of MAP Kinase by 1α,25-(OH)$_2$D$_3$ Is Mediated Through PLC and PKC in Growth Zone Chondrocytes. D. D. Dean,[1] V. L. Sylvia,[1] H. Ehland,*[2] F. Del Toro,*[1] B. D. Boyan,[1] Z. Schwartz.[3] [1]Orthopaedics, Univ Texas HSC, San Antonio, TX, USA, [2]Periodontics, Wilford Hall Med. Ctr., Lackland AFB, TX, USA, [3]Periodontics, Hebrew Univ, Jerusalem, Israel.

Recent studies indicate that a membrane receptor for 1α,25(OH)$_2$D$_3$ [1,25-mVDR] in growth plate chondrocytes regulates rapid responses of growth zone (GC) cells, resulting in increased activity of protein kinase C (PKC). 1α,25(OH)$_2$D$_3$ (1α,25) directly stimulates cell membrane PKCα via phospholipase C (PLC), increasing diacylglycerol and inositol-1,4,5-tris-phosphate production. The effect of 1α,25 on PKC is blocked by Ab99, which is specific for the 1,25-mVDR. Proliferation and differentiation of the GC cells are mediated by this receptor as well. Others have shown that PKC can activate mitogen-activated protein kinase (MAPK) and that MAPK can phosphorylate transcription factors, modulating gene expression. In this study, we tested the hypothesis that MAPK activity and protein production are regulated by 1α,25 and that the sub-family of MAPKs, ERK1/ERK2, are involved in mediating the effects of 1α,25 on GC cell proliferation and differentiation. Further, we examined whether PLC, PLD, PKC, and PKA are involved in the pathway leading to the activation of MAPK. GC cells were isolated from rat costochondral cartilage and cultured to fourth passage. Cells were treated for 9 to 720 min with 10^{-10}-10^{-8} M 1α,25 and MAPK specific activity, as well as the immunoreactive level of ERK1/ERK2 using Western analysis, was measured. The role of ERK1/ERK2 in mediating the effect of 1α,25 on cell proliferation was assessed by adding the specific ERK1/ERK2 inhibitor, PD98059, to the cultures for 24 h in the presence/absence of 1α,25 and then measuring [^{3}H]-thymidine incorporation. Similarly, cultures were treated and then examined for effects on differentiation by measuring alkaline phosphatase specific activity (ALPase) and [^{35}S]-sulfate incorporation. Specific inhibitors of PLC, PLD, PKC and PKA were used to examine the pathways responsible for mediating the effect of 1α,25 on GC cells. 1α,25 caused a dose-dependent increase in MAPK which was significant at 10^{-9}-10^{-8} M. The effect was significant at 9 and 90 min after the addition of 1α,25. Western analysis showed a dose-dependent increase in ERK1/ERK2 at 90 min. Inhibition of ERK1/ERK2 blocked the effect of 1α,25 on [^{35}S]-sulfate incorporation, but had no effect on [^{3}H]-thymidine incorporation or ALPase. The effect of 1α,25 on MAPK was mediated by PLC and PKC, since the effect of 1α,25 was blocked by PLC and PKC inhibitors, but not by PLD or PKA inhibitors. This indicates that the 1,25-mVDR regulates some, but not all, of the genomic effects of 1α,25 in GC cells via PKC and MAPK-mediated mechanisms.

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The PPAR-gamma ligand 15delta-prostaglandin J2 Is a potent endogenous regulator of hsp70-Related Intracellular Vitamin D Binding Protein Expression. M. A. Gacad,[1] R. Chun,[1] S. Wu,[1] J. S. Adams.[2] [1]Burns and Allen Research Institute, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA, [2]Division of Endocrinology, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA.

New World primate species are distinguished from their Old World counterparts by the presence of a vitamin D and gonadal steroid hormone-resistant phenotype. Resistance results from the overexpression in hormone target cells of a series of dominant-negative-acting hormone response element binding proteins in the hnRNP family. Partial compensation for the resistant phenotype is achieved by overproduction and the presence of high circulating hormone levels. Further compensation results from the overexpression of a family of intracellular hormone binding proteins in the heat shock (hsp) family which act to chaperone hormones to receptors and/or sites of intracellular metabolism. The signals generated in vivo to promote a high level of expression of these hsp-related intracellular binding proteins remain unknown. Because 1] we recently determined that the PPARg ligand 15 delta-prostaglandin (15δPGJ2) can specifically bind to hsp70-related molecules (Gacad et al, unpublished) and 2] reports that 15δPGJ2 can enhance expression of hsp-70-related molecules (Santoro et al., PNAS, 86:8407, 1989), we theorized that the PPARγ ligand 15δPGJ2 is responsible for the overexpression of some or all of the hsp-70-related intracellular hormone binding proteins. Overnight incubation of COS-7 and human kidney cell line (HKC) with PPARg-activating concentrations of 15δPGJ2 (10-1000 uM) increased specific intracellular 25-OHD3 binding up to 8-fold. Western blot analysis of various hsp-70-related proteins disclosed that the majority of the increase in 25-OHD3 binding could be accounted for by an increase in expression of intracellular vitamin D binding proteins related to hsp-70 and grp-78, chaperone proteins known to reside largely in the cell cytoplasm. Time course analysis indicated that hsp-70 and grp-78 protein levels peaked at 24 h post-exposure to 15δPGJ2 in COS-7 and HKC cells. Under the influence of 15δPGJ2, upregulation of expression of hsp-70 was most marked in HKC cells (5-8 fold) which corresponded to a >20-fold increase in steady-state hsp-70 mRNA levels. We conclude that the PPARg ligand, 15δPGJ2, is a major transcriptional regulator of hsp-70-related intracellular vitamin D binding/chaperone proteins. We theorize that transactivation is mediated through an increase in heat shock transcription factor expression in target cells.

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Local Production of 17β-Estradiol by Growth Plate Chondrocytes Is Regulated by 1α,25(OH)$_2$D$_3$ in a Gender-Specific Manner. V. L. Sylvia,[1] Z. Schwartz,[2] I. C. Gay,*[1] R. Gomez,*[1] D. D. Dean,[1] B. D. Boyan.[1] [1]Orthopaedics, U Texas HSC, San Antonio, TX, USA, [2]Periodontics, Hebrew U, Jerusalem, Israel.

Recently, a new class of membrane receptor for 17β-estradiol (E_2) was described in growth plate chondrocytes that is only active in female cells, whereas both male and female cells have classical nuclear E_2 receptors. The presence of membrane receptors in addition to classical nuclear receptors for estrogen suggests that female chondrocytes may produce and secrete E_2 and respond to E_2 in an autocrine/paracrine manner. This study tested the hypothesis that growth plate chondrocytes produce E_2 and that production is differentially regulated by 1α,25-(OH)$_2$D$_3$ [1,25], 24R,25-(OH)$_2$D$_3$ [24,25], and rhTGF-β1 in a gender-specific and cell maturation-dependent manner. Aromatase gene expression was examined by RT-PCR and northern analysis of total RNA from male and female resting zone (RC) and growth zone (GC) cells. Aromatase specific activity was measured in cell layer lysates of confluent cultures treated for 24 h with 10^{-10}-10^{-8} M 1,25 (affects primarily GC cells), 10^{-9}-10^{-7} M 24,25 (affects primarily RC cells), and 0.01-10 ng/ml TGF-β1 (differentially regulates hydroxylase activity in RC and GC cells). An RIA kit was used to measure E_2 in the conditioned media. Female RC cells expressed the highest levels of aromatase mRNA compared to the other chondrocyte cultures. Aromatase activity was present in male and female cells, but activity was 1.6X greater in female RC v. female GC cells, male RC and male GC cells. All cultures produced E_2 but female RC cells produced 2.5-fold more E_2 than any other cell type. 1,25 caused a dose-dependent increase in E_2 production by female RC cells (1.5X) and GC cells (3X). In contrast, 1,25 had no effect on male GC cells and only increased E_2 production by 10% in male RC cells at the highest concentration of 1,25 used. Neither 24,25 nor TGF-β1 had an effect. This demonstrates that growth plate chondrocytes produce E_2 and the highest basal levels are by female RC cells. E_2 production was regulated only by 1,25, a steroid hormone shown previously to have most of its effects on GC cells. Interestingly, female GC cells showed the greatest increase in E_2 production in response to 1,25. The effects of 1,25 on RC cells have been primarily associated with proliferation; present data suggest that this is potentially via changes in E_2. Regulation of E_2 production by 1,25 was gender-dependent, although other effects of 1,25 on growth plate chondrocytes do not differ with the sex of the rat. This suggests that locally produced E_2 may have both autocrine and paracrine effects in cells from female rats that are mediated by membrane-associated mechanisms.

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Involvement of Estrogen Receptor Signaling Pathways in the Bone Metabolism. M. Fujita,*[1] S. Ogawa,*[1] H. Fukuoka,[2] T. Tsukui,*[3] Y. Ouchi,*[1] S. Inoue.[4] [1]Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, [2]Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, [3]Department of 2nd Biochemistry, Saitama Medical School, Saitama, Japan, [4]CREST, Saitama, Japan.

Although it is well known that estrogen deficiency causes osteoporosis and estrogen replacement prevents bone loss, the involvement of estrogen receptor (ER) in the bone metabolism is still unclear. Therefore, we have generated transgenic rats harboring a dominant negative ERα, inhibiting both ERα and ERβ signaling pathways. We have shown that growth rate of primary osteoblasts derived from transgenic rats was significantly reduced compared with that of wild-type osteoblasts. Mitogen-activated protein kinase (MAPK) phosphorylation was increased within 10 min after 17β-estradiol (E_2) treatment in wild-type osteoblasts as a consequence of a rapid and non-genomic action of estrogen. Interestingly, in osteoblasts derived from transgenic rats, the MAPK phosphorylation was severely impaired, suggesting that this non-genomic action of estrogen is also mediated by ER-dependent pathway. Then, we have studied the involvement of ER in the male bone metabolism. Administration of E_2 for 60 days increased bone mineral density (BMD) of the femur of wild-type male rats (0.225 ± 0.008 g/cm^2 vs. 0.246 ± 0.014 g/cm^2, mean ± SD, p=0.02) but did not increase BMD of transgenic rats significantly (0.225 ± 0.004 g/cm^2 vs. 0.229 ± 0.012 g/cm^2, p=0.4). Taken together with that we have shown that the effect of estrogen on treatment of ovariectomy-induced bone loss was mediated by ERs (S. Ogawa, M. Fujita *et al.*: J. Biol. Chem., 275, 21372-21379, 2000), we here demonstrated that the effect of estrogen on the bone metabolism in both male and female rats is mediated by ER signaling pathways.

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Differential Phosphorylation of Estrogen Receptor α and β in Human Osteoblasts. M. Subramaniam,[1] D. J. Rickard,[1] S. A. Johnsen,*[1] D. G. Monroe,[1] K. Rasmussen,*[1] S. Khosla,[2] B. L. Riggs,[2] T. C. Spelsberg.[1] [1]Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA, [2]Endocrinology, Mayo Clinic, Rochester, MN, USA.

Estrogen (E) is an important anabolic hormone in bone and plays a pivotal role in bone growth and remodeling. Both estrogen receptor isoforms ER(α and β) have been identified in osteoblasts and osteoclasts, and direct effects of E on these cells have been demonstrated. Regulation of target gene expression by ERα and β is mediated by ligand-induced conformational changes and the differential recruitment of co-regulators, causing activation or inhibition of the transcription complex. It has been previously shown that the activity of ERα is modulated through phosphorylation via both E-dependent and alternative (i.e., cytokine/growth factor-stimulated) signaling pathways. Presently, it is unknown whether ERβ activity is also controlled by similar phosphorylation events mediated by multiple pathways. To characterize the basal and estrogen-stimulated levels of phosphorylation of the ERα and β isoforms in osteoblasts, human fetal osteoblast (hFOB) cells were transiently transfected with expression constructs encoding N-terminally FLAG tagged ERs. After transfection, the cells were treated with vehicle or 10^{-8} M 17β-estradiol (17β-E) for 40 minutes in the presence of [32P]-ortho-phosphate, the cells lysed and then the ERs immunoprecipitated using an anti-FLAG antibody and analyzed by SDS-PAGE. Interestingly, the basal phosphorylation level of ERβ was higher than that of ERα. Moreover, 17β-E induced the phosphorylation level of ERα by approximately 3-fold but had no effect on ERβ phosphorylation. In contrast, treatment with 10^{-8} M 4-OH-tamoxifen had no effect on the phosphorylation of either ERα or β. Epidermal growth factor (EGF), which has previously been shown to elicit certain ER-mediated actions in other cell types and to induce the phosphorylation of ERα through the mitogen-activated protein kinase (MAPK) pathway, increased the level of phosphorylation of both ERα and β to a similar degree. Pre-treatment of transfected cells with PD98059, a MAPK pathway inhibitor, blocked both E and EGF induced phosphorylation of ERα and EGF induced phosphorylation of ERα and β. Our results suggest that the phosphorylation of ERα by E and EGF is mediated through MAPK pathway. Interestingly, E did not induce phosphorylation of ERβ, suggesting that significant differences exist in ERα and β signaling. In conclusion, these results suggest that the differential effects of ERα and β on target gene transcription may be explained, in part, by differences in phosphorylation status of each ER isoform which are generated by the combination of E-dependent and alternative signaling pathways.

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Estrogen Receptor α is the Major Receptor Regulating Bone Response to Estradiol in Gonadectomized Female Mice and Testosterone Plays a Role in Intact Male and Female ERα Knockout Mice. N. A. Sims,[1] P. Clement-Lacroix,[2] D. Minet,*[2] S. Dupont,*[3] A. Krust,*[3] M. Resche-Rigon,[2] M. Gaillard-Kelly,[2] P. Chambon,*[3] R. Baron.[4] [1]Yale University School of Medicine, New Haven CT, USA and St Vincent's Institute of Medical Research, Fitzroy, Australia, [2]Aventis Pharma, Paris, France, [3]IGBMC, Strasbourg, France, [4]Yale University School of Medicine, New Haven, CT, USA and Aventis Pharma, Paris, France.

Our previous results have shown that while only estrogen receptor α (ERα) regulates bone mass in male mice, both ERα and ERβ are involved in females. Moreover, their roles may depend on circulating estradiol (E2) and/or testosterone (T) levels that were markedly and differentially affected by deletion of either or both ERs.To determine whether signaling from gonadal hormones affects bone volume in ER knockout mice, female and male ER knockouts were ovariectomised (OVX) or orchidectomised (ORX), respectively. Significant trabecular bone loss was observed in both male and female ERαKO and ERβKO mice. In male ERαKO and ERαβKO mice, where T levels and trabecular bone volume (BV/TV) are high and E2 normal, significant bone loss was observed after orchidectomy, suggesting that it is the high levels of T in these mice that inhibit bone turnover and maintain a high BV/TV in the absence of any ER signaling. In female ERαβKO mice however, where bone mass and circulating levels of T were already very low and E2 remained near normal, no bone loss was observed after OVX; BV/TV and BMD were equivalent to intact ERαβKO females, thereby precluding any effect of circulating E2 through other receptors in these mice.To determine through which ER bone mass is maintained by estradiol in female mice, ovariectomised wild type, ERαKO and ERβKO mice were treated with estradiol from 0.1 to 100μg/day for 4 weeks by subcutaneous implant or daily subcutaneous injection (2 independent experiments). In wild type mice, low doses of E2 (0.1μg/day and 1μg/day) prevented the OVX-induced elevation in bone turnover and trabecular bone loss. Higher doses had an anabolic effect on BV/TV. The response of female ERβKO mice was similar to wild type mice, indicating that ERα is responsive to low levels of E2, and suggesting that bone loss prevention by E2 in normal mice is exerted mainly via ERα. In female ERαKO mice, E2 treatment also prevented trabecular bone loss but only partially. It was most effective at the high dose of 10μg/kg/day, which restored circulating estradiol to the very high level (10X) seen in the intact female ERαKO mouse. Thus ERβ (the only known ER expressed in ERαKO mice) requires a higher level of E2 to prevent bone loss, and even at these high doses is less effective than ERα.

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Skeletal Changes in Female bLH-bata-CTP Transgenic Mouse Model of Functional Hyperandrogenism. N. Ohashi,*[1] J. M. Pulcini,*[2] S. Murthy,*[2] I. Leyvand,*[2] C. H. Turner,[1] D. B. Burr,[1] J. H. Nilson,*[3] J. M. Hock.[1] [1]Indiana University Medical School, Indianapolis, IN, USA, [2]Indiana University Dental School, Indianapolis, IN, USA, [3]Case Western Reserve University, Cleveland, OH, USA.

Androgens have been implicated as important regulators of skeletal development. Female transgenic bLH-beta (bLHb)-CTP mice, engineered to express a long-lived LH construct, exhibited functional hyperandrogenism due to ovarian hyperstimulation by LH. Serum testosterone and androgen/estrogen ratio increased in mice more than 2 weeks old, and there were significant effects in reproductive tissues. To determine if the functional hyperandrogenism and precocious puberty were correlated with a skeletal phenotype, we investigated the bone mass, bone strength and histomorphometry, using conventional technology, on male and female wildtype control (WT) and transgenic mice.Transgenic female bLHb-CTP mice exhibited a distinctive skeletal phenotype that was significantly different from that of either WT or transgenic males or female WT mice. There were no differences in the skeletal measures of WT and transgenic male mice at 8.5 weeks of age. Skeletal phenotype of transgenic female mice 8.5 and 12 weeks old was equivalent. Compared to age-matched WT female controls, transgenic female bLHb-CTP mice increased bone mineral density of humeri by 22%, and femur ash weight by 10%. Force to fracture was increased by 36% in femurs. Cortical bone thickness of the proximal tibia was increased by approximately 50%, due to retention of endosteal bone. As there was no difference in periosteal or endosteal bone formation measures, the increased thickness was likely due to inhibition of resorption on the endosteal surface during modeling required for bone development. The femur metaphysis was osteosclerotic with little marrow retained. The skeletal effects of androgens in female transgenic mice were site-selective as there were no differences from WT in periosteal circumference. In summary, functional hyperandrogenism in transgenic female bLHb-CTP mice increased cortical and trabecular bone volume, bone mass and bone strength via regulation of modeling on endosteal surfaces in the developing skeleton during puberty.

Disclosures: Eli Lilly & Co.,2.

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Comparative Study of the *in vivo* Action of Different Doses of 17β-Estradiol and Dihydrotestosterone on Bone in an Aged Male Orchidectomized Rat Model. L. Vandenput,*[1] J. V. Swinnen,*[1] E. Van Herck,*[1] S. Boonen,[2] R. Bouillon,[1] D. Vanderschueren.[1] [1]Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Leuven, Belgium, [2]Leuven University Centre for Metabolic Bone Diseases, Katholieke Universiteit Leuven, Leuven, Belgium.

Testosterone deficiency results in concomitant loss of bone and muscle mass and gain of fat mass. Previous experiments showed that even low doses of testosterone (T) (11.5 μg/day) prevented orchidectomy (orch)-induced bone loss in aged (12-month-old) male Wistar rats during a 4-month experimental period. This bone-sparing effect of T was in part determined by its preservation of lean body mass ($r = 0.54$, $p < 0.0001$). The extent to which the actions of T on both bone and body composition are dependent on its aromatization in 17β-estradiol (E2) remains unknown.In this study, the effects of different doses of both E2 (0.15 μg/day, 0.30 μg/day and 1.50 μg/day) and the non-aromatizable androgen dihydrotestosterone (DHT) (45 μg/day, 75 μg/day and 150 μg/day), administered via sc silastic implants, were compared in the same rat model.The highest dose of DHT (150 μg/day) was able to prevent the orch-induced decrease of trabecular bone density of the femur but not the thinning of the cortex, as assessed by pQCT. High-dose DHT also resulted in significant hypertrophy of both ventral prostate and seminal vesicles. DHT, in contrast with T, had no effect on body composition, as measured by DXA. All doses of E2 prevented both trabecular bone loss and cortical thinning, but only the highest dose (1.50 μg/day) appeared to be fully effective. Additionally, E2 also inhibited the orch-associated increase of fat mass, which was associated with decreasing levels of serum leptin. E2 replacement did not prevent the decrease of lean body mass, except for the highest dose. Lower doses of DHT and E2 significantly inhibited the orch-induced rise of bone turnover markers (serum osteocalcin and urinary deoxypyridinoline) to a similar extent. Interestingly, DHT also lowered serum IGF-I levels (which seemed associated with its inhibitory effect on osteocalcin, $r = 0.65$, $p < 0.001$), whereas this growth factor was stimulated by E2.In conclusion, DHT is less effective in preventing orch-induced bone loss than either E2 or T. Furthermore, in the aged orchidectomized rat model, DHT does not prevent loss of lean body mass. Only E2 effectively prevents the orch-related increase of fat mass.

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Phosphorylation of Calbindin-D_{28k}: Evidence for A Role of Phosphorylation in Calbindin-D_{28k}-Caspase 3 Interaction. X. Peng,*[1] K. A. Gengaro,*[1] R. Donelly,*[2] H. Li,*[1] R. McCain,*[1] V. Randolph,*[1] E. B. Cunningham,*[1] S. Christakos.[1] [1]Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School, Newark, NJ, USA, [2]Pathology and Laboratory Medicine, UMDNJ-New Jersey Medical School, Newark, NJ, USA.

Bone formation is regulated not only by the rate of osteoblast formation but also by the rate of osteoblast apoptosis. The vitamin D dependent calcium binding protein calbindin-D_{28k} is expressed in osteoblasts and suppresses their apoptosis, at least in part, by binding to caspase 3, a key modulator of apoptosis and a common downstream effector of multiple apoptotic signaling patways (Bellido et al. J. Biol. Chem. 275:26328, 2000). To investigate molecular mechanisms involved we examined the effect of protein kinase C (PKC) on calbindin-D_{28k} phosphorylation and the role of phosphorylation in modulating calbindin-D_{28k} - caspase 3 interaction. Phosphorylation experiments were performed in vitro using [γ-^{32}P] ATP and purified protein kinase C from rat brain in the presence of $CaCl_2$ (1.6 mM), $MgCl_2$ (5 mM) and phospholipids. Using SDS-polyacrylamide gel electrophoresis and autoradiography, we found that rat calbindin-D_{28k} is phosphorylated in a substrate concentration dependent manner by PKC, consistent with conserved consensus PKC sites at Thr^{106} and Thr^{233}.Trypsin digestion followed by MALDI mass spectrometry indicated that the rat calbindin-D_{28k} consensus PKC sequence at amino acids 106 - 124 is phosphorylated. To evaluate the role of phosphorylation, the binding of phosphorylated and unphosphorylated forms of calbindin to caspase 3 was examined using the protein-protein interaction methodology of Ciphergen's Protein Chip System and SELDI mass spectrometry. Although specific calbindin-D_{28k} - caspase 3 interaction was noted by the protein chip technology, when calbindin-D_{28k} was dephosphorylated by incubation with calf intestinal phosphatase (CIP), purified caspase 3 was unable to bind to calbindin-D_{28k}. Calbindin-D_{28k}, similarly incubated in reaction buffer but in the absence of the phosphatase, bound to caspase 3. No interaction was observed between CIP and caspase 3. These findings provide a linkage between phosphorylation of calbindin and calbindin's ability to bind to caspase 3. In summary our findings indicate that rat calbindin-D_{28k} is a substrate for phosphorylation by PKC. In addition this study suggests that this post translational modification can enhance the association of calbindin with target proteins involved in the cellular function of calbindin.

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Regulation of Mesenchymal Stem Cells Differentiation by Exposure to Vitamin D. G. Duque,*[1] R. Kremer.[2] [1]Geriatrics and Calcium research laboratory, McGill University, Montreal, PQ, Canada, [2]Calcium Research Laboratory, McGill University, Montreal, PQ, Canada.

Age related decrease in bone mass is accompanied by a linear increase in the number and size of marrow adipocytes. This effect is most pronounced in long bones where up to 90% of the marrow cavity is occupied by adipocytes in individuals 65 years of age and older. This inverse relationship between bone mass and adipose tissue formation may be the result of an imbalance in the differentiation of bone forming versus fat-forming cells. Because 1,25 dihydroxyvitamin D3 (1,25(OH)2D3) is a potent prodifferentiative agent in many cellular systems we investigated its effect on mesenchymal stem cells (MSC) differentiation into either adipocytes or osteoblast. Non-committed and committed MSC were analyzed prior to and following addition of 10-8 M 1,25 (OH)2D3 using specific markers of adipocytes and osteoblast differentiation. For this purpose cells were stained with either alizarin red, a marker of osteoblastogenesis, or red oil for adipocytes identification. In addition alkaline phosphatase and leptin were quantified in cellular extracts.Here we demonstrate that 1,25 (OH)2D3 potentiates adipocyte differentiation in MSC treated with adipogenesis induction media and induced mild adipogenesis in MSC treated with an osteoblast induction media. Uncommitted MSC had a fibroblast-like phenotype but did not stain with either red oil or alizarin red. In contrast uncommitted MSC treated with 1,25 (OH)2D3 had a mixed phenotype and expressed both biochemical markers of osteoblasts and adipocytes differentiation. Our results therefore indicate that 1,25 (OH)2D3 induces both adipocyte and osteoblast differentiation in MSC and potentiate adipogenesis in MSC already committed to adipogenesis.

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Molecular Regulation of Vitamin D Activation Involves Transcriptional Suppression of the Renal Vitamin D Receptor. A. Bajwa,* C. Yin,* M. J. Beckman. Biochemistry, Virginia Commonwealth University, Richmond, VA, USA.

The active form of vitamin D-1,25 dihydroxyvitamin D3 [1,25-(OH)2D3]- is converted from 25-hydroxyvitamin D3 mainly in the kidney proximal tubules and is catalyzed by the enzyme 1a-hydroxylase (1a-OHase). The synthesis of 1,25-(OH)2D3 is regulated by dietary calcium, by parathyroid hormone (PTH) to increase 1a-OHase and decrease 24-hydroxylase (24-OHase), and by 1,25 dihydroxyvitamin D3 which regulates its own synthesis through a 1,25 dihydroxyvitamin D3-receptor (VDR)-dependent negative feedback effect on both 1a-OHase and preproparathyroid hormone production. This study examines the molecular mechanism of renal vitamin D activation induced by dietary calcium restriction (hypocalcemia), and tests the hypothesis that the activation process is the result of negative feedback regulation blockade at the transcriptional level. Rats were fed diets containing 0.02% calcium (-Ca) or 0.47% calcium (+Ca). Some of the -Ca and +Ca rats were given daily oral doses of different levels of vitamin D3, 0 (-D), or 4µg D3/day, (+D). In the +Ca+D rats, additional exogenous administration of 1,25-(OH)2D3 markedly suppressed renal 1a-OHase gene expression and increased renal 24-OHase gene expression. In contrast, rats fed a -Ca+D diet had extremely elevated serum 1,25-(OH)2D3 concentrations, which occurred by endogenous production. The -Ca+D group also had high 1a-OHase and low 24-OHase gene expressions, whereas, intestinal 24-OHase gene expression was increased. Low calcium diet also resulted complete down-regulation of VDR concentrations in -Ca+D rat kidney with minimal to no change in intestine VDR concentrations. Differential display RT-PCR was carried out using RNA purified from the two groups that demonstrated extremes in regulation of 1a-OHase gene expressions, the -Ca+D group and the +Ca+D/1,25(OH)2D3 group. A panel of 24 different combinations of anchor and arbitrary primers produced general gene products that relate to 1a-OHase gene expression. Authentic differentially regulated gene products in the two samples were confirmed by the real time RT-PCR method. Two sets of gene products were identified, those that participated in increased 1a-OHase expression and those that participated in 1a-OHase suppression. The gene product results identified in the differential display RT-PCR screening will be discussed. We conclude, however, that persistent tissue-specific down-regulation of VDR under hypocalcemic conditions can block 1,25(OH)2D3 feedback on 1a-OHase activation. The phenomenon that blocks 1,25(OH)2D3 feedback regulation is in part mediated indirectly via gene products produced during hypocalcemia and not produced in the presence of VDR transcript.

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Effects of 1, 25 (OH)2D3 and EB1089 on Bone Cells and Bones of Hindlimb Unloaded Adult Male Rats. R. Narayanan,*[1] M. R. Allen,*[2] J. L. Stafinsky,*[2] S. A. Bloomfield,[2] C. L. Smith,[1] N. L. Weigel.[1] [1]Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA, [2]Health and Kinesiology, Texas A&M University, College Station, TX, USA.

Skeletal unloading causes bone loss in astronauts, in bed rest patients, and in rats subjected to hindlimb unloading (HU). This loss is accompanied by decreases in 1,25(OH)2D3 (1,25D), the active metabolite of vitamin D. Although Halloran et al., (Endocrinology 118:948-954, 1986) found that administering 75 pmol 1,25D (0.21 µg/kg) / day to young HU unloaded rats did not block bone loss, rats with rapidly growing bones are not a good model for a mature adult skeleton. To determine whether 1,25D or a less calcemic analog, EB1089 (Leo Pharmaceutical Products), can prevent bone loss in mature rats, 5 month old male rats were placed on a vitamin D deficient diet containing 0.5% calcium and subjected to HU for 28 days. Animals were treated with vehicle (n=13), 0.1 µg/kg 1,25D (n=11), or 0.1 µg/kg EB1089 (n=13) administered by Alzet pump. The proximal metaphysis of the tibia were analyzed by peripheral quantitative computed tomography (pQCT; Stratec Research-M, Norland Corp.) before and after HU; Total bone mineral content (BMC), total bone mineral density (BMD), and BMD of the trabecular compartment (BMDt) were determined. The combination of altered diet and hindlimb unloading significantly decreased the BMC, BMD, and BMDt. Remarkably, treatment of animals with 1, 25D significantly increased BMC, BMD, and BMDt relative to initial values (+11%, 7%, and 23% respectively). EB1089 was not as effective as 1,25D. Although it prevented loss in total BMC, both BMD and BMDt were reduced. However, the reduction in BMDt (12%) was significantly less than in the vehicle treated animals (19%). A number of other differences were noted. 1,25 D significantly increased both serum and urine calcium levels as well as serum osteocalcin levels, whereas EB1089 did not. 1,25D but not EB1089 increased the urine pyridinium crosslinks suggesting that 1,25D stimulated osteoclast activity. Studies in bone marrow cell cultures were consistent with these findings. Whereas EB1089 treatment in vivo or in vitro was more effective than 1,25D in increasing the percent ALP+ preosteoblast colonies, only 1,25D increased the number of TRAP positive multinucleated putative osteoclasts in cell cultures. Moreover, 1,25D increased the protein levels of RANKL, a protein important in osteoclast differentiation, whereas EB1089 did not. These data suggest that vitamin D receptor agonists have tissue and cell specific effects and that in adults, 1,25D may be able to prevent bone loss due to skeletal unloading.

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LC-MS Based Substrate Specificity Studies of Wildtype and Mutagenized Versions of Vitamin D 25-hydroxylase, CYP27A1. S. Masuda, J. A. Bates,* D. E. Prosser,* Y. D. Guo,* G. Jones. Biochemistry, Queen's University, Kingston, ON, Canada.

Previous studies have shown that CYP27A1 hydroxylates 1α-OH-D_3 and 1α-OH-D_2 at different carbons of the side chain favoring C-25 for 1α-OH-D_3 and C-24 for 1α-OH-D_2. It is assumed that two structural differences in the D_2 side chain: a C22-C23 double bond and C-24 methyl group must make the side chain adopt a different conformation within the active site of CYP27A1. In this study, we have used wildtype and a V515L mutant of CYP27A1 in transiently transfected COS-1 cells to explore the effect of changes in residues lining the binding pocket of CYP27A1 on substrate specificity and rate. We employed 1α-OH-D_4 in addition to 1α-OH-D_3 and 1α-OH-D_2, since it has the C-24 methyl group only. Assay of products utilized UV diode-array detection while peak identification was based upon LC-MS-MS in the positive electrospray mode. These substrates gave products hydroxylated at a variety of side chain positions including C-24, C-25 and C-26(C-27). Products were distinguished by differences in retention time on two LC systems and by molecular weight (MS1) and fragmentation patterns (MS2) from MH^+, sodium adducts and dehydration products. With 1α-OH-D_3 as substrate, we found that while the wildtype enzyme performed both 25- and 27 hydroxylation efficiently (ratio 25/27-OH = 3.05), the mutant enzyme had a dramatically decreased ability to 27-hydroxylate whereas its efficiency of 25-hydroxylation was unaffected (ratio 25/27-OH = 10.68). With 1α-OH-D_2 as substrate, we found that while the wildtype enzyme completely lost its ability to 25-hydroxylate the substrate and exhibited lower 27 hydroxylation efficiency, it gained the ability 24-hydroxylate the substrate (ratio 24/27-OH = 1.83). In contrast, the V515L mutant enzyme not only lost its ability to 25-hydroxylate 1α-OH-D_2 but had a 50% decreased ability to 24- and 27-hydroxylate substrate relative to wildtype. With 1α-OH-D_4 as substrate, the wildtype enzyme gave a similar hydroxylation pattern to that seen for 1α-OH-D_2 with no 25-hydroxylation and similar rates of 24- and 27 hydroxylation (ratio 24/27-OH = 0.73). With 1α-OH-D_4 the V515L mutant enzyme had a slightly reduced ability to 27-hydroxylate whereas its efficiency of 24-hydroxylation was unaffected (ratio 24/27-OH = 1.25). We conclude that the valine at 515 in CYP27A1 plays a crucial role in 27-hydroxylation such that mutation to leucine reduces activity. On the other hand the insertion of the C-24 methyl group into the side chain of 1α-OH-D_3 profoundly affects the rate of 25-hydroxylation. These studies illustrate the effect of subtle changes in both amino-acid residues lining the substrate binding pocket and the vitamin D side chain on rates of

hydroxylation.

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Interactions of VDR With Co-regulators by New Vitamin D Anologues, OCT and ED-71. K. Takeyama,*[1] M. Kim,*[2] A. Murayama,*[2] S. Kato.[1] [1]IMBC, The University of Tokyo/CREST, Bunkyo-ku, Japan, [2]IMBC, The University of Tokyo, Bunkyo-ku, Japan.

A variety of vitamin D analogues has been developed with characteristic actions derived from diverse biological actions of 1α, 25$(OH)_2D_3$. Most of vitamin D actions are believed to exert through VDR-mediated transcriptional control of target genes. VDR is a member of the nuclear receptor superfamily as a ligand-inducible transcription factor. Upon ligand binding, VDR dissociates from co-repressor complexes containing NCoR/SMRT, and histone deacetylases (HDACs), and recruits two classes of co-activator complexes. A co-activator complex contains p160 family co-activators (SRC-1/TIF2/AIB1), and p300/CBP, all of which harbor histone acetylase (HAT), while the another DRIP/TRAP complex has no HAT activity. As receptor binding of SERM, but not estrogen (E_2), makes ERs recruit NCoR/SMRT, probably for their antagonistic actions, we tested an idea that co-regulator interactions with VDR differ among vitamin D analogues. By a transient expression assay with receptor expression vectors, and Luc. reporter plasmids containing DR3 for transcriptional activation and hPTH negative VDRE for repression, potency of OCT and ED-71 (kind gifts from Chugai Pharm.) with 1α, 25$(OH)_2D_3$ in transcriptional controls were examined. Both of OCT and ED-71 were active as 1α, 25$(OH)_2D_3$ in transactivation, but degrees of transrepression depended on ligand-type. To explore the molecular mechanism of different potency of vitamin D anologues between transactivation vs transrepression, interactions of co-regulators (p160 family proteins, CBP/p300, DRIP205/TRAP220 and NCoR/SMRT) with VDR induced by ligand bindings in vivo and in vitro are under study.

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1,25-Dihydroxyvitamin D3 Facilitates RANKL-Stimulated Osteoclast Formation In Vitro Via Vitamin D Receptor-Dependent Expression of M-CSF. K. M. Dienger,* A. C. Bendixen, N. K. Shevde, J. W. Pike. Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA.

Osteoclastogenesis is a growth factor- and cytokine-regulated process whereby osteoclast progenitors undergo differentiation and then fusion into multinucleated cells capable of bone resorption. This process is modulated by a variety of systemic hormones including estrogen, androgens, thyroid hormone, retinoids and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3). The actions of several of these hormones, in particular 1,25(OH)2D3, are exerted indirectly through their ability to modulate production of osteoclastogenic factors such as RANKL from supportive stromal cells or osteoblasts. The ability of 1,25(OH)2D3 to induce macrophage differentiation, however, suggests the possibility that osteoclast precursors might be directly sensitive to this hormone. To test this hypothesis, we used the macrophagic cell line RAW264.7 that differentiates into functional osteoclasts in response to RANKL. Treatment of these cells with soluble RANKL (2 nM) induces efficient osteoclast differentiation beginning at 24 hr, as assessed by the appearance of transcripts for tartrate resistant acid phosphatase (TRAP), and osteoclast formation beginning at 48 hours, as assessed by the presence of multinucleated, TRAP-positive cells. Osteoclast formation by these cells is enhanced approximately 2-fold through the addition of M-CSF (5 ng/ml). Interestingly, 1,25(OH)2D3 was just as effective as M-CSF in enhancing RANKL- induced osteoclast formation, acting at concentrations as low as 10-12 M. This action of 1,25(OH)2D3 was mediated through the vitamin D receptor, which was detected by western blot analysis, and genomic in nature, based upon the ability of the transcriptional antagonist ZK-159222 to suppress 1,25(OH)2D3-stimulated osteoclast formation. To explore the mechanism, we tested the possibility that 1,25(OH)2D3 might function to stimulate M-CSF production, thereby enhancing RANKL-induced osteoclast formation. Accordingly, treatment of RAW264.7 cells with 1,25(OH)2D3 led to a modest upregulation of M-CSF expression using RT-PCR analysis. In addition, neutralizing antibodies to M-CSF were also effective in blocking the potentiating effects of 1,25(OH)2D3 on osteoclast formation. These findings suggest that 1,25(OH)2D3 induces the expression of M-CSF through a direct action on monocytic-macrophagic precursor cells thus facilitating the formation of osteoclasts in the presence of RANKL. These findings suggest a dual role for 1,25(OH)2D3 in regulating osteoclast production, one directed at the osteoclast precursor and the other towards supportive cells such as stromal cells and osteoblasts.

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Muscle Strength and Mobility Are Related to Vitamin D Levels in Vitamin D Deficient Geriatric Patients. H. J. J. Verhaar,* H. J. C. Janssen,* M. M. Samson,* J. A. Raymakers,* S. A. Duursma.* Geriatric Medicine, University Medical Centre Utrecht, Utrecht, The Netherlands.

The objective of the study was to test the hypothesis that muscle strength and mobility are related to serum vitamin D levels in vitamin D deficient geriatric patients.Thirty women (age 81 + 6.7 yr) referred to the geriatric outpatient department for various reasons, who appeared vitamin D deficient (25-hydroxyvitamin D < 20 nmol/l), and were able to walk and follow simple instructions, were included. Body weight, serum 25-hydroxyvitamin D (25OHD), 1,25-Dihydroxyvitamin D (1,25OHD), parathyroid hormone (PTH), calcium, and albumin were determined. Handgrip strength (HGS), isometric knee extension strength (IKES), and leg extension power (LEP) were measured. To quantify mobility, timed-get-up-and-go (TGUG), and a 2-Minute walking test (2-Min) were recorded. For analysis, product-moment-correlations and multiple-stepwise-linear-regression were used.HGS, IKES, and 2-Min were significantly correlated with 25OHD (respectively: r = 0.47, p < 0.05; r = 0.49, p < 0.05; r = 0.42,p < 0.05). No significant correlation was found between any of the outcome parameters and age, body weight, PTH, calcium, and 1,25OHD. After correction for albumin, HGS and IKES remained significantly associated to 25OHD (respectively: r = 0.47, p < 0.05 and r = 0.40,p < 0.05). Correlation between LEP and 25OHD was 0.30 (p = 0.08). No significant correlation was found between TGUG and 25OHD.It can be concluded that deficits in muscle strength and mobility were significantly associated with vitamin D levels in vitamin D deficient geriatric patients.

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Determinants of Vitamin D Status in Young Females. E. J. Ha,[1] N. E. Badenhop-Stevens,[1] J. D. Landoll,[1] S. L. Mobley,[1] B. Hollis,[2] L. Nagode,*[3] V. Matkovic.[1] [1]Bone and Mineral Metabolism Laboratory, OSU, Columbus, OH, USA, [2]University of South Carolina, Charleston, SC, USA, [3]Veterinary Pathobiology, OSU, Columbus, OH, USA.

Maximal calcium (Ca) economy is important during growth and skeletal development for the acquisition of peak bone mass. To a large extent this economy depends on vitamin D status of young individuals. In a cohort of young females (N=1734) from central Ohio, participants of a 7-year long skeletal development study, we measured 25$(OH)D_3$ (calcidiol) and monitored potential modifiers of vitamin D status over time. This includes factors from environment (seasonal influences, dietary intake) as well as endogenous factors (obesity). Serum 25$(OH)D_3$ was measured by a radioimmunoassay (RIA) with ^{125}I-labeled tracer (Hollis et al. Clin Chem 39/3,529, 1993). Season was represented in a month of blood draw/year. Dietary vitamin D intake was obtained from 3-day food records utilizing Nutritionist III software. Body composition was measured by DXA (Lunar, Madison, WI). Biochemical marker of obesity, serum leptin, was assessed by RIA (Linco, St. Charles, MO). Forward stepwise regression analysis (multiple Rsq 16%, F to enter 3.0) selected season, leptin, time since menarche, and body fat (BF) as the main determinants of vitamin D status in the model with Rsq change of 9.6%, 3.2%, 1.6%, 0.7% respectively. The association with leptin and BF was negative. Dietary vitamin D intake had borderline significance, presumably due to errors in estimating vitamin D content of foods. Summary: Obese young females have lower concentration of 25$(OH)D_3$ as compared to nonobese individuals. The data are similar to the previously reported findings in adults. In addition, the study suggests that leptin may influence vitamin D metabolism, however, this may require a direct confirmation by an intervention study.

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Threshold of Serum Vitamin D in Young Females. N. E. Badenhop-Stevens,[1] R. Alexandritis,*[2] J. D. Landoll,[1] E. J. Ha,[1] S. L. Mobley,[1] B. Hollis,[3] L. Nagode,*[4] P. Goel,*[2] V. Matkovic.[1] [1]Bone and Mineral Metabolism Laboratory, OSU, Columbus, OH, USA, [2]Statistics, OSU, Columbus, OH, USA, [3]University of South Carolina, Charleston, SC, USA, [4]Veterinary Pathobiology, OSU, Columbus, OH, USA.

Serum vitamin D (25$(OH)D_3$) threshold indicating a transition between vitamin D surplus and insufficiency for children and adolescents is currently unknown. The threshold is based on vitamin D status as related to circulating parathyroid hormone (PTH) level. To determine this threshold we measured serum 25$(OH)D_3$ and intact PTH in 1937 blood samples collected in a group of young females, participants of the 7-year follow-up study of bone mass measurements (ages 9-20 y). Serum 25$(OH)D_3$ was measured by a radioimmunoassay (RIA) with ^{125}I-labeled tracer (Hollis et al. Clin Chem 39/3,529, 1993) while PTH was measured using Nichols kits. Serum was stored in a freezer on -80^{o}C and stability of the PTH was checked by repeated assay on the subsample (N=10) in 1994 and 2000, revealing agreement of 99.1%. Subsequent mathematical modeling (S-Plus) revealed Line-Line as the best fit model for the data set of PTH vs. vitamin D with the highest Rsq (4.42%) and the inflection point set up at 31.5 ng/ml of serum vitamin D. The equation of the model for vitamin D <=31.5 ng/ml is: PTH = 35.1 - 0.36 * 25$(OH)D_3$ and for vitamin D >31.5 ng/ml is: PTH = 28.2 - 0.14 * 25$(OH)D_3$. 95% CI for the slope of the line determined by the first equation is: -0.15, -0.14. The inflection point for the PTH-25$(OH)D_3$ relationship for individuals with low dietary calcium (Ca) intake (1500 mg/day). The results of this research indicate that serum vitamin D threshold for teenagers is similar to the previously published figures for adults (~30 ng/ml) and that dietary calcium intake is a potent mediator of this relationship. To what extent vitamin D insuffiency (levels < 31.5 ng/ml) during growth may influence bone calcium accretion and peak bone mass formation remains to be established.

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A Direct, Non-extraction Enzyme Immunoassay for Measurement of 25-Hydroxyvitamin D. M. J. Gardner,* A. K. Barnes,* D. Laurie,* R. T. Duggan. IDS Ltd, Boldon, United Kingdom.

Measurement of serum or plasma 25-hydroxyvitamin D (25-OH D) is the preferred method for establishing a patient's vitamin D status, including identification of vitamin D insufficiency. Previously, all 25D measurements have required extraction of sample to remove protein prior to analysis. This has applied equally to all methods of analysis, including HPLC, protein binding assays and immunoassays. IDS has developed a displacer reagent that enables measurement of 25-hydroxyvitamin D by immunoassay using serum samples directly, without the need for extraction.The direct 25-OH D assay comprises dilution of 25uL of serum or plasma with 1mL of a reagent containing binding protein displacers and a vitamin D - biotin conjugate. A portion of the diluted sample (200uL) was added to an antibody-coated microtitre plate and incubated for 2 hours at room temperature. After a wash, avidin-peroxidase reagent (200uL) was added, incubated 30 minutes and washed. TMB substrate (200uL) was added and colour allowed to develop for 30 minutes. After addition of 0.5M HCl (100uL) the absorbance was recorded at 450nm. A calibration curve was prepared from the absorbance values for 25OH-D serum standards (4 - 250nM) using a 4-parameter curve fit. The sensitivity of the direct 25-OH D EIA (the concentration corresponding to the mean minus two standard deviations of the "0" calibrator) was less than 3nM. The assay gave good precision with samples; intra-assay precision was 4.6%, 3.1% and 5.0% at 22.1nM, 62.0nM and 118nM respectively; inter-assay precision was 7.5%, 11.5% and 8.6% at 20.7nM, 56.5nM and 104nM respectively. Linearity was good with a mean for observed / expected of 117% for samples diluted 1:2 and 1:4 with the "0" calibra-

tor. Cross-reactivity (at 50% displacement) for 25-OH D2 was 74%.Correlation against an extraction radio immunoassay (FDA 510k cleared assay, IDS Gamma B RIA, AA-35F1) was carried out with 530 patient samples. Samples were measured in both the direct 25-OH D EIA and the extraction RIA method in a back-to-back assay set up. The comparison of methods by Passing-Babcock gave an equation of: Direct 25-OH D EIA = 1.00 x 25-OH D RIA - 4.0 nM. A linear regression analysis gave a correlation coefficient of R2 = 0.80.This technology provides a rapid, precise and sensitive non-isotopic assay for the measurement of 25D without the need for samples to be extracted.

SU529

Functional Interactions between the Vitamin D Receptor and STAT1. M. Vidal,*[1] C. V. Ramana,*[2] A. S. Dusso.[1] [1]Renal Division, Washington University, St Louis, MO, USA, [2]Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA.

In human monocytes and macrophages, interferon-gamma (IFN-γ) treatment strongly inhibits 1,25-dihydroxivitamin D (1,25D) transcriptional activity. Conversely, 1,25D further enhances the transcription of IFN-γ responsive genes. The present study examines the mechanisms responsible for these findings.Immunoprecipitation of nuclear extracts from the human monocytic cell line THP-I with anti-VDR antibody showed that endogenous VDR and Stat-1 physically interact in a 1,25D and IFN-γ independent manner. Furthermore, immunofluorescence microscopy showed that IFN-γ treatment increases nuclear VDR content, suggesting that activated Stat1 drives unliganded VDR to the nucleus. GST pull down studies utilizing in vitro translated [^{35}S]-Stat1 and different GST-VDR fusion constructs confirmed that VDR/Stat1 interaction is 1,25D independent. This experiment also showed, in an *in vitro* system, that no additional cellular factor is necessary for the interaction and mapped it to the DNA-binding domain of VDR (DBD). In fact, addition of recombinant Stat1 specifically abolishes the binding to DNA of both VDR-RXR heterodimers and the DBD alone, in a dose-dependent manner. Clearly, the inhibitory effect of IFN-γ on 1,25D transcriptional activity is a consequence of the impairment of VDR binding to DNA by nuclear Stat1.Transient transfection of VDR in the fibroblastic cell line 2fgth showed that, in contrast to the inhibitory effect of Stat-1 on VDR binding to DNA, the binding of Stat1 homodimers to DNA is increased in cells overexpressing VDR. Western blot with anti-pY701 Stat1 antibody showed that the increase in DNA binding is due to an increase in tyrosine phosphorylation of Stat1, with no changes in total nuclear Stat1. Immunofluorescence microscopy confirmed that individual cells that overexpress VDR have a dramatic increase in the amount of nuclear phosphorylated Stat1. Since Stat1 dephosphorylation plays an important role in the down regulation IFN-γ signaling, VDR-Stat1 interaction, by protecting Stat1 from dephosphorylation, may prolong the response of target cells to IFN-γ.

SU530

Comparison of the Nichols Advantage and the Conventional Competitive Protein Binding Assay for the Measurement of Circulating Concentration of 25-Hydroxyvitamin D in Human and Non-human Primates. T. C. Chen,[1] J. Mathieu,*[1] D. Thomas,*[2] J. Tran,*[2] M. Garrity,*[2] M. F. Holick.[1] [1]Vitamin D, Skin, and Bone Research Laboratory, Endocrine Division, Department of Medicine, Boston University School of Medicine, Boston, MA, USA, [2]Nichols Institute Diagnostics, San Juan Capistrano, CA, USA.

The circulating concentration of the combination of 25-hydroxyvitamin D_2 [25(OH)D_2] and 25-hydroxyvitamin D_3 [25(OH)D_3] is a summation of the vitamin D that comes from sunlight and the diet. This measurement is considered to be the best indicator of vitamin D status and has become an important diagnostic tool for metabolic bone disease. To determine its concentration, 25(OH)D [25(OH)D_2 and 25(OH)D_3] is first extracted from serum or plasma with absolute ethanol, followed by a protein-binding assay using the serum vitamin D-binding protein which has high affinity for 25(OH)D. Nichols Institute Diagnostics has developed an automated assay using the combination of biotin/acridinium chemiluminescence technology and Nichols Advantage Speciality System. The purpose of this study was to compare these two methods for the analysis of 25(OH)D. We found that the correlation coefficients between these two assays range between 0.89 (n=60) and 0.93 (n=16) for human serum. For the non-human samples, a correlation coefficient of 0.97 was obtained for new world and old world primates (n=9). The Advantage assay is equally specific for 25(OH)D_2 and 25(OH)D_3. The detection limit for the Nichols Advantage is about 1ng/ml. However, the functional sensitivity is approximately 5 ng/ml. The within run imprecision at 10.6, 19.3, 47.6, and 56.8 ng/ml are 5.9, 4.1, 3.0, and 3.5 %CV, respectively. The total imprecision at 10.6, 19.3, 47.6, and 56.8 ng/ml are 10.8, 7.8, 5.1, and 8.5 %CV, respectively. Parallelism results are between 80-120%. Spike and recovery results are between 90-110%. The Nichols Advantage assay takes only 1-2 hours to complete 100 samples, compared to the conventional 25(OH)D assay which requires overnight incubation and takes two full days to measure 100 samples. We conclude that the Nichols Advantage automated system is suitable for the determination of circulating levels of 25(OH)D in the serum of human and non-human primates.

M001

Gender Differences in Axial and Appendicular Muscle Strength and Body Mass Index with Aging. M. Sinaki,[1] N. Nwaogwugwu,*[1] M. P. Mokri,*[2] B. E. Phillips.*[3] [1]Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN, USA, [2]Student, Gustavus-Adolphus, St. Peter, MN, USA, [3]Student, Mayo Medical School, Rochester, MN, USA.

Complex models have been considered to account for fracture risk that has not been explained through adjustment for hormonal deficiency and aging. Vertebral fractures have the highest incidence among osteoporotic fractures, but few studies are available on the supportive axial muscle strength in men and women. The objective of this study was to document differences in muscle strength in healthy men and women of various ages. Subjects included 70 men and 72 women, ages 21 to 89 years. Level of daily physical activity was evaluated in each subject. Back extensor strength (BES), grip strength (GS), knee extensor strength (KES) and body mass index (BMI) were measured and recorded. Across the decades up to age 90, from peak to lowest level, grip strength in men decreased 45% on the dominant side and 48% on the non-dominant side. Women's grip strength decreased 47% on the dominant side and 50.5% on the non-dominant side. Men's KES decreased 36% on the right and 35% on the left while women's KES decreased 38% on the right and 42% on the left. BES ranged from 21 to 187 pounds in men and 16 to 99 pounds in women. BES peaked in the 4th decade for men and in the 5th decade for wmmen, then steadily declined. Women's BES at different decades ranged from 54% to 76% that of men's. In the 4th decade, BES in women was 54% that of men and then increased to 70% to 76% that of men in the 8th and 9th decades, respectively. There was a 64% loss of BES in men from peak in the 4th decade (125.1 pounds) to the lowest level in the 9th decade (45.3 pounds). Women experienced a 50.4% loss from peak in the 5th decade (68.9 pounds) to the lowest level in the 9th decade (34.2 pounds). Men had a greater loss of BES than women with increasing age. BMI increased in the 4th through 6th decades in men and then decreased. Women's BMI increased from the 4th decade on except for a decrease at the 6th decade. Increased BMI in women coincided with reduction of BES in older age. In this study we compared mean values for BES, GS, KES and BMI in healthy men and women ages 21 to 89. Our data showed that men lose more BES with aging than women; BMI decreases with aging in men but not in women; and increased BMI in women correlates with reduced level of physical activity and decreased BES with aging.

M002

Hand Densitometry as the Method for Assessment of Bone Age - Preliminary Results. P. Pludowski,*[1] M. Lebiedowski,*[1] P. Chadzynski,*[2] J. Kowalska,*[1] R. S. Lorenc.*[1] [1]Children's Memorial Health Institute, Warsaw, Poland, [2]Medical Academy, Warsaw, Poland.

The assessment of skeletal age is an important factor for the analysis of biological maturity, growth and its disorders. The classical method is based on the recognition of changes in the radiographic appearance of the maturity indicators in hand - wrist roentgenograms by comparison with reference Atlas. The major weakness of this method appears to be the lack of standardized technique, poor reproducibility of the radiography method and irradiation (100 μSv).The aim of our study was the evaluation of hand densitometry method for the assessment of bone age. Densitometry taking advantage of lower radiation doses (8 μSv), stability of measurement conditions and digitalized imaging processing.During our study we have measured 70 healthy children (6 - 20 years old) and 30 with bone disorders (6 - 20 years old). All measurements were performed using Expert - XL and DPX - L densitometers (Lunar Corp.). To assess skeletal status we performed Total Body measurements by DPX - L. Hand densitometry performed by Expert - XL machine was used for the evaluation of bone age. Parallel, 20 hand - wrist roentgenograms were evaluated as a reference data. All hand scans were saved as a standard pictures (.tiff format) and then sent to independent observers for the evaluation of bone age on the basis of the main maturity indicators: extremitas distalis radii et ulnae, os pisiforme, os capitatium, os hamatum, os sesamoidea, phalanx media digitus medius. Bone age values estimated by the observers did not vary more then 0.5 year. Total Body measurements were performed and Z - scores values as standard diagnostic parameters in paediatric densitometry were calculated. It was noticed in several cases that low values of Z - scores (< -0.5) corresponded with lower bone age in comparison with chronological age. On the basis of this study we conclude that hand densitometry was the method less invasive then the radiography and can be used as an alternative method for the assessment of skeletal maturation. By the usage of hand densitometry parallel with bone mineral density measurements it became possible to evaluate additive information that leads to more objective diagnosis.

M003

Quantitative Ultrasonometry at Radius and Tibia Shows Different Age and Puberty Related Changes in Girls and Boys (Results of SOS Measurements in 570 Healthy Caucasian Children, Aged 6 – 18 Years). O. Bock,[1] T. Biedermann,*[2] A. Oldenburg,*[1] M. Berndsen,*[1] D. Felsenberg.[1] [1]Center for Muscle and Bone Research, University Hospital Benjamin Franklin, Free University Berlin, Berlin, Germany, [2]Dept.of Rheumatology, 2nd Pediatric Clinic, Klinikum Buch, Berlin, Germany.

To investigate the capabilities of quantitative ultrasound (QUS) for monitoring of bone changes in children with juvenile idiopathic arthritis the Sunlight Omnisene Bone Sonometer (Sunlight Medical Ltd., Rehovot, Israel), a multisite QUS device measuring the maximum speed of sound (SOS) in bone, was used. At first we measured 570 healthy Caucasian children - 335 girls (mean age 12.65 y ± 3.13 y) and 235 boys (mean age 12.56 y ± 2.95 y) - to evaluate an age and sex specific normative data base. Intra- and inter-observer variation had been tested before in 15 volunteers. The short-term intra-observer CV was 0.35 % and 0.44 %, the inter-observer CV was 0.79 % and 1.03 % at radius and tibia. Questionnaire based information have been obtained about calcium intake, physical activity, family history of osteoporosis, diseases interfering with bone metabolism, and overall health. In addition height and weight measurements as well as Tanner staging have been performed. A statistically significant correlation ($p < 0.001$) was found between age and maximum SOS at radius ($r = 0.64$ in girls, 0.62 in boys) and tibia ($r = 0.65$ in girls, 0.54 in boys).In girls significant increases in the maximum SOS were found at the tibia between Tanner stages II-V and at the radius between Tanner stages III-V. In comparison the boys showed marked and significant increases in the maximum SOS values at both measurement sites only between Tanner stages IV-V. None of the other parameters obtained showed any significant influence on the maximum SOS values after correction for age and Tanner stage - with exception of the grade of physical activity in female adolescents.In conclusion, girls and boys in the age of 6-18 years showed different age and puberty related changes in bone. Girls developed a slower but earlier and continuous increase of SOS values in comparison with boys. However, the morphological or functional entity represented by SOS measurements in bone is still unclear and remains a topic of further research.

M004

Bone Remodeling Markers, Insulin Growth Factor I (IGF1) and Broadband Ultrasound Attenuation (BUA) in Healthy Argentinean Children Aged 2 to 6 Years. S. N. Zeni,[1] P. Rodriguez,*[2] N. Sosto,*[2] E. Bermudez,*[2] M. Santarelli,*[2] A. Gamba,*[2] J. Somoza,*[1] S. Friedman.*[2] [1]División Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Cátedra de Bioquímica General y Bucal, Facultad de Odontología, Universidad de Buenos Aires, Buenos Aires, Argentina.

Although primarily designed for diagnosis and follow-up of metabolic bone diseases in adults, bone markers are being increasingly used by pediatricians. Bone metabolic activity in children is determined by both skeletal growth rate and bone remodeling. To estimate the bone turnover activity independent of growth processes, the bone marker levels must be compared to the actual skeletal growth. The purpose of this study was to obtain reference data as a function of age, sex and body growth length in a group of healthy Argentinean children attending a kindergarten in the Buenos Aires Province (Argentina) from 8am to 5pm. A total of 65 children (33 boys, 32 girls) aged 2 to 6 years were included. Total and bone alkaline phosphatase (ALP and bALP), serum b-crosslaps (CTX) and IGF1 were determined. BUA (QUS2, Metra Biosystems) was determined in the non-dominant os calcis. Anthropometric determinations were also included: height/age (H/A) z-score and adequacy percentage of H/A respect to the mean population (PMHA). Results are expressed as mean ±SD(ES).No significant differences were found as regards sexes, but CTX and IGF1 showed a tendency to increase with age ($p<0.058$). BUA did not show significant differences between sexes or among age groups: 52.6±6.6(1.2).Although further investigation on a larger study sample is necessary, the clinical relevance of our study is that it provides a potential tool for monitoring normal bone remodeling and longitudinal skeletal growth, as well as normal reference values for the diagnosis and follow-up of children with metabolic bone diseases.

M005

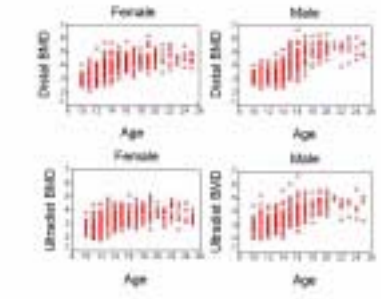
Rodent Model for Investigating the Effects of Estrogen on Skeletal Response to Voluntary Muscle Forces During Growth. L. Wang,[1] A. McMahan,*[2] J. Banu,[1] M. C. Okafor,*[1] D. N. Kalu.[1] [1]Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [2]Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

It has been reported that from about 11-12 years of age in humans, bone mass began increasing faster in girls than in boys with the same muscle mass. By 14-15 years of age, bone mass per unit mass of muscle was significantly higher in girls than in boys. Because around 15 years is the beginning of reproductive age in women, it was suggested that estrogen was possibly involved in the higher bone mass (Schiessl H., et al. Bone, 22:1-6; 1998). However, progress in this important area of research is quite slow, in part, because there is no acknowledged animal model for studying the musculoskeletal effects of estrogen that may occur in humans during puberty. We have previously demonstrated that male Sprague Dawley (SD) rats are suitable animal model for studying age-related bone loss in men (Bone: 2001, in press). Therefore, the current study was undertaken to see if bone mass per unit muscle mass will be higher in the female than in the male SD rat during growth as has been reported for humans and consequently, whether these SD rats are suitable for studying the musculoskeletal effects of estrogen as may occur in humans during puberty. Female and male SD rats aged 1 to 6 months were studied at the L4 vertebra using peripheral quantitative computed tomography (pQCT). In this study, muscle cross-sectional area was measured as a surrogate for muscle mass and several pQCT bone indices were measured as surrogate for bone mass. The area of the paraspinal muscles surrounding the L4 vertebra, total bone mineral content (BMC), cortical BMC, and cancellous BMC of the L4 vertebra of female and male SD rats increased rapidly from 1 to 3 months of age. Total BMC, cortical BMC and cancellous BMC increased faster in female than in male SD rats with the same muscle area. At 3 and 6 months of age, the above vertebral indices of bone mass in female SD rats were significantly higher than in male SD rats with the same muscle area as has been reported in humans during puberty. Since female rats can reproduce at 3 and 6 months of age, the higher bone mass per unit muscle area seen in female SD rats at these ages was possibly due to the musculoskeletal effects of estrogen as has been suggested by Schiessl et al. in humans. In conclusion, we have shown that 3 and 6-month-old female SD rats have significantly higher bone mass per unit area of muscle than male SD rats with the same muscle area. These findings suggest that the young female and male SD rats can be used as appropriate model for studying the effects of estrogen on skeletal response to voluntary muscle forces as has been reported in humans.

M006

Forearm Distal and Ultradistal Peak Bone Mass in a Venezuelan Oopulation, G. Riera Espinoza, G. Velasquez,* R. Carvajal,* M. Naressi,* Y. Cordero,* J. Ramos.* UNILIME, Universidad de Carabobo. Hospital Universitario Dr. Angel Larralde, Valencia, Venezuela.

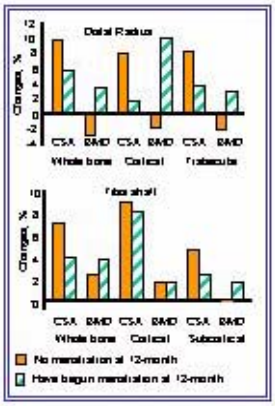
Measuring peripheral BMD during the development of Peak Bone Mass (PBM) by DEXA has been less investigated than central measurements of BMD. To our knowledge there is no report of such a study in Latin America.876 healthy subjects, 10-25 year-old were recruited for Bone Mineral Density assessment. BMD was measured by DEXA (Osteometer-200. VC less than 1.5%) at distal and ultradistal forearm.Subjects were divided into two groups: 410 males and 466 females. In the age range 10-25 year-old, males reached the highest bone mass at age 21 in both areas distal and ultradistal forearm, 80% of that value was obtained at age 16 year-old. Women did so at older age, 22 year-old also at both sites: distal and ultra distal, and 80% of PBM was reached at younger age than men, 13-14 year-old.Conclusion. Forearm Peak Bone Mass in a Venezuelan population 10-25 year-old is achieved at 21 year-old in men (Distal BMD: 0.556±0.01 gr/cm2, Ultradistal 0.503±0.006 gr/cm2) and at 22 in women (Distal BMD: 0.471±0.02 gr/cm2, Ultradistal BMD 0.409±0.02 gr/cm2). 80% of that PBM is achieved later in men, 16 year-old than women, 13-14 year-old.



M007

The Effect of Menarche and Changes in Muscularskeleton Growth in 10-13 Year-old Girls During One-year Follow-up. S. Cheng,[1] F. Tylavsky,[2] M. Kemikangas,*[1] P. Salo,*[1] A. Mahonen,[3] A. Koistinen,*[4] H. Suominen,[1] H. Kröger.[3] [1]Univ of Jyväskylä, Jyväskylä, Finland, [2]Univ of Tennessee, Memphis, USA, [3]Univ Of Kuopio, Kuopio, Finland, [4]Central Hospital, Jyväskylä, Finland.

The purpose of this study is to evaluate effect of menarche and changes in muscular-skeleton growth during a one-year period in Finnish girls as measured by pQCT. The subjects were 10-13 year-old girls (n=98) who presented with Tanner stage I-II and enrolled in an intervention study (the CALEX-study). Bone and muscle mass assessments were performed with pQCT (XCT 2000, Stratec) at the baseline and 12 months from lower leg shaft (65% of the leg length section) and distal forearm (4% of the forearm length section). The data was then analyzed with BonAlyse software (BonAlyse Oy). Our results indicate that the subjects increased their body height on average 4.1% and weight 12.6% during one-year period. The increases in cross-sectional area (CSA) of the lower leg shaft was 10.2% for total cross-section, 11.6% in muscle, 7.2% in subcutaneous fat and 7.3% in bone. The increase in volumetric bone mineral density (BMDv) was 1.8% in the cortical part and 0.29% in subcortical part at the tibia shaft. At the distal radius, the trabecular CSA increased 7.5% while no increase in BMDv of trabecular bone (-1.1%) was found. When we compared the percent change in bone and muscle for those girls who began menstrual cycles during the 12-month follow-up period to those who did not begin menstrual cycles, we found there was an increase in bone size, with an apparent loss or no change in BMDv of the radius and tibia (transient osteoporosis) in the girls who had not begun their menstrual cycles (Fig 1). In contrast, those who began menstrual cycles have a smaller increase in size but larger gains in BMDv ($p<0.05$). No significant difference was found in change in muscle CSA between the two groups. Our results have implications that it may be important for the primary prevention and the interpretation of research studies to concern the timing and type of intervention. As suggested by other studies the influence of diet and exercise pre-menarchial may require different assumptions. The requirement of calcium, vitamin D and other minerals may be a limiting factor for the secondary consolidation of mineral after the onset of menarche.



M008

Patterns of Bone Growth and Its Maintenance Throughout Life. A Segmented Mixed Longitudinal Study Among 787 Females From Childhood to Senescence with 6,914 Cumulative Years of Follow-up. V. Matkovic,[1] J. D. Landoll,[1] N. E. Badenhop-Stevens,[1] K. Kostial-Simonovic,*[2] P. Goel.*[3] [1]Bone and Mineral Metabolism Laboratory, OSU, Columbus, OH, USA, [2]Institute for Medical Research, Zagreb, Croatia, [3]Statistics, OSU, Columbus, OH, USA.

To evaluate the patterns of bone growth and its maintenance throughout life, we used a segmented mixed longitudinal study consisting of cohorts of females from childhood to senescence. Bone mass measurements were done in teenage females followed for 7 y (ages 10.9±0.9 y, N=185), young adult women followed for 6-10 y (ages 34.6±1.9 y, N=84), and adult women followed for 9-10 y (ages 42.3±1.7 y, N=162; 49.3±2.9 y, N=144; 59.2±3.1 y, N=114; 70.0±3.6 y, N=101). DXA measurements of the whole body (TBBMD) and of the forearm were done annually among teenage females and at baseline and after 6 y of follow-up in a cohort of young adult women. Radiogrammetry measurements (CA/TA) of the 2nd metacarpal bone were done among the same individuals at ages 11, 15, and 18 y and among groups of adult women 9-10 years apart. Individuals were stratified into the quartiles of TBBMD and CA/TA at baseline and at the end of the study and the relative distribution of those remaining in the same position after follow-up was counted. The table below presents % of individuals who remain in the same quartile at the end of the follow-up periods.

	Age 11-18	Age 34-46	Age 42-52	Age 49-59	Age 59-69	Age 70-80
Quartile 1	65.2	86.0	68.3	58.3	62.1	52.0
Quartile 2	46.2	79.5	41.0	28.9	43.5	28.0
Quartile 3	41.5	66.5	57.1	32.3	44.1	46.0
Quartile 4	60.0	80.0	75.0	55.6	57.1	52.0

Most females remain in the same quartile of bone density distribution over time. As the bone modeling phase finishes with strong tracking characteristics in bone mineral acquisition and with minimal disturbance in bone tracking dynamics before menopause, early skeletal development may predetermine the risk of osteoporosis later in life. Presenting data according to menarche/menopause may further stabilize the patterns of bone growth and bone loss and improve tracking characteristics. These results may have implications with regard to the timing for osteoporosis screening and primary prevention

M009

Urinary Excretion of Calcium in Relation to Urinary Sodium is a Determinant of Bone Mass in Pre-adolescent Children. F. A. Tylavsky,*[1] K. M. Ryder,[2] C. Womack,[2] J. Norwood,[2] L. D. Carbone.[2] [1]Preventive Medicine, Univ. of TN, Memphis, TN, USA, [2]Medicine, Univ. of TN, Memphis, TN, USA.

Urinary sodium (Na) has been shown to be a determinant of calcium (Ca) excretion and bone mass in adults and white pre-adolescent females. The objectives of this study were to determine if race and/or sex affects the relationship between 24-hr. urine Ca and Na excretion and if there were consequences on bone mass in children who presented as Tanner stage II. 120 children collected 24-hour urine samples and had bone mineral content (BMC) and density (BMD) of the whole body (WB), and lumbar spine (LS) using a QDR 2000 (Hologic, Inc). The average age was 10.4 ± 1.3 (SD), body weight (38.6 ± 8.7 kg), and height (141.5 ± 8.9 cm). There were 72 white females, 29 white males, 12 black females and 8 black males. When we regressed urinary Na on urinary Ca per liter of volume for all children the equation was Ca (mg)= 28 + 0.03(Na, mg.)), $p<0.0001$. This positive relationship remained significant ($p=0.04$) after controlling for race, sex and creatinine. On average, 33 mg of Ca was excreted per 1000 mg of sodium (Ca/Na) ., with a minimum of 1 and maximum of 102 per liter. The cohort was divided into three groups according Ca/Na excretion per liter (low, 1-19; Medium 20-40; high>40) to examine the effects of the level of Ca/Na ratio on urinary calcium output and Area, BMC and BMD of the WB, and LS. Levels of Ca/Na, Na and Ca excretion among the groups were compared

using analysis of variance. Differences in Area, BMC and BMD were compared *adjusting for race, sex, age, and creatinine excretion. There were no differences in Area or BMC among the Ca/Na groups.

Variable N	Low(1-19) 36	Med.(20-40) 44	High(>40) 40	P-value
Ca/Na (per liter)	12.1±1.7	29.0±1.6	56.6±1.7	0.0001
Na (mg/liter)	2970±210	3077±190	2965±199	>0.05
Ca (mg/liter)	38.5±7.5	89.5 ±6.9	161.7±7.2	<0.001
*WB BMD	0.840±0.01	0.849±0.01	0.820± 0.01	0.07
*LS BMD	0.671±0.02	0.680±0.02	0.628±0.64	0.07

Our data suggests that a critical factor that may modulate the effects of Na intake on bone mass is the quantity of Ca excreted in response to Na excretion. Whether the effect is due to the inability to compensate via increases in gut absorption and/or modulation of calcitropic hormones requires additional investigation. The long-term effect of this cross-sectional relationship between urinary Ca and Na and bone mass over time remains to be determined

M010

Leptin, Body Composition and Bone Mineral Density in Premenopausal Women. M. Blum, S. S. Harris, A. Must,* S. M. Phillips,* B. Dawson-Hughes. Tufts University, Boston, MA, USA.

Numerous studies have demonstrated a positive association between body weight and bone mass in subjects of all age groups. It is unclear, however, whether body composition, that is the percent of total weight that is fat tissue (%fat), is associated with bone mineral density (BMD) independently of weight. Leptin is produced in fat tissue, and has recently been shown to be inversely related to bone mass in mice, but its role in predicting human bone mass is uncertain. We investigated associations of %fat and leptin with BMD in a cohort of 153 healthy premenopausal women. BMD (gm/cm^2) of the total hip, lumbar spine and total body and body composition were measured by dual energy X-ray absorptiometry (DXA). Leptin assays (ng/ml) were performed on serum using a commercial competitive binding assay. Leptin and weight were logged in linear analyses. The mean age of the women was 41.6 ± 0.8 years, mean weight was 65.8 ± 14.0 kgs and mean %fat was 33 ± 9%. Leptin levels ranged from 1.7 to 47.5 ng/ml, with a median value of 11.2. Leptin was highly correlated with body weight (r = 0.75) and with %fat (r = 0.89). The table below presents results from 4 multivariable regression models (labelled A through D) in which weight, %fat and/or leptin were included as independent variables. We also report the variability in BMD explained by the independent variables as a group (R^2). Data for the total hip are presented, and similar findings were observed at the lumbar spine and total body. As expected, weight was positively associated with BMD (A) and explained about 29.8% of the variability in BMD. When %fat was added to this model (B), it was negatively associated with BMD and increased the R^2 modestly. When we substituted leptin for %fat (C), we found leptin was also negatively associated with BMD and similarly increased the R^2 modestly. When both leptin and %fat were included (D), neither variable remained statistically significant. Associations of Weight, Leptin and %Fat with Total Hip BMD

	r	R^2
(A) Weight	0.53*	0.298
(B) Weight	0.44*	
%Fat	-0.21*	0.324
(C) Weight	0.48*	
Leptin	-0.19*	0.321
(D) Weight	0.45*	
%Fat	-0.11	
Leptin	-0.07	0.328

All correlations are adjusted for height; r represents partial correlation coefficients. *P < 0.05.

In conclusion, these data suggest that for a given body weight, both a higher proportion of fat and a higher serum leptin concentration have negative associations with BMD. Since leptin is produced by fat cells, the inverse association of %fat with BMD may be mediated by leptin, however we were not able to identify an independent effect of this hormone on BMD.

M011

The Expression of Smad Interacting Protein (SIP)-1 and its Effects on BMP Signal and Myoblastic Differentiation. W. Xia,*[1] K. Nakayama,[1] S. Fukumoto,[2] Y. Takeuchi,[1] T. Fujita.*[1] [1]Department of Internal Medicine, University of Tokyo, School of Medicine, Tokyo, Japan, [2]Department of Laboratory Medicine, University of Tokyo, School of Medicine, Tokyo, Japan.

Bone morphogenetic proteins (BMPs) promote osteoblastic differentiation,while they inhibit myoblastic differentiation. BMPs signal through Smad proteins, which regulate the expression of target genes. However, themechanism of transcriptional regulation by Smad proteins is not well-understood. SIP-1 is a Smad-binding protein which belongs to transcription factor δEF1/Zfh-1 family, but its physiological function is not known. SIP-1 binds to 5'-CACCT sequence, which is a part of E-box, orthe binding site for basic helix-loop-helix (bHLH) class transcription factors, and suppresses transcription. SIP-1 may, therefore, regulate thetranscriptional activities of Smads as well as bHLH class transcription factors, such as MyoD that plays a key role in myoblasitc differentiation. In order to clarify the role of SIP-1 in osteoblastic and myoblastic differentiation, we examined the expression of SIP-1 in mouse osteoblastic cell line MC3T3-E1 and myoblastic cell line C2C12, treatedwith BMP-2 by Northern blot analysis. To analyze the effect of SIP-1 on BMPsignal, we measured luciferase acitivity after co-transfection of MC3T3-E1 cells with full length SIP-1 and a BMP responsive element-containingreporter construct, 3GC2-lux, which is derived from Smad6 promoter. Similarly, in order to study the role of SIP-1 in myoblastic differentiation, we evaluated the promoter activity of muscle creatinine kinase (MCK), a muscle marker, by luciferase assay after the overexpression of full length SIP-1 in C2C12 cells.We detected the expression of SIP-1 in both MC3T3-E1 and C2C12, which was induced by BMP-2. Enhancement of luciferase activity of 3GC2-lux by BMP-2 was almost completely abolished by the overexpression of SIP-1. SIP-1 also suppressed the activation of MCK promoter induced after myoblastic differentiation. However, SIP-1 had no effect on 25-hydroxyvitamin D-1α-hydroxylase promoter activity.Our results suggest that SIP-1 is expressed in osteoblastic and myoblasticcells, and inhibits BMP signal mediated by Smads and myoblastic differentiation. As there is no 5'-CACCT sequence in 3GC2, the inhibitoryeffect of SIP-1 on Smad transcriptional activity may be mediated by theinteraction between Smads and SIP-1, but not by that between SIP-1 and DNA.Since BMP-2 induces the expression of SIP-1 in C2C12, SIP-1 may be involvedin the inhibition of myoblastic differentiation by BMP-2. Results of this study also indicate that SIP-1 may play a role in the negative feedbacksystem against the direct action of BMP.

M012

Bone Morphogenetic Protein-2 Induces Cyclooxygenase-2 in Osteoblasts via a Cbfa1 Binding Site: Role in Osteoblast and Osteoclast Differentiation. D. Chikazu,[1] X. Li,[1] H. Kawaguchi,[2] K. Hoshi,[2] O. S. Voznesensky,[1] H. R. Herschman,[3] L. G. Raisz,[1] C. C. Pilbeam.[1] [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Medicine, University of Tokyo, Tokyo, Japan, [3]Biological Chemistry, UCLA School of Medicine, Los Angeles, CA, USA.

Bone morphogenetic protein (BMP)-2, a member of the transforming growth factor (TGF)-beta superfamily, is a potent osteogenic factor. Because TGF-beta induces cyclooxygenase-2 (COX-2) and prostaglandin (PG) production in osteoblasts, we hypothesized that some effects of BMP-2 might be mediated via COX-2/PGs. BMP-2 induced COX-2 mRNA and PG production in cultured primary murine calvarial osteoblasts. In calvarial osteoblasts from mice transgenic for -371/+70 bp of the murine COX-2 promoter fused to a luciferase reporter (Pluc) and in the murine osteoblastic cell line MC3T3-E1 stably transfected with Pluc, BMP-2 dose-dependently increased luciferase activity up to 4-fold. On sequential deletion analysis in stably transfected MC3T3-E1 cells, site(s) mediating BMP-2 stimulation of luciferase activity lay between -300 and -213 bp of the COX-2 5'-flanking region. Two bp mutation of a putative Cbfa1 binding site (5'-AACCACA-3') at -267/-261 bp decreased BMP-2 stimulated-luciferase activity in transiently transfected MC3T3-E1 cells by 82%. On Northern analysis, Cbfa1 mRNA was constitutively expressed. On electrophoretic mobility shift analysis, constitutive binding to an oligonucleotide spanning the COX-2 Cbfa1 site was competed by the unlabeled oligonucleotide, inhibited by specific antibody to Cbfa1, but unaffected by competition with the unlabeled oligonucleotide carrying the Cbfa1 mutation. To examine the role of COX-2 induction in BMP-2 regulation of osteoblast differentiation, calvarial osteoblasts were cultured from COX-2 knockout (-/-) and wildtype (+/+) mice. BMP-2-stimulated alkaline phosphatase activity was reduced 53% in COX-2-/- cells compared to COX-2+/+ cells, and a similar reduction was seen when COX-2+/+ cells were treated with NS-398, a selective COX-2 inhibitor. In addition, BMP-2 induced osteoclast formation in bone marrow cultured from COX-2+/+ mice but not in marrow from COX-2-/- mice. We conclude that BMP-2 transcriptionally induced COX-2 expression via a Cbfa1 binding site and that the induction of COX-2 may mediate or enhance effects of BMP-2 on osteoblast and osteoclast differentiation.

M013

Histology Indicates Bisphosphonate Limits Transient Resorption Without Decreasing Subsequent Bone Induction in Nonhuman Primate Core Defects Treated With rhBMP-2/ACS. H. J. Seeherman, X. Li, C. Blake,* D. Gavin,* J. M. Wozney, M. L. Bouxsein. Genetics Institute, Andover, MA, USA.

Previous studies using recombinant human bone morphogenetic protein-2 (rhBMP-2)/ absorbable collagen sponge (ACS) in sheep and nonhuman primate femoral core defects demonstrated new bone induction was preceded by transient osteoclastic resorption (Seeherman et al, Trans ORS 1998, 2000). This study was designed to determine if systemic bisphosphonate could be used to limit initial bone resorption without altering subsequent bone induction. Bilateral 3.5 mm distal femoral core defects in 6 cynomolgus monkeys were implanted with rhBMP-2 (360 μg)/ACS (BMP) or buffer/ACS (Buffer). In addition, bilateral proximal femoral core defects treated with similar implants were created 1 week (n = 3) and 2 weeks (n = 3) prior to sacrifice at 6 months. Ibandronate (0.15mg/kg IV) was given 1 month prior to surgery and was continued monthly. Histomorphometry was performed on non-decalcified sections. As was the case in the nonhuman primate study without bisphosphonate, the predominant cells at 1 week in the Buffer defects were osteoblast precursors and osteoblasts initiating osteoid formation at the defect rim. The BMP defects in both studies contained large numbers of monocyte/macrophages, osteoclasts, giant cells and osteoblast precursors. Although giant cells were degrading ACS in both studies, in contrast to the first study, osteoclasts did not appear to be initiating bone resorption in this study. At 2 weeks, there was bone formation at the defect rim in the Buffer limbs, with lit-

tle resorption of the ACS or surrounding trabecular bone in both studies. Despite the presence of large numbers of osteoclasts, there was minimal resorption of surrounding trabecular bone in the BMP defects. In the first study, there was considerable bone resorption at 2 weeks. Giant cell degradation of ACS was observed, and bone formation was initiating at the defect rim in both studies. At 6 months, trabecular bone volume (BV/TV) was greater in BMP than Buffer limbs ($p < 0.05$) within the defects (48.3 ± 9.8% vs 24.2 ± 11.7%) and surrounding trabecular bone (36.2 ± 5.1% vs 30.3 ± 4.0%) in the current study. Despite the initial resorption, BV/TV was greater in BMP than Buffer limbs within the defects (41.1 ± 13.9% vs 16.1 ± 12.6%) and surrounding trabecular bone (33.7 ± 8.9% vs 28.6 ± 6.5%) at the end of the first study ($p<0.05$). Combining the results of these two studies, there was a significant increase in BV/TV in BMP than Buffer limbs ($p< 0.05$). No difference in BV/TV was observed with or without bisphosphonate. This study indicates systemic bisphosphonate can be used to limit initial bone resorption without interfering with subsequent bone induction.

Disclosures: Genetics Institute,3.

M014

Partial Rescue of the Embryonic Lethal Phenotype of the BMP 4 Knockout with a 1.1 kb Proximal BMP 4 Promoter Region. J. Q. Feng,[1] J. Zhang,*[2] M. Harris,*[3] X. Tan,*[4] Y. Pi,*[2] S. E. Harris.[3] [1]Oral Biology, School of Dentistry, Univ. of Missouri-Kansas City, Kansas City, MO, USA, [2]Oral Biology, School of Dentistry, University of Missouri-Kansas City, Kansas City, MO, USA, [3]Medicine/Endocrinology, UT Health Science Center at San Antonio, San Antonio, TX, USA, [4]School of Dentistry, University of Missouri-Kansas City, Kansas City, MO, USA.

BMP4 is a crucial signaling molecule for mouse embryonic development. Precise control of the level, as well as the spatial and temporal expression patterns of BMP4 is important for the normal development of a variety of tissues. Previously we have cloned the mouse Bmp4 gene, and generated transgenic mice carrying different fragments of the BMP 4 gene, driving reporter gene and human Bmp4 transgene in order to study function of BMP4 and its control mechanisms. In this study, we first analyzed expression patterns of transgenic mice harboring lacZ driven by murine BMP4 promoter fragments (0.26 kb, 1.1 kb and 2.4 kb) at different developmental stages and compared these patterns with the Bmp4 lacZ knocked-in mice in which the pattern represents expression from all control domains in the BMP 4 gene. At the 10.5-to-12.5-dpc developmental stages, the endogenous BMP4 is active in many tissues such as brain, somites, limbs, tooth, and hair follicles. However, the 1.1 proximal promoter is active mainly in somites and neural tissues. The expression domains for BMP4 expression in the rest of primordial tissues are not present in this promoter region. At the later developmental stage (13.5 to neonates), the 1.1 kb promoter can control BMP4 expression in osteoblasts, ameloblasts, hair shaft and matrix, but not in mesodermal derivatives such as dental pulp cells, odontoblasts, and dermal papilla. Bmp4 null mutants die in utero before embryonic day 9.5 (between E7.5 to E9) due to a failure in extraembryonic mesodermal communication to several areas of the early embryo. We then tested if crossing the mice bearing the human Bmp4 transgene driven by this 1.1 kb promoter to null mutant mice could correct defects caused by absence of BMP4. Our initial data demonstrate that partial rescue of development can be seen up to 11.5 dpc in the null mutant embryos carrying the human BMP4 transgene directed by the 1.1 kb proximal promoter. Based on above observations, we proposed that 1) the 1.1kb BMP 4 proximal promoter contains DNA elements that control expression of BMP 4 very early in development at the 7.5 to 11.5 dpc stage; 2) the 1.1 kb BMP 4 proximal promoter is missing a large sets of control regions required for BMP4 expression.

M015

Complete Healing of Critical Size Rat Calvarial Defect by Stromal Cells Transduced with BMP-4 Retroviral Vector. R. Gysin, J. E. Wergedal, M. H. C. Sheng, Y. Kasukawa, N. Miyakoshi, S. T. Chen,* H. Peng,* K. H. W. Lau, S. Mohan, D. J. Baylink. Musculoskeletal Disease Center, J.L. Pettis VA Memorial Center and Loma Linda Univ., Loma Linda, CA, USA.

In order to develop an effective and safe gene therapy system for the healing of bone defects, we developed a retroviral vector system with high transduction efficiency, namely an MLV-based vector expressing the human BMP-4 transgene regulated by a LTR promoter. Syngenic rat stromal cells were transduced with this vector system at an efficiency of > 60%. BMP-4 production *in vitro* was verified by Western blot analysis. The bone formation potential of BMP-4 transduced cells was tested by embedding $2.5x10^6$ stromal cells in a gelatin matrix that was then placed in a critical size defect in rat calvariae. Two control groups were used: 1) gelatin matrix without cells; and 2) gelatin matrix with untransduced stromal cells. The defect area was completely filled with new bone in experimental rats after 4 weeks, while no significant bone formation occurred in either control group. Bone mineral density (BMD) of the defect as assessed by DEXA of the gene therapy group was 119±10% (Mean±SD, n=4) of the control BMD surrounding the defect; whereas BMD of rats implanted with gelatin carrier was 6-fold lower (20±16%) compared to rats implanted with cells expressing BMP-4 ($p<0.001$). Furthermore, implants of stromal cells without BMP transgene produced no significant increase in BMD compared to gelatin carrier alone (24±13%), thus suggesting that the increased BMD in the experimental group is due to the BMP-4 transgene. Histological analysis indicated that bone formation was initiated at multiple sites after gene therapy, whereas, in the controls, bone formation was limited to the edges of the defect and a thin layer along the dura. Prolonged exposure of stromal cells to BMP before implantation did not stimulate bone formation above that of controls. We therefore predict that the large amount of bone formation in the gene therapy group was not produced by the inoculated stromal cells but from the recruitment of new host osteoprogenitor cells. Time course studies revealed that there was a linear increase in BMD between 2-4 weeks after inoculation of the critical size defect with $2.5x10^6$ implanted cells expressing the BMP-4 transgene. Cells expressing human BMP-4 were detected by immunohistochemistry up to 2 weeks after implantation, thus suggesting that BMP-4 transgene is expressed at the defect site *in vivo*. In conclusion, the gene therapy system that we have developed has the potential for safely and effectively regenerating human skull bone defects and possibly other skeletal defects in the clinical arena.

M016

Vascular Endothelial Growth Factor Isoform VEGF165 Stimulates Bone Morphogenetic Protein-2 Expression in Human Osteoblast-like Cell Lines. T. Nakamura,[1] M. Yamamoto,[1] K. Suthin,*[1] S. Tatsuyama,*[2] K. Tomita,*[2] K. Matsushita,*[2] Y. Izumi.*[1] [1]Department of Periodontology, Kagoshima University Dental School, Kagoshima, Japan, [2]Department of Operative Dentistry and Endodontology, Kagoshima University Dental School, Kagoshima, Japan.

Vascular endothelial growth factor (VEGF) is known to play a pivotal role in angiogenesis. Recently, VEGF was reported to participate in endochondral bone formation, in which vascular formation is essential step for recruitment of osteoclast and osteoblast. In addition, it was clarified that VEGF has a function in osteoclastogenesis, similar to M-CSF. Thus, VEGF is recognized to be indispensable factor in bone metabolism. However, VEGF function in osteoblast is less elucidated comparing to in osteoclastogenesis. The purpose of this study is to identify the effect of VEGF on cellular phenomenon and gene expression in cultured human osteoblast-like cell line, MG-63 and Saos-2. To examine if VEGF affects on osteoblast phenomenon, recombinant human VEGF isoform 165 or 121 (VEGF165, VEGF121) were added to cell culture system of MG-63. The cell was stained for alkaline phosphatase after 2 or 4 days of culture periods with VEGF165 (100ng/ml) or VEGF121 (100ng/ml), showing increased alkaline phosphatase activity in MG-63 cultured with VEGF165 for 4days, not increased with VEGF121. In order to detect changing mRNA expression levels in osteoblast-like cells stimulated with VEGF, total RNA was isolated from cells stimulated for 0, 4, 8, 12, 24, and 48 hours. Semi-quantified RT-PCR was performed to compare transcription levels correlating to osteoblast differentiation such as bmp-2, bmp-4, bmp receptor IA, bmp receptor IB, bmp receptor II, osteocalcin and house keeping gene GAPDH. In MG-63 stimulated with VEGF165, bmp-2 level at 12 hours was increased 6.3-fold above at 0 hour, whereas no up-regulation was seen in cell with VEGF121. Bmp-2 expression levels were slightly elevated in saos-2 cell line stimulated with VEGF165 for 48 hours (1.9-fold) and in the same cell with VEGF121 for 4 hours (1.9-fold). Transcription levels of bmp receptor IB and osteocalcin were raised in MG-63 with VEGF165, showing 1.9-fold at 4 hours and 2.3-fold at 8 hours, respectively. On the other hand, bmp-4, bmp receptor IA and receptor II expressions were not altered or slightly decreased in MG-63 with VEGF165. In conclusion, these data suggest that VEGF165 promotes osteoblast differentiation through enhancing alkaline phosphatase activity, bmp-2, bmp receptor IB, and osteocalcin in osteoblast, and may be possible to be involved in osteoblast differentiation.

M017

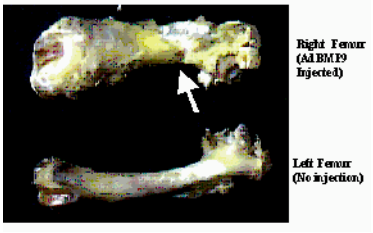
Protein Kinase C mu Mediates BMP-2 Induced Activation of p38 and JNK which are Essential Downstream Effectors for Osteoblastic Cell Differentiation. J. Lemonnier,*[1] J. Guicheux,*[2] G. Palmer,[1] J. P. Bonjour,[1] J. Caverzasio.[1] [1]Division of Bone Diseases, University Hospital of Geneva, Geneva, Switzerland, [2]INSERM 99-03, School of Dental Surgery, Nantes, France.

BMP-2 exerts a wide range of biological effects in bone forming cells, most of which involve the activation of the SMAD pathway. Cooperative interactions between the SMAD and the mitogen-activated protein kinases p38 and JNK pathways have recently been observed by members of the TGFb family. A recent observation indicates that BMP-2 induces activation of p38 and JNK in osteoblast-like cells. However, the cellular mechanisms by which BMP-2 activates p38 and JNK are not known and the role of these pathways in BMP-2 induced cell differentiation remains unclear. We adressed these two questions in MC3T3-E1 (E1) preosteoblastic cells using immunoprecipitation with specific antibodies and an in vitro kinase assay for assessing p38 and JNK activities. Osteoblastic cell differentiation was determined by measurements of mRNA levels of alkaline phosphatase (ALP) and type I collagen (coll I) as well as ALP activity and coll I deposition. In confluent cells, BMP-2 (50 ng/ml, 3h) induced a 23-25 fold stimulation of p38 and JNK kinases. Pretreatment of E1 cells for 24 h with 1 µM PMA did not influence the effect of BMP-2 on p38 and JNK activation suggesting that conventional PKC isoforms are not involved in this response. In contrast, the selective PKCmu inhibitor G06976 (10 µM) completely blocked activation of p38 and JNK induced by BMP-2 whereas this agent did not influence BMP-2 induced SMAD1,5 phosphorylation. Of interest, BMP-2 enhanced the formation of phosphorylated Ser-916 PKCmu (activated form) with a maximal effect at 3 h (3.3 x) and this response was completely prevented by G06976. In addition, the time-dependent stimulation of PKCmu precisely correlated with p38 and JNK activation induced by BMP-2. It was detected after 0.5-1 h exposure and maximally expressed after 2-3h. G06976 not only blocked activation of p38 and JNK but also completely inhibited the increase in mRNA levels of ALP and coll I as well as the enhanced ALP activity and coll I deposition induced by BMP-2. In conclusion, data presented in this study describe a new SMAD-independent signaling pathway activated by BMP-2 in osteoblastic cells. This pathway involves a PKCmu-dependent activation of the MAP kinases p38 and JNK which, in addition to SMADs, appears to be essential for the effect of BMP-2 on osteoblastic cell differentiation.

M018

Development of a Intra-Femoral Gene Delivery Model in the Rat Using Recombinant Human Adeno-BMP9 Virus. B. M. Bhat,[1] V. E. Coleburn,*[1] V. L. Dell,*[1] L. Borella,[1] Y. P. Kharode,[1] D. D. Pittman,*[2] F. J. Bex.[1] [1]Bone Metabolism and Osteoporosis Research, Women's Health, Wyeth-Ayerst Research, Radnor, PA, USA, [2]Genetics Institute, Andover, MA, USA.

Bone marrow provides a unique microenvironment to study the effect of a given purified protein or a gene of interest directly on bone remodeling in vivo. Effective gene delivery to the bone marrow is a challenging process. Since Adenovirus can be purified as a highly concentrated stock and can infect a variety of mammalian cells in vitro or in vivo, it provides an excellent viral gene delivery vector system. In this study, recombinant Adeno-BMP9 was injected into the femoral bone to evaluate the potential of this approach as an in vivo model for assessing candidate genes. The right femur of ten male mature Sprague-Dawley rats per group were injected intra-osseously with 25ul of CsCl gradient purified Adeno-BMP9 virus (1x 10 12 virus particles/ml). Rats receiving inactivated control virus were included as controls. Three weeks after an injection of a single dose of virus, both right (injected) and left femurs were isolated and analyzed by pDEXA. The right femur injected with active AdenoBMP9 virus showed a dramatic increase in the overall size. This was reflected by significant increase in whole femur BMC. The mean BMC value of right femurs injected with the active virus was 0.441 while that of uninjected left femur was 0.396 ($p < 0.05$). Interestingly there was no significant increase in BMD values. Further analysis of the bones by histology will hopefully shed light on the nature of the dynamics of bone changes induced by BMP9. Nonetheless, this experiment demonstrated that one can introduce active AdenoBMP9-like recombinant directly into the bone marrow and the Adenovirus can infect the cells of the marrow cavity. This in turn can lead to the expression of a recombinant gene that can induce a generalized osteogenic effect on the entire femur and not just at the injected region. The absence of osteogenic induction in the left femur of the same animal indicates that a majority of the virus infection was contained within the marrow cavity of the injected femur.



M019

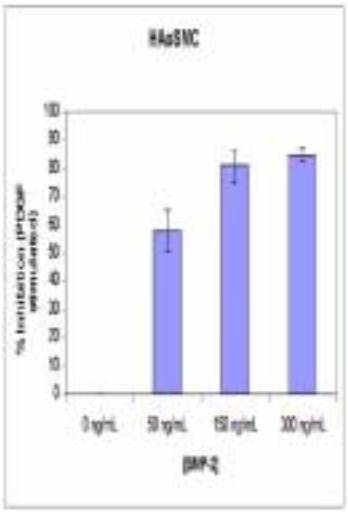
The Downstream of Smad1 Is the Key Target of the Inhibitory Effect of Smad7. T. Katagiri, M. Imada,* T. Suda, N. Takahashi, R. Kamijo. Biochemistry, Showa University School of Dentistry, Tokyo, Japan.

BMP-2 inhibits myogenic differentiation of C2C12 myoblasts and induces their differentiation into osteoblasts. Type I receptors for BMP-2 phosphorylate two serine residues of Smad1/5/8 at their carboxy terminals (SVS motif). These phosphorylated Smads form oligomer complexes with Smad4, move into nuclei, and activate transcription of their target genes. Smad7 has been reported to inhibit the signals of BMP, TGF-beta and activin by stably interacting with their type I receptors and therefore inhibiting phosphorylation of Smads. In this study, we examined the molecular mechanism of osteoblastic differentiation of C2C12 cells and of the inhibitory effect of Smad7 using constitutively active Smad1.Mutant Smad1(DVD) and Smad1(AVA) expression vectors were constructed by replacing two serine residues in the SVS motif of Smad1 with aspartic acid residues and alanine residues, respectively. These mutants and the wild type Smad1 were cotransfected with Smad4 in C2C12 myoblasts, C3H10T1/2 fibroblasts, and ST-2 bone marrow stromal cells. In the presence of Smad4, both wild type Smad1 and Smad1(DVD) but not Smad1(AVA) induced a number of ALP-positive cells in the cultures. Transfection of Smad1(DVD) alone also induced ALP-positive cells in the cultures. We also constructed IdWT4F-luc reporter plasmid, which had 4 copies of the Smad1/Smad4-binding element identified in the human Id1 promoter. Although both wild type Smad1 and Smad1(DVD) activated the luc activity when they were cotransfected with Smad4, they failed to activate the luc activity in the Smad4-deficient MDA-MB468 breast cancer cells. Cotransfection of Smad7 suppressed the induction of ALP activity by Smad1(DVD) in C2C12 cells. Transfection of increasing amounts of Smad4 reduced the inhibitory effect of Smad7 on the ALP-inducing activity of Smad1(DVD) in a dose-dependent manner. Taking together, we conclude that Smad1(DVD) as a constitutively active form of Smad1 is capable of inducing osteoblast differentiation independent of the BMP receptor activation. Since Smad7 inhibited the Smad1(DVD) activity, we propose that the downstream of Smad1, rather than BMP receptor-Smad interaction, is the key target of the inhibitory effect of Smad7.

M020

Bone Morphogenetic Protein-2 Is Expressed by Atherosclerotic Aortic Tissue and Is Associated with Decreased Vascular Smooth Muscle Cell Proliferation. G. A. Wong,[1] V. Tang,*[1] D. R. Haudenschild,*[2] R. H. Weiss,*[3] L. Howard.[3] [1]Endocrinology, Clinical Nutrition and Vascular Medicine, UC Davis School of Medicine, Davis, CA, USA, [2]Orthopedic Surgery, UC Davis Medical Center, Sacramento, CA, USA, [3]Nephrology, UC Davis School of Medicine, Davis, CA, USA.

Bone morphogenetic protein-2 (BMP2) has been shown to elicit wide ranging effects from osteogenic differentiation to apoptosis in a number of cell systems. The purpose of this study was to establish the presence of BMP2 expression in atherosclerotic aortic tissue, and to evaluate the effect of BMP2 on aortic smooth muscle cell proliferation.Total RNA was extracted from rat descending aorta exhibiting atheroma grossly, and subjected to quantitative RT-PCR TaqMan analysis for BMP2 expression after we designed primers and a fluorescently labeled probe for rat BMP2 mRNA. BMP2 message was detectable in rat male aorta, female aorta and heart and liver of both sexes. Detection was linear over wide concentration ranges using GAPDH as an internal control.Cultured rat and human aortic smooth muscle cells were rendered quiescent in serum-poor media, then stimulated to proliferate with 40ng/ml platelet derived growth factor (PDGF) in the presence of varying concentrations of human recombinant BMP2. Cell proliferation was quantitated by 3H-thymidine incorporation. Human recombinant BMP2 at 50ng/ml, 150ng/ml and 300ng/ml inhibited PDGF-induced human aortic smooth muscle cell proliferation by 58%, 81% and 85% respectively as compared to controls. Lower concentrations of BMP2 were associated with a mild stimulatory effect on PDGF-stimulated rat vascular SMCs, but a 44% decrease in PDGF-induced proliferation of rat vascular SMCs was seen with 300ng/ml BMP2. These results demonstrate that BMP2 is present in arterial tissue and may play an inhibitory role in vasculoproliferative processes.



M021

Attenuation of Dexamethasone Suppression of Osteocalcin Gene Expression by Bone Morphogenetic Protein-2. G. A. Wong. Endocrinology, Clinical Nutrition and Vascular Medicine, UC Davis School of Medicine, Davis, CA, USA.

The major mechanism of glucocorticoid-associated bone loss is thought be a derangement of osteoblast-mediated bone formation. Osteocalcin, a marker of terminal osteoblastic differentiation, is highly regulated at the transcription level and has been shown in mature osteoblasts to be downregulated by glucocorticoids and stimulated by vitamin D. We assessed the potential of bone morphogenetic factor-2 (BMP2) to protect against dexamethasone-mediated suppression of osteocalcin gene expression in vitro. Primary cultures of rat osteoblasts were prepared from 22-day pregnant Long Evans rats.Seventy-two hours prior to harvesting, human recombinant BMP2 at a concentration of 150ng/ml was added to designated cultures. Forty-eight hours prior to harvesting, cells were treated with 100 nM dexamethasone alone, 10nM 1,25dihydroxy vitamin D3 alone, 100nM dexamethasone plus 10nM 1,25dihydroxy vitamin D3, or 100 nM dexamethasone plus 10 nM 1,25dihydroxy vitamin D3 plus (i.e. continued incubation with) 150 ng/ml BMP2.Total cellular RNA was extracted and steady state levels of osteocalcin mRNA were measured by Northern blot analysis using GAPDH as a control.Osteocalcin mRNA levels were low in Day 8 osteoblasts prior to mineralization, but increased rapidly by Day 16. In all treatment groups osteocalcin mRNA levels were highest in Day 22 osteoblasts. 1,25dihydroxy vitamin D increased osteocalcin mRNA levels in Day 22 osteoblasts by 50%. Dexamethasone treatment was associated with decreased osteocalcin mRNA levels at Days 22 and 16 in both vitamin D treated and vitamin D untreated cells. The degree of suppression by dexamethasone in vitamin D treated cells was 75% at Day 16 and 55% at Day 22. BMP2 (150 ng/ml) partially abrogated the dexamethasone-related suppression of osteocalcin mRNA levels at Days 16 and 22 in vitamin D treated cells. When osteoblasts were pretreated with BMP2, osteocalcin message levels increased by 205% at Day 16 and by 59% at Day 22 over non-pretreated dexamethasone-suppressed cells. In summary, the osteoinductive protein, BMP2, appears to confer a significant protective effect from dexamethasone-mediated suppression of osteocalcin expression in vitamin-D stimulated osteoblasts.

M022

Roles of BMP-induced Transcription Factors, Msx2 and Dlx5, in Cranial Suture Development. H. Kim,*[1] M. Park,*[1] J. M. Wozney,[2] Y. Kim,*[3] M. Lee,*[3] S. Nam,*[1] Y. Kim,*[1] H. Ryoo.[3] [1]Pediatric Dentistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [2]Genetics Institute, Inc, Cambridge, MA, USA, [3]Biochemistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea.

Mutation in the homeobox gene Msx2 causes Boston-type human craniosynostosis and the phenotype of Dlx5 knockout mouse presents craniofacial abnormalities including a

delayed ossification of calvarial bone. Further, it has been known that transcriptional regulation of osteocalcin, a mature osteoblast marker, is reciprocally regulated by the homeodomain proteins MSX2 and DLX5. These results suggest a pivotal role of Msx2 and Dlx5 genes in the calvarial bone and suture devleopment. In order to elucidate the function of these molecules in the early morphogenesis of mouse calvarial sutures, we have first analyzed the expression of Msx2, Dlx5 and osteocalcin genes by in situ hybridization in developing parietal bones and sagittal suture of mouse calvaria during the embryonic stage (E15-E18). Msx2 mRNA was intensely expressed in the sutural mesenchyme, osteogenic fronts and mildly expressed in the dura mater during the embryonic stage. Dlx5 mRNA was intensely expressed osteogenic fronts and the periosteum of parietal bones. Osteocalcin mRNA was found in the periosteum of parietal bones from E15, and gradually more highly expressed with cranial bone development. To further examine the upstream signaling molecules regulating Msx2 and Dlx5 genes, we have done ex vivo organ culture experiments with E15.5 mouse calvarial explants. Msx2 and Dlx5 expression was strongly induced around the BMP2- or BMP4-soaked beads after 48 h of the application of the beads onto the osteogenic fronts. On the other hand, application of beads soaked with TGF-beta1, GDF-5, -6, -7, FGF-2, -4 and Shh could not induce the expression of Msx2 and Dlx5. Taken together, these data indicate that Msx2 and Dlx5 play central roles in the osteogenesis of calvarial bones and the maintenance of cranial sutures in response to BMP signaling. Furthermore, the different expression patterns between Msx2 and Dlx5 suggest their specific functions in the osteoblast differentiation, ie, role of Msx2 is in the earlier stage of osteogenesis of calvarial bone development and maintenance of cranial sutures while that of Dlx5 is in the later stage of osteoblast differentiation and obliteration of the sutures.

M023

BMP-Induced Osteogenesis Is Impaired in Human Marrow Stromal Cells. D. L. Diefenderfer,* P. S. Leboy. Biochemistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA.

The relatively poor induction of osteogenesis in human marrow stromal cells by BMP is in marked contrast to its potent osteogenic effect in rat or mouse stromal cells. We are attempting to understand why this phenomenon exists. Human stromal cells were isolated from femoral marrow obtained during total hip replacement surgeries. The marrow was washed to remove the fatty fraction and nucleated cells were concentrated on Ficoll-Paque® (Amersham Pharmacia). The nucleated cell fraction was cultured for the isolation of adherent stromal cells either in the presence or absence of dexamethasone (dex; 10^{-7} M). First passage cultures supplemented with ascorbate phosphate were used for osteogenic studies. A portion of these first passage cultures were supplemented with the osteogenic inducers BMP-2 (100ng/ml) or dex (10^{-7} M). The osteogenic response was determined by alkaline phosphatase (AP) activity assay and RT-PCR for osteogenic markers. In first passage cultures derived from dex-treated primaries, BMP-2 significantly increased AP activity compared to that seen in BMP-treated first passage cultures derived from primaries not treated with dex ($p = 0.01$). BMP-2 actually inhibited AP activity in most first passage cultures derived from untreated primaries isolated from female patients. Interestingly, RT-PCR analyses indicated that BMP-2 consistently induced both its own message and noggin, irrespective of the osteogenic response. We conclude that while BMP signaling is functional in human marrow stromal cells, it is relatively ineffective in inducing osteogenesis in the absence of dex pre-treatment.

M024

Static and Dynamic Histomorphometry of Iliac Crest Biopsies from 46 Normal Women. D. E. Jewison,*[1] J. A. Burgess,*[1] C. M. Rowland,*[2] D. M. Ilstrup,*[2] J. D. Sibonga.[1] [1]Orthopedic Research, Mayo Clinic, Rochester, MN, USA, [2]Biostatistics, Mayo Clinic, Rochester, MN, USA.

Archived specimens of transiliac crest biopsies from normal, healthy women were pooled from 4 different Mayo Clinic IRB-approved studies. Normal values for static and dynamic histomorphometry were generated from a total of 46 women. Biopsies were obtained from 13 premenopausal (30-45 years) and 33 postmenopausal women (50-63 years). All subjects had received tetracycline over a 13±2 day labeling period for fluorochrome-based estimations of bone formation/mineralization. Histomorphometry was performed with the OsteoMeasure analysis system (OsteoMetrics Inc., Atlanta, GA) which employs a digitizing mouse and graphics tablet to trace bone perimeters and fluorescent labels on an image of the biopsy section transmitted to a video monitor. Primary and derived data are generated by the software in accordance with standardized nomenclature and formulae (Parfitt et al. J Bone Miner Res 2(6):595, 1987). The total cancellous bone tissue area that was measured ranged between 11 and 68 mm2; total cancellous bone perimeter measured ranged between 30-161 mm. Data for the common structural, dynamic, perimeter-based and cellular-based histomorphometry were not normally distributed. The mean, median, range and the 2.5 and 97.5 percentile limits will be reported. Distributions that varied significantly with age were noted: osteoid width, osteoid maturation time and the percent of eroded surface all negatively correlated with age; there were positive correlations for osteoclast # per 100 mm of bone surface and for the percent of osteoid surface. This database of normal values is distinct from other similar reports in that biopsies were obtained from healthy subjects, measurements were tetracyline-based, and 67% of the subjects were postmenopausal. In conclusion, we can report mean values for histomorphometry parameters of iliac crest biopsies from 46 normal women. This database from both pre- and postmenopausal aged women represents normative values from the largest set of healthy women recruited specifically for this purpose.

M025

The Relationship of Physical Fitness at Adolescence to Adult Bone Mineral Density. R. A. Faulkner,* R. L. Mirwald,* A. Baxter-Jones,* D. A. Bailey.* University of Saskatchewan, Saskatoon, SK, Canada.

Several studies have reported a positive relationship between physical activity during childhood and adolescence on adult bone mineral density (BMD). Most of these data, however, involve retrospective assessment of physical activity, and there is little data on the impact of physical fitness during adolescence on adult BMD. In addition, accurate assessment of physical activity is problematic in children. Physical fitness, however, is an objective measurement that may be a better indicator of overall physical activity than subjective measures. The purpose of this study was to investigate the relationship of physical fitness during adolescence on adult total body BMD. Subjects were from the Saskatchewan Growth and Development Study (SGDS). This longitudinal (boys) and mixed-longitudinal (girls) study involved yearly physiological, and anthropometric measurements between 1964-1973. Subjects from the original study were contacted for follow-up assessments between 1997-1998 – when their age range was about 38-40 years (males, n= 58; females, n=31). In addition to the original physiological tests, DXA bone scans (Hologic 2000, array mode) were also done at follow-up. Physical fitness was determined from a composite score of aerobic power, muscular strength and endurance and body composition measurements done yearly over the growing years and at follow-up. The composite fitness scores were then converted to T-scores. Peak height velocity (PHV) was used as the biological landmark to control for maturity and growth effects on physical fitness. The fitness scores for the years surrounding PHV (3- 4 years depending on subject) were averaged to construct one overall fitness score for each subject. The relationship of physical fitness at adolescence to adult BMD was determined using partial-correlation controlling for adult weight and physical fitness scores. In girls, results showed a positive and significant relationship ($r=.51$; $p<.005$) between physical fitness at adolescence and adult BMD; but there was no relationship found in boys ($r=.13$; $p<.32$). The results suggest that there may be gender differences in the relationship between adolescent physical fitness and adult BMD; however, there may also be factors such as study design or other variables affecting the relationship. Further investigation thus is warranted in order to determine why a relationship was found in girls but not boys.

M026

Analysis of Pyrrolic Crosslinks in Bone Collagen Using a Biotinylated Ehrlich's Reagent. J. D. Brady,* S. P. Robins. Rowett Research Institute, Aberdeen, United Kingdom.

Stabilization of bone collagen through lysyl oxidase-mediated crosslinking results in the formation during maturation of both pyridinium and pyrrolic bonds. The pyridinium compounds, pyridinoline (PYD) and deoxypyridinoline (DPD), have been well characterized but only recently (1) have suitable reagents been developed to allow isolation and identification of corresponding pyrrolic crosslinks, pyrrololine (PYL) and deoxypyrrololine (DPL). The aim of this study was to utilize a novel biotinylated reagent to quantify pyrrolic crosslinks in bone and other tissues and to assess the changes that occur with age.Decalcified (EDTA) bone, cartilage or skin samples were solubilized with trypsin following brief heat denaturation. Collagen content of each digest was estimated by hydroxyproline analysis. The peptides produced were immobilized through covalent attachment of their free amino groups using succinimide-activated microtiter plates. The plate was treated with a biotinylated Ehrlich reagent (1) in acid solution which allows reaction with the pyrrole-containing peptides: the biotin was detected using streptavidin peroxidase. The amounts of pyrrole crosslink in the sample was assessed from a standard curve constructed using phorphobilinogen, a model amine-containing pyrrole, attached to the plate according to the same reaction scheme.The results for bone digests showed a linear response with concentrations up to about 5 μM collagen, with curves parallel to those generated with phorphobilinogen. Digests of both cartilage and skin showed no reaction above background, indicating good specificity for pyrrolic crosslinks. This was corroborated by the finding of negligible pyrrolic crosslink concentrations in digests of bone from a patient lacking telopeptide lysyl hydroxylase activity (2). The concentrations of pyrrole crosslinks in bone from children were about 10-fold higher than those in adult bone and analysis of a range of ages (n=12) indicated a rapid decline in the second decade, with a relatively consistent concentration after 20-years-old (about 2 mM porphobilinogen equivalents per μM collagen). We conclude that the assay using biotinylated Ehrlich's reagent provides a specific assessment of the relative concentrations of pyrrolic crosslinks in collagenous tissues. The results confirm the absence of these crosslinks in collagen from skin and cartilage. The changes with age in bone collagen suggest more complex processes than might be expected from crosslink maturation alone, and the possibility of further modification of pyrrole crosslinks with age cannot be excluded.(1) Brady and Robins (March 14, 2001) J. Biol. Chem. 10.1074/jbc.M009506200(2) Bank et al (1999) PNAS 96, 1054-1058

M027

FTIR Microspectroscopic Imaging analysis of Critical Sized Defects healed using Composite Allografts in the Rabbit Ulna Model. C. Abjornson,* E. Paschalis, E. Tomin,* E. DiCarlo,* J. M. Lane. Hospital for Special Surgery, New York, NY, USA.

Earlier formulations of demineralized bone matrix(DBM)had the limitation of offering no structural support, however with the addition of segments of bone, composite allografts with structural support can be acheived. The purpose of this study was to investigate the quality and quantity of the new bone formed in the fracture callus at given locations using FTIR-I. The hypothesis was that processed allograft materials has superior new bone formation and more advanced healing properties than the classic standard, ABG, in a critical sized segmental model.Twenty-five 6 month old (3.5-4.0 kg) male New Zealand white rabbits were used in this study. The animals underwent surgery to create bilateral 2 cm ulnar

defects. Two defects were left empty to prove the defect to be of critical size. The 48 remaining defects were randomly assigned one of the following treatments; grafting with cortico-cancellous morcelized bone harvested from the iliac crest (ABG), DBM fibers combined with demineralized cortical bone segment (1.7-2.8mm in diameter) (DBM-Dcort), DBM fibers with non-demineralized cortical bone segments(DBM-cort), DBM Putty with non-demineralized cortico-cancellous bone segments (DBM-C/C). A 1 cc volume of graft material was utilized in all defects. All animals were sacrificed at 12 weeks.FTIR-I analysis was performed on 2 micron thick histological sections. Infrared vibrations of both the mineral phase and the matrix phase can be monitored simultaneously via different representative peaks. The defects that received DBM-Dcort histologically showed the most newly deposited bone with normal mineral to matrix ratios. FTIR-I analysis of this group showed high correspondence between the areas of new mineral and collagen content. The DBM-cort group also displayed new bone formation but was not able to maintain its three dimensional space. The was confirmed by the FTIR-I results which revealed an almost equivalent amount of formation and resorption occuring with formation slightly ahead. In the third allograft group, DBM-C/C, both histologic and FTIR-I results showed new bone formation but like the DBM-cort, there was still considerable resorption occuring. The last group, ABG, showed the most resorptive activity with large areas of unresorbed dead bone from the graft. ABG had the least new bone formation at the center of the defect. Unlike radiographs, FTIR-I allows the investigator to differentiate the quality of the bone. A fully demineralized composite material has the advantage of being able to begin anabolic healing as soon as it implanted unlike the other materials which must undergo varying degrees of resorption activities as well as formation.

Disclosures: Osteotech, Inc.,2.

M028

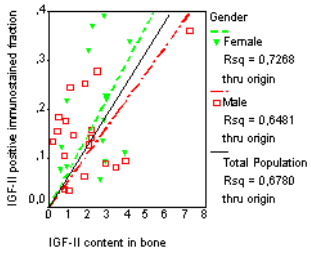
Direct Measurement of Osteocalcin mRNA in UMR106 Cells Via Branched DNA. H. A. Bullock,* K. Bramlett,* T. P. Burris,* A. G. Geiser, V. Krishnan. Gene Regulation, Bone, and Inflammation, Eli Lilly and Company, Indianapolis, IN, USA.

We have developed a high throughput assay for the direct measurement of osteocalcin mRNA in a rat osteoblast cell line. Osteocalcin (OCN) is a specific marker for osteoblast activity. Unlike traditional methods of mRNA quantification such as RT-PCR, branched DNA (bDNA) does not measure amplified target but instead measures message directly through a series of hybridization reactions and subsequent luminescent signal amplification similar to an ELISA. B-DNA also obviates the need for cumbersome RNA extraction that is inherent in most traditional methods. Probes were designed specific to rat osteocalcin mRNA using the ProbeDesigner software (Bayer Diagnostics). UMR 106.1 cells were seeded into 96-well tissue culture plates and treated with PTH (1-38) at various concentrations. Twenty-four hours post treatment, the samples were assayed for the presence of osteocalcin message through bDNA using the Quantigene High Volume Kit (Bayer Diagnostics). An 8-10 fold increase in OCN mRNA was observed after 24 hrs of PTH (1-38) treatment. The dose response and the kinetics of PTH (1-38) indicate that maximal increase in OCN mRNA is observed in 24 hrs using 10 nM PTH. In conclusion, we have successfully developed a novel high throughput method to directly measure OCN mRNA in cell culture.

M029

Quantitative Image Analysis of IGF-II Immunostained Bone Biopsies Can Determine In Vivo IGF-II Content. T. Andersen,*[1] L. Sørensen,*[1] M. Bünger,*[1] A. Baatrup,*[1] M. Lind,*[2] C. Bünger,*[2] E. F. Eriksen.[1] [1]Dept. of Endocrinology and Metabolism, Aarhus University Hospital, Aarhus C, Denmark, [2]Orthopaedic Research Lab., Aarhus University Hospital, Aarhus C, Denmark.

Quantitative image analysis has been used in bone research, but none has determined whether a measured positive fraction reflects real protein content. We investigated the relation between IGF-II content in trabecular bone sections determined by image analysis and IGF-II content in whole bone determined by ELISA.34 spine surgery patients (19 males and 15 females, mean age 40, range 13-76) had two 8 mm unicortical bone biopsies taken from the posterior iliac crest prior to graft harvesting. Biopsy 1 was stored in 70% alcohol, embedded undecalcified in MMA by a modified Erben procedure. One section was stained for IGF-II and analysed with a quantitative image analysis system which uses an Olympus BH-2 microscope and a Sony XC-003P 3CCD colour camera. 10 images from one section were used. They were processed with histogram stretch and increase 20% on green in Paint Shop Pro 6.0 and thresholded with the colour threshold function in SigmaScan Pro 4.0. Biopsy 2 was stored in liquid N2 until RNA isolation was performed for other purposes using Trizol in accordance to the manufacturers protocol. Proteins were isolated from the protein-trizol suspension by precipitation with acetone. The precipitated protein pellet was redissolved in demineralised water by sonication. IGF-II content was measured as free + bound IGF-II using an Active Non-extraction ELISA kit (DSL Inc.). IGF-II content was calculated as ng IGF-II/biopsy weight in g, or adjusted for the total protein content (ng IGF-II/mg protein) measured with the Lowry method.IGF-II content in bone sections determined with image analysis correlated well IGF-II content in whole bone determined by ELISA, both when calculated as fraction of biopsy weight, r2=0.68, p<0.001 (se figure) and when adjusted for total protein content r2=0.73, p<0.001. No significant difference between genders where seen in either of the two groups.In conclusion IGF-II content in bone matrix determined from immunostained sections of bone biopsies reflects the in vivo protein content. This is likely to be the case for other growth factors and proteins in bone matrix. Thus, quantitative image analysis can be a used as a tool for matrix protein measurements in bone research.



M030

Visualization of Osteogenesis Imperfecta Collagen Fibers Using Second Harmonic Generation. D. A. Redford-Badwal,*[1] M. Stover,*[2] P. J. Campagnola,*[3] A. Millard,*[3] D. W. Rowe.[2] [1]Pediatric Dentistry, MC 1610, University of Connecticut Health Center, Farmington, CT, USA, [2]Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA, [3]Physiology, University of Connecticut Health Center, Farmington, CT, USA.

Osteogenesis imperfecta (OI) is a heterogeneous disorder of bone where the severity depends on the molecular defect in type I collagen. The defect results in either the underproduction of normal collagen or the incorporation of an abnormal protein product within the developing collagen trimer producing a protein structurally inferior to the normal collagen fiber. However, it has been difficult to predict severity of clinical outcome based on the underlying genetic mutation probably because it does not translate directly into the quantity and quality of extracellular collagen matrix. The visualization of the abnormal protein in the extracellular matrix has not been easily performed but a recent technique called second harmonic generation (SHG) makes this possible. SHG is a form of non-linear excitation that has been adapted for high resolution imaging on confocal-like timescales for data acquisition. In this process, a sample is excited with a near infrared laser line, and subsequently a wave is emitted precisely at the second harmonic frequency. The use of this optical method confines excitation to the plane of focus and thus provides intrinsic 3 dimensionality. We have utilized this technique to image murine tissues and found that collagen fibers in the interstitial matrix generated an extremely strong signal. This study was initiated to evaluate this technique for imaging the extracellular matrix in human fibroblasts from normal/OI individuals with characterized mutations. We examined fibroblasts grown to confluence in tissue culture from patients with characterized mutations. Fibroblasts making normal collagen fibers demonstrate a mesh of fibers with cell bodies visible as holes within this cross-matrix. Fibroblasts from an individual with a lethal form of OI show scant, delicate looking fibers. Fibroblasts from an individual with a moderate form of OI demonstrate uniformly directional fibers with fewer cell bodies visible within the matrix. Fibroblasts from an individual having mild OI show areas of the collagen fibers, which look more like the normal, however there also appears to be areas with less cross-matrix as well. This examination allowed for a quantitative and qualitative evaluation of the matrix produced by living cells without destroying the integrity of the cells and matrix examined. SHG offers a novel method of examining the fibers made from mutations within the collagen genes, which may be predictive of disease severity.

M031

Expression of the Col1a1 Promoter/Green Fluorescent Protein Transgenes During Odontoblast Differentiation. A. Braut,* I. Kalajzic,* Z. Kalajzic,* E. J. Kollar,* D. W. Rowe, M. Mina. University of Connecticut Health Center, Farmington, CT, USA.

Type I collagen is the major structural protein of many tissues including bone and dentin. In developing teeth, dentin is formed by odontoblasts which are post-mitotic polarized cells. After teeth are fully formed, the odontoblasts continue to form secondary dentin. In addition, reparative/reactionary dentin is formed at specific sites in response to injury. Mild stimuli lead to formation of reactionary dentin by surviving odontoblasts. Strong noxious stimuli leading to destruction of odontoblasts are followed by formation of reparative dentin by new odontoblast-like cells. Although there is evidence suggesting that new odontoblast-like cells originate from a progenitor population that resides within the pulp tissue, the exact origin of these cells is not known. The long term objective of our studies is to be able to directly examine the origin of these progenitor cells. Toward this goal, we have examined the temporal and spatial pattern of expression of a Green Fluorescent Protein (GFP) in developing teeth and supporting tissues (E13, E18, 1-13 days postnatal) of transgenic mouse lines that carry a GFP reporter gene fused to 3.6 kb or 2.3 kb segments of the rat Col1a1 promoter (pOBCol3.6 GFP and pOBCol2.3 GFP). Our results show that GFP expression directed by both constructs is not detected in the dental papilla at early stages of odontogenesis (E13) or in non-polarized odontoblasts (E18 and 1-day-old tooth germs). Strong GFP expression directed by the 3.6 (but not the 2.3) construct is first detected in condensing preosteogenic mesenchyme of the mandible (E13), and in odontoblasts located at the tips of the developing cusps of the mandibular first molar in late bell staged tooth germ (E18). As development progresses strong expression of both Col 3.6 GFP and Col 2.3 GFP appears in osteoblasts, osteocytes, and in polarized odontoblasts. These observations indicate that the 3.6 kb segment directs the expression of GFP in both differentiating and differentiated odontoblasts and osteoblasts, while the 2.3 kb segment directs the expression of GFP only in differentiated odontoblasts and osteoblasts. These observations suggest that different populations of cells within the odontoblast and osteoblast lineage can be distinguished based on the pattern of transgene expression of these two rat promoters. Therefore, cells from these transgenic animals can provide a powerful tool for determining the origin

of odontoblasts giving rise to new or ectopic dentin in various experimental settings. Supported by NIH grant DE13363.

M032

Extracellular pH Determines Extent of Mineralization by Human Osteoblasts. M. van Driel,* M. Koedam,* C. J. Buurman,* H. A. P. Pols, J. P. T. van Leeuwen. Internal Medicine, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands.

Metabolic acidosis induces the loss of bone calcium and retardation in growth, either via a direct effect on the dissolution of bone mineral or via a stimulation of osteoclast-mediated bone resorption and an inhibition of osteoblast-mediated bone formation. On the other hand, an alkali-rich diet stimulates bone formation, and decreases the rate of bone resorption resulting in an improved mineral balance in osteoporosis patients.To study the significance of extracellular pH on osteoblast differentiation and action, we cultured the in vitro differentiating human osteoblastic cell line SV-HFO in media with three different pHs: pH 7.2, 7.5 or 7.7.Under normal culture conditions (pH 7.5), these cells proceed through different stages of osteoblast development, reflected by the interrelated synthesis of extracellular matrix proteins, allkaline phosphatase activity and mineralization. During the first week of culture, osteocalcin production is maximal, in the second week the collagen I expression and the alkaline phosphatase activity reach a peak level and at the same time mineralization starts, which progressively increases during the third week of culture. When these cells are cultured in either acidic (pH 7.2) or alkaline medium (pH 7.7), data so far don't show differences in the expression of matrix proteins osteocalcin and collagen I, alkaline phosphatase activity and DNA content during differentiation. However, in acidic medium mineralization of the extracellular matrix was significantly reduced, while in alkaline medium a significant increase was found.The magnitude of the 1,25(OH)2D3 (10-8 M) stimulation of mineralization by the SV-HFO cells was also strongly dependent on the extracellular pH, with the lowest stimulation at pH 7.2 and the highest stimulation at pH 7.7.In conclusion, this study shows that only small differences in extracellular pH within a clinical meaningful range dramatically affect mineralization by human osteoblasts, and also have an effect on hormonal regulation of this mineralization. This demonstrates that changes in bone metabolism observed in metabolic acidosis may be partly due to direct effects of pH on osteoblast function. Finally, it provides new insights into the control of hormone action in bone formation.

M033

Vitamin K Insufficiency Is a Risk Factor of Aortic Calcification. M. Miyao,[1] M. Shiraki,[2] Y. Shiraki,[2] T. Aoki,[2] S. Ogawa,[3] T. Hosoi,[4] S. Inoue,[3] Y. Ouchi.*[3] [1]Division of Endocrinology and Metabolism, Kanto Central Hospital, Tokyo, Japan, [2]Research Institute and Practice for Involutional Diseases, Nagano, Japan, [3]Department of Geriatric Medicine, Univ. of Tokyo, Tokyo, Japan, [4]Endocrinology section, Tokyo Metropolutan Geriatric Center, Tokyo, Japan.

Aortic calcification was considered as a passive terminal result of arteriosclerosis. However, recent studies showed that the calcification caused many severe clinical complications. Furthermore, several regulatory factors in bone metabolisms were found in artheriosclerotic plaque, such as bone morphogenetic protein2a, parathyroid hormone (PTH), osteopontin, osteocalcin (OC), matrix gla protein(MGP) and so on. OC and MGP are vitamin K (Vit.K) dependent proteins. MGP-deficient mice exhibit inappropriate calcification and die within two months as a result of arterial calcification, which leads to vessel rupture. Those findings made us assume that aortic calcification is an active and regulated processes by calcium regulating factors. In this study, we analyzed the association between the aortic calcification and calcium regulating hormones as well as lipids and blood pressure. In addition, we analyzed the effects of Vit.K on aortic calcification in same population. We investigated 545 Japanese women (mean age; 63.5+10.7years). Calcification of the abdominal aorta was assessed using lateral radiograph of lumbar spine, and the length of calcified area parallel to the anterior edge of the spine was measured. The severity of calcification was graded according to the lengths of the area involved. We divided them into two groups (mild group and severe group) by the median point. Serum levels of cholesterol, triglyceride, high-density lipoprotein cholesterol, 1-25 dihydroxy vitamin D, 25-hydroxy vitamin D and intact-PTH were measured. Serum Vit.K concentrations (phylloquinone; K1and menaquinone-7; MK-7) was also measured. The severe group was elder (71.6 vs. 61.8 years old). They had higher blood pressure (sys150.0 vs131.2, dia 84.2 vs78.2mmHg), and Vit.K was significantly lower (K1; 1.08 vs1.61, MK-7; 3.35vs6.00). In addition, the effect of Vit.K to aortic calcification remained still clear after adjusting age and blood pressure. On the other hand, lipids and calcium regulating hormones were not related to aortic calcification. Our results suggest that Vit.K insufficiency is a risk factor for aortic calcification but lipids or calcium regulating hormones were not. Intervention for Vit.K insufficiency may lead to a novel way for preventing aortic calcifiction.

M034

Quantitative Analysis of Matrix Gla Protein and Bone Gla Protein in Normal and Abnormal Human Calcifications. P. A. Price, W. F. Figueira,* R. Patel,* M. K. Williamson. Division of Biology, University of California, San Diego, La Jolla, CA, USA.

The present investigations were carried out to determine the quantitative levels of two vitamin K-dependent bone matrix proteins in bone and in abnormal calcifications of human tissues. The midshaft region of the tibia and the hardened, bone-like regions of atherosclerotic plaques and stenotic aortic heart valves were dried, ground to particles less than 1mm diameter, and weighed. Normal aorta media samples were dried and ground. Each sample was extracted with 10% formic acid and the levels of matrix Gla protein (MGP) and bone Gla protein (BGP; osteocalcin) were determined by radioimmunoassays specific for each protein, and the levels of calcium and phosphate were also measured in each extract.

Human Tissue	MGP, μg/g	BGP, μg/g	Ca, mmol/g	PO_4, mmol/g
Bone (n=5)	120 ± 20	450 ± 90	6.4 ± 0.6	4.0 ± 0.2
Atherosclerotic Plaques (n=10)	200 ± 80	6 ± 2	6.6 ± 0.6	3.9 ± 0.3
Stenotic Valves (n=8)	290 ± 90	5 ± 2	6.8 ± 0.5	3.9 ± 0.2
Normal Aorta Media:				
Age 60 - 99 (n=28)	170 ± 100	< 1	0.34 ± 0.17	0.24 ± 0.12
Stillborns (n=3)	1.3 ± 0.6	< 1	0.02 ± 0.01	0.02 ± 0.01

As seen in the table, MGP is a prominent constituent of all human calcifications examined, while BGP is a major constituent only of bone. On a per g dry weight basis, the highest levels of MGP were found in the hardened regions of atherosclerotic plaques and stenotic heart valves. The MGP in these calcifications was isolated by Sephracryl S300 filtration and shown to have a molecular weight and N-terminal protein sequence identical to the MGP isolated from human bone.

Surprisingly high levels of MGP were also found in the apparently normal regions of the human aorta media. As is well established from earlier studies, the human aorta media contains innumerable small calcium phosphate crystallites that increase in amount with age. We have confirmed the presence of these diffuse calcifications in adult aorta media samples, and their apparent absence in stillborns (see Table). If MGP is, as we suspect, associated with these crystallites, the ratio of MGP to mineral in the aorta media is about 12-fold greater than found in any other human tissues. We believe that the physical association of MGP with mineral phases is the mechanism by which the protein inhibits calcification, and that the MGP found in the diffuse calcifications of the normal human artery media plays a direct role in the inhibition of artery calcification.

M035

X-ray Absorption Microtomography and Phase Contrast X-radiography of the Structure of Sea Urchin Teeth. S. R. Stock,[1] W. K. Lee,[2] K. Fezzaa,[2] J. Barss,[3] T. Dahl,*[4] A. Veis.[4] [1]School of Materials Science and Engineering, Georgia Institute of Technology, Wilmette, IL, USA, [2]Advanced Photon Source, Argonne National Laboraotry, Argonne, IL, USA, [3]Dept. of Cell & Molecular Biology, Northwestern Univ. Medical School, Chicago, IL, USA, [4]Northwestern Univ. Medical School, Chicago, IL, USA.

Sea urchin teeth are complex composite structures with remarkable mechanical properties and are of interest as models for both biomineralization mechanisms and biomimetic constructs. The objective of this work is to determine the relationship between the organic and mineral phases and understand how these phases interact. The microstructure of intact sea urchin teeth was studied by X-ray absorption microtomography and by x-ray phase contrast radiography; the noninvasive interrogation revealed a low attenuation region within the tooth which appeared to be less highly mineralized than the surrounding tissue and to be associated with portions of the tooth in which staining revealed a high concentration of protein. A Scanco microtomography apparatus and associated software were used for the absorption-based imaging; synchrotron x-rays at station 1-ID C (SRI-CAT) at the Advanced Photon Source (APS) and the propagation technique were used for the phase-enhanced radiography. The different contrast mechanisms yielded complementary information about the morphology of the calcite reinforcement phase as it varied from the soft, apical, lightly mineralized zone to the stiff, hard incisal end of the tooth. These data reveal a picture more complete than previously provided by polished sections. This is particularly true for the mineral distribution within the soft tissue portion of the tooth where mineralization is initiated.

M036

Osteoblasts Transfected with Cx43 Antisense Oligonucleotides to Block Gap-Junctional Communication Do Not Mineralize Their Extracellular Matrix In Vitro. G. A. Howard, B. A. Roos, G. D'Ippolito, P. C. Schiller. GRECC and Research Service, Veterans Affairs Medical Center, and University of Miami School of Medicine, Miami, FL, USA.

We have previously reported that blocking connexin 43 (Cx43)-mediated gap-junctional communication (GJC) with reversible, chemical inhibitors (e.g., 18-α-glycyrrhetinic acid) results in inhibition of the ability of osteoblasts to mineralize their extracellular matrix (ECM) in vitro. Because the respective chemical substances may not be specific in their inhibition of GJC, we report here on experiments using a genetic approach to evaluate the necessity for GJC in the process of osteoblast mineralization of the ECM. Although not generally used for mineralization experiments in vitro, it has been reported that rat ROS 17/2.8 osteosarcoma cells can form a mineralized matrix in culture under the appropriate conditions. Hence we obtained ROS 17/2.8 cells and ROS 17/2.8-derived sublines expressing Cx43 antisense sequences from Dr. H. Donahue (Vander Molen, M.A., et al., J. Biol. Chem. 271:12165, 1996). These sublines, RCx4 and RCx16, when maintained in medium containing Geneticin (G-418) showed a drastically reduced GJC as determined by the ability of lucifer yellow (LY) to transfer from a single injected cell to surrounding cells. Moreover, the cAMP increase in response to PTH stimulation is significantly decreased in the antisense-transfected sublines compared to controls. These results have been confirmed in our laboratory. In addition we evaluated these changes in a medium that allows mineralization of the ECM (α-MEM, 10% fetal bovine serum, ascorbic acid, β-glycerophosphate) in

the parental, non-transformed ROS 17/2.8 cell line and showed the results to be identical to those reported by Vander Molen et al. using Ham F-12 medium with 10% fetal bovine serum. In contrast to results obtained with the parental ROS 17/2.8 cells in which there is substantial cell-cell GJC (14±3 cells coupled / injected cell, mean±SD, n=20 injections) as well as antibody-labeled Cx43 at cell-cell interfaces, the subline RCx4, where Cx43 cannot be detected (as judged by the lack of immunostaining of Cx43 on the cell surfaces), and GJC (as quantified by LY cell-cell transfer) is essentially absent (1±1 cells coupled / injected cell), does not mineralize the ECM under identical culture conditions. In the subline RCx16, where expression of Cx43 is not completely inhibited and cells remain coupled to a slight extent (4±1 cells coupled / injected cell), the level of mineralization is significantly reduced (>50% vs parental cell line) but not completely abolished. These results indicate Cx43 and/or GJC is/are involved in osteoblast-mediated mineralization of the ECM.

M037

Iron Deficiency Results in Lower Bone Density and Lower Tibial Cortical Thickness in Growing Female Rats. A. B. Arquitt,*[1] B. H. Arjmandi,[1] M. P. Akhter,[2] [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Osteoporosis Research Center and Biomedical Engineering Center (CUBE), Creighton University, Omaha, NE, USA.

Iron plays an important roll in health and has been identified as an essential element for Type I collagen formation. The present study was designed to investigate the effects of four levels of dietary iron on bone properties in growing female rats. Forty weanling Sprague Dawley rats were randomized into dietary treatments as follows: 6 ppm, 12 ppm, 35 ppm or 150 ppm iron. At 18 weeks of age the rats were fasted for 12 hours, killed and tissues were collected. There were no significant differences in starting or final body weights between dietary treatment groups. The 6 ppm iron-fed rats were iron deficient as indicated by significantly lower hemoglobin concentrations. Whole body bone mineral density (BMD) as determined by dual energy absorptiometry (DXA) was significantly lower in the 6 ppm iron-fed rats than in the 12 or 150 ppm iron-fed rats. Whole body bone mineral content (BMC) was not significantly different among the groups ($p<0.08$). Left tibia histomorphometric examination revealed significantly lower cortical thickness both in the 6 ppm compared to the 35 and 150 ppm iron-fed rats and the 12 ppm compared to the 150 ppm iron-fed rats. There were no differences in marrow cavity or bone area. Results similar to the histomorphometric data were found for DXA scans of the left tibia: BMC was significantly lower in the 6ppm fed rats compared to 35 ppm or 150 ppm Iron-fed rats, and BMD was significantly lower in the 6 ppm fed rats than in the35 ppm or 150 ppm iron-fed rats. We conclude that iron deficiency results in low bone mass and that iron slightly more than 4 times the AIN-93 dietary recommendations for rodents may not negatively impact bone during growth. (Sponsored by OCAST Grant #98-015 and OAES OKLO2132)

M038

Effects of Halofuginone on Chicken Chondrocytes in Culture. N. C. Rath, H. Xie,* G. R. Huff,* W. E. Huff,* J. M. Balog.* USDA, ARS, Fayetteville, AR, USA.

Halofuginone (HAL) is widely used in poultry production as an coccidiostat. However, its negative effect on collagen synthesis has been linked to skin tearing, and recently it has been shown to be a potent suppressor of endothelial function and angiogenesis. Rapidly growing, meat-type poultry, exhibit a skeletal problem called tibial dyschondroplasia (TD) where a part of the growth plate fails to undergo endochondral bone transformation rendering the growth plate fragile, prone to breakage and deformation. This defect is associated with the lack of focal vascularization at the growth plate, and extensive apoptosis of pre hypertrophic chondrocytes, and death of the capillary vessels. The etiology of TD is unknown. Because of the influence of HAL on angiogenesis, an event that is critical to endochondral bone formation, we were interested to study if it influences chondrocyte survival, matrix metalloproteinase (MMP), and cytokine production. The chondrocytes were isolated from healthy 3-4 week-old broiler chickens by overnight digestion of growth plate cartilages with collagenase and hyaluronidase. The cells were plated in DMEM/F-12 medium with conventional antibiotics, antimycotic, and supplement such as ascorbic acid and 10% FBS at a concentration of 2 $X10^5$cells/ well in 48 wells and allowed to establish a confluent monolayer for three days. The cells were then replaced with medium containing serum free supplement TM-235, and different concentrations of HAL (0, 0.01, 0.1, 0.5, 1 and 5 µg/ ml) for 24 and 48 hrs. The conditioned medium was used to determine interleukin-6 (Il-6) with B9 cell bioassay and MMP production by gelatin substrate zymography. The viability of cell layers was assessed using MTT reduction assay, and apoptosis using terminal deoxynucleotide transferase (TdT) mediated nick end labeling (TUNEL) assay of collagenase dissociated cells. In a different set of assays, ^{3}H-thymidine incorporation was measured using non confluent chondrocytes (2 X 10^4/ well in 96 well) in complete medium during the first 48 hr of plating, that were exposed to ^{3}H-thymidine during final 22 hrs of the assay. The results showed that, in concentrations at 0.1 and 0.5 µg/ ml, HAL inhibited the production of Il-6 and MMP without affecting the viability and mitogenic potential of chondrocytes. At more than 1µg/ ml, the cells showed significant loss of viability indicated by low ^{3}H-thymidine incorporation, decreased MTT reduction, and increased apoptosis and necrosis indicated by TdT mediated incorporation of fluorescin-dUTP into nuclei, and loss of membrane integrity. Although these observations do not link HAL as a causative factor for TD, it is likely that localized increase in the levels of this antibiotic may be disruptive to chondrocyte function.

M039

Expression of Cbfa1 Is Dependent on the Three-Dimensional Tissue Structure in Human Articular Chondrocytes in Vitro. A. Battmann,*[1] M. Schaller,*[1] J. Rose,*[1] M. Bayer,*[1] J. Kühling,*[1] H. Stracke,[2] R. M. Bohle,*[1] L. Fink,*[1] A. Schulz,[1] U. Stahl.*[1] [1]Institute of Pathology, Justus Liebig University, Giessen, Germany, [2]III. Med. Dept., Giessen, Germany.

Cbfa1 is a gene encoding a transcription factor of the runt-superfamily. It is one of the earliest markers for osteoblastic differentiation. Recent studies have shown as well its importance for the maturation of chondrocytes as well as for enchondral ossification in Cbfa1 deficient mice. However, regulation of Cbfa1 gene expression is not yet understood. We tested the hypothesis, if Cbfa1 is present in human articular chondrocytes and if a regulation of its expression can be achieved changing from two to three dimensional in vitro conditions. Human articular chondrocytes have been isolated out of samples obtained at joint surgery as described by Brittberg 1996. Patients with inflammatory joint disease were excluded. Chondrocytes were cultured in DMEM/Ham'sF12 medium supplemented with ITS, 10% fetal calf serum and 1% penicillin/ streptomycin at an atmosphere of 5% CO_2, and 37°C. To evaluate chondrocytic differentiation we performed an immunocytochemical staining. Polyclonal antibodies to collagen typ I, II and III (Ibby Dunn) were applied, staining was visualized by using an ABC-Elite Kit with diaminobenzidine as substrate. Cells were grown either in monolayer cultures or in an alginate bead system, providing a three-dimensional cell arrangement. mRNA preparation and cDNA synthesis were prepared according to standard procedures. Real time PCR (SDS 7700, Perkin Elmer) was performed to relatively quantitate Cbfa1 mRNA in relation to a housekeeping gene (HPRT). Specific and intron spanning primer pairs were used in combnation with SYBR-Green (Perkin Elmer) for flourescence detection. The immunohistochemical stainings revealed differentiated chondrocytes as determined by an expression of collagen type II. Those results given, we assumed an in vitro model which maintains typical chondrocyte like properties. In all human articular chondrocytes examined, a Cbfa1 expression could be demonstrated. A significant increase of Cbfa1 mRNA was detectable during subsequent passages of the monolayer cultures in vitro. Providing a three dimensional tissue structure in vitro, the Cbfa 1 mRNA level was maintained or even decreased.This is the first study to demonstrate a regulation of Cbfa1 expression dependent on a two- or three- dimensional tissue structure in human articular chondrocytes in vitro. This observation suggests that for establishing and maintainance of a stable cell differentiation in articular chondrocytes the tissue architecture might be of major importance. The underlying mechanism needs to be elucidated yet.

M040

Analysis of Murine HIP/RPL29 Expression During Skeletogenesis and Tooth Development: A Marker for Early Organogenesis and Differentiation of Mineralized Tissues. C. B. Kirn-Safran, D. Baraniak, M. C. Farach-Carson, D. D. Carson.* Biological Sciences, University of Delaware, Newark, DE, USA.

HIP/RPL29 is a 24 kDa, highly basic, heparin/heparan sulfate binding protein identical to ribosomal protein L29 and is differentially expressed in a variety of adult tissues and cell types. Using in situ hybridization and immunohistochemistry we report the normal expression pattern of HIP/RPL29 in developing bone and tooth in conjunction with relevant markers for chondrogenesis, osteogenesis and odontogenesis. HIP/RPL29 is expressed, at both mRNA and protein levels in proliferating chondrocytes, but is absent in hypertrophic chondrocytes. The identity of the HIP/RPL29-expressing cells was determined by analyzing the expression of perlecan, type II collagen, and type X collagen proteins on serial sections. In addition, HIP/RPL29 mRNA and protein are highly expressed in calcifying chondrocytes, and in epiphyseal and periosteal osteoblasts synthesizing bone sialoprotein (BSP). Finally, the expression of HIP/RPL29 message in dental papilla and enamel organs of both molar and developing incisors is markedly increased at the epithelio-mesenchymal junction in preameloblasts and in functional odontoblasts expressing BSP mRNA. In conclusion, these data indicate that HIP/RPL29 might participate in both the early distribution and proliferation, and the terminal differentiation of developing mineralized tissues. Additional studies will define the intracellular and extracellular expression patterns of HIP/RPL29 as well as its potential role in chondrocyte proliferation and differentiation. (supported by 2000 Lalor grant to C.B.K.S., and NIH grants HD 25235 [to D.D.C.] and DE13542 [to D.D.C. and M.C.F.C.]).

M041

ATF-2 Dooperates with Smad 3 in Transducing TGF-ß Effects on Chondrocyte Maturation. A. M. Ionescu,* C. M. Ferguson,* E. M. Schwarz, J. E. Puzas, R. N. Rosier, R. J. O'Keefe. Center for Musculoskeletal Research, University of Rochester, Rochester, NY, USA.

TGF-ß is an important regulator of chondrocyte phenotype during endochondral ossification. TGF-ß signaling occurs through a Smad-mediated pathway but also involves a parallel pathway mediated by TAK/p38, leading to activation of the transcription factor ATF-2. This study examines ATF-2 activation, interactions with Smad signaling molecules, and its role in chondrocyte differentiation. ATF2 phosphorylation was induced in embryonic chick cephalic sternal chondrocytes stimulated with TGF-ß within 5 minutes. Levels peaked by 15 minutes, and returned to basal levels by 1 hour, while total protein levels of ATF-2 were unchanged. ATF-2 effects on Smad signaling was investigated in chondrocytes using both gain and loss of ATF-2 function experiments. TGF-ß is a known stimulator of p3TP-lux promoter activity, and resulted in a 5.2-fold increase, while co-transfection with wild type Smad 3 and 4 constructs mimicked the effects of TGF-ß (6.0-fold), and further potentiates the effect of TGF-ß (10.2-fold stimulation). Co-transfection of wild type ATF-2 did not alter basal promoter activity, but enhanced TGF-ß effects (42% enhance-

ment), Smad 3 effects (52% enhancement, p<0.005), and Smad 3 and TGF-ß effects (46% enhancement, p<0.005). Loss of function was performed using both a dominant negative ATF-2 and a p38 inhibitor, SB 203580. SB 203580 resulted in a 50% inhibition of ATF-2 phosphorylation and TGF-ß stimulated p3TP-lux activation. The dominant negative ATF-2 completely blocked Smad 3 mediated luciferase stimulation in both control and TGF-ß treated cultures. Thus, basal activity of ATF-2 is necessary for Smad-mediated signaling. Next, a series of gain and loss of function experiments were performed to determine whether ATF-2 is a functionally important mediator of TGF-ß phenotypic effects in chondrocytes. Smad 3 and ATF-2 infections were performed using RCASBP with two different envelopes (A and B), that permit high levels of expression of both proteins simultaneously. Similar to TGF-ß, both wild type Smad 3 or ATF-2 alone inhibit col X expression, and in combination, result in a level of suppression comparable to TGF-ß. In contrast, both dominant negatives alone partially block TGF-ß effects on col X, and in combination, completely block the inhibitory effect of TGF-ß on col X. The current findings demonstrate that ATF-2 is activated in parallel with the Smad signaling pathway and the combination of these signaling events is necessary to mediate TGF-ß effects on chondrocytes. A more complete understanding of this transcription factor and its target genes will further define the events involved in endochondral bone formation.

M042

Ca^{2+}-Sensing Receptors Mediate High Extracellular Ca^{2+}-Induced Chondrocyte Differentiation. W. Chang, T. H. Chen,* S. A. Pratt,* C. Tu,* D. Shoback. Endocrine Unit, VAMC, University of California San Francisco, San Francisco, CA, USA.

Rickets results from deficiencies of Ca^{2+} and vitamin D and produces a disorganized and demineralized growth plate (GP) and growth retardation. Matrix protein production and matrix mineralization are defective in rachitic cartilage. To test the hypothesis that changes in the availablilty of Ca^{2+}, reflected by changes in the extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$), directly affect the function of chondrocytes, we tested the effects of different $[Ca^{2+}]_o$ on the expression of markers of differentiation in cultured rat chondrogenic RCJ3.1C5.18 (C5.18) cells and bovine GP chondrocytes. Raising $[Ca^{2+}]_o$ from 0.4 to 6 mM in C5.18 cells dose-dependently suppressed levels of RNA encoding the early chondrogenic markers aggrecan and type II collagen and increased RNA levels for the terminal differentiation markers osteopontin (OP), osteonectin, and osteocalcin by Northern blotting. In both C5.18 cells and GP chondrocytes high $[Ca^{2+}]_o$ suppressed proteoglycan accumulation, assessed by alcian green staining, and promoted the accumulation of alizarin red-stainable minerals. In GP chondrocytes high $[Ca^{2+}]_o$ also increased the production of matrix vesicles visualized by EM. Our previous studies showed that C5.18 cells and GP chondrocytes express "parathyroid-like" CaRs by in situ hybridization and immunocytochemistry (Endocrinology 140: 5883,1999). We tested whether altering CaR function could block extracellular Ca^{2+}-mediated changes in gene expression in C5.18 cells. Stable expression of a dominant–negative CaR mutant (Phe --> Trp 707-CaR) in C5.18 cells increased alcian green staining by 2-fold and aggrecan RNA levels by 3-fold and reduced OP RNA levels by > 50 % compared to vector controls. In contrast, cells stably transfected with wild-type (wt) CaR cDNA demonstrated > 50% reductions in aggrecan RNA and alcian green staining. OP expression was increased by > 3-fold in these cells, indicating that altering CaR function had profound effects on gene expression. Furthermore, overexpression of wt CaRs in C5.18 cells by adenoviral infection enhanced their sensitivity to suppression of matrix protein production by high $[Ca^{2+}]_o$ and increased mineral deposition by these cultures. Taken together, these studies support the idea that raising $[Ca^{2+}]_o$ promotes chondrocyte differentiation by modulating matrix gene expression and mineral deposition and that CaRs are likely to play a role in mediating this sensitivity to $[Ca^{2+}]_o$ in GP chondrocytes.

M043

Direct Adenovirus-mediated BMP-2 gene Delivery Accelerates Fracture Healing Locally and Contralaterally in a Mouse Experimental Fracture Model. H. Uusitalo,*[1] A. Hiltunen,*[2] E. Vuorio.[1] [1]Department of Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland, [2]Department of Surgery, University of Turku, Turku, Finland.

Bone morphogenic protein-2 (BMP-2) is a growth factor capable of inducing endochondral bone formation. We have investigated the capacity of direct adenovirus-mediated BMP-2 gene delivery to enhance fracture healing. Fractures injected with BMP-2 were compared with those in contralateral limbs injected with adenovirus carrying LacZ reporter gene (RAdLacZ), and with those in control animals injected with RAdLacZ virus only. For this purpose a recombinant adenovirus (RAdBMP-2) harboring the entire coding sequence of the human BMP-2 under the control of cytomegalovirus IE promoter was constructed. RAdBMP-2 viruses were injected into a mouse diaphyseal fracture, which was produced into a prenailed tibia, immediately after the operation. Fractures in control animals, as well as fractures in the contralateral limbs of the BMP-2 injected animals, were injected with RAdLacZ. The healing process was followed at 5, 7 and 14 days using immunohistochemistry, in situ hybridization, Northern blotting as well as RNase protection assays for determination of the expression levels of proa1(II) collagen and transcription factors L-Sox5, Sox6 and Sox9, which regulate chondogenesis.Using LacZ as a reporter gene, we demonstrated that adenovirus effectively infects cells in fracture callus. The expression peaked within the first week and persisted up to 3 weeks, but had no effect on fracture healing.After local injection of RAdBMP-2, transcriptional activation of Sox9 and type II collagen genes was increased and occurred earlier than during normal healing indicating acceleration of chondrogenesis. Surprisingly, similar positive effects of BMP-2 gene transfer were also seen in the fractures in the contralateral limbs. This acceleration of fracture healing on the contralateral side was approximately 50% of that seen in fractures injected with RAdBMP-2.Our study demonstrates that BMP-2 gene transfer in vivo upregulates production of type II collagen and chondrogenetic transcription factors during fracture healing. We also present evidence that the local injection of BMP-2 gene transfer to fracture not only accelerates fracture healing at the site of the injection, but also in the contralateral limb. Explanations for the latter finding include traffic of cells carrying the BMP-2 gene from one side to the other, or spread of protein or adenovirus through circulation to the other site. Thus, further studies are warranted to determine the mechanism by which the local gene therapy using BMP-2 stimulates healing also at distant fracture sites.

M044

Noggin Regulation of Chondrogenic Differentiation in a Mesodermal Stem Cell Line C1 and Skeletal Cells. A. Nifuji,[1] O. Kellermann,[2] M. Noda.[1] [1]Dept. Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]UPR CNRS 1983, Villejuif, France.

Osteoblast and chondroblast are derived from common mesenchymal progenitors. BMP induces mesenchymal cell differentiation into both osteogenic and chondrogenic pathways in vitro. Its inihibitor, noggin, is expressed during the chondrogenic differentiation of mesodermal C1 cells cultured in the presence of dexamethasone, but it is not expressed during the osteogenic differentiation of C1 cells cultured in the presence of ascorbate and beta-GP. We hypothesize that noggin may function in lineage-specific manner to block BMP action. To test this hypothesis, we constructed an recombinant adenovirus to express noggin (Ad/noggin). C1 cells are derived from an embryonal carcinoma cell 1003 and have characteristics of mesodermal cells. When C1 cells are cultured in aggregate form in differentiation medium, they differentiate into chondrogenic, osteogenic and adipogenic cells. When we infected Ad/noggin into C1 cells before induction of differentiation, endogenous alkaline phosphatase level in these cells was not altered compared with Ad/lacZ infected C1 cells. When Ad/noggin-infected C1 cells were induced to differentiate into chondrogenic lineage, expression of Sox9 and type X collagen mRNAs was reduced, showing noggin inhibition of chondrogenesis. In contrast, upon induction of C1 cells into osteogenic lineage, expression levels of osteoblast phenotypic markers, osteocalcin and alkaline phosphatase mRNAs, did not differ between Ad/noggin and lacZ- infected cells. We further examined if noggin may affect skeletal tissue development by using organ cultures of embryonic long bones. When we infected Ad/noggin into 15.5dpc hind limb bones, chondrogenic growth was inhibited compared with Ad/lac Z infected limb bones. These results suggest that noggin is a regulator of chondrogenic differentiation, rather than osteogenic differentiation, of mesodermal stem cell line C1 and skeletal cells.

M045

Regulation of Cartilage Matrix Genes by Pro-inflammatory Cytokines. C. A. Seguin,* K. M. W. Elder,* S. M. Bernier. Dept. of Anatomy and Cell Biology, The University of Western Ontario, London, ON, Canada.

Articular cartilage matrix is distinguishable from other connective tissues by its rich content of proteoglycan and type II collagen that is reduced and not readily replaced in response to inflammation. The purpose of this study was to define the pattern of loss of key components regulating the integrity of cartilage matrix following exposure to inflammatory cytokines (TNFα and IL-6). Articular chondrocytes isolated from rat femoral condyles expressed type II collagen, aggrecan, the major proteoglycan, and link protein, the molecule responsible for stabilizing the interaction between aggrecan and hyaluronan. Although stable for a few passages, loss of type II collagen mRNA expression preceded that of link protein and aggrecan mRNA with greater passages. The articular chondrocytes were treated with TNFα or IL-6/sIL-6r and changes to the steady state mRNA levels of the three cartilage matrix molecules were examined. Within 24 h, the expression level of mRNA for all three matrix molecules was reduced following treatment with TNFα but not IL-6, with the loss of link protein and aggrecan mRNA being greater than that for type II collagen. A brief exposure to TNFα (1 h) was sufficient to induce a significant reduction of type II collagen and link protein mRNA at 24 h. The stability of the mRNAs was not altered by TNFα suggesting regulation at the level of transcription. TNFα and IL-6 activated distinct signaling pathways in the articular chondrocytes. Use of pharmacologic inhibitors identified a key role for the MAPK pathway in the regulation of type II collagen mRNA by TNFα and for the MAPK and NF-kB pathways in link protein gene expression. Co-treatment or pretreatment of chondrocytes with IL-6 did not protect from the TNFα-mediated loss of matrix gene expression but appeared to act synergistically with TNFα to further reduce type II collagen mRNA expression. Using a washout protocol, expression of link protein mRNA recovered more readily than that of type II collagen upon withdrawal of TNFα. In conclusion, cytokines can act alone (TNFα) or can contribute to an existing cellular response (IL-6) disrupting in a non-coordinate and partially reversible manner the expression of three key cartilage matrix components thereby reducing the ability of chondrocytes to repair cartilage following inflammation.

M046

c-Fos Expression in Chondrocytes in Vitro Modulates Their Responsiveness to Exogenous Growth Factors. D. P. Thomas, I. Anagnostopoulos,* A. Sunters,* A. E. Grigoriadis. Dept Craniofacial Dev, KCL, Guy's Hospital, London, United Kingdom.

The c-Fos proto-oncogene is involved in the control of differentiation and transformation of chondrocytes, as demonstrated by *in vivo* models. We have recently used inducible c-Fos expression in clones of the ATDC5 chondroprogenitor cell line to demonstrate that exogenous c-Fos inhibits chondrocyte differentiation. To further disect the possible mechanisms of c-Fos action, we have investigated whether exogenous c-Fos expression alters the responsiveness of these cells to growth factors that are known to affect the growth and differentiation of chondrocytes. We have previously reported, using an ATDC5 clone with tetracycline-regulatable c-Fos expression (DT12.4), that ectopic c-Fos upregulates the expression of the fibroblast growth factor receptor 1 (FGFR1) from early differentiation stages, implicating FGF signalling pathways as potential mediators of c-Fos. We show here

that this elevated expression of FGFR1 is maintained throughout differentiation and that c-Fos-dependent upregulation of FGFR1 is seen in other ATDC5 clones (DT7.1, DT8.6) and in an osteoblastic clone (AT9.2). Moreover, high expression of FGFR1 is seen in a cell line (wT2) isolated from a c-Fos-induced chondrosarcoma. We have further investigated the response of DT12.4 cells to treatment with FGF ligands. Both FGF-1 and FGF-2 inhibited chondrocyte differentiation in the absence of c-Fos induction. However, dose-response experiments with FGF-2 showed that ectopic c-Fos expression reduced the cells' responsiveness to ligand, suggesting that c-Fos may lie downstream of FGF-signalling in the inhibition of differentiation. FGF-2 also inhibited chondrocyte proliferation as measured by BrdU incorporation, and this effect was enhanced by exogenous c-Fos expression. Moreover, FGF-1 and FGF-2 induced changes in cellular morphology from polygonal to spindle-shaped, and FGF-2 caused the formation of foci only in the presence of c-Fos. Interestingly, c-Fos-transformed wT2 cells, which express elevated FGFR1 levels, form foci spontaneously without exogenous FGF-2, implicating c-Fos and FGF in chondrocyte transformation. Finally, we have looked at the response of DT12.4 cells to BMPs. Increasing doses of BMP-2 promoted the differentiation of DT12.4 cells, whilst high doses (>100ng/ml) were inhibitory. Interestingly, BMP-2 rescued in part the c-Fos-induced block in DT12.4 differentiation, however, a significantly higher dose of BMP-2 was required. These data demonstrate that ectopic c-Fos expression in chondrocytes can modulate their responsiveness to FGFs and BMPs and that this may contribute to the observed effects of c-Fos on chondrocyte differentiation.

M047

Gene Expression of Periodontal Ligament Cells after TGF-b Treatment. J. Jeong,*[1] J. Kim,*[2] S. Kim,*[3] H. Ryoo,[4] J. Kim,*[5] J. Kim,[6] I. Kim,*[1] J. Choi.*[1] [1]Biochemistry, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, [2]Orthodontics, Catholic University, Seoul, Republic of Korea, [3]Orthopedics, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, [4]Biochemistry, School of Dentistry, Kyungpook National Univesity, Daegu, Republic of Korea, [5]Immunology, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, [6]Biochemistry, School of Dentistry, Dankook University, Chunan, Republic of Korea.

Periodontal ligament (PDL) is unmineralizing soft tissue between tooth and alveolar bone under the physiological condition. It has been shown that PDL cells have osteoblast-like phenotypes in vitro culture system. Transforming growth factor-b (TGF-b) has a strong inhibitory effect on osteoblastic differentiation when cells were treated with TGF-b at early stage during the osteogenic differentiation culture condition. To identify the genes that involved in TGF-b mediated inhibition of osteoblastic phenotype in PDL cells, we analyzed gene expression profile using cDNA microarray technology. Primary cultured PDL cells were obtained from patient who visit for orthodontic treatment and cells were treated with 5 ng/ml of TGF-b for 24 h. Poly A (+) RNA from control and TGF-b treated group was reverse transcribed and labeled with different fluorescent probes either Cy3-dUTP or Cy5-dUTP. The fluorescent-labeled cDNA mixture was allowed to hybridize to the 3K human cDNA chip that included 3000 unigenes obtained from mesenchymal stromal cell cDNA library. The results showed that twelve transcripts were highly upregulated. These genes included cell signaling related gene such as connective tissue growth factor, gene expression related genes such as plasminogen activator inhibitor I, transcription factor E2A (TCF3), CCAAT enhancer-binding protein delta, cell structure and motility related genes such as osteonectin, fibronectin, BIGH3 (TGF-beta induced gene product) and others such as gastrin binding protein, lysyl hydroxylase isoform 2, p311. We also identified two upregulated novel genes that were not shown in Genebank. The down regulated genes were phosphatidic acid phosphatase type 2b, and cathepsin K. Taken together, up- or down-regulated genes by TGF-b may be involved in maintenance of unmineralizing soft tissue in PDL in vivo.

Disclosures: IBEC of KOSEF of Korean government.,2.

M048

Forced Expression Of Runx2/Cbfa1 Driven By The –3.6 Kb Collagen Type I Promoter Results In A Normal Phenotype In Transgenic Mice. J. Pratap,* C. J. Lengner,* A. Javed, S. Dalamangas,* A. J. vanWijnen, J. L. Stein,* J. B. Lian, G. S. Stein. Cell Biology, UMASS Medical School, Worcester, MA, USA.

Runx2/Cbfa1 is an essential transcription factor for skeletogenesis and osteoblast differentiation. To assess the ability of early expression of Runx2 to induce osteogenesis in collagen Type I-containing tissues, we generated transgenic mice using the -3.6 kb Type I Collagen promoter driving expression of wild type Runx2 (til-1/MASNS isoform). This -3.6 kb promoter expresses at high levels in bone and is also active in skin and tendon. Transient transfection assays revealed that the transgene is expressed in osseous (ROS 17/2.8) and non-osseous (HeLa) cell lines and that the encoded protein is predominantly nuclear, similar to endogenous Runx2, as detected by in situ immunofluorescence. We selected three transgenic lines and determined developmental expression of the Runx2 transgene from 13.5 days post-coital (dpc) up to post natal age day 10. We find that the expression level of transgene is comparable to endogenous Runx2 at day 13.5 dpc and increases up to 3 fold (2 lines) and 6-7 fold (1 line) at day 10 post natal. Radiography of the newborns and adult mice up to 6 weeks showed no evidence of ectopic bone formation in any of the transgenic lines. Histologic examination of bone showed normal periosteum, cortical bone, growth plate organization and trabeculae volume in the metaphysis relative to wild type littermates. Taken together our findings indicate that increased expression of Runx2 by Col Type I promoter neither caused any gross abnormalities in bone formation nor induced ectopic calcification. These findings are in contrast to the phenotype observed for collagen Type II (cartilage) driven Cbfa1 expression in vivo by others. We suggest that the precise timing for introducing perturbations of Runx2 activity is critical for observing pathological manifestations.

M049

Does Glutamate Differentially Regulate GLAST-1 and its Splice Variant GLAST-1a? J. F. Huggett,* D. J. Mason. School of Biosciences, Cardiff University, Cardiff, United Kingdom.

GLAST-1 is a Na+ dependent glutamate transporter that was initially characterised in the central nervous system where it is expressed, predominantly by astrocytes, at the synapse to terminate excitatory signals. Since GLAST-1 was isolated as a candidate gene for mechanical regulation in osteocytes in vivo (Mason 1997), there have been many reports in support of a role for glutamate bone cell signalling (Skerry 2001). We have demonstrated that the entire open reading frame (ORF) of GLAST-1 is expressed in bone in vivo but have also identified a splice variant of this gene in which exon 3 is excluded (Huggett 2000). GLAST is a transmembrane protein and loss of the 46 amino acids encoded by exon 3 effectively removes a single transmembrane domain. We believe that this will reorientate the transporter within the cell membrane, possibly facilitating glutamate release. Whilst we have detected a protein of the correct molecular weight for GLAST-1a by immunoblotting of membrane proteins extracted from rat cerebellum, we have not yet been able to detect this in bone extracts. In order to confirm that GLAST-1a mRNA is translated and assembled in the plasma membrane of bone cells, we generated GLAST-1 and GLAST-1a ORF constructs (pD2EGFP-N1, Clontech) with C-terminal GFP tags and transiently transfected SaOS-2 cells (Effectene transfection reagent, Qiagen). Fluorescent microscopy of transfected cells showed that both transporter proteins are expressed and localise to the cell membrane although the distibution of GLAST-1a appears more focal. Since GLAST-1 was originally identified in vivo in response to osteogenic mechanical stimuli and recent data suggests that binding of glutamate to NMDA and AMPA receptors on osteoblasts causes bone formation in vitro, control of extracellular glutamate concentrations may be critical in balanced bone remodelling. There is evidence from other tissues that GLAST-1 expression is directly regulated by extracellular glutamate concentration, thus controlling glutamate levels for signalling. To test this we have cultured SaOS-2 osteoblast-like cells in with various concentrations of glutamate (0,1,10,100 and 1000µM) and detected relative amounts of GLAST-1 and GLAST-1a by RT-PCR. Primers designed to exons 2 and 4 yield a 210bp product from GLAST-1a and a 345bp products from GLAST-1. Our data shows that the relative amount of GLAST-1a mRNA is up-regulated at extracellular glutamate concentrations of 10-100µM in SaOS-2 cells. If our ongoing studies prove that GLAST-1a is reversed within the cell membrane the differential regulation of the 2 variants of this gene in bone in response to glutamate could represent a novel mechanism controlling glutamate-mediated osteogenesis.

M050

Hedgehog Signaling Enhances Expression of RANKL and Noggin Gene in Chondrocytes. M. Takamoto,[1] K. Tsuji,[1] H. Sasaki,*[2] A. Nifuji,[1] Y. Taketani,*[3] T. Komori,[2] M. Noda.[1] [1]Dept. of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Osaka University, Osaka, Japan, [3]University of Tokyo, Tokyo, Japan.

Hedgehog signaling is considered to play a crucial role in chondrogenesis by acting through a network of cytokine actions. Indian hedgehog (Ihh), expressed predominantly in prehypertrophic chondrocytes, regulates chondrocyte functions. However, downstream targets of Ihh have not yet been fully understood. This paper examines the effects of hedgehog on the genes expressed in chondrocytes to understand the downstream targets of the chondrocyte differentiation factors including hedgehog signaling. Chondrocytes were prepared from costal cartilage of newborn mice by enzymatic digestion. These chondrocytes constitutively expressed Patched and Gli, key components in hedgehog signaling, and the mRNA levels of these genes were upregulated by the hegehog treatment, indicating that hedgehog signaling is active in these cells. These chondrocytes expressed osteopontin mRNA constitutively, but alkaline phosphatase mRNA expression was not observed. To examine hedgehog effects on osteopontin, the cells were treated with sonic hedgehog starting from 5 days after separation. Northern blot analysis indicated that hedgehog treatment suppressed osteopontin mRNA expression in these cells. Alkaline phosphatase mRNA levels were upregulated by the hedgehog treatment, however osteocalcin mRNA expression was not observed either in the absence or the presence of hedgehog. We also examined by RT-PCR whether hedgehog has any effects on the factors involved in the regulation of osteoclast differentiation and activity. RANKL, a critical stimulator of osteoclast differentiation and activity, is constitutively expressed in these chondrocytes and hedgehog treatment upregulated its expression level. RANK mRNA expression was not observed in the Northern blot analysis, however, it was induced by the hedgehog treatment. OPG mRNA was constitutively expressed and its level was not altered, indicating that hedgehog signaling could stimulate osteoclast activity via the activation of RANKL and RANK expression in chondrocytes. Noggin, an inhibitory factor for BMP signaling, was expressed in these chondrocytes and its mRNA level was also enhanced by hedgehog treatment. These data indicate that hedgehog signaling plays a key role not only in the positive regulation of chondrogenesis but also in its negative regulation such as activation of RANKL expression which may lead to chondroclastic degradation of cartilage and suppression of BMP actions via promotion of Noggin expression.

M051

Decreased Skeletal Growth in Dopamine Beta-Hydroxylase Null Mutants. P. Patterson-Buckendahl,*[1] K. Dennison,*[2] S. A. Thomas.*[3] [1]Center of Alcohol Studies, Rutgers University, Piscataway, NJ, USA, [2]Stockton College, Pomona, NJ, USA, [3]Pharmacology, University of Pennsylvania, Philadelphia, PA, USA.

There have been numerous reports of receptors for neurotransmitters on bone cells, including epinephrine (E) and norepinephrine (NE), two hormones which are also integral to the response to psychological stressors. Previous research showed that under acute restraint immobilization, a rapid increase in rat plasma osteocalcin (OC) returned to normal levels in the absence of E, but remained elevated if NE was eliminated by sympathetic neural blockade. Deletion of the gene in mice responsible for synthesis of dopamine beta-hydroxylase (DBH) results in the loss of both E and NE. Homozygous mutants (DBH-/-) can be rescued during critical early developmental stages with dihydroxyphenylserine, which is converted to NE by alternative pathways, allowing normal morphological development to adulthood. However, body mass of adult male DBH-/- is 80% and females 88% that of DBH+/- littermates, reflecting a lower growth rate of mutants. To determine whether DBH deletion affected skeletal size, we studied post-cranial carcasses from 10 female and 12 male mice, half of which were DBH-/- and half DBH+/-, approximately 3 mo. of age when killed. Bones were cleaned by dermestid beetles prior to triplicate measurement with digital calipers of length and midshaft width of femurs, length of tibia, humerus, ilium, and ischium to pubis. Bones were then freeze-dried and weighed. OC levels were also determined in plasma from 10 females of each genotype and 18 DBH+/- and 15 DBH-/- males. Long bone length in male DBH-/- mice averaged 96% of DBH+/- males ($p < 0.05$). Femur width was about 90% of DBH+/-. However, female DBH-/- were not significantly different from +/- littermates in any dimension. Bone weights of DBH-/- males averaged 79% of DBH+/- ($p < 0.01$), while there were no significant differences among females. There was striking symmetry between contralateral bones in both length and weight. There was a trend toward lower OC levels in mutants which reached marginal significance in males, $p = 0.08$. However, there was a pronounced gender difference in OC: DBH+/- 87 ± 4 vs. 122 ± 12; DBH-/- 77 ± 4 vs. 109 ± 7, male vs. female respectively ($p < 0.01$). We conclude that loss of E and NE during post-natal development adversely affects skeletal growth in male mutants, but not in females. It is possible that estrogen has a protective effect on the skeleton of the female mutants, over-riding the influence of neurotransmitter deletion.

M052

Osteogenic Regulation of Macrovascular Calcification: Dysmetabolic Signals Promote Heterotopic Osteoblast Gene Expression in Aortic Mesenchymal Cells. M. Bidder, A. Loewy, J. Shao, T. Latifi, G. Ferguson, C. F. Semenkovich, D. A. Towler. Washington University Medical Center, St. Louis, MO, USA.

The ascending aorta and aortic valve are predisposed to calcification in response to mechanical stressors and dysmetabolic states (hypercholesterolemia, diabetes). High fat diets typical of western societies induce dyslipidemia and diabetes in lipoprotein receptor (LDLR)-deficient mice, with concomitant vascular calcification revealed by von Kossa staining. Image analysis reveals involvement of ca. 0.1% of valve leaflet area with calcium deposition within 5 weeks of dietary manipulation. Analyses of aortic mRNA identifies heterotopic activation of an osteogenic gene expression program that includes bone sialoprotein (BSP) and osteopontin (OPN) in response to diabetogenic diets. Expression of BMP2 --and its target, Msx2 -- is also induced, within 2 weeks of dietary manipulation. In situ hybridization reveals expression of BMP2 and Msx2 in peri-aortic adventitial and valvular fibrosa cells. OPN expression is seen in adventitial cells, vascular smooth muscle cells (VSMC), and foam cells. To identify the mechanisms whereby osteoblast genes are aberrantly expressed in aortic mesenchymal cells in response to relevant dysmetabolic stimuli, we cloned the 2 kb (-1976 to +78) mouse OPN promoter, and mapped elements supporting OPN promoter activity (LUC reporter) in A7r5 rat aortic VSMCs cultured under basal and dysmetabolic conditions. The DNA-protein interactions that support ca. 90% of basal OPN promoter activity in VSMCs map to nucleotides -135 to -61 relative to the start site of transcription, encompassing cognates for Runt, Ets, bHLH, Krox/Sp1, Oct/POU, and AP1 factors. This 75 bp OPN promoter fragment -135 to -61 augments activity of the RSV minimal promoter ca. 8-fold, and also confers glucose responsiveness. No response to 25-hydroxycholesterol is observed. Gel shift analyses using extracts obtained from aortas of LDLR -/- mice fed chow vs. high fat diets reveal constitutive protein-DNA interactions at the POU/Oct and AP1 elements important for basal activity in A7r5 VSMCs. Thus, aortic calcification -- in response to physiologically relevant dysmetabolic stimuli -- is an active process, driven by the expression of powerful bone morphogens (BMP2), osteoblast transcription factors (Msx2), and bone extracellular matrix molecules (BSP, OPN). This process recruits osteoprogenitors from peri-aortic adventitial adipocytes or pericytes, valvular fibrosa cells, and medial VSMC populations. Hyperglycemia promotes expression of OPN in aortic medial VSMCs, augmenting transcription in part via DNA-protein interactions in the OPN promoter region -135 to -61.

Disclosures: Merck & Co.,5.

M053

A Gene Expression Profile for hBMP-2-Induced Endochondral Bone Formation in Mouse Quadriceps. B. M. Clancy,* J. D. Johnson,* D. D. Pittman.* Musculoskeletal Sciences, Wyeth-Ayerst Research/Genetics Institute, Andover, MA, USA.

Endochondral bone formation has been fairly well characterized from a morphological perspective and yet this process remains largely undefined at a gene transcriptional level. In vitro and in vivo studies have shown bone morphogenetic protein-2 (BMP-2) plays an important role in bone formation. In this study, the osteogenic activities of hBMP-2 protein were evaluated in mouse quadriceps. RNA samples from affected muscles were used to create a transcriptional profile of the bone formation process.Adult female C57BL/6J SCID mice were used in this study. Mice received a 50 ul intramuscular injection of either formulation buffer (control) or hBMP-2 protein (50 ug) in both quadriceps. On days 1,2,3,4,7, and 14 mice were euthanized and muscles were collected for either histological evaluations or an analysis of changes in gene transcription. Total cellular RNA was isolated from the entire muscle. Labeled mRNA solutions were prepared and hybridized to an oligonucleotide array designed to monitor 1,300 murine genes. Changes in gene expression associated with hBMP-2 were determined from time-matched comparisons between buffer and hBMP-2 samples.Histological evaluations of muscle sections from mice injected with formulation buffer showed no significant changes. In contrast, sections from hBMP-2 muscles showed morphological changes entirely consistent with endochondral bone formation by day 14. Moreover, x-ray images at day 14 revealed radio-opaque masses in the quadriceps of mice injected with hBMP-2. A gene expression profile was created with the compilation of all genes for which there were greater than four-fold changes at at least one time point. This list contains 217 genes, many of which have been previously associated with bone formation and showed significant increases in expression, e.g., aggrecan, Cbfa1, osteocalcin and collagens type II and X. In addition, there were at least 64 genes that have yet to be associated with bone formation. In conclusion, oligonucleotide arrays enabled a broader view of endochondral bone formation than has been reported to date. An increased understanding of the roles played by these gene products will improve our understanding of skeletogenesis, fracture repair and pathological conditions such as osteoporosis and osteopetrosis.

M054

Inhibition of Expression of Endogenous Genes Within Cells of the Osteoprogenitor Lineage. P. Liu, M. S. Kronenberg, D. W. Rowe. Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA.

Understanding the function of a gene, especially as it affects bone cell biology, requires methods to rapidly and consistently inhibit the output of that gene in a model system that can be adapted to assess the activity of the osteoblast lineage. We have previously found that the U1snRNA gene can be modified to change its primary role in RNA splicing to inhibit the output of a transfected target RNA probably by interfering with the polyadenylation of the transcript. (Beckley, S. A., et.al. Mol Cell Biol. 21, 2815-2825, 2001). This study was initiated to determine if the modified U1snRNA could inhibit the output of an endogenous target gene in commonly used osteoblastic cell lines.In the first case, the 10 bp donor sequence of U1snRNA was changed to one colinear with sequence in the terminal exon of the endogenous rat osteocalcin gene. ROS 17/2.8 cells were transfected and selected in hygromycin (present in vector backbone) and RNA from individual or pools of G418 resistant colonies were examined by Northern hybridization. In multiple experiments and clones, the level of the OC transcript was inhibited between 60-90% compared to cells transfected and selected with the parental U1snRNA construct. Expression of type I collagen was unaltered. The inhibitory effect was also apparent in transiently transfected ROS 17/2.8 cells.A second series of experiments were performed in MC3T3 E1 cells. U1snRNA was modified to recognize a sequence in the terminal exon of the murine Runx2 gene. After transfection and hygromycin selection, individual clones or pools of resistant clones were examined under culture condition optimal for bone nodule formation. The cultures derived from the U1antiRunx2 transfected cells grew more slowly than control cultures and their morphology became fibroblastic in appearance. The Runx2 inhibited cultures failed to develop alkaline phosphatase (AP) staining while the control cultures are strongly AP positive. Northern analysis showed a 60-80% reduction in Runx2 expression. Gel mobility shifts with nuclear extracts of these cells showed a loss of binding activity for a Runx2 consensus oligonucleotide relative to control levels. Endogenous osteocalcin expression was completely inhibited in the Runx2 depleted cells and this effect remained after multiple passages of the transfected cell lines. These exploratory experiments suggest that a modified U1snRNA could be used to assess the role of genes crucial to molecular pathways that control the expansion and differentiation of the osteoprogenitor lineage.

M055

Macrophages at the Skeletal Tissue/Device Interface of LoosenedProsthetic Devices Express a Range of Bone-Related Genes and their Products. H. Zreiqat,*[1] B. MArkovic,*[1] B. Zicat,*[2] C. R. Howlett.*[3] [1]School of Pathology, University of New South Wales, Sydney, Australia, [2]Mater Misericordiae Hospital, Sydney, Australia, [3]Pathology, University of New South Wales, Sydney, Australia.

Aseptic loosening of cemented arthroplasty is the most common reason for implant failure in adult orthopaedic reconstruction. At the interface of aseptic loosened prostheses there is an abundance of particle-activated macrophages and other inflammatory cells. The role of macrophages in bone and connective tissue healing in this pathological condition is poorly understood. In situ hybridization and immunohistochemistry were performed to examine molecular signalling by the mesenchymal cells and the mononuclear inflammatory cells that reside in the interfacial tissues between the cement and the bone of an aseptically loosened joint prosthesis. A range of matrix collagenous and non-collagenous matriceal proteins, including osteopontin, osteocalcin, bone sialoprotein and type I collagen were produced not only by osteogenic cells but also, unexpectedly, by foamy macrophages found embedded in the dense fibrous tissue and in the pseudomembrane closely apposed to the device. In contrast, mesenchymal cells in the dense fibrous tissue zone between the pseudomembrane and the osteogenic layers failed to express such mRNAs and proteins. To the authors knowledge, this study is the first to report the expression of bone-related genes and proteins in this critically important setting of bone healing and repair.

M056

Differences in Phenotype Shown in Endothelial NOS and Neuronal NOS Knockout Mice. N. Moradi-Bidhendi,Miss,,[*1] L. Mancini,Dr,,[*1] M. C. O'Shaughnessy,Miss,,[*2] P. Forte,Dr,,[*1] P. L. Huang,Dr,,[*3] L. D. Buttery,Dr,,[2] J. M. Polak,Professor,,[2] N. Benjamin,Professor,,[*1] I. MacIntyre,Professor,.[1] [1]William Harvey Research Institute, London, United Kingdom, [2]Imperial College School of Medicine, London, United Kingdom, [3]Harvard Medical School, Boston, MA, USA.

Nitric oxide (NO) has been implicated in the local regulation of bone metabolism. The contribution made by specific NO synthase (NOS) enzymes is unclear. The results obtained using NOS inhibitors have been inconsistent probably arising from their lack of isoform-specific selectivity. We have studied endothelial NOS (eNOS(-/-)) and neuronal NOS (nNOS(-/-)) knockout mice to help clarify this situation and to define more clearly the contribution made by a specific NOS isoform.In our studies we have found that mouse NOS isoform knockout phenotypes differ markedly. eNOS (-/-) knockout mice are hypertensive, transiently osteoporotic and have oestrogen-resistant osteoblasts. nNOS (-/-) mice are hyper-aggressive and their total NO output is only 8% that of intact animals. eNOS (-/-) knockout mice (with intact nNOS) show a normal increase in urinary nitrate with oestrogen demonstrating that oestrogen stimulates nNOS. This finding suggests the possibility that the effect of oestrogen on the central nervous system may be NO-dependent. The differences observed in this study provide clear information on the important roles of both eNOS and nNOS isoforms.

M057

The Effect of a Vitamin K Supplement on Bone Loss Induced by Skeletal Unloading. M. Thierry-Palmer,*[1] M. Pasquali,*[2] P. Hatch,*[3] S. B. Arnaud.*[3] [1]Biochemistry, Morehouse School of Medicine, Atlanta, GA, USA, [2]Pediatrics, Emory University, Atlanta, USA, [3]Ames Research Center, Moffett Field, USA.

Deficiency of vitamin K has been implicated in the pathogenesis of osteoporosis (Vermeer et al, J. Nutrition 1996). To determine the potential of a vitamin K supplement to prevent the loss of bone from skeletal unloading, we fed mature Sprague Dawley male rats diets enriched with menaquinone-4 (0.8 mg/kg diet) during 4 weeks exposure to a space flight model (HKS). Controls were fed the same food (AIN-76A) with (HKC) or without added vitamin K (NKC and NKS). Rats were housed in metabolic cages and weekly 24 hr urine specimens collected for collagen cross-link analyses by a chromatographic method to estimate bone turnover. Average body weights of all 4 groups were similar at the start (435 g). At the end of the study, HKC weighed more than NKC (468 ± 18 vs. 450 ± 12 g, $p<0.05$). Body weights in HKS and NKS were similar (469 ± 20 vs. 461 ± 29 g). Heart, liver, spleen, kidney, and adrenal weighed the same in both control groups. However, the livers in NKS and HKS were larger than controls. Other organ weights were similar. Mineral content of the femurs in NKC and HKC was similar (0.51 ± 0.03 vs. 0.54 ± 0.04 g) and greater ($p<0.001$) than that of the unloaded femurs (NKS and HKS). There were no differences in NKS and HKS femoral bone mineral content (0.44 ± 0.02 vs. 0.44 ± 0.02 g). Although HKS and NKS showed similar losses of mineral from the unloaded femur, excretion of urinary deoxypyridinoline did not significantly differ from that of ambulatory controls (HKC and NKC) on day 7 or day 28. Vitamin K supplementation, thus, had no effect on the loss of femoral bone mineral content or the gain in liver weight of suspended compared with ambulatory rats. Assuming that vitamin K absorption was unaffected by the space flight model, these data indicate that this level of vitamin K supplementation does not prevent or reduce bone loss induced by skeletal unloading in mature rats.

M058

hPTH (1-38) Stimulation of Bone Resorption in Murine Calvariae Is Mediated in Part by Its Ability to Decrease OPG Secretion. R. J. S. Galvin, T. R. Fuson,* X. Yang,* A. K. Harvey, S. Chandrasekhar. Lilly Research Labs, Indianapolis, IN, USA.

Osteoprotegerin (OPG) plays a critical role in osteoclast differentiation and function by acting as a decoy receptor to receptor activator for NF-κB ligand (RANKL). Previous *in vivo* and *in vitro* studies have demonstrated that OPG mRNA expression is suppressed by PTH. The purpose of this study was to evaluate the effects of hPTH (1-38) on bone resorption and OPG protein synthesis and secretion in the complex calvarial model of bone resorption. The calvariae were isolated from Swiss Albino ICR mice (aged 3-5 days) and cultured in DMEM containing 15% horse serum, 2.8 mM L-glutamine, 100 units/ml penicillin, 100 ug/ml streptomycin, 10 units/ml sodium heparin, and 200 ng/ml indomethacin in sealed tubes with a 50% O_2, 5% CO_2, and 45% N_2 atmosphere. The calvariae were preincubated (washout) for 24 h in control medium prior to treatment. Following a 24-72 h of treatment with hPTH (1-38) in the presence or absence of OPG, the culture medium was removed and ionized calcium (Ca^{2+}) and pH were evaluated using a Ciba-Corning Ca^{2+}/pH analyzer. In some experiments medium OPG levels were evaluated by an ELISA, using OPG-specific antibodies. hPTH (1-38) treatment increased Ca^{2+} release to the medium 1.5 to 2.5 fold following 72 h of treatment and this increase was blocked with salmon calcitonin confirming that the effects on Ca^{2+} release are the result of osteoclastic bone resorption. Bone resorption was stimulated by hPTH (1-38) in a time and concentration-dependent manner (EC_{50} of approximately 2 nM). Exogenous OPG blocked the hPTH (1-38)-induced Ca^{2+} release in a concentration dependent manner with an IC_{50} of approximately 700 pM. The pH of the medium decreased with increasing concentrations of hPTH (1-38). Further, treatment with hPTH (1-38) resulted in a concentration-dependent decrease in OPG synthesis and secretion with an approximately 6-fold decrease at 10 nM hPTH (1-38). In summary, these results demonstrate that OPG inhibits hPTH (1-38)-induced bone resorption in neonatal mouse calvariae and that hPTH (1-38) inhibits OPG release to the culture medium. Since OPG functions as a decoy receptor for RANKL, decreasing OPG secretion would make more RANKL available to support osteoclast differentiation and resorption. The mechanisms by which PTH stimulates bone resorption in the calvarial model are mediated in part by decreased OPG secretion.

M059

Novel Porous Hydroxyapatite Ceramics Exhibited Excellent Osteoconduction through Inter-pore Connections. N. Tamai, K. Kuriyama, A. Myoui, T. Nakase, T. Tomita,* T. Ochi,* H. Yoshikawa. Orthopaedic Surgery, Osaka University Medical School, Suita,Osaka, Japan.

Calcium Hydroxyapatite Ceramics (CHA) have been clinically used as a bone substitute. However, most of them failed to be fully incorporated in host bone even after several years of implantation, probably because of its poorly organized structure and the limited connectivity between pores. Recently, we successfully developed a fully inter-connected porous hydroxyapatite ceramics (IP-CHA) by adopting "foam-gel" technique which utilizes foaming reagent to yield uniform bubbles in slurry and cross-linking reagent to stabilize the foamy structure. Structural analysis by scanning electron microscopy (SEM) revealed that IP-CHA had even sized spherical pores which were interconnected by window-like holes in their walls. The surface of the wall was smooth and hydroxyapatite particles were bound tightly to one another. According to the results of mercurry porosimetry, most of the inter-pore connections of IP-CHA ranged from 10 to 80 mm in diameter(average; 40 mm), which are ideal size for cells to pass through. The porosity of IP-CHA was 75%, and 90% of the pores were effectively connected each other by interconnections more than 10mm in diameter. When a cylindrical IP-CHA (diameter; 6 mm, height; 15 mm) was implanted into a rabbit femoral condyle, mineralized bone and bone marrow with abundant vessels formed deep in the pores through the inter-pore connections. Within a period of 6 weeks, such tissue penetration reached as deep as 3mm from the surface of the IP-CHA implant. Initial compression strength was 12MPa which is equivalent to that of cancellous bone. Of note, a compression test at 9 weeks revealed that the implanted IP-CHA steadily increased in strength to more than three times the value of the initial test. These results indicate that fully opened inter-pore connections allowed cell tissue penetration into the deeper pores of IP-CHA, which in turn, accomplished a superior osteoconduction. In future, artificial organs would be in need of the combination of biomaterials and tissue engineering. Taking the advantage of IP-CHA into consideration, IP-CHA would be very useful for artificial bone tissue engineering as a scafold, into which osteogenic cells, growth factors or genes could be introduced for its fully opened interconnectivity.

Disclosures: NEDO, JAPAN,2.

M060

Perichondrium Acts to Induce Apoptosis in Epiphyseal Chondrocytes in In Vitro Organ Cultures But Its Proapoptotic Activity Is Blocked in In Ovo Organ Cultures. Y. Maeda,* M. Noda. Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan.

Perichondrium (PRC) has been suggested to suppress differentiation and proliferation of chondrocytes during endochondral bone formation based on previous in vitro organ culture studies in which removal of diaphyseal and metaphyseal PRC from embryonic chick tibiae resulted in over-growth of epiphyseal cartilage. However, these in vitro organ cultures did not allow the growth of diaphyseal bone collar and thus the effect of PRC in normal environment where bone collar development is taking place has not yet been fully understood. Furthermore, no mechanism has been known regarding the perichondrium function. Therefore, we examined the effect of PRC on the cartilage development using chicken long bone organ cultures on chorio-allantoic-membrane (CAM) in ovo and further investigated the mechanism of the regulation by PRC. Embryonic chick tibiae were cultured with intact PRC or without PRC on chick embryonic CAM which supported bone collar development. In this system, we were able to examine the effect of PRC removal in a condition where chondrocytes were in a more physiological condition and bone collar could develop. After the cultures, tibiae were subjected to histological analysis. TUNEL assay and TRAP activity assay were conducted by using these bones. In contrast to the previous reports using in vitro organ cultures, removal of PRC did not result in over-growth of epiphyseal cartilage in in ovo organ cultures. Although the development of the bone collar in the absence of PRC was slightly less than that in the presence of PRC, bone collar formation, which was not observed in in vitro cultures even in the presence of PRC, was clearly observed in the organ cultures on CAM. TUNEL assay indicated the presence of significantly high levels of apoptosis in the epiphyseal chondrocytes in in vitro organ cultures in the presence of intact PRC. Strikingly, no such apoptosis was observed in the epiphyseal chondrocytes of bones in in vitro organ cultures when PRC was removed. Furthermore, such difference was no longer observed in the epiphyseal chondrocytes in bones organ cultured in ovo, as virtually no apoptosis was observed in the epiphyseal chondrocytes regardless of the presence or absence of PRC. These results indicated that the formerly-suggested PRC derived activity that suppressed cartilage development is to induce apoptosis in the epiphyseal chondrocytes in vitro. This proapoptotic activity could be counter-balanced in ovo where bone collar development coexists. Thus, chondrogenesis in long bone is coordinately regulated by both PRC and possibly by adjacent bone collar through the modulation of apoptosis in the chondrocytes.

M061

Accelerated Apoptosis and Suppressed Proliferation of Chondrocyte Associated with the Aberrant Cartilage of Klotho Mutant Mice. Y. Asou,*[1] N. Amizuka,[2] K. Kashimada,[1] T. Yamashita,[1] Y. Nabeshima,*[3] H. Ozawa,*[2] M.

Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Niigata University, Niigata, Japan, [3]Kyoto University, Kyoto, Japan.

Klotho mutant mice show abnormal morphology of growth plate and ectopic ossification in joint cartilage. These observations suggest that klotho protein may regulate the proliferation and/or differentiation of chondrocytes. To investigate the functions of klotho protein in the maturation of chondrocytes both in the growth plate and in the joint, we examined the cartilage of klotho mutant (kl/kl) mice immunohistochemically. 8-9 weeks old wild type (WT) and kl/kl mice were examined histomorphometrically or immmunohistochemically. To evaluate the presence of vessels, sections were stained immunohistochemically using anti CD31 antibody. For the evaluation of proliferation, anti-PCNA antibody was used to stain the section. For the evaluation of apoptosis in chondrocytes, TUNEL assay was performed. Hypertrophic chondrocytes in the growth plate indicated apoptosis signals in both of the genotypes, however, the number of apoptotic cells was more in kl/kl mice. In the resting zone, apoptotic cells were few in WT mice, whereas in kl/kl mice, numerous apoptotic cells were observed in resting zone and even in proliferating zone. Examination on the proliferation of chondrocytes indicated that PCNA expression in the growth plate was reduced in kl/kl mice. These observations on accelerated apoptosis and impaired proliferation could explain the abnormal morphology of the chondrocytes in the growth plate of kl/kl mice including the reduction in the number of chondrocyte columns and the width of proliferating zone and impaired maturation of hypertrophic chondrocytes. Evaluation on the angiogenesis revealed that the number of CD31 positive vessels at the end of hypertrophic zone was decreased in growth plate of klotho mutant mice. This observation suggests that angiogenic activity was suppressed by the absence of klotho protein. The impaired angiogenesis could result in the abnormally wide and irregular shape of the primary trabeculae in kl/kl mice. At the joint region, apoptosis of chondrocytes was also accelerated especially in the deep layer of joint cartilage in kl/kl mice, suggesting relationship to the previous reported ectopic ossification in the joint cartilage of kl/kl mice. These phenotypes appear to resemble those of osteoarthritis. Our observations suggest that klotho protein regulate survival and proliferation of chondrocytes both in growth plate and joint cartilage.

M062

Modifying Proteins for an Increased Bone Affinity. H. Uludag,[1] J. Yang,[1] T. Gao,[1] N. Kousinioris,[1] G. Wohl,*[2] R. Zernicke.*[2] [1]University of Alberta, Edmonton, AB, Canada, [2]University of Calgary, Calgary, Canada.

Growth factors are endogenous proteins capable of stimulating new bone formation when delivered locally (i.e., into a bony site) or systemicly (i.e., when delivered into the circulation). The therapeutic benefit of the growth factors could be significantly increased if they are delivered specifically to bones. For this to happen, it is critical that the proteins exhibit a significant affinity to bone. This could be achieved by derivatizing proteins with ligands that exhibit a high bone affinity (e.g., bisphosphonates). To demonstrate the feasibility of this approach, 1-amino-1,1-diphosphonate methane (aminoBP) was conjugated to a model protein, albumin. The conjugation was performed by (1) converting the amino group of aminoBP to a thiol group using 2-iminothiolane, (2) derivatizing the albumin amino groups with a thiol-reactive sulfosuccinimidyl-4-(N-maleimidomethyl)-1-cyclohexane carboxylate, and (3) reacting the derivatized albumin with thiolated aminoBP. One to 4 aminoBP molecules per albumin were typically obtained as a result of the described chemistry. The conjugated albumin exhibited a high affinity to hydroxyapatite that was proportional to the extent of conjugation. The conjugates were shown to exhibit a high affinity to bone matrix in vitro in a serum-containing medium. One bound to bone matrix, the conjugates were found to desorb slower than the unmodified albumin, especially from bone whose organic matrix was removed by ashing. The bone affinity of the modified protein was tested in normal and ovariectomized (OVX) Sprague-Dawley rats. Initial (3 hour) retention of BSA and aminoBP-BSA were found to be equivalent when injected into the medullary cavity of tibia. After 1 day, an 8- and 12-fold higher tibiae retention of the protein was obtained in normal and OVX rats as a result of aminoBP conjugation. A similar result (~12-fold difference) was also obtained in OVX rats after 3 days. We concluded that BP conjugation to BSA imparted a high bone affinity and enhanced bone-retention of proteins in normal and OVX rats. This technique can be used to engineer growth factors with a high affinity to bone. Growth factors with a high affinity to bone could serve as novel therapeutic agents for local and systemic stimulation of bone formation.

Disclosures: Innovative Medicines Inc.,4.

M063

Biglycan Deficient Mice Have Delayed Bone Formation after Bone Marrow Ablation. X. Chen,[1] M. R. Allen,*[2] S. A. Bloomfield,[2] T. Xu,*[1] M. F. Young.[1] [1]CSDB, NIDCR, Bethesda, MD, USA, [2]Department of Health & Kinesiology, Texas A&M University, College Station, TX, USA.

Biglycan (bgn) is a small leucine rich proteoglycan (SLRP) that is enriched in bone and other skeletal connective tissues. Previously, we generated bgn-deficient (knockout, KO) mice and showed that they developed age-dependent osteopenia. Further in-vitro analysis showed that bone marrow stromal cells isolated from bgn deficient mice had multiple defects including increased apoptosis, reduced numbers of colony forming units (CFUf) as well as decreased collagen mRNA and protein. In the present study, we tested the hypothesis that bone formation capability in response to a physiological stress was compromised in bgn-KO mice. To test this hypothesis, we used a bone marrow ablation assay to evaluate the effect of the bgn deficiency on bone formation in vivo. Bone marrow ablation was used because it is a highly reproducible way to evaluate bone formation and resorption in a relatively short time span in vivo. Six-week old wild type (wt) and bgn-KO mice were first anesthetized, and a 25G needle was introduced into the marrow cavity at the intercondylar regions of the femora, and the marrow was flushed with sterile PBS. The mice were sacrificed at day 7, 10, and 17 postsurgery, and the radiograph of femur was taken by Faxitron X-ray, and the bone mineral density (BMD) was measured in vivo at the proximal, midshaft, and distal femur using peripheral quantitative computed tomography (pQCT; Stratec Research M; Norland Corp.). On each animal, the right femur was used for the marrow ablation, while the left femur was untreated and served as an internal control. The data were presented as ratio (ablation/control) of the total trabecular BMD. The radiographs clearly showed that bone marrow ablation in femora induced vigorous new bone formation in the animals within 10 days, which was followed by rapid bone resorption in the next 7 days where the regenerated trabecular bone marrow returned to normal levels. The results of pQCT suggested that wt and bgn-KO mice had similar bone densities at day 7 and day 17 post-surgery in all 3 sites. However, at day 10 post-surgery the trabecular BMD in the midshaft femur was significantly higher in wt (2.1 ± 0.32) than bgn-KO (1.3 ± 0.21). From these data, we conclude that the absence of bgn caused impaired bone formation, but had no significant effect on bone resorption. These results support our previous observation that the defective bone marrow stromal cells from the bgn-KO may contribute to diminished bone formation and, further, provides insights about the role of bgn in bone tissue structure and function in vivo.

M064

Over Expression of Bone Sialoprotein Enhances Nodule Formation and Calcification in Vascular Smooth Muscle Cells. S. Harrington, T. O'Brien,* L. A. Fitzpatrick. Endocrine Research Unit, Department of Medicine, Mayo Clinic, Rochester, MN, USA.

The role of bone matrix proteins in the process of ossification is not fully elucidated. Vascular smooth muscle cells (VSMCs) are an integral part of the atherosclerotic plaque and are capable of producing markers of bone formation including alkaline phosphatase, osteopontin, osteocalcin, bone sialoprotein and type I collagen. Calcification within an atherosclerotic plaque is an active and regulated process with similarities to ossification in bone. Bone matrix proteins play an integral role in both processes. We explored the possible role of 2 matrix proteins, osteopontin (OPN) and bone sialoprotein (BSP) in the process of calcification by over-expression of these 2 proteins in VSMCs. VSMCs were isolated from the coronary arteries of sexually mature pigs. A replication defective adenovirus (AD) human serotype 5 was constructed with either OPN (AD-OPN), BSP (AD-BSP), or null (AD-Null) inserts driven by a CMV promoter. VSMCs were infected and maintained for 26 days in DMEM. VSMCs were fixed and stained for calcium using the Von Kossa method and nodules and calcium were quantified. Cultures infected with AD-BSP showed a 3-fold increase in nodule number compared to AD-OPN or control ($p<0.00005$). The amount of calcium in AD-BSP was 12 times greater than in AD-OPN or control ($p<0.02$). These findings suggest that BSP over-expression contributes to increases in both nodule formation and calcification and provide evidence for a unique role of BSP in ossification associated with atherosclerotic plaque development.

M065

Osteopontin-Deficiency Suppress Joint Swelling, Chondrocyte Apoptosis and Joint Destruction in Antibody-Induced Rheumatoid Arthritis Model in Mice. K. Yumoto,[1] M. Ishijima,[1] S. R. Rittling,[2] K. Tsuji,[1] Y. Tsuchiya,*[3] S. Kon,*[3] A. Nifuji,[1] T. Uede,*[4] D. T. Denhardt,*[2] M. Noda.[1] [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Rutgers University, Piscataway, USA, [3]IBL, Gumma, Japan, [4]Hokkaido University, Sapporo, Japan.

Osteopontin is expressed in synovial cells in rheumatoid arthritis and also in chondrocytes. However, the function of osteopontin in the pathogenesis of rheumatoid arthritis has not yet been elucidated. To investigate the roles of osteopontin in the pathogenesis of the inflammatory process that leads to destruction of articular cartilage in rheumatoid arthritis, we examined the effect of osteopontin-deficiency on antibody-induced arthritis in mice. Anti-type II collagen antibody cocktail /LPS injection induced swelling in fore and hind limb paw joints in wild type mice within 7days. Arthritis score reached up to 12 as reported previously. In contrast, such swelling in joints induced by anti-type II collagen antibody / LPS injection was significantly suppressed in OPN -/- mice, where arthritis score did not exceed 4. Toluidine blue staining of the articular cartilage was markedly reduced by antibody-cocktail /LPS injection in wild type mice indicating loss of proteoglycan and destruction of cartilage matrix. In contrast, such histological changes were not observed at all in OPN -/- mice after the induction of arthritis. Scanning electron microscopic survey demonstrated that articular chondrocytes were destroyed all over the joint surface in wild type mice after induction of arthritis by injection with antibody / LPS, however, no such destruction was observed in OPN -/- mice even after the antibody cocktail /LPS injection. To understand the mechanism underlying the resistance to destruction of articular cartilage even after induction of arthritis by antibody-cocktail /LPS injection in the OPN -/- mice, TUNEL assay was conducted. The levels of apoptosis in the chondrocytes in articular cartilage in the wild type mice were enhanced in the arthritic joints induced by antibody cocktail /LPS injection (12.9 +/- 2.6). In contrast, the levels of apoptosis were significantly suppressed in OPN -/- mice even after the antibody-cocktail /LPS injection (2.3 +/- 0.5, $p<0.05$). These results indicated that OPN plays a critical role in the promotion of articular cartilage destruction in the rheumatoid arthritis mouse model by increasing apoptosis in chondrocytes.

M066

Bone Matrix Production in Human Bone and Soft Tissue Tumors – An Immunhistochemical Approach. B. Knoblauch,*[1] B. Loreth,*[1] A. Battmann,*[1] U. Stahl,*[1] L. Fisher,[2] A. Schulz.[1] [1]Institute of Pathology, Justus Liebig University, Giessen, Germany, [2]NIDH, Bethesda, USA.

Osteosarcomas produce an extracellular matrix (ECM), the so called tumor osteoid.

This osteoid is also the microscopic hallmark of these tumors. It is composed of collagenous proteins like collagen type I and non-collagenous bone proteins like osteopontin, osteonectin or osteocalcin. The distinction of tumor osteoid from other formations of the ECM in intra- and extraskeletal bone and soft tissue tumors and tumor like lesions can be very difficult. Special osteologic stainings can increase the reliability of the diagnosis, but do not identify the type of tumor conclusively. Those stainings reflect physiochemical features of the extracellular matrix but do not identify its specific molecular components. In this study, the immunohistochemical application of specific antibodies against molecular bone matrix components is tested as additional technique to identify specific ECM components in bone and soft tissue tumors.107 benign and malignant primary bone and soft tissue tumors and tumor like lesions of bone, including 35 osteosarcomas, have been analysed. For visualisation, standard immunohistochemical protocols using the APAAP and DAB technique had been used. A panel of polyclonal and monoclonal antibodies against COL-I-C-peptide, osteopontin (OPN), osteonectin (ON), osteocalcin (OC) and decorin was applied.The biochemical composition of the bone matrix in benign and malignant primary bone and soft tissue tumors and tumor like lesions of bone is reflected by the corresponding immunhistochemical results. The tumor matrix of osteoblastic bone tumors and tumor like lesions show an identical pattern of matrix immunohistochemistry. Soft tissue tumors contain only single components of the osteoid matrix, but do not show the typical pattern of immunohistochemistry seen in bone cell derived tumors.In conclusion, immunohistochemistry of bone matrix proteins using polyclonal and monoclonal antibodies against COL-I-C-peptide, osteopontin (OPN), osteonectin (ON), osteocalcin (OC) and decorin is a useful tool for the differential diagnosis of the osteoid matrix in matrix producing bone and soft tissue tumors and tumor like lesions of bone.

M067

Acid Phosphatase Deficiency Leads to Accumulation of Osteopontin in the Subosteoclastic Resorption Area. V. Everts,[1] A. Suter,*[2] K. von Figura,*[2] W. Beertsen,[3] P. Saftig.*[2] [1]Cell Biology, AMC, Amsterdam, The Netherlands, [2]University Goettingen, Goettingen, Germany, [3]ACTA, Amsterdam, The Netherlands.

Bone-resorbing osteoclasts are characterized by the expression of high levels of acid phosphatase, in particular the tartrate-resistant isoform: TRAP. Yet, the function of this enzyme is still unclear. In an attempt to elucidate its role in osteoclastic bone resorption, bones from mice deficient in lysosomal acid phosphatase (LAP, Acp1) and/or TRAP (Acp5) were analysed. Metacarpal and calvarial bones of 5 d old wild type, single or double knock-out mice were collected and fixed in 4% paraformaldehyde with or without 1% glutaraldehyde in 0.1 M sodium cacodylate buffer. The bones were embedded in epoxy resin or LR-white and processed for light and electron microscopic examination and for immunolocalization of osteopontin. Microscopic examination revealed in osteoclasts of all phosphatase deficient mice the presence of large intracellular vacuoles filled with fine filamentous material. In osteoclasts of TRAP -/- and LAP/TRAP -/- mice these vacuoles contained also mineral crystallites. Immunolocalization studies revealed that the latter vacuoles also contained osteopontin. This protein was also found in the extracellular space adjacent to the ruffled border of osteoclasts. Quantitative evaluation revealed that the highest level of osteopontin immunolabeling was found adjacent to osteoclasts of the double knock-out mice. Our findings indicate that acid phosphatase deficiency results in an accumulation of osteopontin in and adjacent to osteoclasts. The present observations provide direct evidence for a role of TRAP (possibly in conjunction with LAP) in osteoclastic bone resorption. We propose that TRAP (and LAP) dephosphorylates osteopontin during bone resorption and that an insufficient processing results in an extra- and intracellular accumulation of this protein and associated mineral crystallites.

M068

Sites of Enzyme Synthesis Can Be Distinguished from Sites of Enzyme Activity by the "Two Domain Approach", as Demonstrated by Immunohistochemical Localization of Gelatinase B and Collagenase-3 in Growing Rat Tibia in Areas Undergoing Cartilage Resorption. E. R. Lee,[1] L. Lamplugh,*[1] B. Kluczyk,*[1] J. S. Mort,*[2] G. Murphy,*[3] C. P. Leblond.*[1] [1]Electron Microscopy Unit, Shriners Hospital for Children, Montreal, PQ, Canada, [2]Joint Diseases Laboratory, Shriners Hospital for Children, Montreal, PQ, Canada, [3]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom.

Since proteases are usually synthesized as proenzymes bearing a propeptide domain, antibodies can be prepared against the propeptide (anti-propeptide domain antibodies) and used to detect the proenzyme in tissues by immunostaining, presumably at its cellular source. Since removal of the propeptide results in functional activation, antibodies against the new N-terminus (now present as the catalytic domain), can be used to detect, by immunostaining, the sites in which the enzyme exerts its activity (anti-activated or catalytic domain antibodies). This "two domain approach" was used to identify the synthesis and activity sites of two matrix metalloproteinases: gelatinase B (MMP-9) and collagenase-3 (MMP-13). Thus, the two types of antibodies were prepared for each enzyme and each was applied to frozen tissue sections from 5-day old rat tibial epiphyses, that is, in animals from an age when cartilage is actively resorbed for secondary ossification. In general, the immunostaining results were similar for both enzymes whether the anti-propeptide or the anti-activated antibodies were applied. Thus anti-propeptide antibodies showed that, next to every cartilage site committed to resorption, a mononuclear cell identified as "septoclast" was immunostained by both antibodies and was, therefore, taken to be the source of both enzymes. As for the anti-activated antibodies, they revealed that both enzymes were active in cartilage resorption sites, such as: a) the blind end of canals dug into the epiphysis for the entry of blood vessels; b) the last transverse septae of the primary growth plate in the zone of vascular invasion where cartilage is resorbed for endochondral bone formation. In conclusion, the two enzymes are synthesized by mononuclear cells and, after activation, are localized in sites destined for cartilage resorption. It is proposed that, in these sites, they cooperatively degrade type II collagen and thus participate in the critical transition from cartilage to bone.

M069

Gelatinase Activity in Synovial Fluid of Degenerative Joint Disease Resulting from Canine Elbow Dysplasia. S. W. Volk,*[1] A. S. Kapatkin,*[1] M. D'Angelo.[2] [1]Clinical Studies School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA, [2]Anatomy and Cell Biology, Temple University, Philadelphia, PA, USA.

Matrix metalloproteinases (MMPs), a group of zinc-dependent endopeptidases, have been shown to be synthesized intraarticularly in cartilage damage associated with joint disease. In this study we measured the activity of MMPs in synovial fluid obtained from a dog examined for elbow dysplasia and compared this MMP activity to progression of degenerative joint disease (DJD) of the elbow as documented by radiographs, computed tomography and arthroscopy from age 8 through 20 months. Synovial fluid samples were obtained from anesthetized patients prior to surgical procedures (arthroscopy or arthrotomy) or from research dogs with no arthritis or cartilage abnormalities at the time of sacrifice for reasons unrelated to this project. Utilizing casein and gelatin-containing polyacrylamide gels, MMPs were identified based on molecular weight, substrate specificity and western blot analysis. A 62kD MMP was found to be present in synovial fluid from all samples but increased in samples obtained from joints with known DJD. A doublet at 88kD was induced in synovial samples from osteoarthritic joints. Western blot analysis confirmed that these were gelatinases, MMP 2 and MMP 9, respectively. Specific MMP activity profiles did not differ in synovial samples obtained from the elbow dysplasia compared to osteoarthritis secondary to other disease processes. Although imaging studies documented progressive disease in our research animal with elbow dysplasia, zymography failed to reveal a correlation between the extent of disease and absolute amount of MMP activity. This study confirms that higher levels of active gelatinases are present in synovial fluid from dogs with osteoarthritis, indicating important parallels between human and canine osteoarthritis. Though further research is required to investigate the role of MMP activation during the progression of joint diseases, these studies support the hypothesis that therapeutic agents targeting specific MMP activities can be used as a clinical treatment option for patients with osteoarthritis.

M070

DDR2, a Collagen Receptor, Interacts with Jab-1 and Activates MMP1 Promoter Through AP-1. A. Hishiya,* K. Mizuno,* Y. Niikura,* A. Sasaki,* K. Ikeda, K. Watanabe. Dept of Geriatric Res, Natl Inst for Longevity Sci, Obu, Japan.

Recognition of collagen matrix is essential for osteoblastic differentiation and function. It has been recognized that alpha2beta1 integrin on osteoblasts plays a central role in their recognition of and adhesion to collagen matrix. Discoidin domain receptor (DDR)-1 and -2 have been identified as a novel class of collagen receptors, which belong to a receptor tyrosine kinase family. It has recently been reported that DDR1 knockout mice exhibit severe skeletal defects. We observed that DDR2 is also expressed on osteoblasts and that its expression is induced by BMP, raising a possibility that DDR2 may play a role in osteoblast differentiation and/or function. Treatment of DDR2-expressing cells with collagen upregulated the expression of MMP1 with an unusually slow and sustained kinetics, however, molecular mechanism of DDR-mediated intracellular signal transduction remains unknown. In order to clarify the signaling pathway(s) downstream of DDR2, a reporter construct, MMP1-luc, was made by cloning a 0.5-kb promoter region of the mouse MMP1 gene into pGL3-basic. When full-length DDR2 was overexpressed in human embryonic kidney cell line 293, an elevated luciferase activity was observed. The MMP1-luc construct contains two Ets-binding sites and a proximal AP-1 motif. When the Ets-binding sites were deleted (with an intact AP-1 motif), DDR2-dependent transcription activation was still observed. DDR2 also activates AP-1-luc reporter, suggesting that DDR2 activates MMP1 promoter via AP-1. To further elucidate the molecular mechanism of AP-1 activation by DDR2, we carried out yeast two-hybrid screening using the cytoplasmic domain of DDR2 as bait. A Jun-associated AP-1 activator, Jab-1, was isolated as a positive clone. When expression vectors for DDR2 and Jab1 were introduced into 293 cells, Jab1 was coprecipitated with DDR2. These results suggest that the cytoplasmic domain of DDR2 interacts with Jab-1, thereby activating target genes, such as MMP-1, through AP-1.

M071

Enhanced Bone Mineralization in Plasminogen Activator-Deficient Mice. E. Daci,* S. Torrekens,* R. Bouillon, G. Carmeliet. Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Katholieke Universiteit Leuven, Leuven, Belgium.

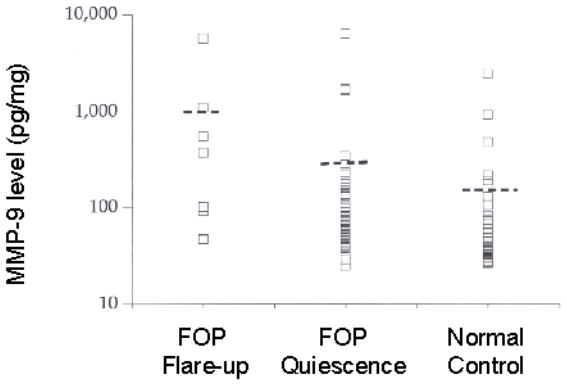
Proteolytic pathways are suggested to play a role in endochondral ossification. To elucidate the involvement of the plasminogen activators tPA and uPA in this process, the long bones of 1-week-old tPA-/-:uPA-/- and WT mice were analyzed morphologically and biochemically. The length of bones, as of the ossification centers, was significantly increased in plasminogen activator-deficient mice. Histomorphometric analysis of the proximal tibial metaphysis revealed a significantly increased trabecular bone volume (by 25%) in tPA-/-:uPA-/- mice compared to WT mice. Neither the morphology of the growth plate, nor bone vascularization, were altered in the plasminogen activator deficient mice. The morphological data, suggesting an enhanced bone mineralization in plasminogen activator-deficient mice, were confirmed by biochemical analysis of the long bone composition. The mineral fraction, osteocalcin and fibronectin content, were significantly increased in tPA-/-:uPA-/- bones. Real time quantitative RT-PCR showed higher osteocalcin mRNA levels in bones of

tPA-/-:uPA-/- mice. Previous results have shown no effect of plasminogen activator-deficiency in osteoclast formation and activity. We therefore investigated whether alterations in osteoblast proliferation, differentiation and mineralization are responsible for the enhanced bone mineralization in plasminogen activator-deficient mice. These processes were investigated in vitro in cultures of primary osteoblasts. Osteoblast proliferation, assessed by [^{3}H]-thymidine incorporation, was significantly increased in tPA-/-:uPA-/- cultures both in the presence or absence of fetal calf serum. Analysis of osteoblast differentiation, studied over a 4-week culture period, showed that in tPA-/-:uPA-/- osteoblast cultures, type I collagen content in the extracellular matrix, the osteocalcin secretion in medium and the deposition of calcium and phosphorus in the matrix, were significantly higher compared to WT osteoblasts. In addition, gene expression of collagen Iα1 and osteocalcin was significantly increased in tPA-/-:uPA-/- osteoblasts compared to WT osteoblasts. In conclusion, this study shows that combined deficiency of the plasminogen activators enhances bone mineralization in vivo, and suggests that the plasminogen activators inhibit osteoblast proliferation, in vitro mineralization, and the expression of osteoblast-specific markers.

M072

MMP-2 and MMP-9 Activity in Fibrodysplasia Ossifican Progressiva (FOP). Y. Wu,* P. C. Billings,* R. Patel,* E. M. Shore, F. S. Kaplan. Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, USA.

FOP is a disabling genetic disorder of progressive heterotopic ossification characterized by highly angiogenic pre-osseous fibroproliferative lesions which progress to form mature hetrotopic bone through an endochondral process. Although heterotopic angiogenesis is important in the pathogenesis of FOP lesions, the mechanism of heterotopic angiogenesis in FOP patients is not well understood. Matrix metalloproteinases (MMPs) are a family of Zinc-dependent endopeptidases. MMP-2 and MMP-9, two well characterized MMPs, have been shown to be critical for extracellular matrix degradation which is neccessary for endothelial cell invasion and for new capillary sprouting in wound healing and a variety of diseases. We hypothesized that MMP-9 and MMP-2 may also play a role in the angiogenesis of pre-osseous lesions in FOP. To test this hypothesis, spot urine samples from thirty-eight normal controls, forty three FOP patients during disease quiescence, and eight FOP patients during active disease flare-up were collected and assessed for MMP-9 and MMP-2 activity by zymography. The results were quantitated by densitometry scanning and normalized to urine creatinine. Urinary samples from FOP patients with flare-up exhibited the highest level of MMP-9 activity. Consistent with zymography, MMP-9 levels in FOP patients during flare-ups were significantly higher than those in normal controls as measured by ELISA (Fig.; $p<0.001$). Interestingly, MMP-2 levels were only significantly elevated in urine from the quiescent-phase patient group. Our data suggest that MMP-2 and MMP-9 may play a role in angiogenesis during the development of FOP heterotopic ossification and possibly serve as a marker for anti-angiogenic therapy.



M073

Development of the Mouse Cranium Is a Metamorphic Process Dependent on the Matrix Metalloproteinase MT1-MMP. K. Holmbeck,*[1] P. Bianco,*[2] K. Chrysovergis,*[1] H. Birkedal-Hansen.*[1] [1]Matrix Metalloproteinase Unit, Division of Intramural Research, National Institute of Dental and Craniofacial Research, Bethesda, MD, USA, [2]Dipartemento di Medicina Sperimentale e Patologia Anatomia Patologica, Universita La Sapienza, Rome, Italy.

Development of the mouse cranium is a tightly controlled process involving endochondral ossification at the base, and what is commonly known as membranous ossification at the vault. Here we describe a novel paradigm governing the removal of substantial unmineralized cartilaginous primordia of the embryonic mouse cranial vault and their coordinated replacement with bone of membranous origin. Detailed analysis of parietal bone development in wildtype and MT1-MMP deficient mice reveals that this bone, which develops (in part) from a soft connective tissue also relies on the formation, and subsequent efficient degradation, of a cartilaginous primordium. An integral feature of this process is the timely MT1-MMP dependent dissolution of unmineralized cartilage matrix. In this process a subset of chondrocytes undergo immediate programmed death while other cells actively divide upon release from a matrix that is undergoing degradation, and appear to differentiate into osteogenic cells, which migrate to the surface of the parietal bone. Disruption of the matrix dissolution in MT1-MMP deficient mice leads to arrest of this process, persistence of a cartilage vestige into adulthood and interference with cranial morphogenesis. Although cells in the cartilage vestige gradually die by apoptosis, other cells remain viable, synthesize DNA, and undergo cell division. The latter demonstrates that a dual fate of either demise, or survival and growth is slated for cartilage cells downstream of their release from the matrix. This process is normally linked to MT1-MMP activity, but is now blocked in MT1-MMP deficient mice. Together these findings suggest that cartilaginous primordia of the cranium, which precede membranous bone structures, are removed in a timely manner as part of the normal modeling and growth of the cranium via a process distinct from endochondral ossification. More importantly, cartilaginous primordia actively contribute to morphogenesis not only as topographical templates for the subsequent osseous cranium, but also as a reservoir of dividing cells which become topologically associated with osteogenesis. These data demonstrate a set of events in bone development of mammals, which is not accounted for by the endochondral/membranous paradigm, and fall in the realm of metamorphic processes common in post-natal development of other classes of vertebrates.

M074

Differences in the Degree to Which Osteoclasts from Various Parts of the Skeleton Employ MMPs and Cathepsin K for Bone Resorption. S. Shorey,*[1] J. N. M. Heersche.[2] [1]Pharmacology, University of Toronto, Toronto, ON, Canada, [2]Faculty of Dentistry, University of Toronto, Toronto, ON, Canada.

Osteoclasts (OCs) resorb bone by demineralization and subsequent degradation of the demineralized organic matrix. Degradation of the organic matrix involves the activity of cathepsin K and matrix metalloproteinases (MMPs). Current literature suggests that site-specific differences may exist in the skeleton with respect to resorption of bone by OCs: OCs derived from calvarial bone (intramembranous bone) have been reported to differ from OCs found in long bone (endochondral bone) with regard to the degree to which MMPs and cathepsin K are involved. Specifically, MMPs appear to be predominant in the degradation of bone matrix by calvarial OCs, while cathepsin K activity has been shown to be primarily involved in matrix degradation by long bone OCs.We have studied the involvement of cathepsin K and MMPs in the resorptive activity of OCs derived from intramembranous and endochondral bone by analyzing the effects of selective low molecular weight inhibitors of MMPs (CT-1746, CellTech, 10 μM) and a propeptide inhibitor of cathepsin K (PCK, 16 nM) on organic matrix degradation. OCs isolated from the scapula (intramembranous bone) and long bones (endochondral bone) of newborn rabbits were cultured for 48 hours on bovine cortical bone slices in the presence or absence of inhibitors. OCs were counted after TRAP staining. The addition of inhibitors had no effect on OC numbers. Resorption pits were stained using an anti-collagen type I antibody combined with diaminobenzidine staining and the area of pits was measured. The total amount of matrix degraded was quantitated by measuring collagen fragments released into media using CrossLaps for Culture (Osteometer®, Biotech). Scapular OCs and long bone OCs had similar resorptive activity (number of pits per OCs, area of pits, amount of collagen released). Addition of CT-1746 decreased the total area of bone resorbed by scapular OCs by 32 fold ($p<0.01$), but had no effect on resorption by long bone OCs. The amount of collagen released by scapular OCs was reduced by 6 fold ($p<0.04$). PCK reduced the area resorbed by scapular OCs by two fold ($p<0.08$) and also decreased the amount of collagen released by two fold ($p<0.03$), but had no effect on resorption by long bone OCs. This outcome was unexpected and requires further exploration. Our results indicate that organic matrix degradation by intramembranous bone OCs involve MMPs to a greater extent than by endochondral bone OCs.

M075

Role of CSF-1 in Breast Cancer Cell-Mediated Osteolytic Lesions. S. L. Abboud, K. Woodruff,* N. Ghosh-Choudhury. Pathology, University of Texas Health Science Center and South Texas Veterans Health Care System, San Antonio, TX, USA.

CSF-1 is a key regulatory factor for the proliferation, differentiation and survival of monocytes and osteoclasts. Human breast cancer cells express CSF-1 in vitro and in vivo that my act in an autocrine and/or paracrine fashion to influence tumor growth. In patients with breast cancer, high serum levels of CSF-1 are associated with a poor prognosis and increased rate of metastasis. The precise role of CSF-1in local tumor growth and bone metastasis is unclear. To address this issue, we established a breast cancer model in nude mice that mimics breast cancer in humans and reproducibly results in lytic bone lesions. Human breast cancer cell lines were screened and MCF-7 cells were found to produce low levels of CSF-1 protein and lack the CSF-1 receptor. Cells were transfected with an expression vector containing the CSF-1 cDNA or with an empty vector (control). Stable CSF-1-producing transfectants (MCF-7/CSF-1) that secreted abundant amounts of CSF-1 in conditioned medium (> 2,000 pg/ml) compared to control cells (500 pg/ml) were isolated. 5 x 106 MCF-7/CSF-1 or control cells were directly injected into the right and left upper breast pads. Mice were followed for 28 days, sacrificed and analyzed for local tumor growth and lytic bone lesions by x-ray. The average tumor volume in the breast pad of mice bearing MCF-7/CSF-1 tumors was approximately 1.5-fold greater than controls. Osteolytic lesions were detected by day 20, and the lesion area measured in the hindlimbs was larger in MCF-7/CSF-1 mice (.145mm2) compared to controls (.055 mm2), with this effect persisting up to 28 days. Tumor was microscopically confined to the bone, without other organ involvement and none of the mice developed hypercalcemia. These findings indicate that tumor produced by local breast pad injection of MCF-7 is a useful model for evaluating the mechanisms involved in breast cancer metastasis. Release of CSF-1 from breast cancer cells may be critical for modulating macrophage recruitment and cytotoxicity, local tumor invasion, metastatic potential and osteoclast activation in the bone microenvironment. Elucidation of the role of CSF-1 and its receptor in breast cancer growth may provide novel therapeutic strategies designed to reduce tumor burden, local invasion and the morbidity associated with bone metastasis.

M076

The Metastatic Phenotype of MDA-MB-231 Human Breast Cancer Cells. D. Gaddy-Kurten,[1] T. Mon,*[1] D. C. Montague,[1] N. S. Akel,*[1] T. A. Guise,[2] L. J. Suva.[1] [1]Center for Orthopaedic Research, Orthopaedic Surgery/Physiology and Biophysics, UAMS, Little Rock, AR, USA, [2]Medicine/Endocrinology, University of Texas, Health Science Center, San Antonio, TX, USA.

The most damaging event during cancer progression is the switch from a locally growing tumor to a devastating metastatic lesion. The precise mechanism for this switch is unknown, but is thought to involve multiple changes that allow the tumor cells to complete the complex series of events leading to metastasis. To date, relatively few genes have been directly implicated in these events. In this study, we have used an in vivo selection scheme to identify highly metastatic human breast cancer cells from a less metastatic (MDA-MB-231) parental cell line. MDA-MB-231 cells were injected in the left ventricle of 3 week old nude mice (bg-nu-xid) and metastases were isolated from bone, ovary and liver after ~6 weeks. In this model, tumor-bearing mice exhibit features similar to those associated with human metastatic bone disease, such as bone destruction. The metastatic cells harvested from the target tissues were expanded in culture and re-injected into nude mice. The entire in vivo selection process was repeated 4 times, before sub-lines of the parental MDA-MB-231 cells with an enhanced metastatic potential to bone were identified (MDA-MET). We then compared the phenotype and behavior of the MDA-MET cells with the parental MDA-231 cells in vivo and in vitro. Following inoculation of cells (100,000/0.1 ml) into nude mice, bone tumors are evident in MDA-MET cells within 3 weeks (by x-ray and histology), but not in parental MDA-231 cells. Bone (and other tissue) tumors eventually develop in the parental cells (~6 weeks). In vitro, the MDA-MET cells have a similar growth rate to the parental MDA-231 cells, yet demonstrate distinct adhesive and invasive phenotypes. MDA-MET show decreased adhesion to a variety of substrates, including type I collagen, type IV collagen, laminin and fibronectin, suggesting selectivity for certain extracellular matrix (ECM) components. Invasion through the ECM is an important component of the metastatic process. In matrigel invasion assays, MDA-MET cells have a markedly diminished invasion index compared to MDA-231 cells. In addition, the cell surface expression of syndecan-1 (a heparan sulfate proteoglycan expressed on the cell surface that mediates cell-cell and cell-ECM adhesion) is also significantly decreased in MDA-MET cells. We have developed a human breast cancer cell line (MDA-MET) with an enhanced bone metastatic phenotype. These cells will allow us to identify families of genes involved in metastasis and may highlight which molecular and cellular events determine the metastatic phenotype of human breast cancer cells.

Disclosures: GlaxoSmithKline,1.

M077

Endothelin-1 (ET-1) Mediates Pathological but Not Normal Bone Remodeling. K. S. Mohammad,[1] J. J. Yin,[1] B. G. Grubbs,*[1] Y. Cui,*[1] R. Padley,*[2] T. A. Guise.[1] [1]Medicine, UTHSCSA, San Antonio, TX, USA, [2]Abbott Laboratories, Abbott Park, IL, USA.

Osteoblastic metastases cause morbidity in patients with breast and prostate cancer. ET-1 may play a causal role in these metastases. It is unknown whether ET-1 has a function in normal bone remodeling, although it is expressed by many cell types in the bone microenvironment. Human breast cancer lines, ZR-75-1, MCF-7 and T47D, cause osteoblastic metastases in nude mice. All secrete ET-1, a potent peptide vasoconstrictor. ET-1 is also produced by cancers of the breast and prostate and is a potent stimulator of osteoblast activity. Selective ETA receptor antagonists, as well as non-selective ETA/B receptor antagonists, completely inhibited ET-1 and ZR-75-1-mediated new bone formation in neonatal mouse calvariae cultures, while an ETB antagonist had no effect. In nude mice, administration of an orally active ETA receptor antagonist (ABT-627, 20mg/kg/day) significantly reduced the osteoblastic metastases caused by ZR-75-1. The data suggest that tumors metastatic to bone cause osteoblastic responses by secreting ET-1, which activates ETA receptors on bone cells. To determine the role of chronic ETA receptor blockade on normal bone remodeling, we determined bone mineral density and histomorphometric parameters of bone remodeling in intact female nude mice, as well as those bearing ZR-75-1 mammary fat pad tumors. Mice received ABT-627, 20mg/kg/d, in drinking water for 140 days and were given fluorescent labels of tetracycline and calcein 7 days apart and sacrificed 3 days later. Total body, femur and tibia bone mineral density did not differ between normal or ZR-75-1 mammary fat pad tumor bearing mice or between those which received ABT-627 vs control. Histomorphometric analysis revealed no differences in trabecular bone volume of tibial secondary spongiosa, osteoclast number, and bone formation and mineral apposition rates between ABT-627-treated mice and controls. No metastases from the primary site were detected in mice bearing ZR-75-1 mammary fat pad tumors.Taken together, these data indicate that chronic ETA receptor blockade has no effect on normal bone remodeling in intact mice without bone metastases. In mice with breast tumors confined to the mammary fat pad, long-term ETA receptor blockade again had no effect on bone mineral density at any site measured. Therefore, the predominant role of ET-1 in bone remodeling appears to be in pathologic states such as those associated with metastatic bone disease. The implications of these findings are: 1) ETA receptor blockade should benefit those patients with osteoblastic bone metastases and 2) ETA receptor blockade should not result in bone loss when used to prevent osteoblastic bone metastases.

M078

Annexin II Expression in Breast Cancer Cells and Enhancement of Osteoclast Formation. S. Takahashi,[1] G. D. Roodman,[2] E. Ogata.[3] [1]Dep. of Medical Oncology, Cancer Institute Hospital, Japanese foundation for Cancer Research, Tokyo, Japan, [2]Dep. of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [3]Dep. of Internal Medicine, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan.

We previously cloned annexin II from cDNA library of human osteoclast-like cells as an autocrine factor which promotes osteoclast formation and bone resorption. Annexin II is first cloned as a cytoplasmic protein, but is recently found to be expressed on the outer side of cell membranes of many types of cells including cancer. We postulated that breast cancer cells also express annexin II protein on their membranes or secrete annexin II and stimulate osteoclast formation and osteolysis through annexin II. Several human breast cancer cell lines (MCF-7, MDA-MB-231, ZR-75-1, BSY-1, HBC-4, HBC-5) expressed annexin II mRNA detected by Northern blot, and expressed and secrete annexin II protein detected by western blot. Some of the cell lines also expressed annexin II on their cytoplasmic membrane detected by flowcytometry. Treatment of bisphosphonates, YM529 and pamidronate decreased annexin II protein expression. Expression of annexin II mRNAs in primary breast cancer samples was also detected by RT-PCR. Murine breast cancer cell line MMT060562 enhances formation of osteoclasts from mouse bone marrow cells, and MMT060562 cell lines transfected with annexin II cDNA enhanced formation of osteoclasts further more. These results suggest that breast cancer cells might stimulate osteoclast formation and bone resorption through annexin II expression.

M079

A Novel Arabinocytidine-Bisphosphonate Has Potent Effects on Human Osteoblasts and Breast Cancer Cells: Role of the Mevalonate Pathway. G. G. Reinholz,[1] B. Getz,*[1] E. S. Sanders,*[1] M. Y. Karpeisky,*[2] N. S. Padyukova,*[2] S. N. Mikhailov,*[2] J. N. Ingle,*[3] T. C. Spelsberg.[1] [1]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA, [2]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation, [3]Department of Oncology, Mayo Clinic, Rochester, MN, USA.

Bisphosphonate-treatment reduces the formation and progression of bone metastases, and decreases the resulting complications. However, the mechanism of bisphosphonate action is not completely understood, and there is a continued search for more potent analogues. Here we report the potent effects of a novel bisphosphonate analogue, the anhydride formed between arabinocytidine 5' phosphate and etidronate (Ara-CBP), on human fetal osteoblasts (hFOB) and MDA-MB-231 breast cancer cell proliferation and hFOB cell mineralization. The effects of Ara-CBP are compared to etidronate, the anhydride formed between AMP and etidronate (ABP), pamidronate, zoledronate and the HMG-CoA reductase inhibitor, mevastatin. ABP is a reported metabolite of etidronate. Etidronate and ABP had little effect on hFOB or MDA-MB-231 cells. Ara-CBP was the most potent inhibitor of hFOB and MDA-MB-231 cell proliferation followed by mevastatin, zoledronate and pamidronate. Ara-CBP was also the most potent stimulator of hFOB cell mineralization followed by zoledronate and pamidronate. In contrast, mevastatin was a potent inhibitor of hFOB cell mineralization.The role of the mevalonate pathway was examined using the mevalonate pathway intermediates mevalonate, farnesol and geranylgeraniol. Mevalonate reversed the effects of mevastatin on both the hFOB and MDA-MB-231 cells suggesting that inhibition of HMG-CoA reductase activity is the mechanism of mevastatin action on these cells. Inhibition of hFOB cell proliferation by Ara-CBP and zoledronate was partially reversed by mevalonate pathway intermediates, and the stimulation of mineralization was completely reversed by geranylgeraniol. However, none of the mevalonate pathway intermediates reversed the inhibition of MDA-MB-231 cell proliferation by Ara-CBP, zoledronate, or pamidronate. In summary, Ara-CBP is a potent inhibitor of hFOB and MDA-MB-231 cell proliferation and stimulator of hFOB cell mineralization. The main mechanism of Ara-CBP action in hFOB cells appears to be inhibition of the mevalonate pathway, whereas in the MDA-MB-231 cells other mechanisms are involved. Interestingly, while Ara-CBP, zoledronate and mevastatin inhibit the mevalonate pathway in hFOB cells, Ara-CBP and zoledronate have the opposite effect of mevastatin on hFOB cell mineralization.

M080

Expression of OPG, ODF and RANK in Myeloma Cells. C. Silfverswärd,*[1] H. Penno,*[1] A. Frost,[1] H. Brändström,*[2] O. Winqvist,*[2] O. Nilsson,*[1] Ö. Ljunggren.[2] [1]Inst. of Surgical Sciences, Uppsala, Sweden, [2]Inst. of Medical Sciences, Uppsala, Sweden.

The B-cell neoplasm multiple myeloma (MM) has affinity to and excert profound effects on bone tissue. These effects, generating osteolysis, are mediated by osteoclasts although there is little knownledge about the specific interactions between MM and osteoclasts in bone micro-environment. In recent research the ODF-RANK pathway has been elucidated as a major pathway of differentiation and proliferation of osteoclasts. In the present study we have investigated the expression of ODF, RANK and OPG in human myeloma cell-lines (EJM, Karpas and LP-1). By RT-PCR we have detected constitutive mRNA expression of ODF in EJM and Karpas. Constitutive mRNA expression (RT-PCR) and secretion of the OPG protein (Elisa) was found in all three cell-lines but seems to be most pronounced in EJM. OPG formation could not be regulated by IL-4, IL-6, IL-13, IL-1ß or TNF-ß. Nor did conditioned media from human osteoblasts affect OPG formation. However treatment with dexamathasone inhibited OPG formation and PGE2 induced a slight stimulation. Constitutive expression of RANK mRNA was seen in EJM and LP-1. In conclusion: as osteoclasts express RANK these results indicate that the ODF-RANK axis is a possible pathway of interaction with myeloma explaining it´s osteolytic potential. Furthermore, two of the cell-lines express RANK themselves, which concerning EJM suggests a possible way of autostimulation. All studied cell-lines also express OPG which makes it possible that the relation between OPG and ODF expressed by MM regulate the osteolysis.

M081

Extracellular Matrix Protein-stimulated Breast and Prostate Cancer Cell Lines Affect Osteoblast Activity and Differentiation. G. Schmidt,*[1] R. Lomri,[2] O. Hoffmann.[1] [1]University of Vienna, Vienna, Austria, [2]INSERM, Paris, France.

Bone provides a microenvironment that supports the homing, migration and growth of metastatic tumours. The factors governing this specific metastatic migration are not completely understood. However, there is evidence that the microenvironment plays an important role, for example matrix molecules, among them collagen I, collagen IV, laminin and fibronectin interact with integrins on tumour cells. It is thus conceivable in the early stages of metastases that specific interactions with extracellular matrix proteins induce tumour cells to modulate osteoblast activity. To test this hypothesis, we studied the effect of supernatants from cultures of tumour lines grown on plates coated with collagen I, collagen IV, laminin and fibronectin on the human osteoblast line AHTO-7. We chose to examine breast and prostate tumours because of their high frequency of bone metastasis and the osteosclerotic and osteoporotic nature of the resulting bone lesions. We investigated rapid cell activation by assessing protein tyrosine phosphorylation and alkaline phosphatase activity, which is a measure of osteoblast differentiation. We observed that the breast tumor cell line MDA-MB-231 and the prostate tumor line PC-3 are differentially influenced by matrix molecules. Both breast tumour and prostate cancer line supernatants induced protein tyrosine phosphorylation after incubation with collagen I, collagen IV, laminin and fibronectin. We found that there was specific, transient phosphorylation of ERK2, which is a kinase important for cell growth, that peaked between between 5 and 30 minutes of incubation. Alkaline phosphatase activity was inhibited by the interaction of breast tumour cells with collagen I and collagen IV. Supernatants from the culture of prostate cells and collagen I lead to a 20% increase, while incubation with collagen IV resulted in a 30% decrease in alkaline phosphatase activity. Culture supernatants from both cell lines with fibronectin and laminin did not affect alkaline phosphatase activity significantly. Moreover, supernatants from cultures of NIH3T3 fibroblasts stimulated with extracellular matrix proteins lead to minimal induction of protein tyrosine phosphorylation but no induction of alkaline phosphatase activity. This suggested that there is a specific effect on the differentiation of osteoblasts by these extracellular matrix protein-stimulated tumour cells. Taken together, these observations generated in a human model system demonstrate that the interaction of extracellular matrix proteins with breast and prostate tumour cell lines results in secreted factors able to affect osteoblast activity.

M082

TGFβ Signaling in Osteolytic Cancer Cell Lines: Stimulation of IL-6, IL-11, PTHrP, and VEGF Through MAP Kinase Pathways. S. Kakonen,[1] K. S. Selander,[2] M. R. Carreon,*[1] Y. Cui,*[1] M. Neal,*[1] J. M. Chirgwin,[1] B. G. Grubbs,*[1] T. A. Guise.[1] [1]Medicine, UTHSCSA, San Antonio, TX, USA, [2]University of Alabama, Birmingham, AL, USA.

TGFβ, stored in bone matrix and released during osteoclastic resorption, plays a central role in stimulating tumor osteolysis by inducing PTHrP in the human breast cancer cell line MDA-MB-231. However, it is unclear whether this is true for other cancer cell lines and whether factors other than PTHrP contribute to the osteolysis. Here, we report that TGFβ also regulates production of VEGF and IL-11 in a variety of osteolytic cancer cell lines. TGFβ signaling to induce tumor PTHrP secretion is both Smad-dependent and -independent in MDA-MB-231 cancer cells. p38 MAP kinase pathway is a major component of Smad-independent signaling, since the combination of p38 inhibition and dominant-negative Smad blockade completely inhibited the TGFβ-induced PTHrP production. Therefore, we determined the role of the MAP kinase pathways in TGFβ regulation of tumor factors implicated in osteolytic bone metastases.We tested MEK (MAP kinase kinase of Erk) (PD98059) and p38 (SB202190) inhibitors on PTHrP, VEGF, IL-11 and IL-6 secretion by 5 osteolytic cancer lines +/- TGFβ: breast MDA-MB-231, MDA-MB-435S, BT549; prostate PC-3; and lung RWGT2. TGFβ stimulated PTHrP production in all cell lines, and this was significantly reduced by p38 inhibition. Complete blockade was observed in PC-3 cells. p38 inhibition reduced basal PTHrP in MDA-MB-231 and MDA-MB-435S breast cancer. TGFβ stimulated the secretion of VEGF by all cancer lines; this was totally blocked by p38 inhibition in MDA-MB-231. IL-11 was produced by MDA-MB-231, PC-3 and MDA-MB-435s cell lines and was significantly stimulated by TGFβ. p38 inhibition reduced the basal IL-11 secretion, but had no effect on the ratio of TGFβ-stimulated to basal IL-11 secretion in MDA-MB-231. In contrast to p38 inhibition, which significantly reduced TGFβ induction of PTHrP and VEGF in most cancer lines, MEK inhibition reduced only the basal, and not TGFβ-stimulation of these factors. All the cell lines studied produced IL-6. TGFβ induced its secretion only in PC-3 and RWGT2 cell lines.Taken together, these data indicate that the p38 MAP kinase pathway contributes significantly to TGFβ signaling in cancers which cause osteolytic metastases. However, the relative importance of the MAP kinase pathways in the TGFβ induction of PTHrP varied among cell lines. The data support a major role for bone-derived TGFβ in stimulating cancer metastasis to the skeleton, in particular by inducing tumor expression of PTHrP, VEGF, and IL-11. p38 MAP kinase signaling pathway regulates the secretion of these factors and may provide molecular targets for anti-osteolytic therapy.

M083

BMD at Diagnosis of Primary Operable Breast Doesn't Influence the Incidence of Skeletal Metastases. R. U. Ashford,[1] T. Powles,*[2] S. Paterson,*[3] M. Beneton,*[1] J. Kanis,*[1] T. Spector,[4] L. Pylkkänen,[5] J. Nevalainen,*[5] E. McCloskey.[1] [1]WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, Sheffield, United Kingdom, [2]Royal Marsden Hospital, London, United Kingdom, [3]Tom Baker Cancer Centre, Calgary, Canada, [4]St. Thomas' Hospital, London, United Kingdom, [5]Leiras Oy, Helsinki, Finland.

Given the "seed and soil" hypothesis for bone metastases, we wished to examine whether low BMD at diagnosis was related to the subsequent incidenceof bone metastases in breast cancer.Femoral neck and spine BMD (Hologic QDR1000) were measured in a subset of 343 women at the Royal Marsden Hospital at enrolment to a randomised controlled trial of adjuvant clodronate in breast cancer. Women were randomised to receive either clodronate 1600 mg (Bonefos®)per day or identical placebo, for 2 years, starting within six months of primary treatment(carried out according to local protocols). This analysis remains blinded to treatment. Radiological assessments for bone metastases were repeated at 24 and 60 months and also when clinically indicated. Vertebral collapse required confirmation of bone metastases by magnetic resonance imaging. Baseline measurements were compared to those obtained in a randomly selected healthy control group of 776 women (the Chingford Study).Mean age, height, weight and BMI were not statistically significantly different between the women with primary breast cancer and the control population (p-values ranged from 0.24-0.80). Femoral neck BMD was also comparable (0.79±0.12 vs. 0.78±0.12 g/cm^2, p=0.24). Spine BMD tended to be higher in the women with primary breast cancer but this was only of borderline statistical significance (0.99±0.14 vs. 0.97±0.16 g/cm^2, p=0.053). During a median follow-up of 5.9 years, 43 of the women with primary breast cancer (12.5%) developed skeletal metastases. No differences in age, height or weight were observed between these 43 women and those remaining free of skeletal metastases. Mean spine and femoral neck BMD was also similar at baseline in the two groups (LS-BMD 1.00±0.14 vs. 0.99±0.14 g/cm^2, p=0.62; femoral neck BMD 0.80±0.12 vs. 0.78±0.12 g/cm^2, p=0.477). The median time to first bone metastasis was also similar across tertiles of baseline spine BMD (p=0.66).We conclude that spine and femoral neck BMD at the time of diagnosis of breast cancer is comparable to that in age-matched healthy controls. Furthermore, BMD at the time of diagnosis appears to exert no influence on the subsequent incidence of skeletal metastases. Further analyses are necessary to determine if there is any influence of changes in BMD after diagnosis on the subsequent risk of skeletal metastases.

Disclosures: Leiras Oy,2.

M084

Bisphosphonates Activate p38 Mitogen-Activated Protein Kinase in Human Breast Cancer Cells. K. S. Selander,[1] K. W. Harris,*[1] L. Kallman,*[1] M. Merrell,*[1] K. Väänänen.[2] [1]Medicine, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Anatomy, University of Turku, Turku, Finland.

Bisphosphonates (BPs) have been shown to effectively prevent osteolytic complications of bone metastases. Studies in experimental animals have suggested that BPs may also prevent cancer spread to bones. Results from clinical studies, where primary breast cancer patients were treated with adjuvant clodronate have been, however, controversial. BPs do not inhibit the growth of MDA-MB-231 breast cancer cells in vitro. BPs also do not inhibit the growth of established breast cancer bone metastases in vivo. We show here that BPs (clodronate, etidronate, pamidronate, alendronate and risedronate) induce phosphorylation of the p38 mitogen-activated protein kinase in human estrogen receptor negative (ER-) MDA-MB-231 breast cancer cells in vitro. p38 phosphorylation was detected with Western blots using the antibody directed against the active, phosphorylated p38. BP-induced p38 phosphorylation resulted in increased p38 activity, as detected with in vitro kinase assays. Similar BP-induced increase in p38 activity was also detected in the ER+ human breast cancer cell line MCF-7. The effect of BPs on p38 activation was indirect, since addition of the drugs directly to the kinase assay did not inhibit the ability of the immunoprecipitated p38 to phosphorylate its downstream target ATF-2. To further characterize the functions of p38 in MDA-MB-231 breast cancer cells, we transfected the cells with the cDNA encoding the dominant negative p38 and established single cell clones stably expressing the protein. Proinflammatory cytokines (IL-1b, TGF-b and TNF-a) induced interleukin-6 (IL-6) production in the control clone expressing the empty pcDNA 3 vector. In the dominant negative p38-expressing clones, TGF-b-induced IL-6 production was blocked. Clodronate or risedronate did not affect the basal in vitro growth rates of the pcDNA3 expressing control clone or the p38 dominant negative expressing clone. In summary, BP treatment results in phosphorylation and activation of p38 in human breast cancer cells in vitro. Since p38 mediates proinflammatory cytokine-induced production of IL-6 in breast cancer cells in vitro, it is possible that BPs affect the IL-6 production of breast cancer cells.

M085

Skeletal Effects of Two Different Cancer Cell Lines, MDA-MB-231 and EL4, in Mice. B. A. Lechowska,* F. L. McCabe,* M. N. Whitacre,* S. M. Blake, C. McMonigal,* P. Liang, G. B. Stroup. GlaxoSmithKline, King of Prussia, PA, USA.

Osteolytic metastases are a significant clinical problem associated with a variety of cancer types, notably, breast cancer. Anti-resorptive therapies offer the possibility of intervention in this disease by preventing the breakdown of bone. This has the secondary effect of decreasing the local concentration of factors such as TGFb that promote the progression of tumors in the marrow space. Appropriate animal models to evaluate these therapies are needed. Here, we evaluate the skeletal effects of two distinct tumor cell lines, MDA-MB-231 (human breast cancer) and EL4 (murine lymphoma) in mice. MDA-231 cells (1x10^5 per mouse) were injected into the left ventricle of 5-week old female nude mice, while EL4 (5x10^5 per mouse) were injected into the tail vein of 16-week old female C57BL/6 mice. One group of animals in each study received alendronate twice weekly (1mg/kg s.c.). To measure the effects on bone, digital x-ray images of tibiae were taken at baseline, week 2, and 3 in MDA-231 study and at baseline and day 11 in EL4. The images of left proximal

tibia were later analyzed with image analysis software. Volumetric BMD was analyzed ex-vivo by pQCT. Whole blood ionized Ca^{++} was measured at term in both studies. Digital X-rays showed the development of multiple discrete lesions in tibiae of MDA-injected mice that were absent in vehicle-injected controls. Alendronate treatment reduced the bone lesion area by 98% ($p<.05$). BMD increased in all three groups from baseline to week 2. However, during the final week a 9% decline in BMD was observed in animals that received only MDA cells. Alendronate treated animals showed a significant increase during this period. Whole blood Ca^{++} levels were significantly ($p<.05$) higher in mice that received MDA cells alone (1.32mM) compared to animals receiving vehicle (1.24mM) or MDA + alendronate (1.25 mM). Discrete lesions were not detected in X-rays of the proximal tibia of EL4 injected animals. However, image analysis of these bones showed that BMD was significantly lower in EL4-injected mice receiving vehicle (0.688 mg/mm^2) relative to mice receiving alendronate (0.788 mg/mm^2). Whole blood Ca^{++} was significantly higher in untreated mice (1.37mM) than mice treated with alendronate (1.32mM) or normal controls (1.23mM). Both lines of tumor cells had a detrimental effect on the skeleton of mice resulting in lowered BMD, hypercalcemia, and paralysis. The use of MDA-231 cells is more technically challenging due to the requirement of ventricular administration. However, this method results in endpoints that are very similar to those observed in the most common form of metastatic bone disease in humans and is therefore more appropriate.

Disclosures: GlaxoSmithKine,3.

M086

Comparison of Korean Female and Male Reference Data Using DXR. S. O. Yang,[1] S. Y. Ham,*[1] Y. I. Kim,*[1] T. H. Jeong,*[1] S. K. Lee,*[2] K. B. Johansen,*[3] A. B. Helboe.*[3] [1]Ulsan University Hospital, Ulsan, Republic of Korea, [2]Kangnam General Hospital Public Corporation, Seoul, Republic of Korea, [3]Pronosco A/S, Vedbaek, Denmark.

The Pronosco X-posure System^{TM} estimate forearm BMD using the Digital X-ray Radiogrammetry (DXR) technique from radiographs of the hand, without the use of aluminium wedges. It has been shown to have good correlation to DXA and to have very low precision error. We report here the results of a comparison of the reference data for Korean females and males.The system calculates BMD using a weighted average of cortical and bone width measurements at the second through fourth metacarpal. The output is an absolute BMD estimate called DXR-BMD as well as the metacarpal index (DXR-MCI), cortical thickness (CT) and bone width.A total of 363 Korean women and 120 Korean men were recruited from the Seoul Area (only men) and Ulsan area. Women and men with a history of bone diseases (except osteoporosis), medication impacting bone mineral density were excluded, as were those who had undergone prolonged hospitalisation or immobilisation.The included women and men had their non-dominant hand radiographed.The age distribution and the age-specific means and SD's for DXR-BMD at the forearm were:

		DXR-BMD		
	Number Women/Men	Women Mean ± SD	Men Mean ± SD	P
20 - 29	67/26	0.534 ± 0.044	0.568 ± 0.038	0.0007
30 - 39	78/27	0.554 ± 0.047	0.589 ± 0.050	0.0011
40 - 49	68/16	0.578 ± 0.044	0.594 ± 0.045	0.2070
50 - 59	55/19	0.537 ± 0.059	0.581 ± 0.046	0.0037
60 - 69	50/17	0.473 ± 0.044	0.572 ± 0.055	<0.0001
70 - 79	45/15	0.427 ± 0.050	0.537 ± 0.067	<0.0001
All	363/120	0.525 ± 0.068	0.575 ± 0.052	<0.0001

The above results show that relative to the Korean male population, the Korean females have significantly lower DXR-BMD across the age decades as expected. For both women and men, the peak DXR-BMD is reached in the forties and in women and men over the age of 50, a decline in mean DXR-BMD with age is demonstrated, where a more rapidly decline is seen in women compared to men.For DXR-MCI the results show the same tendency as for DXR-BMD, as the Korean women have significantly lower DXR-MCI compared to the Korean men

M087

Digital X-ray Radiogrammetry: Establishment and Comparison of Indian Female and Male Normative Reference Data. K. C. Pande,[1] K. B. Johansen,*[2] A. B. Helboe.*[2] [1]Research Centre & PG Institute of Ortopaedics, Sushrut Hospital, Nagpur, India, [2]Pronosco A/S, Vedbaek, Denmark.

The purpose of the study was to establish normative reference databases for Indian female and male population and compare the two.Forearm BMD estimated by the Pronosco X-posure System^{TM} has been shown to have good correlation to DEXA and good in vivo reproducibility. The system estimates BMD (DXR-BMD) using digital x-ray radiogrammetry from radiographs of the hand, without the use of aluminium wedges. A composite of 3 metacarpal measurements of cortical and bone widths are weighted and averaged. The DXR-BMD measurement has been shown to be constant even with changes in the capture conditions. BMD varies with ethnic origin and as of today reference ranges for DXR-BMD for this system have been established for North-American Caucasian, Scandinavian, German, Hispanic, Chinese, British and Korean females and Korean males.A total of 262 Indian women and 178 Indian men were recruited from two different centres in India (Nagpur and New Delhi). Subjects with a history of bone diseases (except osteoporosis), medication impacting on bone mineral density were excluded. Also excluded were subjects with a history of prolonged hospitalisation or immobilisation of the arm. Plain radiographs of the non-dominant forearm and hand were taken under standardised conditions and analysis was performed by the Pronosco X-posure System.The age distribution and the age-specific mean and SD for DXR-BMD at the forearm is shown in the following table:

		DXR-BMD		
Age	Number Women/Men	Women Mean ± SD	Men Mean ± SD	P
20 - 29	30/40	0.560 ± 0.058	0.586 ± 0.046	0.0461
30 - 39	45/37	0.570 ± 0.056	0.600 ± 0.056	0.0200
40 - 49	75/34	0.569 ± 0.057	0.602 ± 0.056	0.0056
50 - 59	74/33	0.529 ± 0.064	0.589 ± 0.052	<0.0001
60 - 69	33/23	0.485 ± 0.047	0.559 ± 0.052	<0.0001
70 - 79	5/11	0.440 ± 0.050	0.540 ± 0.043	0.0010
All	262/178	0.544 ± 0.066	0.586 ± 0.054	<0.0001

The peak expected DXR-BMD in women was 0.5825 g/cm^2 occurring at age 35, and in men it was 0.6029 g/cm^2 occurring at age 42. An age dependent decrease in BMD is shown in both women and men over the age of 50 years.In conclusion, normative reference databases for the Pronosco X-posure System is generated for Indian women and men. As expected, the DXR-BMD in Indian women is significantly lower than that in Indian men across the age decade range

M088

Bone Mineral Density (BMD) Assessment with DEXA and Quantitative Ultrasound (QUS) of 47 Dialysis Patients. P. Guggenbuhl,*[1] I. Cozic,*[1] E. Laruelle,*[2] v. Joyeux,*[2] F. Lamer,*[1] P. Le Pogamp,*[2] G. Chalès.*[1] [1]Rheumatology, University Hospital, Rennes, France, [2]Nephrology, University Hospital, Rennes, France.

There is a contreversy about osteoporosis and risk factors in renal diseases, particularly when dialysis is performed. We investigated 47 patients (33 with hemodialysis (HD) and 14 with dialysis peritonal ambulatory (DPA)) between 1997 april and 2000 july. For each patient a BMD measurement was made with DEXA and with QUS (BUA and SOS). Mean age was 45.8 years ± 13.7 ans (20-75) ; they were 32 men (68 %) and 15 women (32 %). The mean dialysis duration was 25.9 months (1-252). The mean DEXA values were : at the lumbar spine (LS) in 42 patients, 0.935 ± 0.15 g/cm2 (T score : - 1.3 ±1.3 SD), at the femoral neck (FN) in all patients, 0.760 ± 0.13 g/cm2(T-score : -1.7 ± 1.2 SD). Osteopenia (T score <-1 SD) was found in 50% at LS and in 59.5% at FN ; osteoporosis (T score <-2.5 SD) was found in 22.5% at LS and in 27.6% at FN. With QUS, osteopenia was found in 65.9% with BUA and 68.2% with SOS. There was no significant differences according to dialysis duration, type of dialysis, renal disease, tobacco consumption. Women with menopause had a significant lower BMD than non menopausal women at FN (0.626 vs 0.771 g/cm2, p = 0.02) and at total hip (0.697 vs 0.865 g/cm2 , p = 0.03) ; this was also found in BUA and SOS. Concerning corticosteroids use, a trend to a lower BMD was found only in men at LS (0.838 vs 0.985 g/cm2, p = 0.09) and at total hip (0.792 vs 0.939 g/cm2, p = 0.08) ; when T scores were considered, the difference became significant at LS (-2.7 vs +0.9 SD, p = 0.01) and nearly significant at total hip (-2.1 vs +0.9 SD , p = 0.06). Five patients had a fracture, with a mean BMD not different from the other patients.There was a good correlation between DEXA and QUS (r = 0.5 to 0.67, p < 0.01) but a poor concordance (Kappa test = 0.2). In conclusion, we found a high rate of osteopenia but a poor number of fractures in dialysis patients. Menopause in women and corticosteroid use in men remained major risks factors. We failed to demonstrate any influence of the type or duration of dialysis on BMD. Both DEXA and QUS were useful for BMD assessment but they seemed to measure different parameters.

M089

Women With Silent Osteoporosis: Impact Baseline Data. C. Roux,[1] H. Pols,[2] J. Ringe,[3] B. Giraudeau,[4] L. Van De langerijt,[5] D. Cahall,[6] P. Delmas.[7] [1]Rheumatology, Cochin Hospital, Paris, France, [2]Erasmus University Medical School, Rotterdam, The Netherlands, [3]Klinicum Leverkusen, Leverkusen, Germany, [4]INSERM U 444, Tours, France, [5]Aventis Pharma, Hoevelaken, The Netherlands, [6]Aventis Pharma, Bridgewater, NJ, USA, [7]University Claude Bernard, Lyon, France.

Many osteoporotic women remain undiagnosed until they sustain a fracture. Clinical risk factors for low bone density have been identified in previous studies. We assessed three key risk factors (RFs) and osteoporotic status in women included in the IMPACT trial (Improving Measurements of Persistence of ACtonel Treatment), designed to prospectively evaluate the impact of reinforcement with bone marker data on patient persistence.We studied 7,166 ambulatory women, between 65 and 80 years of age, not previously diagnosed for osteoporosis, and not receiving glucocorticoids. Three key RFs were assessed : low body weight (< 57 kgs) (LW), history of a low-trauma fracture (LTF) after the age of 45 years, and maternal history of hip (MHF) fracture. Bone mineral density was measured at lumbar spine and hip. In osteoporotic women (T < - 2.5 at either site), and osteopenic women having LTF, thoracic and lumbar spine lateral X-rays were performed, using a standardized procedure. Vertebral deformities were diagnosed using a semi-quantitative assessment. All the data were analysed in a central facility.In this analysis, 6 654

women were included from 172 centers in 21 countries.Lateral spine X-rays are available in 1850 patients, and 592 (32 %) had at least one vertebral deformity. In this unselected global population of osteoporotic women, 65-80 years old, the prevalence of "silent osteoporosis", i.e. no key clinical risk factors or prevalent deformities and confirmed by bone densitometry, was in 1 in 5 women (21%). This supports that a significant proportion of undiagnosed osteoporotic women requires bone densitometry for confirmation of their osteoporosis.

M090

Case Finding and Opportunistic Screening: A Mathematical Approach. C. E. D. De Laet,*[1] A. Odèn,*[2] O. Johnell,[3] B. Jönsson,*[4] J. A. Kanis.[5] [1]Institute for Medical Technology Assessment, Erasmus University Rotterdam, Rotterdam, The Netherlands, [2]Consulting Statistician, Gothenberg, Sweden, [3]Department of Orthopaedics, Malmo General Hospital, Malmo, Sweden, [4]Department of Economics, Stockholm School of Economics, Stockholm, Sweden, [5]Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Sheffield, United Kingdom.

Fracture risk assessment can be based on the use of Clinical Risk Factors (CRF's), on bone mineral density (BMD) measurement or on a combination of both where the presence of a number of CRF's is the trigger to perform BMD measurement (opportunistic screening). The value of this risk assessment depends upon the overall performance of the resulting risk score. We present a mathematical approach to evaluate the performance of these risk scores.When only dichotomous CRF's are used a risk score will have discrete values, but when combined with continuous measurements such as weight or BMD this risk score becomes continuos. Risk can then be expressed as a risk gradient, i.e. the RR per standard deviation (SD) change in risk score. When we assume that this risk score is distributed normally in the population, such as BMD, it is possible to calculate risk of individuals compared to the average risk in the population (of the same gender and age). For example, when a RR of 2.6/SD is assumed we can calculate that only 32 % of the population will have a higher than average risk and that people with the median risk score will have a fracture risk that is half that of the average population. Under the same assumptions 11 % will have a doubled risk and only 5% will have a risk that is three times that of the average population. When the assumption of a RR of only 2/SD is used the proportions are 36%, 9% and 3% respectively for average, double and triple risk. When it is assumed that the RR is 4/SD (such as for a combination of CRF's and BMD) those risks change to 24%, 12% and 7 %. But, while the change of the proportion of the population detected to be at high risk is small, the performance of a test will be better when the RR per /SD is higher. This can be shown by the average risk in those identified at high risk. When the average population risk is used as a threshold value the average risk in the test-positive category will be 1.7 with a RR of 2/SD risk gradient. At a risk gradient of 4/SD this average risk becomes 3.1 at the same threshold value. When the threshold is a risk twice the population risk those average risks become 2.9 and 5 respectively.CRF's and direct bone density assessments combined can greatly increase the efficacy of case finding strategies. More research is needed to validate the combined predictive power of different risk indicators.

M091

Comparison of the X-Posure System for Radiogrammetry of the Metacarpals with Standard Techniques and DXA. J. D. Landoll,[1] N. E. Badenhop-Stevens,[1] R. May,*[2] T. Hangartner,[3] J. L. Roehrig,*[1] V. Matkovic.[1] [1]Bone and Mineral Metabolism Laboratory, OSU, Columbus, OH, USA, [2]PronoscoAmerica, Pittsburgh, PA, USA, [3]Wright State Univ., Dayton, OH, USA.

This preliminary study investigates the X-posure system (ver. 2.0, PronoscoAmerica) for measuring radiogrammetry of the metacarpals. X-posure digitizes standard hand radiographs and measures bone width (BW) and cortical thickness (CT) of the 2nd, 3rd, and 4th metacarpals; results are presented as weighted averages of BW, CT, and metacarpal index. This study examines the internal reproducibility of the X-posure system and correlates its measurements with those for the 2nd metacarpal by a well-established automated procedure, with manual analysis (needle-tipped caliper), and with DXA forearm measurements (Lunar DPX-L). Two hand radiographs taken 4 years apart from each of 51 Caucasian females (baseline age 10.9±0.9y) were analyzed. Internal precision of the X-posure system is investigated by analyzing the same radiograph 10 times each with and without repositioning between each scan. The coefficients of variation for the static and repositioned measurements are 0.0% and 0.2% for BW and 0.5% and 0.6% for CT, respectively. This indicates that the X-posure system is quite robust and provides a reliable estimate of radiogrammetry parameters. The correlations of X-posure data with those of the other techniques are given in the table below ($p \leq 0.0001$ for all; CA/TA = Cortical Area/Total Area).

Independent Variable	Dependent Variable	R^2 (%)
BW (Auto)	BW (Man)	75.7
BW (Auto)	BW (X-posure)	71.1
CA/TA (Auto)	CA/TA (X-posure)	61.4
Radius BMC (33% site, DXA)	CA/TA (X-posure)	27.3
CA/TA -Baseline (X-posure)	CA/TA - Year 4 (X-posure)	75.6
CA/TA -Baseline (Auto)	CA/TA - Year 4 (Auto)	70.1
CA/TA -Baseline (Man)	CA/TA - Year 4 (Man)	68.6
Radius Areal BMD (33% site, DXA)	Radius Areal BMD (33% site, DXA)	44.9

X-posure reports average measurements so direct comparisons are not possible; however, the correlation of BW between the X-posure and automated system is comparable to that between the automated and manual measurements. Furthermore, the correlation of CA/TA at baseline and 4 year follow-up is good (considering growth) and is best for the X-posure system. Although further investigation is necessary, including obtaining full radiogrammetry output, the above data show the X-posure system appears to be a very reliable method for obtaining radiogrammetry measurements

M092

In Vitro Short Term Reproducibility Evaluation of a Flash Beam X-Rays Bone Densitometer. V. Boudousq,*[1] J. M. Dinten,*[2] R. Grando,*[3] M. Darboux,*[2] L. Gaucher,*[3] C. Robert-Coutant,*[2] G. Gonon,*[2] C. Barreau,*[1] P. O. Kotzki.*[1] [1]CHU Nimes, Service de Medecine Nucleaire, Nîmes, France, [2]CEA, Laboratoire d'Electronique et de Traitement de l'Information, Grenoble, France, [3]DMS, Montpellier, France.

The LEXXOS Flash Beam DXA (FB-DXA) system takes profit of the nowadays available digital radiological flat panels. This system associates such a detector with a cone-beam collimated X-Rays generator. One of its main advantages is to realize spine, hip, forearm exams in two X-Rays flashes, in an acquisition time of less than 2 seconds, without any scanning. However, with FB-DXA, the acquisitions contain significant X-rays scatter, when compared to collimated pencil or fan beam. Therefore, a specific methodology, dedicated to X-rays scatter processing, has been developed and enables to realize bone density measurement. First characterization result of this new generation of bone densitometer is presented hereafter. The short term reproducibility has at first been evaluated by the use of one geometric phantom taken from "Groupe de Recherche et d'Information sur les Osteoporoses" evaluation kit : it is composed of five 3mm height sheets of PVC in the form of five steps (6cm wide and 3cm deep), included in a Lucite container filled with water. The total thickness is 20cm. The five steps represent five different bone density values (respectively 0.3; 0.6; 0.9; 1.2; 1.5 $g.cm^{-2}$). This configuration has been measured twenty times and a CV has been computed for each step, the values are :

Step density ($g.cm^{-2}$)	0.3	0.6	0.9	1.2	1.5
CV	4%	0.84%	0.76%	0.65%	0.77%

Then to take into account influence of ROI determination, same short term reproducibility has been evaluated on two phantoms representative of lumbar spine: the Quality Control Hologic phantom composed of four uniform density vertebras, and an anthropomorphic phantom composed of three true vertebras included in a Lucite container. The CV have been measured for each vertebra and a mean CV has been computed for each phantom. Mean CV are respectively : 0.45% for the Hologic phantom and 0.72% for the anthropomorphic phantom. The new generation FB-DXA system improves significantly examination comfort (no scan, very fast acquisition). These results prove that, associated with a dedicated processing methodology, it presents short term reproducibility performances in the same range as the one provided by pencil and fan beam scanning systems.

M093

Digital X-Ray Radiogrammetry (DXR) on Hand X-Ray Images Using Computed Radiography (CR). A. M. Hussain,* N. Baadegaard,* O. Wendt,* A. Rosholm.* Pronosco A/S, Vedbaek, Denmark.

The Pronosco X-posure System™ estimates bone mineral density (BMD) on the distal forearm by a combined computerized radiogrammetric analysis of the 2nd, 3rd and 4th metacarpals using plain X-ray radiographs of the hands. The system has so far been restricted to the use of conventional X-ray radiographs. A new feature has recently been introduced allowing for the use of computed radiography (CR) imaging capturing devices. A series of technical studies using anthropomorphic phantoms have been conducted in order to determine the correlation between BMD estimates obtained from CR imaging plates and conventional X-ray radiographic films. In addition, the sensitivity of the BMD measuring algorithm to the settings and conditions of the X-ray equipment during CR capture has also been tested. The settings and conditions tested were the use of different imaging plates, different exposure levels (mAs), different tube voltage settings (kV), different filtering of the X-rays, the presence of additional soft-tissue, rotation of the hand on the imaging plate and finally site variation, i.e. the reproducibility between different X-ray setups. The study used as reference the settings corresponding to the settings recommended in the Pronosco X-posure System User Manual.
The BMD estimates obtained from CR imaging plates correlated extremely well with radiographic films, as the correlation was found to be higher than 0.99. In addition, the sensitivity studies concluded that realistic deviations from a standard protocol for the capturing conditions did not influence the estimated BMD value, when using the CR imaging plates. The maximum estimated BMD deviation from the reference using the settings in the protocol was seen to be below 1%.

Thus the Pronosco X-posure System has been successfully proven to be able to use computed radiography (CR) imaging plates in addition to conventional X-ray radiographic films.

Disclosures: Pronosco A/S,3.

M094

Dominant Distal Forearm Is Weaker Than Non-Dominant as Assessed by Bone Mineral Density (BMD). T. G. Palferman, P. J. Whelan.* Somerset Osteoporosis Service, Yeovil District Hospital, Somerset, United Kingdom.

We sought to determine whether the convention which dictates that the non-dominant hip as the preferred side for BMD measurement, on the assumption of it being the more fragile, can be extended to distal forearm scanning.All women aged between 60 and 80 years in two family practices were invited to attend for bilateral wrist BMD measurements by means of an Osteometer DTX-200 DXA bone densitometer (MediTech A/S, Denmark) with an in-vivo precision error of 1%.A total of 1174 women attended for ultradistal forearm scanning (2348 wrists). Number of right-hand dominant women 1076, left-hand dominant 98. Mean ages of total 68.9 years, no difference between right and left-hand dominant subjects.Mean T-scores: left wrist -1.56, right wrist -1.69; dominant side -1.71, non-dominant -1.54.Right lower than left, mean difference -0.13, p<0.001 (paired t-tests, 95% CI -0.16 to -0.09).Dominant side lower than non-dominant, mean difference -0.18, p<0.001 (95% CI -0.21 to -0.14).Right minus left mean T-scores among right-hand dominant women -0.17; for left dominant 0.27, p<0.001 (unpaired t-tests).Dominant minus non-dominant mean T-scores -0.17 and -0.27 for right and left-hand dominant women respectively, p=0.09.Mean Z-scores: left wrist 0.61, right 0.51; dominant side 0.5, non-dominant 0.63. Right hand lower than left, mean difference -0.1, p<0.001 (95% CI -0.13 to -0.06).Dominant lower than non-dominant side, mean difference -0.14, p<0.001 (95% CI -0.17 to -0.1).Right minus left mean Z-score -0.13 among right-hand dominant women and 0.22 for left-hand dominant, p<0.001.Dominant minus non-dominant mean Z-scores for right and left-hand dominant subjects -0.13 and -0.22 respectively, p=0.13.Considering both T and Z-scores, dominant is lower than non-dominant wrist whether the woman is right or left-handed. Right is lower than left generally, but only because dominant is lower than non-dominant and most people are right-handed.Dominant wrist, therefore, is genuinely weaker than the non-dominant as assessed by BMD. These findings have implications in the choice of side when only one wrist is used to diagnose osteoporosis and assess fracture risk.

M095

Placement of Regions-of-Interest for the DXR Method in Metacarpals. A. Rosholm, H. Thodberg.* Pronosco A/S, Vedbaek, Denmark.

Evaluation of the cortical thickness (radiogrammetry) in the metacarpals is a well-established method for diagnosis of osteoporosis. For example the metacarpal index has been used in many years. Recently radiogrammetry has been revitalised and brought into line with current emphasis on BMD by means of the DXR-BMD technique.A main advantage of radiogrammetry is the high precision made possible through the large number of cortical thickness measurements, which can be performed inside the region of interest (ROI). However, a prerequisite for this precision is a precise method of placing the ROI itself. This work reports the state-of-the-art in placement of the ROIs for measuring cortical thickness in the metacarpals.We use three ROIs rather than one to get higher precision. The three ROIs are placed in metacarpal 2, 3 and 4 by "coupling" them to each other and sliding them together up and down until the average bone width in the three regions is minimal.The traditional method for defining location of measuring is to take the midpoint. The new method is - in contrast to the traditional - independent and insensitive of any anomaly present outside the shafts of bone. Thus the ROI placement works smoothly aver a wide range of images, including: Subjects with severe arthritis, images with over-exposed distal ends, images taken in mammography equipment, which gives larger contrast, women, men as well as children down to the age of ten. Long term precision of the ROI placement was investigated and found to be 0.7 mm relative to the distal end of the metacarpals.This method of ROI placement is an important element of the high precision of DXR-BMD and MCI.

M096

Predicting Factors of BMD Variations Induced by Parathyroidectomy in Primary Hyperparathyroidism. C. Chappard,[1] C. Roux,[2] M. Paillard,*[1] P. Houillier.[1] [1]Physiology, Hopital Georges Pompidou, Paris, France, [2]Rhumatologie B, Hopital Cochin, Paris, France.

Factors determining a bone density gain after radical treatment in Primary Hyperparathyroidism (PHPT) are not well known. To evaluate these factors, we studied 19 patients with PHPT (3 men, 16 women) with age of 57.4±11.3. The diagnosis was made on biological criteria (high iPTH level with high calcemia) and confirmed by parathyroidectomy (PTX). All patients were measured for BMD both before and after surgical removal of adenoma. The duration between DXA_1 and DXA_2 was 21 ±6.6 months. At baseline, the following biological data were collected: ionized calcemia, iPTH, phosphorus, creatinine, fasting calciuria, 25 OH D_3, Calcitriol, Osteocalcin and Deoxy-Pyridinolin. The mean variation of BMD obtained after PTX was expressed as a percentage per year and compared to zero using a Wilcoxon test. For correlation, we used Spearman coefficients.

	BMD	Mean variation / year (%)	p
Spine	Lumbar	+2.0±3.8	0.03
Femur	Neck	+0.5±2.5	ns
	Trochanter	+4.1±5.2	0.00001
	Intertrochanter	+2.2±3.7	0.007
	Total	+1.3±5.1	0.009
Radius	Ultradistal	-0.6±2.5	ns
	MID	+0.6±1.7	ns
	1/3 proximal	+0.3±1.3	ns
	Total	+0.5±1.4	ns
Whole body	Total	+0.7±1.9	0.05

The BMD variation of lumbar spine was correlated with BMD variation of trochanter (r =0.59, p=0.007), UD radius (r = 0.48, p=0.04) and with whole body (r =0.70, p=0.001). The BMD variation of femoral neck was only correlated with other femur sites. The BMD variation of 1/3 proximal radius was not correlated with any sites. The BMD variation of MID radius was correlated only with whole body BMD variation (r=0.61, p<0.01). The change of BMD depends on the initial Z score at the same site for femoral neck (r=-0.58, p=0.008), total femur (r =-0.45, p=0.05) and total radius (r =-0.47, p=0.05). Only BMD variation of 1/3 proximal radius was correlated with age (r =0.46, p=0.05) and with fasting calciuria (r=-0.46, p=0.05). There is no variation of BMD related to other biological data. Conclusion : The variation of BMD induced by PTX was more pronounced in trabecular sites of weight-bearing bone (lumbar spine, trochanter) than in non bearing-bone (ultradistal radius) or cortical sites (femoral neck, 1/3 proximal radius). One hypothesis is that bone remodeling is more important in trabecular bone; therefore, the BMD increase is probably due to the mineralization of new bone present mainly in trabecular weight-bearing sites. The main factors affecting BMD variation were the initial Z score at femoral neck, total femur and total radius. No biological data are predicting of BMD variation excepted fasting calciuria for 1/3 proximal radius. These results emphasized the benefits of PTX on bone when the initial BMD is low according to the Z score.

M097

Comparison of the Bone Density Data of Two Hips. S. Yaturu,* M. G. Alferos, C. DePrisco. Endocrinology, Overton Brooks VAMC/LSUHSC, Shreveport, LA, USA.

Dominant and non-dominant differences in bone mineral density (BMD) have been observed in the upper extremities. However for the proximal femur, the distinction between dominant and non-dominant hips is not clear. The purpose of this study is to evaluate left to right variations in femoral BMD. We reviewed the bone density studies of 840 individuals. Correlations were done comparing both hips at corresponding sites. The results are expressed as mean plusor minus standard error (SE).

Site	Right:Mean±SE	Left:Mean±SE	Correlation
Neck	0.727±0.005	0.727±0.005	0.86
Trochanter	0.657±0.005	0.658±0.005	0.85
Intertrochanteric	1.030±0.007	1.038±0.007	0.89
Total	0.872±0.006	0.872±0.006	0.92
Ward's	0.53±0.006	0.530±0.006	0.88

We conclude that the BMDs of the two hips highly correlate at relevant measurement sites and there does not appear to be a dominant hip as there is a dominant forearm.

M098

Prediction of Osteoporotic Fracture by Digital X-ray Radiogrammetry and DXA. A Case-control Study of Three Different Fracture Types. P. Bach-Mortensen,*[1] K. Hindsø,*[2] K. Jensen,*[3] T. Jensen,*[2] L. Hyldstrup.[1] [1]Endocrine Unit, Hvidovre Hospital, Hvidovre, Denmark, [2]Orthopedic Surgery, Hvidovre Hospital, Hvidovre, Denmark, [3]Radiology, Hvidovre Hospital, Hvidovre, Denmark.

Digital X-ray Radiogrammetry (DXR) applied to hand and forearm x-rays can provide a BMD estimate with a high short-term precision. It is the aim of the present study to compare DXR with conventional DXA measurements with respect to identification of women with a recent osteoporotic fracture. A study group consisting of 264 postmenopausal women was consecutively sampled at admission to hospital. Only women accepting participation and meeting inclusion and exclusion criteria were included. The study included 139 patients with distal forearm fractures, 56 patients with proximal humeral fractures and 69 patients with proximal femoral fractures. Within the first week of the fracture, measurements of bone mineral density by DXR (Pronosco X-posure System) and by DXA of the lumbar spine, total hip and distal and ultradistal forearm (Norland XR-26) were performed. As controls a normative database sampled in the same region was used. A logistic regression model was used for calculations of odds-ratios per SD for each group of patients as well as the entire group. Results are given in the table below.In conclusion, the highest odds-ratios was not surprisingly seen for total hip BMD measurements in the hip fracture group, but DXR measurements were comparable to DXA measurements at the spine and forearm in all fracture groups. Digital X-ray Radiogrammetry may therefore be useful as a simple and cost-effective alternative to conventional densitometry.

Disclosures: Pronosco AS,2.

M099

Discrepant Areal and Volumetric Bone Density Measurements in a Young Woman With Low Bone Mineral Density. D. H. Schussheim, M. R. Rubin,* E. Shane. Medicine, Columbia University, New York, NY, USA.

Although osteoporosis predominantly affects postmenopausal women, reduced bone mineral density (BMD) has also been described in young premenopausal women. The majority of these affected young women have a secondary cause for their low bone density. Some, however, have no identifiable underlying disorder. A 34-year-old Caucasian woman was found to have low BMD in the course of an evaluation for back pain. She was 5' 2" tall, 104 pounds and otherwise in good health. BMD by dual energy x-ray absorptiometry (DXA) fulfilled World Health Organization criteria for osteoporosis in postmenopausal women. T scores at the lumbar spine (LS) and femoral neck (FN) were -2.9 and -3.1, respectively. Daily dietary supplementation with 1500 mg of elemental calcium and 600 IU of vitamin D daily was started and the patient was referred to our Metabolic Bone Diseases Unit for consideration of alendronate therapy. History, physical and biochemical evaluation for secondary causes of bone loss were unrevealing. The possibility that small bone size might be causing a spuriously low BMD measurement was considered. Bone mineral apparent density (BMAD), a measurement made by dividing the areal bone mineral content (BMC) by the square root of the projected area, was calculated. BMAD has been shown to minimize the effect of bone size on BMD. Although T scores improved, bone mass remained more than 2 standard deviations below the age-matched mean (LS = -2.38 and FN = -2.15). In contrast, quantitative computed tomography (QCT) of the lumbar spine, which measures true volumetric BMD, revealed a much higher T score of -1.6. The disparity between DXA T scores (even when corrected for bone size by BMAD) and QCT measurement is most likely due to this patient's small frame. This case highlights the difficulties in the assessment of bone mass in young women. QCT proved helpful in clarifying the spuriously low BMD found in this patient, even after correcting with the BMAD calculation. Anti-resorptive therapy was not recommended and is rarely advisable in this patient population in the absence of ongoing bone loss and/or fragility fractures. Interpretation of BMAD results is currently hampered by the lack of large population-based normative data. QCT, which is often not considered in this patient population, perhaps should play a more prominent role in the evaluation of young women with abnormal BMD.

M100

A New Classification of Osteoporosis in Children and Adolescents. E. Schoenau,[1] C. M. Neu,*[1] B. Beck,*[1] F. Manz,*[2] F. Rauch.[1] [1]Children's Hospital, Koeln, Germany, [2]Research Institute of Child Nutrition, Dortmund, Germany.

In adults, the diagnosis of osteoporosis relies on bone densitometry. In children and adolescents, however, densitometric data are often difficult to interpret, due to large inter- and intraindividual variations in bone size. Here we propose a functional approach to bone densitometry which addresses two questions: First, is bone strength normally adapted to the largest physiological loads (i.e., muscle force)? Second, is muscle force adequate for body size? To implement this approach, forearm muscle cross-sectional area (CSA) and bone mineral content (BMC) of the radial diaphysis were measured in 349 healthy subjects from 6 to 19 years of age (183 girls), using peripheral quantitative computed tomography. Reference data were established for height-dependent muscle CSA (an indicator of muscle force) and for the variation with age in the BMC/muscle CSA ratio. Based on the results of these muscle and bone analyses four diagnostic categories arise: 'Normal': Muscle force normal for height, and BMC adequately adapted to muscle force. 'Primary bone defect': Muscle force normal for height, but BMC low for muscle force. 'Secondary bone defect': Muscle force low for height, but BMC adequate for muscle force. 'Mixed bone defect': Muscle force low for height, and BMC low for muscle force. This diagnostic algorithm was used to evaluate results from three pediatric patient groups. Children who had sustained multiple fractures without adequate trauma (n = 11) and renal transplant recipients (n = 15) were found to have a primary bone defect. In children with preterminal chronic renal failure (n = 11) the musculo-skeletal system was normally adapted to their (decreased) body size. This functional approach to pediatric bone densitometric data allows for a rational diagnostic of osteoporotic conditions in childhood and adolescence.

M101

Age Specific Discordance in Osteoporosis Detection by DXA at Spine and Hip. A Retrospective Study of 13,663 Females in the UK. S. A. Steel, S. Howey,* D. W. Purdie. Centre for Metabolic Bone Disease, Hull Royal Infirmary, Hull, United Kingdom.

The aim of this study was to determine the effect of measuring bone mineral density by DXA at either spine or hip on the proportion of patients who would thus be defined as osteoporotic (T score <-2.5).13,663 adult females were referred to this specialist centre for bone mineral density evaluation. All referrals complied with locally agreed high-risk criteria. Bone densitometry of the spine and hip was carried out using DXA. The table below presents a retrospective age-specific analysis of the prevalence of osteoporosis detected by scan site.

Prevalence of Osteoporosis by Scan Site and Age

Age (years)	Osteoporotic at Spine (%)	Osteoporotic at Hip (%)	Osteoporotic at Spine or Hip (%)
20 to 29	7.6	0.6	7.6
30 to 39	3.5	1.1	4.1
40 to 49	3.1	1.6	4.0
50 to 59	9.4	3.5	10.9
60 to 69	29.3	14.5	33.9
70 to 79	39.1	27.9	48.4
over 80	38.4	37.9	52.5

Overall, 2877 (21%) of women were defined osteoporotic at hip or spine, 5343 (39%) as osteopenic and 5443 (40%) had normal BMD. There was a significant discordance in individual results for spine and hip and the degree of this discordance was age dependent. Across all ages, more females were defined osteoporotic at the spine than at the femoral neck. The addition of a femoral neck assessment in those below 70 years of age adds very little to the overall number defined as osteoporotic. Proposals that densitometry should be confined to hip evaluation may thus fail to disclose a significant burden of disease

M102

Accuracy of GE Lunar Prodigy Compared with Corrected DPX Scans and Chemical Analysis of Neonatal Pigs. T. D. Crenshaw,[1] D. K. Schneider,[1] R. H. Nord.[2] [1]Animal Sciences, University of Wisconsin, Madison, USA, [2]GE Medical Systems-Lunar, Madison, USA.

Bone mineral content (BMC) of neonatal pigs can be accurately predicted by dual energy x-ray absorptiometry (DXA) scans using the Lunar DPX with small animal software (detailed, medium scan mode, Lunar version 1.0 c) and correction factors generated by regression analysis (Crenshaw et al., 1997). The current objective was to evaluate the accuracy of a new instrument and software, GE Lunar Prodigy with v. 3.60 software for assessment of small animal body composition. Accuracy was based on prediction of reference values from scans of a phantom composed of various amounts of saline, fat and hydroxapatite (calcium tribasic phosphate) to provide a range in BMC (29 to 107 g), fat (28 to 1206 g), lean (2165 to 4517 g), and total mass (2222 to 5248 g). Results were validated by comparisons with chemical analysis of five dissected pigs ranging from 0.84 to 6.3 kg. Prodigy scans under-predicted BMC of phantoms by 3 + 2.1% and over-predicted pig BMC by 4 + 2.1%, but these predictions represent major improvements compared with DPX predictions of BMC (36 to 45% over-prediction of phantom and pig composition). Use of regression correction factors in the current study confirmed the accuracy of DPX scans for BMC (0.4 + 5% over-prediction). DPX does not accurately predict fat composition. Prodigy scans of the phantom under-predicted fat and total mass by 1.0 + 1.4% and 1.0 + 1.0% respectively. Prodigy scans of pigs over-predicted pig fat content by 2 + 6.3% and lean content by 9 + 2.7%. Total pig mass was under-predicted by 0.5 + 0.3% using Prodigy. In conclusion, Prodigy scans predict BMC, fat, lean and total mass composition of neonatal pigs between 1 and 6 kg within the range of accuracy detectable by traditional chemical analysis and dissection methods.

Disclosures: GE Medical Systems-Lunar,2.

M103

Digitial X-ray Radiogrammetry in Assessing Age-related Loss, Fracture Discrimination, and Diagnostic Classification. J. A. Shepherd, B. Fan, Y. Chen,* C. F. Njeh, H. K. Genant. Radiology, University of California at San Francisco, San Francisco, CA, USA.

Purpose: This study assessed Digital X-ray Radiogrammetry (DXR) across three defined female populations to examine its ability to reflect age- and menopause-related bone loss, discriminate osteoporotic fractures, and classify patients diagnostically. These results were also compared and interrelationships found to other peripheral bone assessments devices. Methods: A total of 47 healthy premenopausal (33 ± 7 years), 41 healthy postmenopausal (64 ± 9 years), and 36 osteoporotic postmenopausal (70 ± 6 years) women were examined with the following DXR measurements: DXR bone density of the metacarpals (DXR BMD), metacarpal index (DXR MCI), and metacarpal porosity (DXR POR). The radiographs were originally acquired using a radiographic absorptiometry protocol. The comparative peripheral measurements were: quantitative ultrasound of the calcaneus for speed of sound (QUS CALC SOS) and broadband ultrasound attenuation (QUS CALC BUA), radial dual x-ray absorptiometry bone density (DXA RAD BMD), peripheral quantitative computed tomography of the radius (pQCT BMD), metacarpal bone density using radiographic absorptiometry (RA METC BMD), phalangeal bone density using radiographic absorptiometry (RA PHAL BMD).Results: Of the three DXR measurements, DXR MCI showed the largest age-related change between the each subject group and the largest correlation to age (r=0.67, p<0.0001) The strongest correlations between DXR MCI and DXR BMD were for RA METC BMD (r=0.81 and 0.82 respectively) and RA PHAL BMD (r=0.93 and 0.79 respectively) while the weakest correlations between DXR MCI and DXR BMD were for QUS CALC BUA (r=0.20 and 0.17). All inter-device r values for DXR POR were statistically insignificant. The odds ratios (confidence interval) to discriminate osteoporotic fractures were 1.5 (0.7,3.0), 1.4 (0.7,2.7), and 1.33 (0.7,2.4) for DXR BMD, DXR MCI, and DXR POR respectively. This compared to a high odds ratio of 2.2 (1.1,4.4) for DXA RAD TOTBMD and a low odds ratio of 1.0 (0.6,1.5) for QUS SOS CALC. Kappa score analysis (using -2.0 T score as a cutoff value for osteopenia and -2.5 T score for osteoporosis) showed that in general the diagnostic agreement between the peripherals devices is low but highest between DXR MCI and RA METC BMD (osteoporotic: k=0.72, osteopenic: k=0.51) Conclusions: DXR BMD and MCI had a similar ability to discriminate fracture subjects, similar age-related change, and similar diagnostic classification to other peripheral devices.

Disclosures: Pronosco Inc.,2.

M104

Do Different X-ray Techniques Affect the Measurement Parameters of Digital X-ray Radiogrammetry on Scanned Radiographs of the Hands? Y. Chen,* J. A. Shepherd, B. Fan, X. G. Cheng, H. K. Genant. Radiology, UCSF, San Francisco, CA, USA.

Purpose: To investigate the affect of different X-ray techniques on measurement parameters in digital X-ray radiogrammetry.Materials and Methods: Ninety-nine women (56.65 ± 18.30 years of age) were involved in this study, including 30 healthy premenopausal (PRE, mean age 31.5 ± 7.5 years), 35 healthy postmenopausal (POST, mean age 65.60 ± 8.19 years), and 34 osteoporotic postmenopausal (OSTEO, mean age 70.18 ± 8.30 years) women. All participants had two radiographs of the non-dominant hand, one with 50kvp, 400mA, 0.8s (50kv), and the other with 60kvp, 300mA, 0.5s (60kv). The radiographs were taken as part of a standard radiographic absorptiometry protocol and retrospectively analyzed using an X-posure System (Pronosco, Version 2. Denmark), a computer-based diagnostic tool that calculates bone status from an X-ray image of the hand. Bone mineral density (BMD), metacarpal index (MCI), cortical thickness (CT) and porosity of the second to the fourth metacarpals were automatically calculated as comparable measurement parameters. Results: Mean values, standard deviations and t-test results (Student's t and p values) for the difference between the 50kv and 60kv radiographs are shown in Table. Two sets of parameters (BMD, MCI, CT) were highly correlated (r from 0.996 to 0.999) for the entire study population. When the statistics were applied to the three different groups of women, the same results (r from 0.993 to 0.999) were found. There were statistically significant differences between BMD, MCI and CT in the three groups and these differences were similar for the two different X-ray techniques. However, porosity was poorly correlated when comparing the data from the 50kv radiographs to those of the 60kv radiographs (r from 0.271 to 0.513). Porosity also showed little ability to discriminate among the three groups.

	Mean	Std Dev	t value	p value
BMD (50KV)	0.511	0.067	-149	0.1401
BMD (60KV)	0.512	0.066		
MCI (50KV)	0.423	0.088	0.66	0.5134
MCI (60KV)	0.423	0.087		
CT (50KV)	0.165	0.031	-0.07	09466
CT (60KV)	0.165	0.030		
PORO (50KV)	9.025	1.204	3.79	0.0003
PORO (60KV)	8.640	1.230		

Conclusion: In estimating bone status from retrospective radiographs acquired using radiographic absorptiometry protocol, radiogrammetric parameters—except porosity—will not vary much and seem to be unaffected by variations in the X-ray source

Disclosures: Pronosco,2.

M105

The Distal Femur: An Alternative Site for Assessing Bone Mineral Density (BMD). H. H. Kecskemethy,[1] H. T. Harcke,*[2] L. Saltzman,*[1] R. K. Lark,*[3] R. C. Henderson.[3] [1]Research, duPont Hospital for Children, Wilmington, DE, USA, [2]Medical Imaging, duPont Hospital for Children, Wilmington, DE, USA, [3]Orthopedics, University of North Carolina, Chapel Hill, NC, USA.

For patients with deformity and/or metal hardware in place, it is difficult, if not impossible, to obtain meaningful measurements at the hip and/or spine sites. We sought to verify that Dual X-ray Absorptiometry (DXA) determinations of BMD from the lateral distal femur can serve as a substitute for traditional sites when these can not be used. Eighty-eight children aged 7 - 18 years of age who were undergoing studies of the lumbar spine had scans of their distal femur(s) performed in the lateral projection. The femoral scan was analyzed using 9 combinations of regions which varied by location (metaphysis to diaphysis) and composition (cortical and medullary). Regional BMD vales were compared to standard lumbar spine (L1 - 4) values using regression coefficients (Pottoff). Standardized regression (beta) coefficients for 9 femoral regions indicated that spine BMD can be predicted by these measurements. Beta values ranged from 0.673 to 0.804 (p values ranged from 0.01 to 0.001). The best predictor of spine BMD was a metadiaphyseal area of interest containing both cortical and medullary bone. A number of regions were not statistically different from this region, however, this method of measuring femur BMD was never inferior to predicting spine BMD and the region of interest was easy to place. For children who can not have spine and hip BMD measured because of metal hardware or deformity, a metadiaphyseal area of the lateral distal femur provides a valid predictor of lumbar spine BMD and therefore can be used as an alternative site for monitoring.

M106

Digital X-ray Radiogrammetry in the Assessment of Bone Status in Children. C. F. Njeh, B. Fan, M. Grigorian,* J. A. Shepherd, X. Cheng,* H. K. Genant. Radiology, University of California San Francisco, San Francisco, USA.

Bone mineral density (BMD) measurement is a useful tool for the assessment of skeletal status. The Prosnosco X-posure system estimates forearm BMD using digital x-ray radiogrammetry from radiographs of the hand. The system also provides an estimate of the porosity of the cortical bone and the level of striation at the endosteal surface. The purpose of the current study was to characterize the influence of age, anthropomorphics, and systematic lupus erythematosus (SLE) on BMD, porosity and striation. Healthy female children (n=108, mean age 14.7± 4.3years) and girls with SLE (n=51, mean age 15.8±3.4 years) of age range 7- 20 years were recruited. Ethnicity was diverse and included African American, Caucasian, Asian and Hispanics. BMD was derived from standard hand radiographs using the Prosnosco X-posure system. Forearm BMD was also measured by DXA (Hologic QDR 4500).Among healthy subjects BMD was positively correlated to age ($r = 0.62$, $p < 0.001$), while porosity was inversely related to age ($r = -0.48$, $p < 0.001$). BMD ($r = 0.89$, $p < 0.001$) and porosity ($r = -0.55$, $p<0.01$) were significantly correlated with forearm BMD by DXA. However striation was not associated with age or BMD by DXA. There were no significant differences in height and weight among the different ethnic groups, although Hispanic subjects tend to be heavier. Ethnicity influenced both BMD and porosity values with a trend towards higher BMD and lower porosity for African American subjects and lower BMD and higher porosity for Asians subjects. Statistically significant differences between ethnic groups may not have been observed because of the limited sample size. However, in a multiple linear logistic regression analysis with BMD as the dependent variable, age, ethnicity and weight were significant independent predictors. Similar results were found for porosity. SLE subjects were older, shorter and heavier than healthy subject, but this did not reach statistical significance. BMD was significantly lower in the SLE subjects than healthy subjects ($p = 0.03$). Surprisingly porosity was also significantly lower in the SLE subjects compared to healthy subjects ($p = 0.002$). We can conclude that BMD measured using the Prosnosco X-posure system is a useful approach to monitor skeletal development in children.

M107

The Clinical Utility of Spine Bone Density in Elderly Women. D. L. Schneider, R. Bettencourt,* E. L. Barrett-Connor. University of California San Diego, La Jolla, CA, USA.

It is common clinical practice to obtain bone mass measurement at both the hip and spine to evaluate for osteoporosis. We examined the association of spine osteoarthritis and bone mineral density in 1082 community-dwelling ambulatory older women aged 50-96 years. At a 1992-1996 research clinic visit, a standard medical history was obtained, height and weight were measured, and bone mineral density (BMD) was measured at the hip and AP and lateral lumbar spine using DXA (Hologic 2000). Spine osteoarthritis was identified on the AP lumbar spine DXA print out images by a musculoskeletal radiologist. Osteoporosis was defined by the WHO criteria of T-score =< -2.5 using normative data from NHANES for the hip DXA and Hologic female normals for spine DXA. Forty percent of women had evidence of osteoarthritis (OA) in the lumbar spine. Women with spine OA had mean age of 77.4 years (95% CI, 76.5-78.2) and were significantly older than women without spinal OA (mean age 66.8; 95% CI, 65.9-67.7). Women with spine OA in comparison with those without spine OA were less likely to use current estrogen (38.4% vs. 47.4%, p< 0.05) and were more likely to use thyroid hormone (25.1% vs. 18.7%, p< 0.05). Mean BMD at the total hip, AP spine, and lateral spine adjusted for age, body mass index, current estrogen and thyroid hormone use was significantly higher in women with spine OA. The prevalence of osteoporosis by measurement site is shown in table.

	All women	No spine OA	Spine OA
Site	%	%	%
Total hip	17.4	13.9	32.2
AP spine	20.3	24.5	14.4
Lateral spine	53.9	55.7	51.3

In summary, over half of the women were identified as osteoporotic based on lateral spine DXA. Although the effect on BMD of spine OA appears to be removed in the spine lateral projection, lateral spine DXA is not routinely performed in clinical practice. In elderly women who are likely to have spine OA and other degenerative changes, DXA of the hip only is recommended for identification of osteoporosis.

M108

Precision of Hip Axis Length and Upper Neck BMD Measurements in Normal Subjects. M. K. O'Connor,*[1] G. Sieck,*[1] K. Hammel,*[1] D. Settergren.*[2] [1]Mayo Clinic, Rochester, MN, USA, [2]GE Lunar, Madison, WI, USA.

Purpose: To determine the precision of hip axis length and upper neck BMD measurements in normal subject. Background: Most densitometric techniques perform equally well when predicting the risk of an osteoporotic fracture at any site. Site-specific measurements, however, improve the prediction of fracture at that particular skeletal site. Hip fracture constitutes the most serious type of osteoporotic fracture in terms of mortality, morbidity, and expense. Research has shown that the risk of hip fracture increases approximately 1.5 fold for each standard deviation decrease in BMD at the spine or radius, but site-specific measurement at the proximal femur results in a gradient of risk between 2.5 and 3. Further improvements in predicting fracture risk at the hip might constitute an important clinical advance. Various suggestions have been proposed for more completely utilizing information available from a densitometry scan of the proximal femur. BMD of the proximal femur provides the best predictive ability for fracture at the hip, but aspects of femur geometry and distribution of BMD within the femoral neck site provide additional information that might lead to improved prediction of fracture risk. Hip axis length , a measurement extending from the base of the greater trochanter along the hip axis to the inner pelvic brim, has been shown to be associated with the risk of hip fracture in several independent studies.

Additionally, the distribution of BMD in the upper half of the femoral neck has been shown to improve the prediction of cervical hip fractures. Methods: We determined the short-term precision of HAL and upper neck BMD, as an initial step in evaluating the feasibility of utilizing additional information readily accessible from a DEXA analysis. Thirty normal subjects were measured twice on a PRODIGY (GE Lunar), a narrow angle (4°), fan-beam densitometer. Two problematic scans that required manual point typing were not included in BMD results. Results: Precision (CV) calculated using the root-mean square method was 0.9% for HAL and 2.4% for upper neck BMD. By comparison, total neck BMD had a precision of 1.4%. Conclusions: Measurement of HAL appears to be reproducible in normal subjects, with a precision comparable to that seen in total spine and total hip BMD measurements. Upper neck is considerably less reproducible which diminishes its potential usefulness as an indicator of fracture risk.

	Scan 1	Scan 2	Mean	SD*	%CV
HAL	11.37	11.38	11.37	0.07	0.91
Upper Neck	0.899	0.896	0.898	0.019	2.41
Total Neck	1.067	1.055	1.060	0.016	1.38

Average within subject SD

M109

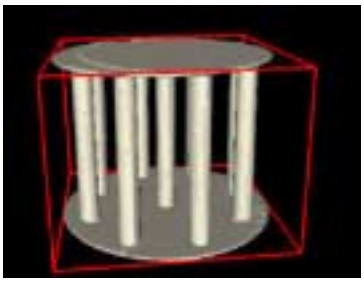
Cost-Effective Methods in Identifying Systemic Lupus Erythematosus Patients With Low Bone Densities. Y. Lu,*[1] J. A. Shepherd,[1] E. Von Scheven,[2] H. K. Genent.[1] [1]Department of Radiology, University of California, San Francisco, San Francisco, CA, USA, [2]Department of Pediatrics, University of California, San Francisco, San Francisco, CA, USA.

Pediatric systemic lupus erythematosus (SLE) patients often develop low bone mineral density (BMD). To identify low BMD patients, we applied a modified recursive partitioning algorithm (RPA) to develope cost-effective classification rules based on clinical, laboratory, and readiological results. We recruited 56 SLE patients aged 8 to 22 (mean=15.74) yrs old. Clinical assessments (ethnics, gender, age, disease duration, Tanner score, bone age, etc.), laboratory results (urine and serum tests), QUS parameters (SOS, BUA, etc.), BMD measured by DXA at forearm, AP and lateral spine, hip, whole body, as well as BMD measured by QCT were collected. Low BMD status was determined by Z-score below -1 in QCT volumetric spine BMD (in reference to 118 age-matched normals). Fifteen of 56 patients had low QCT BMD. RPA is a sequence of binary splits of patients. It selects the best splitting variables and cut-off thresholds automatically to optimize the classification accuracy. We modified the conventional RPA (CRPA) with consideration of the costs of the splitting variables. We grouped predictive variables into 4 levels based on their costs: clinical assessment (lowest cost), laboratory tests (lower cost), QUS (higher cost), and DXA (most expensive). First, we found best splits among variables in four levels. The CRPA-selected split (statistically optimum) was compared to the best splits in the lower cost levels. McNemar test and bootstrap confidence intervals were used to determine the statistical equivalence of these splits (at 5% type I error rate). We chose the equivalent split at the lowest cost level as our modified split. Two classification rules were derived in this paper. A1 was the result of CRPA that utilized DXA hip scans for all patients and whole body DXA scans for 41% patients with low BMD in Ward's Triangle and were younger than 17 yrs. A2 resulted from the modified RPA that applied hip DXA scans to 64% patients who had disease duration more than 1.5 yrs. Table 1 shows their statistical properties. Although A1 was better statistically, it was also more expensive. Bootstrap results showed no significant differences between 2 rules in sensitivity, specificity, and accuracy at a significant level of 5%. The most cost-effective classification for low BMD among childhood SLE can be achieved by testing DXA hip BMD only for patients with longer disease duration.

M110

Validation of a Micro-CT System for the 3-D Measurement of Bone. P. A. Chmielewski,*[1] T. E. Dufresne,*[1] K. H. Combs,*[2] B. Borah,*[1] M. W. Lundy.[1] [1]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [2]Procter & Gamble, Cincinnati, OH, USA.

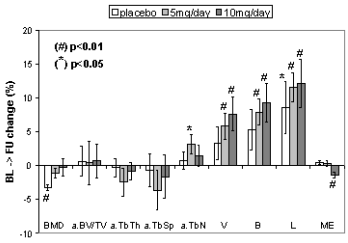
3-D x-ray micro-computed tomography (µCT) has emerged in recent years as a powerful non-invasive technology for analyzing bone architecture of both human bone biopsies and whole small animal bones directly in 3D. Comparing µCT to conventional histomorphometry, we have shown strong correlations for BV/TV(R2=0.90), TbTh-(R2=0.61), TbN-(R2=0.88),and TbSp-(R2=0.91) [1]. In order to demonstrate the accuracy and reproducibility of µCT for use in analysis of pre-clinical and clinical biopsies, we have successfully brought the µCT system (mCT20 3-D x-ray CT scanner manufactured by Scanco Medical and associated validation software) into GLP (Good Laboratory Practices) compliance. The use of physical phantoms was an important component in the validation to establish the accuracy and precision of the system throughout its operation. One phantom, an embedded aluminum wire with a known thickness measure traceable to NIST standards, verified the accuracy of the system. For our system, this yielded a 2.08 % error in thickness from the known reference. The other phantom, consisting of nine concentric aluminum tubes connected to aluminum endplates (as shown in the figure),verified the precision of the system based on the volume and surface area measures determined on each tube. In a typical multi-week study this yielded a maximum error of 1.83%. The reproducibility of the system was assessed by evaluating intra-sample variability and inter-operator variability in the volume and surface area measures in two bone types (human iliac crest biopsy and minipig vertebra core). The resulting % coefficient of variation ranged from 0.11 to 2.03 in the biopsies and from 0.07 to 1.64 in the cores. The GLP system now in place allows for higher confidence in current measurements and provides a procedural mechanism for maintaining the integrity of the µCT system for its on-going use in bone structural research. [1], T.Dufresne, P. Chmielewski, B. Borah, M. Lundy, K. Combs, Novel 3-D Image Analysis Methods in mCT Bone Research, Symposium on Bone Architecture and the Competence of Bone, Ittingen, Switzerland 1998.



M111

New Descriptors of Trabecular Bone Microarchitecture: An in vivo Longitudinal Study Using Magnetic Resonance Imaging. L. Pothuaud,[1] D. C. Newitt,[1] C. Chesnut,[2] H. K. Genant,[3] B. MacDonald,[4] S. Majumdar.[1] [1]Magnetic Resonance Science Center, University of California, San Francisco, USA, [2]University of Washington, Seattle, USA, [3]Osteoporosis and arthritis group, University of California, San Francisco, USA, [4]Smith Kline Beecham Pharmaceuticals, PA, USA.

The in vivo evaluation of Trabecular Bone Microarchitecture (TBM) could contribute to understanding the results of different bone therapeutic interventions. The aim of this study was to apply new 3D parameters to in vivo Magnetic Resonance (MR) images of TBM, and check the potential use of these parameters in monitoring the anti-resorber agent (idoxifene). Thirty postmenopausal women were recruited and randomly assigned into 3 groups: placebo (n=9), 5mg/day (n=11), and 10mg/day (n=10). MR images (156x156x500microns) and Bone Mineral Density (BMD) measurement of the ultradistal radius were performed at the beginning of the study (BL) and after 1 year of treatment (FU). A standardized process was applied to the MR images in order to align BL and FU images, select a region of analysis (7.5mm length starting at 12mm from the distal-end), and apply a global threshold. Apparent morphological parameters were evaluated as bone volume fraction (a.BV/TV), Trabecular Thickness (a.TbTh), Trabecular Spacing (a.TbSp) and Trabecular Number (a.TbN). 3D Line Skeleton Graph Analysis (LSGA) was used, and topological parameters such as the densities of vertices (V), branches (B) and loops (L) were calculated. Finally, the complexity of TBM was quantified by Maximal Entropy (ME). Relative changes between BL and FU in each of the 3 groups are reported on the figure. The statistical difference between BL and FU data was checked for each parameter using paired t-test inside each group (symbol is reported on the figure when this difference is significant). The statistical difference between the 3 groups was checked using Kruskal-Wallis test, and was not significant for any of the investigated parameters and for both BL and FU evaluations. Linear correlations with a.BV/TV were: R^2=0.58 (a.TbTh), 0.85 (a.TbSp), 0.57 (a.TbN), 0.30 (V), 0.19 (B), 0.37 (L), 0.01 (ME) and 0.10 (BMD).The present study has shown that 3D-LSGA topological and ME parameters, new descriptors of TBM recently applied to MR in vivo images, could potentially be used in the monitoring of bone therapeutic intervention to complement BMD measurement.



M112

Trabecular Bone Microarchitecture Derived from High-resolution MRI of the Ultradistal Radius: Relationship to Osteoporotic Status. L. Pothuaud, D. C. Newitt, S. Majumdar. Magnetic Resonance Science Center, University of California, San Francisco, USA.

Trabecular Bone Microarchitecture (TBM) evaluation may be potentially important in predicting fracture risk. The aim of this study was to apply new TBM indicators to in vivo Magnetic Resonance (MR) images of TBM, and check the potential use of these parameters for the diagnosis of osteoporosis.127 postmenopausal women with (n=70) and without vertebral osteoporotic fracture were included in this study. Subjects without fracture were classified into normal (n=21) and osteopenic (n=36) groups following standard spine or femur BMD T-score criterion. MR images (156x156x500microns) of the ultradistal radius were obtained, and a standardized process was used in order to select a region of analysis (7.5mm length starting at 12mm from the distal-end of the radius), and apply a global threshold. Apparent morphological parameters were evaluated as bone volume fraction (a.BV/TV), Trabecular Thickness (a.TbTh), Trabecular Spacing (a.TbSp) and Trabecular Number (a.TbN). Furthermore, 3D Line Skeleton Graph Analysis (LSGA) was used, and some topological parameters were evaluated as the densities of vertices (V), branches (B) and loops (L). Finally, the complexity of TBM was quantified by Maximal Entropy (ME). Mean values of TBM parameters are reported in the Table for each of the 3 groups. Linear

correlation were checked (R^2-coefficient) between a.BV/TV and other parameters. The statistical difference between the 3 groups was tested (p-value) with a Kruskal-Wallis rank test. The same group-test was applied after a manual adjustment of a.BV/TV, the corresponding results being reported in brackets.3D-LSGA topological and ME parameters have been recently applied to in vivo high-resolution MR images. This new TBM evaluation has potential interest in clinical application for osteoporosis diagnosis and fracture risk prediction.

	a.BV/TV	a.Tb.Th	a.Tb.Sp	a.Tb.N	ME	L	V	B
normal n=21(7)	0.373	0.218	0.372	1.707	4.597	2.199	2.963	5.124
osteopenic n=36(14)	0.357	0.213	0.384	1.687	4.610	2.174	2.938	5.072
osteoporotic n=70(21)	0.365	0.219	0.384	1.670	4.698	2.126	2.849	4.934
R^2 with a.BV/TV	1.00	0.28	0.66	0.38	0.05	0.22	0.08	0.14
Kruskal-Wallis test	0.311	0.097	0.419	0.376	**0.0001**	0.751	0.428	0.541
(a.BV/TV adjustment)	(0.94)	**(0.032)**	(0.245)	(0.056)	**(<0.0001)**	**(0.031)**	**(0.015)**	**(0.016)**

M113

Micro-CT Evaluation of Structural Changes in Arthritic Knees. V. V. Patel,*[1] A. Burghardt,*[2] A. Laib,*[2] M. Ries,*[1] S. Majumdar.[2] [1]Orthopaedic Surgery, University of California, San Francisco, CA, USA, [2]Radiology, University of California, San Francisco, CA, USA.

Though the effect of osteoarthritis (OA) on articular cartilage is well known, the reaction of subchondral trabecular bone has not been adequately evaluated. Micro-CT is an effective tool for such study.Six cadaver knee specimens were harvested at the time of autopsy. The joint surfaces were evaluated as follows: 4 normal and mild OA specimens (minimal cartilage fraying), 1 moderate OA specimen (cartilage degeneration, no exposed bone) and 1 severe OA specimen (cartilage degeneration, exposed bone). 72 bone cores were taken from the patella, trochlea, medial and lateral tibial plateaus and femoral condyles. Micro-CT was performed to obtain 30 µm resolution three dimensional images. Vendor software was modified to evaluate on a per mm basis: trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular connectivity (Conn.D.), trabecular bone volume/ total volume (BV/TV), trabecular separation (Tb.Sp), and structural model index (SMI) (a measure of rod-like vs. plate-like structure). Medial compartments were compared with lateral, as were normal and mild OA with moderate and severe OA groups. Tb.N was increased in OA knees and in the medial compartment regardless of OA. Tb.Th was lower in arthritic knees though higher in the subchondral regions of all the medial compartments relative to lateral. Conn.D. was increased in arthritic knees with no significant difference between medial and lateral compartments. BV/TV was increased in the subchondral bone of arthritic tibias, and higher in the medial compartments relative to lateral. Tb.Sp was lower in arthritic knees and in the medial compartments. Finally the SMI had no significant difference in arthritic knees but was slightly lower (more plate-like) in the subchondral medial compartments. Data results also revealed the greatest divergence in different groups near the subchondral surface and convergence with increasing distance from the joint surface.The decrease in Tb.Th. and Tb.Sp. with an increase in Tb.N and Conn.D in the arthritic bone may be explained by an increase in bone turnover. Increased stresses likely stimulate remodeling of the subchondral bone, which, in turn stimulates increased resorption and formation of trabeculae. This would explain the increased number of thinner trabeculae with decreased space between them, and the observation that differences are greatest near the joint surface. This theory also correlates well with increased uptake in arthritic knees during nuclear medicine imaging.

M114

Precision Error of Leg and Arm Muscle and Cortical Bone Cross-sectional Areas Using Peripheral Quantitative Computed Tomography in Girls. R. L. Seip,* D. Falk,* E. Dennis.* Human Performance Laboratory, University of Nebraska at Kearney, Kearney, NE, USA.

Peripheral QCT is valuable for longitudinal studies of children because it can simultaneously assess cross-sectional areas of muscle, cortical bone, and trabecular bone in the limbs. In the present study, cross-sectional areas of leg and arm tissue in nine normal-sized children, ages 9-12, were assessed using a Norland/Stratec XCT2000 on three different days within three weeks to evaluate pQCT reliability. Images of the leg were obtained at 66% of the distance from the medial maleolus to the medial condyle of the tibia, and those of the arm at 66% of the distance from the styloid process of the ulna to the olecranon process. Standard scan parameters (voxel size = 0.8 mm; scan speed = 30) were used. Average scan time was 90 seconds. All scans were of high quality. Summary data for muscle (M) and cortical (C) bone tissue areas and their ratio (C/M) are shown below. The overall mean was a group mean derived from the individual means of the three measurements for each subject. The precision error terms (Root Mean Square Standard Deviation, RMSSD; and coefficient of variation, CV) were calculated according to the method of Gluer et al (Osteo. Int. 5: 262-270, 1995).

	Leg			Arm		
	M	**C**	**C/M**	**M**	**C**	**C/M**
Overall Mean (mm^2)±sd	4436 ± 928	234 ± 40	0.0530 ± .0069	2165 ± 417	113 ± 19	0.0527 ± 0.0054
RMSSD (mm^2)	101.2	4.05	0.0017	64.6	3.02	0.0018
CV (%)	2.28	1.73	3.30	3.00	2.68	3.37

The data show that pQCT error in children in early or pre-puberty ranges from 1.7 to 3.4%. Precision is greater (i.e., CV is less) for leg muscle and cortical bone compared to arm, indicating greater precision in leg measurement due perhaps to the larger mass of the leg. The cortical bone-to-muscle ratio error is similar in arm and leg. We conclude that the pQCT is reliable for muscle and cortical bone tissue measurement in children.

M115

Anisotropy Measurement of Trabecular Bone Radiographic Images Using Spectral Analysis. B. Brunet-Imbault, G. Lemineur,* S. Poupon,* E. Lespessailles, C. L. Benhamou. Equipe INSERM ERIT-M0101, Centre Hospitalier Régional d'Orléans, Orleans, France.

The definition of osteoporosis involves both a decrease of bone mass and alteration of trabecular bone microarchitecture. The anisotropy of trabecular bone is a very important microarchitectural property of this tissue, it depends upon the strength applied to bone : gravity and muscular tractions. The aim of this work was to study the anisotropy of the trabecular bone projection using spectral analysis.This preliminary study was performed on calcaneus radiographs in a region of interest rich in transverse and longitudinal trabeculae. 28 women with fractures were studied. 10 women having a Bone Mineral Density (BMD) < -2.5 SD (osteoporotic BMD cases) were compared to 18 women having a BMD > -2.5 SD (normal BMD cases). The low frequency noise was filtered from the radiographic images and the Fast Fourier Transform (FFT) was used to perform the spectral analysis. The shape of the Fourier transform was a cross-like pattern in all cases, one branch corresponding to the longitudinal trabeculae and the other one to the transverse. The FFT permits to determine the orientation and the proportion of both longitudinal and transverse trabeculae. The amplitude integration of the Fourier transform corresponds to the total amount of the projected trabeculae and therefore the longitudinal and the transverse trabeculae amount can be determined. The maximum length (Rmax) with regards to the minimum length (Rmin) of the FFT pattern directly reflects the anisotropy of the structure. The more Rmax / Rmin is close to 1, the more the structure is isotropic. Difference in Rmax / Rmin values was found between the two groups of subjects : Rmax / Rmin = 1.334 ± 0.295 for osteoporotic BMD cases versus Rmax / Rmin = 1.138 ± 0.094 for normal BMD cases, p = 0.034. AL / AT = 1.444 ± 0.359 for osteoporotic BMD cases versus AL / AT = 1.179 ± 0.118 for normal BMD cases (p was not significant). This preliminary study was performed with a small population, this fact possibly explaining the lack of statistical significance with AL / AT. The trend of AL / AT may suggest a loss of transverse trabeculae with osteoporosis, leading to an increase of anisotropy. The variation of Rmax / Rmin showed the same anisotropy evolution with osteoporosis. We have previously shown the ability of a fractal analysis of bone texture to discriminate osteoporosis cases from control cases. This preliminary study is promising to characterize the microarchitectural organization of the trabeculae.

M116

Similar Prevalence of Vertebral Deformities despite Differently Derived Data. P. M. Szulc.* Epidemiology os Osteoporosis, INSERM, Lyon, France.

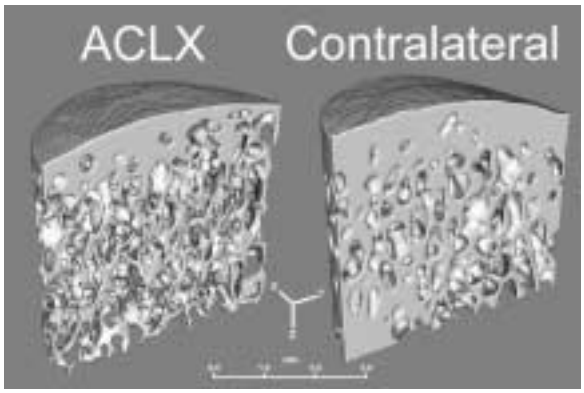
Similar prevalence of vertebral deformities despite differently derived reference data.P. Szulc, F. Munoz, P.D. Delmas; INSERM Research Unit 403, Lyon, FranceMorphometric diagnosis of vertebral deformities (VD) requires definition of reference values. In previous studies, reference values have been attained in young healthy adults or in adults of various age in whom abnormal radiographs were excluded either by a trained observer or by using statistical algorithms. In this study, we used four sets of morphometric reference data obtained in: A. 102 early postmenopausal women with normal bone mineral density and normal radiographs of spine belonging to the OFELY cohort, B. 102 women aged 51 to 85 years with normal spine radiographs selected among women recruited for the EVOS study (radiographs of poor quality and those presenting pathology of spine were excluded), C. 260 women aged 51 to 85 years recruited for the EVOS study using Melton's algorithm (Melton et al., Osteoporos Int, 1993, 3, 113), D. the same 260 women using Black's algorithm (Black et al. J Bone Miner Res, 1991, 6, 883). Using the above four sets of reference data, we applied two diagnostic criteria (mean – 3SD and 0.85*mean) in two groups of women: 260 women recruited for the EVOS study in whom we diagnosed 63 VD using Genant's semiquantitative method (EVOS cohort) and 176 osteoporotic women (OP group) aged 54 to 88 years in whom we diagnosed 285 VD using Genant's method. In the EVOS cohort, number of VD diagnosed using different reference values varied from 53 to 57 for the 0.85*mean criterion and from 94 to 117 for the mean – 3SD criterion. In the OP group, number of VD diagnosed using different reference values varied from 255 to 273 for the 0.85*mean criterion and from 253 to 404 for the mean – 3SD criterion. For the 0.85*mean criterion, agreement of diagnosis was excellent both in OP group ( = 0.96 – 0.98) and in EVOS cohort ( = 0.96 – 0.99). For the mean – 3SD criterion, agreement of diagnosis was very good and only slightly higher for the OP group ( = 0.80 – 0.93) than for the EVOS cohort ( = 0.79 – 0.84). Agreement of diagnosis was lower when two morphometric criteria were compared using reference values obtained in the same set.Conclusion: Reference data derived using different methods give similar rates of prevalence of VD. Differences of prevalence of VD can be real due to differences between populations or spurious due to different diagnostic criteria or due to different quality of radiographs as we have previously shown (Szulc et al., Bone, 2000, 27, 841).

M117

Mechanical and Architectural Bone Adaptation in Early-Stage Experimental Osteoarthritis. S. K. Boyd,*[1] R. Mueller,[1] R. F. Zernicke.[2] [1]Institute for Biomedical Engineering, ETH & University Zuerich, Zuerich, Switzerland, [2]McCaig Centre for Joint Injury and Arthritis, Univ Calgary, Calgary, AB, Canada.

The purpose of this study was to quantify mechanical and architectural changes to knee joint periarticular subchondral cancellous bone in early-stage experimental osteoarthritis (OA). Unilateral anterior cruciate ligament transection (ACLX) was performed on 10 dogs that were randomly assigned to two groups: 3 or 12 wk post ACLX. Two additional dogs were used as normal unoperated controls. Cylindrical bone cores excised from the medial condyle of the distal femur after euthanasia were scanned using high resolution computed

tomography (μCT) (Fig. 1)
and subsequently failed under unconstrained uniaxial compression. The apparent-level elastic modulus was less in the ACLX femur compared to the contralateral control, and the 45% decrease was significant ($p<0.05$) by 12 wk post ACLX. A finite element (FE) analysis based on μCT data simulated the uniaxial compression tests on a specimen-by-specimen basis to determine tissue modulus. No change in tissue was detected, and a single tissue modulus of 5100 MPa (95% confidence interval ±600 MPa) explained the apparent-level modulus changes observed in the disease-related bone adaptation. The three-dimensional connectivity was evaluated from the original μCT data to quantify architectural alterations in contrast to tissue alterations. Significantly increased connectivity occurred as early as 3 wk post ACLX and was as high as 127% by 12 wk post ACLX in the distal femur. These measured changes indicated that architectural adaptation predominated over tissue modulus changes affecting apparent-level elastic modulus in the early-stage of experimental OA, and that to maintain normal cancellous bone following a traumatic injury, early intervention should focus on preventing the substantial architectural alterations.



M118

Solid Components of Bone Matrix Measured by Proton Magnetic Resonance Imaging. Y. Wu,*[1] D. A. Chesler,*[2] J. L. Ackerman,*[2] J. Wang,*[1] M. J. Glimcher.[1] [1]Laboratory for the Study of Skeletal Disorders and Rehabilitation, Department of Orthopaedic Surgery, Children's Hospital and Harvard Medical School, Boston, MA, USA, [2]NMR Center, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

The degree of mineralization of bone can be expressed as the ratio of a volumetric bone mineral density and a volumetric bone matrix density. We have previously shown that the 3D bone mineral density of both cortical and cancellous bone can be noninvasively measured by solid state phosphorus 31 magnetic resonance imaging (MRI). We now demonstrate that bone matrix density can be measured by solid state proton MRI. When combined, these two techniques can measure true extent of bone mineralization.The total proton MRI signal can be modeled as arising from two tissue compartments: a. solid (mineral crystals and molecularly immobile cellular and extracellular molecules) and b. fluid (mainly tissue water, lipid, and other molecularly mobile constituents). Most of the constituents of soft tissue and bone marrow fall into the fluid classification. Proton MRI separates these compartments on the basis of the T2s (spin-spin relaxation times) of their molecular constituents by measuring both a free induction decay (FID) signal and an echo signal. The solid compartment will contribute to the FID signal but not to the echo signal, while the fluid compartment will contribute to both signals. Because almost all of the solid state protons are in the matrix, the solid state proton signal is a measure of bone matrix content. This initial simple model offers a means to measure noninvasively the bone organic matrix density as a 3D image. Sections 1 cm long were cut from freshly dissected femoral cortices of 8 week old postnatal calves. Cancellous bone were cut form the femoral head. Bovine gastroc tendon was also studied. All specimens were examined wet.The initial results were shown in Table 1. Note that the signal ratio does not directly represent the mass in gram units. However, the trend is clear and consistent. Our study has demonstrated that proton MRI provides a novel means to measure solid phase of bone matrix noninvasively.

Specimen	Slid Signal/Total Signal
Tendon1	0.42
Tendon2	0.43
Tendon3	0.51
Cortical1	0.69
Cortical2	0.59
Cortical3	0.64
Cancellous1	0.08
Cancellous2	0.09
Cancellous3	0.05

M119

Utilization of Fractal Analysis as a Diagnostic Tool in the Progression of Periodontal Disease. K. H. Lee,* Y. Lu, G. Armitage, N. Lane, M. Khan, S. Majumdar. UCSF, San Francisco, CA, USA.

Periodontal disease results in alveolar bone loss and clinical loss of attachment. Digital subtraction radiography (DSR) and standard dental radiographs have traditionally been used to assess alveolar bone changes in this disease. Recently, fractal analysis, a method of assessing trabecular bone indices, has become available. The purpose of this study was to assess if fractal analysis could be used as a reliable method to detect the progression of alveolar bone loss in periodontitis. We performed a retrospective, operator-blinded study on a group of 32 periodontally healthy and a group of 31 periodontitis diseased patients. Standardized clinical vertical bitewing radiographs were obtained at baseline and after an interval of 6-months of observation without standard treatments. In addition, changes in clinical measurements including gingival index, bleeding on probing, suppuration, probing depth, clinical attachment level, and relative attachment level measured with an automated disk probe, were compared to the changes in fractal analysis. Bone change, categorized as loss, no change, or gain, was assessed by DSR. For fractal analysis, radiographs were digitized by using Epson Expression scanner (1600 model EU-35) at 1000 dpi with an 8 bit gray scale. The region of interest was defined as a circle with a radius limited by the cortical border between the roots of two teeth an the alveolar crest. Fractal dimension (FD) was calculated using the Fourier transform method. Our data revealed that fractal dimension was significantly different ($P<0.05$) between the healthy and the diseased group of patient. In the diseased group of patients, fractal dimension was significantly ($P<0.03$) associated with radiographic bone loss by DSR, but not in the healthy group. Manual probing depth was also significantly associated with fractal dimension ($P<0.01$) in the healthy group. Comparing the different radiographic bone changes, fractal dimension was significantly associated ($P<0.03$) with detected bone loss. These data suggest that fractal analysis can differentiate between healthy and diseased patients. In addition, fractal analysis is able to detect bone loss as assessed by DSR.

M120

Radial Bending Breaking Resistance Derived by Densitometric Evaluation Predicts Femoral Neck Fractures. D. Gatti, E. Sartori,* V. Braga, F. Corallo,* M. Rossini,* S. Adami. Rheumatology Unit, Valeggio S/M, Italy.

Bone Mineral Density (BMD) as measured by DXA is the best predictor of osteoporotic fracture risk. Bone cross-sectional area that is also an important determinant of both bone compression strength and of bending breaking resistance but it is taken into account only in part by DXA-BMD. From DXA measurements of proximal radius (Osteoplan ®, NIM, Verona, Italy) we obtained the projected outer diameter (D) and the mean diameter of the medulla (d) making the assumption that volumetric cortical bone density is constant and then by an algorithm validated by the good correlation found in 21 cases (r=0,8) between "d" calculated from DXA and that actually measured by peripheral quantitative tomography pQCT (XCT960; Stratec, Unitrem, Italy) at the same radial site. From the "D" and "d" values we calculated a bending breaking resistance index (BBRI): (D^4-d^4/D) that is a component of the cross-sectional moment of inertia. In sixty-eight women with either prior femoral neck (no. 41) or pertrochanteric fracture (no. 27) DXA measurements at proximal and ultradistal radius, lumbar spine and femoral neck were obtained together with the evaluation of proximal radius BBRI. The diagnostic accuracy of BBRI was somewhat comparable to that of spine and femoral neck BMD and significantly superior to that of ultradistal and proximal radius BMD, from which it was derived. The diagnostic accuracy of BBRI was better for neck fractures. BBRI is significantly more predictive for femoral neck fracture respect ultradistal and proximal radius BMD. Odds Ratios (95% CI) for a 1 SD decrease is 3,01 (1,61-5,62) for BBRI while it is 0,80 (0,52-1,23) and 1,16 (0,88-1,54) for ultradistal and proximal radius BMD respectively. The proportion of patients with Z-score lower than 0 in the group with femoral neck fracture is higher for BBRI (80%) respect ultradistal radius BMD (59%) and proximal radius BMD (63%) and it is equal that for femoral neck BMD (80%). In conclusion we have shown that a simple re-elaboration of the data obtained by peripheral radial densitometry may achieve diagnostic accuracy for femoral neck BMD comparable to that of the direct measure of the BMD of the femoral neck. These results give additional support to the view that bone geometry, particularly for long compact skeletal segments, is a determinant of its strength at least as important as bone density.

M121

Diurnal Variation in the Serum Concentration of the C-terminal Telopeptide of the Type I Collagen (Serum CrossLaps). C. Christiansen,[1] B. J. Riis,*[1] S. Christgau,[2] P. Qvist.[2] [1]Center for Clinical and Basic Research, Ballerup, Denmark, [2]Osteometer Biotech, Herlev, Denmark.

Markers of bone resorption including urinary CrossLaps are subject to significant diurnal variations of about 100% with maximum in the morning (0500-0800) and minimum in late afternoon. The Serum CrossLaps One Step ELISA is a sandwich assay using two monoclonal antibodies specific for a beta-aspartate form of the epitope EKAHDGGR derived from the carboxy-terminal telopeptide region of type I collagen alpha1-chain. The inter- and intra-assay imprecision is <8%. This paper reports data from studies of the diurnal variation of serum CrossLaps (see table). Samples were collected every three hours over 27 hours in all the studies, and 103 participants underwent this procedure.

Study Objective	N
Diurnal variation in pre- and postmenopausal women with and without osteopenia.	9 premenopausal women 12 early postmenopausal women 12 late postmenopausal women 12 osteopenic women

The diurnal variation in serum CrossLaps in normal men	11 Normal men
Diurnal variation upon five days of bed rest.	9 premenopausal women
Morning or evening administration of nasal calcitonin? Effects on biochemical markers of bone resorption	9 postmenopausal women
Circadian variation in bone resorption in relation to serum cortisol	10 female and 1 male either hypophysectomized (n=7) or adrenalectomized (n=4)
The effect of acute fasting on the diurnal variation of bone resorption	11 premenopausal women
Does the daylight cycle determine the diurnal variation in bone turnover	Blind women (4) and men (3)

Serum CrossLaps changes over the 24-hour with a maximum of 140 % at about 05.00 in the morning and a minimum of 50 % at about 14.00 in the afternoon. The diurnal variation was independent of age, postmenopausal status and bone mineral density. Furthermore, 5 days of bed rest made no difference in terms of diurnal variation of serum CrossLaps. A stable serum cortisone level did not affect the diurnal variation of serum CrossLaps as it was in those participants with no internal cortisone production. Also blind participants, with no day/night fluctuation in the production of melatonine, had a normal fluctuation in serum CrossLaps. Participants that were kept fasting for 10 hours before and throughout 24 hours had a reduced variation that on average was less than 1/3 of the non-fasting participants. It is concluded that serum CrossLaps has the same diurnal variation as bone resorption markers measured in the urine and that this is partially offset by fasting. Due to the short plasma half-life of CrossLaps this marker is useful for studies of the pathophysiology of osteoporosis.

M122

A Novel Immunoassay for the Determination of Tartrate-resistant Acid Phosphatase 5b from Rat Serum. S. L. Alatalo,*[1] Z. Peng,[1] J. M. Halleen,[1] H. Kaija,*[2] P. Vihko,*[2] H. K. Väänänen.[1] [1]Department of Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland, [2]Biocenter Oulu, WHO Collaborating Centre for Research on Reproductive Health, University of Oulu, Oulu, Finland.

Bone-resorbing osteoclasts express high amounts of tartrate-resistant acid phosphatase 5b (TRAP 5b) and secrete it into the circulation, suggesting that serum TRAP 5b may be a useful marker of bone resorption. We have developed a novel immunoassay for rat TRAP, and studied serum TRAP 5b as a marker of bone resorption in orchidectomized (ORX) rats. In the immunoassay, we used a monoclonal antibody specific for rat TRAP as a capture antibody, and recombinant rat TRAP as a standard protein. Trabecular bone mineral density (BMD) was measured from the left tibia shaft of ORX and sham operated rats with peripheral Quantitative Computed Tomography (pQCT) before the operation and 5, 11, 17, 24, 40, 70, 110, 150 and 180 days after the surgery. The results of the ORX group were compared with the results of the sham-operated control group. Tail blood samples were taken at the same time points and serum TRAP 5b activity was measured with the immunoassay. Intra-assay variation of the immunoassay was 4.5%, inter-assay variation 3.8% and recovery 99.1 ± 5.8%. Serum TRAP 5b activity was significantly elevated at 5 days after ORX, returned to the control level at 17 days after the operation, and decreased below the control level at all later time points. BMD was decreased after ORX, the decrease being statistically significant ($p < 0.05$) already at 11 days after the operation. Trabecular bone volume was approximately 80% decreased at 180 days after ORX, and osteoclast number per trabecular bone area was slightly increased, suggesting that further bone loss still occurs at this time point. However, the absolute number of osteoclasts in trabecular bone was significantly decreased, and correlated with serum TRAP 5b activity at the same time point. These results suggest that absolute bone resorption is increased within the first week after ORX. Later, absolute bone resorption is decreased because there is less bone to be resorbed, whereas relative bone resorption (compared with the amount of bone left) is increased. We conclude that our immunoassay for rat TRAP 5b is a useful method to monitor changes in the bone resorption rate in rat ORX model.

M123

Monitoring Response to Cyclic Etidronate Therapy for Osteoporosis Using a Point-of-Care Device for Urinary CrossLaps. R. Branton,[1] R. A. Hannon,[2] D. A. Percival,*[1] R. Eastell.[2] [1]Research and Development, Provalis Diagnostics Ltd, Flintshire, United Kingdom, [2]Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Point-of-care measurement of biochemical markers of bone turnover may give valuable information on response to anti-resorptive therapy before it is possible to detect a bone density response. The aim of this study was to evaluate the use of Osteosal™ (Provalis Diagnostics Ltd, Deeside, UK), a rapid test for urinary C-telopeptide of type I collagen (CTX), to monitor the response of patients to cyclical etidronate therapy (400 mg/day etidronate for 2 weeks, 1200 mg/day calcium carbonate for 11 weeks) in the setting of clinical practice. Twenty six patients were recruited (21 female, 5 male, mean age 71 years, range 48 - 87). Diagnosis for the patients was: 11 primary osteoporosis and 15 secondary osteoporosis. Patients gave two baseline urine samples (second void) before commencement of therapy, then a third sample six months after commencement of therapy. CTX, measured by Osteosal, was normalised for urinary creatinine level using a dipstick (Bayer Corp, Elkhart, USA) and expressed as a T-score (compared to young healthy women). There was a significant ($p<0.01$, paired t-test) response at six months compared to baseline measurements.

Mean T-score

Baseline	6 month	Change
1.6	0.3	-1.3 (SD 2.3)

We conclude that the ease of use and the responsiveness to bisphosphonate therapy could make Osteosal a useful means of monitoring osteoporosis therapy in the setting of the metabolic bone clinic.

M124

Concentration of P1NP in Pagetic and Normal Plasma, Comparison Between an Automated ECLIA Method and a RIA Method. B. H. Durham, J. J. Dutton, W. D. Fraser.* Clinical Chemistry, Royal Liverpool University Hospital, Liverpool, United Kingdom.

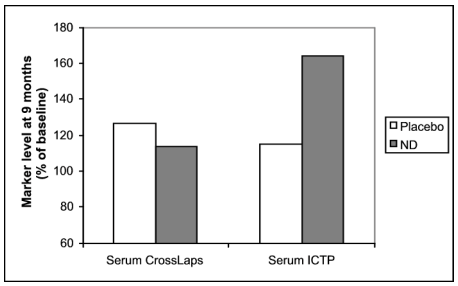
The N-terminal extension peptide of type 1 procollagen [P1NP] can be found in the circulation and its estimation in plasma can be used as an indicator of bone collagen formation. We have compared 2 methods for estimating P1NP in normal and pagetic plasma; one is a well established RIA kit from Orion Diagnostica [Finland] and the other is a newly formulated ECLIA for the automated Elecsys platform [Roche Diagnostics, Germany]. The maximum measurable concentration without dilution is 250 mcg/L for the RIA with a minimum detectable concentration of 3 mcg/L and for the ECLIA the maximum is 1000 mcg/L with a minimum of 4 mcg/L, CVs over the measurable range were <5% RIA, <2.5% ECLIA. Plasma from three groups of subjects was analysed, A) normals [n=40], B) Paget's pre-treatment [n=54] and C) Paget's post APD therapy [n=24]. Mean of total alkaline phosphatase concentration for group B was 278U/L with a range of 158-927U/L, in all group C subjects total alkaline phosphatase had decreased to <125U/L [upper limit of reference range]. Mean [SD, range] of concentrations in mcg/L from the RIA method [x] were group A: 32 [10.2, 17-56]; group B: 301[208, 62-1045]; group C: 52 [19.5,20-91]; and from the ECLIA method [y] were group A: 48 [11.9,31-78]; group B: 293 [159, 69-950]; group C: 67 [23.5,35-115]. The correlation overall $y= 0.915x + 23.5$, $r=0.988$, $p<0.05$. To facilitate the comparison between the two methods the ratio ECLIA/RIA was calculated for each sample and the mean [SD] for each group were A) 1.54[0.15], B) 1.05[0.16], C) 1.33[0.15]. From the results it appears that the assays differ in their estimation of P1NP produced in normal and treated Paget's subjects but in untreated Paget's subjects both assays give similar results. The ECLIA has some technical advantages over the RIA :- 1) fourfold increase in maximum measurable concentration compared with RIA, 2) lower CVs than in the manual RIA method and 3) because of automation larger batches of samples can be assayed. The fourfold increase in the maximum measurable concentration will be more useful in samples from untreated Paget's disease and also paediatric and children's samples where the concentration of P1NP often exceeds the upper limit for the RIA method. In our opinion the automated ECLIA for P1NP is an excellent robust assay suitable for the analysis of large batches of samples in the routine laboratory setting.

M125

Serum CrossLaps Is a Biochemical Marker of Bone Resorption and Is Not Influenced by Anabolic Steroid-Induced Increase in Soft Tissue Turnover. P. Qvist,[1] J. C. Lovejoy.*[2] [1]Osteometer Biotech A/S, Herlev, Denmark, [2]Pennington Biomedical Research Center, Baton Rouge, LA, USA.

Apart from their effects on lipids, anabolic steroids have been postulated to prevent bone loss in postmenopausal women. To study this further, various biochemical markers of type I collagen turnover have been measured during such treatments, e.g. with nandrolone decanoate (ND) (1-2). It has been demonstrated that whereas synthesis of type I collagen, as reflected in serum levels of carboxy-terminal propeptide of type I procollagen (PICP), was unaffected by one year of treatment every third week with ND (1), degradation of mature collagen, as detected by the carboxy-terminal pyridinoline cross-linked telopeptide of type I collagen (ICTP), increased 90% during similar treatment (2). We therefore investigated if a new bone resorption marker, Serum CrossLaps, which detects isomerised C-telopeptide fragments of type I collagen generated during osteoclastic bone resorption, was influenced by the steroid-induced increase in mature type I collagen degradation.Fasting morning blood samples were obtained at baseline and every three months for nine months from postmenopausal, obese women receiving either 30 mg of ND every two weeks (n=10), 75 mg/day of spironolactone (SP) (n=10), or placebo (n=10). The study has been described in detail elsewhere (3). For the present study the baseline and nine months serum samples were available, and only Serum CrossLaps and ICTP results from the ND and placebo arm is reported here.The data showed a significant (p=0.005) increase in ICTP compared to baseline after prolonged treatment with ND, in agreement with previous observations (2). In contrast, Serum CrossLaps remained unaffected by ND treatment indicating that while ND has pronounced effects on the soft tissue lean body mass it has little, if any, effects on degradation of skeletal tissue.(1) Hassager et al., Metabolism (1991) 40:205-208.(2) Hassager et al., Calcif Tissue Int (1994) 54:30-33.(3) Lovejoy et al., J Clin

Endocrinol Metab (1996) 81:2198-2203.



M126

Validation of Type II Collagen Cleavage Assay for the In Vivo Monitoring of a Rabbit Model of Inflammatory Arthritis. L. Chouinard,*[1] N. Rouleau,*[1] A. Leyshon,*[1] C. Woodland,*[1] P. Oldfield,*[1] G. Lulham,*[1] M. Wood,*[2] S. Y. Smith.[1] [1]CTBR, Senneville, PQ, Canada, [2]British Biotech, Oxford, United Kingdom.

The in vivo monitoring of drug efficacy in animal models of arthritis is limited to clinical observations (limb size), blood parameters measuring cell populations to evaluate inflammation, or is dependant on expensive and prohibitive imaging techniques. Unlike serum and urinary biochemical markers of bone (collagen type I) turnover, which are used routinely to monitor disease progression and treatment, current markers of articular cartilage collagen type II turnover are lacking in reliability, sensitivity and specificity. The utility of a specific marker of articular collagen degradation measured in synovial fluid (SF) would permit longitudinal monitoring in vivo of disease progression and the evaluation of potential chondroprotective agents. The purpose of this study was to validate the use of a commercially available new marker of collagen degradation, the collagenase-generated neoepitope of type II collagen, COL2-3/4Clong mono (C2C) (HDM Diagnostics & Imaging Inc.), in a rabbit model of experimental inflammatory arthritis.Six male rabbits, 6 months of age, sensitized with ovalbumin and challenged with an intra-articular injection into the stifle joint of both hindlimbs, were monitored for 21 days. A control group of six age-matched unsensitized males were injected with sterile saline. SF was collected by lavage, under isoflurane anesthesia, from the right knee every 4 days and from the left knee 6 and 20 days following intra-articular injection. Animals were euthanized Day 21 and tissues and SF collected from both knee joints of each animal.Typical macroscopic and microscopic lesions of antigen-induced inflammatory arthritis were induced in all sensitized rabbits and were consistent with pathologic changes seen in the human disease. Increases in C2C of at least 2-fold were observed on each sampling occasion compared to controls, with the highest mean concentration of C2C observed Day 4. Control samples and a few samples from sensitized animals fell below the limit of detection for the assay (LOD). C2C concentrations in the range from the LOD to 12 ng/mL were obtained for controls and up to 25 ng/mL for antigen challenged animals. There were no meaningful differences in data with the multiple sampling or reduced sampling schedules. Articular cartilage collagen degradation assessed by increases in C2C concentrations in SF were consistent with the histology observation of proteoglycan depletion (assessed by Safranin-O staining). This assay will provide a useful tool for the early detection of cartilage degradation and evaluation of chondroprotective agents in this animal model.

M127

Discriminant Analyses of Bone Biomarker Data. V. S. Sethuraman,*[1] A. K. Mathur,[1] K. J. Simpson,*[2] H. S. Chou,*[3] M. Schultz,*[3] S. J. Kovacs,*[4] U. S. Prabhakar.*[3] [1]Biomedical Data Sciences, GlaxoSmithKline Pharmaceuticals, Philadelphia, PA, USA, [2]Biomedical Data Sciences, GlaxoSmithKline Pharmaceuticals, Oakville, ON, Canada, [3]Clinical Pharmacology & Experimental Medicine, GlaxoSmithKline Pharmaceuticals, Philadelphia, PA, USA, [4]GlaxoSmithKline Pharmaceuticals, Philadelphia, PA, USA.

The length and cost of developing compounds demands the use of surrogate biomarkers for finding drug activity so that an early indication of efficacy can be ascertained. Logistic regression, discriminant analyses and receiver operator characteristic (ROC) analyses are methods used commonly to compare discriminatory ability of variables. We use them to compare bone biomarkers to discriminate between placebo and known active control (L-triiodothyronine). Based on the analyses a set of "good" bone biomarkers are obtained. Odds Ratio (OR), Area under ROC curves (AR) for normalized urine CTx bone biomarker are given below.

Summary Measure	OR	AR
AUC	18.71	0.868
Cmin	15.5	0.850
Tmin	2.63	0.821
D10	2.61	0.721

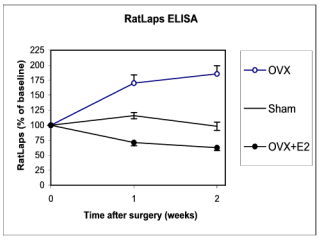
Similar results will be presented for other bone biomarkers.

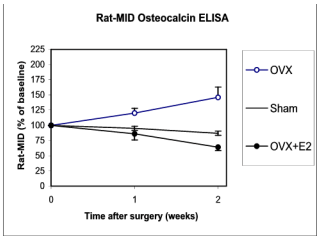
Disclosures: GlaxoSmithKline Pharmaceuticals,3.

M128

Rapid Assessment of Bone Resorption and Bone Formation in the Ovariectomized Rat. P. Qvist,[1] A. Heegaard,[2] M. K. Jespersen.*[1] [1]Osteometer Biotech, Herlev, Denmark, [2]OSTEOPRO, Herlev, Denmark.

The ovariectomized rat is an appropriate, experimental model of postmenopausal osteoporosis, and the FDA requires that effectiveness and safety of any new anti-osteoporotic drug be investigated in this model. The increase in bone turnover associated with surgical removal of the ovaries can be assessed by numerous techniques, including biochemical parameters of bone formation and resorption as well as measurement of bone density and bone strength. Biochemical assessment has until now been difficult by the lack of reliable markers applicable for serum measurements as well as guidelines for minimising factors causing variability, e.g. diurnal variation.Two biochemical tests for application on rodent serum samples, i.e. the RatLaps ELISA for detection of C-telopeptides released during bone resorption and the Rat-Mid ELISA for detection of osteocalcin generated by the osteoblasts, were evaluated in the ovariectomized rat model. Three months old female Sprague-Dawley rats were randomly allocated to one of three groups: SHAM (n=10), ovariectomy (OVX) (n=10) and OVX+E2 (n=10), where E2 represents subcutaneous placement of an estradiol pellet (0.5 mg, corresponding to 0.008 mg/day) on the day of operation. Blood samples were collected at baseline and one and two weeks after operation. To minimise dietary interference on bone resorption, all blood samples were collected at 2 pm after 6 hours of fasting.One week after OVX the marker levels detected by RatLaps ELISA and Rat-Mid ELISA had increased significantly, i.e. p=0.0001 and p=0.019, respectively, compared to SHAM. Administration of E2 to the OVX rats completely abolished the OVX induced increase in bone formation and resorption.In conclusion, the RatLaps and Rat-Mid ELISA could rapidly detect the OVX induced increase in bone resorption and formation, respectively. For both markers this increase reached statistical significance after one week being more pronounced after two weeks. The rapid response detected by RatLaps ELISA and Rat-MID ELISA to changes in the skeletal metabolism of the rat could increase the usefulness of this important model in the study anti-osteoporotic drugs.





M129

Changes in Bone Biomarkers and IGF-I in Time Since Menarche. S. L. Mobley,[1] J. D. Landoll,[1] N. E. Badenhop-Stevens,[1] E. J. Ha,[1] M. Andon,*[2] C. Rosen,[3] L. Nagode,*[4] V. Matkovic.[1] [1]Bone and Mineral Metabolism Laboratory, OSU, Columbus, OH, USA, [2]Procter and Gamble Co., Cincinnati, OH, USA, [3]Center for Osteoporosis Research, Bangor, ME, USA, [4]Veterinary Pathobiology, OSU, Columbus, OH, USA.

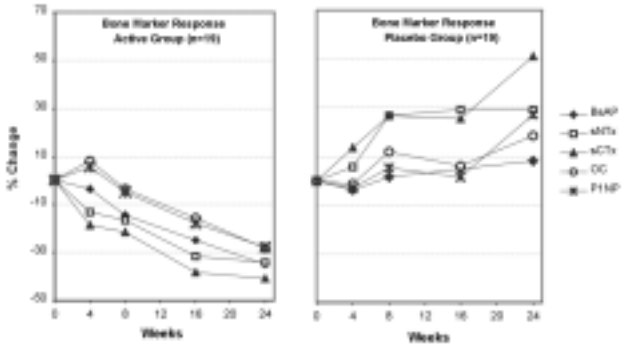
Menarche (M) marks the acquisition of fertility in young women and is characterized by the increased secretion of estrogens (E) by the ovaries. As E play a dominant role in skeletal physiology we, therefore, evaluated its relationship with the changes in bone biomarkers and insulin like growth factor 1 (IGF-I) in a cohort of 185 young females followed from prepuberty (age 10.9±0.9 y) to late adolescence (17.9±0.9 y). The goal was to present the data in TSM. All participants were premenarcheal (pubertal stage 2, ages 8-13 y) at the beginning of the study. Blood and 24-hr urine samples were obtained annually, and the onset of menarche recorded. Serum was analyzed for total alkaline phosphatase (AP), osteocalcin (OC), and IGF-1. Urine was analyzed for creatinine and N-telopeptide (uNTP). All measurements were done by the standard chemistry and radioimmunoassays. The onset of M was marked as time (0) with (-) being premenarcheal, and (+) postmenarcheal. The average onset of M in the cohort of these women was 12.8±1.0 y. For almost all variables studied, the TSM was the best descriptor of the events associated with puberty although behavior was different among the various biomarkers. AP is the highest 2 years before the menarche (320±71 IU) while peak OC concentration (23.2±7.1 ng/ml) is present at the onset of menarche (time 0). uNTP excretion is the highest 1 year before the menarche, while IGF-I reaches a very high level at time 0 and remains at that range for 2 years since menarche, before it starts to decline. The results of this research show that M is a powerful marker of skeletal physiology and bone growth in young women and indicates the transition between bone modeling and skeletal consolidation. The discrepancy in behavior between various biomarkers and the onset of menarche is currently unknown and remains to be established.

M130

Serum Bone Turnover Markers in Chronic Renal Insufficiency Patients Treated With Doxercalciferol (1-Alpha-Hydroxyvitamin D2). E. T. Leary,[1] C. W. Bishop,[2] L. W. LeVan,*[2] L. L. Douglass,*[2] T. K. Aggoune,*[1] M. K. McLaughlin,*[1] T. H. Carlson,*[1] J. W. Coburn,*[3] J. S. Lindberg.*[4] [1]Pacific Biometrics, Inc., Seattle, WA, USA, [2]Bone Care International, Middleton, WI, USA, [3]VA Medical Center, West Los Angeles, CA, USA, [4]Ochsner Clinic, New Orleans, LA, USA.

Chronic renal insufficiency (CRI) is often associated with bone disorders, including

secondary hyperparathyroidism (SHP) and osteoporosis. This study investigated the responses of five serum bone turnover markers (BTM) to oral doxercalciferol (1-alpha-hydroxyvitamin D_2 ;Hectorol ®) in patients with mild to moderate CRI and SHP. 38 patients between 18-80 years with entry PTH > 85 pg/ml, serum creatinine between 1.6 - 5.0 mg/dl completed a 24-week multi-center double-blind placebo-controlled study. Following an 8-week washout period, patients were randomized to placebo or doxercalciferol for 24 weeks with dosage increments to achieve desired PTH levels. Sera collected at baseline, week 4, 8, 16 and 24 were stored frozen until analysis in batch mode for the following BTM: Two markers of resorption, C-telopeptide (sCTx; serum b-Crosslaps, Roche Diagnostics) and serum N-telopeptide (sNTx; Osteomark, Ostex International); and three markers of formation, bone specific alkaline phosphatase (BsAP; Alkphase B, Metra Biosystems/Quidel), osteocalcin (OC; N-MID Osteocalcin, Roche Diagnostics), and N-terminal propeptide of type 1 procollagen (P1NP; Roche Diagnostics). sCTx, OC and P1NP were analyzed on the automated Elecsys 2010 analyzer (Roche Diagnostics). PTH fell in response to doxercalciferol (n=19) by approx. 60% at week 24. There was no change in the placebo group (n=19). All baseline BTM values were several times that of normal healthy adults. There was a significant decrease in all BTM for the doxercalciferol group by week 16. At week 24, sCTx decreased by 41%, sNTx by 34%, BsAP by 35%, OC by 28% and P1NP by 28%. OC and P1NP increased initially at week 4 followed by a steady decline. In the placebo group, BTM response was more variable with increasing variability as study progressed. There was a positive mean increase in all BTM in the placebo group by week 24. In summary, in addition to suppressing PTH in CRI patients with SHP, doxercalciferol decreased bone turnover significantly as demonstrated by decreased serum BTM. Serum BTM are useful tools in monitoring response to therapy in CRI patients.



M131

Changes in Serum Levels of NTx, PINP and Total Body Bone Mineral Density in Healthy Elderly Women Over a Six-Year Period. J. K. Scariano,[1] P. J. Garry,*[1] G. Montoya,*[2] R. N. Baumgartner.[2] [1]Pathology, University of New Mexico School of Medicine, Albuquerque, NM, USA, [2]Medicine, University of New Mexico School of Medicine, Albuquerque, NM, USA.

Biochemical markers of bone turnover and bone mineral density measurements were monitored in a population of 86 healthy, non-institutionalized women (age = 66 - 91 yrs.) over the course of six years. Thirty five of the women experienced a significant decrease in total body bone mineral density (TBMD) over the course of the six-year period, and the remaining 49 women had no significant change in TBMD. Changes in serum levels of a biochemical marker of bone resorption (NTx) and an index of type-I collagen synthesis (PINP) correlated significantly with change in BMD over a six year period. Women who lost TBMD experienced mean increases in serum NTx of 5 nmol BCE/L and 8.1 ug/L in serum PINP levels over 6 years, while women whose TBMD remained stable manifested a mean NTx increase of only 0.8 nmol BCE/L ($p = 0.0006$) and a mean decrease of 8.7 ug/L in serum PINP levels ($p = 0.01$). Over the six-year period the change in serum NTx and PINP levels correlated inversely with change in total body BMD ($r = -0.33$, $p = 0.002$) and ($r = -0.22$, $p = 0.04$) respectively. The change in NTx and PINP levels were highly correlated ($r = 0.62$, $p < 0.0001$) Baseline levels of NTx and PINP were not significantly associated with change in TBMD, however, baseline levels of ICTP, another resorption marker, correlated positively with change in TBMD ($r = 0.34$, $p = 0.001$). Baseline PINP levels also were positevly correlated with change in spine BMD ($r = 0.25$, $p = 0.03$). At the end of the study, serum levels of NTx and PINP values were significantly higher in those who lost TBMD (19.0 v 14.1 nM for NTx, $p = 0.0004$ and 55.0 v 31.8 ug/L for PINP, $p < 0.0001$) as compared with those whose TBMD did not change significantly. These data indicate that increasing serum levels of NTx and PINP reliably predict bone loss in elderly women.

M132

Clinical Utility of Ultrasound and Grip Strength to Predict Incident Fracture in Elderly Women. R. L. Prince, A. Devine, I. Dick,* A. Marangou,* R. Naheed,* S. Dhaliwal.* Dept of Medicine University of Western Australia, Sir Charles Gairdner Hospital, Perth, Australia.

In light of improved methods of fracture prevention it is important to improve future fracture prediction. Both skeletal integrity and neuromuscular function contribute to fracture propensity. In 1998 we recruited 1499 women over the age of 72 directly from the population. At two years 8% had sustained at least one incident fracture. We determined whether baseline measurements of skeletal strength and neuromuscular function predicted fracture. Skeletal strength was measured by the Lunar Achilles heel ultrasound machine. Neuromuscular function was assessed by grip strength using a hand held dynamometer. Incident clinical fractures were verified from x-ray reports after the event. The mean age at baseline was 75 yrs, mean weight was 69kg, 34% had a prevalent fracture, the mean grip strength was 20. In univariate logistic regression analysis incident fracture was predicted by prevalent baseline fracture, grip strength the timed up and go test and the ultrasound parameters bone ultrasound attenuation (BUA), speed of sound (SOS) and stiffness but not age, socio-economic status or weight. In multivariate logistic regression only SOS (Relative Risk (RR) per SD 1.4, 1.1-1.7) and grip strength (RR per SD 1.3, 1.0 - 1.5) remained significant after adjustment for other demographic variables. There was no interaction between SOS and grip strength. The table shows the two-year RR (c.f. SD of 0) and the model predicted two-year actual fracture risk (AR (%)) by SD of grip strength or SOS. Also shown is the proportion of the population in each SD (PP (%)).

Grip\SOS	SD 0	SD -1	SD -2
SD 0	RR 1.0, AR 7.4, PP 13.8	RR 1.4, AR 10.1, PP 12.2	RR 2.0, AR 13.5, PP 1.6
SD -1	RR 1.2, AR 9.1, PP 8.1	RR 1.8, AR 12.3, PP 6.2	RR 2.4, AR 16.4, PP 1.4
SD -2	RR 1.6, AR 11.2, PP 1.4	RR 2.2, AR 15.0, PP 2.1	RR 3.1, AR 19.7, PP 0.4

The high baseline fracture risk in this population is illustrated as is the important role of simple measures of bone strength and neuromuscular function in defining a group of patients who have up to a 20 % risk of fracture in just two years. However the proportion of potential fracture patients detected as a proportion of all fractures is small because of small numbers in the high-risk groups.

M133

Can a Large Caucasian Population Based Recruitment Without Exclusion Criteria Be Used to Build a Reference Database Compared to Well Defined Normative Population? D. B. Hans, C. Perron,* L. Genton,* G. Conicella,* D. O. Slosman. Nuclear Medicine, Geneva University Hospital, Geneva, Switzerland.

Establishing a reference data curve for bone assessment devices has always been a difficult and controversial task leading to uncertainties in clinical interpretation. Concerns were raised regarding the appropriate sample size and the eligibility criteria. In most of the data collection sets strictly eligibility criteria introduce and found to be essential to avoid increasing the sample size. Sample size for the Sunlight Omnisenseä (Omnisense) reference data curve was calculated based upon the minimum number of patients per age group required achieving a stable mean and standard deviation. The requirements were n=150 per age group, allowing a mean deviation of ±20 m/sec from a SD of 100 m/sec, with a 5% significant level and 80% power. Eligibility criteria were very strict i.e. excluding of those suffering from metabolic bone disease or any clinical condition likely to affect bone mineral metabolism , as well as subjects taking medications know to affect the bones.This study aims to show the validity of Omnisense reference data curve of the by compare it to a very large data set collected from different countries, by different devices and without any eligibility criteria. More than 6000 women were measured by 14 Omnisense devices at the Distal 1/3 Radius. Data obtained from eight countries in Europe, USA and Israel. A reference database curve was calculated from this cohort. Both curves (system and new cohort) were compared by their mean and SD. As can be observed from this comparison both curves are superimposed, and no statistical differences were noticed between the two data sets.

	System RDB (n=1132)			Cohort RDB (n=6021			p
	N	Mean	SD	N	Mean	SD	<0.05
20 - 29	182	4108	95	113	4139	90	0.06
30 - 39	185	4161	101	267	4169	111	ns
40 - 49	266	4167	98	1222	4157	110	ns
50 - 59	145	4115	128	2148	4104	133	ns
60 - 69	160	3989	151	1398	3996	141	ns
70 - 79	145	3931	129	758	3925	146	ns
80 - 89	49	3879	159	114	3877	151	ns
Total	1132	4082	151	6021	4066	156	ns

The population-based cohort overlays the 'selected' system reference database. In this population the medical conditions and medication known to affect the bone either positively or negatively seems to balance the effect of each. A confirmation of the validity of the system sample size may be drawn from this analysis

Disclosures: Sunlight Medical Ltd,2.

M134

Influence of Trabeculae Orientation on Ultrasound Propagation Through Decalcified Specimens of Equine Vertebrae. F. Cavani,*[1] F. de Terlizzi,*[2] L. Ciminelli,*[2] M. Fini,*[3] G. Giavaresi,*[3] S. Ortolani,[4] R. Cherubini,*[4] V. Canè,[1] R. Cadossi.[2] [1]Department of Morphological and Medicolegal Sciences, University of Modena, Modena, Italy, [2]Biophysics Lab, IGEA, Carpi, Italy, [3]Department Experimental Surgery, Istituti Ortopedici Rizzoli, Bologna, Italy, [4]Bone Metabolism Unit, Istituto Auxologico Italiano, Milan, Italy.

Ultrasound (US) propagation through trabecular bone tissue is influenced by density,

elasticity, structure. In this study we investigated the relationship between propagation of ultrasound pulses and trabeculae orientation in three orthogonal planes. 15 cylindrical specimens (8 mm Ø, 9 mm height) were obtained in a fixed volume of interest from equine thoracic vertebrae with the same trabeculae orientation. All samples were measured before, during and at the end of the decalcification performed with 0.2M EDTA. For each sample we measured apparent density (dry weight/volume), BMD (g/cm2, DXA) in the axial direction of the cylinder, elasticity by mechanical non destructive tests in the same direction of US propagation. Ultrasound investigation (1.25 MHz) foresaw 2 orthogonal measurements in the transversal direction of the cylinder and one along the longitudinal axis. The results showed a strong relationship between apparent density and BMD (r=0.95, p<0.0001). Different behaviour of US parameters was observed in the 3 directions: SOS (Speed of Sound) is related to bone density when US propagation is parallel to the trabeculae orientation in transverse transmission (r=0.88, p<0.0001 vs apparent density, r=0.91, p<0.0001 vs BMD) and in axial transmission (r=0.92, p<0.0001 vs apparent density, r=0.93, p<0.0001 vs BMD). In the same experimental set-up ultrasound FWA (amplitude of fastest wave) is related to a lower degree to apparent density (r=0.72, p<0.0001 transversal, r=-0.43, p<0.005 axial) and BMD (r=0.78, p<0.0001 transversal, r=-0.51, p<0.005 axial). On the contrary, along the other transverse direction, no significant association has been found between SOS and apparent density (r=0.27, n.s.) or BMD (r=0.13, n.s.), whilst high correlation levels have been found for FWA and bone density (r=0.83, p<0.0001 vs apparent density and BMD). By testing the combined influence of densitometric and US variables in the prediction of elastic properties of the bone, we found that in all directions at least one of the US parameters is independent predictor of mechanical properties of bone.These results show that the propagation of US through trabecular architecture of bone is strongly affected by trabeculae orientation. Furthermore trabeculae orientation influences the results of the correlation among US parameters (SOS and FWA) and bone density (BMD and apparent density).

M135

Fractures in Dialysis Patients. S. A. Jamal,* C. Chase, G. A. Hawker. Osteoporosis Research Program, Women's College Ambulatory Care Centre, Toronto, ON, Canada.

To assess if bone mineral density (BMD) testing and/or calcaneal ultrasound measurements were associated with fractures, we studied 72 men and 33 women, 55 years and older, who had been on hemodialysis for at least one year. We inquired about prior low trauma fractures and risk factors for fractures. A chart review gave data on medication use and most recent laboratory tests. Patients underwent bone mineral density (BMD) testing of the lumbar spine and femoral neck using a Lunar DPX-L densitomer. Heel ultrasound gave measurements of bone stiffness, bone ultrasound attenuation (BUA), and speed of sound (SOS). Lateral and thoracic radiographs of the spine were obtained and incident vertebral fractures were identified by vertebral morphometry. We examined the relationships between fracture (incident vertebral, self-reported low trauma and/or both), lumbar spine and femoral neck BMD using logistic regression. We considered BMD in g/cm^2 and classified subjects as having osteopenia (T score at either the lumbar spine or femoral neck between -1 and -2.5) or osteoporosis (T score at either the lumbar spine or femoral neck less than or equal to -2.5). We examined the relationship between fracture and calcaneal ultrasound measures. We considered stiffness as %, BUA as dB/MHz, and SOS as m/s, and classified subjects as having low ultrasound if either of stiffness, BUA, or SOS were in the lowest quintile. Analyses were adjusted for age and weight. The mean age of patients was 69 ± 9 years, the mean weight 72 ± 15 kg, and most (76) were Caucasian. The average duration of dialysis was 3.1 ± 2.6 years. The most common cause of renal failure was diabetes (42 subjects). The mean levels of calcium, phosphate, and alkaline phosphatase, were normal. The mean level of intact PTH was 36 ± 41 pmol/L. There were no differences by gender or between patients with and without fractures. Incident vertebral fractures were found in 34 patients, a history of low trauma fracture in 28, and either a spine fracture and / or a low trauma fracture in 54 patients. Mean lumbar spine BMD was 1.3 ± 0.2 g/cm^2 and femoral neck BMD was 0.9 ± 0.2g/cm^2. The number of patients with osteopenia was 72 and with osteoporosis 25. The mean stiffness was 74 ± 16 %, the mean BUA 106 ± 12 dB/MHz, and the mean SOS 1514 ± 33 m/s. The number of patients with values of stiffness, BUA and/or SOS in the lowest quintile was 31. There was no association between fracture (vertebral, nonvertebral or both) and BMD. Patients with calcaneal ultrasound measurements in the lowest quintile were 2.6 times more likely to have a low trauma fracture (95% CI 1.1 to 6.2; p=0.03). Our findings suggest that calcaneal ultrasound measures may identify patients with dialysis dependent renal failure at risk for fracture but BMD may not.

M136

Influence of Time Since Fracture on Hip Fracture Discrimination Assessed by Quantitative Ultrasound of the Calcaneum. S. Allaoua,* D. B. Hans, L. Genton,* C. Perron,* M. Delmi, R. Rizzoli, H. Vuagnat, C. Pichard,* D. Slosman. Geneva University Hospital, Geneva, Switzerland.

In a previous study conducted within our division and comparing several QUS devices in discriminating hip fracture patients versus age matched controls, we found that the OR of all studied QUS parameters were in general different than reported by the literature. Because all these QUS measurements were performed only within a few days after hip replacement surgery, the previous conclusions could have been influenced by this short time interval between fracture and measurement. The aim of the current study is to re-measure a subset of patients about 9 months after the hip replacement surgery and to check the impact of this time since fracture on the OR derived from different ultrasound technologies.We re-measured the same calcaneum of 14 out of 30 hip fractured female patients which were compared to 30 age matched controls (already measured during the previous study). Ultrasound measurements at the same non-dominant side were acquired around 9 ± 1.2 months (visit 2) after the first ones (visit 1), using the Achilles-Plus (GE-Lunar, USA), Sahara (Hologic, USA) and UBIS 5000 (DMS, France). Heel width was also measured at the two visits to seek an effect of potential oedema. Discrimination of fracture versus control cases at the two visits was assessed using logistic regression analysis (expressed as odds ratios -OR- per standard deviation decrease with 95% confidence interval). An average of 9.8% increase of heel width (~ 4 mm) has been observed between the two visits probably due to oedema linked to a lack of activities.

	Visit 1		Visit 2	
	OR	**95% CI**	**OR**	**95% CI**
Sahara - QUI	4.7	1.5 - 14.7	6.3	1.7 - 22.8
Sahara - BUA	5.3	1.7 - 16.1	4.6	1.6 - 13.3
Sahara - SOS	4.0	1.3 - 12.1	7.2	1.8 - 28.4
Achilles - STI	2.6	1.1 - 6.2	2.5	1.1 - 5.8
Achilles - BUA	3.7	1.2 - 11.0	3.3	1.2 - 9.3
Achilles - SOS	2.0	1.0 - 4.2	2.0	1.0 - 4.4
UBIS - BUA	2.7	1.2 - 6.5	2.7	1.2 - 6.1
UBIS - SOS	1.9	0.9 - 4.2	2.6	1.1 - 5.9

No impact of time since fracture (or oedema as a co-founding factor) has been observed on the Lunar Achilles whatever the QUS parameters used. The most clear impact has been seen on the Hologic Sahara device principally on the SOS and QUI parameters. Since OR are based on an exponential, a small difference in absolute QUS values could lead to large difference. Oedema will mainly impact dry system rather than wet system (unfocussed). While the design of cross-sectional studies should take this into account, because the sample size is small, results should neverthesess be interpreted with caution

M137

Pulse-echo Normalization of QUS Parameters for Bone Width : Accuracy, Precision and Clinical Assessment. P. Laugier,*[1] R. Porcher,*[2] C. Roux.[3] [1]LIP UMR 7623 CNRS-Université Paris 6, CNRS-Université Paris 6, Paris, France, [2]DBIM, Hopital Saint Louis - Université Paris 7, Paris, France, [3]CEMO, Hopital Cochin - Université Paris 5, Paris, France.

QUS technology has potential for fracture risk prediction in osteoporosis. QUS parameters are usually measured in transmission, however when applied to the calcaneus, clinical devices generally do not determine the calcaneal thickness. The aim of this study was to explore a new technological development of the QUS imaging device UBIS5000 (DMS, France), adding the measurement of the heel width using pulse echo technique as part of the ultrasound measurement, normalizing BUA and SOS for bone size and to compare the clnical value of both normalized and non-normalized QUS parameters. We examined 30 healthy premenopausal controls (group I), 150 menopausal women without fractures (group II), and 60 with osteoporotic fractures (group III). All subjects had hip BMD measurements. To test the accuracy of pulse-echo bone width measurements (Us.Th), the calcaneal thickness was measured using MRI (MRI.Th) in an additional group of 20 subjects. High correlation coefficients were found between Us.Th and MRI.Th (r=0.91). The slope of the linear fits was not significantly different from 1. The reproducibility for calcaneal thickness evaluated in 49 women was 1.68%. Bone width was 2.81±0.24 cm in group I, 2.88±0.28 cm (group II) and 2.95±0.29 cm (group III). The standardized coefficients of variation were comparable between normalized (nBUA : 3.03%; nSOS: 3.43%) and non-normalized parameters (BUA : 2.96%; SOS: 3.40%). The odds ratio (OR) for fracture discrimination adjusted for age was 2.77 [1.72:4.46] for hip BMD, 1.75 [1.24:2.47] (nBUA) and 2.94 [1.70:5.01] (nSOS) for normalized QUS, and 1.92 [1.34:2.77] (BUA) and 2.90 [1.72:2.4.90] (SOS) for non-normalized QUS. After adjustment for hip BMD, ORs were still significant for SOS and nSOS only. Age and BMD adjusted area under ROC (0.76-0.78) were comparable for normalized and non-normalized QUS. We have demonstrated the feasibility of accurate and precise pulse-echo measurement of bone width. Normalized and non-normalized QUS parameters at the calcaneus have equal adequate ability to discriminate osteoporotic patients from controls. However, in this study, normalized QUS is not superior to non-normalized QUS. This may be due to the narrow range of variation of calcaneal thickness found in our population. As the normalization for bone width may enhance the precision and the accuracy and may lead to the dissemination of the technique to other fields like osteoporosis in men or pediatrics, additional studies are required to evaluate the clinical value of normalized parameters in different groups (e.g., children or men).

M138

Does a Combination of Different Quantitative Ultrasound Variables from Different Devices and BMD Improve Fracture Discrimination? Results from the OPUS Study. R. Barkmann,[1] D. Felsenberg,[2] C. Roux,[3] C. C. Glüer,[1] D. M. Reid,[4] R. Eastell.[5] [1]University Hospital Kiel, Kiel, Germany, [2]Free University Berlin, Berlin, Germany, [3]René Descartes University, Paris, France, [4]University of Aberdeen, Aberdeen, United Kingdom, [5]University of Sheffield, Sheffield, United Kingdom.

Dual x-ray absorptiometry (DXA) and quantitative ultrasound (QUS) methods can be used to estimate the risk of osteoporotic fractures. We tested whether vertebral fracture discrimination can be improved by combining several QUS variables or by combining QUS and DXA results. In the Osteoporosis and Ultrasound (OPUS) study, 2206 women age 55-80 were recruited from random population samples at 5 centers in Aberdeen, Berlin, Kiel, Paris, and Sheffield. A subgroup of 1720 postmenopausal women had complete results for DXA of the spine and hip, the Achilles+ as an example for a QUS heel device, the DBM-

Sonic BP, a QUS device measuring the finger phalanx, and vertebral fracture status assessed by lateral radiography of the spine. We tested whether the area under the receiver operating characteristic (ROC) curve of combined QUS/QUS or QUS/DXA models was significantly larger compared to the ROC areas of models with single age-adjusted variables ($p<0.05$, one tailed test). 271 women had at least one osteoporotic vertebral fracture. On the Achilles+, SOS was the best variable with no significant independent contribution of BUA. Stiffness did not perform better than SOS alone. On the DBMSonic BP, AD-SoS performed best and the best additional variable (finger width) did not improve the result significantly. No improvement was observed for adding a finger variable to SOS as the best heel variable or for adding a QUS variable to spine or hip BMD.

Base model	Area ROC	Added variable	Added Area ROC (95%C.I.)
Heel SOS, age	0.660	Heel BUA	0.000 (-0.003, 0.005)
Finger AD-SOS, age	0.647	Finger width	0.002 (-0.006, 0.009)
Heel SOS, age	0.660	Finger AD-SOS	0.006 (-0.003, 0.014)
Spine BMD, age	0.680	Heel SOS	0.008 (-0.007, 0.016)
Spine BMD, age	0.680	Finger AD-SOS	0.007 (-0.003, 0.015)
Hip BMD, age	0.678	Heel SOS	0.006 (-0.002, 0.020)
Hip BMD, age	0.678	Finger AD-SOS	0.006 (-0.003, 0.018)

Conclusion: Combinations of several QUS variables measured at the heel or the finger or combinations of these QUS variables with DXA measurements of the spine or hip offer no improvement for discrimination of women with and without vertebral deformities.

Disclosures: Igea,2.

M139

Theoretical Prediction of Ultrasound Attenuation in Trabecular Bones Using a Combined Model of Poroelasticity and Scattering Theories. F. Padilla, P. Laugier. LIP, CNRS - Universite Paris 6, Paris, France.

QUS measurements represent an established means of bone status assessment in osteoporosis. However, to date, the exact physical mechanisms underlying ultrasound attenuation in cancellous bone have not been clearly documented. This is a crucial point in order to define the proper QUS parameters relevant in predicting ultimate bone strength. Attenuation includes absorption and scattering losses. This paper proposes a model for calculating absorption and scattering losses and accounting for total attenuation losses. To test this model, QUS measurements of attenuation and backscattering were performed on 25 human calcaneus specimens. Then the 3D m-architecture of the specimens was analyzed with synchrotron radiation mCT. Trabecular bone is a porous material and the Biot's theory may be used to predict the absorption coefficient. This theory requires the knowledge of several parameters describing the density, the elasticity and the geometry of the material. In previous attempts, the calculated viscous absorption coefficient was always much lower than the experimental one. This was due to incorrect values of the parameters required by the model. In this paper, the 3D m-architecture of each specimen was used as a feedback to adjust several parameters such as porosity, structural Young's modulus and permeability.The scattering losses were derived using a model based on scattering medium autocorrelation function. The parameters describing the characteristics of the scatterers were extracted from the 3D m-structure analysis. The attenuation coefficient was finally computed by summing the absorption and the scattering coefficients.The scattering model yields good agreement between predicted and experimental backscatter coefficient for both frequency dependence (theoretical f^3.48 and experimental f^3.38) and magnitude (accuracy of 10 %). Comparison of experimental and theoretical attenuation coefficient show a reasonable agreement for both frequency dependence (theoretical f^1.72 and experimental f^1.14) and magnitude (accuracy of 28 %). In addition we show that absorption and scattering contribute to 70 % and 30 % approximately of total attenuation losses. It is the first time that a combined model of scattering and viscous absorption using data frommCT is used to account for observed ultrasound attenuation in trabecular bones. This model provides improved predictions compared to previous studies and suggests the importance of both absorption and scattering losses. This theoretical development provides a better knowledge on interaction mechanisms and may be used to define new QUS parameters.

M140

Comparison of Calcaneal SOS and BUA Between Healthy Chinese and Caucasian Women. X. G. Cheng,[1] B. Fan,[1] Y. Xue,[2] C. F. Njeh,[1] H. K. Genant.[1] [1]Radiology, UCSF, San Francisco, CA, USA, [2]Biochemistry, Ji Shui Tan Hospital, Beijing, China.

It is generally believed that Chinese women have lower osteoporotic hip fracture rates than their Caucasian counterparts. Previous studies have shown that Chinese women have lower hip bone mineral density (BMD) than Caucasian women, although after adjustment for body size this difference is insignificant. To determine whether these differences can be found in ultrasound measurements also, we compared the differences of speed of sound (SOS) and broadband ultrasound attenuation (BUA) at the calcaneus between healthy Chinese and Caucasian women.373 healthy Chinese women were recruited at the Beijing. The SOS and BUA were measured with a UBIS 3000 ultrasound scanner. 212 healthy Caucasian women were recruited at San Francisco. A UBIS 5000 scanner was used in San Francisco. The two UBIS systems have similar hardware and are calibrated by the manufacturer to give comparable results. We have not yet performed our own cross-calibration.We also calculated T-scores of SOS (T-sos) and BUA (T-bua) for each group separately by taking the means and SDs of 20-40 year old subjects as the young normals.The Table shows the differences between Chinese and Caucasians women. Statistical analysis revealed that BUA and T-bua, but not SOS and T-sos, were affected by weight and height; after adjusting for weight and height the differences in BUA an T-bua between the two groups were significant. The table also shows the T scores of SOS and BUA for each group by decade, both groups followed a similar pattern in terms of SOS and BUA T score changes with age, reaching peak values in the 5th decade, then declined with age.This study shows that BUA and T-bua, but not SOS and T-sos, are affected by weight and height; after adjusting for weight and height the differences in BUA and T-bua between the two groups were significant. This study indicates that Chinese women have lower SOS, BUA and their T scores than Caucasian women, but the differences are small. Both SOS and BUA T score reach peak values in the 5th decade, then decline with age. These results indicate that there are small differences between these two ethnic groups, and it may be inappropriate to use Caucasian women as a reference base for Chinese women.

	Chinese			Caucasians		
Age	N	T-sos	T-bua	N	T-sos	T-bua
<40	115	-0.01(1.0)	0.01(1.0)	82	0.02(1.0)	0.02(1.0)
40-49	57	0.17(1.4)	0.65(1.4)	32	-0.17(0.7)	0.46(0.9)
50-59	114	-1.12(1.8)	-0.32(1.1)	30	-0.54(0.7)	-0.45(1.3)
60-69	74	-1.8(1.7)	-0.58(1.4)	30	-0.68(0.8)	-0.66(1.1)
>69	13	-1.96(2.0)	-0.27(1.0)	38	-1.05(0.6)	-0.87(1.4)
Total	373	-0.74(0.1)a	-0.12(0.06)b	212	-0.38(0.1)	-0.24(0.08)
	N	SOS	BUA	N	SOS	BUA
	373	1507(23)a	63(7)b	212	1514(35)	63(5)

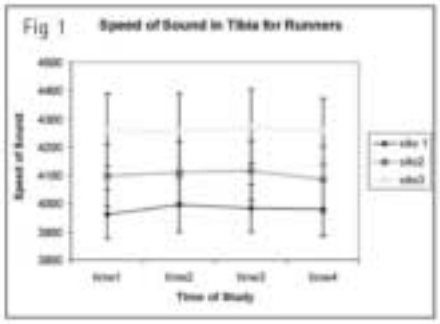
a: $p<0.01$, b: not significant differences between Chinese and Caucasians

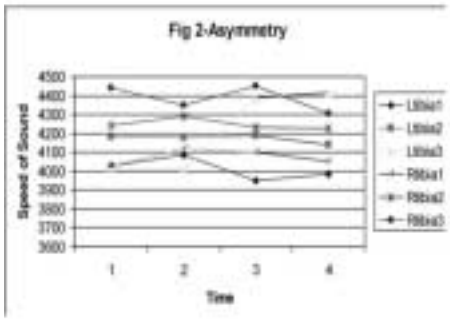
M141

Assessment of Tibial Speed of Sound Measurement in Cross-country Sunners. J. P. Dickey,*[1] J. Holliday,*[1] M. B. Hurtig,*[2] S. Gawron.*[3] [1]Human Biology, University of Guelph, Guelph, ON, Canada, [2]Clinical Studies, University of Guelph, Guelph, ON, Canada, [3]Health & Performance Center, University of Guelph, Guelph, ON, Canada.

The purpose of this study was to study quantitative ultrasound bonemeasurements in healthy young subjects to provide baseline measurescharacterizing the changes associated with regular running exercise.Twenty-eight varsity cross-country runners participated. Four quantitative ultrasound measures were obtained at monthly intervals from December 2000 through March 2001. These measures were taken from three sites along both the right and left tibias. The three sites were spaced along the tibia, with "site 1" corresponding to the mid-tibia location recommended by the instrumentation manufacturer (Sunlight Medical Ltd., Israel). Sites 2 and 3 were progressively more distal than site 1. Appropriate validation procedures were performed at the start of each data collection session using a phantom and temperature compensation. A three-way repeated measures ANOVA (site x leg x time) was performed. Plots of SOS data for each subject were made and subjective assessments of left/right symmetry at each measurement site were made. For this study, symmetry was defined as a SOS difference less than <150m/sec between corresponding sites in each tibia. We observed a clear difference in speed of sound (SOS) with site along the tibia (Figure 1; $p<0.0001$). The SOS increased distally in a predictable pattern. The time*site interaction reached a level of statistical significance (Figure 1; $p=0.0412$) but this may not be biologically significant. SOS measurements showed left/right limb symmetry in 17/28 runners none of who reported pain associated with running and asymmetry in 11/28 runners (Fig 2) of which 4 had pain with radiographically or scintigraphically confirmed stress fractures. The significant differences in SOS between the three sites along the tibia have immediate application to studies performing ultrasound measurements on the tibia, and precise landmarking is essential. Asymmetry in SOS measurements at comparable levels in the tibia may be an indicator of an impending injury or compensation for an existing injury. Studies are needed that control for factors such as running history, speed,

gait anomalies, weekly mileage are needed.





Disclosures: Sunlight Technologies,2.

M142

Fracture of the Distal Forearm: Association With Quantitative Ultrasound Measurements and Bone Density. J. A. Clowes, N. F. A. Peel, R. Eastell. Clinical Sciences (North), University of Sheffield, Sheffield, United Kingdom.

It is unclear whether the strength of association between distal forearm fracture and measurement of the peripheral skeleton are similar to those of the central skeleton. We recruited 75 consecutive women (ages 55 to 80 years) who had sustained a distal forearm fracture. These were compared to a population-based sample of 500 women (ages 55 to 80 years) from the Sheffield center of the osteoporosis and ultrasound study (OPUS study). All subjects had measurements of the lumbar spine and total hip using dual x-ray absorptiometry (DXA) and QUS of the heel on 4 devices (Lunar Achilles+, Osteometer DTU One, Metra QUS 2, and DMS UBIS 5000) and of the proximal phalanges (IGEA DBM Sonic BP). In the fracture cohort the contralateral forearm was measured. We examined the association of these measurements with distal forearm fractures by calculating standardized odds ratios (sOR, per one SD decrease of population variance), OR adjusted for age, and compared devices by calculating the area under the receiver operating characteristic (ROC) curve. The difference between the area ROC at the lumbar spine was compared to the area ROC for each parameter.

Parameter	sOR	Age Adj. sOR	Area ROC	P < (ROC)
DXA spine	1.72	1.71 (1.32-2.24)	0.661	-
DXA hip	1.56	1.59 (1.23-2.06)	0.642	0.49
Achilles BUA	1.64	1.66 (1.28-2.15)	0.639	0.53
DTU One BUA	1.60	1.59 (1.31-1.94)	0.655	0.70
UBIS 5000 BUA	1.60	1.64 (1.26-2.14)	0.632	0.28
QUS 2 BUA	1.77	1.84 (1.39-2.44)	0.653	0.68
Achilles SOS	1.66	1.68 (1.28-2.21)	0.627	0.37
DTU One SOS	1.77	1.77 (1.37-2.30)	0.658	0.80
UBIS 5000 SOS	1.49	1.52 (1.14-2.03)	0.609	0.11
DBM Ad SoS	1.18	1.13 (0.83-1.53)	0.537	0.02

The BUA and SOS measurements of the heel had ROC areas similar to DXA measurements of the lumbar spine (p>0.05). Heel QUS variables showed significant associations with distal forearm fracture, however small but significant differences in performance were observed. Optimal results were achieved with both BUA and SOS parameters measured at the calcaneus with a discriminatory power comparable to DXA.

M143

Relationship Between Quantitative Ultrasound, Physical Activity and Anthoprometric Measures in College Age Adults: Tufts Longitudinal Health Study. A. C. Wetter,* C. D. Economos. School of Nutrition Science and Policy, Tufts University, Boston, MA, USA.

We report on the relationship between quantitative ultrasonometry (QUS) of the os calcis and field measures of body composition and physical activity in a longitudinal study of lifestyle behaviors and biomarkers of chronic disease risk in college aged adults. Study participants are undergraduate students enrolled in the Tufts Longitudinal Health Study. Data were collected at the annual assessments on consenting first and second year students (17-21 years old). To limit confounding due to ethnicity, data on Causcasians only were used in the following analyses (n=53 men; n=95 women). Body composition was assessed by bioelectrical impedance (BIA), muscular strength was assessed by hand grip dynamometry, aerobic fitness was assessed by Queens College step test, and physical activity was assessed by self report on several surveys using different formats. Skeletal muscle mass was calculated from BIA parameters using the equation developed by Janssen, et al (J Appl Physiol 89:465-71;2000). Gender differences were found in the relationship between anthropometric measures and QUS. In men, no relationship was seen between bone Stiffness Index (SI) and any of the anthropometric measures. In women, fat free mass ($r=0.275$; $p<0.01$) and skeletal muscle mass ($r=0.277$; $p<0.01$) were more strongly related to SI than body weight ($r=0.211$; $p<0.05$). Furthermore, this positive relationship between SI and body weight persists when body fat mass is controlled, but disappears when fat free or skeletal muscle mass are controlled, indicating that, in our population of young women, lean mass contributes more than fat mass to the relationship between body weight and bone quality. Finally, we found that the strongest correlate to SI in women and the only correlate to SI in men was muscular strength. In women, weight training, team or vigorous sports participation, increasing physical activity, and increasing aerobic fitness were all associated with significantly greater SI. In men, some of these relationships tended to occur, but none were significant. Important findings are that assessments of anthropometry, muscular strength and physical activity that have been previously correlated to BMD and BMC are similarly related to QUS in women. Further studies are warranted in young adult men to better characterize predictors of QUS measures in bone.

M144

Interdependent Relationship Between Trabecular Bone Quality and Ultrasound Attenuation and Velocity Using a Scanning Confocal Acoustic Diagnostic System. Y. Qin,* W. Lin,* C. Rubin. Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, NY, USA.

Early diagnosis of musculoskeletal complications, i.e., osteoporosis and/or the delayed union of fractures leads to prompt treatment. It is hypothesized that such a musculoskeletal disorder, i.e., osteoporosis, is not only changing the structure and the mineral density (BMD), but the modulus of the bone. Recently, advents in ultrasonic techniques provide an intriguing method for characterizing the material properties of bone in a manner which is non-invasive, non-destructive, repeatable, safe and relatively accurate. Limitations with this approach, however, leave non-invasive ultrasound - in its current configuration - as a first order screening tool, rather than a highly accurate diagnostic. While ultrasonic techniques provide both structural and property information of bone, the objective of this work is to evaluate multiple ultrasound parameters in predicting bone's mineral density and material strength using newly developed scanning confocal acoustic diagnostic (SCAD) system.17 trabecular bone cubes (1 x 1 x 1 cm), harvested from sheep distal femoral condyle has been tested using micro-CT, contact mechanical strength, and ultrasonic (US) attenuation and velocity in three orthogonal directions, i.e., longitudinal, med-lat and ant-post. SCAD determined US velocity and attenuation are correlated with micro-CT identified BMD, and with bone's material modulus measured in MTS machine. A new parameter that combined US attenuation and velocity was used in the linear regression correlation. While both US attenuation and velocity correlate well to BMD individually with correlation coefficient values higher than R=0.6, the correlations are significantly improved using combined parameters of US attenuation and velocity, i.e., yielding R>0.8 between US prediction and micro-CT determined BMD and R>0.71 between US prediction and bone stiffness (Table).These results suggest that SCAD can provide much detailed information in predicting bone mass and strength. Considering bone's complex architecture, combining US attenuation and velocity can provide better prediction of bone's quality in determining osteogenic conditions. Thus, a well-established database using this new developed system may provide an insight in non-invasive diagnostic of osteoporosis and bone quality using ultrasound.

M145

Use of Quantitative Ultrasound in Managing Renal Osteodystrophy. G. R. Haynatzki,[1] R. W. Dunlay,*[2] J. M. Lappe,[1] M. R. Stegman,[1] R. R. Recker.[1] [1]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [2]Medicine, Creighton University, Omaha, NE, USA.

This is a report on a longitudinal (24 mos) observational study on osteodystrophy in patients with end-stage renal disease who are on dialysis, either hemodialysis or continuous ambulatory peritoneal dialysis. The purpose is to compare the use of Quantitative Ultrasound (QUS) with Dual Energy X-ray Absorptiometry (DEXA) for assessment of bone status. QUS is considered simple, inexpensive, non-invasive, and safe, with some studies indicating that QUS measures both bone mass quantity and bone structure, i.e. the overall bone quality. Serial measurements were taken at 6-month intervals on the following 21 variables: 12 forearm densitometer (BMC, BMD and Area) of (Radius, Ulna, Distal and Ultradistal); 4 calcaneus ultrasound (QUI, BUA, SOS and BMD); and 5 serum biomarkers (PTH, Alkaline Phosphatase, Calcium, Phosphorus, Creatinine, and BUN). We modeled the percent change in these 21 variables (excluding PTH) as dependent on patient Age,

Gender, Race, Time on Dialysis, and Diagnosis, as well as their interactions. Diagnosis was having three levels: Adynamic Bone Disease (0 MedianPTH 150); Normal Bone Formation (150 MedianPTH 250); Osteitis Fibrosa (MedianPTH>250). A general linear model was used with repeated measures that accounts for all factors, as implemented in the statistical package SAS 8.0. Interestingly, only Ultradistal BMC, Ultradistal BMD, QUS SOS and BUN were associated (at level 0.05) with the so-defined Diagnosis, while Radius BMD was marginally associated with Diagnosis. In this model, factor Gender is significant for all BMC and BMD endpoints while factor Race is significant for all BMD endpoints. We conclude that, based on the current status of our data set, the QUS cannot replace DEXA as the main tool in assessing bone status, and only QUS SOS could be used for diagnosis of the prevalent forms of renal bone disease.

M146

Investigation of Bone Quality in Ly-6A(Sca-1) Knock-out Mice. D. Liu,*[1] W. L. Stanford,*[1] W. G. Beamer,*[2] M. D. Grynpas.[1] [1]Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, ON, Canada, [2]Jackson Laboratory, Bar Harbor, ME, USA.

Ly-6A (Sca-1) is a murine antigen, which is expressed on most peripheral lymphocytes, thymocytes as well as osteoblast from bone marrow, and involved in homeostasis between osteoclasts and osteoblasts. Ly-6A (Sca-1) null mice, which were healthy and had a normal body weight compared to wild type mice were produced by gene targeting methods. 4 groups of 10 Ly-6A mice, 2 months old wild type (WT) and null (N) as well as 12 months old WT and N were studied for bone mineral density (BMD) and bone mineral content (BMC) using Dual Energy X-ray Absorptiometry (DEXA). Bone density and architecture of the cortical and trabecular bone was studied by peripheral quantitative computed tomography (pQCT), and mechanical properties were determined by three point-bending tests. DEXA results showed that at two months, WT and N mice had similar body weight, body BMD and BMC. However at 12 months, body BMD and BMC of N mice were significantly lower than WT mice, even though the body weight and the size of these mice were still quite similar. These mice were sacrificed two months after the whole body DEXA test, and the femurs and vertebrae were dissected. These isolated bone samples were tested again using DEXA, and similar results were found. The left femurs were tested by pQCT. The bone density measurements were consistent with the DEXA results. No significant differences were found in the trabecular area, but the cortical area and cortical thickness of the old N mice were significantly lower than the WT mice, while those of the young N mice were still similar to the WT mice. Three point-bending tests results on right femurs showed that there were no significant differences found within the young group in all the geometric, structural and material parameters, except for the thickness of the shaft and the maximum strain of WT was higher than the N mice. But in the older group almost all the geometric, structural and material properties of the N mice were significantly lower than that of the WT mice, except for the femur length, flexural modulus and maximum strain. From these results we concluded that although the bone mineralization, geometrical parameters and mechanical properties of the N mice were similar to the WT at two months, there were no changes with age in N mice as oppose to normal aging changes in the WT mice. We are further investigating the mineralization, histomorphometry and mechanical properties of vertebrae in these mice.

M147

Autosomal Dominant High Bone Mass: The Phenotype. R. R. Recker,*[1] M. L. Johnson,[1] K. M. Davies,[1] S. M. Recker,*[1] R. P. Heaney.[1] [1]Creighton University, Omaha, NE, USA.

The purpose of this report is to describe the phenotype occurring in an extended kindred resulting from an autosomal dominant mutation (High Bone Mass, HBM) on chromosome 11 (11q12-13). We encountered an 18 year-old female and her mother with spine Z-scores (Z{BMD}) of 5.70 and 4.98 respectively. The maternal kindred contained 37 members (2 deceased, 17 affected and 20 unaffected), who were parents, offspring or siblings of an affected member. Mean Z{BMD}of the spine, hip, total body and forearm for affected members ranged between +2.72 (± 0.89) at the mid radius and +4.91 (± 1.41) at the total body, and for unaffected members, between -0.26 (± 0.83) at the hip and +0.81 (±1.65) at the total radius. Total spine and hip areas were greater in the affected vs unaffected adult females (61.0 ± 4.1 vs 52.7 ± 3.1 and 32.2 ± 3.8 vs 30.1 ± 2.1 cm2, respectively, P<0.05) and in the affected vs unaffected adult males (73.5 ± 4.8, vs 63.3 ± 6.0 and 41.2 ±4.3 vs 39.6 ± 1.5 cm2, respectively, P<0.05). Skeletal radiographs in 10 affected individuals were normal except for increases in cortical thickness. Clinical laboratory examination revealed no significant abnormalities, and there was no morbidity associated with the trait. The trait appeared in childhood, was sustained throughout life, and appeared to confer resistance to fracture. We speculate that the mutation caused increased sensitivity to mechanical loading, thus resulting in over-adaptation to normal mechanical loads. Further study of this mutation should yield information on the regulation of peak bone mass in humans.

Disclosures: Genome Therapeutics Corporation,1,2,7; American Home Products,2,7.

M148

High Heritability of Bone and Muscle Mass Among Afro-Caribbean Men. J. M. Zmuda,[1] J. A. Cauley,[1] C. H. Bunker,*[1] A. L. Patrick,*[2] V. W. Wheeler,*[2] D. Hill,*[1] R. E. Ferrell.*[1] [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]The Tobago Regional Health Hospital, Scarborough, Trinidad and Tobago.

Men of African ancestry have greater bone and muscle mass than Caucasian men. These interethnic differences contribute to a lower lifetime risk of osteoporotic fracture among men of African descent. Little is known about the relative importance of genetic and environmental influences on bone and muscle mass among men of African ancestry. Thus, we estimated the familial resemblance and heritability of bone and muscle mass in 86 Afro-Caribbean brothers (43 independent full-sib pairs) aged 40-83 years (mean±SD; 61±11 yrs) residing on the West Caribbean island of Tobago. Hip and whole body bone mineral density (BMD) and appendicular muscle mass were measured by dual energy x-ray absorptiometry (Hologic QDR 4500). Familial resemblance was assessed by intra-class correlation coefficients and heritability estimated as twice the intra-class correlation. After adjusting for age, weight, and height, the heritability (±standard error) of total hip and whole body BMD was 0.88±0.24 and 0.76±0.26, respectively (P<0.01 for both). Similar results were observed for the sub-regions of the hip. After adjusting for age and height, the heritability of arm and leg muscle mass was 0.80±0.26 and 0.72±0.26, respectively (P<0.01 for both). These heritability estimates are consistent with those reported from studies of Caucasians. Our results suggest that genetic factors may explain a considerable proportion of the variation in bone and muscle mass among men of African descent.

M149

Explaining the Familial Clustering of Hip Fractures: A Twin Study. J. D. Wark,[1] K. Hill,*[2] A. Cassano,*[3] N. El Haber,*[3] R. MacInnis.*[3] [1]Medicine (Royal Melbourne Hospital), University of Melbourne, Parkville, Australia, [2]National Ageing Research Institute, Parkville, Australia, [3]University of Melbourne, Parkville, Australia.

A maternal history of hip fracture doubles hip fracture risk. Bone mineral density (BMD) is highly heritable, even in older females. We investigated factors that might explain why hip fractures run in families by seeking evidence of a genetic influence on gait and balance functions that are known to predict falls and fractures. The proportion of familial fracture risk that could be attributed to heritability of hip BMD, hip axis length (HAL), and gait/balance function was estimated using the theoretical model of Hopper & Carlin (1992). Clinical and laboratory tests of gait/balance were performed in 35 pairs of monozygotic (MZ) and 39 pairs of dizygotic (DZ) female twins aged 46-82 years. Within-pair MZ correlations were moderate-to-high (0.48 - 0.86) for multiple test outcomes (adjusted activity scores, muscle strength, stride velocity, double support duration, Lord's balance test, Chattecx balance testing, step test).Within-DZ pair correlations tended strongly to be lower, suggesting moderate-to-strong additive genetic influence. Modelling indicated that heritability of BMD, gait/balance function and HAL might explain 20%, 15-20% and 10%, respectively, of the familial clustering of hip fractures.These observations should improve understanding of the genetic epidemiology of osteoporotic fractures. They suggest also that we need to learn more about why fractures run in families.

M150

Cluster Analysis Predicts Whole Bone Function in Inbred Mouse Strains Using Non-Invasive Measures of Bone Geometry and Composition. K. J. Jepsen,[1] O. Akkus,*[1] M. Warman,[2] J. H. Nadeau.*[2] [1]Orthopaedics, Mount Sinai School of Medicine, New York, NY, USA, [2]Genetics and Medicine, Case Western Reserve University, Cleveland, OH, USA.

Inbred mouse strains are used to identify the genes regulating peak bone mass with the assumption that reduced bone mass is predictive of increased fracture risk. However, bone mass alone does not fully characterize whole bone mechanical function since this measure does not account for mass distribution and tissue mechanical properties which are known to contribute substantially to function. In this study, we conducted a cluster analysis to determine if using bone mass (Area), mass distribution (Moment of Inertia), and bone composition (Ash Content) resulted in a better segregation of whole bone function compared to using bone mass alone.Female mice (n=10-11/strain) from 129/SvJ, A/J, AKR/J, BALB/cByJ, C3H/HeJ, C57BL/6J, DBA/2J, and SM/J were sacrificed at 15 weeks of age. Cross-sectional area (A) and polar moment of inertia (J) were quantified for the femoral diaphyses from plastic sections. Whole bone stiffness, max load, work to fracture, and post-yield deflection (PYD; a measure of brittleness) were assessed from 4-point bending tests using the contralateral femurs. Ash content was assessed for the femurs failed in bending. A K-means cluster analysis was conducted using A/weight, J/weight, and ash content. Femurs segregated into 4 clusters with 80-100% of the femurs from a given inbred strain segregating into a common cluster. BALB was the only strain that did not segregate into one cluster. Inbred strains with similar geometry and composition clustered together and showed similar whole bone function. By including mass distribution and composition, strains with similar bone mass (SM/J, BL/6, A/J, DBA) segregated into different clusters exhibiting significant differences in failure modes. These results indicated that long bones from inbred strains can be segregated on the basis of 3 physical properties that can be measured non-invasively and that these properties predict whole bone mechanical properties in a more comprehensive manner. Importantly, this segregation not only predicted variations in whole bone stiffness and max load, but also predicted variations in bone toughness and brittleness. The phenotypic clusters may provide clues to the identity of independent genetic traits and the strains that are likely to share similar controls of bone biology.

M151

Densitometry in Men 2A. F. Aguiló.* Medicine, University of Puerto Rico, San Juan, Puerto Rico.

MEN 2A, described by Sipple in 1961, comprises the triad of MTC (medullary thyroid carcinoma), PHPTH (primary hyperparathyroidism) & Pheos (pheochromocytomas, mostly bilateral). It arises from mutations in the Ret oncogene cysteine-rich region, codons 609-634 of exon 10. We follow a family having a new mutation in codon 624 (Asp----Asn). The posible role of the 2 major hormones regulating bone accretion/resorption (i.e., calcitonin & PTH), might be inferred by DEXA (Hologic QDR 1000) of suitable ROI's such as radius & proximal femur (mainly cortical) vs. L2 - L 4 spine (mostly trabecular). Sixteen affected subjects from 3 generations (out of 4), were studied at about "expected peak " val-

ues, with mean values 24-30 years. Concomitant blood samples and treatment status were recorded. There were 7 males; 4 clinically affected(+) and 9 females (3 clinically apparent), for a total of 16, screened for the Ret mutation. Correlative & statistical analyses revealed the following results. (See table.)

Site	n	Forearm	Lumbar	Hip
Males	4+	0.66 ±.05	1.01 ± 0.05	1.01 ± 0.08
Males	3-	0.62 ± 0.06	1.04 ± 0.14	0.95 ± 0.21
Total males	7	0.64 ± 0.05	1.02 ± 0.09	1.04 ± 0.16
SD		-0.84	-0.80	-0.14
Females	3+	0.54 ± 0.02	1.06 ± 0.04	1.03 ± 0.08
Females	6-	0.52 ± 0.02	0.93 ± 0.12	0.96 ± 0.18
Total females	9	0.53 ± 0.04	0.99 ± 0.11	0.99 ± 0.14
SD		-0.86	-0.73	+0.39

Thus, having (+) or not (-) the clinical manifestations, disclosed no significant differences or correlation with BMD, nor with actual serum Ca, P, or Alk. P'tase, Lowest values in forearm might be attributed to PTH action at an earlier, sub-clinical stage not in keeping with a proposed initial calcitonin-mediated hypocalcemia, as the initiating pathogenic factor, as this would be expected to increase BMD pre-clinically

M152

The Relationships among COL1A1/COL1A2 Genotype, Bone Histological Phenotype and Apparent Bone Mineral Density in Thirty-Two Patients With Osteogenesis Imperfecta Types I to IV. L. M. Ward, F. Rauch, R. Travers, L. Lalic,* P. Roughley,* F. H. Glorieux. Genetics Unit, Shriners Hospital, McGill University, Montreal, PQ, Canada.

Osteogenesis imperfecta (OI) is a heritable disease of bone with low bone mass and bone fragility. A proportion of patients with OI have abnormalities in the genes encoding type I collagen, COL1A1 and COL1A2. The purpose of this study was to determine if there were relationships among the type I collagen genotype, histological phenotype and areal bone mineral density (aBMD) in patients with OI.Thirty-two children with OI types I to IV who had mutations in the COL1A1/COL1A2 genes affecting glycine residues were included in the study (10 in COL1A1, 22 in COL1A2). Trans-iliac bone biopsies were performed following dual-tetracycline labeling. Areal bone mineral density (aBMD) was determined at the lumbar spine (L1 to L4).Bone histomorphometric parameters and aBMD were converted to age- and sex-matched z scores and were compared with the distance of the mutation from the n-terminal end of type I collagen, which has been associated with phenotypic severity. None of the histomorphometric parameters reflecting disease severity were associated with the distance of the mutation from the n-terminal end of type I collagen.The mean aBMD z score was -5.2(±1.3) for patients with mutations in COL1A1 and -5.1(±1.3) for COL1A2. There was no significant correlation between aBMD and the glycine substitution distance from the n-terminal end.Conclusion: No evidence was found for a relationship between the distance of the glycine substitution from the N terminus and histomorphometric or densitometric parameters of disease severity.

M153

Gender-Specific Determinants of Bone Breaking Strength, Geometry and Material Properties. E. S. Orwoll, M. Shea, M. Serang,* R. J. Turner,* J. K. Belknap,* R. F. Klein. Oregon Health Sciences University, Portland, OR, USA.

In both males and females, skeletal development is strongly influenced by genetic factors, and yet there are distinct gender differences in bone composition and fracture risk. We have previously reported marked gender effects on chromosomal determinants of BMD and femoral cross-sectional area. To extend these observations we examined the gender influence on other essential measures of structural and material properties in an additional population of mice. We studied mid-diaphyseal femurs in 16-week-old male and female mice from a panel of 18 BXD recombinant inbred (RI) strains, derived from a cross between C57BL/6 and DBA/2 progenitors. The distribution of failure load (a composite index of fracture resistance that is influenced by both geometric and material properties) among the strains revealed estimated heritabilities of 53% and 39% in male and female mice, respectively. Similarly, genotype (RI strain) had a significant impact on all measured geometric and material properties ($p < 0.0001$), indicating a strong genetic component. Comparison of RI strain results (adjusted for body weight) by two-way ANOVA showed significant strain-by-gender interactions for failure load, as well as for key geometric measures that were related to failure load, including femoral shaft moment of inertia, cortical area, cortical thickness, stiffness ($p < 0.0001$). However, indices of material properties were not influenced by gender (modulus, strength and energy, $p>0.05$). Quantitative trait locus (QTL) analysis of the BXD RI strain series provisionally identified eight chromosomal sites linked to femoral failure load in males and ten regions in females. In five cases, the provisional chromosomal loci were shared between genders (chromosomes 1, 5, 8, 12 and 15), but in many cases they were distinct - 3 male-specific QTLs (chromosomes 4, 11 and 13) and 5 female-specific QTLs (chromosomes 4, 9, 13, 14 and 19). Thus, genetic factors exert a robust effect on skeletal strength in this model, including BMD, size, material properties and, ultimately, failure load. Gender strongly affects the genetic determinants of failure load, primarily via effects on geometric rather than material properties. The genetic loci that determine biomechanical properties in males and females are to some extent shared, but other loci act via gender-specific mechanisms. Gender must be considered in the design of studies intended to discover and characterize genes affecting bone biology. Elucidation of the nature of these effects could provide the basis for novel diagnostic and therapeutic approaches to osteoporosis.

Disclosures: Merck & Co,2,5; Eli Lilly,2,5; Aventis,5; Procter and Gamble,2,5.

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Mapping and Expression Analysis of Candidate Genes in the Vicinity of the Mouse gl Osteopetrosis Mutation. N. Benachenhou,[*1] N. Chalhoub,[*1] J. Vacher.[1] [1]Montreal Clinical Research Institute, Montreal, PQ, Canada.

Grey-lethal (gl) is a recessive mutation that maps to the proximal portion of mouse Chromosome (Chr) 10 and is an established model for osteopetrosis. Homozygous mice exhibit an osteopetrotic phenotype with reduced bone marrow, failure of tooth eruption and early death. To facilitate the positional cloning of the gl gene, both cDNA selection and assignment of sequence-tagged-sites from the human transcript map have been used to identify genes within the gl interval. Together with previous physical mapping, this allowed us to characterize and localize 10 transcription units within an approximately 0.5-Mb region that spans the gl genetic interval. This map includes genes that encode an integral endoplasmic reticulum membrane protein required for translocation of presecretory proteins (Sec63), a sorting nexin probably involved in several stages of intra-cellular trafficking (Snx3), a ligand-activated nuclear receptor-type transcription factor (Mtll), a ribosomal protein-like (Rpl3 like), and several expressed-sequence-tags and genes of unknown function (AI156858, H15310, AA046445, HSPC019, M78981 and N57482). Importantly, expression analysis by RT-PCR and Northern blot indicated that a subset of these genes is expressed in the osteoclast. Finally, this region of mouse Chr 10 represents a conserved linkage group with genes on human chromosome 6q21, a region that is frequently altered in human cancers and that harbors loci for several pathologic conditions. Consequently, analysis of the gl interval may provide important tools to understand how the corresponding region of human Chr 6q21 contributes to disease, in addition to defining a key gene product essential for bone resorption.

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Tests of Linkage and/or Association of Genes for Vitamin D Receptor, Osteocalcin, and Parathyroid Hormone with Bone Mineral Density (BMD). H. T. Zhang,*[1] H. W. Deng,[1] H. Shen,*[1] F. H. Xu,*[1] Y. Li,*[1] T. Conway,[2] R. R. Recker.[2] [1]Biomedical Sciences Department and Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Bone mineral density (BMD) is a major determinant of osteoporotic fractures. The heritability of BMD ranges from 50-90% in human populations. Extensive molecular genetic analyses have been performed through traditional linkage or association approaches to test and identify genes or genomic regions underlying BMD variation. The results, particularly those concerning the vitamin D receptor (VDR) gene, have been inconsistent and controversial. In this study, we test linkage and/or association of the genes for VDR, osteocalcin (also known as bone Gla protein, BGP), and parathyroid hormone (PTH) with BMD in 630 subjects from 53 human pedigrees. Each of these pedigrees was ascertained through a proband with extreme BMD value at the hip or spine (Z-score $\leq$ -1.28). For the raw BMD of the hip and spine, adjusting for significant covariate effects of age, sex, and weight, we performed tests for linkage alone, association alone, then for both linkage *and* association using the QTDT and SOLAR programs. For the spine BMD, at the two markers (*Apa*I and *Fok*I) inside the VDR gene, we found evidence for linkage (p <0.05), and for both linkage and association by the transmission disequilibrium test (TDT, p<0.05); association was detected (p<0.05) with regular ANOVA which assumed independence of samples but not detected (p>0.37) with QTDT which accounted for nonindependence among subjects. Significant results were also found for association alone (p=0.05), linkage alone (p=0.0005), and for linkage and association (p=0.0019) for the intragenic marker, *Hind*III, of the BGP gene for the hip BMD. Other unspecified results are generally not significant. For the first time, through testing for association, linkage and for linkage and association simultaneously, our data support the VDR gene as a QTL underlying spine BMD variation and the BGP gene as a QTL underlying hip BMD variation. However, our data do not support the PTH as a QTL underlying hip or spine BMD variation. This is the first study in the broad field of bone genetics that tests candidate genes as QTLs for BMD by testing simultaneously association alone, linkage alone, and association and linkage (via TDT).

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Bone Loss in Aged Intact Female Sprague Dawley Rats. J. Banu, L. Wang,* D. N. Kalu.* Physiology, UTHSCSA, San Antonio, TX, USA.

The aim of this study is to see if senile bone loss occurs in aged intact female Sprague Dawley (SD) rats. Bone loss was assessed by scanning bones in different age groups of female SD rats (9, 12 and 18 months) using pQCT densitometer, both in vivo and in vitro. In vivo scans were performed on the left tibia, while in vitro scans were performed on the L4 vertebra, left femur and left tibia. Cancellous bone and cortical bone were analyzed at the proximal tibial metaphysis (PTM) and vertebra. Lumbar Vertebra: In 18 months old animals, cortical BMC (Ct. BMC), cortical BMD (Ct. BMD) and cortical thickness (Ct. Th), decreased by 12.4%($p<0.05$), 2.6% ($p<0.05$) and 11.0% ($p<0.05$), respectively, when compared to 9 months old animals. No significant change was observed in the Periosteal perimeter (Peri PM). Endocortical perimeter (Endo. PM) increased by 3.1% ($p<0.05$) when compared to 9 months old animals. At 18 months of age, cancellous bone mineral content (Cn. BMC) and cancellous bone mineral density (Cn. BMD) decreased by 12.0% ($p<0.01$) and 10.4% ($p<0.01$) respectively, when compared to 9 months old animals. Neck of the Femur: In 18 months old animals, Ct. BMC, Ct. BMD and Ct. Th decreased by 31.4%

(p<0.0001), 18.9% (p<0.0001) and 59.5% (p<0.0001) respectively, when compared to 9 months old animals. Endo. PM increased by 268.9% (p<0.0001) when compared to 9 months old animals. At 18 months of age, Cn. BMC and Cn. BMD decreased but not significantly, when compared to 9 months old animals. Proximal Tibial Metaphysis: In 18 months old animals, Ct. BMC, Ct. BMD, Ct. Th and Peri. PM decreased by 17.6% (p<0.05), 5.5% (p<0.05), 18.2% (p<0.05) and 6.6% (p<0.001) respectively, when compared to 9 months old animals. Endo. PM increased by 2% (p<0.001) when compared to 9 months old animals. At 18 months of age, Cn. BMC and Cn. BMD decreased by 31.0% (p<0.001) and 21% (p<0.01) respectively, when compared to 9 months old animals. In summary significant bone loss occurred in cortical and cancellous bone in the vertebra and proximal tibial metaphysis. We conclude that intact female SD rats loose bone with age.

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Evidence for a Major Gene for Bone Mineral Density/Content in Human Pedigrees Identified Via Probands with Extreme Bone Mineral Density. F. H. Xu,*[1] H. W. Deng,[1] G. Livshits,*[2] K. Yakovenko,*[2] T. Conway,[3] K. M. Davies,[3] H. Y. Deng,*[3] R. R. Recker.[3] [1]Osteoporosis Research Center and Biomedical Sciences, Creighton University, Omaha, NE, USA, [2]Anatomy and Anthropology, Tel Aviv University, Tel Aviv, Israel, [3]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Bone mineral content (BMC) and/or bone mineral density (BMD, i.e., BMC scaled by bone size) are major determinants for osteoporosis, which is a serious health problem. It has been established that the major determination of the variation of BMD/BMC is genetic. A limited few studies are inconsistent in the identification and even in the existence of major genes for BMD/BMC. In 51 human pedigrees with 941 individuals (526 measured for phenotypes) identified via probands with extreme BMD values, we performed complex segregation analyses to test the existence of a genetic locus with a major effect on BMD/BMC variation. We analyzed BMD and BMC at spine, hip and wrist jointly by employing, as the studying phenotype, factor scores (FS) of the principle component that explains ~75% of the total BMD/BMC variation at the three skeletal sites. The results indicated that there exists a major gene with codominant effect that is responsible for ~16% of the FS variation when adjusted for significant effects of sex, body weight and age. A significant genotype x sex x age interaction was found, which may explain ~14% of the FS variation after adjusting for body weight. Testing of various models did not provide support for shared familial environmental effects but support existence of residual polygenic effects, which may explain ~56% of the FS variation when adjusted for sex, body weight and age. This study indicates a promising aspect to identify a major gene for BMD/BMC variation in our pedigrees identified via extreme probands.

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Testing of Association and/or Linkage for Candidate Genes with Bone Mass in Chinese Nuclear Families. Y. J. Qin,*[1] X. Y. Mo,*[2] Q. R. Huang,[1] Z. K. Cao,*[2] Q. H. Wang,*[1] M. Y. Liu,*[2] Q. Zhou,*[1] J. H. Lu,*[1] J. W. He,*[1] F. H. Xu,*[3] H. W. Deng.[2] [1]Center for Preventing and Treating Osteoporosis, Shanghai Sixth People's Hospital, Shanghai, China, [2]College of Life Sciences, HuNan Normal University, Changsha, China, [3]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Abstract: Our long-term goal is to test, via a contemporary and powerful approach of the transmission disequilibrium test (TDT), all important candidate genes for association and/or linkage with bone mass variation in at least 400 Chinese nuclear families, each of which is composed of both parents and at least one healthy female child aged 25-40. Numerous molecular genetic studies have been conducted to search for genes underlying bone mass variation. The majority of these studies are conducted in Caucasian populations either to test for association of candidate genes or to identify linkage of major genomic regions underlying bone mass variation, with the results being largely controversial or unconfirmed. Association and linkage approaches, while valuable, both suffer some methodological problems in generating false positive results and/or having low power. TDT is a new approach that is known to be robust and powerful for testing both linkage and association. We have so far recruited and phenotyped 150 nuclear families, 121 of which have one female child, 27 with 2 female children and 2 with 3 female children. We genotyped restriction fragment length polymorphism (RFLP) markers for three candidate genes [*Apa*I inside vitamin D receptor (VDR) gene, *Pvu*II and *Xba*I inside the estrogen receptor (ER) gene, and *Hind*III for the osteocalcin (BGP) gene]. Using the program QTDT, we did not detect significant population stratification in our sample, although the *Pvu*II and *Xba*I marker genotypes significantly deviate (p<0.001) from Hardy-Weinberg equilibrium. At the spine, associations are found between the BGP marker with peak bone mass in female children aged 25-40 (p=0.02), between the VDR *Apa*I genotypes with bone mass in mothers of the nuclear families (p<0.05), and between the ER *Xba*I genotypes with bone mass in nuclear families (p=0.01). At the hip, our preliminary data suggest both association and linkage of the ER markers with BMD variation (p<0.05). Statistical power analyses suggest that ~400 nuclear families are needed in order for the TDT to detect both association and linkage for a gene responsible for at least 10% of the bone mass variation. Therefore, although our results are preliminary, they are encouraging and suggest that our targeted sample will provide a powerful basis for testing linkage of the candidate genes that show association in our sampled Chinese nuclear families.

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Type I Alfa 1 Collagen Gene Polymorphism is Associated with Bone Density, Muscle Strength and Susceptibility for Fractures in Elderly Women. P. Geusens,[1] C. Vandevyver,*[2] P. Stinissen,*[2] J. Vanhoof,*[2] J. Raus,*[2] L. Michiels.*[2] [1]Limburg University Center & University of Maastricht, Maastricht, The Netherlands, [2]LUC, Diepenbeek, Belgium.

The dimorphisms of type I collagen gene locus Sp1 and its association with bone density and fracture history were investigated in 213 unrelated postmenopausal Caucasian women of 70 years and older. The overall distribution of the Sp1 alleles in this study was similar to that previously reported in Caucasian study populations (SS 68.1%, Ss 26.3% and ss 5.6%). Compared to the presence of SS dimorphism, carriage of at least one "s" allele was associated with a significant lower bone density [mean difference: 0.034 mg/cm2 (95% Confidence Interval (C.I.): 0.001, 0.069 mg/cm2) in the spine, 0.029 mg/cm2 (95% C.I.: 0.004, 0.055 mg/cm2) in the hip and 0.021 mg/cm2 (95% C.I.: 0.002, 0.039 mg/cm2) in the proximal radius]. In women over 75 years of age with the "s" allele grip strength was significantly lower than in women with the SS dimorphism [17.4% (95% C.I.: 3.3, 32.1)]. After adjustment for age, muscle strength and bone density, the "s" allele genotype was associated with previous fracture of the wrist [odds ratio (OR) 2.32 (95% C.I.: 1.09, 4.93)], but not with other fractures. We conclude that in elderly women Sp1 dimorphism is associated with fracture risk, independent of coexisting differences in bone density and muscle strength.

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Association of Collagen type 1 alpha 1 (COL1A 1) to Femoral Neck Bone Mineral Density in 1044 Swedish Women 75 Years Old. H. Brändström,*[1] P. Gerdhem,*[2] F. Stiger,*[1] K. Obrant,[2] H. Melhus,[1] Ö. Ljunggren,[1] A. Kindmark,[1] K. Åkesson.[2] [1]Department of Medical Sciences, Uppsala, Sweden, [2]Department of Orthopedics, Malmö, Sweden.

Genetic factors are known to play a central role in the pathogenesis of osteoporosis and several candidate genes have been studied for association to bone mineral density. One of the most studied is a polymorphism in the collagen 1A1 gene, the polymorphism affects a binding site for the transcription factor Sp-1. The Sp-1 polymorphism has been associated with bone mineral density and has been suggested to predict bone quality /bone strength in some studies, whereas other studies have refuted an association. In this study we have investigated the Sp-1 polymorphism in a well characterised cohort of 1044 postmenopausal females, all aged 75 years (The Malmö OPRA-study). The participants in the cohort were examined by DXA for bone mineral density at spine, hip and total body. Also, quantitative ultrasound of the calcaneus was performed and quantified as speed of sound, broadband attenuation and stiffness index. Quantitative ultrasound measurements have been suggested to measure microarchitecture and elasticity of bone, and have been proposed as a predictor of bone quality. The genotype of each individual was determined using solid-phase minisequencing with biotinylated forward primer. The PCR products were captured in streptavidin coated microtiter plate wells and rendered single stranded. The polymorphic nucleotide was detected in the captured DNA strand by extension of a complementary primer with 3H labelled nucleotides, complementary to the polymorphic site. The genotype of each individual was defined by the ratio between the incorporated 3H nucleotides. The Sp-1 genotype frequencies were SS 70%, Ss 27%, ss 3% and were in agreement with a Hardy-Weinberg equilibrium. The SS homozygous individuals had a significantly higher BMD at femoral neck compared to individuals with the presence of the s allele (0.754 g/cm2 vs. 0.734 g/cm2, n=851, P-value=0.035). No other differences in BMD measurements at any site were found. No association between the Sp-I polymorphism and variation in the ultrasound measurements were identified. In this cohort of 1044 Swedish women 75 year old, individuals who were homozygous for the SS genotype had higher bone mineral density at the femoral neck compared with women with a presence of the s allele. No other differences in bone mineral density or measurements of bone quality assessed by quantitative ultrasound of the calcaneus could be linked to differences in Sp-1 genotype.

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No Association between Collagen IA1 Sp1 Polymorphism and Geometrical Properties of the Skeleton in Middle-Aged Women. P. H. Kann,[1] A. G. Uitterlinden,[2] Y. Fang,*[2] E. Bungert,*[1] A. Hofer,*[1] J. Sorgel,*[1] H. A. P. Pols,[2] J. Beyer.*[1] [1]Endocrinology, Johannes Gutenberg University Hospital, Mainz, Germany, [2]Department of Internal Medicine, Erasmus University, Rotterdam, The Netherlands.

Bone mineral density and fracture risk are under strong genetic control. An association of the G to T polymorphism of the Sp1 binding site of the collagen Ia1 gene with the risk for fractures has been previously reported. This association is only in part explained by differences in bone mineral density (1). Furthermore, there is evidence that elastic properties of bone tissue as in vivo characterized by measurement of ultrasound transmission velocity independently associated to collagen Ia1 polymorphism contribute to fracture risk (2). This study addresses the question whether there might also be geometrical properties of the skeleton associated to collagen Ia1 polymorphism linking to fracture probability. In a sample of 207 women without growth disorders and skeletal dysplasias with a mean age of 55 + 15 years living in the Rhine-Main area in Germany, we determined collagen Ia1 genotype and body height (BH), ulna length (UL) using a ruler, lateral thickness of the midshaft of the middle phalanx of the third finger (MP), lateral thickness of the ground phalanx of the third finger head (HP), and lateral thickness of the heel (TH) with a high precision caliper. There were 129 subjects in the "GG"-group, 68 in the "GT"-group, and 10 in the "TT"-group. Similar to other cohorts investigated previously, allelic frequency for "G" was determined 79 % and for "T" 21 % and thus comparable to other cohorts reported in the literature. In the "GG"/"GT"/"TT"-groups, BH was 1.64+0.07/1.64+0.07/1.64+0.07m, UL

25.7+1.3/25.9+1.2/25.6+0.6cm, MP 13.2+0.9/13.5+1.2/13.1+0.9mm, HP 16.9+1.0/ 17.3+1.1/ 16.7+1.0mm, and TH 44.9+4.1/44.5+4.1/45.3+3.7mm (no significant differences for all parameters [ANOVA], even after adjustment for age, height, and body mass [ANCOVA]) Based on these data, there is no evidence for an association of collagen Ia1 Sp1 polymorphism to geometrical parameters of the skeleton as obtained in this study in middle-aged women. (1) Uitterlinden et al., NEJM 1998 (2) Kann et al., Calcif Tissue Int, in press.

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PTH Genotypes and Rate of Bone Loss -Response to Treatment with Estrogen and Calcitriol in Postmenopausal Women. P. B. Rapuri,[1] J. C. Gallagher,[1] J. A. Knezetic,*[2] K. L. Ryschon.*[3] [1]Bone Metabolism Unit, Creighton University, Omaha, NE, USA, [2]Biomedical Sciences, Creighton University, Omaha, NE, USA, [3]Ryschon Health and Technology Services, Valentine, NE, USA.

The association of polymorphisms in the PTH gene and bone mineral density (BMD) is not well established. In a longitudinal study, we examined the association of polymorphisms in the PTH gene (TaqI restriction enzyme) and rate of bone loss, changes in biochemical indices, in relation to treatment with estrogen, calcitriol and combination of both, in 336 normal elderly women. Subjects were randomized into four treatment groups, calcitriol (0.5mcg/d, C), estrogen (CEE 0.625 mg/MPA 2.5 mg, E), calcitriol+estrogen (E+C) and placebo (P). The percent changes in BMD over baseline after 36 months of treatment at different skeletal sites and changes in biochemical indices were calculated and compared between the PTH genotypes. A significant association was observed between the rate of bone loss and PTH gene polymorphisms. Women with tt genotype of placebo group had higher rate of bone loss at spine compared to women with TT genotype. No association was found at other skeletal sites measured. The treatment response varied significantly between the genotypes. In the group treated with calcitriol, women with TT genotype gained significantly ($p<0.05$) more bone mass compared to those with tt genotype at total femur. On treatment with estrogen, women with tt genotype gained more BMD at total body ($p<0.05$) compared to that of women with TT genotype. No significant differences were seen between the PTH genotypes on combination treatment. Women with TT genotype on calcitriol treatment and women with tt genotype on estrogen had significantly higher calcium absorption compared to tt and TT genotypes respectively, which probably explains the mechanism. In conclusion, our results suggest a significant association between the rate of bone loss and polymorphisms in PTH gene. Women with TT genotype respond better to calcitriol and women with tt genotype respond to estrogen.The effect in both, is mainly due to increased calcium absorption.

Percent change over baseline

Genotype (n)	trt	spine	total body	total femur	calcium absorption
TT(15)	P	4.46±2.51	-2.08±0.85	-1.39±1.49	-13.89±5.67
tt (32)	P	-2.04±1.06*	-1.36±0.64	-1.57±1.10	-8.84±3.96
TT(12)	C	2.04±1.35	0.76±0.69	0.89±1.36	29.98±10.98
tt(35)	C	1.18±1.04	-0.84±0.57	-3.22±1.22*	5.89±9.60*
TT(23)	E	4.70±1.41	-0.33±0.98	2.38±1.10	-10.64±7.04
tt(25)	E	6.95±1.24	2.61±0.50*	3.93±0.97	9.78±6.23*
TT(11)	E+C	8.72±1.73	2.29±0.80	7.01±1.59	19.41±5.72
tt(33)	E+C	6.96±1.19	1.87±0.52	5.53±1.09	-0.75±5.93*

Values are adjusted means±SEM. *$p<0.05$ compared to TT genotype.calcium absorption expressed as % actual dose/L blood, corrected for BMI

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A Functional Interleukine-6 Gene Polymorphism Predicts Peak Bone Mineral Density, but Not Postmenopausal Bone Loss and Bone Turnover in Women: The OFELY Study. P. Garnero,[1] O. Borel,*[2] E. Sornay-Rendu,*[2] P. Woo,*[3] P. D. Delmas.[2] [1]Inserm Unit 403, Synarc, Lyon, France, [2]Inserm Unit 403, Lyon, France, [3]Windeyer Institute of Medical Science, London, United Kingdom.

It has been recently shown that interleukin- 6 (IL-6) -174 G/C allelic variants were associated with peak bone mineral density (BMD) in young men (Lorentzon et al., 2000) and bone resorption in elderly women (w.) (Ferrari et al., 2001). The CC genotype was associated with a higher total body, hip and spine peak BMD in men and a lower bone resorption assessed by serum C-terminal telopeptide of type I collagen (CTX) in w. We assessed the relationships between IL-6 gene polymorphism, peak BMD, rate of postmenopausal BMD loss and bone turnover. BMD was measured in 255 healthy premenopausal (pre MP) w. aged 31 to 57 yr. BMD loss at the forearm was measured over 4 yr in 302 (372 w. at baseline) healthy untreated postmenopausal women (Post MP) w. 50 to 88 yr (mean :64 yr). We also measured levels of serum osteocalcin, bone alkaline phosphatase and N-propeptide of type I collagen for bone formation and 3 markers of bone resorption: urinary and serum (by ELISA and automated analyser) CTX and urinary N-terminal telopeptide of type I collagen (NTX) in Pre MP and Post MP w. at baseline. The distribution of IL-6 genotypes was similar to previous reports with 14% and 16% CC, 49% and 48% CG and 37% and 36% GG in pre MP and post MP, respectively. Pre MP and Post MP in the 3 genotypes did not differ for age, years since menopause (post MP), weight, height, physical activity and calcium intake. In pre MP we found a significant association between IL-6 genotypes and BMD at the whole body (p trend =0.020), femoral neck (p=0.026), trochanter (p=0.008), ward (p=0.015) and total hip (p=0.004), subject with the CC genotypes having a 3% to 6% higher BMD levels than their GG counterparts. BMD values were 2.8% higher at the spine in CC compared to GG (p=0.18). In post MP w., the mean rate of mid and distal radius bone loss and baseline BMD values at all sites did not differ between genotypes. However, the differences of BMD were higher in those w. within 10 yr of menopause (n=136, mean age: 56 yr) than in the older w. (n=235, mean age: 68 yr). For example, the differences between CC and GG genotypes were +2% vs -1.1% for whole body and +2.6% vs -1.5% for total hip BMD in w. within 10 yr and more than 10 yr of menopause, respectively. We found no significant difference between genotypes and any bone turnover markers either in Pre or Post MP. We conclude that this new functional IL-6 polymorphism is associated with the level of peak BMD, but not with the rate of postmenopausal bone loss in healthy French women. Whether or not this polymorphism is related to fracture risk remains to be determined.

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Risk of Loosening After Total Hip Arthroplasty and Single Nucleotide Polymorphisms in the Promoter Region of the Tumour Necrosis Factor Gene. J. M. Wilkinson,[1] I. Stockley,*[2] I. R. Scott,*[3] D. A. MacDonald,*[4] A. G. Wilson,*[5] G. W. Duff,*[5] R. Eastell.[1] [1]Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom, [2]Department of Orthopaedics, Northern General Hospital, Sheffield, United Kingdom, [3]Department of Orthopaedics, Chesterfield Royal Hospital, Chesterfield, United Kingdom, [4]Department of Orthopaedics, St James's University Hospital, Leeds, United Kingdom, [5]Division of Genomic Medicine, University of Sheffield, Sheffield, United Kingdom.

Tumour necrosis factor (TNF) is thought to play a role in aseptic loosening, the major factor limiting implant survival following total hip arthroplasty (THA). Single nucleotide polymorphisms (SNP) at the -238 and -308 positions in the promoter region are the most common polymorphisms of the TNF gene in caucasian populations. We tested whether carriage of the rare alleles at these sites are risk factors for aseptic loosening in 482 white caucasians (216 with failed versus 266 with radiologically intact implants) at 11.7±4.1 years following primary cemented THA for osteoarthritis. Genomic DNA was extracted from peripheral blood samples and genotyped using the Taqman 5' nuclease method. Carriage rates were calculated and analysed using the χ^2 test.

Carriage Rate of Rare Allele (G to A transition)

	-238		-308	
Population	**Carriage (%)**	**Odds Ratio (95% CI)**	**Carriage (%)**	**Odds ratio (95% CI)**
Intact THA (n=266)	10.9		33.1	
Cup or stem loose (n=216)	17.1	1.7 (1.0 to 2.9)	37.5	1.2 (0.8 to 1.8)
Cup and stem loose (n=122)	20.5	2.1 (1.2 to 3.8)*	36.9	1.2 (0.8 to 1.9)

*χ^2 $P<0.05$ Using the multivariate Cox proportional hazards model with backwards-stepwise exclusion of non-significant variables, risk factors for loosening of both implant components included gender (P=0.005), age at THA (P=0.005), implant type (P=0.002), and carriage of the -238A allele (P=0.015). Carriage of this allele was associated with a 2.1 increase in the rate of aseptic loosening of both prosthetic components. The frequency of this allele in the loose and non-loose populations was 10.7% and 5.5% respectively, and was in Hardy-Weinberg equilibrium with the common allele.This data provides the first reported evidence to show that genetic, as well as environmental factors, influence failure of prosthetic joint replacements. Whether the -238A SNP causes a biological change that predisposes to loosening, or is in linkage disequilibrium with such a locus, is not yet known.

M165

Polymorphisms in the Promoter of the Interleukin-6 Gene Are not Associated with Osteoporotic Fractures or Bone Mass. B. L. Langdahl, M. Carstens,* L. Stenkjaer,* E. F. Eriksen. Endocrinology & Metabolism, Aarhus University Hospital, Aarhus C, Denmark.

Interleukin-6 (IL-6) is a potent stimulator of bone resorption and IL-6 levels are elevated in postmenopausal women compared to premenopausal or HRT treated women. It has previously been shown that an AT rich VNTR polymorphism in the 3' UTR of the IL-6 gene is associated with bone mineral density in both pre- and postmenopausal women. Three polymorphisms have been identified in the promoter region:G^{-597}-A, G^{-572}-C and G^{-174}-C. G^{-174}-C has been demonstrated to be associated with production and serum levels of IL-6. We therefore wanted to investigate if these polymorphisms affect the prevalence of osteoporotic fractures and bone mass in 342 osteoporotic patients and 369 normal controls. The polymorphisms were examined by RFLP using Mnl I, Aci I and Nla III after PCR. BMD was examined by DXA. The G^{-597}-A polymorphism was not found in our population. The genotype distribution of G^{-572}-C was 0% CC, 6.3% GC and 93.7% GG in osteoporotic patients and 0.8% CC, 7.7% GC and 91.5% GG in normal controls (χ^2=2.69, p=0.26). The genotype distribution of G^{-174}-C was 26.5% GG, 54.4% GC and 19.2% CC in osteoporotic patients and 29.4% GG, 49.2% GC and 21.4% CC in normal controls (χ^2=1.43, p=0.49). Both polymorphisms were in Hardy-Weinberg equilibrium and in linkage equilibrium. Combining the genotypes resulted in 6 different haplotypes which were

distributed similarly in osteoporotic patients and normal controls (χ^2=4.63, p=0.46). BMD of the lumbar spine (ls), femoral neck (fn) and total hip (th) in the different genotypes are presented below (mean±SD). BMD was not different between individuals with the different genotypes or combined genotypes at any site.

	No	BMD(ls)(g/cm^2)	BMD(fn)(g/cm^2)	BMD(th)(g/cm^2)
-572:CC	3	0.870±0.077	0.724±0.086	0.851±0.124
-572:GC	43	0.898±0.164	0.734±0.099	0.828±0.130
-572:GG	547	0.868±0.184	0.702±0.123	0.817±0.153
ANOVA		0.59	0.25	0.85
-174:GG	169	0.883±0.180	0.710±0.116	0.829±0.150
-174:GC	304	0.859±0.182	0.698±0.124	0.811±0.158
-174:CC	130	0.885±0.180	0.713±0.121	0.825±0.143
ANOVA		0.25	0.38	0.51

In conclusion: Three polymorphisms in the promoter of the IL-6 gene are not associated with osteoporotic fractures or BMD at the spine or hip.

M166

Relation of Aromatase Gene Polymorphism and Hormone Replacement Therapy to Serum Estradiol Values, Bone Mineral Density Values and, Fracture Risk in Early Postmenopausal Women. T. Salmén,*[1] A. Heikkinen,*[2] A. Mahonen,[1] H. Kröger,[3] M. Komulainen,*[2] H. Pallonen,*[1] S. Saarikoski,*[2] R. Honkanen,[4] P. H. Mäenpää.[1] [1]Dept. of Biochemistry, University of Kuopio, Kuopio, Finland, [2]Dept. of Obstetrics and Gynecology, Kuopio University Hospital, Kuopio, Finland, [3]Dept. of Surgery, Kuopio University Hospital, Kuopio, Finland, [4]Research Institute of Public Health, University of Kuopio, Kuopio, Finland.

After menopause estrogen synthesis from androgens and androgen precursors by the aromatase enzyme is the principal source for circulating estrogens. Therefore, we studied whether aromatase gene (CYP19) polymorphism affects circulating estradiol (E2) levels, bone mineral density (BMD), BMD change, or fracture risk in a 5-year randomized hormone replacement therapy (HRT) trial on 331 early postmenopausal women (subgroup of the population-based OSTPRE study, Kuopio, Finland, n=13 100).The participants consisted of two treatment groups: the HRT group (n=151) received a sequential combination of 2 mg estradiol valerate and 1 mg cyproterone acetate with or without vitamin D3, 100-300 IU + 93 mg calcium as lactate/day, and the non-HRT group (n=180) received calcium 93 mg alone or in combination with vitamin D3, 100-300 IU/day. BMD was measured from spine and hip (DXA). All new symptomatic, radiographically defined fractures were recorded. Polymorphism at aromatase (TTTA repeats) gene was evaluated.In the analyses, CYP19 polymorphism was divided into three groups: short (repeat length of 7 or 8 in both alleles; n=135), long (repeat length of 11 or higher in both alleles; n=47), and medium (rest of the genotypes; n=149) genotypes. The mean baseline age was 52.7±2.3 years. Of the baseline characteristics, only physical activity was associated with CYP19 polymorphism (p=0.039), also a trend was observed with previous fractures (p=0.051). In the HRT or non-HRT group 5-year E2 change was not associated with CYP19 polymorphism (p=0.872 and 0.736, respectively). CYP19 polymorphism did not modulate lumbar or femoral neck BMD change during the 5-year follow-up in the HRT (p=0.709 and 0.263, respectively) or non-HRT (p=0.919 and 0.789, respectively) group. In all, 28 women sustained 33 fractures during the follow-up. In the HRT or non-HRT group, the CYP19 polymorphism was not significantly associated with fracture risk (p=0.894 and 0.230 respectively; Cox proportional hazards model). The mean number of TTTA repeats, grouped into low, medium, or high number of TTTA repeats, was not associated with E2 values, BMD values, or fracture risk.In conclusion, it appears that in these early postmenopausal women CYP19 gene polymorphism is not associated with E2 values, BMD values, or fracture risk.

M167

Vitamin D and Estrogen Receptor Gene Polymorphisms and Dietary Calcium intake in Korean Healthy Young Women: Evidence for Multiple Genes and Dietary Calcium Contribution to Peak Bone Mass. H. Kim,*[1] S. Kim,*[2] E. Kim,*[3] G. Kim.[2] [1]Department of Internal Medicine, University of Gachon College of Medicine, Incheon, Republic of Korea, [2]Division of Endocrinology, Asan Medical Center, Seoul, Republic of Korea, [3]Department of Internal Medicine, Incheon Sarang Hospital, Incheon, Republic of Korea.

Previous studies have reported that strong genetic effect was observed for the premenopausal peak bone mass. We studied the possible association of peak bone mass with complex interaction of multiple candidate genes for osteoporosis considering dietary calcium intake in young premenopausal women having their peak bone mass.The associations between bone mineral density (BMD) and polymorphisms of vitamin D receptor (3'-end region by BsmI restriction enzyme and start codon by FokI restriction enzyme), estrogen receptor (by PvuII and XbaI restriction enzyme), and type I collagen α1 (Sp1 binding site by MscI and BalI restriction enzyme) genes were examined in 100 Korean healthy young women (age 20-35 years). BMD was measured by dual energy X-ray absorptiometry, and dietary calcium intake was estimated by food frequency questionnaire.The frequencies of B allele in vitamin D receptor (VDR) gene BsmI polymorphism and X allele in estrogen receptor (ER) gene XbaI polymorphism were lower in Koreans than those in Caucasians. In contrast, the allele frequencies of VDR gene FokI polymorphism and ER gene PvuII polymorphism were similar to those of Caucasians. We could not find the polymorphism in Sp1 binding site of type I collagen α1 gene in our subjects. No significant association was found between BMD and VDR genotype by BsmI or FokI polymorphism. There was also no significant relation between PvuII or XbaI polymorphism of ER gene and BMD. The associations between BMD and cross-genotypes combining VDR gene (BsmI and FokI) and ER gene (PvuII and XbaI) polymorphisms were analyzed. Among the subjects lacking the Bf haplotype of the VDR gene, the BMD of femoral neck area was significantly higher in subjects lacking Px haplotypes of the ER gene than those having Px haplotype ($p < 0.05$). In the group having low calcium intake (< 500 mg/day), the subjects lacking Bf and Px haplotypes had significantly higher BMD in femoral neck ($p < 0.01$), Ward's triangle ($p < 0.05$), and trochanteric area ($p < 0.05$) than those lacking Bf but having Px haplotype.These data suggest that complex interaction of vitamin D and estrogen receptor gene with dietary calcium intake, rather than polymorphism of a single gene, influence peak bone mass in Korean healthy young women.

M168

Apa I and TaqI VDR Genotype Is Associated with Lumbar Spine BMD in Spanish Postmenopausal Women. X. Nogues,[1] A. Enjuanes,*[1] N. García-Giralt,*[2] J. Puig,*[1] S. Balcells,*[2] L. Mellibovsky,*[1] A. Garrido,*[1] D. Grinberg,*[2] A. Díez-Perez.[1] [1]URFOA, Hospital del Mar UAB, Barcelona, Spain, [2]Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain.

Previous investigations showed significant associations between polymorphisms of the vitamin D receptor (VDR) gene and bone mineral density (BMD).Patients and methods: One hundred and ninety-six postmenopausal Spanish women, aged 50.2 ± 4 (mean ± SD) years, all of them with Spanish ancestors, were recruited from the Menopausal Unit in the Hospital del Mar. Bone mineral density (BMD) was measured in lumbar spine (L2-L4) using dual-energy X-ray absorptiometry (Hologic QDR 4500 SL). Genomic DNA was obtained from blood leukocytes, VDR gene polymorphisms Fok I, Bsm I ApaI and TaqI were investigated using polymerase chain reaction followed by enzymatic digestion. We examined the association of these polymorphisms, separately and in combination, with bone mineral density (BMD)Results: Only the combination between Apa I and TaqI VDR genotype was associated with BMD of the lumbar spine in the multivariate regression analysis p=0,048. Women with AATT genotype had higher BMD 0,998 + 0.15. On the other hand, there was not significant association between any of the VDR polymorphisms and BMD, when taken separately. Conclusions: We conclude that Vitamin D receptor polymorphisms genotype AATT, defined by TaqI and ApaI, are significantly associated with higher BMD in postmenopausal women. Our results support the idea that the vitamin D receptor polymorphisms could at least partially account for same of phenotype expression of BMD.

M169

Association Between TNF Beta Gene Polymorphism and Bone Mineral Density in Postmenopausal Japanese Women. K. Noriko,* F. Takafumi,* F. Itsuko,* F. Seiichiro.* Obstetrics and Gynecology, Hokkaido University school of Medicine, Sapporo, Japan.

The purpose of the present study was to investigate the relationship between TNF beta gene polymorphism and bone mineral density (BMD) at lumbar spine, bone metabolic markers and cytokines. Ninety-nine women not more than sixty years old, who visited Hokkaido University Hospital and whose BMD was measured, gave informed consent for blood and urine sampling. None had medical complications or any history of bone diseases and was under medical treatments that were known to affect bone metabolism. The mean age of the subjects was 55.2 years and the mean body mass index (BMI) was 22.5 kg. Genomic DNAs were extracted from white blood cells. DNA fragments including NcoI restriction site within the first intron of the TNF beta gene were amplified by polymerase chain reaction (PCR). The RFLPs were represented as b1, b2, signifying the presence and the absence of restriction site, respectively. BMD at the lumbar spine (L2-4) was measured by dual-energy X-ray absorptiometry (DEXA) using a Hologic QDR 2000. Serum Osteocalcine (OC), unine Deoxypiridinoline (DPD), serum TNF alpha and TNF beta were measured by ELISA. The women with b1/b1 genotype tended to have lower BMD Z-score values than those with other genotype (p=0.09 vs b1/b2 type, p=0.07 vs b2/b2 type). The women with b1/b1 genotype had significantly lower BMD Z-score values than those with the others. There were no association between TNF beta gene polymorphism and OC, DPD, TNF alpha or TNF beta levels. This study suggested that BMD Z-scores of postmenopausal women not more than sixty years old with b1/b1 genotype were lower than those with other genotypes. This is the first report suggests that TNF beta gene polymorphism can be considered as one of the genetic markers for predicting bone loss in postmenopausal women at high risk for osteoporosis.

M170

***Klotho* Promoter Polymorphism Associated with Bone Density of Aged Postmenopausal Women in Caucasian and Japanese Populations.** K. Kawano,[1] M. Chiano,*[2] N. Ogata,[1] M. Uchida,[1] T. Hosoi,[3] H. Orimo,[3] S. Inoue,[1] K. Nakamura,[1] M. Kuro-o,*[4] H. Kawaguchi.[1] [1]Univ. of Tokyo, Tokyo, Japan, [2]Gemini Genomics plc, Cambridge, United Kingdom, [3]Tokyo Metropolitan Geriatric Ctr., Tokyo, Japan, [4]Univ. of Texas SW, Dallas, USA.

Because mice deficient in *klotho* gene expression exhibit multiple aging phenotypes including low-turnover osteopenia, we explored the possibility that the *klotho* gene may be involved in human osteoporosis by examining the association between the human *klotho* gene polymorphism and bone density in Caucasian (U.K.) and Japanese populations. Initially, we screened for single-nucleotide polymorphisms (SNPs) in and around the coding regions of the human *klotho* gene. Eight SNPs in the Caucasians and six SNPs in the Japa-

nese were identified, and three of them: one in the promoter region (G-395A) and two in exon 4 (C1818T & C2298T) were common in both populations. None of these common SNPs was associated with menopausal status, age, height or weight in either population. In the association analysis of unrelated Caucasian women (n=1,187, 47.1±12.0 yrs.), none of the common SNPs was associated with bone density (the whole body BMD) in the overall or premenopausal (n=506) women; however, in postmenopausal women (n=364, 57.9±6.7 yrs.) C1818T SNP showed a slight association with bone density (p=0.029). We further divided the Caucasian postmenopausal women into three age groups: those ≤54, 55-64, and ≥65 years old. None of the SNPs was associated with bone density in the two younger subpopulations. In the oldest subpopulation (≥65 yrs.), however, G-395A SNP (p=0.002) showed stronger association than C1818T SNP (p=0.014), and women with G->A substitution exhibited lower bone density. In the analysis of unrelated Japanese postmenopausal women (n=215, 72.9±5.5 yrs.), G-395A and C1818T SNPs were associated with bone density (the forearm BMD; p=0.023 and 0.035, respectively) and the associations became stronger (p=0.002) in women ≥80 years old. Because the two SNPs in exon 4 were not accompanied by amino acid substitutions, the possible functional relevance of the G-395A SNP was explored. An electrophoretic mobility shift analysis using synthetic oligonucleotides spanning the G-395A site revealed that G->A substitution markedly decreased the binding activity to nuclear extracts from human kidney 293 cells that were confirmed to express *klotho*, implying that this promoter polymorphism may affect the expression of the *klotho* gene. It is concluded that a polymorphism in the human *klotho* promoter is associated with bone density of aged postmenopausal women independent of race. The *klotho* gene may therefore be involved in the pathophysiology of bone loss with aging in humans.

M171

Association Between IL-6 Production in Bone Marrow Cultures and IL-6 Polymorphism in Elderly Women with Fracture. D. Feng,*[1] Y. Koshihara,[1] H. Ishibashi,*[2] S. Yamamoto,[2] N. Ota,*[3] M. Emi,*[3] T. Hosoi,[2] H. Orimo.[2] [1]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan, [2]Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan, [3]Department of Molecular Biology, Institute of Gerontology, Nippon Medical School, Kawasaki, Japan.

Emi et al. have recently reported a common –571 G>C allelic variant which significantly relates to bone mineral density (BMD). To investigate how this polymorphism relates to BMD, we investigated the relationship among Interleukin-6 (IL-6) levels, osteoclast-like multinucleated cells (MNCs) formation, and BMD in Japanease elderly women. Femoral bone marrow was obtained as surgical waste from subjects undergoing proximal joint replacement surgery for fracture in 47 patients (female, aged 80 ± 1.3 yr). MNCs were prepared according to the method of Koshihara et al. IL-6 level in the conditioned medium was measured by ELISA. After 2 weeks from starting culture, when MNCs formation was seen, isolated stromal cells were treated with IL-1alpha for 24h, and then IL-6 production was determined. Genomic DNA was extracted from an aliquot of monocytes in bone marrow, which were simultaneously used for MNCs formation. The PCR was used to detect the IL-6 BsrBI RELP. Forty-five subjects had a C at position –571 of the IL-6 gene, 20 heterozygotes (C/G) and 25 homozygotes (C/C). Two subjects were homozygotes of G at the position, therefore we examined the following experiment in only GC genotype and CC genotype. There were no significant differences among IL-6 levels, MNCs formation and BMD, when comparing between the two groups defined by the IL-6 genotype. In the CC genotype, there were negative correlation between IL-6 and femoral neck BMD (p=0.016), radius BMD (p=0.001), lumbar spine BMD (p=0.0092), whereas in the GC genotype IL-6 levels showed tendency of relationship with BMD. The stromal cells in the CC genotype were shown higher IL-1 alpha-stimulated IL-6 production than that in GC genotype. In this study, the CC genotype was 52% in the distribution of IL-6 alleles, conversely it was reported that in no fracture females was only 6%. Therefore, ours study suggest that IL-6 gene promoter polymorphism, as G/C replacement, may be susceptible to influence the fracture, and regulate promoter activity in response to IL-1alpha.

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Effect of COL1A1 Polymorphism on Bone Resorption Markers in Early Postmenopausal Scottish Women. H. M. Macdonald,*[1] W. D. Fraser,*[2] F. E. McGuigan,*[1] S. A. New,[3] A. J. Black,[1] S. H. Ralston,[1] D. M. Reid.[1] [1]Department of Medicine & Therapeutics, University of Aberdeen, ABERDEEN, United Kingdom, [2]Department of Clinical Chemistry, University of Liverpool, LIVERPOOL, United Kingdom, [3]School of Biomedical & Life Sciences, University of Surrey, GUILDFORD, United Kingdom.

The collagen I alpha 1 sp1 polymorphism has been associated with reduced BMD and increased fracture risk. We recently reported that early postmenopausal women with the "ss" genotype lost significantly more bone at the lumbar spine (LS BMD) compared with "SS" and "Ss" women (1). The mechanism by which women with the "ss" genotype have greater bone loss is not clear. An association between "ss" genotype and reduced levels of serum C-terminal extension propeptide of type I collagen has been reported (2). Also, the "s" allele is associated with an abnormal ratio of collagen α1 to α2 chains (3). In 1990-1993, 1064 pre-menopausal women (aged 45-49 yrs) were invited randomly from the population to undergo a BMD scan. Between 1997 & 1999, 907 women returned for reassessment (response rate 85%) and over 80% of these provided a blood sample for DNA analysis. Analysis of urinary bone resorption markers by HPLC has now been carried out for 533 of the 734 women who were genotyped. As expected, current HRT users had lower levels of free pyridinoline (fPyd/Cr) and deoxypyridinoline (fDpd/Cr) cross-links than non users and past users (p=<0.001), and lower rates of bone loss (p=0.001).

	n	Pyd/Cr (mean [SD])	Dpd/Cr (mean [SD])	n	LS BMD (%change/yr [SD])
Current HRT users	SS	102	17.4 [1.8]	4.6 [1.0]	157	-0.36 [0.9]
	Ss	58	15.4 [1.7]	4.0 [1.0]	72	-0.46 [0.8]
	ss	8	13.7 [2.3]	3.6 [0.9]	10	-0.69 [0.8]
Significance			p=0.021	p=0.009		p=0.422
Postmenopausal no HRT	SS	185	20.6 [2.2]	5.7 [1.2]	250	-1.54 [1.0]
	Ss	82	19.8 [2.0]	5.5 [0.9]	117	-1.38 [0.9]
	ss	10	19.4 [2.0]	5.0 [0.9]	14	-2.29 [1.
Significance			p=0.517	p=0.471		p=0.004

No differences in fPyd/Cr and fDpd/Cr were observed according to genotype for postmenopausal women who did not use HRT, even though bone loss was significantly increased in the "ss" group (p=0.016). HRT users carrying the "s" allele (i.e. Ss and ss women) had significantly lower levels of fPyd/Cr and fDpd/Cr than "SS" homozygotes. The genotype-related difference in response of urinary crosslinks to HRT indicates that individuals who carry the "s" allele may be more responsive to the anti-resorptive effects of HRT, suggesting that COLIA1 genotyping could be of clinical value not only in identifying fast bone losers after the menopause but also patient subgroups who have an enhanced response to HRT. 1) Macdonald et al J Bone Miner Res 2001 (in press) 2) Garnero et al J Bone & Miner Res 1998 13 813-8. 3) Mann et al (2001) J Clin Invest (in press

M173

A Novel Titreplate Based Assay for the Detection of the COLIA1 Sp1 Binding Site Polymorphism Associated with Increased Risk of Osteoporotic Fracture. C. M. Scott,*[1] S. Cribbes,[1] G. Zajicek,*[2] S. Ralston,*[3] P. Wallace.*[1] [1]Discovery R&D, Axis-Shield Diagnostics Ltd, Dundee, United Kingdom, [2]Axis-Shield Diagnostics Ltd, Dundee, United Kingdom, [3]University of Aberdeen, Aberdeen, United Kingdom.

INTRODUCTIONGenetic factors have been found to be very important in the pathogenesis of osteoporosis. Studies have shown that 50-85% of population variance in bone mineral density (BMD) can be attributed to genetic factors.Recently, a single nucleotide polymorphism (SNP) has been described in the Sp1 binding site of the type I collagen a1 gene (COLIA1). This G to T transition at nucleotide +2046 of the COLIA1 gene occurs at relatively high frequencies, with 30-35% of most populations being heterozygous and 3-5% homozygous for the polymorphism.A number of studies indicate that the COLIA1 polymorphism is associated with decreased BMD and increased risk of fracture. Statistical analysis has shown that risk of vertebral fracture is increased 2 fold in heterozygotes and around 3 fold in homozygotes for the COLIA1 SNP. This increase in risk is independent of BMD.We have developed a titreplate based assay for the detection of the COLIA1 polymorphism. The method utilises allele specific PCR amplification of patient DNA followed by hybridisation capture onto a titre plate. Biotinylated PCR product is then detected using standard ELISA based techniques.This method offers an economical alternative to the Taqman assay, and is less cumbersome and more rapid than conventional RFLP and sequencing methods. Results can be visually determined or recorded by a microtitre plate reader.Up until now genetic testing for the COLIA1 polymorphism has been a complicated process requiring highly skilled staff and expensive specialised equipment. It has therefore been performed almost exclusively in dedicated molecular biology laboratories.Our method simplifies the whole genotyping process, allowing non-specialist research laboratories to perform COLIA1 genotyping in large or small batches when required. The technology also opens the way to the development of many other genetic marker detection kits, which will simplify the process of genetic profiling.

Disclosures: Axis Shield Diagnostics Ltd,3.

M174

Vitamin D Receptor Genotypes in Chronic Renal Failure. M. Pazianas,[1] E. Mercer,*[2] K. Colston,[2] J. Eastwood.*[2] [1]Medicine, University of Pennsylvania, Philadelphia, PA, USA, [2]St. George's Hospital Medical School, London, United Kingdom.

Renal bone disease is a major complication of chronic renal failure (CRF). Central to the development of the skeletal abnormalities are changes in the metabolism and actions of vitamin D. Vitamin D receptor (VDR) polymorphisms have been implicated in important aspects of bone biochemistry and structure. We have studied normal individuals and patients with CRF to investigate the significance of these polymorphisms in the development of renal bone disease.We assessed 88 normal caucasian subjects (M39, F49, mean age 43 [range 19-88], creatinine 150mmol/L). Serum analyses included albumin, corrected calcium (cCa), phosphate, alkaline phosphatase (AP) and intact parathyroid hormone (iPTH). Bsm1 restriction enzyme was used to identify a polymorphic site at the VDR locus. Patients with CRF had significantly higher AP, phosphate and creatinine levels. There was no age related difference in VDR genotype. However a significant difference in genotype frequency between controls and patients was noted (x2 test, p <0.02)

VDR Genotype	Bb	BB	bb
Controls	45	16	39
Patients	50	29	21

Within the patient group no significant differences were observed between cCa or AP in

relation to VDR genotype. Histological evidence of bone disease in CRF is associated with iPTH levels >25pmol/L. We therefore evaluated the relationship between VDR genotype and iPTH. 19% of bb patients had iPTH values >25pmol/L compared to 4% and 8% for BB and Bb patients respectively.Further studies are underway to elucidate the functional significance of VDR polymorphisms in relation to progression of hyperparathroidism and renal bone disease

M175

Influence of Calcium Intake on the Interaction of Vitamin D Receptor Genotypes and the Rate of Bone Loss in Elderly Women. P. B. Rapuri,[1] J. C. Gallagher,[1] J. A. Knezetic,*[2] K. L. Ryschon.*[3] [1]Bone Metabolism Unit, Creighton University, Omaha, NE, USA, [2]Biomedical Sciences, Creighton University, Omaha, NE, USA, [3]Ryschon Health and Technology Services, Valentine, NE, USA.

Dietary calcium intake has been reported by some investigators to influence the relationship between the vitamin D receptor (VDR) genotypes and bone mineral density (BMD). In a longitudinal study of postmenopausal elderly women, we examined the role of calcium intake on the association between the VDR genotypes (defined by TaqI and BsmI) and the rate of bone loss. The study population is comprised of 87 Caucasian women on placebo treatment, recruited for an osteoporotic intervention study. The percent change in BMD (spine, femoral neck, total body and total femur) from baseline and the percent change in biochemical indices (serum PTH, serum 25 hydroxy vitamin D, serum osteocalcin, urine N-telopeptides) from baseline were calculated after 36 months of follow-up. These variables were compared between the VDR genotypes defined by TaqI (TT, Tt and tt) and BsmI (BB, Bb and bb) in both the low and high calcium intake groups using a Univariate General Linear Model after adjusting for smoking, alcohol intake, baseline BMD and other significant covariates. Calcium intake was found to have a marginally significant influence on the rate of bone loss between VDR genotypes at spine (p=0.06) and total body (p=0.09). Women with tt genotypes were noted to have a higher rate of bone mass loss compared to that of women with TT genotype at spine and total body when their calcium intake was less than 600 mg/day. A similar observation was made with regard to the VDR genotypes defined by BsmI. No influence of calcium intake was observed on the association of the VDR genotypes and the changes in biochemical indices. In summary, the results presented suggest that genetic influence on bone loss appears to be modified by the calcium intake level.

Genotype (n)	**Percent change in BMD over baseline**			
	spine	**femoral neck**	**total body**	**total femur**
Low calcium (<=600 mg/day)				
TT(9)	3.03±1.71	2.43±1.06	-3.63±1.06	-1.85±1.95
Tt(13)	-0.96±1.36	-2.60±1.56	-2.43±0.84	-2.82±1.58
tt(5)	-5.62±2.85	-1.76±2.80	-6.86±1.75	-0.24±2.78
High calcium (> 600 mg/day)				
TT(29)	-0.22±1.03	2.55±1.33	-1.57±0.65	-0.76±1.17
Tt(25)	-1.10±1.04	-0.94±1.23	-2.00±0.65	-0.41±1.20
tt(6)	-0.34±2.10	-3.75±3.40	-1.96±1.28	-1.32±2.31

Values are adjusted means±SEM

M176

Genetic Variants of Aromatase (CYP19): Association with Bone Density Among Men of African Ancestry. J. M. Zmuda,[1] J. A. Cauley,[1] C. H. Bunker,*[1] L. H. Kuller,[1] A. L. Patrick,*[2] V. W. Wheeler,*[2] R. E. Ferrell.*[3] [1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA, [2]The Tobago Regional Health Hospital, Scarborough, Trinidad and Tobago, [3]Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA.

The aromatase enzyme is expressed in peripheral tissues such as bone, where it catalyzes the conversion of androgens to estrogen. Natural (human) and experimental (knock-out mice) models of aromatase deficiency have revealed an important role for estrogen in the control of bone metabolism in males. In the present study, we tested whether common genetic variants of aromatase (CYP19) are associated with bone mineral density (BMD) in 250 Afro-Caribbean men (age 41-91 yrs; mean±SD, 63±9) on the West Caribbean island of Tobago. Hip BMD was measured with a Hologic QDR 4500 densitometer. Men were genotyped for a tetranucleotide [(TTTA)n] repeat polymorphism in intron 4 and a single base substitution (C/T) in exon 7 which creates an Arg/Cys amino acid replacement at codon 264. We averaged repeat length over the 2 chromosomes and tested for differences in hip BMD across the 4 most common repeat lengths. We found a significant monotonic increase in BMD at all regions of the hip associated with increasing repeat length that was independent of age, weight and height (Table). For example, femoral neck BMD was 12% or 0.8 standard deviations greater among men with the longest compared to shortest repeat length (*P*=0.003). Repeat length explained 4.4% of the phenotypic variation in femoral neck BMD (*P*=0.001). The Arg264Cys polymorphism was not associated with hip BMD. Our results reinforce the concept that estrogens have important skeletal effects in men and suggest that aromatase polymorphisms contribute to the polygenic control of BMD among men of African descent.

Repeat length (bp)	**298**	**300**	**306**	**308**
No. of men	**155**	**23**	**37**	**18**
BMD (g/cm^2)[a]				
Total hip	1.10 (.16)	1.13 (.17)	1.17 (.15)	1.21 (.13)
Femoral neck	.93 (.15)	.97 (.16)	.98 (.13)	1.04 (.13)
Trochanter	.85 (.14)	.86 (.15)	.91 (.13)	.94 (.11)
Intertrochanter	1.29 (.19)	1.31 (.20)	1.36 (.18)	1.40 (.16)
Wards	.75 (.19)	.79 (.16)	.83 (.17)	.89 (.21)

[a]Values are mean (SD) and adjusted for age, weight and height. All P<0.01 (ANOVA).

M177

Performance of Colia1, Vdr and Er Polymorphism to Identify Postmenopausal Women with Osteoporosis. M. E. Muñoz-Torres,[1] P. Mezquita-Raya,*[1] F. Lopez-Rodriguez,*[1] J. Luna,*[2] J. Quesada,*[3] F. Luque-Recio,*[3] F. Escobar-Jimenez.*[1] [1]Endocrinology Unit, Hospital Clinico, Granada, Spain, [2]Facultad de Medicina, Granada, Spain, [3]Endocrinology Unit, Hospital Reina Sofia, Cordoba, Spain.

Genetic factors are now recognized to be one of the most important determinants of bone mineral density (BMD), and several studies have shown that 50-85% of the population variance in bone mass is under genetic influence. Some studies have associated diverse genetic polymorphisms with decreased BMD and increased risk for osteoporotic fractures, although others did not observe similar associations. Experience with previous candidate genes suggests that such results recquire confirmation in other populations.AIMS: to examine the association of the COLIA1, VDR and ER genotypes with the risk of osteoporosis in ambulatory postmenopausal spanish women.SUBJECTS AND METHODS: We determined the COLIA1 (MlsI), VDR (BsmI, TaqI, FokI) and ER (Xba, Pvu) polymorphisms by PCR and BMD by dual X-ray absorptiometry in 135 postmenopausal women (61±7 yrs). RESULTS: there was a significant overrepresentation of the "s" allele in osteoporotic women (p=0.006). After adjusting for age, time since menopause and body mass index, COLIA1 polymorphism was the most strongly variable associated with osteoporosis (OR=2,82[1,4-5,6] per copy of "s" allele) followed by years since menopause (OR=1,16[1,06-1,27]). None of the remainding genotypes entered the logistic regression model.CONCLUSION: our study shows that COLIA1 polymorphism is associated with osteoporosis independently of anthropometrics parameters, VDR and ER genotypes. These data indicate that the Sp1 polymorphisms in the COLIA1 gene might improve our current methods of assessing the risk of fracture in postmenopausal women.

M178

Bone Formation Induced By Ad5-LMP-1: Histological and Immunohistochemical Analysis. A. Minamide,* L. Titus, M. Viggeswarapu, C. Oliver,* G. Hair,* S. D. Boden. Dept of Orthopaedic Surgery, Emory Univ Sch Medicine, Atlanta, GA, USA.

LIM mineralization protein-1 (LMP-1), an intracellular osteoinductive protein, is thought to induce secretion of soluble factors that convey its osteoinductive activity. Although evidence suggests LMP-1 to be a critical regulator of osteoblast differentiation in vitro and in vivo, little is known about its mechanism of action. The purpose of the present study was to describe the time sequence of histologic changes during bone formation induced by LMP-1. Sixteen athymic rats (4-5 weeks old) each received four subcutaneous implants on the chest. Rabbit or human buffy coat cells from peripheral blood were used to deliver the LMP-1 cDNA. One million cells were infected with recombinant (-E1E3) type 5 human adenovirus (MOI = 4.0) for 10 minutes, placed on a collagen disc and implanted. The buffy coat cells contained either Ad-CMV-LMP-1 or Ad-CMV-Bgal (negative control). The animals were sacrificed at 1, 3, 5, 7, 10, 14, 21 and 28 days after surgery and explants were analyzed by undecalcified histology (H+E, Goldner trichrome) and immunohistochemistry for BMP-4 and BMP-7, two potential intermediate messengers. By Day 3, an increased number of cells were seen at the periphery of the Ad-LMP implants. By Day 5, the number of cells surviving in the center of the implant was diminished, especially in the controls. Matrix could be seen deposited near the cells in the periphery by Day 7 in the Ad-LMP implants. By Day 10, osteoblast-like cells were observed in the gaps between the collagen fibers. Osteoid and some mineralized bone was consistently seen by Day 14 growing inward from the edge of the implant. By Day 28, secondary trabeculae were seen with osteoblasts, osteoclasts, and bone marrow elements. Immunohistochemistry revealed some positive BMP-4 and BMP-7 staining on Day 3 in Ad-LMP-1 specimens within cells near the outer edge of the implant. Later (Day 10-28), the positive staining for BMP-4 and BMP-7 was more prevalent as additional osteoblastic cells were present. Ad-LMP-1 transfected buffy coat cells induce direct intramembranous bone formation ectopically. It appears that BMP-4 and BMP-7 may be early intermediate signals that can recruit and differentiate additional mesenchymal cells. Ex vivo gene transfer of LMP-1 to cells can induce the osteoinductive cascade in vivo.

Disclosures: Medtronic Sofamor Danek,2,5.

M179

Leptin Mediated Changes in Bone Phenotype. M. A. Kacena,[1] D. W. White,[2] F. F. Chehab,*[3] C. M. Gundberg,[1] M. C. Horowitz.[1] [1]Yale University School of Medicine, New Haven, CT, USA, [2]Millennium Pharmaceuticals, Cambridge, MA, USA, [3]University of California, San Francisco, CA, USA.

Leptin has proven to be a complex regulator of multiple neuroendocrine pathways. Lep ob/ob (leptin deficient) and Lepr db/db (leptin receptor deficient) mice have been reported to have a high bone mass phenotype. To better understand the role of leptin in bone remodeling, ob/ob, db/db, leptin overexpressing transgenic, and C57BL/6 control mice were examined. Histomorphometric analysis showed that ob/ob and db/db bones contained significantly more trabecular bone (~4-fold increase) with increased bone formation indicies (7.5-8-fold increase in the number of osteoblasts per total area) compared to C57BL/6 controls. Osteoclastic/bone resorption parameters were similar or slightly elevated in the ob/ob and db/db mice compared to controls. Interestingly, while trabecular bone measurements were increased, cortical bone measurements were significantly decreased (15% decline as assessed by pQCT). Further, serum osteocalcin and urinary Dpd concentrations were reduced. In contrast, the leptin overexpressing transgenic mice exhibited no change in either bone formation or resorption parameters compared to littermate controls. In an attempt to clarify the mechanism by which leptin signaling can effect bone homeostasis, we transfected cells of the osteoblast lineage with either the long (OB-RL) or short (OB-RS) form of the leptin receptor and a recorder responsive to ligand stimulation of OB-R. These cells were then either mock stimulated or treated with leptin. Non-transfected cells were un-responsive to leptin treatment, indicating that there were no endogenous functional receptors on the cells tested. In contrast, cells transfected with OB-RL were responsive to the leptin treatment. Taken together, our data indicate that: 1) there are no endogenous functional leptin receptors on cells of the osteoblast lineage; 2) cells of the osteoblast lineage transfected with the long signaling form of the receptor are responsive to leptin stimulation; 3) trabecular bone volume/area/density and bone formation parameters are increased in Lep ob/ob and Lepr db/db mice compared to controls; 4) bone resorption parameters are similar between ob/ob, db/db, and control mice; 5) cortical bone density is reduced in ob/ob and db/db mice compared to controls; and 6) there are essentially no histomorphometric changes in leptin overexpressing transgenic mice compared to controls. These data suggest that leptin acts through a yet unidentified mechanism to control bone mass and that the bone response to leptin can plateau beyond a certain threshold level of this circulating molecule.

M180

Glutamatergic Regulation of Megakaryocyte Function. I. S. Hitchcock, M. R. Howard,* T. M. Skerry, P. G. Genever. Biology, University of York, York, United Kingdom.

Although the primary role of megakaryocytes is the production of platelets, they assert directional control within the bone marrow microenvironment by secreting numerous cytokines and growth factors that regulate differentiation of mesenchymal and haematopoietic precursor cells. We have shown that osteoblasts, osteoclasts and various marrow cells express functional components of the glutamate signaling pathway that can direct multicellular glutamate-mediated communication in a manner similar to glutamatergic activity in the central nervous system. Recent evidence indicates that megakaryocytes also express N-methyl-D-aspartate (NMDA)-type glutamate receptors and that these cells are centrally involved in this signaling hierarchy. Here we provide further compelling evidence demonstrating the fundamental importance of glutamate signaling in the function and differentiation of primary human megakaryocytes. $CD34^+$ cells were immunoisolated from human umbilical cord blood and cultured for 14 days in the presence of 25ng/ml thrombopoietin to promote megakaryocyte differentiation. RT-PCR analysis identified expression of NMDAR1 and NMDAR2 receptor subunits in these cells, as well as the NMDA signaling accessory proteins Yotiao, PSD-95 and chapsyn-110. In functional studies, addition of a selective concentration of NMDA receptor antagonist, MK-801 (50μM), induced a 9-fold decrease in the number of proplatelet forming structures compared to controls ($P<0.001$) after 14 days in culture. Propidium Iodide staining indicated that MK-801 did not affect cell viability, but did induce a marked decrease in the percentage of polyploid cells. At the ultrastructural level, MK-801 treatment prevented the formation of indented multilobed nuclei; dramatically restricted demarcated membrane dilation and markedly inhibited alpha granule number and platelet shedding, characteristics of an undifferentiated phenotype. MK-801-treated cells also exhibited large cytoplasmic cisternae and were approximately half the size of control cells ($P<0.001$), as determined by quantitative image analysis of cytospin preparations. Following exposure to MK-801, expression of the megakaryocytic cell surface markers CD61 (early), CD41 (intermediate) and CD42a (late) was analysed by flow cytometry after 14 days of culture. MK-801 significantly inhibited expression of CD61 from 72.5% to 49.6%, CD41 from 73.5% to 49.6% and CD42a from 30.3% to 17.3% compared to controls. These data demonstrate the importance of glutamate signalling in the regulation of megakaryocyte differentiation which may significantly influence intercellular interactions within the bone marrow compartment.

M181

Serum Levels of VEGF and bFGF During Early Stages of Distraction Osteogenesis. Preliminary Report. G. Benke,* W. Glinkowski,* A. Górecki,* S. Zarek,* J. Macias.* Department of Orthopedics and Traumatology of Locomotor System, Medical University of Warsaw, Warsaw, Poland.

Angiogenesis is essential to both normal and pathological bone physiology. Vascular endothelial growth factor (VEGF) has been implicated in angiogenesis, whereas basic fibroblast growth factor stimulates mitogenesis, chemotaxis, differentiation, angiogenesis and accelerate fracture healing. It is also considered that bFGF promotes the fracture healing in by the stimulation of bone remodeling. In patients with isolated bone fractures a transient increase of bFGF was observed only during the 2nd week after injury. Longer lasting increase was determined in the sera of patients with head injury. Single local injection of fibroblast growth factor-2 stimulates the healing of segmental defects. PURPOSE of the study was to determine early changes of serum levels of VEGF and bFGF during early stages of distraction osteogenesis.Seven patients were treated by distraction osteogenesis to egalize legs with Ilizarow method. Blood samples were obtained from patients immediately before surgery and then after 1, 2, 6 and 12 weeks after surgery. Serum level of VEGF significantly increase was observed one week after bone corticotomy as well as after 2 weeks than decrease after 6th postoperative week. Serum level of bFGF did not rise significantly after one or two weeks after bone corticotomy but significant decrease was observed after 6th postoperative week.

M182

Transcriptional Regulation of RANKL Gene Expression by Protein Kinase C (PKC). H. Yang, X. Feng. Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Receptor activator of nuclear factor kappa B ligand (RANKL, also known as OPGL/ODF/TRANCE) plays a pivotal role in osteoclastogenesis. Osteoblasts/stromal cells support osteoclastogenesis by expressing RANKL in response to various factors such as 1alpha, 25-(OH)2D3, Glucocorticoids, PTH, PGE2 and IL-11. In addition, several intracellular signals including those mediated by PKC and intracellular calcium are also implicated in regulating RANKL expression in osteoblasts/stromal cells. However, the transcriptional mechanisms of PKC/calcium-mediated RANKL expression remain unclear. To investigate the molecular mechanisms by which PKC/Calcium-mediated signals regulate RANKL gene transcription, we subcloned 1.1-kb murine RANKL promoter (from -953 to +150), amplified by PCR using high fidelity thermal Polymerase based on published sequence, into pGL3-basic plasmid to generate reporter construct named RL(-953)Luc. RL(-953)Luc was transiently transfected into ST2 cells (passages 8-11) and transfected cells were then either untreated or treated with phorbol 12-myristate 13-acetate (PMA, an activator of PKC), cyclopiazonic acid (CPA) or ionomycin (CPA and ionomycin are both able to elevate intracellular calcium concentration). The transfection results indicated PMA up-regulated the 1.1-kb RANKL promoter activity while CPA/ionomycin failed to do so. Thus, the 1.1-kb RANKL promoter region contains a response element(s) mediating PKC-dependent upregulation of RANKL gene expression. Furthermore these results also suggested that intracellular calcium-mediated signal stimulates RANKL gene expression by either stabilizing RANKL mRNA or up-regulating RANKL gene transcription via a response element(s) located in RANKL promoter regions other than this 1.1-kb region. To further locate the response element(s) mediating PKC-mediated RANKL gene transcription, we generated 7 deletion mutants of the 1.1-kb RANKL promoter: RL(-753)Luc, RL(-553)Luc, RL(-403)Luc, RL(-253)Luc, RL(-153)Luc, RL(-103)Luc and RL(-53)Luc. These mutants contain RANKL promoter regions starting from different 5' positions (indicated by the numbers in parentheses) and ending at the same 3' site (+150). These mutants were then used to repeat the previous transfection assays. Among these mutants, only RL(-753)Luc was able to mediate PKC-dependent RANKL gene transcription, suggesting a 200-bp region (-953 to -753) contains a cis-element(s) mediating PKC-dependent regulation of RANKL gene. In conclusions, our studies have demonstrated that PKC-mediated intracellular signal enhances RANKL gene transcription via a cis-element(s) located between -953 to -753 in the RANKL promoter.

M183

Insulin-like Growth Factor-1 Induces Bone IGF-Binding Protein-5 in Vivo in an Autocrine-Paracrine Manner. M. M. Rutter,*[1] E. Markoff,*[1] L. Clayton,*[1] G. Zhao,*[2] T. L. Clemens,[2] S. D. Chernausek.*[1] [1]Department of Pediatrics, Children's Hospital, Cincinnati, OH, USA, [2]Department of Medicine, University of Cincinnati, Cincinnati, OH, USA.

We have previously shown that osteoblast-targeted overexpression of IGF-1 in transgenic mice stimulates bone formation in vivo. Since IGFBPs are believed to modulate IGF-1 action, we postulated alterations in IGFBP abundance might be involved in the paracrine action of IGF-1 in bone. The study objective was to determine whether overexpression of IGF-1 in osteoblasts influenced IGFBP abundance in bone in vivo. Femurs were obtained from 3 and 6 week old transgenic mice overexpressing IGF-1 under the control of the human osteocalcin gene promoter and wild-type littermates. IGFBPs were identified by immuno and radioligand blotting of extracted bone protein. Ligand blot of bone extract showed a predominant 30 kDa band, which was increased fivefold in transgenic bone compared with controls ($p<0.001$) at 3 weeks. At 6 weeks the difference was still apparent albeit less so at 1.5 times more abundant in transgenic bone compared to controls ($p <0.01$). Immunoblotting experiments revealed the difference in the 30 kDa band intensity could be explained by a change in IGFBP5, and that IGFBP5 abundance declined markedly with age in normal animals. No difference was seen in IGFBP4 between the groups. Thus, bone IGFBP5 displays a distinctive developmental pattern, with greater abundance in younger animals. Furthermore, it is regulated in bone by IGF-1 via autocrine-paracrine mechanisms. These data suggest that IGFBP-5 may be involved in the stimulatory effects of IGF-1 in bone. In support of this, we have found that osteoblast-targeted IGF-1 overexpression markedly stimulates bone formation rate at 3 weeks of age but declines thereafter coincident with the reduction in bone IGFBP-5 abundance. Thus, abundant IGFBP5 may be required for the maximal stimulatory effect of IGF-1 on bone, as seen in the younger animals, with the age-related decline of IGFBP5 explaining the diminishing capacity of IGF-1 to stimulate bone formation with maturation.

M184

Paracrine Overexpression of IGFBP-4 in Osteoblasts of Transgenic Mice Results in Global Growth Retardation. M. Zhang,*[1] M. Faugere,[2] H. Malluche,[2] C. J. Rosen,[3] S. V. Chersausek,*[4] T. L. Clemens.[1] [1]Department of Medicine, University of Cincinnati, Cincinnati, OH, USA, [2]Department of Medicine, University of Kentucky, Lexington, KY, USA, [3]Department of Medicine, St. Joseph's Hospital, Bangor, ME, USA, [4]Department of Pediatrics, Children's Hospital, Cincinnati, OH, USA.

Insulin-like growth factor (IGF1) binding protein 4 (IGFBP-4) is abundantly expressed in bone and is generally believed to function as an inhibitor of IGF action. To investigate the function of locally produced IGFBP-4 in bone in vivo, we targeted overexpression of IGFBP-4 to osteoblasts using a human osteocalcin promoter to direct transgene expression. IGFBP-4 protein levels in transgenic (OC-BP4) mice, as measured by western ligand blot, were increased by as much as 25 fold over the endogenous level, whereas serum levels were unchanged. Interestingly, levels of IGFBP-5 were decreased in the OC-BP4 mice, possibly due to a compensatory alteration in IGF1 action. At birth OC-BP4 mice were of normal size and weight but exhibited a striking postnatal growth retardation. The body weights of adult transgenic animals were ~70-75% of control and the crown to rump lengths were ~88% of control. Organ allometry (mg/g body weight) analysis revealed that calvarial and femoral wet weights were disproportionally small (~70% and 80% of control respectively). Most other organs exhibited a proportional reduction in weight with the exception of brain and kidney, which were disproportionally large compared with controls. pQCT measurements showed a decrease in femoral length and total bone volume in transgenic animals compared to the controls. Quantitative histomorphometry at the distal femur disclosed a striking reduction in bone turnover in the OC-BP4 mice. Osteoblast and osteoclast number/bone length, and bone formation rates in OC-BP4 mice were approximately half that seen in control mice. Bone formation rate expressed per osteoblast was also significantly decreased in the transgenic mice compared to controls. In conclusion, paracrine overexpression of IGFBP-4 in the bone microenvironment markedly reduced cancellous bone formation and turnover and severely impaired overall postnatal skeletal and somatic growth. We attribute these effects to the sequestration of IGF1 by IGFBP-4 and consequent impairment of IGF1 action in skeletal tissue.

M185

Calvariae From Transgenic Mice With Osteoblast-Targeted Insulin-like Growth Factor-1 Show Evidence of Increased Bone Resorption and Formation. J. Jiang,[1] G. Gronowicz,[2] F. Ledgard,*[2] S. k. Lee,[1] J. Lorenzo,[1] S. H. Clark,*[3] A. C. Lichtler,[3] B. E. Kream.[1] [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT, USA, [3]Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA.

To explore the local effect of insulin-like growth factor-1 (IGF-1) on bone remodeling, we previously generated transgenic mice expressing Col1a1-driven murine IGF-I cDNA in osteoblasts (pOB3.6FLAG-IGF-1). The initial founder line (98-572) had a marked calvarial phenotype characterized by increased bone width, marrow area and osteoclast number/bone area, suggesting that trangenic IGF-1 enhanced both bone formation and resorption. The goals of the present study were to correlate the level of transgene expression with the calvarial phenotype in three independent founder lines and to assess bone resorption in ex vivo assays. The ratio of transgene mRNA was 10:2:1 in lines 99-324-7 (HIGH), 98-572 (INT), 99-324-9 (LOW). Percent collagen synthesis (PCS) in calvarial cultures was significantly greater in both the HIGH and INT lines than in wild-type (34±1.3 vs 23±0.8% and 29±0.7% vs 23±0.7%, respectively, $p<0.01$). However, PCS of the LOW line was not different than wild-type (31±2.1% vs 27±1.9%). Static histomorphometry showed that 8 week-old HIGH and INT calvariae were significantly wider and had greater marrow area and osteoclast number/bone area than wild-type calvariae; however, LOW and wild-type calvariae were similar. In the INT line, transgene mRNA was strong in calvariae of 16-day embryos, 7-day and 1-month old mice and thereafter gradually decreased; at 5-months, transgene mRNA was barely detectable by Northern blot analysis. However, 7-month old INT calvariae still were significantly wider with greater bone marrow area and osteoclast number/bone area than wild-type. To assess bone resorption, calvariae from 2-4 day-old neonatal mice were labeled in utero with ^{45}Ca and cultured ex vivo. In 5 day cultures, the release of ^{45}Ca was significantly higher in INT calvariae compared to wild-type calvariae (17.5 ± 0.4% vs 14.8 ± 0.3%, $p<0.01$). To assess osteoclastogenesis, bone marrow cells were cultured in the presence of 30 ng/ml of M-CSF and 10 ng/ml of RANKL for 7 days. The formation of TRAP-positive multinucleated cells in INT marrow cultures was nearly 2-fold greater than wild-type, suggesting that transgenic mice had a greater number osteoclast progenitors in bone marrow. In summary, pOBCol3.6-IGF mice show a sustained dose-dependent calvarial phenotype that likely reflects stimulation of bone resorption and formation by osteoblast-targeted IGF-1 expression.

M186

The Regulation of Multiple Insulin-like Growth Factor-I Transcripts by Exogenous IGF-I in Human Primary Bone Cells. R. D. Jackson, J. Sun.* Division of Endocrinology, Diabetes and Metabolism, The Ohio State University, Columbus, OH, USA.

Human insulin-like growth factor-I is a six-exon, single copy gene which is transcribed by adjacent promotors into nascent RNA with different 5' leader sequences (exon 1 and exon 2) resulting in two kinds of mRNAs: class 1 and class 2. Both classes undergo alternative RNA splicing and differential polyadenylation at the 3' end to yield multiple mature transcripts (IGF-I Ea and Eb). These diverse IGF-I mRNA eventually yield the same IGF-I protein. Previous studies have shown that class 1 transcripts in humans are more sensitive to growth hormone(GH) regulation in comparison to class 2 transcripts. It has also been shown that total IGF-I gene expression can be regulated transcriptionally by IGF-I. It is the goal of this study to extend these observations on IGF-I mRNA regulation further by delineating the role of exogenous IGF-I on the expression of the multiple mRNA transcripts in human bone cells. Eleven human primary bone explant cultures (hOB) derived from the distal femur of men and women undergoing elective knee arthroplasty (age 48-84 yrs) were prepared as previously described. The osteoblastic phenotype of hOB was confirmed by the expression of osteocalcin and bone-specific alkaline phosphatase. Recombinant human IGF-I (rhIGF-I) was added to serum-free monolayers at concentrations of 0, 10, 50 or 250 ng/ml for 48 hrs. Competitive reverse transcription polymerase chain reaction was used to determine the relative quantities of the multiple IGF-I transcripts. IGF-I class 1 transcripts were more responsive to exogenous rhIGF-I than class 2 transcripts. IGF-I class 1-Ea mRNA was suppressed by 71.6% at 10 ng/ml ($p = 0.0388$); no further suppression of IGF-I expression was seen at higher doses. IGF-I class 1-Eb was also significantly suppresssed (- 83.9%; $p = 0.0368$). In contrast, there was no significant change in expression of IGF-I class 2-Ea at any dose of rhIGF-I. IGF-I class 2-Eb could not be detected in any samples in this study using this methodology. In summary, exogenous rhIGF-I suppresses expression of IGF-I class 1 transcripts whereas class 2 transcripts are independent of this negative feedback on transcription. Future studies exploring the regulation of IGF-I in relation to local and systemic factors that could influence IGF-I levels should assess both class 1 and class 2 expression to delineate the molecular regulation of IGF-I gene expression.

M187

Expression of Insulin-like Growth Factor System Constituents in Differentiating Osteoprogenitor-containing Rat Vertebral Cell Cultures. D. Jia, J. N. M. Heersche. Faculty of Dentistry, University of Toronto, Toronto, ON, Canada.

Osteoprogenitors (OPs) present in cells isolated from adult rat bone tissue proliferate and differentiate to form discrete bone nodules under appropriate culture conditions. The glucocorticoid dexamethasone (Dex) and insulin-like growth factor-I and -II (IGF-I and -II) stimulate OP proliferation and differentiation in this system. We tested the hypothesis that Dex-stimulated OP proliferation and differentiation is associated with changes in gene expression of the IGF system components by comparing mRNA levels for IGF-I and -II, the type 1 and 2 IGF receptor and 6 IGF binding proteins (IGFBPs) between differentiating (Dex-treated) and non-differentiating (control) cultures using reverse transcription-polymerase chain reaction and Northern blot analysis. Cells were isolated from the outgrowth of vertebral explants of 3-month-old rats and cultured for 20 days in the presence of either Dex or vehicle. Total RNA was extracted at day 8 (end of rapid proliferation in both control and Dex-treated cultures and start of osteoblastic colony formation in Dex-treated cultures), day 14 (increase of osteoblastic colony formation and start of bone matrix accumulation in the presence of Dex) and day 20 (mineralization of bone matrix). IGF-I mRNA levels in Dex-treated cultures were lower than in control at all three time points, with the greatest decrease at day 20. Levels for IGF receptors were also lower in Dex-treated cultures, but with the greatest decrease at day 8. In Dex-treated cultures, mRNA levels for IGFBP-3 were higher than in control at day 8 and 14 but lower at day 20. In contrast, the levels for IGFBP-4 were lower at day 8 and 14 but higher at day 20 when compared with control cultures. Dex-treated cultures also exhibited lower levels for IGFBP-1 but higher levels for IGFBP-2 at all three time points. There was no difference in IGFBP-5 mRNA levels between Dex-treated cultures and control cultures. Signals for IGFBP-6 were undetectable. Our results indicate that proliferation of OPs and formation of osteoblastic colonies at early stage cultures (day 8) may be associated with upregulation of IGFBP-3 (stimulatory binding protein for IGF action) and downregulation of IGFBP-4 (inhibitory binding protein), while formation and maintenance of mature osteoblasts at later stages (day 14 and 20) are associated with downregulation of IGF-I and IGFBP-3 and upregulation of IGFBP-4.

M188

Increased Osteoblast Activity in the C3H/HeJ(C3H) Mice Compared to the C57BL/6J (B6) Mice Is Associated with a Higher Serum IGF-1 Level and a Greater Response in Collagen Production to IGF-1 and TGF-beta 1. M. H. C. Sheng, D. J. Baylink, H. W. Lau, J. E. Wergedal. MDC (151), VAMC, Loma Linda, CA, USA.

PQCT analysis showed that the C3H mice had a 53% higher peak bone mass than the B6 mice at 16 weeks. This difference was in part due to a higher bone formation rate, which was in turn a consequence of 25 to 68% higher mineral apposition rate (MAR) at 6 to 12 weeks in the C3H mice than that in the B6 mice. Based on our earlier finding that serum IGF-1 was 25 to 35 % greater ($P<0.05$) in the C3H than B6 mice between 4 to 8 weeks, at a time when osteoblast activity was increased, we proposed to test the hypothesis that this increase in serum IGF-1 could explain in part the increase in osteoblast activity in the C3H compared to the B6 mice. To test the hypothesis, we determined if the osteoblast activity *in vitro* was responsive to IGF-1 treatment in the C3H and B6 mice. We determined the % of collagen production by measuring the incorporation of [^{3}H] proline into collagenase digestible protein in the presence of 50 mg/ml of ascorbate and 25 µg/ml of b-amino-proprionitrile. Under a basal condition, the C3H mice did not show a significantly higher collagen production compared to the B6 mice. Because of the negative results, we evaluated the response of bone cells from the two mouse strains to the treatment with growth factors that increase collagen production. IGF-1 (100 ng/ml) increased the % of collagen production by 133±7 % in the C3H mice and by 33±5 % in the B6 mice. The difference in responses to IGF-1 between the two mouse strains was statistically significant ($n=4$, $P=0.006$). Similarly, TGF-b1 (100 pg/ml) increased the % of collagen production by 399±59 % in the C3H while increasing it only 103±14 % in the B6 mice and the difference in the response of the two strains to TGF-b1 was also statistically significant ($n=4$, $P=0.009$). In summary, the C3H compared to the B6 mice showed: 1) no difference in basal

levels of osteoblastic collagen production *in vitro*; 2) highly significant increased response in collagen production to TGF-β1 and IGF-1. In conclusion, the combination of a higher serum IGF-1 level and a greater response to IGF-1 and TGF-β1 may in part explain our in vivo observation of higher MAR in the C3H compared to that in the B6 mice.

M189

Soy Protein Relieves Symptoms of Osteoarthritis and Increases Circulating Levels of Insulin-Like Growth Factor-I. B. H. Arjmandi,[1] D. A. Khalil,[1] E. A. Lucas,*[1] S. Juma,[1] M. E. Munson,*[1] A. Svanborg,*[1] A. B. Arquitt,*[1] R. Tivis,*[2] R. A. Wild.*[3] [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Center for Alcohol and Drug Related Studies, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA, [3]Obstetrics and Gynecology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

The effects of soy protein supplementation on pain and limited physical abilities in osteorthritis were examined in a three-month long double-blind clinical study that included 131 men and women with self-reported or diagnosed knee osteoarthritis. The subjects were randomly assigned to receive 40g/day of either soy protein (providing 90 mg isoflavones/d; n = 65) or milk-based protein (n = 66) for three months. Assessment of active flexion, extension, and range of motion (sum of flexion and extension) of both knees as well as assessment of pain were obtained before initiation of treatment and monthly thereafter. Pain and quality of life were assessed using a self-administered questionnaires modified from McGill Pain Questionnaire, SF-36 Health Survey, and the Medical College of Wisconsin Noncancer Pain Questionnaire. Soy protein supplementation and not the control protein resulted in significant ($P<0.05$) improvements in the range of motion of the knees and significant ($P<0.05$) reductions in pain intensity, pain frequency, discomfort caused by pain, severity of pain, hindrance of activities because of pain, and improvements in the ability to climb several flights of stairs, as reported by subjects. Soy protein supplementation improved self-described pain parameters and the effects became more pronounced as the treatment duration progressed. The improved symptoms of osteoarthritis observed in soy-supplemented individuals, in part, may be due to increased circulating concentrations of insulin-like growth factor-I (IGF-I), which was increased by 2.7-fold above those of casein-supplemented individuals. Higher IGF-I levels have been associated with cartilage regeneration. The mechanisms by which soy protein with its isoflavones exerts beneficial effects on osteoarthritis need to be investigated. Supported by grants from Oklahoma Center for the Advancement of Science and Technology and Protein Technologies International

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Microgravity Induces Apoptosis and Increases Interleukin 6 Expression in Osteoblast-like Cells. N. Rucci,*[1] S. Migliaccio,[1] B. Zani,*[1] A. Taranta,*[2] A. Teti.[1] [1]Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy, [2]Department of Histology and Medical Embryology, University "La Sapienza", Rome, Italy.

Mechanical loading is mandatory for physiologic skeleton homeostasis. Weightlessness induces bone loss in humans and animals, up to 19% of weight–bearing bone. To evaluate the effects of microgravity on osteoblasts, we employed the NASA-approved Rotating Wall Vessel bioreactor (RWV) in which a rat osteoblast-like cell line (ROS.SMER#14) was grown for 48 to 72 hr under a microgravity condition of 0.008xg. Control cells were cultured in tissue culture dishes (TC) or in non rotating RWV (N-RWV) at unit gravity. Our results showed that microgravity induced a significant increment in alkaline phosphatase gene expression (+3.4-fold vs N-RWV; +1.7-fold vs TC; n=3; $P<0.05$) and activity (+2.3-fold vs N-RWV; +1.7-fold vs. TC; n=3; $P<0.005$), and an increased expression of the bone-matrix protein osteopontin (+1.5-fold vs N-RWV; +1.6-fold vs TC; n=3; $P<0.05$) and of the bone morphogenetic protein (BMP)-4 (+2.3-fold vs N-RWV; +1.8-fold vs TC; n=3; $P<0.05$). In contrast, no changes in osteonectin, bone sialoprotein II and BMP-2 levels were noticed. A remarkable increase in the expression of protein kinase C alpha (+2.8-fold vs N-RWV; +4-fold vs TC; n=3; $P<0.03$), epsilon (+4-fold vs N-RWV; +3-fold vs TC; n=3; $P<0.05$) and zeta (+4.6-fold vs N-RWV; +4.4-fold vs TC; n=3; $P<0.05$) was also observed under microgravity conditions. These results are consistent with a marked osteoblast phenotype. However, in osteoblasts cultured in RWV we observed a reduction of cell proliferation together with an increased apoptosis as demonstrated by bis-benzimide staining of DNA, and by oligonucleosome-size DNA ladder. Nevertheless, the p53 and bcl-2/bax pathways were not altered. As bone loss could also be attributed to enhanced osteoclast function, we evaluated whether microgravity induced transcriptional regulation of osteoblast-derived, osteoclast-stimulating factors. Semiquantitative RT-PCR analysis showed a transcriptional increment of the potent osteoclast stimulating cytokine, interleukin-6 (IL-6) (+1.9-fold vs N-RWV; +4-fold vs TC; n=3; $P<0.05$) but no changes in IL-1beta mRNA. IL-6 was able to enhance osteoclast formation by 2 fold, and bone resorption by 3.5 fold in murine bone marrow cultures. On the basis of these results we conclude that, under microgravity, reduced osteoblast-life span and enhanced IL-6 expression may result in inefficient osteoblast- and increased osteoclast-activity, respectively, thus contributing to bone loss in individuals subjected to weightlessness.

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A Role for Annexin V in Bone Cell Mechanotransduction. T. L. Haut Donahue,*[1] H. J. Donahue,[1] C. R. Jacobs,[2] C. E. Yellowley.[1] [1]Orthopaedics, Musculoskeletal Research Laboratory, Penn State College of Medicine, Hershey, PA, USA, [2]Mechanical Engineering, Stanford University, Stanford, CA, USA.

$Ca^{2+}{}_i$ is a second messenger that has been implicated in the mechanism by which physical signals exert biological effects on bone cells. How $Ca^{2+}{}_i$ signals are generated in response to physical signals is unclear. The aim of this study was to examine the role of Annexin V (Anx V), a Ca^{2+} dependent phospholipid binding protein, in the Ca^{2+} response to oscillating fluid flow (OFF) in human osteoblastic MG 63 cells. Anx V has a number of attributes that suggest it is ideally suited for a role as a mechanoreceptor, including its ability to function as a Ca^{2+} selective ion channel and to interact with both extracellular matrix and cytoskeletal elements. In addition, we studied the cellular relocation of Anx V in response to changes in the level of $Ca^{2+}{}_i$. $Ca^{2+}{}_i$ imaging: MG 63 cells cultured in monolayer were loaded with FURA-2AM and placed on a parallel plate flow chamber. Following a 1 minute no flow period, cells were exposed to OFF at 1 Hz and a peak shear stress of 20 dynes/cm^2 for 3 minutes. Prior to OFF, cells were exposed to anti-Anx V antibody (40 ug/ml for 24 hours) to disrupt Anx V activity, anti-cFOS antibody (40 ug/ml for 24 hours) as a control, or standard media for 24 hours. $Ca^{2+}{}_i$ transients of 80 nM or greater were considered responses. Anx V relocation: MG63 cells were exposed to the Ca^{2+} ionophore, ionomycin (10 mM) or standard media (control) for 20 minutes, after which plasma membrane, cytosol, nuclear extract and nuclear membrane fractions were isolated. Expression of Anx V in each fraction was assessed by Western blot. Densitometry was used to quantitate differences in Anx V expression between control and ionomycin stimulated cell fractions. The percent of cells responding to fluid flow with an increase in $Ca^{2+}{}_i$ was significantly attenuated in cells exposed to anti-Anx V (27.3 ±11.3 %) compared to either control (75.5 ± 3.9 %, $p=0.002$) or anti-cFOS (63.6 ± 12.8 %, $p=0.024$) treated cells. There was a 12.1% increase in Anx V expression in the plasma membrane and a 15.4 % decrease in the cytosol for cells stimulated with ionomycin compared to controls. Interestingly, both the nuclear membrane and the nuclear extract showed increases of 25.1% and 39.6% in Anx V expression for cells stimulated with ionomycin compared to controls, respectively. These data suggest that Anx V may be involved in the mechanism by which mechanical signals are detected by bone cells, and that $Ca^{2+}{}_i$ levels may determine the cellular location of Anx V. Future studies will examine whether Anx V is functioning as a Ca^{2+} selective channel in bone cells and whether its activity is influenced by cellular location.

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Regulation of Cx43 Phosphorylation and Gap Junction Communication in Osteocytic MLOY-4 Cells by Oscillating Fluid Flow. A. I. Alford,*[1] C. R. Jacobs,[2] H. J. Donahue.[1] [1]Department of Orthopaedics and Rehabilitation, Pennsylvania State University College of Medicine, Hershey, PA, USA, [2]Biomechanical Engineering Division, Stanford University, Stanford, CA, USA.

Osteocytes are hypothesized to sense mechanical deformation of bone in the form of oscillating fluid flow within the canalicular network. The presence of gap junctions between osteocytes and osteoblasts suggests that biochemical signals elicited by shear stress may be conveyed from osteocytes to osteoblasts via intercellular communication. The purpose of the present work was to investigate the effects of oscillating fluid flow on the gap junction protein connexin (Cx) 43 and on gap junction intercellular communication (GJIC) in the osteocytic cell line MLOY-4 (Kato et al. 1997). Cells were exposed to oscillating shear stress at ± 10 dynes/cm^2 using a parallel plate fluid flow apparatus. Control cells were incubated in the parallel plate chamber without being exposed to shear stress. In order to ensure adequate availability of nutrients during the fluid flow interval, all groups were exposed to a low magnitude (0.1 dynes/cm^2) steady flow. Immediately following exposure of MLOY-4 cells to oscillating fluid flow, phosphoserine content of Cx43 was analyzed by immunoprecipitation with a polyclonal anti- Cx43 antibody followed by Western blot analysis using a monoclonal anti-phosphoserine antibody. Phosphoserine content was normalized to Cx43 protein abundance determined on the same blots. In order to study the effect of oscillating shear stress on GJIC, a standard dye transfer assay was employed. Compared to control cells, serine phosphorylation of Cx43 increased approximately two fold during one hour of oscillating fluid flow. Conversely, Cx43 protein abundance did not change in response to shear stress. Functional analysis of GJIC indicated that in contrast to control cells, MLOY-4 cells exposed to oscillating fluid flow established more gap junctions with dye labeled cells. These results indicate that oscillating fluid flow regulates the ability of MLYO-4 cells to establish new intercellular contacts and gap junctions possibly via increased phosphorylation of Cx43 on serine residues.

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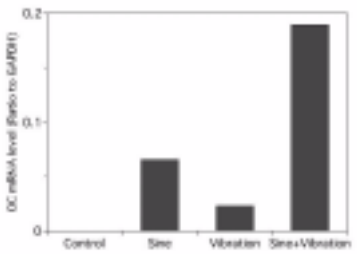
Mechanical Stimulation with Broad Frequency Vibration Promotes Differentiation of Osteoblasts in 3D-Culture. S. Tanaka,*[1] J. Li,*[2] R. L. Duncan,[1] D. B. Burr,[2] C. H. Turner.[1] [1]Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA.

Broad frequency vibration enhances the tactile sensation in the fingertips. Here, we postulate that vibration also enhances mechanosensitivity of osteoblasts. MC3T3-E1 cells were seeded in rat tail type I collagen gels (1 mg/ml) with α-MEM with 10% FBS and 1% antibiotics at a density of 1 x 10^6 cells/ml and were placed into a mechanical stimulator. The gels were anchored by porous polyethylene plates and strained by piezoelectric actuators for 7 days (3 min/day). Gels were grouped as follows: Group 1) no stimulation (control); Group 2) haversine wave at 3 Hz (3000 μstrain p-p); Group 3) broad frequency

vibration (gaussian distribution up to 50 Hz) with mean of 300 µstrain; and Group 4) sine wave plus vibration. After 8 days in culture, the collagen gels were dissolved using 0.34% collagenase solution and cells were collected to measure cell number, ALP activity and mRNA expression levels. Cell counting using a hemacytometer demonstrated that mechanical stimulation reduced the number of cells, compared to control. In particular, cell number was reduced significantly in Group 4 (sine plus vibration) ($p<0.05$). There were no significant differences among groups for ALP activities per protein level. Semi-quantitative RT-PCR was performed to measure mRNA levels for osteocalcin (OC), type I collagen, osteopontin (OPN) and connexin 43 (Cx43). No significant changes from control were observed after mechanical stimulation for mRNA levels of type I collagen, OPN or Cx43. However, OC mRNA was up-regulated after mechanical stimulation and OC expression was greatest in the sine plus vibration stimulation group. These results suggest that sine wave or vibration by themselves do not significantly change proliferation and differentiation of 3D-cultured MT3T3-E1 cells. However, sine wave combined with vibration suppresses proliferation and promotes OC expression. These data suggest that osteoblasts can be induced to differentiate in response to sine wave plus vibration.



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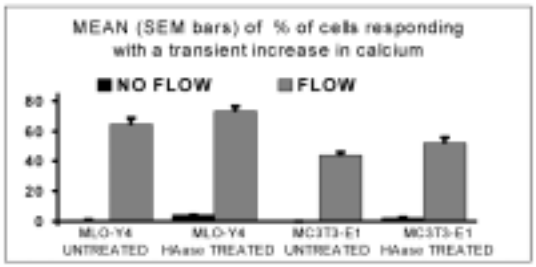
Manipulation of the Glycocalyx of an Osteocytic and an Osteoblastic Cell Line Does Not Affect the Intracellular Calcium Increase in Response to Fluid Flow. G. C. Reilly,[1] T. R. Haut,*[2] C. E. Yellowley,[2] H. J. Donahue,[2] C. R. Jacobs.[3] [1]Biochemistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA, [2]Orthopaedics and Rehabilitation, Penn State College of Medicine, Hershey, PA, USA, [3]Biomechanical Engineering Division, Stanford University, Stanford, CA, USA.

We have previously shown that the osteocytic cell line, MLO-Y4 and the osteoblastic cell line MC3T3-E1 have a hyaluronic acid (HA) rich glycocalyx (cell coat). It has been hypothesized that in vivo the osteocyte glycocalyx will contribute to the detection of mechanical loading via fluid flow, with drag forces on the long proteoglycan chains increasing strains at the cell membrane (You et al. BED-43, ASME 1999). We examined whether manipulation of the glycocalyx would affect the transient intracellular calcium concentration $[Ca^{2+}]_i$ increase that occurs in these cells in response to an oscillatory fluid flow stimulus. MLO-Y4 cells, a gift from Dr. L.F. Bonewald and MC3T3-E1 cells were grown on collagen coated and uncoated quartz slides, respectively, for 2 days, to 80% confluence. Prior to flow experiments cells were pretreated for 1hr with either 160U/ml of hyaluronidase (HAase) or vehicle. Staining with fluorescently labeled HA binding protein confirmed that the treatment removed HA from the glycocalyx. Cells were subjected to oscillating flow at a maximum shear stress of 1Pa for MLO-Y4 cells and 2Pa for MC3T3-E1 cells (Jacobs et al. J. Biomech. 31, 1998). The flow chamber was mounted on a fluorescent microscope and $[Ca^{2+}]_i$, was recorded using the indicator dye Fura-2AM. 20-60 cells per slide were imaged (7 slides for each treatment for MLO-Y4 and 4 for MC3T3-E1), over a 3 minute pre-flow and 3 minute flow period. A $[Ca^{2+}]_i$ increase over 2 x the mean pre-flow levels was considered to be a response. There was no difference in the percentage of cells responding with an $[Ca^{2+}]_i$ increase between HAase treated and untreated cells, in either bone cell line, or in the magnitude of the intracellular calcium increase. The level of disruption caused to the glycocalyx by this treatment does not affect the intracellular calcium response to fluid flow. As of yet it is unclear whether this is because the HAase treatment does not digest the glycocalyx to a sufficient extent, or because the calcium response of bone cells to fluid flow does not involve mechanotransduction via the glycocalyx.



Disclosures: NIH grants AR45985 and AG13087,2; US Army Medical Research and Materiel Command DAMD17-98-1-8509,2.

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Long Term Perfusion Loading of Trabecular Bone Cores and Formation Rate. E. L. Smith,[1] U. Boudriot,*[2] B. Daume,*[3] M. Kratz,*[3] D. B. Jones,[3] D. M. Cullen.[4] [1]Preventive Medecine, University of Wisconsin, Madison, WI, USA, [2]Surgery, Klinik für Kinderheilkunde, Marburg, Germany, [3]Exp. Othopädie & Biomechanik, Phillips- Universität, Marburg, Germany, [4]Medicine, Creighton University, Omaha, NE, USA.

The purpose of this study was to document bone formation in trabecular bone cores from a human femoral head with and without mechanical loading in a Perfusion Loading System (PLS). The PLS had a computer controlled high-voltage piezoelectric crystal stack able to precisely load bone specimens while measuring force and compression simultaneously. Sixteen trabecular bone cores (5 mm thick and 10 mm in diameter) were obtained from the femoral head of a 68-year-old male patient who had hip replacement for coaxarthrosis. Bone cores were perfused using an Ismatec pump at 3.2-3.4 µl/hr in Dulbecco Modified Eagle Medium (DMEM-high glucose) containing: 10% fetal calf serum (FCS), 72.2mg/l Ca and 28.5mg/l P, 2 mM glutamine, 5 mM ß-glycerophosphate, streptomycin and penicillin G at 50,000 U/l each, vitamin C 10 µg/ml, 0.12g/l of NaHCO3 and 10 mM Hepes. The bone cores were maintained at 37 o C, in a controlled temperature room at a pH of 7.2-7.3 throughout the study period. The medium was changed at 48-hour intervals. Bone cores were randomly assigned to three groups: control non-loaded (N=5), loaded at 750 micro-strain (N=5) and at 1500 micro-strain (N=6). Loads were applied in the PLS for 300 cycles/day at1 Hz for 49 days. Bone cores were labeled on days 19, and 34 with Calcein for two hours, and on day 44 with Alizarine Red for 4 hours. Histomorphometric endpoints included bone area, mineralizing surface (MS/BS), and mineral apposition rate (MAR).There were no differences among groups detected by ANOVA, therefore results are reported as mean (SD) for all groups. The relative bone volume of the cores averaged 26.6 (9.8)% of total volume. Alizarin Red label on day 44 was extensive with 79 (23)% labeled surface. Double labeled surface from day 34 and 44 averaged 15 (14)%. During this period the MAR averaged 0.60 (0.31) µm/day. Only two sections had double calcein labels (day 19 + 34). Stained sections show abundant osteoid, osteoblasts, and scattered osteoclasts indicating continued bone turnover. We conclude that viable bone cores can be maintained through 49 days in the PLS system. In addition, these ex vivo bone cores demonstrate mineral apposition rates similar to those from iliac crest biopsies labeled in vivo. These studies also demonstrate that 750 and 1500 micro-strain are not sufficient to alter bone formation in the PLS system. Future studies will examine different loading strains and patterns.

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Increase in EGF Receptor Protein Induced in Osteoblast-like Cells After Flow of Culture Media. T. Ogata. Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

To investigate how bone cells respond to mechanical loading, we subjected osteoblast-like cells to fluid flow, which is a candidate for mediators transmitting mechanical loading to bone cells. We had previously found that in osteoblast-like cells, flow of culture media generated by shaking culture dishes induced increase in egr-1 mRNA levels and tyrosine-phosphorylation of many proteins containing ERK1/2 and Shc and that the induction did not occur in non-serum media but was recovered by the addition of EGF. As the role of EGF in this response, two possibilities were considered. The first is that EGF receptor is activated directly by the flow. The second is that the signal induced by the flow stimulates the signaling pathway from EGF. Therefore, we examined whether tyrosine-phosphorylation of EGFR is enhanced by the flow, but we could not obtain clear results. However, we found that the amount of EGFR protein increased within 2 minutes after shaking culture dishes for 1 minute, peaked at about 10 minutes and returned to the basal level at 60 minutes, using Western blotting of whole cell lysate. This short time from stimulation to response suggests that the increase in EGFR protein may be caused by decreased proteolysis. Pretreatment with AG1478 (EGFR kinase activity inhibitor) or MG-132 (proteasome inhibitor), which was performed to find a clue to the mechanism, did not block the increase in EGFR. Recently EGFR was reported to be maintained in an activated form bound by EGF in cytoplasm, as well as activation of EGFR on cell membrane. Although the mechanism of EGFR increase remains to be clarified, this result suggests that proteolysis may be involved in signal transduction of mechanical strain.

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Bone Marrow Derived Osteogenic Cells, but not Mature Osteoblasts/ Osteocytes, are the Target Cells for the Anabolic Response to Therapeutic Low-Intensity, Pulsed Ultrasound. Y. Mikuni-Takagaki,[1] K. Naruse,*[2] A. Miyauchi,[3] Y. Oonuki,*[2] T. Izumi,[2] M. Itoman.*[2] [1]Oral Biochemistry, Kanagawa Dental College, Yokosuka, Japan, [2]Orthopedic Surgery, Kitasato University, Sagamihara, Japan, [3]Internal Medicine, National Hyogo-Chuo Hospital, Sanda, Japan.

Cells in bone are equipped with machineries to sense diverse physical forces and transduce signals so that they respond to loading and adjust themselves to their environment. Studying osteocytes' stretch-sensing mechanisms suggested that certain types of mechanical stress are received only by certain stages of osteogenic cells. In search of mechanotransduction pathways unique to osteoblasts and of crosstalk among different pathways, we studied anabolic responses of osteogenic cells to low-intensity, pulsed ultrasound, a non-invasive therapeutic treatment for fracture repair and distraction osteogenesis. The effects of 20-min exposure to a 200-µs burst of pressure pulses repeated every msec(sine wave of 1.5 MHz repeated at a 1.0kHz frequency), were examined in mouse bone marrow-derived ST2 cells, and primary rat bone- and bone marrow-derived cells. The intensity was 30 mW/cm^2, same as that of clinical devices (Exogen Inc.). By using conventional and semi-quantitative RT-PCR analyses, ST2 cells cultured with ascorbate and exposed to the ultrasound showed that steady state levels of immediate early genes such as c-fos or cox 2 were greatly upregulated in a less differentiated population of ST2 cells. The appearance of ALP and osteocalcin, osteoblast differentiation markers, was accompanied by some loss of responsiveness. IGF-I, osteocalcin as well as c-fos and cox 2 messages were upregulated to some extent in the more differentiated population. The result was similar in rat bone marrow derived osteogenic cells. On the other hand, mature osteoblasts and osteocytes, derived from new born rat tibia and fully expressing osteocalcin, were almost insensitive to the pulsed ultrasound. Compared to the stretched osteocytes, which exhibited stretch-activated and PTH-potentiated Ca influxes, none of these cells showed evidence of Ca internalization upon exposure to the ultrasound. Moreover, inhibitors of MAPK and upstream kinases blocked cox 2 upregulation by the exposure, also distinct from the response to stretching. Analysis of differential expression levels demonstrated the involvement of transcription factors such as Egr-1, potential target of MAPKs. Our findings suggest that accel-

erated fracture repair and distraction osteogenesis by the low-intensity, pulsed ultrasound depend on the stimulation of cells at relatively early stages of osteogenic lineage that, in turn, influences more mature cells in a paracrine manner.

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A Novel In Vivo Perfusion Chamber for the Study of Bone Cell Signaling in Response to Mechanical Stimulation. M. R. Moalli, S. Wang,* N. J. Caldwell,* S. A. Goldstein. Orthopaedic Surgery, University of Michigan, Ann Arbor, MI, USA.

The effects of mechanical stimulation on the skeleton are highly complex and difficult to replicate by in vitro loading experiments. Furthermore, the particular intracellular signaling pathways that bone cells utilize to transduce mechanical signals remain poorly characterized in an in vivo context. We developed a perfusion chamber in which an intact bone extracellular matrix is treated with biochemicals prior to the application of a controlled mechanical load in vivo. The purpose of this study was to utilize the model to test the inhibition of load-induced tyrosine phosphorylation of two candidate signaling molecules, focal adhesion kinase (FAK) and src, following perfusion with U0126 and herbimycin, respectively.

Bone chambers equipped with perfusion caps were surgically implanted into the proximal tibia of adult dogs. The perfusion cap has a swedged-on 22 gauge needle, which is centrally positioned and extends to the bottom of the chamber. A cylindrical core of trabecular bone grows within the chamber and surrounds the needle. The hub of the needle is occluded and radial fenestrations drilled along its length allow for uniform delivery of the perfusate. An access port is buried within the subcutaneous tissue of the medial thigh and is connected to the perfusion cap by 5-6 cm of polyurethane tubing. Thus the direction of flow of the injected inhibitor is from the access port reservoir, through the tubing, into the cap and out the fenestrated needle to the bone tissue.

In separate experiments, either U0126 (20 μM) or herbimycin (1 μg/ml) was perfused into one of the tibial chambers, while the contralateral chambers were perfused with DMSO/PBS vehicle, 24 hours and 2 hours prior to loading. In some dogs, a mechanical load stimulus (17.8 N@ 89N/s, for 1800 cycles, @1 Hz) was simultaneously applied to both chambers. In other dogs, the inhibitor-infused chamber was loaded, while the contralateral chamber which received vehicle, served as the unloaded control. Specimens were harvested immediately after loading and prepared for immunoblot analysis using MAPK or src phosphospecific antibodies. As expected, there was abundant MAPK activation in response to mechanical stimulation with perfusion of vehicle alone. Perfusion of U0126 prior to loading significantly decreased MAPK activation to well below basal, unloaded control levels. Src kinase activity was inhibited in a similar manner.

We conclude that the in vivo perfusion chamber can be utilized to evaluate specific components of signaling pathways that occur in response to mechanical loading. In addition, it may provide a valuable way to study bone cell activity following local delivery of novel compounds.

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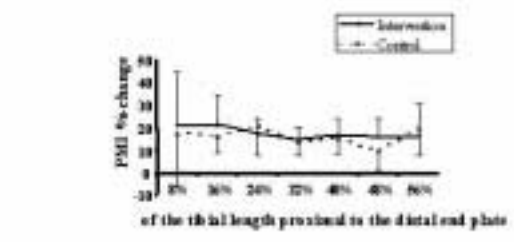
Relationship Between Prostaglandins and Cyclooxygenase-2 in Response to Mechanical Stimuli and Their Role in Load-induced Bone Formation. J. Li,*[1] S. M. Norvell,*[2] S. M. Ponik,*[2] F. M. Pavalko,[2] D. B. Burr,[1] C. H. Turner.[1] [1]Orthopedic Surgery, Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN, USA.

Exposure of bone tissue to mechanical loading increases extracellular prostaglandins and cyclooxygenase-2 (COX-2) protein expression, both of which mediate adaptive bone formation. However, cellular mechanisms related to the production of prostaglandins and COX-2 induced by mechanical loading are unknown. We tested the effect of the COX-2 inhibitor NS398 in cell culture and in vivo using the rat tibia loading model. Adult rats were divided into four groups: one vehicle group and 3 groups treated with NS398 (10 mg/kg, i.p.), given at 3 hr or 20 min before loading, or 20 min after loading. Loading at 360 loading cycles (2 Hz, 54 N peak force) significantly increased bone formation rate on the endocortical surface of the tibia ($p<0.001$). Treatment with NS398 3 hrs before loading suppressed load-induced bone formation by 72% ($p<0.0001$). Treatment with NS398 20 min before loading decreased bone formation rate by only 39% ($p<0.05$) even though the serum levels of NS398 in this group had peaked at the time of loading. Thus, we concluded that new prostaglandin synthesis was not the primary mechanism mediating load-induced bone formation. When NS398 was given 20 min after loading bone formation was suppressed by 29%, suggesting that PG synthesis occurring several minutes or a few hours after loading does contribute to bone formation. These studies suggest that a primary cellular mechanism of bone formation following brief bouts of mechanical loading involves secretion of intracellular prostaglandins from an existing pool, rather than new prostaglandin synthesis associated with increased COX-2 protein levels or increased COX-2 enzymatic activity. To investigate the cellular mechanisms involved, MC3T3-E1 osteoblasts were treated with NS398 for 30 minutes prior to measuring intracellular levels of PGE2 and PGI2. NS398 reduced intracellular PGE2 and PGI2 levels by 80% compared to untreated controls and suppressed release of prostaglandins after a mechanical stimulus. Thus, when inhibitors of prostaglandin synthesis are given prior to loading in vivo, intracellular stores of prostaglandins may be decreased and prostaglandin secretion may be suppressed.

M200

High-Impact Exercise and Tibial Polar Moment of Inertia in Pre- and Early Pubertal Girls: A Quantitative MRI Study. A. O. Heinonen,*[1] H. A. McKay,[1] K. J. MacKelvie,*[1] K. P. Whittall,*[2] B. B. Forster,*[2] K. M. Khan.[1] [1]School of Human Kinetics, UBC, Vancouver, BC, Canada, [2]Department of Radiology, UBC, Vancouver, BC, Canada.

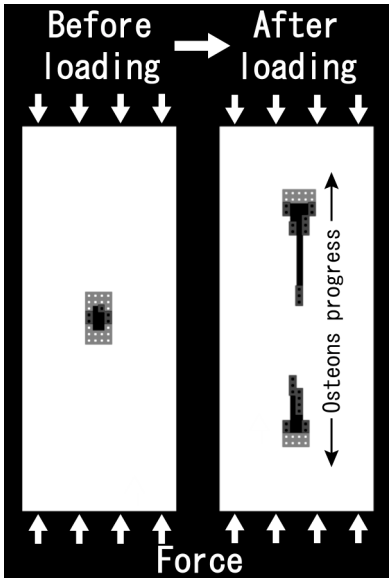
Bone response to mechanical loading may vary along the length of a loaded long bone. Therefore, we studied the effect of a seven-month, 3x/week, progressive high-impact (weight-bearing) circuit program on tibial polar moment of inertia (PMI) in 9-11 yr old girls (n = 8 exercise, n = 8 non-training controls). We used a 1.5T MR system (GE Medical Systems) with a quadrature head coil to measure PMI at slices located proximal to the distal tibia end plate by 8%, 16%, 24%, 32%, 40%, 48% and 56% of the total tibial length. The sequence was T1-weighted, spin echo in the aforementioned seven transverse (tibial) planes, 3.0 mm sections. There were no differences in calcium, or physical activity between the intervention and the control groups at baseline, but controls were taller and heavier. These parameters changed similarly for both groups over 8 months. The lower limb explosive performance capacity (standing long jump, cm) changed 6% and 4%, in the intervention and control groups, respectively, but the intergroup difference was not significant. Weight change-adjusted PMI changes with 95% CIs in the seven tibial segments (mean of the left and right) are shown in the Figure. There was a small trend in favor of the intervention group at the distal tibia (site of greatest impact) and mid tibia (site of maximal bending). In summary, this novel imaging modality does not reveal PMI changes along the tibia following a 7-month exercise intervention in 9-11 yr old girls. However, further studies are needed in larger numbers of subjects, before drawing conclusions about the utility of this novel, non-irradiating technique to measure changes in bone strength in children.



M201

Mechanical Stress Induces Cortical Osteon and Trabecular Osteon in a Computer Simulation Program. **Y.** Tamura,*[1] S. Fukomoto,[2] Y. Takeuchi,[1] T. Fujita.*[1] [1]Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo, Japan, [2]Department of Laboratory Medicine, University of Tokyo School of Medicine, Tokyo, Japan.

"Mechanostat Theory" (Frost HM, 1986) is a well-known hypothesis that explains the mechanism of bone remodeling responding to local mechanical stress. Recently some researchers succeeded in constructing trabecular bones by computer simulation based on Mechanostat theory (e.g. Huiskes R, Nature, 2000). These results suggest that structure of trabecular bone is actually determined by mechanical stress. However, these methods have not been applied to other microstructure of bones. Therefore, we performed computer simulation to examine whether mechanical stress can create cortical osteons (haversian system) and trabecular osteons (packet, hemi-osteon).At first, we developed a computer simulation program using FEA (finite element analysis). When mechanical stress is applied to a virtual 'bone' in this program, it calculates local stress in all small regions of the bone by FEA. Then, according to this local stress, virtual 'osteoclasts' and 'osteoblasts' are activeted. The rule of the activation is based on Mechanostat theory. Second, we performed simulations of cortical and trabecular osteons by this program. In the simulation of a cortical osteon (Figure), we supposed that there was a hole in a 'bone cortex' at first as shown in the left part of the figure. When we compressed the 'bone', the hole divided into two parts, progressed to the direction of the force, and formed a structure similar to a cortical osteon. In the simulation of a trabecular osteon, it was supposed that there was a hollow on the surface of a column of trabecula. When we compressed the column, the hollow moved along the surface and became a structure similar to a trabecular osteon. In both simulations, resorption preceded formation and activities of 'osteoclasts' and 'osteoblasts' mimicked BMU (basic multicellular unit).From these results, we conclude that cortical and trabecular osteons may be formed by mechanical stress. Considering the results by other researchers, mechanical stress seems to be an essential factor that determines both micro- and macro-structure of bone through regulating activities of osteoclasts and osteoblasts.



M202

A Three-Year Longitudinal Study of the Effect of Physical Activity on the Accrual of Bone Mineral Density in Healthy Adolescent Males. A. Gustavsson,* K. Thorsen, P. Nordström. Sports Medicine Unit, Department of Surgical and Perioperative Sciences, Umeå, Sweden.

The purpose of the present study was to examine the effect of physical activity on peak bone mass in male badminton players (n=12), ice hockey players (n=24) and controls (n=24) at 16 and 19 years of age. The groups were matched according to age, pubertal stage, and height. The bone mineral density (BMD, g/cm2) of the total body, lumbar spine (L2-L4), dominant and non-dominant humerus, head and femoral neck was measured twice with a 3-year interval by dual energy X-ray absorptiometry (DEXA). In addition, at the femoral neck, volumetric bone mineral density (vBMD, mg/cm3) was estimated. At baseline, the badminton players were found to have significantly higher total body (p= 0.008), dominant humerus (p=0.001) and femoral neck BMD (p= 0.003) compared to the controls after adjusting for weight. These findings were consistent at the three-year follow up where the vBMD of the femoral neck were significantly higher as well (p=0.04). The ice hockey players exhibited a higher BMD at the non-dominant humerus compared to the controls at baseline (p= 0.003) and at the three year follow up, they also had a significantly higher BMD of the total body (p= 0.006). To be an athlete was found to be independently associated with a higher increase in femoral neck BMD (beta=0.26) and vBMD (beta=0.32) compared to the controls during the three year study period. Furthermore, estimated femoral neck vBMD did only increase significantly in the athletes during the study period. In summary, the novel finding of the present study is that physical activity seems to be necessary to increase BMD of the femoral neck after puberty.

M203

24 Hours of Immobilization Increases Bone Resorption as Shown by Bone Resorption Markers. N. Kamps,* C. Mika,* A. Boese,* M. Heer. Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany.

Long-term bedrest and space mission studies have shown that immobilization as well as microgravity induce increased bone resorption while bone formation tends to decrease. In order to analyze the kinetics of short-term changes in bone turnover we studied in a randomized, strictly controlled crossover design the effects of 6 days 6° head-down tilt bedrest (HDT) in 8 male healthy subjects (mean body weight (BW): 70.1 ± 1.88 kg; mean age: 25.5 ± 1.04 years) in our metabolic ward. Two days before arriving in the metabolic ward the subjects started with a diet consisting of an energy content of 10 MJ/d, 2000 mg Calcium/d, 400 i.U. Vitamin D, 200 mEq Na+ and 50 ml water/kg BW/d. The diet was continued in the metabolic ward. The metabolic ward period (11days) was divided into 3 parts: 4 ambulatory days, 6 days either HDT or control and 1 recovery day. Continuous urine collection started on the first day in the metabolic ward to analyze calcium excretion and bone resorption markers, namely C-telopeptide (CTX) and N-telopeptide (NTX). On the 2nd ambulatory day in the metabolic ward and on the 5th day in HDT or control blood was drawn to analyze serum calcium, parathyroid hormone, and bone formation markers (bone Alkaline Phosphatase (bAP), Procollagen-I-Propeptide (P-I-CP). Both study phases were identical with respect to environmental conditions, study protocol and diet. Urinary calcium excretion was as early as the first day in immobilization increased ($p<0.01$). CTX- and NTX-excretion stayed unchanged the first 24 hours in HDT compared to the control. But, already on the 2nd day of immobilization both bone resorption markers significantly increased. NTX-excretion was increased by 28.7 ± 14.0% ($p<0.05$), while CTX-excretion rose by 17.8 ± 8.3% ($p<0.01$). Both, the CTX-excretion as well as the calcium excretion keep the significantly higher level during the HDT period, and even continued through the first day of recovery. However, NTX excretion, descended from day three until the end of HDT. But, the level of NTX excretion during HDT was always higher than during control. In contrast to the bone resorption markers, the formation marker P-I-CP tended to increase as early as the fifth day of immobilization ($p<0.10$). Serum calcium-, parathyroid hormone-, as well as bAP concentrations were unchanged. We conclude from these results of a pronounced rise of bone resorption markers that already 24 hours of immobilization induce a significant rise in osteoclasts activity in healthy subjects. Therefore, short-term confinement in bed in older people, who often suffer from osteoporosis, may exacerbate their fracture risk. Further studies are mandatory to investigate the underlying mechanisms and respective countermeasures.

M204

Bone Metabolism in Obese Rats : Influence of Physical Activity. J. Mathey,*[1] M. Horcajada-Molteni,*[2] B. Chanteranne,*[1] C. Picherit,*[1] P. Lebecque,*[1] M. Davicco,*[1] V. Coxam,[1] D. Courteix,[2] J. Barlet.*[1] [1]INRA, Theix, France, [2]UFR STAPS, Orléans, France.

Obese Zucker fa/fa rats are characterized by obesity associated with non insulin dependent diabetes. This condition results from hyperphagy due to leptin resistance following inactivating mutation in the leptin receptor. The aim of this work was to compare bone mass in obese fa/fa rats and their +/+ controls to observe influence of endurance running.One hundred and twenty 2.5 month-old rats, 60 male [30 Zucker (MZ), 30 controls (MC)] and 60 females [30 Zucker (FZ) and 30 controls (FC)] were used. On day 90, in each group, ten animals were killed and used as initial controls. Among the 20 others, ten (10 RMZ, 10 RMC, 10 RFZ, 10 RFC) were trained for treadmill running (60% VO2max, 1h/day, 6 days/week for 67 days). Other males (10 SMZ, 10 SMC) and females (10 SFZ, 10 SFC) were sedentary controls. Glycosuria demonstrated that all Zucker rats were diabetic on day 130. Body composition was determined by dual energy X-Ray absorptiometry (DEXA) on day 175. Rats were killed on day 180. The heart was weighed. The right femur was used to measure femoral failure strength (3-point bending test). Total (T-BMD), diaphyseal (D-BMD ; cortical bone) and distal metaphyseal (M-BMD ; trabecular bone) left femoral density was assessed by DEXA.In obese or control male or female rats, running increased heart weight (g) (RMZ 1.49 ± 0.04 vs SMZ 1.36 ± 0.09 ; $p<0.05$; RMC 1.46 ± 0.07 vs SMC 1.28 ± 0.07 ; $p<0.05$). Physical activity decreased fat mass (% from total body weight) (RFZ 58.3 ± 0.9 vs SFZ 64.6 ± 0.6 ; $p<0.05$; RFC 22.7 ± 0.9 vs SFC 24.9 ± 0.6 ; $p<0.05$), while lean mass was higher (% from total body weight) (RMZ 41.3 ± 0.4 vs SMZ 35.8 ± 1.3 ; $p<0.05$ RFC 74.3 ± 0.9 vs SFC 24.9 ± 0.6 ; $p<0.01$). On day 90, no significant difference concerning BMDs was observed. On day 180, T-BMD was lower in male (SMZ 0.2099 ± 0.0006) or female Zuckers (SFZ 0.2045 ± 0.0013) than in controls (SMC 0.2392 ± 0.0013 ; $p<0.05$ vs SMZ ; SFC 0.2233 ± 0.0010 ; $p<0.05$ vs SFZ). Endurance running increased T-BMD in obese male (RMZ 0.2189 ± 0.0010 ; $p<0.05$ vs SMZ) or female (RFZ 0.2261 ± 0.0012 ; $p<0.05$ vs SFZ) rats. Differences concerning M-BMDs, D-BMDs and femoral failure strength paralleled those observed for T-BMDs. In both Zucker and controls male and female rats, treadmill running significantly decreased urinary deoxypyridinoline excretion, a marker for bone resorption, without any significant effect on plasma osteocalcin concentration, a marker for osteoblastic activity.In conclusion, our results demonstrate that bone mass is lower in diabetic obese Zucker rats than in homozygotous controls. In obese as in control rats, treadmill running increased bone mass, mainly by inhibiting bone resorption.

M205

Bone Mineral Density Increases With 16 Weeks of Training in Collegiate Swimmers. H. L. Petersen,*[1] C. T. Peterson,*[2] A. St. Germain,*[1] K. B. Hanson,*[1] R. L. Sharp,*[3] D. L. Alekel.[1] [1]Food Science & Human Nutrition, Iowa State University, Ames, IA, USA, [2]Statistics, Iowa State University, Ames, IA, USA, [3]Health & Human Performance, Iowa State University, Ames, IA, USA.

Several reports indicate that swimmers have no greater bone mineral density (BMD) than controls or runners, but have lower BMD than gymnasts, body builders, or volleyball players. Although one study indicates a slight BMD loss at the lumbar spine and femoral neck in 20 year-old female swimmers over 12 months, no data are available on changes from preseason to postseason. This study documented changes (paired t-test) in whole body, lumbar spine, and femoral BMD (g/cm2, ±SD) using Hologic QDR 2000 in eumenorrheic female (19.5 [18.0-21.9] yrs; median age at menarche was 13.0 yrs) collegiate swimmers (N=18) and divers (N=6) during their 16-week training period. Swimmers and divers were combined because BMD values did not differ. We also correlated the change in biochemical markers of bone formation (serum bone-specific alkaline phosphatase [BAP]), bone resorption (urinary cross-linked N-telopeptides [N-Tx]), and a marker of bone turnover (N-Tx / BAP ratio) with the change in whole body BMD values in 18 swimmers. Training (16 weeks) consisted of 3 days/wk of dryland (resistance+flexibility, 1.5 hr/day) and 6 days/wk of in-water (endurance, nine 2-hr sessions/wk) exercises.

BMD Measure	Preseason	Postseason	P value	% increase
Whole Body	1.065±0.073	1.072±0.078	0.0004	0.8
Lumbar Spine	1.054±0.125	1.070±0.128	0.0004	1.5
Total Femur	1.018±0.130	1.025±0.134	0.027	0.7
Femoral Neck	0.899±0.117	0.902±0.120	0.59	NA

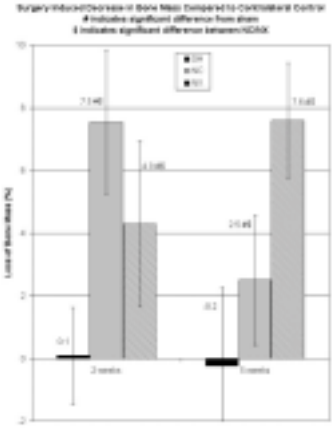
From preseason to postseason, urinary N-Tx did not change ($p=0.20$), serum BAP decreased ($p<0.0001$), and N-Tx/BAP increased ($p=0.03$), whereas these changes were not significantly ($p>0.90$) related to the change in whole body BMD. Although swimming is not considered an osteogenic form of exercise, we found small but significant increases in whole body, lumbar spine, total proximal femur BMD during 16 weeks of training. Our bone results should be confirmed in a larger sample size of swimmers training for a longer period and to determine if the observed changes were due to the dryland or to the in-water swimming training

M206

Comparison of Two Nerve Damage Disuse Models in Mice: Sciatic Nerve Crush and Sciatic Neurectomy. H. Wang,* V. L. Ferguson, S. J. Simske, T. A. Bateman. BioServe Space Technologies; University of Colorado, Boulder, CO, USA.

This study compared two sciatic nerve damage disuse osteoporosis models in female C57BL/6J mice: nerve crush (NC) and neurectomy (NX) two and five weeks post surgery. The primary difference between these two models is that the disuse associated with crush is temporary, allowing a period of skeletal recovery. It is this recovery that is being examined five weeks post surgery. Eighty 18-week-old mice were assigned to one of six groups (n=13-14/group): NC, NX and sham (SH) groups with half sacrificed two-weeks post surgery and the other half five-weeks post surgery. The right sciatic nerve was crushed midway between the sciatic notch and patella for 30 seconds with mosquito forceps for the NC animals, while 3 mm of the sciatic nerve was removed from the same area for the NX animals. For sham operations, the forceps were placed around the nerve, but no pressure was applied. The left leg, which was not operated on, served as a contralateral control. X-ray analysis was performed on right and left femora to measure relative bone densities and dry masses (Dry-M) of the whole bone were taken. Nerve crush resulted in a greater loss of bone Dry-M (7.5 ± 2.3%) compared to NX (4.3 ± 2.6%) and SH (0.1 ± 1.6%) 2-weeks post surgery. At 5-weeks, recovery for the NC group was evident (2.5 ± 2.1%) while the bone loss in the NX (7.6 ± 1.8%) group continued to increase (SH:-0.2 ± 2.5%). The figure represents the loss of bone mass in the surgery limb relative to the contralateral control limb (mean ± sd). Dry-M for the ipsilateral (crushed) bones were significantly different for NC and NX compared to SH ($p<.001$) for both the 2 and 5-week groups. Analysis of the x-rays at the proximal and distal epiphysis of the femur indicated a significant difference for NX at both 2 and 5-weeks post surgery compared to the contralateral control, with a trend towards a difference for NC at 2-weeks ($p=.11$). The increased loss in bone mass associated with sciatic nerve crush compared to neurectomy was an unexpected result. It is possibly associated with a greater immune/healing response to crush, though this hypothesis is yet to be examined. At five weeks post surgery the NC bones were in a period of recovery

from disuse, while NX continued to contribute to bone loss.



M207

$1,25(OH)_2D_3$ and EB1089 Produce Higher Femoral Cancellous Bone Density After 28-d Hindlimb Unloading While Only $1,25(OH)_2D_3$ Enhances Material Properties. H. A. Hogan,[1] M. R. Allen,[2] W. E. Rogers,*[1] N. L. Weigel,[3] S. A. Bloomfield.[2] [1]Mechanical Engineering, Texas A&M University, College Station, TX, USA, [2]Health & Kinesiology, Texas A&M University, College Station, TX, USA, [3]Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA.

Hindlimb unloading (HU) in rats produces site-specific significant declines in the bone mineral density (BMD) of the unweighted hindlimb bones; proximal tibia cancellous BMD declines ~20% over 28d in mature adult male rats (unpublished data). The current study evaluated the effectiveness of $1,25(OH)_2D_3$, the active form of vitamin D, and EB1089, a vitamin D analog that produces less hypercalcemia, in attenuating changes of BMD and mechanical properties in adult male rats after 28d of HU. All animals were fed a vitamin D deficient/0.5% calcium diet to eliminate exogenous vitamin D intake. On day 0 of HU, an Alzet osmotic pump was implanted to deliver one of three treatments: vehicle (VEH; n=13), EB1089 (EB; 0.1 µg/kg/day; n=13), or $1,25(OH)_2D_3$ (D; 0.1 µg/kg/day; n=11). After 28d HU and the right femora were analyzed ex vivo with pQCT (Stratec Research M, Norland Corp.) at the distal metaphysis (3 slices centered at 6.5mm from the distal condyles) to determine total BMD, cortical and cancellous compartment BMD, and bone area. Next, reduced-platen compression (RPC) tests were used to determine cancellous bone material properties. Specimens were cut to match the pQCT location (5.75-7.25mm from distal condyles) and compressed using quasi-static loading on the cancellous bone compartment. After 28d of HU, all groups had significantly ($P < 0.05$) different total BMD (VEH: 524 ± 8 mg/cm^3; EB: 581 ± 14; D: 788 ± 12) and cancellous BMD (VEH: 280 ± 7 mg/cm^3; EB: 306 ± 10; D: 388 ± 9). Also, D animals had higher total area (24.94 ± 0.64mm^2) compared to both EB and VEH (22.25 ± 0.82 mm^2 and 22.83 ± 0.45, resp.) while marrow area was lower (D: 7.2 ± 0.38 mm^2; EB: 14.03 ± 0.66; VEH 15.54 ± 0.38). D animals had greater ultimate stress, US, (D: 13.3 ± 2.2 MPa; EB: 1.12 ± 0.37; VEH: 0.76 ± 0.13) and elastic modulus, EM (D: 405 ± 62 MPa; EB: 54 ± 15; VEH: 41 ± 8) compared to both EB and VEH animals. Both EM and US were significantly correlated ($P < 0.0001$) to cancellous BMD ($r^2 = 0.478$ and 0.502, resp.). These data show the benefits of both $1,25(OH)_2D_3$ and EB1089 in enhancing cancellous BMD above VEH in unloaded bones, however, $1,25(OH)_2D_3$ indicates a higher potential based upon cancellous bone material properties. Follow-up studies should verify these results using rats fed a regular maintenance chow diet as well as a more physiological dose of $1,25(OH)_2D_3$.

M208

Biomechanical Testing as a Rapid, Primary Efficacy Endpoint in a Murine Model of Postmenopausal Osteoporosis. S. Bain,[1] S. Tellefson,*[1] R. Leininger,*[1] M. Heggem,*[1] J. Iuliucci,[2] V. Shen.[1] [1]Skeletech, Inc., Bothell, WA, USA, [2]ARIAD Pharmaceuticals, Cambridge, MA, USA.

As the goal of osteoporosis therapies is to reduce the incidence and rate of bone fractures, it follows that preclinical models must address bone strength endpoints. Biomechanical testing has therefore been used in ovariectomized (OVX) rat and primate models to assess drug effects on bone strength. While these techniques are well established in rats and primates, less is known about the utility of biomechanical endpoints in murine models. Given the increased application of murine models in drug efficacy testing, we have evaluated the utility of biomechanical testing techniques in a short-term (5 week), murine osteoporosis model. Experimentally, four groups of Swiss Webster mice were either sham-operated or ovariectomized (OVX) at the age of three months. One group each of sham or OVX animals was treated with either vehicle or alendronate for 5 weeks. Femurs and lumbar vertebra were collected at necropsy. Three different biomechanical tests were performed: 1) a compression test of the lumbar vertebral body; 2) a four point bending test of the femoral midshaft; and 3) a cantilever compression test of the femoral neck. The bone strength, maximum load, stiffness and energy absorbed, were found to be significantly reduced in the vertebral body in Ovx animals and alendronate treatment prevented this loss of bone strength. Similar decreases of strength were also noted in femoral neck in OVX animals and again the decrease was prevented by alendronate. Four-point bending of the femoral shaft did not show any significant changes. In summary, we conclude that biomechanical testing can be used to assess fracture efficacy of drugs in the murine model. Furthermore, the changes in bone strength in the murine model occur in a temporal framework that allows them to be used as a primary endpoint in short-term efficacy studies.

M209

Exercise Countermeasure to Disuse Osteoporosis. L. C. Shackelford,*[1] A. LeBlanc,[2] A. Feiveson,*[1] S. M. Smith,*[1] D. Feeback,*[1] M. Greenisen.*[1] [1]NASA JSC, Houston, TX, USA, [2]Baylor College of Medicine, Houston, TX, USA.

Cosmonauts on space missions of four months or longer have lost an average of between 6% and 8.6% of their original bone density in the spine, pelvis, femoral neck, and greater trochanter, despite use of a cycle ergometer, a treadmill, elastic cords, and an isokinetic dynamometer. Bed rest with no countermeasures has historically produced similar patterns of bone loss. Although microgravity does have a direct effect upon cells in culture, a large part of the changes experienced in space flight is believed to be due to insufficient loading during exercise. Intense resistive exercise has been shown to promote bone formation in healthy young adults and was found to be effective in maintaining positive calcium balance and decreasing regional bone loss in a 17-week bed rest pilot study in 1995.Between October 1997 and September 2000, 5 men and 4 women completed 17-weeks bed rest with 1 - 1.5 hour bouts of intense resistive exercise six days a week. Urinary calcium excretion dropped to below baseline values. Serum bone specific alkaline phosphatase and osteocalcin levels and urinary n-telopeptide excretion were elevated during the 17-weeks bed rest with resistive exercise compared to pre- bed rest baseline measurements, indicating a higher metabolic rate with an overall pattern of bone formation. Five female and 3 male bed rest control subjects (no exercise) had similar increases in n-telopeptide excretion with no increase in bone specific alkaline phosphatase or osteocalcin during bed rest. Compared to bed rest controls, the exercising bed rest subjects increased density in the lumbar spine and maintained calcaneus bone mineral density. Effectiveness of the exercises in maintaining bone mineral density varied with bone region, indicating the effect of resistive exercise upon bone maintenance is influenced by regional loading patterns.

M210

Endothelin-1 (ET-1) Appears to Inhibit Bone Resorption Through Upregulation of Osteoprotegerin (OPG). H. L. Guenther, W. Hofstetter, S. Shaw.* Dept.of Clinical Research, University of Berne, Berne, Switzerland.

Bone is a highly vascularized tissue a circumstance that prompted numerous laboratories to evaluate the effects of endothelial cell-derived products, such as endothelin-1 (ET-1) a 21-amino acid vasoactive peptide, on the function of both osteoblasts (Ob) and osteoclasts (Oc). Indeed, with ET-1 monoclonal antibodies, intense immunostaining has been detected over osteoclasts, osteoblasts and osteocytes. Other studies revealed that Ob express ET-1 receptors and that the hormone acts upon the osteoblastic phenotype. In addition, ET-1 was found to reduce osteoclastic bone resorption, presumably by inhibiting Oc motility. Present knowledge indicates that Ob play a central role in regulating osteoclastic bone resorption. Hence, the objective of the present study was to assess whether ET-1 achieves its antiresorptive effect on bone through mediation of osteoblasts.As target cells, we used the rat osteoblastic cell line CRP10/30, and for the assessment of the antiresorptive response, the osteoclast resorption pit assay was employed. Expression of transcripts encoding ET-1 receptors, OPG and RANKL in CRP10/30 cells were determined by RT-PCR. Confluent cells were incubated for 24-h in phenolred-and serumfree MEM containing different concentrations of ET-1 (10-10 to 10-8M) or an equivalent amount of vehicle solution. The effects of media conditioned by ET-1-treated osteoblasts on osteoclastic resorption were compared to CM of untreated cells and to media that contained ET-1 but no cells. Examination of the various CM on isolated Oc revealed that compared to controls, media of ET-treated cells caused an inhibition upon both pit and TRAP+ MNC formation. The induced effect was biphasic, generating maximum inhibition at an ET-1 dose of 10-10M. The fact that the ratios, number of pits formed per TRAP+ MNC, of control and ET-1 -treated cells were about the same suggests that the inhibitory effect is on osteoclast recruitment rather than on the Oc resorption activity. Employing RT-PCR, evidence was obtained that CRP10/30 cells express transcripts encoding the ET-1 A receptor. Moreover, applying semi-quantitative RT-PCR, data were collected indicating that ET-1 upregulates the expression of OPG transcripts without affacting those encoding RANKL. 1,25-dihydroxy vitamin D3 (10-8M) was found to abrogate the ET-1-mediated increase of OPG, and at the same time to enhance the production of transcripts encoding RANKL.In conclusion, the present data suggest that the antiresorptive action of ET-1 on bone resorption may be achieved by ET-1 eliciting osteoblasts to enhance OPG expression, which by virtue of acting as decoy receptor for RANKL causes an inhibition of osteoclast formation.

Disclosures: University of Bern,2.

M211

Stimulation of RANKL Gene Transcription and mRNA Stability in Stromal/Osteoblastic Cells by PTH: A Direct Effect Mediated by the Protein Kinase A Pathway. Q. Fu,* R. L. Jilka, S. C. Manolagas, C. A. O'Brien. Div. of Endo/Metab, Center for Osteoporosis & Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

PTH maintains calcium homeostasis in part by binding its receptor on stromal/osteoblastic cells, resulting in enhanced formation and activity of osteoclasts. Since PTH stimulates the expression of RANKL in these cells, and since RANKL, together with M-CSF, is sufficient for osteoclast formation, it has been proposed that RANKL is the major target of PTH action with respect to osteoclastogenesis. However, the signaling pathways and molecular mechanisms responsible for PTH stimulation of RANKL remain unknown. Further, some studies have demonstrated that IL-6 is required for PTH-induced osteoclast formation or bone resorption suggesting that PTH may stimulate RANKL indirectly via IL-6. Therefore, the goal of this study was to identify the signaling pathways utilized by PTH to stimulate expression of RANKL mRNA, and to determine whether PTH-regulated expression of another gene mediates its effect on RANKL synthesis. For these studies, UAMS-32 murine stromal/osteoblastic cells were stably transduced with the PTH/PTHrP receptor since these cells normally express low levels of this receptor. The transduced cells, desig-

nated UAMS-32-P, supported robust osteoclast formation in response to bovine PTH(1-34) when co-cultured with non-adherent bone marrow cells. Consistent with this, PTH strongly stimulated RANKL mRNA expression in UAMS-32-P cells after 2-24 hours of treatment as assessed by Northern blot. Pretreatment with the protein synthesis inhibitor cycloheximide did not block PTH stimulation of RANKL either in UAMS32-P cells, or in murine primary bone marrow cultures, indicating that intermediate gene expression is not required for PTH stimulation of RANKL mRNA. Mechanistic studies revealed that PTH increased RANKL gene transcription by approximately 4-fold in UAMS32-P cells, as determined by nuclear run-on, and increased the stability of its mRNA by approximately 2-fold, as determined by actinomycin D treatment. Dibutyryl-cAMP and PTH stimulated similar levels of RANKL, whereas TPA had no effect. Consistent with this, RP-cAMP, an inhibitor of protein kinase A (PKA), blocked the effect of PTH, indicating that PTH induces RANKL via activation of PKA in these cells. The finding that PTH directly stimulates RANKL gene expression in stromal/osteoblastic cells suggests that the previously observed requirement of IL-6 for PTH-induced bone resorption may involve IL-6 stimulation of osteoclast precursor proliferation or osteoclast lifespan rather than induction of RANKL.

M212

VIP Regulates the Expression of Osteotropic Cytokines in Mouse Calvarial Osteoblasts. E. Persson,* U. H. Lerner. Oral Cell Biology, Umeå University, UMEA, Sweden.

Signalling molecules in skeletal nerve fibers have been shown to regulate bone cell activities. Thus, the neuropeptide vasoactive intestinal peptide (VIP) stimulates bone resorption in mouse calvariae, causes a transient inhibition of isolated rat osteoclast activity followed by a stimulation of their resorptive activity and inhibits osteoclastogenesis in mouse bone marrow cultures [Mukohyama et al. BBRC 271:158, 2000; Lundberg et al. Bone 27:803, 2000]. These effects are likely to be due to direct effects on bone cells since osteoclasts and osteoblasts express different VIP receptor subtypes [Ransjö et al. BBRC 274:400, 2000; Lundberg et al. Endocrinology 142:339, 2001]. In the present study, we have investigated the effects of VIP on the expression in osteoblasts of cytokines known to be involved in regulation of osteoclastogenesis and osteoclast activity. Mouse calvarial osteoblasts were isolated and incubated in the absence and presence of VIP (10-6 M) for 48 hr. Total RNA was isolated and used for analysis of the mRNA expression of IL-6, RANKL, OPG and M-CSF with a semi-quantitative approach using GAPDH as house keeping gene. In addition, the effects of VIP on IL-6R and gp130 were assessed. The effects of VIP were compared with those of IL-1b (100 pg/mL). VIP and IL-1b both stimulated the mRNA levels of IL-6 and RANKL, with synergistic effects obtained when the peptides were combined. OPG mRNA was decreased by IL-1b, whereas VIP was without effect. VIP downregulated M-CSF mRNA, whereas IL-1 b had no effect. IL-6 protein (ELISA) was increased by both VIP and IL-1b with a synergistic response obtained by co-treatment with VIP and IL-1b. VIP decreased mRNA expression of IL-6R and gp130. In contrast, IL-1b enhanced the expression of these proteins, an effect that was decreased by VIP. These data demonstrate that VIP stimulates the release of IL-6, but decreases the expression of the two proteins (IL-6R, gp130) required for recognition and signal transduction by IL-6. The fact that VIP enhanced RANKL without affecting OPG is compatible with that VIP stimulates calcium release in mouse calvariae and activates isolated rat osteoclasts in the presence of stromal cells/osteoblasts, but is in contrast to the effect of VIP in mouse bone marrow cultures. Since osteoclast activity and formation requires the concerted action of several signalling molecules expressed by osteoblast/stromal cells and preosteoclasts/osteoclasts, the importance of the effects of VIP on IL-6, IL-6R and gp130, RANKL and M-CSF on bone resorption requires further studies. The data further supports the view that neuro-osteogenic interactions are important in bone metabolism and further indicates the possibility of neuro-immune interactions in bone.

M213

PTH and TNFα Induce Differential Patterns of IL-6 mRNA and Protein Production in Osteoblasts. J. C. Dai, E. M. Greenfield. Orthopaedics, Case Western Reserve University, Cleveland, OH, USA.

The goal of this study was to compare the effects of PTH and TNFα on expression of IL-6 at the mRNA and protein levels. IL-6 mRNA expression induced by PTH is rapid and transient in many osteoblastic cell lines as well as in vivo. We found that the accumulation of IL-6 protein induced by PTH is also rapid and transient. Thus, IL-6 protein accumulated in culture media from MC3T3-E1 cells during the first 2 hours of stimulation with 10 nM PTH and the IL-6 protein levels were relatively constant from 2-24 hours (~80 pg/ml). In contrast, the effects of TNFα were biphasic. During the first 2 hours of stimulation with 10 ng/ml TNFα, IL-6 mRNA and protein were rapidly induced. This initial phase resulted in IL-6 protein levels similar to those induced by PTH. It was followed by a period of declining IL-6 mRNA levels associated with little IL-6 protein accumulation (2-12 hours after stimulation with TNFα). A late phase of increased IL-6 mRNA and protein expression occured 12-24 hours after stimulation with TNFα. The late phase of IL-6 protein accumulation led to a significant increase in the IL-6 protein level after 24 hours of TNFα stimulation (~500 pg/ml) compared with that after 24 hours of PTH stimulation (~80 pg/ml). IL-6 protein levels were also measured during continuous exposure to PTH or TNFα but at relatively short intervals after removing the IL-6 protein that had accumulated earlier. PTH stimulated significant IL-6 protein release during the first 2 hour time period (~80 pg/ml) but not during the later time periods (<10 pg/ml). In contrast, TNFα stimulated equivalent amounts of IL-6 protein release from 0-6, 12-18, or 18-24 hours after stimulation (~200 pg/ml) but significantly less from 6-12 hours (~50 pg/ml). The different rates of IL-6 protein accumulation are most likely due to different rates of translation induced by the concominant changes in mRNA levels. Thus, blocking the increases in IL-6 mRNA with the transcriptional inhibitor, DRB (50 μM), completely eliminated the IL-6 protein accumulation induced during the early phases of stimulation by either PTH or TNFα. Moreover, transiently exposing MC3T3-E1 cells to TNFα for 5 minutes induced a transient increase in both IL-6 mRNA and protein, similar to that induced by continuous exposure to PTH. Taken together, our results show that PTH and TNFα induce IL-6 mRNA and protein with markedly different kinetics. The more extensive effect of TNFα likely reflects that TNFα stimulates IL-6 production and bone resorption in pathological situations while PTH acts in physiological situations where it is important to minimize the potential adverse effects of high levels of IL-6 on bone and/or surrounding tissues.

M214

Interleukin-1 β Stimulation of Osteoprotegerin Synthesis in Osteoblastic Cells Is Dependent on the p38 Mitogen-activated Protein Kinase and NF-kB Pathways. N. Franchimont,[1] V. Deregowski,*[2] M. Bentires-Alj,*[2] B. Relic,*[1] V. Bours,*[2] M. Malaise.*[1] [1]Rheumatology, CHU Sart-Tilman, Liège, Belgium, [2]Oncology, CHU Sart-Tilman, Liège, Belgium.

Interleukin-1 β is a proinflammatory cytokine that enhances bone resorption. IL-1 β increases the expression of receptor activator of NF-kB ligand (RANKL), a factor produced by osteoblasts and required for osteoclastogenesis. IL-1 β also stimulates osteoblast production of RANKL decoy receptor, osteoprotegerin (OPG). OPG inhibits RANKL effects on osteoclast differentiation and activation. The aim of this work was to identify signaling pathways regulating OPG expression following IL-1 β stimulation in osteoblastic cells. We measured OPG protein levels by ELISA in Saos-2 and MG-63 osteoblastic cell lines. IL-1 β at 10 ng/ml induced a 1.5 to 3-fold and a 8 to 25-fold increase in OPG levels after 24 h in Saos-2 and MG-63 cells, respectively. We next studied if the p38 mitogen-activated protein kinase (p38), a kinase required for osteoclast development mediated by RANKL, was necessary for IL-1 β induction of OPG. IL-1 β induced a rapid increase in p38 phosphorylation in Saos-2 as shown by Immunoblot. SB203580 (10 μM), a known inhibitor of the p38 signaling pathway, decreased OPG basal protein levels in control cultures by 25 to 35 % in MG-63 and Saos-2 cultures. In addition, IL-1 β stimulation of OPG was inhibited by 50 to 75 % in both cell lines by SB203580. We further examined if p38 influences the activation of NF-kB, a nuclear transcription factor activated by IL-1 β. The effects of IL-1 β in the presence or absence of SB203580 were tested in Saos-2 osteoblastic cells transiently transfected with a luciferase reporter gene under the control of NF-kB consensus site. SB203580 (10 μM) did not inhibit the luciferase activity in control or IL-1 β treated cultures. Furthermore, SB203580 did not decrease NF-kB binding activity in nuclear extracts of Saos-2 cells treated with IL-1 β as determined by mobility shift assay. However, OPG protein levels were dose-dependently inhibited by the IkB-α phosphorylation inhibitor BAY at 1 to 10 μM in Saos-2 and MG-63 cultures treated with IL-1 β, indicating that NF-kB is directly or indirectly required for OPG synthesis in osteoblastic cells. NF-kB requirement was confirmed in MG-63 cells infected with an adenovirus expressing a dominant negative IKB-α inhibitor that blocks NF-kB activation. When SB203580 (10 μM) was used simultaneously with BAY (10 μM), an additive inhibition of OPG protein levels was found in control and IL-1 β treated MG-63 and Saos-2 cultures. All together these data suggest that both p38 and NF-kB signaling pathways are independently required for OPG stimulation by IL-1 β in osteoblastic cells.

M215

Longitudinal Growth of the Cortex: Increased Osteoblasts in the Peripheral Spongiosa Causes Coalescence of Trabeculae to Create New Cortical Bone. R. I. Gafni,[1] E. R. Cadet,*[2] E. F. McCarthy,*[3] D. R. McCray,*[3] J. Baron.*[1] [1]Developmental Endocrinology Branch, National Institutes of Health, Bethesda, MD, USA, [2]Howard Hughes Medical Institute - NIH, Bethesda, MD, USA, [3]Department of Pathology, Johns Hopkins Hospital, Baltimore, MD, USA.

In mammalian long bones, radial growth of the cortex occurs by intramembranous bone formation at the periosteum. The mechanism responsible for longitudinal growth of the cortex, however, is not well understood. To investigate the underlying mechanisms, we administered oxytetracycline to growing New Zealand White rabbits to label newly formed bone. In the femoral diaphysis, the fluorescent lines parallel to the periosteum confirmed that diaphyseal cortical bone was formed by subperiosteal apposition. The metaphyseal cortex, however, showed multiple fluorescent closed curves outlining enlarging trabeculae. Thus, trabeculae generated by endochondral bone formation at the periphery of the growth plate enlarge and coalesce to create new cortical bone. In contrast, trabeculae under the central portion of the growth plate are resorbed to form the medullary cavity. Therefore, the balance of bone formation and resorption in the spongiosa differs, depending on location. To explore the mechanism responsible for this difference, we compared metaphyseal bone under the central and peripheral regions of the growth plate using computer-assisted histomorphometry. As the distance from the growth plate increased, bone volume and trabecular thickness decreased in the central metaphyseal bone; in contrast, these parameters increased in the peripheral metaphyseal bone ($p < 0.001$, central vs. peripheral). Osteoclast surface (osteoclasts per bone surface) did not differ between the peripheral and central regions ($p = 0.1$). Osteoblast surface (osteoblasts per bone surface), however, was significantly greater in the peripheral bone compared to the central metaphyseal bone (14 ± 3% vs. 28 ± 3%, mean ± SEM, central vs. peripheral , $p < 0.001$). In conclusion, longitudinal growth of the cortex is due to the coalescence of trabecular bone formed at the periphery of the growth plate. Our data suggest that this coalescence is the result of increased numbers of osteoblasts in the peripheral spongiosa. We speculate that this increase may be due to an inductive effect of the periosteum.

M216

Orthopaedic Particulate Debris Increase Osteoclast Maturation by a RANKL Dependent Mechanism. J. T. Ninomiya,* J. A. Struve.* Department of Orthopaedic Surgery, Medical College of Wisconsin, Milwaukee, WI, USA.

Aseptic loosening remains the primary cause of failure in total joint arthroplasty, and is the major factor limiting long-term surgical success. Although many proteins such as cytokines, prostaglandins, and proteases participate in this process, little is known about the role of the proteins RANKL and OPG in implant loosening. Therefore, we hypothesized that orthopaedic particulate debris stimulate osteoclast maturation by altering the expression of RANKL and OPG in osteoblasts, resulting in enhanced bone loss. Particles consisting of CP titanium were added to confluent cultures of murine MC3T3-E1 osteoblasts. Samples were determined to be free of endotoxin using the Limulus amebacyte

lysate assay. Controls consisted of cells which were cultured in the absence of particles. Following incubation, the conditioned media were collected, and cell membrane proteins were prepared following lysis in hypertonic sucrose. Gene expression of RANKL and OPG was determined by RT-PCR, and alterations in protein secretion were determined by Western blot analysis. The effects of particulate debris on osteoclast maturation were assessed using a murine bone marrow osteoclast maturation assay. Time course experiments with titanium particulate debris demonstrated a rise in RANKL expression at 4 h, which then peaked at 12 h and rapidly declined to baseline levels by 24 h. The greatest increase in RANKL gene expression was 5-fold, which was seen at the 12 h time point. In contrast, OPG expression increased only slightly over basal levels following up to 24 hours incubation. The titanium particles produced a dose-dependent increase in the expression of RANKL but not OPG. Western blot analysis did not reveal any detectable alterations in RANKL protein in the cell membrane protein fractions below the threshold sensitivity level of 25ng, and there were no changes in OPG levels in the conditioned media compared to unstimulated controls. Aliquots of the cell membrane protein fraction were added to murine bone marrow cells, and following incubation the cells were stained for TRAP activity, and counted. The addition of cell membrane proteins produced a 6 fold increase in the formation of TRAP positive multinucleate cells compared to unstimulated controls. The observed increases in osteoclast maturation were blocked by the addition of anti-RANKL antibodies. Our data demonstrate that orthopaedic particulate debris alter the gene expression and secretion of RANKL in osteoblasts, a pivotal protein in the regulation of osteoclast maturation and bone resorption. These findings suggest that both RANKL and OPG may play an important role in the generation of osteolysis and orthopaedic implant loosening.

M217

Bone-Specific Expression of RANKL After LPS Administration to Mice. C. A. O'Brien, Q. Fu,* S. C. Manolagas. Div. of Endo/Metab, Center for Osteoporosis & Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

The expression level of RANKL in stromal/osteoblastic cells is a major determinant of osteoclast number. The finding that bone is one of the few tissues that expresses high levels of RANKL suggests that this gene may be regulated by stromal/osteoblast-specific factors. Alternatively, restricted RANKL expression may result from lack of the appropriate stimulus to non-expressing tissues. Therefore, we sought to determine whether systemic activation of pathways that stimulate RANKL expression in stromal/osteoblastic cells would lead to RANKL expression in other cell types. To accomplish this, C57BL/6 mice were injected i.p. with LPS, which is known to stimulate RANKL both directly and indirectly via cytokines such as IL-6, IL-1, and TNF. RANKL mRNA in various tissues was then quantified by Northern blot. Under basal conditions, RANKL was detected only in calvaria and thymus. However, 2 hours after LPS injection, RANKL was highly induced in tibia, calvaria and spleen. After 8 hr, RANKL levels continued to increase in tibia and calvaria but returned to baseline in the spleen. RANKL mRNA in the thymus was unaffected by LPS treatment and was undetectable in heart, liver, lung, or kidney at any timepoint. All tissues examined were capable of responding to the LPS injection as demonstrated by increased IL-6 mRNA in each case. Cell-specificity was also reflected in a set of 4 different cell lines in response to the cytokine oncostatin M (OSM), a known stimulator of RANKL. OSM strongly induced a control mRNA, Tis11, in each cell line indicating that they all possessed OSM receptors. However, OSM induced RANKL only in UAMS-32 cells, the only one of the 4 that supports osteoclast formation. Another mechanism that regulates the cell-specificity of some genes is CpG methylation, which can inhibit gene promoter activity. Therefore we examined the methylation status of a CpG island in the first exon of the RANKL gene using genomic DNA from expressing and non-expressing cell lines and tissues. Southern blot analysis with methylation-sensitive restriction enzymes revealed that the CpG island was hyper-methylated in non-expressing cell lines but not in UAMS-32 cells or in any of the tissues examined. We conclude that both basal and stimulated RANKL expression are limited to certain tissues in vivo and that lack of expression in some cell lines, but not tissues, may be due to promoter hyper-methylation. Taken together, these results suggest that RANKL expression in cells of osteoblastic lineage may involve cell-specific factors, which may thus constitute a molecular linkage between osteoblast and osteoclast formation.

M218

Role of 521-41, a Novel Splicing Variant of Runx1/Cbfa2, in the Regulation of Runx2/Cbfa1 Promoter Activity. K. Tsuji,[1] H. Harada,[2] T. Komori,[3] M. Noda.[1] [1]Dept. of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Sumitomo Pharmaceuticals Research Center, Osaka, Japan, [3]Osaka University, Osaka, Japan.

Runx2 (Cbfa1/Pebp2aA/Til-1/Osf-2) is a transcription factor which belongs to Drosophila pair rule gene runt family and is considered to play critical roles during chondrocytes and osteoblastic maturation based on the knock out and transgenic mice experiments. Recently, we identified a novel splicing variant of Runx1, 521-41, which contains novel sequences in the C-terminal trans-activation domain and is expressed in the early stage during mouse embryonic development at the time before Runx2 starts to express. Since seven Runx2 binding motifs exist around the transcriptional starting site of Runx2 and Runx2 itself suppresses the Runx2 promoter activity by binding to them, we examined the function of 521-41 in the regulation of Runx2 promoter. Three Runx2 promoter-Luciferase reporter constructs were used, i.e., (4384 to 6651)-Luc (1M), (4384 to 6703)-Luc (+45), and (4384 to 7052)-Luc (2M) (the number indicate the sequence positions of Runx2 genome (DDBJ/Genbank/EMBL #AB013129)). 1M contains 1.8kb of Runx2 promoter, and +45 and 2M contain additional 45bp and entire 5'UTR sequences (395bp) respectively. 1M, +45, and 2M contain three, six, and seven Runx2 binding motifs respectively.Previous paper reported that the three Runx2 binding motifs present within the first 45bp of 5'UTR also act as suppressor elements in ROS17/2.8 cells. We first examined the endogenous promoter activities of three reporter constructs (1M, +45, and 2M) in MC3T3E1, C3H10T1/2, and ATDC5 cells. Promoter activity of +45 was less than that in 1M promoter in mouse osteoblast-like MC3T3E1 cells and non-osteoblastic mesenchymal cell line, C3H10T1/2 cells. Compared to these cell lines, +45 promoter activity was two fold more in chondrocytic ATDC5 cells. In all cells tested, 2M promoter activity was less than +45 promoter activity, suggesting that novel suppressor element(s) exist in the latter half of 5'UTR (+45 to +395). Runx2/Pebp2aA-coexpression in these cells resulted in suppression of the activities of all promoter constructs. Coexpression of 521-41 also suppressed all three promoter activities, but its suppression efficiency was less than that in Runx2/Pebp2aA coexpression. These data suggest that 521-41 would be involved in the regulation of Runx2 gene expression.

M219

Expanded Identification and Functional Analysis of Runx2 Sites in the Rat Col1a1 Promoter. M. S. Kronenberg,[1] A. Ivkosic,*[2] B. E. Kream,[2] A. C. Lichtler.[1] [1]Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA, [2]Medicine, University of Connecticut Health Center, Farmington, CT, USA.

Runt domain factor (Runx2/Cbfa1) is a critical element in osteoblast maturation and is known to activate the osteocalcin gene in bone. However, the influence of Runx2 on Col1A1 gene expression is much less clear. We previously identified three Runx2 binding sites downstream of –1719 bp in the rat Col1a1 promoter at –1376, -622 and –257 bp. These sites specifically bind Runx2 present in nuclear extracts from ROS 17/2.8 cells. We showed that mutation of these sites in ColCAT constructs caused an increase in ColCAT activity in transfected ROS 17/2.8 cells, and that cotransfection of wild-type ColCAT constructs with a Runx2 expression vector decreased promoter activity. These studies suggested that binding of Runx2 may inhibit Col1a1 promoter activity. However, the three Runx2 sites originally studied were identified using relatively restrictive search criteria. Thus, it was possible that the Col1a1 promoter contained other Runx2 binding sites that could stimulate promoter activity. In addition, the effect of mutations in Runx2 binding sites on the ability of co-transfected Runx2 to inhibit Col1a1 promoter activity had not been determined. Using a less restricted search, we have identified two additional Runx2 sites at –949 and –282 bp. These latter two sites, whose core consensus sequences are identical to those at –257 and –1376 bp respectively, exhibit stronger binding of Runx2 than the previously identified sites. Mutation of the consensus sequence of the –949 and –282 sites blocked Runx2 complex formation in gel mobility shift assays. However, the activity of ColCAT1719 constructs mutated in the three original sites plus those at –282 and -949 showed no change relative to wild type ColCAT1719 in transiently transfected ROS 17/2.8 cells. In addition, overexpression of Runx2 decreased ColCAT activity irrespective of whether some, all or none of these Runx2 sites were mutated. We have never observed a consistent decrease in Col1a1 promoter activity upon mutation of any or all currently identified Runx2 sites. Moreover, overexpressed Runx2 consistently inhibited Col1a1 promoter activity even with five mutated Runx2 sites. Therefore, we conclude that Runx2 binding is not required for rat Col1a1 promoter activity in ROS 17/2.8 cells. We speculate that high level expression of Runx2 may inhibit Col1A1 expression in osteoblasts indirectly or through an as yet unidentified site.

M220

Activation of PPAR-gamma Inhibits Expression of Osteoblast Differentiation Markers. A. Mukherjee,* E. Khan,* Y. Zhang,* Y. Abu-Amer. Orthopaedics, Washington University School of Medicine, Saint Louis, MO, USA.

Osteoblasts and adipocytes diverge from common mesenchymal origin. It has been shown that the nuclear molecule peroxisome proliferator-activated receptor-gamma (PPAR-gamma) is critical for phenotype determination at early differentiation stages of these cells. Activation of nuclear receptor PPAR-gamma is readily attainable with addition of the ligand 15-Deoxy-Prostaglandin J2 (PGJ2). PGJ2 administration recruits cellular mediators such as 5- and 12-Lipoxygenases into the nucleus. These enzymes are believed to cause PPAR-gamma activation. Activated PPAR-gamma inhibits gene expression in part by antagonizing the activities of several transcription factors. In this study we examine inhibitory mechanisms of osteoblast differentiation markers by activating PPAR-gamma. Our data indicate that PGJ2 dose-dependently inhibits expression of alkaline phosphatase, the osteoblastic differentiation marker, by primary stromal cells and by cell lines such as ST2 and MC3T3 cells. Next, we turned to examine the possible mechanisms of this inhibition. Using immunoblots we show that PGJ2 treatment leads with time to decrease in cytoplasmic levels of 5-Lipoxygenase and 12-Lipoxygenase, which coincides with increased expression of PPAR-gamma in the nuclear compartment. Beta-Glycerophosphate and ascorbic acid induce in vitro osteoblast differentiation. Examination of the down-stream signaling reveals that induction of osteoblast differentiation by beta-Glycerophosphate and ascorbic acid involves activation of the transcription factors NF-kB and cbfa-1, the latter is critical for osteoblast differentiation. Thus, we asked if inhibition of alkaline phosphatase expression by activated PPAR-gamma reflects attenuation of transcriptional activity. To address this issue, DNA-protein binding assays for NF-kB and cbfa-1 were performed using oligonucleotides derived from the kB3 site of the TNF promoter (for NF-kB) and the mouse OSE2 (cbfa-1). Electrophoretic mobility shift assay revealed that beta-Glycerophosphate-induced DNA binding activity of both NF-kB and cbfa-1, is abrogated by inclusion of PGJ2. These findings were consistent in primary stromal cells as well as in the cell lines ST2 and MC3T3 cells. Thus, activation of PPAR-gamma by PGJ2 inhibits DNA binding activity of the transcription factors NF-kB and cbfa-1, leading to inhibited expression of osteoblast differentiation markers.

M221

Trafficking of Runx1/Cbfa2 and Runx2/Cbfa1 in Living Cells Defines a Dynamic Subnuclear Domain. K. S. Harrington,* A. Javed,* S. K. Zaidi,* H. Drissi, A. J. van Wijnen, J. B. Lian, G. S. Stein, J. L. Stein.* Cell Biology Department, University of Massachusetts Medical School, Worcester, MA, USA.

The runt related transcription factors (Runx/Cbfa/AML) are key regulators of tissue specific gene expression in skeletogenesis (Runx2) and hematopoiesis (Runx1). We have previously demonstrated that Runx factors are targeted to transcriptionally active nuclear domains. This localization requires a 31 amino-acid nuclear matrix targeting signal (NMTS) located in the carboxy terminus of Runx1 (amino-acid 351-381) that, based on crystallographic analysis, adopts a loop-turn-loop structure. We have shown by mutational analysis that a region in Runx2 (amino-acid 388-414) required for nuclear matrix association has significant homology to the Runx1 targeting signal. Deletion of the NMTS reduces transcriptional activity of Runx2 target promoters (Osteocalcin, Bone Sialoprotein and TGFbeta-1 receptor), but does not interfere with nuclear import of Runx2. Because Runx1 and Runx2 are both expressed in osteoblasts, we fused these proteins to enhanced green fluorescent protein to assess intra-nuclear trafficking in real time. Our results show that Runx1 and Runx2 localize to the same punctate subnuclear domains in live osteoblastic cells. Over a 30 minute period these punctate foci are positionally stable within the nucleus. Fluorescence recovery after photobleaching (FRAP) demonstrates that both Runx1 and Runx2 dynamically associate with the foci, yet are less mobile than GFP alone or GFP fused to a mutant Runx protein lacking the NMTS. Our findings suggest that the unique intranuclear trafficking signal of Runx factors targets these proteins to stable subnuclear structures to support bone-tissue specific gene regulation.

M222

Changes in Bone Cell Gene Expression in Mice Early After Cadmium Gavage. M. H. Bhattacharyya,[1] A. K. Wilson,[2] D. Glesne,*[1] A. Regunathan,*[1] T. Flores.*[1] [1]Argonne National Laboratory, Argonne, IL, USA, [2]Benedictine University, Lisle, IL, USA.

An in vivo model for cadmium-induced bone loss has been developed in our laboratory in which a mouse begins to excrete a significant amount of bone mineral in feces starting at 8 h after a single gavage administration of cadmium. The bone response starts at a blood cadmium concentration of 5 µg Cd/l, below current OSHA standards for industry. Following the in vivo model, female mice from three strains were placed on a low calcium diet for two weeks to decrease fecal calcium excretion to a constant, low level. The three strains were CF1, metallothionein-wildtype (MT+), and metallothionein1,2-deficient (MT-), chosen because they differ in the extent of their bone response to cadmium. Cadmium was gavaged at 8am, during the animal's active feeding cycle (12:12 LD cycle: L 6pm-6am; D 6am-6pm). Each mouse received 200 µg Cd in 0.2 ml 0.1N HCl, or HCl only for vehicle controls. In eight mice per strain (4 +Cd , 4 -Cd mice), the bone response was documented by collecting feces daily for 9 days, starting 3 days before cadmium gavage, and analyzing for fecal calcium by atomic absorption spectrometry. For CF1 mice, a gene expression time-course was investigated in which bones for RNA isolation were taken from four groups (8 mice/group): Cd-exposed and vehicle-controls at 2h after gavage; the same groups at 4h after gavage. For the MT+ and MT- strains, bones were collected for RNA from +Cd and -Cd groups (8 mice/group) only at 4 h after gavage. At sacrifice, femura and tibiae were rapidly removed, cleaned of muscle, cut on both ends, and flushed to removed all marrow. Marrow-free shafts were immediately frozen in liquid nitrogen. Total and polyA+ RNA were isolated from the bones of each group (8 groups). PolyA+ RNA samples were submitted to IncyteGenomics for analysis on their mouse gene array (GEM2). Fecal analysis results confirm that the three strains differed in the amount of bone calcium excreted in response to cadmium (mean+/-SE, n=4; CF1, 0.24+/-0.08 mg; MT+, 0.92+/-0.22 mg; MT-, 1.7+/-0.4 mg). Gene array results demonstrate that the messages for 4 genes (out of ca. 10,000) were upregulated by cadmium in all three strains and time points: MT-1, MT-2, cysteine-rich protein 61, and glutamine synthase pseudogene. An additional 8 genes were Cd-induced in three of the four hybridization pairs, including transferrin receptor, MAP Kinase 14, and a vacuolar proton pump. Results are being further investigated regarding identification of specific pathways involved in the early bone loss response to cadmium.

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Adenovirus-mediated Expression of Cbfa1/Runx2 in C3H10T1/2 Mesenchymal Cells Induces Mineralization and Expression of Some, but not all Osteoblast Markers. D. Wei,* S. Yang,* D. Wang,* R. Franceschi. The University of Michigan, Ann Arbor, MI, USA.

The Cbfa1/Runx2 transcription factor is essential for osteoblast differentiation and bone-specific gene expression. Transient overexpression of Cbfa1 in mesenchymal cells has been reported to stimulate certain osteoblast-related genes including osteocalcin. However, no previous studies have determined the extent to which Cbfa1 can convert mesenchymal cells into functional osteoblasts in vitro or in vivo. To address this issue, an adenovirus containing cDNA for the Runx2 isoform of Cbfa1 (AdCMV-Cbfa1) was constructed using Cre/lox recombination (Hardy, 1997, J. Virology 71:1842). AdCMV-Cbfa1 was used to transduce C3H10T1/2 mesenchymal cells that do not normally express Cbfa1 and preosteoblastic MC3T3-E1 cells which are Cbfa1-positive. C3H10T1/2 cells transduced with AdCMV-Cbfa1, but not control virus containing lacZ cDNA, expressed Cbfa1, alkaline phosphatase, osteocalcin and other osteoblasts markers. Like primary osteoblasts, marker expression in Cbfa1 transduced cells was further stimulated by conditions conducive to ECM synthesis (i.e. growth in the presence of ascorbic acid). AA-treated Cbfa1-transduced cells also formed mineralized nodules after growth in the presence of 5 mM beta-glycerol phosphate. Their ability to form bone in vivo will also be reported. Although Cbfa1-transduced C3H10T1/2 cells share many similarities with osteoblasts, certain important differences were apparent; cells did not express detectable levels of bone sialoprotein mRNA or protein and type I collagen levels were very low. In contrast, AdCMV-Cbfa1 transduction of MC3T3-E1 preosteoblast cells increased the already high levels of all osteoblast markers examined including bone sialoprotein. These results suggest that accessory factors present in osteoblasts, but not mesenchymal cells are necessary for Cbfa1 to activate the full repertoire of osteoblast-related genes.

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Intra-femoral Delivery of AdenoCBFA1 Virus Leads to Increased Bone Formation in Adult Rat Femur. B. M. Bhat, V. E. Coleburn,* V. L. Dell,* L. Borella, Y. P. Kharode, J. A. Robinson, F. J. Bex. Bone Metabolism and Osteoporosis Research, Women's Health, Wyeth-Ayerst Research, Radnor, PA, USA.

The importance of CBFA1 in vivo during bone development is well established by knock out studies in mice demonstrating the lack of endochondral and intramembranous ossification. In humans mutations (missense or deletions) within the CBFA1 coding region leads to the syndrome of Cleidocranial Dysplasia with tooth abnormalities, short stature and dysplasia of the clavicle. Recent studies indicate that transgenic mice expressing CBFA1 in chondrocytes show progressive replacement of cartilage by bone (Takeda et.al. JBMR suppl. Vol.15, 2000, pp. S145). In order to address the in vivo osteogenic effect of CBFA1 directly on mature bone, we utilized the recombinant human adenovirus vector approach to express abundant human CBFA1. The recombinant can infect a variety of human and non-human cells but is replication defective in most of them except in human embryonic kidney-293 cells. The purified AdenoCBFA1 (1x 10 12 virus particles/ml) was injected in a 25ul volume into the right femur of ten mature Sprague-Dawley rats. Rats receiving UV-inactivated AdenoCBFA1 served as controls. After three weeks, both injected right femur and non-injected left femur of all the animals were removed and pDEXA BMD analysis was carried out on (a) whole femur, (b) 17mm section from distal end, (c) 10mm section from proximal end, and (d) ~10mm section around the injected region. There was a statistically significant BMD increase (~10%) in total, proximal and distal region values between live and UV-inactivated virus injected right femurs as well as to their respective contralateral uninjected femurs. The whole femur mean BMD value of active virus injected femur was 0.193 and that of UV-inactivated virus injected femurs was 0.175 ($p < 0.05$). There was no difference in BMD values of the 10mm region around the injected site. In addition the pQCT values of 5 slices of trabecular bone taken at the distal end near the growth plate of the femur revealed a statistically significant increase (~30%) in BMD. This study validates the intra-femoral adenoviral injection approach to evaluate the in vivo bone activity of candidate genes when given directly into the bone environment. More importantly this study demonstrates that a CBFA1 mediated osteogenic effect can be observed in vivo when expressed in the bone marrow of mature rats.

M225

Retroviral Gene Delivery of Cbfa1/Runx2 to Enhance Osteoblast-specific Gene Expression and Matrix Mineralization. B. A. Byers,*[1] G. K. Pavlath,*[2] A. J. Garcia.*[1] [1]Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [2]Department of Pharmacology, Emory University, Atlanta, GA, USA.

This work focuses on the forced expression of Cbfa1/Runx2 in potential target cells for bone tissue engineering applications. Expression of Cbfa1, a transcriptional activator of osteoblast differentiation, is essential for bone mineralization.Cbfa1 cDNA was cloned into a retroviral vector that produces both Cbfa1 and a zeocin resistance-eGFP selectable marker. Forced expression of Cbfa1 was investigated in NIH3T3 fibroblasts, C3H10T1/2 pluripotent fibroblasts, and MC3T3-E1 osteoblast-like cells. NIH3T3 and C3H10T1/2 cells are non-osteoblastic as neither express osteoblast-specific genes nor produce a mineralized matrix. MC3T3 cells served as model osteoblasts. Retroviral packaging and mammalian cell infections were performed using standard techniques. Target cells were infected with Cbfa1 or empty vector and grown in MEM supplemented with fetal bovine serum, penicillin-streptomycin, glycerophosphate, and ascorbic acid. Osteoblast-specific gene expression was examined by RT-PCR, alkaline phosphatase (AP) activity was quantified using a biochemical assay, and mineralization was visualized by von Kossa staining.High transduction efficiencies (>95%) and sustained Cbfa1 gene expression were observed in all target cells. Cbfa1-infected NIH3T3 and C3H10T1/2 cells expressed osteocalcin (OCN), whose osteoblast-specific transcription is regulated by Cbfa1. Forced expression of Cbfa1 in MC3T3 cells resulted in significant upregulation of osteoblast-specific genes. OCN was detected in Cbfa1-infected MC3T3 cells as early as 1 day post infection, whereas endogenous expression was not present in controls until day 7. AP activity increased 4-fold in MC3T3 cells infected with Cbfa1 at 7 days compared to controls. Cbfa1-expressing C3H10T1/2 cells expressed a high level of AP activity, whereas activity in controls was not detectable. Cbfa1-infected MC3T3 cells exhibited 2 to 3-fold upregulation in matrix mineralization, while Cbfa1-expressing NIH3T3 fibroblasts did not produce mineralized matrix. Detectable mineralization was observed in C3H10T1/2 cells in 2 of 8 separate experiments. This variability may be explained by differences in viral titer or the nonspecific pluripotency of the cell line. Forced and sustained expression of Cbfa1 via retroviral gene delivery induced robust expression of osteoblast-specific genes and cell-type dependent upregulation in matrix mineralization. Results indicate that although Cbfa1 expression is essential, it is not sufficient for mineralization.

M226

Identification and Characterization of the Gene Encoding Human Osteoactivin. T. A. Owen,[1] S. L. Smock,*[1] T. A. Castleberry,[1] B. Lu,*[1] S. N. Popoff,[2] F. F. Safadi.[2] [1]Cardiovascular and Metabolic Diseases, Pfizer Global Research & Development, Groton, CT, USA, [2]Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA.

Osteoactivin (OA) was discovered as a cDNA more highly expressed in RNA isolated from both calvaria and long bones of osteopetrotic mutant rats *(op/op)* than the bones of their normal littermates. Rat OA was demonstrated to be the homologue of a human cDNA

(*nmb*) previously reported as being preferentially expressed in melanoma-derived cell lines with a low metastatic potential. While OA appears to be distantly related to a family of melanocyte-derived proteins termed pMEL/gp100, the function of OA remains unknown. In order to obtain more insights into the potential role of OA, especially in bone metabolism, we characterized the human OA gene locus. Southern blot analysis suggested that OA is a single copy gene in the human genome. A search of GenBank using the rat OA cDNA as query revealed the presence of the human OA gene on BAC clone RG271G13. Alignment of the human OA/ *nmb* cDNA sequence with this BAC demonstrated that the human OA transcript is encoded by 11 exons spanning 28.3 kb. These exons range in size from 95 bp to 1019 bp. FISH analysis, radiation hybrid mapping, and bioinformatic localization all have placed the human OA gene on chromosome 7p15.1. No other genes involved in bone metabolism have been reported at this locus. OA is expressed in human osteoblasts in culture as a single transcript of approximately 2.4 kb. 5' RACE analysis of human OA in both human osteoblast and kidney mRNA demonstrated that the same transcriptional initiation site was used in both tissues and that this site mapped to the end of the human *nmb* cDNA as previously reported. The dramatic overexpression of OA in the *op* mutant rat skeleton suggest that it may be secondary to the uncoupling of bone resorption and formation resulting in dysregulation of osteoblast gene expression and function. Given this evidence from the rat, as well as the high levels of expression observed in human osteoblasts, we propose that osteoactivin may play a previously unreported role in normal human skeletal modeling/remodeling.

M227

Cloning of the Human N-Cadherin Promoter in Osteoblasts: Functional Analysis of a G.C-Rich Region and Regulatory Elements. S. Le Mée,* P. J. Marie. Hopital Lariboisiere, INSERM U349, Paris Cedex 10, France.

Recent studies indicate that some cadherins may play an important role in bone cell-cell adhesion and in the osteoblast differentiation program. We recently showed that N-cadherin is expressed by human calvaria osteoblasts and is required for the promoting effect of bone morphogenetic protein-2 on osteoblast differentiation marker genes. We also found that N-cadherin is transcriptionally up-regulated by Fibroblast Growth Factor-2 in human osteoblasts. In order to determine the mechanisms of transcriptional regulation of N-cadherin expression in osteoblasts, we have cloned the human N-cadherin promoter and determined essential regulatory elements. We isolated and analyzed a genomic DNA sequence from a clone from a human BAC library containing 3.7 kb of the 5' flanking region of the previously characterized N-cadherin gene. To define sequences that are essential for the promoter activity of this region, various fragments of the 3.7 kb sequence were fused to the luciferase (LUC) reporter gene cloned into the pGL3-Basic vector. All constructs were transiently transfected in Immortalized Human Neonatal Calvaria (IHNC) cells which express osteoblast phenotype. Deletion analyses revealed that a 318 bp sequence mediates maximal LUC activity. The active promoting sequence is G.C rich, does not contain TATA box, and differs from the known chicken N-cadherin promoter sequence. Nucleotide sequence identities between human/mouse and human/chicken are 80% and 53%, respectively. Several regulatory elements present in the cloned human N-cadherin promoter sequence were identified and the functionality of these elements was tested by electrophoretic mobility shift assay (EMSA). At least two regulatory elements were found to bind nuclear proteins specifically. This identifies for the first time the functional sequence of the human N-cadherin promoter, and suggests the presence of putative regulatory elements in human osteoblasts.

M228

Cell-cell/cell-matrix Interactions and Hormonal Bone Modulators Regulate the mOST-PTP Gene in MC3T3 Osteoblasts. L. J. Mauro, D. C. Tranter,* M. K. Townsend,* M. Wheeler,* E. Kishel.* Animal Science and Biochemistry, Molecular Biology & Biophysics, University o**f Minnesota, Twin Cities, MN, USA.**

Tyrosine phosphorylation is a delicate balance between tyrosine kinase and phosphatase (PTP) activities that is critical for all cellular processes. The factors that regulate PTP function during osteogenesis are largely unknown. We previously reported a unique bone PTP gene known as osteotesticular PTP (mOST-PTP); a receptor-like enzyme with extracellular motifs found in adhesion molecules. We have determined the expression pattern of this gene during differentiation of MC3T3 osteoblasts (OBs) and explored the role of cell interactions and hormones in its regulation. Northern analysis revealed dramatic changes in mOST-PTP mRNA from low-nondetectable levels during proliferation (days 1-2); elevations during differentiation/ matrix maturation (~10 fold > on day 9 vs.day 2) and a return to baseline levels by mineralization. No changes in mRNA were observed for structurally similiar receptor PTP, LAR, or intracellular PTP, PTP1B. Transient transfections of differentiating cells with an mOST-PTP promoter-luciferase construct revealed steadily increasing luciferase activity during days 1-6 of culture. Interference with differentiation by perturbation of cell-matrix interactions has striking effects on mOST-PTP expression. Culturing in 'differentiation-deficient' medium (DDM; no ascorbic acid, b-glycerol phosphate) delays and attenuates increases in mOST-PTP mRNA. Transfection of cells in DDM with the luciferase reporter construct eliminates increases in luciferase activity observed during differentiation. Inhibition of procollagen processing using dihydroxyproline (0.5 mM; days 1-9) produces an effect similar to DDM cultures. As mOST-PTP expression was not abolished, cell density was manipulated to test cell-cell interaction effects. Plating cells at 100% confluency (vs. 50% confluency mimicking day 1 cultures) results in increased mOST-PTP mRNA within 8 hrs post-plating to levels comparable to that observed in early cell cultures grown in DDM. Finally, we tested effects of parathyroid hormone (PTH) and vitamin D3 (D3), modulators of OB differentiation. Treatment of differentiating cells with PTH analogs (i.e.10 nM rPTH (1-34), 10 min on day 6 or 12) or active vitamin D3 (10 nM, 48 hrs, day 6) results in a dramatic suppression of mRNA expression. In conclusion, mOST-PTP expression is upregulated during OB differentiation, in part, due to transcriptional activation and appears to be dependent on an interaction of cell-cell/cell-matrix adhesive events. In addition, regulators of osteogenesis, PTH and D3, serve as potent repressors of the expression of the mOST-PTP gene.

M229

Serotonin Receptor Expression and Function in Osteoblasts. M. Bliziotes,[1] X. Zhang,[2] A. Eshleman,[1] K. M. Wiren.[1] [1]OHSU and Portland VAMC, Portland, OR, USA, [2]OHSU, Portland, OR, USA.

We have previously reported mRNA expression for several serotonin (5-HT) receptors in osteoblastic cells. Here we further characterize protein expression and functional activity of the receptors. Western blot analysis confirmed protein expression of the 5-HT1A, 5-HT2A and 5-HT2B receptors in the osteoblastic cells. For the 5-HT2A receptor, a monoclonal antibody raised against a fusion protein containing amino acids 1-72 of the human 5-HT2A receptor recognized a band of ~61 kDa in rat hippocampus, ~72 kDa in ROS 17/ 2.8 and UMR 106-H5 cells, and ~65 kDa in day 32 primary rat calvalrial osteoblast (rOB) cells. A monoclonal antibody against human 5-HT2B receptor recognized a doublet of 80-85 kDa in rat hippocampus, ROS, and UMR; a single band of ~85 kDa was seen in day 25 rOB. A smaller band of 53 kDa was also seen in rat hippocampus, and additional bands ranging from 47 to 74 kDa were seen in ROS and UMR cells. An anti-peptide antibody against a carboxy-terminal sequence of the human 5-HT1A receptor recognized a band of ~65 kDa in extracts of rat hippocampus, ROS, and UMR cells. No bands were detected in day 25 rOB cells. We next explored the effects of 5-HT on AP-1 activity using a transient transfection assay with a chloramphenicol acetyltransferase (CAT) reporter gene fused to tandem AP-1 binding sequences in UMR 106-H5 osteoblastic cells. We also investigated the effect of 5-HT on AP-1 activity induced by PTH in the same model. Six hours of incubation with 1 µM 5-HT produced a non-significant 1.7+0.1-fold increase (mean+S.E.M.) in AP-1 activity, whereas bPTH(1-34) (100 ng/ml for one hour) produced a similar effect. However, preincubation for 5 hours with 5-HT (1 µM) followed by one hour of 100 ng/ml PTH produced a significant 2.3-fold increase of AP-1 activity over control ($p<0.05$). No additional effect was seen at 10 µM 5-HT. Thus 5-HT potentiates the PTH-induced increase in AP-1 activity in osteoblastic cells. In summary, we have demonstrated that 1) at least three different 5-HT receptors are expressed in osteoblastic cells, and 2) 5-HT potentiates the PTH-induced increase in AP-1 activity in osteoblastic cells. This has physiologic relevance since PTH induces collagenase production in osteoblastic cells through a mechanism which involves an AP-1 consensus-binding sequence, and thus 5-HT may play a role in the regulation of this metalloproteinase as well.

M230

A Model System for Proteomic Analysis of Osteoblastic Function. J. R. Neale,* W. M. Pierce. Pharmacology and Toxicology, University of Louisville, Louisville, KY, USA.

The goal of this study was development of a system for proteomic analysis of osteoblastic function. This study is a composite of cell culture, electrophoresis, mass spectrometry, and informatics analyses. A characterized cell line [SV40-transformed human fetal osteoblast cells (hFOB 1.19)] was purchased from ATCC and raised in culture. Following controlled cell proliferation, differentiation to the osteoblastic phenotype was assessed. hFOB cells responded in a dose dependent manner to parathyroid hormone with increased intracellular cyclic AMP and to 1,25(OH)2D3 with increases in intracellular alkaline phosphatase and secreted osteocalcin. Confluent hFOB cells were then taken for proteomic analysis of cytosolic contents. Cytosolic proteins were resolved using two-dimensional electrophoresis, using isoelectric focusing (pH range 4-9) followed by SDS-PAGE (12%). Gels were visualized using silver staining and then protein containing regions of interest were excised from the gels. Samples were taken for reduction and alkylation of sulfhydryls and then for in-gel limited hydrolysis using trypsin. Peptide mixtures were analyzed using matrix-assisted laser (N2-337 nm) desorption ionization followed by time of flight mass spectrometry (MALDI-TOF) using ion mirror kinetic energy focusing. Ion currents were collected at 2 GHz and transformed into centroided mass measured peaks. For the informatics portion of the analysis, mass spectra were compared to protein and genomic data bases that were indexed by species, pI, and molecular weight. Tryptic hydrolysate patterns were compared to data base in silico digest patterns. A variety of proteins were identified, including collagen precursors, cytoskeletal proteins, and enzymes involved in carbohydrate metabolism. The utility of this model has been verified, and it is useful for study of expression proteomics (functional genomics) and for study of molecular mechanisms of osteoblastic function without the confounding variables introduced by extracellular proteins. This model is readily adapted to studies of hormone, cytokine and drug action, signal transduction, and post-translational modifications.

M231

Expression and Differentiation-Dependent Regulation of the 'Brain-Specific', Sodium-Dependent Inorganic Phosphate Cotransporter (BNPI) in Osteoblasts. P. S. Bhangu, P. G. Genever, T. S. Grewal,* T. Skerry. Biology, University of York, York, United Kingdom.

Glutamate (Glu) is involved in regulated intercellular communication in bone. Previously we have shown that osteoblasts are able to take up Glu in a regulated manner and direct its release through regulated vesicular exocytosis. To date however, understanding of the specific mechanisms for intracellular vesicular packaging of Glu for targeted and focal release either in the CNS or in bone has been limited. Increased understanding of these mechanisms could allow specific control of Glu signalling in bone by manipulating vesicular loading and hence modulating Glu release.Recently in the CNS, the essential molecular component responsible for vesicular packaging of Glu has been identified as the 'brain-specific', sodium-dependent inorganic phosphate cotransporter (BNPI). BNPI encodes a membrane protein with 6-8 putative transmembrane domains localised exclusively to Glu containing synaptic vesicles. These studies have confirmed conclusively that BNPI functions as the vesicular Glu transporter with its expression "sufficing to define the glutamatergic phenotype in neurons". Interestingly, expression of BNPI in neurons that do not normally release Glu is sufficient to make them do so (1). Therefore, the aim of this study was to determine if a similar mechanism for vesicular glutamate packaging exists in osteoblasts analogous to the CNS.Using primers directed to rat and human BNPI sequences, RT-PCR amplified single band products for BNPI in cDNA derived from rat

calvarial osteoblasts and human osteoblasts undergoing differentiation in osteogenic media. Cloning and sequencing confirmed the identity of the PCR amplicons to be 100% homologous to neuronal BNPI. Also, using an antibody raised to the C terminus of the BNPI transporter, western blot analysis revealed protein expression in rat calvarial osteoblasts detecting a ~ 60kDa band corresponding identically to that observed in rat brain. Northern blot analysis of total RNA extracted from whole rat tissues revealed that BNPI mRNA is expressed predominantly (if not exclusively) in bone and brain. Expression levels of BNPI increased during differentiation consistent with previous studies showing elevated glutamate release during osteoblastic differentiation of MC3T3-E1 cells (2nmoles/mg protein on day 3 of culture as compared to 5nmoles/mg protein on day 8). These data suggest that the ability of osteoblasts to release Glu is modulated during osteoblast development. In conclusion we have shown that osteoblasts express BNPI, the key molecular component required to store Glu in vesicles and release it by exocytosis upon stimulation. (1). Nature. 407, 189-194 (2000).

M232

Nitric Oxide Represses RANKL Expression at a Transcriptional Level in Stromal Cells. J. Rubin, T. Murphy,* L. Zhu,* M. S. Nanes, X. Fan. Medicine, VAMC and Emory University, Decatur, GA, USA.

Nitric oxide (NO) is known to have effects on bone remodeling. Several groups have shown that NO decreases osteoclast recruitment in vitro, that inhibition of NO production potentiates bone resorption in rats, and that mice lacking endothelial nitric oxide synthase (eNOS) have decreased bone density. Endogenous NO production also appears to necessary for some of the anabolic effects of loading in the skeleton. We therefore postulated that NO might have a role in regulating osteoclast recruitment through the expression of RANKL. The local expression of RANKL in bone determines the entry of monoblastic precursors into the osteoclast lineage and subsequent bone resorption. RANKL expression is susceptible to both hormonal and mechanical control, as others and we have previously shown. Both primary murine marrow stromal cells and the ST2 stromal cell line were treated with 10 nM vitamin D to raise RANKL expression for use in our studies. Deta-NONOate was used as a long-acting NO donor. In both cell types deta-NONOate maximally decreased RANKL mRNA expression to less than 25% of control values in a dose dependent fashion. The half-maximal effect for reduction of RANKL expression was ~300 uM and 150uM deta-NONOate respectively in primary cells and in ST2 cells. This effect was not due to decreases in RANKL mRNA half-life. Activity of a 965 nt RANKL promoter driving a luciferase reporter (RANKL-luc) was studied in the presence of deta-NONOate. 300 uM deta-NONOate decreased transcriptional activity of RANKL-luc by 50% in ST2 cells; the promoter activity in the absence of vitD was also decreased by the NO donor. Dibutyryl cGMP did not reproduce the NO effect, nor did inhibition of endogenous guanylate cyclase with ODQ prevent NO effect on RANKL mRNA expression, indicating that NO acted in a cGMP-independent fashion. The mechanism whereby NO decreases RANKL may thus involve nitrosylation of nuclear factors controlling RANKL transcription. Our results show that NO is a potent repressor of RANKL expression by bone cells and suggest that processes which regulate NO production, including mechanical factors, may affect bone remodeling through downstream inhibition of RANKL and subsequent osteoclast recruitment.

M233

Pharmacological Studies and Structure-Activity Relationships of 1-Phosphinico-prostaglandin F Receptor Ligands; The Development of New Bone Anabolic Agents. F. H. Ebetino, D. L. Soper,* M. L. Dirr,* M. W. Lundy, G. Mieling,* P. Chmielewski,* C. Froman,* R. Farmer,* J. A. Wos,* M. A. deLong. Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Prostaglandin F analogs have attracted considerable recent interest as superior agents for the lowering of Intraocular Pressure (IOP) pursuant to glaucoma management and, more recently, as potential bone anabolic agents. Early generation anabolic agents in this class are characterized by relatively short half-lives, likely due in part to the C-1 carboxylic acid, which is subject to beta oxidation and may serve as the recognition element for active metabolism. Guided by molecular modeling, we investigated the replacement of the C-1 acid by various suitable functional groups. Most of these replacements resulted in a significant loss of activity at the human FP receptor, the cellular mediator of the effects of the F analogs. For example, the sodium salt of the phosphonic acid analog of PGF2a, 2-decarboxy-2-phosphono-PGF2a, has only a 1.0 micromolar hFP IC50 in our radioligand displacement assay, as compared to 5.0 nM for PGF2a itself. In contrast, in the phosphinic acid series, the sodium salt of compound (1), 1-nor-2-(P-methyl)-phosphino-16-phenoxy-16-tetranor PGF2a, proved to be potent (hFP IC50 = 42 nM) and selective (IC50's > 10,000 nM at all other tested receptors) and was found to increase bone volume above pretreatment animals at 0.3 and 3mg/kg/day for 60 days in an OVX rat model of osteoporosis. The selectivity of these compounds observed for the FP receptor was unique compared to corresponding carbon analogs, and was predicted as a component of the original drug design, due to differences among the various prostaglandin receptors in the binding region of the C-1 carboxylic acid.

M234

Disruption of the Fgf2 in Mice Impairs Prostaglandin E2 Stimulation of Osteoclast Formation and Cyclo-oxygenase 2 mRNA and Protein Expression. T. Sobue,[1] Y. Okada,[2] X. Zhang,[1] J. D. Coffin,*[3] M. M. Hurley.[1] [1]Endocrinology, University of Connecticut Health Center, Farmington, CT, USA, [2]Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan, [3]Pharmaceutical Sciences, University of Montana, Missoula, MT, USA.

Basic fibroblast growth factor (FGF-2) which stimulates osteoclast proliferation and differentiation, is expressed in osteoblasts and is highly regulated by stimulators of osteoclast (OCL) formation including prostaglandin E2 (PGE2). We assessed the role of endogenous FGF-2 in PGE2 mediated OCL formation in bone marrow cultures and co-cultures of calvarial osteoblasts and spleen cells from 6-9 week old wild type (Fgf2+/+) and mice with disruption of the Fgf2 gene (Fgf2-/-). The formation of tartrate resistant acid phosphatase multinucleated OCLs in response to PGE2 (1 μM) was reduced by 65 % (97 ± 7 vs 34 ± 4) in bone marrow cultures from Fgf2-/- mice and these OCLs formed 62 % fewer bone resorption pits on dentine slices. The addition of FGF-2 (10 nM) stimulated OCL formation and completely reversed the reduced OCL formation in response to PGE2 in bone marrow cultures from Fgf2 (-/-) mice. Co-cultures of calvarial osteoblasts and spleen cells showed that OCL formation in response to PGE2 was reduced by 81 % when osteoblasts were derived from Fgf2 (-/-) mice. There was no reduction in OCL formation when spleen cells from either genotype were cultured with osteoblasts from Fgf2 (+/+) mice. FGF-2 and PGE2 are potent stimulators of receptor activator of NF-kB ligand (RANKL) which is critical for OCL formation as well as cyclo-oxygenase (COX-2) which is critical for maximal stimulation of OCL formation by multiple agonists. To determine the mechanism by which PGE2 regulates OCL formation in Fgf2 (-/-) mice, we examined the expression of RANKL, COX-2 and osteoprotegerin (OPG) mRNA in calvarial osteoblasts and marrow stromal cell cultures from both genotypes. Six h of treatment with PGE2 significantly increased RANKL mRNA expression in bone marrow stromal cells from Fgf2+/+ and Fgf2-/- mice. PGE2 treatment also caused a small decrease in OPG mRNA expression in both genotypes. Two h of treatment with PGE2 (1 μ M) induced COX-2 mRNA 9.3 fold in calvarial osteoblasts from Fgf2+/+ mice but only 3.6 fold in calvarial osteoblasts from Fgf2-/ mice. Western blot analysis showed that 6 h of treatment with PGE2 (1 μ M) increased COX-2 protein 7.6 fold in calvarial osteoblasts from Fgf2+/+ mice but only 4.8 fold in calvarial osteoblasts from Fgf2-/ mice. We conclude that endogenous FGF-2 is an essential co-factor for PGE2 to stimulate COX-2 synthesis in osteoblasts.

M235

Effects of Selective Prostaglandin E Receptor Agonists on Transcriptional Regulation of Cyclooxygenase-2 in Primary Osteoblastic Cells. X. Li,[1] C. C. Pilbeam,[1] D. Chikazu,[1] H. Kawaguchi,[2] C. B. Alander,[1] F. N. Woodiel,[1] L. G. Raisz.[1] [1]University of Connecticut Health Center, Farmington, CT, USA, [2]University of Tokyo, Tokyo, Japan.

Induction of cyclooxygenase-2 (COX-2) enzyme expression is necessary for stimulated prostaglandin responses in bone. Prostaglandin E_2 (PGE_2), the major prostaglandin produced by osteoblasts, can amplify its own production by inducing COX-2 expression in osteoblasts. The purpose of this study was to examine transcriptional regulation of the COX-2 gene by novel and selective prostaglandin E (EP) receptor agonists developed by ONO Pharmaceutical Co., Osaka, Japan. We used confluent primary calvarial osteoblastic cells derived from mice transgenic for -371/+70 bp of the murine COX-2 promoter fused to a luciferase reporter (Pluc). Cells were cultured for 7 days with or without NS-398 (0.1 μM), a selective inhibitor of COX-2 activity, to block endogenous prostaglandin production. Luciferase activity was measured in cell extracts and activity was normalized to total protein. COX-2 mRNA expression was examined by Northern blot analysis. Intracellular cyclic 3', 5' adenosine monophosphate (cAMP) was measured by immunoassay in primary osteoblastic cells treated for 15 min in the presence of a phosphodiesterase inhibitor. In the cells without NS-398 pretreatment, PGE_2 and ONO-AE1-259, a selective EP_2 agonist (EP_2A), at 1 μM, stimulated luciferase activity at 4 h, the time of peak response, by 8-to14-fold and 5- to 8-fold, respectively. ONO-AE1-329, a selective EP_4 agonist (EP_4A), stimulated a maximum luciferase response of 5-fold but sometimes had no effect. Butaprost, another EP_2 agonist, and EP_2A had a similar dose response curve, but butaprost was 10 times less potent than EP_2A. On Northern blot analysis, 2 h of PGE_2 (1 μM), EP_2A (1 μM), or butaprost (10 μM) increased COX-2 mRNA expression 17-, 11- and 9-fold, respectively; however, EP_4A (1 μM) only increased COX-2 mRNA 3-fold. In cells pretreated with NS-398, the luciferase response to EP_4A was increased substantially and was similar to the response to EP_2A.In cells without NS-398 pretreatment, PGE_2 and EP_2A at 1 μM increased cAMP production 90- and 43- fold, respectively, while EP_4A at 1 μM increased cAMP production only 8-fold. However, in cells pretreated with NS-398, EP_4A increased cAMP production 37-fold. Thus, the ability of these agonists to stimulate COX-2 mRNA and luciferase activity paralleled their ability to increase cAMP production. We conclude that PGE_2 autoamplifies COX-2 through both EP_2 and EP_4 receptors by a cAMP pathway in primary murine calvarial osteoblastic cell cultures. However, the EP_4 responses may be markedly downregulated by endogenous prostaglandin production.

M236

Cloning, Expression, and Functional Characterization of Three Non-Human Primate EP4 Subtype Prostaglandin E2 Receptors. S. L. Smock,* B. Lu, T. A. Castleberry, T. A. Owen. Cardiovascular and Metabolic Diseases, Pfizer Global Research & Development, Groton, CT, USA.

Prostaglandin E2 (PGE2) is an important mediator of diverse biological functions in many tissues, including bone, and binds with high affinity to four cell surface receptors (EP1-EP4) that are members of the G-protein coupled receptor superfamily. Cloning of the EP4 receptors from human, dog, rabbit, rat, and mouse has revealed a conserved molecule with 7 potential transmembrane domains and a long intracellular carboxy-terminal region that is functionally coupled to adenylate cyclase, resulting in elevated intracellular cyclic AMP (cAMP) levels upon activation. To further investigate the evolutionary conservation of the EP4 receptor subtype, especially in primates, PCR-based strategies were used to clone this receptor from the chimpanzee, cynomolgus macaque, and baboon. Degenerate PCR primers corresponding to the DNA sequences encoding the human, dog, rabbit, rat, and mouse EP4 receptor protein-coding regions were synthesized. Two independent PCR reactions were performed on cDNA reverse-transcribed from chimpanzee peripheral blood cell RNA and cynomolgus macaque spleen or heart RNA or on DNA isolated from baboon spleen and heart cDNA libraries. PCR products were cloned into an expression vector and at least four clones from each PCR reaction were sequenced. The EP4 protein coding region from all three species was contained in an open reading frame of 1470 bp, potentially encoding a 490 amino acid protein with a predicted molecular weight of 53.4 kD. Analysis of this open reading frame revealed >97% identity within the three monkey species as well as between any of them and the human EP4 cDNA. Analysis of the predicted

protein sequences revealed that the cynomolgus macaque and baboon were identical and >98% identical to the chimpanzee and human EP4 proteins. Strikingly, the chimpanzee and human EP4 proteins revealed a >99% identity, varying by only 3 amino acids. All putative transmembrane domains were identical between all four primate species. A consistent change between the human EP4 protein and all other EP4 proteins, including these non-human primate EP4 proteins, is the lack of two amino acids (valine/x) in the intracellular loop 3 of the human EP4. Competition binding studies demonstrated specific displacement by PGE2 with IC50 values between 1 and 20 nM, but no displacement by either butaprost (EP2 selective) or sulprostone (EP1/EP3 selective). Binding of PGE2 resulted in increased levels of intracellular cAMP, with EC50 values between 15 and 25 nM. Together, these data indicate that the EP4 receptor subtype is extremely conserved within the primates, both in sequence and in functional characteristics.

M237

High Dose Lipopolysacharride Stimulates Bone Resorption, but Low Dose Stimulates Bone Formation in Mice Lacking 5-Lipoxygenase. D. L. Carnes, M. A. Flynn,* J. L. Rosser,* R. Q. Green,* L. F. Bonewald. The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Bone resorption in response to lipopolysacharride (LPS) is associated with increases in arachidonic acid metabolism. It is known that LPS not only stimulates prostaglandin production, but also stimulates the production of metabolites of the 5-lipoxygenase (5LO) pathway known as leukotrienes. In order to determine to what extent the bone resorbing effects of LPS are mediated through 5LO metabolites, 250ng / 20µl LPS (E. coli 0111:B4, Sigma, St. Louis, MO) was injected 3 times-a-day for 3 days into the subcutaneous tissue overlying the parietal bone on the right side of calvaria of 5LO knockout and wild-type mice. Vehicle control injections were carried out for both types of mice. Mice (N = 3-5 / group) were euthanized 64 hours after the last injection and the calvaria removed, decalcified and embedded in paraffin for histomorphometric evaluation. Multiple comparison analysis (ANOVA) using Tukey's procedure as the post hoc test showed no significant differences in osteoclast number or resorption parameters between the control and treated mice whether wild-type or 5LO knockout. Surprisingly, osteoblast numbers were increased four-fold ($p < 0.05$), new bone area increased six-fold ($p < 0.05$) and new bone width increased 2.8 fold ($p < 0.05$) following LPS treatment of 5LO knockout mice. No bone formation was observed in wild type mice at the same dose of LPS. Because no resorption was observed in the previous experiment, a second experiment was carried out using a dose 1000 times greater (250µg / 20µl). At this dose there was no evidence of increased bone formation in 5LO knockout mice. Rather significant increases in marrow area ($p < 0.05$), marrow perimeter ($p < 0.05$), and osteoclast number ($p < 0.01$) compared to the vehicle control indicated that bone resorption was stimulated in 5LO knockout mice. These same parameters were similarly increased in wild type mice. These results indicate that the effects of LPS on bone metabolism in calvaria of 5LO knockout mice are dependent upon dose. In summary, high dose LPS stimulated bone resorption in wild-type mice, whereas low dose LPS had no effect. High dose LPS also stimulated bone resorption in 5LO knockout mice, but low dose LPS stimulated formation, suggesting that this dose normally stimulates production of factors that stimulate bone formation in the absence of 5LO metabolites. We hypothesize that low dose LPS stimulates production of low levels of prostaglandin that are responsible for the observed bone formation. This study has implications for the use of 5LO inhibitors, not only to prevent bone loss, but also to potentiate bone formation, especially in the oral cavity.

M238

Cytokine Modulation of Phospholipase A-2 mRNA Levels in Normal Human Osteoblast-like Cells. P. E. Keeting,*[1] F. J. Secreto,*[2] A. Grover,*[2] J. D. Blaha.*[3] [1]Biology, West Virginina University, Morgantown, WV, USA, [2]West Virginia University, Morgantown, USA, [3]Orthopedics, West Virginia University School of Medicine, Morgantown, USA.

Free arachidonic acid is the precursor of a group of bioactive lipids, collectively referred to as the eicosanoids. Some of these are known mediators of bone biology, such as PGE-2, while others are strongly implicated as mediators, such as leukotriene C-4. Arachidonic acid is typically esterified at the Sn-2 position of membrane glycerophospholipids, and as an ester is not a useful substrate for the eicosanoid producing enzyme systems. The release of arachidonic acid from the glycerophospholipids is, thus, a requisite first step in eicosanoid biosynthesis. It is widely held that the calcium-sensitive, cytoplasmic isoform of phospholipase A-2 (Group IV; cPLA-2) is principally responsible for producing free arachidonic acid for eicosanoid biosynthesis. Accordingly, the effects of several cytokines on cPLA-2 mRNA levels in cultured normal human osteoblast-like cells (hOB) have been studied. The cytokines evaluated here have all been shown to modulate eicosanoid production by hOB cells. Intron-exon spanning primers for human cPLA-2 were designed and used in RT-PCR based experiments. hOB cells stimulated for 6 hrs by 40 pM TGF-beta, 30 nM TNF-alpha, or 300 pM IL-1-beta were tested, and the TNF-alpha and the IL-1-beta stimulated samples exhibited increased steady-state levels of the mRNA for cPLA-2 ($p<0.05$, Wilcoxon rank-sum). cPLA-2 mRNA levels increased by 2.6 + 1- fold following TGF-beta stimulation, 7.6 + 3.1- fold following TNFa stimulation, and 7.9 + 3.1- fold following IL-1-beta stimulation. Parallel studies also demonstrated that the steady-state levels of cyclooxygenase-2 were sensitive to cytokine modulation, including TGF-beta. At least in the case of IL-1-beta stimulated hOB cells the increased mRNA levels were accompanied by an increase in cPLA-2 activity (data not shown). Thus, the responses of cytokine-stimulated hOB cells appears to insure substrate availability for eicosanoid biosynthesis. An increased production of PGE-2 by cytokine-stimulated hOB cells is further accounted for by the apparent induction of COX-2 expression, a finding previously described for many responsive cell types.

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Isolation of Osteoprogenitor Cells from Skeletal Muscle. L. Reading,* A. Scutt. Child Health, University of Sheffield, Sheffield, United Kingdom.

Bone diseases such as osteogenesis imperfecta (OI) occur because of the presence of single mutations. One possible treatment for these diseases is the use of transplanted bone marrow-derived mesenchymal stem cells (MSC) stem cells containing a the correct gene. Allogeneic bone marrow transplantation in children with OI achieved a small degree of engraftment (1-2%) but appeared to be accompanied by some clinical improvement. The risk associated with such a procedure is substantial however, autogenic transplantation might offer a safer and more effective therapy. The withdrawal of bone marrow is an invasive procedure and is unsuitable for children with skeletal disorders such as OI. We have therefore been looking for other sources of MSC, one such source being skeletal muscle. Putative MSC were isolated from soleus muscle from 200g male Wistar rats by 3 methods. 1. The muscle were diced into 0.5mm cubes and allowed to grow as explants in DMEM containing 10% FCS, 10-8M dexamethasone, Pen/Strep, Glutamax and ascorbate. The outgrowing cells were then subcultured into larger culture vessels. 2. The diced muscle was digested in collagenase for 1.5h in 1mg/ml collagenase, the cells washed and then grown in the above medium. 3. The cells obtained from the digests were "preplated" by transferring non-adherent cells daily to new petri dishes leaving the adherent cells to grow further. The cells were then examined for the presence of osteoblast-like cells morphologically and by staining for alkaline phosphatase and calcium deposition. All 3 methods reproducibly produced cells which morphologically resembled stromal or osteoblast-like cells, stained positive for alkaline phosphatase and produced calcified nodules. By preplating the digests, fibroblastic cells were removed in the first 1-2 preplates and thereafter the cultures were progressively enriched with a population of small round slow-adhering cells which given the appropriate conditions adopted an osteoblastic phenotype. This process of preplating has been maintained for up to 21 d and still maintains the capacity to give rise to osteoprogenitor cells.These data show that there exists in skeletal muscle a population of primitive slow adhering cells with osteogenic potential. Whether these are true MSC requires further work.

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Osteogenic and Chondrogenic Precursors Associated With the Embryonic Dorsal Aorta. M. Riminucci,[1] M. Minasi,*[2] A. Caprioli,*[3] B. Berarducci,*[2] A. Innocenzi,*[2] T. Jaffredo,*[3] P. Bianco,[4] G. Cossu.*[2] [1]Medicina Sperimentale, Università dell'Aquila, L'Aquila, Italy, [2]Istologia ed Embriologia Medica, Università "La Sapienza", Rome, Italy, [3]Institut d'Embryologie Cellulaire et Moleculaire, CNRS, Nogent sur Marne, France, [4]Medicina Sperimentale e Patologia, Università "La Sapienza", Rome, Italy.

A number of indirect evidences indicate a close association of skeletal precursor cells with microvascular structures in the post-natal skeleton, a direct osteogenic potential of extraskeletal pericytes , as well as a praeternatural osteogenic capacity of vascular wall cells, only apparent in disease. To investigate a potential developmental link between vasculature and skeletogenic progenitors, we isolated the embryonic dorsal aorta from E3 quail embryos and transplanted fragments thereof in the limb bud of developing chicks. Chimeric tissues were harvested at E19 and labelled with a monoclonal antibody recognizing a quail-specific nuclear epitope. Donor cells were found to form a regular smooth muscle coat in arteries and arterioles of the chimeric wing, and to distribute to a host of peripheral tissues along, and in conjunction with, the branching vasculature. Fully differentiated skeletal muscle cells, smooth muscle cells, and skin fibroblasts of donor origin were observed in close topographical association with the most peripheral branches of the chimeric vasculature. The alkaline phosphatase positive perichondrium/periosteum of developing bone rudiments was repleted with donor cells located in an abluminal position around nascent blood vessels. Fully differentiated osteocytes and chondrocytes of donor origin were also easily identified. Similar experiments were performed with cells derived from mouse embryonic aorta. Cells carrying LacZ under a muscle specific promoter were found to develop into mature striated muscle cells in the chimeric wing, whereas cells labelled with DiI were found in cartilage and other tissues. We conclude from these data that progenitors of diverse mesodermal tissues including bone and cartilage are found in the embryonic vasculature, and can be subsequently delivered to peripheral tissues in the fetal, and perhaps post-natal period, via the branching and growth of vascular structures. While the canonical origin of mesodermal tissues from defined embryonic structures such as somites or the lateral mesoderm would account for the formation of skeletal tissue progenitors during embryonic development, vascular progenitors may be pivotal for fetal and post-natal growth of the same tissues, and perhaps account for the origin of skeletal progenitors found in the post-natal marrow microvascular structures (stromal cells).

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Expression Analysis of Human Primary Osteoblasts Exposed to Ethanol. D. Lin,* J. Dai, E. Keller. Unit Lab Animal Medicine, University of Michigan, Ann Arbor, MI, USA.

Chronic alcoholism induces osteoporosis that is associated with decreased osteoblastogenesis. The molecular mechanisms through which ethanol modulates bone remodeling are unclear. To help define the mechanisms that contribute to ethanol-induced osteoporosis, we evaluated ethanol-induced differential gene expression in osteoblasts and bone marrow stromal cells. Accordingly, we exposed either primary human osteoblasts or primary human bone marrow stromal cells to 0 or 50 mM ethanol for 12 hours. Then total RNA was collected and subjected to PCR-select subtraction hybridization to identify differential gene expression. For both cell strains we reciprocally used either 0 or 50 mM ethanol treated cells as driver for the subtraction procedure. Analysis of the subtraction libraries revealed that (1) in osteoblasts, ethanol upregulated 25 genes and downregulated 72 genes and (2) in bone marrow stromal cells, ethanol upregulated 48 genes and downregulated 23 genes. Some of these genes are known, such as collagen alpha type III, which was induced in osteoblast and others are gene of unknown identify (i.e., expressed sequence tags; ESTs). As these genes are identified, we may find that a subset of these genes represent

novel markers for ethanol-induced osteoporosis screening and prognosis as well as potential targets for future drug therapies.

M242

Wnt 1 Induces the Differentiation of Stromal Cells into Mature Osteoblasts. R. C. Pereira,[1] A. M. Delany,[2] E. Canalis.[2] [1]Saint Francis Hospital and Medical Center, Hartford, CT, USA, [2]Saint Francis Hospital and Medical Center and The University of Connecticut School of Medicine, Hartford, CT, USA.

Wnts are a family of genes encoding signaling molecules that play a central role in cell growth and fate during development and tumor progression. Wnt proteins bind to a transmembrane receptor to initiate a signal transduction cascade involving phosphorylation of the cytoplasmic protein beta-catenin or Arm, which can bind and activate target genes. Although Wnt genes are expressed by stromal cells, there is no information about the function of Wnts in stromal cells and whether they modulate their differentiation into osteoblasts. Recent studies revealed that overexpression of Wnt-1 in 3T3 fibroblasts inhibits their ability to undergo adipogenesis. We postulated that Wnt-1 could regulate the differentiation of stromal cells and direct them towards osteoblastic differentiation and away from the adipocytic pathway. To address this question, we transduced murine ST-2 stromal cells with a retrovirus in which the CMV promoter drives Wnt-1 gene expression, and compared them to cells transduced with retrovirus vector alone (both vectors kindly provided by J. Kitajewski, New York, NY). Following selection, ST-2 cells were cultured in the presence of 10% fetal bovine serum, 5 mM β-glycerolphosphate, and ascorbic acid for up to 27 days following confluence. Cells were cultured in the presence or absence of bone morphogenetic protein (BMP)-2, (kindly provided by Genetics Institute), known to induce osteoblastogenesis, or cortisol, known to induce adipogenesis. Northern blot analysis demonstrated increased expression of retrovirus driven Wnt-1 transcripts throughout the culture period. At the time of confluence (0 days) ST-2 cells overexpressing Wnt-1 had increased levels of alkaline phosphatase activity (APA), which were sustained for the entire 27 days of culture. ST-2 cells transduced with vector alone developed mineralizing nodules, as determined by alizarin red staining, 21 days after confluence. In contrast, cell maturation was accelerated in ST-2 cells overexpressing Wnt-1, which developed mineralizing nodules as early as 12 to 15 days. There were no differences in cell number, assessed by hematoxylin staining of the cultures. BMP-2 at 1 nM accelerated and increased ST-2 cell differentiation towards the osteoblastic pathway and had an additive effect to the overexpression of Wnt-1. In contrast, cortisol at 1 μM opposed the differentiation of ST-2 cells into the osteoblastic pathway and the effect of Wnt-1. In conclusion, Wnt-1 is a novel regulator of stromal cell function, acting in concert with BMPs in the induction of osteoblastic cell differentiation and opposing the actions of cortisol.

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Raloxifene Stimulates Mineralized Bone Nodule (MBN) Formation When Added Intermittently to Cultures of SaOS-2 Cells and Osteogenic Stem cells from Normal Human Bone Marrow Preparations (hBMOSC). L. G. Rao, L. J. F. Liu,* T. M. Murray, E. Schemitsch. Department of Medicine, St. Michael's Hospital and University of Toronto, Toronto, ON, Canada.

Raloxifene is an approved drug for the treatment and prevention of osteoporosis. However, its effect on bone formation in vitro has not been studied in osteoblasts of human origin. This study reports the effects of raloxifene (provided by Eli Lilly and Company, Indianapolis) on the differentiation and MBN formation in SaOS-2 cells and hBMOSC. Osteogenic stem cells were cultured from bone marrow preparations obtained from 31 normal donors; 13 males and 18 females, aged 37 to 90 years. Beginning at day 3 or 8 of culture, varying concentrations of raloxifene were added to the cells either continuously or intermittently for 24 h of each 48 hr cycle of medium changes. At day 17, the cells were either fixed, stained with von Kossa reagent and the MBN number and area quantified by an image analyzer, or sonicated for alkaline phosphatase activity (ALP) assay. The data were analyzed statistically using one-way ANOVA followed by Dunnett multiple comparison test. Continuous treatment of SaOS-2 or hBMOSC cells with raloxifene, added from day 3 or day 8, had no effects on MBN number and area and ALP. However, when added intermittently to SaOS-2 cells from day 3 or 8, raloxifene dose-dependently stimulated MBN number and area at concentrations of 10^{-8} to 10^{-6} M. At 10^{-6} M raloxifene, nodule number increased 1.5-fold ($p< 0.01$) while nodule area increased by 2.2 to 2.5-fold ($p<0.01$). ALP was also stimulated by raloxifene at the same concentrations (at 10^{-8} M, $p<0.05$ and at $1x10^{-6}$M, $p<0.01$) when raloxifene was added from day 8 to SaOS-2 cells. When added from day 3, 10^{-6}M raloxifene stimulated ALP by 1.2-fold ($p < 0.01$). Raloxifene at concentrations from 10^{-8} M to 1 x 10^{-6} M significantly stimulated ALP in a dose-dependent manner both, when it was added from day 3 ($p<0.05$) or day 8 ($p< 0.0001$) to hBMOSC cells. There were no differences in the responses of hBMOSC cells obtained from female donors when compared with those obtained from male donors. Intermittent additions to hBMOSC cells from day 8 also resulted in stimulation of MBN number ($p< 0.005$) and area ($p<0.0005$); a maximum of 4-5-fold stimulation was observed in both cases. In conclusion, intermittent, but not continuous, treatment with raloxifene was found to stimulate both differentiation and bone formation in osteosarcoma SaOS-2 cells and normal human osteogenic stem cells cultured from bone marrow preparations. This is the first report of an anabolic effect of raloxifene in a human osteoblast systems.

Disclosures: Eli Lilly and Company,2.

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Diminished Incorporation of Tritiated Thymidine into DNA of MC3T3-E1 Cells Is Related to Inhibition of Thymidine Transport Cause by Extracellular Matrix. W. J. Peterson,[1] K. H. Tachiki,*[2] D. T. Yamaguchi.*[2] [1]GRECC 691/11G, Greater Los Angeles Healthcare System and UCLA School of Medicine, Los Angeles, CA, USA, [2]Research Service, Greater Los Angeles Healthcare System, Los Angeles, CA, USA.

Extracellular matrix (ECM) regulates the basic biological processes of bone cells including metabolism, development, growth and differentiation. ECM regulation of cell behavior depends upon its composition, ability to act synergistically with growth factors and ability to engage receptors on the cell surface. Through these mechanisms cells are able to rearrange their cytoskeleton, change shape, and initiate signal transduction responses which may lead to gene expression involved in growth and function. It is likely that the *in vitro* activity of cells cultured on tissue culture treated plastic may be different from the activity of cells cultured on their natural ECM substrate. We selected MC3T3-E1 cells to study the effect of ECM on processes associated with proliferation because these cells undergo a progressive developmental sequence of proliferation and differentiation. MC3T3-E1 cells were cultured on plastic or on plastic coated with native ECM, fibronectin (Fn), collagen Type I (Col), BSA, or poly-L-lysine (PLL). Their ability to proliferate was assessed by incorporation of [^{3}H] dT into DNA or by enumeration of cells. Our results show that (1) ECM inhibits incorporation of [^{3}H] dT by MC3T3-E1 cells, (2) Col, but not BSA, PLL or Fn also inhibits incorporation of [^{3}H] dT, (3) the level of ECM inhibition of [^{3}H] dT incorporation is directly related to the number of cells cultured, but unrelated to the cell cycle distribution or endogenous thymidine content, (4) the kinetic profile of [^{3}H] dT uptake suggest that ECM inhibits the transport of [^{3}H] dT from the extracellular medium, and (5) cell counts are similar in cultures whether cells are grown on plastic or ECM. These results suggest that ECM inhibits function of the salvage pathway responsible for transporting [^{3}H] dT into cells thus resulting in decrease incorporation of [^{3}H] dT into DNA. Therefore, incorporation of [^{3}H] dT by cells cultured on ECM is not reflective of cell proliferation.

M245

Microarray Analysis of Fetal Calvarial Cell Differentiation Induced by Dexamethasone and Endothelin-1. H. P. von Schroeder, C. J. Veillette.* Dept. of Surgery, University of Toronto, Toronto, ON, Canada.

Vascular endothelial cell factors, including endothelin-1 (ET), induce osteoblast differentiation and bone production. Dexamethasone (Dex) *in vitro* (in the absence of endothelial cells) also induces osteoblast differentiation, but Dex has a negative effect on bone *in vivo* (in the presence of vascular cells). To account for the contradictory effects of Dex, we hypothesize that Dex interferes with vascular endothelial-osteoblast signalling by altering regulatory factors or by activating different pathways within osteoblastic cells. To test this, and to better delineate the effects of ET on osteoblastic cells, microarray analysis of 1700 gene products was performed on RNA isolated from fetal rat calvarial (FRC) cells. Cultured FRC cells were treated with Dex (10^{-8}M), ET (10^{-8}M) or vehicle alone. RNA was collected after 7 days and 14 days of culture, and labelled with fluoro dyes. Array slides were hybridized, and quantified. Data was normalized and processed using criteria and thresholds established from array data from control RNA. ET treatment resulted in a >two-fold upregulation of 18 gene products, Dex treatment upregulated 89 gene products; of these, 8 were upregulated by both ET and Dex. The latter included microtubule-associated protein, endothelial-associated cell-surface glycoprotein, myogenic factor, and 3 intracellular enzymes. Subarray analysis revealed that ET upregulated TGFβ 3 and 4, tumour necrosis factor receptor 4, corticosteroid-binding globulin, osteoblast cadherin, PDGF receptor, osteonectin, decorin and angiopoietin 1 receptor (Tie 1). Of these, TGFβ 4, corticosteroid-binding globulin, and PDGF receptor mRNA were *down*-regulated by Dex, as were TGFβ receptor III, core binding factor α subunit 22, and insulin-like growth factor binding proteins 2 and 6. Dex upregulated endothelin-converting enzyme, Vitamin D binding protein, osteopontin, osteoblast cadherin, estradiol 17 β-dehydrogenase and bone morphogenic protein 6. Of 89 gene products upregulated by Dex at day 7, 25 were also upregulated at day 14. Our data showed that Dex and ET upregulated several common constitutive genes, but also differentially regulated others. This suggests that Dex and ET stimulate osteoblastic differentiation by both common as well as different intracellular pathways. Dex-regulation of factors associated with vascular (ET)-induced pathways may account for the negative *in vivo* effects of Dex on osteoblastic cells.

M246

Human Osteoblasts Express the Receptor RON and Its Ligand MSP Regulates their Differentiation and Activities. C. Camerino,[1] M. Santoro,[2] G. Mori,*[1] P. Marchisio,*[3] G. Gaudino,*[2] A. Zambonin Zallone.*[1] [1]Department of Human Anatomy, University of Bari, Bari, Italy, [2]Department of Medical Sciences, University of Oriental Piedmont, Novara, Italy, [3]Dibit Department of Biological and Technological Research, San Raffaele Scientific Institute, Milan, Italy.

Macrophage stimulating protein (MSP) is a growth factor with chemotactic activity on murine peritoneal macrophages. MSP is synthesized in an inactive form and can be cleaved by members of the coagulation cascade. His receptor is the RON gene product, a transmembrane tyrosine kinase, member of the hepatocyte growth factor receptor family. Ron is expressed in epithelial cells, keratinocytes, granulocytes, monocytes, and osteoclasts. In this study we examined if MSP/RON have a role in human osteoblast (OB) activities. Using specific anti RON C-terminal antibodies, we detected RON expression in OBs. Binding of MSP to RON induced phosphorylation of RON 150 KDa β-chain within 10 minutes. RON activation caused an increase in MAPK and P-AKT activities. MAPK and AKT are 2 different but synergic pathways, both triggering differentiative and anti-apoptotic effects due to the phosphorylation of BAD. The effect of MSP on OBs was mediated by an intracellular calcium increase ($\delta = 75 \pm 15$), often characterized by two subsequent spikes. MSP treatment induced a time-dependent inhibition of proliferation and a strong decrease of alkaline phosphatase activity compared with untreated control. OBs treated by MSP displayed morphological changes characterized by the formation of long cellular extroflexions resulting in an osteocyte-like shape. This study establishes OB as a new target cell for MSP. The action of MSP on OBs may have implication on cell growth, differentiative processes and protection from apoptosis. Further investigations are required to

understand if MSP has a specific direct effect on the final differentiation of OBs in osteocytes.

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Osteogenic Differentiation of Human Mesenchymal Stem Cell Could Be Regulated by β-Catenin Pathway. S. Maeda,[1] K. Shimo-Onoda,*[1] T. Nobukuni,*[1] K. Hayashi,*[2] H. Koga,[2] S. Matsunaga,*[2] K. Yone,*[2] S. Komiya,*[2] I. Inoue.*[1] [1]Division of Genetic Diagnosis, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]Department of Orthopaedic Surgery, Faculty of Medicine,Kagoshima University, Kagoshima, Japan.

Osteoblasts and adipocytes are differentiated from a common mesenchymal stem cell (MSC) in bone marrow. To investigate the molecular mechanisms that regulate osteogenic differentiation in the early stage, we examined gene expression profile with DNA chip microarray, which covers approximately 8000 genes. 24 hours after treatment of human MSC with osteogenic supplements, 2-fold or more increase of expression was detected in 32 genes and 1/2-fold or less decrease was detected in 62 genes. We focused on the genes, which were reported to interact with or involved in signaling of β-catenin (Wnt5A, FRZB, Dickkopf-1, phosphoinositide 3 kinase (PI3K), laminin, integrin a5, mucin 1) and hypothesized that regulation of β-catenin pathway may play roles in osteogenic differentiation. We quantified gene expression of Wnt5A, FRZB, Dickkopf-1 or related genes during osteogenic and adipogenic induction using ABI7700 Sequence Detector and TaqMan chemistry. Osteogenic and adipogenic differentiation were monitored by histochemistry (alkaline phosphatase staining, von Kossa's staining and Oil Red O staining) and measuring osteoblast- or adipocyte-specific gene expression. Osteogenic-specific increase of gene expression of Wnt antagonists FRZB and Dickkopf-1 and decrease of Wnt5A were observed. β-catenin participates in gene transcription by accumulating in cytosol followed by translocating to nucleus. To investigate the functional association of β-catenin with osteogenic or adipogenic differentiation, MSCs were harvested from day 0 to day 11 after differentiation induction and the proteins were subjected to Western blot analysis. By the analysis of cytosolic fraction, gradient increase of β-catenin during osteogenic differentiation and, in contrast, gradient decrease during adipogenic differentiation were observed. Moreover, continuous accumulation of cytosolic β-catenin by treatment with 10 mM lithium chloride inhibited both osteogenic and adipogenic differenriation. In conclusion, these observations provide a potential mechanism involving β-catenin signaling in osteogenic differentiation of MSC.

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Msx2 Is Required for the Enhancement of Osteogenesis in vitro by Novel Osteoblast Differentiation Dromoting Compound, TAK-778. M. Gotoh,* K. Notoya, Y. Ienaga,* M. Kawase,* H. Makino.* Pharmaceutical Research Laboratories I, Takeda Chemical Industries. Ltd., Osaka, Japan.

TAK-778 [(2R,4S)-(-)-N-(4-diethoxyphosphorylmethylphenyl)-1,2,4,5- tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepin-2-carboxamide: mw 505.52], a novel osteoblast differentiation promoting compound, promotes osteogenesis in vitro and enhances new bone formation during skeletal repair in vivo (Notoya et al., J Pharmacol Exp Ther 290:1054-1064, 1999). In this study, we investigated the effects of TAK-778 on the differentiation of cultured bone marrow stromal cells into osteoblast in the presence of dexamethasone, paying particular attention to transcriptional alteration of markers related to the expression of the osteoblast phenotype. The treatment of TAK-778 for 48 hours resulted in increases in both ALP mRNA and osteocalcin mRNA expression in a dose dependent manner. Twenty-four hour exposure had significant effects and the stimulatory effects continued for the 7 days. Under the culture conditions, TAK-778 also stimulated the expression of TGF-beta2 mRNA and IGF-I mRNA on day 7, while an initial 48 hours exposure had no effect on these indices, suggesting that these autocrine/paracrine growth factors are not the initial targets of TAK-778 on osteoblast differentiation. To determine the first target for TAK-778, we examined the effect of TAK-778 on the expression of transcriptional factors in regulating osteoblast differentiation. TAK-778 stimulated the mRNA expression of Msx2, but not Cbfa1 or Dlx5. An initial 4 hours exposure was long enough to upregulate its expression. Osteogenic cells were also transfected with Msx2-antisense to investigate the relationship between TAK-778-induced osteoblast differentiation and the enhancement of Msx2 mRNA expression, and we found that the inhibition of Msx2 mRNA expression caused a significant reduction in the levels of ALP mRNA expression induced by TAK-778. These results suggest that TAK-778 regulated osteoblast differentiation at the transcriptional level in rat bone marrow stromal cell culture and that Msx2, a homeobox-related gene, is required for the stimulatory effects of TAK-778 on osteoblast.

Disclosures: Takeda Chemical Industries, Ltd.,3.

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Evidence for Linkage Between Osteoblast Post-Confluent Cell Growth and Differentiation. M. J. Birnbaum,*[1] E. Smith,*[2] F. Hores,*[1] T. Diliegro,*[1] B. Frenkel.[2] [1]Department of Biology, Merrimack College, North Andover, MA, USA, [2]Department of Biochemistry and Molecular Biology, Institute for Genetic Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA, USA.

We have previously demonstrated the inhibitory effects that glucocorticoids exert on both MC-3T3 osteoblast post-confluent persistent cell cycling, and on formation of a mineralized extracellular matrix (ECM). Linkage between the two inhibitory effects is suggested by the progressively normal differentiation observed in cultures exposed to dexamethasone (DEX) after 1-3 days of DEX-free post-confluent cell cycling. In the present study, we examine glucocorticoid inhibition of both the MC3T3-E1 osteoblasts post-confluent cell cycle and the formation of mineralized ECM during differentiation with regard to the roles of collagen synthesis, apoptosis, hormone dose, and differentiation-related gene expression. The above-mentioned effects likely do not result from the known inhibitory effect of DEX on collagen synthesis, since cells plated on collagen-coated dishes in the presence of DEX exhibit normal cell cycle progression to confluence, but post-confluent mineralization is fully inhibited. This inhibitory effect of DEX on mineral deposition is likely not related to the known proapoptotic effect of glucocorticoids, because DEX did not induce, but instead inhibited apoptosis in differentiating MC3T3-E1 cultures. Further linkage between the osteoblast persistent cell cycle and differentiation is evidenced by the fact that DEX inhibits these two processes with the same concentration curve: no inhibition is observed at 10 nM DEX, whereas inhibition is close to complete at 100 nM and complete at 1 μM. In addition, Northern analysis indicates that as DEX inhibits the post-confluent cell cycle, it also brings about a 5-fold drop in osteoblast differentiation-specific osteocalcin gene expression. These data suggest that osteoblasts may utilize cell cycle regulatory mechanisms to promote osteoblast-specific transcription and differentiation. To test this idea, we stably transfected MC3T3-E1 cells with an osteocalcin promoter-luciferase construct, and examined promoter activity during the cell cycle, following release from starvation. We observed a 12-fold increase in promoter activity 36 hours after cell cycle stimulation, a decrease back to basal level at 42 hours, and an increase back to 20-fold the initial level at 66 hours. Taken together, these results suggest a functional linkage between the post-confluent cell cycle that promotes osteoblast growth to high density (required for nodule formation) and establishment of the osteoblast phenotype in vitro.

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Phosphodiesterase Expression During Rat Calvarial Cell Differentiation. K. S. Holden,* A. L. Mansolf,* D. N. Petersen, P. A. Krasney.* Department of Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT, USA.

Cyclic adenosine monophosphate (cAMP) is a second messenger for several bone anabolic hormones including parathyroid hormone and the E- and D-series prostaglandins. Subsequent to hormonally-stimulated cAMP elevation, cAMP concentrations are returned to low constituitive steady state levels by the hydrolytic action of cyclic nucleotide phosphodiesterases (PDEs). The purpose of this study was to determine the complement of cAMP-hydrolyzing PDE isoforms in rat calvarial cultures during osteoblast differentiation. PDE expression was assessed by RT-PCR using RNA prepared from rat calvarial cells on days 0-19 after culture in differentiation media. Our results indicate that most of the known cAMP-hydrolyzing PDE isozymes are expressed throughout the timecourse of calvarial cell differentiation.

M251

Temporal Gradients in Shear, but not Ramp Flow, Stimulate the Proliferation of Osteoblast-like cells. G. L. Jiang,* C. R. White,* H. Y. Steven,* M. R. Inzunza,* J. A. Frangos. Bioengineering, University of California, San Diego, La Jolla, CA, USA.

Bone cells are subject to interstitial fluid flow driven by venous pressure and mechanical loading. Dynamic mechanical loading induces transients in interstitial fluid flow, subjecting bone cells to large temporal gradients in fluid shear stress. Previously, it was shown that steady and temporal gradients in fluid shear stress stimulate NO release in osteoblasts through distinct biochemical pathways (*JBMR 1999; 14:930-936*). In the present study, we investigated the ability of steady fluid flow shear and temporal gradients in fluid shear to stimulate osteoblast proliferation. Proliferation was assessed by BrdU incorporation and changes in cell number. One hour after fluid shear, pulsatile flow induced a 91±28% increase in BrdU positive cells compared to sham static controls ($p<0.001$). In contrast, ramp flow stimulated only a 8±10% increase in BrdU uptake relative to sham static controls ($p>0.05$). Pulsatile flow significantly increased relative cell number by 28±16% and 67±16% at 1.5 hr and 24 hrs after flow onset ($p<0.005\sim0.05$). Ramp flow had no effect on cell proliferation (-5±5% and 0±5% of static controls over 1.5 hr and 24 hrs, respectively; $p>0.05$). This suggests that mechanical loading regimes that produce sharp transients in fluid shear stress, such as high impact or high-frequency loading, are potent stimuli of bone cell proliferation.

M252

Glucose-dependent Insulinotropic Peptide Stimulates Proliferation and TGF-beta Synthesis in Osteoblastic-like Cells. Q. Zhong,*[1] K. Ding,*[1] A. L. Mulloy,[2] R. J. Bollag,*[1] C. M. Isales.[3] [1]IMMAG, Medical College of Georgia, Augusta, GA, USA, [2]Medicine, Medical College of Georgia and VAMC, Augusta, GA, USA, [3]IMMAG, Medical College of Georgia and VAMC, Augusta, GA, USA.

Glucose-dependent insulinotropic peptide (GIP) is known to increase alkaline phosphatase activity and collagen type I message in osteoblastic-like cells. The effects of GIP on cell proliferation are not known. We first examined the effect of GIP on ^{3}H thymidine incorporation. GIP stimulated ^{3}H thymidine incorporation by almost two fold (GIP nM-0.01: 107±7.9; 0.1: 117±7.1; 1.0: 154±9.4; 10.0: 180±3.6; 100.0: 186±5.5 % of Control-Mean±SEM). Proliferation and differentiation of osteoblasts is known to be modulated by the bone enriched growth factor TGF-beta and in fact, osteoblasts both secrete TGF-beta and have TGF-beta receptors. Parathyroid hormone has been reported to increase both TFG-beta secretion and mRNA in osteoblasts. In order to examine any potential effect of GIP on TGF-beta we first examined TGF-beta secretion from osteoblasts. We found that GIP stimulated release of this factor into the medium (Control: 127.4+4.2; GIP 0.1-1000 nM respectively: 167.8±10.4; 181.1±10.1; 205.4±22.9; 204.5±13.8; 265.9±4; pg/ml Mean±SEM; GIP 10nM was more potent than PTH 10nM in stimulating TGF-beta secretion). TGF-beta message was also increased by GIP (Densitometry [arbitrary units]-Control:95+29; GIP 0.1-10nM respectively: 129.5±17.5; 177.5±49.5; 195±29; Mean±SEM). We next examined the effect of TGF-beta on GIP stimulated proliferation. GIP stimulated ^{3}H thymidine incorporation was potentiated by a TGF-beta neutralizing antibody (Control: 6699±577, Anti-TGF-beta neutralizing antibody 10ng/ml 7995±210, GIP 10nM 16781±915, GIP 10nM± Anti-TGF-beta neutralizing antibody 10ng/ml 19,572+408 CPM

+Mean+SEM). In summary, GIP stimulates ^{3}H thymidine incorporation and TGF-beta secretion from osteoblastic-like cells. Thus, there appears to be an autocrine feedback system in which GIP stimulated TGF-beta secretion modulates GIP stimulated ^{3}H thymidine proliferation in osteoblastic-like cells.

M253

Insulin-like Growth Factor Binding Protein (IGFBP)-6 Is a Strong Inhibitor of Osteoblast Differentiation: Evidence for an IGF-Independent Intracrine Mechanism. D. D. Strong,[1] T. Yan,*[2] T. A. Linkhart,[1] D. J. Baylink,[1] S. Mohan.[1] [1]MDC, Pettis VAMC, Loma Linda, CA, USA, [2]Mayo Clinic, Rochester, MN, USA.

Recently, we discovered that BP-6 strongly inhibited alkaline phosphatase (ALP) activity (a marker of differentiation) in human osteoblasts (hOBs) without affecting proliferation. This is an important observation because there is no other known cytokine which directly acts to inhibit hOB differentiation. These findings led us to propose and test the hypothesis that BP-6 broadly inhibited hOB differentiation under basal and stimulated conditions. Stable transgenic (TG) SaOS-2 cells expressing increased BP-6 mRNA and BP-6 protein in the conditioned medium (CM) were prepared. All of the clones exhibited a 90% decrease in differentiation measured as ALP activity, and ALP and type I procollagen mRNA under basal and stimulated conditions (IGF-II, 1,25 D and TGF-b). We reasoned that adding sufficient amounts of exogenous IGF-II would eliminate the effect of BP-6 on differentiation in the TG clones. However, doses of IGF-II up to 300 ng/ml did not abrogate the action of BP-6 on differentiation. These unexpected findings led to the second hypothesis that BP-6 acted through an IGF-independent intracrine mechanism to inhibit hOB differentiation. Accordingly, we determined if BP-6 was present inside the cell. Western immunoblots with the CM and cell lysates of TG clones and normal hOBs treated with retinoic acid (a strong stimulator of BP-6) showed that up to 10 fold more BP-6 was in the cell lysates than in the corresponding CM. Furthermore BP-6 was found in nuclear extracts by radioimmunoassay. Because BP-3 and BP-5 have nuclear localization signals (NLSs) and BP-6 has a similar basic region (Pro^{188}-Pro^{211}), we determined if BP-6 was also translocated into the nucleus. Plasmid expression vectors with full-length BP-6 or the putative 24 amino acid BP-6 NLS were fused to red fluorescent protein (RFP). The corresponding 24 amino acid BP-4 region was also fused to RFP as a negative control. These constructs were transfected into SaOS, U-2 OS and normal human bone cells. Live cells examined by fluorescence microscopy showed that only full-length BP-6-, BP-6-NLS- and BP-3-NLS-RFP fusion proteins translocated into the nucleus in all of the hOBs tested. In conclusion, 1) BP-6 is a strong inhibitor of hOB differentiation under basal and stimulated conditions; 2) The action of BP-6 to inhibit hOB differentiation cannot be abrogated by added IGF-II, suggesting an IGF-independent mechanism; 3) BP-6 is translocated into the nucleus where it could mediate its inhibitory effect on hOB differentiation by an intracrine mechanism.

M254

PGE2 Enhances BMP-2-Stimulated Osteoblast Differentiation in Human Bone Cells Through EP2/EP4-cAMP/PKA Signaling Pathway. T. Takiguchi,*[1] M. Kobayashi,*[1] T. Takada,*[1] A. Yamaguchi,[2] K. Hasegawa.*[1] [1]Periodontics, Showa university, Tokyo, Japan, [2]Oral Pathology, Nagasaki university, Nagasaki, Japan.

Bone morphogenetic protein (BMP) stimulates osteoblast differentiation in various types of cells. We also have reported that BMP-2 stimulated osteoblast differentiation in human bone cells isolated from mandibules (HBC), and this action of BMP-2 was differentially modulated by several cytokines and growth factors. Prostaglandins (PGs) are also important local factors with an autocrine/paracrine role in bone metabolism. In this study, we examined the effect of PGE2 on the BMP-2-stimulated osteoblast differentiation in HBC and the mechanisms by which mediated the PGE2 action. Treatment with BMP-2 (500 ng/ml) for 72 hours significantly stimulated alkaline phosphatase (ALP) activity in HBC. The BMP-2-stimulated ALP activity was significantly enhanced by simultaneous treatment with PGE2 (10-10-10-7 M). The stimulatory effect of PGE2 was inhibited by co-treatment with a protein kinase A (PKA) inhibitor, N- [2-(p-bromocinnamylamino) ethyl]-5-isoquinolinesulfonamide (H89; 20 μM), but not by co-treatment with a PKC inhibitor, 3-{1-[3-(dimethylamino) propyl]-1H-indol-3-yl}-4-(1H-indol-3-yl)-1H-pyrrole-2, 5-dione (GF109203X; 2 μg/ml). Conversely, cAMP-elevating agents, such as forskolin (10 μM), dibutyryl (dBt)-cAMP (1 mM) and 3-isobutyl-1-methylxanthine (IBMX; 0.1 mM), significantly enhanced the BMP-2-stimulated ALP activity. HBC constitutively expressed mRNA of 4 PGE receptor subtypes (EP1, EP2, EP3 and EP4). None of 4 EP agonists could cause appreciable changes in ALP activity in HBC. However, treatment with PGE2 (10-8 M), EP2 agonist (ONO-AE1-259-01; 10 μM) or EP4 agonist (ONO-AE1-329; 10 μM) for 10 minutes significantly elevated cAMP accumulation, and simultaneous treatment with these agonists and BMP-2 for 72 hours also significantly enhanced the ALP activity stimulated with BMP-2 alone. The stimulatory effect of these agonists on BMP-2-stimulated ALP activity was inhibited by co-treatment with H89, whereas GF109203X had no effect on it. These results suggest that PGE2 enhanced BMP-2-stimulated osteoblast differentiation in human bone cells isolated from mandibules through EP2/EP4-cAMP/PKA signaling pathway.

M255

Overexpression of PPARgamma2 Down-Regulates Osteocalcin Expression in Osteoblast. M. J. Jeon,*[1] S. H. Kwon,*[1] S. Y. Kim,*[1] J. Y. Choi,[2] C. S. Shin.[1] [1]Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea, [2]Biochemistry, Kyungpook National University School of Medicine, Taegu, Republic of Korea.

Mesenchymal cells are able to differentiate into several distinct cell types, including osteoblasts and adipocytes. The commitment to a particular lineage may be regulated by specific transcription factors. Peroxisome proliferator activated receptor 2 (PPARγ2) in conjunction with C/EBPα has been suggested as a key regulator of adipogenic differentiation. This study was conducted to evaluate whether overexpression of PPARγ2 suppresses osteoblast differentiation and osteocalcin expression in osteoblast. ROS 17/2.8 cells were stably transfected with either PPARγ2-pcNDA3 or pcDNA3. Expression of osteocalcin (OC) mRNA was decreased >50% in cells overexpressiong PPARγ2 relative to vector-transfected cells. Likewise, alkaline phosphatase mRNA was decreased in PPARγ2 expressing cells. Treatment of cells with 5 μM of Troglitazone did not show further changes. Transient cotransfection of OC promoter-luciferase construct and PPARγ2 expression vector demonstrated that OC transcription was down-regulated by PPARγ2 cotransfection in ROS 17/2.8 and MC3T3-E1 cells. Activity of other less specific osteoblast promoters, such as osteopontin and osteonectin, was less sensitive to overexpression of PPARγ2. In conclusion, PPARγ2 negatively regulates the trancriptional activity of OC in osteoblast. This action of PPARγ2 on the OC promoter may be direct or indirect. Further studies are under way to elucidate the precise mechanism of PPARγ action on the transcriptional activity of OC.

M256

The Fetal Origins of Peak Bone Mass: Maternal Protein Deficiency Affects Mesenchymal Stem Cell Activity in the Developing Offspring. R. O. C. Oreffo, B. Lashbrooke,* N. M. P. Clarke,* C. Cooper. Musculoskeletal Research Group, University of Southampton, Southampton, United Kingdom.

Skeletal ageing is associated with a progressive decrease in bone mass, but there is considerable variation in the degree of bone loss as a consequence of genetic, environmental and nutritional factors. Epidemiological studies have suggested that skeletal growth is programmed during intrauterine and early postnatal life. Maternal nutrition appears to be important in determining skeletal size through fetal adaptation of endocrine and metabolic systems. In this study, a rat model of maternal protein insufficiency was used to investigate the cellular mechanisms involved in the programming of bone development. We examined whether colony formation (colony forming unit-fibroblastic, CFU-F), proliferation and differentiation of bone marrow stromal cells from offspring of female rats maintained on normal (18% casein) or low (9% casein) protein diets was altered and / or their response to the key endocrine factors, Growth Hormone (GH), $1,25(OH)_2D_3$ and IGF-1. Dams were fed an 18% casein control diet or 9% casein low protein diet from conception until the end of pregnancy. Offspring (n=40) were fed a normal protein diet until harvest at 4, 8 12 and 16 weeks after birth. The results (see table) indicate that normal proliferation and differentiation

Difference in bone activity: 9% compared to 18% group

Time (weeks)	Total CFU-F	CFU-F AP+ve	Alk. Phos specific activity
4	-21%**	-41%**	-60%***
8	-38%**	-90%**	-92%***
12	+22%	-9%	+220%*
16	+211%***	+349%	+7,287%***

*P<0.05, **P<0.01, ***P<0.001

of mesenchymal stem cells was delayed by restricted maternal nutrition during early life. At 12 weeks, no significant differences were observed in colony formation. Modulation of osteoblast proliferation and differentiation was observed in cells from the 18% group by $1,25(OH)_2D_3$, IGF-1 and GH at 8 weeks and the low protein group at 12 weeks. Bone mineral measurements by DXA confirmed decreased bone mineral content in the low protein offspring. The data indicate that at 16 weeks, with skeletal maturity, "catch-up" or a physiological shift in bone cell activity was observed in the low protein group. In summary, growth trajectory and bone growth appear to be programmed in early life and indicate the key role of maternal nutrition on programming of skeletal development with consequences in later life.

M257

Expression of Signal-Transducing Leptin Receptors on Rat Osteoblasts Indicates a Direct Involvement of Leptin in the Regulation of Bone Formation. Y. J. Lee,*[1] J. H. Park,*[2] J. S. Ko,[1] H. M. Kim.[1] [1]College of Dentistry and Intellectual Biointerface Engineering Center, Seoul, Republic of Korea, [2]Korea Research Institute of Bioscience and Biotechnology, Taejon, Republic of Korea.

Leptin is a 16 kDa non-glycosylated protein that is expressed in adipocytes. It acts in the brain to suppress appetite and to regulate body weight and energy expenditure. Surprisingly, the overall bone mass in leptin-deficient or leptin receptor-deficient mice is dramatically elevated despite their obese and hypogonadal phenotype, of which cause could be tracked back to an increased osteoblast activity. To determine whether leptin might directly act on osteoblasts, in the present study, leptin receptor expression was analyzed in primary rat osteoblasts and an established osteoblast cell line. In addition, the effect of recombinant leptin which was produced in E.coli, on osteoblast differentiation was studied in vitro. The biological activity of recombinant rat leptin was confirmed by i.p. injection into normal SD rats, which showed then loss of appetite and a drastic decrease in body weight. Total RNA was isolated from osteoblasts isolated from neonatal rat calveriae and the ROS 17/2.8 by the acidic guanidium isothiocyanate method. The possibility of contamination with genomic DNA was eliminated by treating the isolated RNA with RNase-free DNase. Reverse transcription-PCR was performed with oligonucleotide primers specific for each of the various leptin receptor isotypes, whereby GAPDH-specific PCR primers were used as control. Identity of every amplified PCR product was confirmed by nucleotide sequencing. A clear signal for leptin receptor expression was observed in rat primary osteoblasts and ROS17/2.8 cells in contrast to preceding reports. Interestingly, both the leptin receptor (OB-R) a- and b-isoform were detected in osteoblasts, which are so far known the only isoforms capable of transmitting leptin-mediated signals into the cell among the 6 reported

OB-R types (a-f). Rat osteoblasts treated with recombinant rat leptin showed a modulation of osteoblast activity. These results indicate that leptin not only binds to but also signals into osteoblasts, which would represent under physiological situation a direct repressive signal of leptin to osteoblasts.

M258

The Role of Immunosuppressant FK506 on Osteoblast Differentiation and Its Application to Tissue Engineering in Bone. T. Uemura,[1] M. Lee,[2] J. Dong,[3] H. Kojima,[4] D. Iejima,*[5] T. Yoshikawa,*[6] H. Ryoo,[2] P. Wang,*[5] T. Tateishi.*[7] [1]Tissue Engineering Research Center(TERC), National Institute of Advanced Industrial Science and Technology(AIST), CREST JST, Tsukuba, Japan, [2]Department of Oral Biochemistry, Kyungpook National University, Taegu, Republic of Korea, [3]Fudan University, Shanghai, China, [4]TERC, AIST, Domestic Research Fellow JST, Tsukuba, Japan, [5]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba Ibaraki, Japan, [6]Nara Medical University, Kashihara, Japan, [7]University of Tokyo, Tokyo, Japan.

Immunosuppressant FK506 is an effective drug for organ transplantation. Recently, Yoshikawa et al. have reported that the osteogenic potential of FK506 on cultured allogenic bone in porous hydroxyapatite(JBMR 15,1147,2000). To further srudy the role of FK506 in osteoblast differentiation, we examined the expressions of CBFA1 and several osteoblast marker genes and intracellular calcium signaling in UMR106 osteoblastic cell line. Furthermore, we examined the osteogenic potential of FK506 as in vitro osteogenic supplemental in implantation model system. Osteoblastic UMR cells were cultured in alpha-MEM with the supplement of Dex(dexamethasone) and FK506, or no treatment. mRNA expressions of rat oseocalcin(OCN), alkaline phosphatase(ALP), osteopontin(OP) and CBFA1 in UMR cells were detected by northern blot analysis. Intracellular calcium in UMR cells was observed by detecting fluorescence of calcium green using confocal microscope. In the implantation model system, rat bone marrow derived osteoblastic primary cells were cultured in vitro with supplement of Dex and/or FK506, then subcutaneously implanted into rat with artificial porous hydroxyapatite. After several weeks, ALP activity, OCN content of harvested implants were examined with histological analysis. Northern blot analysis indicated that FK506 plus Dex induced the highest expression of CBFA1 in UMR cells at day 3. At the same time point, ALP and OP were strongly induced by Dex plus FK506. These results suggest that FK506 signaling induced CBFA1 expression to promote the osteoblastic differentiation. Detailed study is now under progress, comparing with the result of intracellular calcium signaling coming from calcium release from ER. In the implantation model system, at 4 weeks post implantation, the composites with FK506 plus Dex showed highest level of osteogenic parameters(ALP and OCN) and bone formation was observed together with active osteoblasts. At 8 weeks, it still showed higher level of osteogenic parameters, maintaining progressive bone formation. These results suggest that FK506 plus Dex induces osteogenic potential of osteoblast in vitro and in vivo.

M259

Connective Tissue Growth Factor (CTGF) Promotes Osteoblast Differentiation and Stimulates Bone Formation. S. N. Popoff,[1] F. F. Safadi,[1] M. D'Angelo,[1] A. H. Selim,*[1] V. Zakhaleva,*[1] S. L. Smock,*[2] T. A. Castleberry,[2] B. Lu,*[2] S. C. Marks,[3] T. A. Owen.[2] [1]Anatomy and Cell Biology, Temple Univ. School of Medicine, Philadelphia, PA, USA, [2]Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT, USA, [3]Cell Biology, Univ. of Massachusetts Medical School, Worcester, MA, USA.

Connective tissue growth factor (CTGF) is a secreted, extracellular matrix-associated protein that regulates diverse cellular functions. CTGF mRNA expression and protein production has been demonstrated in various cell types including fibroblasts, endothelial cells, chondrocytes, and most recently by our lab, in osteoblasts. In primary cultures of osteoblasts, CTGF mRNA levels exhibit a bimodal pattern of expression being relatively high during proliferation and increasing again to peak levels as the cells terminally differentiate. Furthermore, the protein is synthesized by osteoblasts and secreted into the medium. For this study, we generated recombinant rat CTGF (rCTGF) and examined its effects in primary rat osteoblast cultures. Since the mitogenic effect of CTGF has been universally demonstrated in various cell types, we first examined its effect on cell proliferation and, as expected, rCTGF showed a dose-dependent increase in cell proliferation. We also examined the effects of rCTGF on various functional parameters associated with osteoblast differentiation; rCTGF significantly increased alkaline phosphatase activity, osteocalcin gene expression and calcium deposition/matrix mineralization. Based on the results from osteoblast cultures, we tested its capacity to induce bone formation *in vivo* using a local delivery system that has been used to test the anabolic effect of other known osteoinductive agents. Adult male rats (12-16 weeks of age) were anesthetized, the distal femur was surgically exposed and 1 microgram of rCTGF in 20 microliters saline was injected into the marrow cavity; control femurs were injected with the same volume of saline or 1% BSA. One week later, the animals were euthanized and femurs removed for radiographic or histological analyses. Recombinant CTGF-injected femurs showed increased radiodensity within the marrow cavity compared with control-injected femurs. Histologically, the rCTGF-injected femurs had islands of newly formed woven bone within the marrow cavity; the bony trabeculae were lined with rows of active, cuboidal osteoblasts and labeled intensely with calcein. There was no evidence of an osteogenic response in any of the control-injected femurs. Collectively, data from these experiments establish that CTGF plays an important role in regulating osteoblast differentiation and bone formation.

M260

Osteoactivin: A Novel Factor That Regulates Osteoblast Development and Function. F. F. Safadi,[1] A. H. Selim,*[1] V. Zakhaleva,*[1] R. Kanaan,*[1] A. Ravindra,*[1] M. D'Angelo,[1] S. L. Smock,*[2] T. A. Owen,[2] S. N. Popoff.[1] [1]Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA, [2]Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT, USA.

Osteoblast development is a complex process involving the expression of specific growth factors and regulatory proteins that control cell proliferation, differentiation and maturation. We have previously identified a novel gene, termed osteoactivin (OA), which is more highly expressed in the bones of *osteopetrotic* mutant rats compared to their normal littermates. OA is a glycoprotein of 572 amino acids with a predicted molecular weight of 63.8 kDa and has 13 potential sites for *N*-linked glycosylation. In this study, we further examined OA expression in bone and its role in the differentiation of osteoblasts in primary cultures. In a comparison of long bones and calvaria with other tissues, Northern blot and RT-PCR analyses showed that OA is most highly expressed in bone compared with any of the other non-osseous tissues examined. *In situ* hybridization and immunohistochemical analyses of OA in normal bone revealed that it is primarily expressed in osteoblasts. In primary rat osteoblast cultures, OA showed a temporal pattern of expression, being expressed at highest levels during the later stages of matrix maturation and mineralization. To further study the properties of the OA protein, primary osteoblasts were cultured for 1 week, fixed, and incubated with a polyclonal antibody raised against amino acids 551-568 at the C-terminal end of the protein. OA co-localized with the endoplasmic reticulum and Golgi apparatus, suggesting that OA is a secreted protein. These results were confirmed by the detection of osteoactivin in the conditioned medium of primary osteoblast cultures by Western blot analysis. To test the effects of OA on osteoblast differentiation and function, we used an anti-OA antibody to block the OA that is constitutively produced in culture. Treatment with neutralizing OA-antibody inhibited nodule formation and mineralization in a dose-dependent manner. Collectively our results demonstrate that OA is a novel protein that is produced by osteoblasts and plays an important role in regulating osteoblast development and function.

M261

Adult Human Dental Pulp Stem Cells (DPSCs) Can Differentiate into Neural-like Cells in vitro. S. Shi,* P. G. Robey, S. Gronthos.* Craniofacial and Skeletal Diseases Branch, National Institutes of Health, Bethesda, MD, USA.

In previous studies, we used adult human dental pulp tissue to isolate and characterize a population of cells with stem cell characteristics both in vitro and in vivo. These dental pulp stem cells (DPSCs) can differentiate into functional odontoblasts to regenerate a dentin/pulp-like complex following xenogeneic transplantation. Using human specific probes, odontoblasts formed in the transplants were clearly of human origin. However, due to the histological complexity of the pulp-like tissue, could not be determined with any certainty if DPSCs have the capacity to differentiate into other cell lineages in the transplant. A histological survey of intact dental pulp revealed that it contains prominent nerve fibers, penetrating into the tubular dentin structures along the cellular processes of odontoblasts. This system of nerve fibers infiltrating deep within the dentin matrix allows teeth to receive external stimulation, and most certainly contributes to the pain experienced when the enamel/dentin barrier is compromised by caries or trauma. During development, odontoblasts are presumed to originate from neural crest cells. For these reasons, we asked the question of whether or not DPSCs can differentiate into neural-like cells. A low, but significant percentage of individual ex vivo expanded DPSCs were shown to constitutively express nestin, and glial fibillary acid protein (GFAP), which are characteristic of neural precursor cells and glial cells, respectively, at both the mRNA and protein levels. When cultured under defined neural culture conditions, DPSCs appeared to form long cytoplasmic processes and rounded cell bodies in contrast to their usual bipolar fibroblastic morphology, and the percentage of cells expressing nestin and GFAP increased dramatically. Moreover, DPSCs cultured under neural inductive conditions were found to express the neuron specific marker, neuronal nuclei (NeuN), by immunohistochemical staining. In conclusion, this study provides the first experimental evidence that adult human DPSCs can differentiate into neural-like cells with expression of nestin, GFAP, and NeuN in vitro.

M262

Stimulation of Osteoblastic Activity from Bone Marrow Cells by in Vitro Application of FK506, a Potent Osteogenic Agent. J. Dong,[1] H. Kojima,[2] T. Uemura,[3] T. Yoshikawa,*[4] T. Tateishi.*[5] [1]Tissue Engineering Research Center (TERC), National Institute of Advanced Industrial Science and Technology (AIST), (Fudan University), (CREST JST), Tsukuba, Ibaraki, Japan, [2]TERC, AIST, (Domestic Research Fellow, JST), Tsukuba, Ibaraki, Japan, [3]TERC, AIST, (CREST JST), Tsukuba, Ibaraki, Japan, [4]Nara Medical University, Kashihara, Nara, Japan, [5]University of Tokyo, Tokyo, Japan.

FK506 has been used as immunosuppressive drug in clinical organ transplantation. Many studies have shown that this drug generally cause bone loss when administered at high doses over the long term. The goals of the current study were to assess the in vitro osteogenic differentiation of rat marrow-derived mesenchymal stem cells (MSCs) by FK506, and to characterize the effect of changes in the microenvironment upon the process. MSCs derived from primary passage were cultured for 16 days in α-minimal essential medium Eagle containing 15% fetal bovine serum, antibiotics, 0.05 mM L-ascorbic acid-2-phosphate (AsAP), 10mM β-glycerophosphate (βGP), plus 5 to 5000nM FK506, and with or without 100 nM Dexamethasone (Dex). Cultures were examined using phase-contrast microscope, histochemistry (for alkaline phosphatase activity), bone nodule assay, northern blot analysis of osteocalcin mRNA, calcium assay, cell proliferation assay, scanning electron microscopes. Osteogenic differentiation, as determined by osteoblastic morphology, expression of alkaline phosphatase (APase), modulation of osteocalcin mRNA production, and the formation of a mineralized extracellular matrix containing hydroxyapatite was achieved with FK506. Optimal osteogenic differentiation was achieved with α-modified eagle's MEM plus 0.05 mM AsAP, 10 mMβGP, 100nM Dex, and 50nM FK506. FK506 can collaborate with Dex on osteogenic differentiation.

M263

Inhibition of Adipogenesis by Cytokines with Suppression of PPARγ Function Through TAK1/TAB1-NIK Promotes Osteoblastogenesis. M. Suzawa,[1] I. Takada,*[1] J. Yanagisawa,*[1] Y. Takeuchi,[2] Y. Gotoh,*[1] K. Matsumoto,*[3] S. Kato.[1] [1]IMBC, University of Tokyo/ CREST, Tokyo, Japan, [2]School of Medicine, University of Tokyo, Tokyo, Japan, [3]Department of Molecular Biology, Nagoya University, Nagoya, Japan.

Composition of mesenchymal cells in bone marrow is critically involved in bone formation. Accumulation of adipocytes differentiated from mesenchymal cells along with loss of osteoblasts with advancing age in bone marrow is one of characteristic features in senile osteoporosis. Thus, it is important to elucidate the regulatory mechanisms for differentiation of adipocytes as well as osteoblasts in bone marrow cells. Peroxisome proliferator-activated receptor γ (PPAR γ) is a member of nuclear receptor superfamily that plays a pivotal role for adipocyte differentiation. The present study was undertaken to clarify how bone resorbing cytokines, TNF-α and IL-1, modulate adipogenesis and then influence osteoblastogenesis. To address this issue, we examined effects of TNF-α and IL-1 on PPAR γ transactivation and differentiation of mouse bone marrow stromal cell line ST2 into adipocytes and osteoblasts. TNF-α and IL-1 inhibited adipogenesis induced by a PPARγ-ligand, troglitazone and enhanced osteoblastogenesis only in the presence of BMP-2. These cytokines suppressed the PPAR γ transactivation by troglitazone. To explore molecular mechanisms of the transcriptional suppression, we investigated the responsible intracellular signalling pathway to find that the ligand–induced transactivation of PPAR γ was suppressed by IL-1 and TNF-α. This suppression was mediated by NF-κB, and its upstream signaling cascade of TAK1/TAB1-NIK(NF-κB-inducing kinase) was required for the suppression. NF-κB activation prevented PPAR γ binding to DNA sequence PPRE, which could be a direct molecular mechanism. Our results suggest that IL-1 and TNFα are involved in the down-regulation of adipogenesis and in the subsequent up-regulation of osteoblastogenesis in bone marrow. PPARγ could be a molecular target of these cytokines through the TAK1/TAB1-NIK signaling cascade, and it is suggested that the suppression of PPAR γ transactivation makes mesenchymal cells prone to differentiate into osteoblasts with preventing adipocytic differentiation.

M264

The Effects of Lycopene, a Carotenoid Antioxidant from Tomato, on the Growth and Differentiation of SaOS-2 Cells In-Vitro. L. Kim,*[1] A. V. Rao,*[1] L. G. Rao.[2] [1]Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada, [2]Department of Medicine, St. Michael's Hospital and University of Toronto, Toronto, ON, Canada.

Epidemiological studies have shown that antioxidants including vitamins C, E and beta-carotene may play a role in the prevention of osteoporosis. Lycopene, one of the most potent carotenoid antioxidants naturally present in many plant foods and abundant in tomatoes, has not yet been studied in relation to bone health. The present study reports the effect of lycopene on the proliferation and differentiation of the human osteosarcoma SaOS-2 cells. The cells were cultured in Ham's F-12 medium, supplemented with 10% FBS and antibiotic. After 24hrs, different concentrations (10^{-6}, 10^{-5} and 10^{-4} M) of a water dispersible lycopene (LycoRed Natural Products Industries Ltd., Israel) were added and the cells were further cultured for 24, 48, 96 and 144 hrs. Respective vehicle control of similar dilution for each concentration of lycopene was also evaluated. The cells were counted in triplicate with a hemacytometer at each time point. Our data showed that lycopene treatment had a significantly ($p<0.05$) higher cell number than the corresponding vehicle treatment as follows: after 96 hrs, by 25.36% at $1x10^{-6}$ M and 20.27% at $1x10^{-5}$ M; after 144 hrs, by 21.01% at $1x10^{-6}$ M and 25.36% at $1x10^{-5}$ M. In another experiment, we tested the effect of lycopene on the proliferation and differentiation of SaOS-2 cells at different stages of differentiation in the presence and absence of dexamethasone (Dex). At day 1, 3 or 6 of culture, varying concentrations of lycopene (10^{-7}, 10^{-6} and 10^{-5} M) and their respective vehicles were added, and then further cultured for 24, 48 and 72 hrs. Our results indicate that Dex enhanced alkaline phosphatase activity (ALP) in a time-course manner indicating differentiation of the cells. In the absence of Dex, lycopene had an inhibitory effect on ALP. In the presence of Dex, lycopene was also inhibitory when added at day 1 for 24 to 72 hrs ($p<0.05$ to $p< 0.005$). However, when added at day 3, there were significant stimulatory effects on ALP after 24 hrs (both above 70 % at $1x10^{-6}$ and $1x10^{-5}$ M, $p<0.05$), and at day 6 after 24 hrs (34.29% at $1x10^{-5}$ M, $p<0.05$). In summary, our results showed that lycopene has stimulatory effects on the growth of SaOS-2 cells in a dose dependent manner and an effect on ALP that is dependent on the stage of cell differentiation. This is the first report of the effects of lycopene in osteoblasts of human origin. We conclude that these results may have implication for a significant role for lycopene in regulating osteoblast activity, which may be important in the prevention of osteoporosis due to oxidative stress.

M265

The Effect of FGF-2 on the Proliferation and Differentiation of Human Bone Marrow Stromal Cells to Osteoblastic Lineage Following Bone Marrow Transplantation : Comparison with Normal Subjects. M. Kang,[1] W. Lee,*[1] E. Oh,*[1] K. Oh,*[2] B. Cha,*[1] K. Lee,*[1] H. Son,*[1] S. Kang.*[1] [1]St. Mary's Hospital, The Catholic University Medical College, Seoul, Republic of Korea, [2]Mizmedi Hospital, Seoul, Republic of Korea.

Fibroblast Growth Factor-2 (FGF-2) is known to be a potent mitogen of marrow stromal cells and bone cells. According to our previous study, bone marrow stromal cells (BMSCs) were of recipient origin following BMT (bone marrow transplantation) and osteoblastic differentiation from bone marrow stromal cells was significantly delayed in the bone marrow recipients compared to those of normal subjects. We investigated the effect of FGF-2 on the proliferation and differentiation of human BMSCs in both post-BMT patients and normal subjects. Bone marrow from healthy donors and BMT recipients were aspirated and mononuclear cells including marrow stromal cells were separated and cultured in osteogenic medium. At near confluence, human BMSCs were subcultured. Cells in the secondary culture were divided by two groups according to the period of FGF-2 treatment. One is a group for the early effect of FGF-2 (Day 7-11) and the other is for the late effect of FGF-2 (Day 14-18). In each group, cells were treated with no FGF-2 (control), 5 ng/ml of FGF-2 and 50 ng/ml of FGF-2, respectively. Cell proliferation rate, histochemical staining with cell morphology and alkaline phosphatase activity were observed in each group. The size and numbers of colnonies (CFU-fALP) were significantly lower in post-BMT patients than those of normal subjects. The morphology of osteoblast-like cells differentiating from BMSCs, was different between control and FGF-2 treated groups. Cells cultured in 20% FCS alone became flattened in polygonal shape, whereas FGF-2 treated cells maintained a fibroblast-like elongated spindle shape and more definite cell contour. In primary culture, cells treated with FGF-2 had much larger size of colonies than those of control group in both normal and post-BMT patients. In normal subjects, proliferation rate measured by [3H]-thymidine uptake was higher in FGF-2 treated group than control group, especially in the early period of FGF-2 treatment. But in post-BMT patients, this effect of FGF-2 was observed in both early and late period of FGF-2 treatment. Alkaline phosphatase activities in BMSCs treated with FGF-2 were lower than control during whole culture period in both normal subjects and post-BMT patients. We conclude that the number of marrow CFU-f that include osteoblast precursors were lower in post-BMT patients and FGF-2 treatment in these patients promotes the proliferation of osteoblast-like cells, but could delay the osteoblastic differentiation in this culture system.

M266

Mechanisms of Regulation of Gq/11 Alpha Protein by Dexamethasone in Osteoblastic UMR 106-01 Cells. R. Cheung, J. Mitchell. Department of Pharmacology, University of Toronto, Toronto, ON, Canada.

We have previously demonstrated that in the rat osteosarcoma cell line UMR 106-01 glucocorticoids increase the expression of $G_{q/11}\alpha$ protein and PTH-mediated PLC activation (Mitchell and Bansal Am. J. Physiol. 273:E528, 1997). In order to determine the mechanisms responsible for this effect, we have studied the actions of dexamethasone on both $G_q\alpha$ and $G_{11}\alpha$ proteins. Glucocorticoid treatment slightly increased the level of $G_q\alpha$ protein by 55% but significantly elevated the $G_{11}\alpha$ protein expression by 395%. Therefore, we focused on the effect of dexamethasone on $G_{11}\alpha$ protein, steady-state mRNA level and their turnover rates. Incubation of UMR cells with dexamethasone for various time indicated that $G_{11}\alpha$ protein level was elevated over 72 hrs of treatment. Dexamethasone treatment increased the expression of $G_{11}\alpha$ protein in a dose-dependent manner. Steady-state $G_{11}\alpha$ mRNA level was also increased by glucocorticoid treatment, however, induction occurred relatively slowly, with a lag period between 12 to 24 hrs before any effect was observed. Measurement of $G_{11}\alpha$ mRNA turnover by incubation of cells with the transcription inhibitor 5,6-dichlororibofuranosylbenzimidazole (DRB), indicated dexamethasone slightly increased degradation of $G_{11}\alpha$ mRNA. The dexamethasone induction of $G_{11}\alpha$ mRNA occurred after a time lag 12-24 hrs and was blocked by the protein synthesis inhibitor cycloheximide. Analysis of the rate of decay of ^{35}S-labeled $G_{11}\alpha$ protein demonstrated a slower rate of degradation in the cells treated with dexamethasone. Glucocorticoid treatment significantly increased the half-life of $G_{11}\alpha$ protein from 26.4 to 80 hrs, a three-fold increase compared to the control cells. These results suggest that the dexamethasone-induced rise in $G_{11}\alpha$ protein results primarily from changes in the degradation rate of the protein while changes in $G_{11}\alpha$ mRNA play a smaller role and require *de novo* synthesis of regulatory protein(s).

M267

Treatment With Parathyroid Hormone Analogs and Parathyroid Hormone Related Protein Increase Receptor Activator of NF-kappaB Ligand Protein in the UMR-106 Osteoblastic Osteosarcoma Cell Line. D. A. Dossing,* P. H. Stern. Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL, USA.

Osteoclast maturation and function are regulated by factors expressed and/or secreted by the osteoblast in response to various hormones and cytokines. The factor thought to be responsible for this regulation is receptor activator of NF-kappaB ligand (RANKL). A number of bone resorptive factors, including parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP), have been shown to increase RANKL mRNA expression in stromal cells as well as in osteosarcoma cell lines. While these previous studies examined the effects of PTH on mRNA expression, the effects of PTH as well as the other resorptive factors on RANKL protein expression are just beginning to be examined. The purpose of this study was to investigate the effect of the PTH analogs PTH 1-34 and PTH 3-34 as well as PTHrP on RANKL protein expression. The widely used rat osteoblastic osteosarcoma cell line, UMR-106, was used for these studies. The cells were cultured for 48 hours in the presence of PTH 1-34, PTH 3-34 or PTHrP. After treatment, the cells were lysed and RANKL protein levels were examined using Western blotting analysis utilizing RANKL specific antibodies. The results indicate that PTH 1-34, PTH 3-34 and PTHrP treatment cause an increase in RANKL protein expression in the UMR-106 cell line. The results of cell fractionation studies revealed the presence of two forms of RANKL. A band corresponding to the molecular weight of the full length cell associated form of RANKL, about 40-45 kDa, was observed in the cytosolic fractions, while a band corresponding to the molecular weight of the processed, soluble form of RANKL, about 30 kDa, was observed in the membrane fractions. The results show that the UMR-106 cell line expresses RANKL protein, that two forms of the protein can be observed in these cells and that PTH 1-34, PTH 3-34 as well as PTHrP can regulate the expression of RANKL protein.

M268

Inhibition of TGF-Beta Signaling by Glucocorticoid Receptor. X. Shi, X. Cao. Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

TGF-beta and glucocorticoid have important, but directly opposing effects on bone metabolism. TGF-beta is one of the most abundant of the known growth factors stored within the bone. During the remodeling cycle, osteoclasts resorb bone, TGF-beta is released and it stimulates new bone formation. Glucocorticoid, on the other hand, opposes

this effect. Compelling evidence shows that long term glucocorticoid treatment causes bone loss and results in osteoporosis in 30-50% of the affected patients. To investigate the molecular basis of the antagonistic effect between these two agents, we studied the effect of glucocorticoid receptor (GR) on the TGF-beta signal transduction pathway. TGF-beta induces expression of osteopontin, an osteoblast marker gene. Using osteopontin-promoter luciferase construct as a reporter, we found that in the transient transfection assays, TGF-beta induces osteopontin-promoter activity more than 5-fold. To test whether the DNA binding of Smad3, the mediator of TGF-beta signaling, is required for the transactivation, we performed electrophoretic mobility shift assays. Using affinity purified GST-Smad3 and a labeled osteopontin gene promoter fragment as a probe, we found that Smad3 binds specifically to the osteopontin promoter. Interestingly, GST-GR, which by itself did not bind to this probe, dose dependently inhibited Smad3 binding when coincubated with GST-Smad3 in the binding reaction. Consistent with the binding results, when glucocorticoid receptor was coexpressed with 3TP-Lux, a TGF-beta responsive luciferase reporter, GR repressed TGF-beta-induced reporter activity. These data demonstrate that the inhibition of TGF-beta signaling by glucocorticoid occurs at the step where Smad3 DNA binding is disrupted, and that this disruption most likely is mediated by the interaction between Smad3 and GR.

M269

Parathyroid Hormone Stimulates Insulin-Like Growth Factor Binding Protein-5 mRNA Expression by Both PKA and PKC-δ-dependent Mechanisms in Rat Osteoblast-Like Cells. M. S. Erclik,* J. Mitchell. Department of Pharmacology, University of Toronto, Toronto, ON, Canada.

Parathyroid hormone (PTH) regulation of osteoblasts has been shown to mediate both anabolic and catabolic processes in bone. This dual functionality of PTH is thought to be in part the result of the PTH receptor's coupling to both the protein kinase A (PKA) and protein kinase C (PKC) signal transduction pathways. We have investigated the role of these pathways in PTH's regulation of the production of insulin-like growth factor binding protein-5 (IGFBP-5) in the rat osteoblast-like cell line, UMR106-01. Consistent with previous findings we have demonstrated that rPTH(1-34) was able to induce IGFBP-5 mRNA. Induction was also observed with the PTH analogs (1-84) and (1-31). Our data demonstrates that this induction by PTH is partially mediated by PKA as forskolin and 8Br-cAMP were able to mimick the effect of PTH and H-89 was able to partially block PTH's induction. The involvement of the PKC pathway in PTH-induced IGFBP-5 mRNA was confirmed by the findings that bisindolylmaleimide-I and cherylethrine choride, two non-isozyme specific inhibitors of PKC were able to partially block the effect of rPTH (1-34). To further substantiate the involvement of the PKC pathway, 10^{-7}M b(3-34)PTH, an analog of PTH which activates PKC but not PKA was able to significantly stimulate IGFBP-5 transcript levels. To determine the specific PKC isozyme(s) responsible for PTH's effects, we assessed the ability of PTH to induce the translocation of PKC isozymes between cellular compartments. We have observed that on the basis of subcellular fractionation studies, 100 nM concentrations of the PTH analogs r(1-34), h(1-84), b(1-31), b(3-34) and b(53-84) were all able to stimulate the translocation of PKC-δ from the membrane to the nuclear fraction. PKC-δ's involvement in PTH induction of IGFBP-5 mRNA, was confirmed by the finding that rottlerin, a PKC-δ-specific inhibitor was able to inhibit the PTH effect. These results suggest that the induction of IGFBP-5 by PTH is both PKA and PKC dependent, and that PKC-δ is likely the primary mediator of PTH's effects via the PKC pathway.

M270

Lysophosphatidic Acid-Induced Ca^{2+} Signaling in Osteoblasts Employs Sphingosine-1-Phosphate as a Second Messenger. J. M. Lyons,* N. J. Karin. Biological Sciences, University of Delaware, Newark, DE, USA.

Lysophosphatidic acid (LPA) is a potent mitogen in many cell types, including osteoblastic cells. Our previous results (Lyons and Karin, submitted) demonstrated that LPA elicits rapid elevations in cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) in MC3T3-E1 cells via release of the ion from the endoplasmic reticulum (ER). Furthermore, LPA-induced Ca^{2+} signaling was blocked by pertussis toxin, indicating a role for G protein-coupled plasma membrane receptors. Lysophospholipids are known to bind receptors of the *Edg* family and our earlier studies revealed expression by osteoblasts of *Edg*-1 and *Edg*-5 receptors for extracellular sphingosine-1-phosphate (SPP). We employed RT-PCR to determine that MC3T3-E1 cells express *Edg*-2, *Edg*-4 and *Edg*-7 transcripts that encode LPA-specific, G protein-coupled receptors. G protein-coupled receptors typically are linked to the generation of the intracellular Ca^{2+} signaling agent inositol 1,4,5-trisphosphate (IP_3). However, using the Ca^{2+}-sensitive fluorescent dye, fura-2, we showed that LPA elevated $[Ca^{2+}]_i$ in MC3T3-E1 cells that had been treated with 2-aminoethoxydiphenyl borate, an inhibitor of IP_3-gated Ca^{2+} channels in the ER. Thus, LPA-induced Ca^{2+} signaling in osteoblasts appears not to involve IP_3 as a second messenger. SPP is both an extracellular signaling agent and an intracellular agonist of Ca^{2+} release from the ER that is generated by phosphorylation of sphingosine. Pretreatment of MC3T3-E1 cells for 10 minutes with the sphingosine kinase inhibitor dimethylsphingosine (30μM) led to a greater than 90% inhibition of LPA-induced $[Ca^{2+}]_i$ elevations. Therefore, our data suggest that LPA binds one or more G protein-coupled *Edg* receptor types (2, 4, 7) which leads to the cytoplasmic generation of SPP as an agonist of Ca^{2+} release from the osteoblast ER.

M271

Parathyroid Hormone Stimulates Chemotaxis of Human Osteoblasts via Protein Kinase C Pathway. L. R. Halstead, C. F. Lai, S. L. Cheng. Div. of Bone and Mineral Diseases, Dept. of Med., Washington Univ. School of Med., St. Louis, MO, USA.

Parathyroid hormone (PTH) has been shown to have anabolic effects on bone when administered intermittently at low doses. The mechanisms governing these anabolic effects remain elusive, however. Since osteoblast precursors reside in the bone marrow compartment, their migration from marrow to trabecular bone surfaces constitutes an important process in bone formation. We hypothesized that PTH exerts its anabolic effects in part via increased osteoblast migration and examined the signal transduction mechanisms mediating this effect. PTH 1-34 induces human osteoblast chemotaxis on type I collagen in a dose-dependent manner with optimum chemotaxis occurring at 10^{-10} M. Incubation of osteoblasts with calphostin C to inhibit protein kinase C activity prevents osteoblast chemotaxis induction by PTH 1-34. Conversely, phorbol myristate ester, a protein kinase C stimulator, induces osteoblast migration. PTH 3-34, which activates the protein kinase C pathway without eliciting the cAMP/protein kinase A signaling, also enhances osteoblast chemotaxis. By contrast, forskolin, which activates protein kinase A, inhibits osteoblast migration to well below the control cell levels. These combined data affirm the role of protein kinase C in the PTH induction of osteoblast chemotaxis. Phosphatidylinositol 3-kinase (PI3K), Erk, and p38, which are down-stream effectors of protein kinase C and are stimulated by PTH, also play important roles in chemotaxis. Incubation of osteoblasts with their respective inhibitors (wortmannin, PD-98059, and SB203580) down-regulates PTH effects. Similarly, Cdc42, a Rho GTPase family member responsible for the rearrangement of cytoskeleton and filopodia formation, is essential in PTH-induced chemotaxis. Transduction of osteoblasts with a dominant negative Cdc42 protein (N17Cdc42) conjugated to a TAT sequence inhibits this PTH effect. Integrin α2β1, which mediates cell interaction with collagen, also plays a key role in PTH-induced chemotaxis on type I collagen since PTH fails to stimulate osteoblast migration in the presence of anti-α2 antibody. The α2β1 level on osteoblast surface, however, is not altered by PTH. In conclusion, we have demonstrated that PTH 1-34 serves as a chemotactic agent for osteoblasts and that this property is mediated via protein kinase C, PI3K, Erk, p38, Cdc42, and integrin α2β1 signaling.

M272

Age-Related Changes in Bone Formation in Response to C-Type Natriuretic Peptide (CNP) and the Expression of Receptors for CNP in the Cultures of Calvarial Cells from Rats of Various Ages. H. Kaneki, H. Ide. Pharmaceutical Sciences, Toho University, Funabashi, Japan.

Recently, it has been shown that CNP stimulates the differentiation and mineralization of MC3T3-E1 cells and osteoblast-like cells from newborn rat calvariae. These biological effects of CNP mainly mediate with the production of intracellular cGMP through the guanylyl cyclase-coupled receptor termed NPR-B, and the effect of CNP is suppressed by NPR-C, which is known to play a role in the clearance and metabolism of CNP. The purpose of this study is to determine the age-related changes in the stimulatory effect of CNP on bone formation and the expression of NPR-B and NPR-C in the cultures of rat calvarial osteoblasts-like cells. CNP significantly increased markers for osteoblastic differentiation, alkaline phosphatase activity, collagen synthesis, mineralized bone nodule formation and osteocalcin expression, in a dose-dependent manner in the cells from 5- and 25-week-old rats, although the stimulatory effects of CNP was not observed in the cells form 90-week-old rats. A membrane-permeable cGMP analogue, 8-bromo-cGMP, increased markers for osteoblastic differentiation in a dose-dependent manner regardless of the cell donor. The expression level of NPR-B was decreased and that of NPR-C was increased with aging, although age-related change in the expression level of CNP was not observed. In conclusion, age-related change in the expression of receptors for CNP may play an important role in age-related decrease in osteoblastic functions.

M273

1a, 25-dihydroxyvitamin D3 Enhances and Prolongs Growth Hormone Signaling Via JAK/STAT5 System in Osteoblast-like Cells. O. Morales,*[1] M. Hedengran Faulds,[2] J. U. Lindgren,*[1] L. A. Haldosen.*[2] [1]Department of Orthopedic Surgery, Karolinska Institutet, Stockholm, Sweden, [2]Medical Nutrition, Novum, Karolinska Institutet, Stockholm, Sweden.

Growth hormone (GH) is an important growth factor for normal postnatal longitudinal bone growth. GH is known to activate several intracellular signaling pathways, among them JAK/STAT pathway. In the majority of studies GH has been shown to activate JAK2 and STAT5.We have previously shown that the rat osteoblast-like osteosarcoma cell line UMR 106 expresses functional GH-JAK2/STAT5 signaling (J Bone Miner Res 2000;15:2284-2290). In that study physiological concentrations of GH were shown to activate JAK2 and both isoforms of STAT5, STAT5a and STAT5b. In the present study we have investigated the effects on GH/JAK2/STAT5 signaling in 1a ,25 (OH)2 D3 pretreated UMR 106 osteosarcoma cellsCells were exposed to 100 nM 1a ,25 (OH)2 D3 for 48 h before stimulation with GH. Tyrosine phosphorylation, i.e. activation of JAK2 and STAT5 was analyzed with Western Blot. DNA binding capacity of STAT5 was analyzed with gel electrophoretic mobility shift assay (GEMSA) and STAT5 functional activity with STAT5 regulated reporter gene construct. We found, both with Western blot and GEMSA, that 1a,25 (OH)2 D3 greatly enhanced and prolonged GH signaling via JAK2/STAT5. Interestingly, we also found that pretreatment of cells with 1a ,25 (OH)2 D3 was necessary in order to detect GH-induced activation of STAT5 responsive reporter construct.This study shows for the first time that long term stimulation of UMR 106 osteoblast-like cells with 1a ,25 (OH)2 D3 enhances and prolongs GH signaling via the JAK2/STAT5 pathway and renders these cells the capacity to transcriptionally respond to GH.

M274

Involvement of RhoA in Parathyroid Hormone-Mediated PKCα Signaling in Osteoblasts. J. M. Radeff,*[1] S. M. Sebti,*[2] P. H. Stern.[1] [1]Molecular Pharmacology and Biological Chemistry, Northwestern University, Chicago, IL, USA, [2]Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA.

Protein kinase C (PKC) has been shown to be important for mediating parathyroid hormone (PTH) signaling in osteoblasts. The current studies were undertaken to further characterize factors regulating the PKC response. There is evidence from many studies suggesting that small G protein- and PKC-mediated signaling pathways converge. In previous studies, we reported that PTH elicits membrane translocation of the calcium- and diacylglycerol-dependent PKCα and β isozymes in UMR-106 osteoblastic cells. This effect of

PTH on PKC translocation is inhibited by Clostridium difficile Toxin B, which antagonizes small G proteins of the Rac, Rho, Cdc42 families. We also observed that PTH promotes membrane localization of RhoA, a small G protein that controls cytoskeletal organization. In the current study, we examined the effect of RhoA on PTH-induced localization of PKCα in osteoblastic cells. Immunofluorescence was used to determine localization of endogenous PKCα. Transient transfection of UMR-106 cells with a constitutively active RhoA (RhoA63L) mimicked the effect of PTH 1-34 to promote membrane localization of PKCα. In cells expressing a dominant negative RhoA (RhoA19N), PTH 1-34 failed to induce membrane translocation of PKCα. To determine whether the actions of RhoA on PKCα intracellular localization were mediated upstream or downstream of diacylglycerol generation, we examined the effects of Clostridium difficile Toxin B on the PKCα translocation elicited by the diacylglycerol mimetic phorbol-12,13-dibutyrate (PDBu). Clostridium difficile Toxin B failed to inhibit the effect of PDBu, suggesting RhoA acts upstream of diacylglycerol. To further define the signaling pathway by which PTH acts on PKCα, studies were carried out with PTH 3-34, which does not activate cAMP in UMR-106 cells. PTH 3-34 stimulated translocation of PKCα with a similar dose and time dependence as PTH 1-34. The effects of PTH 3-34 on PKCα were also blocked by Clostridium difficile Toxin B. The findings indicate that a) the small G protein RhoA is involved in the pathway by which PTH stimulates PKCα translocation in UMR-106 osteoblastic cells, b) that RhoA acts upstream of diacylglycerol generation, and c) that these effects are independent of cAMP.

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Adhesion of Primary Osteoblasts to Fibronectin Activates Focal Adhesion Kinase and Promotes Cell Survival Signaling. E. A. C. Almeida,*[1] D. Sutijono,*[1] C. H. Damsky,*[2] R. K. Globus.[1] [1]NASA Ames Research Center, Moffett Field, CA, USA, [2]University of California, San Francisco, CA, USA.

In previous studies we identified fibronectin as a key extracellular matrix component that is required for the differentiation and survival of primary osteoblasts. Our present goal is to investigate the signaling pathways that are activated by the adhesion of primary osteoblasts to fibronectin. We used primary fetal rat calvarial osteoblasts plated onto fibronectin-coated substrates in medium without additional growth factors or serum. Osteoblasts plated onto uncoated plastic for 24h underwent extensive apoptosis (65% apoptotic/total cells), whereas only 10% of the cells plated onto fibronectin-coated dishes were apoptotic. We then examined the function of Focal Adhesion Kinase (FAK), a key molecule associated with activation of the fibronectin/integrin signaling complex. Using immunoprecipitation followed by immunoblotting with phosphospecific antibodies, we showed that FAK was phosphorylated at Y397 during adhesion of osteoblasts to fibronectin. Furthermore, immunostaining revealed that the pY397 kinase-active form of FAK was localized to focal adhesions in a manner that was sensitive to mechanical loads. To disrupt FAK function, we over-expressed in adenoviral expression vectors either dominant negative FAK (Focal Adhesion Targeting region of FAK or GFP-FAT) or full length GFP-FAK. FAT acts by localizing to focal adhesions and displacing endogenous, full-length FAK. We observed that over-expression of GFP-FAT over 72h caused extensive apoptosis of primary osteoblasts (70%). Overexpression of full-length GFP-FAK caused an intermediate level of apoptosis (30%), whereas over-expression of GFP alone had no effect (5%). We interpret these results to indicate that replacing wild-type FAK with GFP-FAT in focal adhesions interrupts kinase-mediated, signaling pathways necessary for cell survival. Over-expression of GFP-FAK may function by sequestering cytoplasmic signaling molecules that bind FAK, thereby preventing their activation by fibronectin in focal adhesions. In conclusion, fibronectin supports survival signaling pathways in osteoblasts via FAK activation within focal adhesions.

M276

Calcium Permeability Changes in Rodent Osteoblasts Treated with $1,25(OH)_2D_3$. J. J. Bergh,* M. C. Farach-Carson. Department of Biological Sciences, University of Delaware, Newark, DE, USA.

1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) elicits both a long-term nuclear receptor-mediated and a rapid membrane response in target osteoblasts. In culture, increases in deposition of osteoid, a bone-specific matrix component, are detectable 24-48 hrs after treatment with $1,25(OH)_2D_3$. Within millisecs of exposure to $1,25(OH)_2D_3$, a rapid increase in Ca^{2+} influx occurs via extracellular calcium influx through L-type voltage-sensitive calcium channels (VSCCs). This increase in influx leads to elevation in intracellular calcium levels. Electrophysiological studies have shown that L-type VSCCs are responsible for most of the Ca^{2+} influx into osteoblasts treated with $1,25(OH)_2D_3$. Previous studies in our laboratory and others showed that expression and activity of the $Ca_V1.2$ subunit of the VSCC modulates permeability to Ca^{2+} in the proliferating osteoblast. In this study, we use RT-PCR and confocal microscopy to show that mRNA and protein levels for the $Ca_V1.2$ subunit decrease more than two-fold following 24 hr exposure to $1,25(OH)_2D_3$. Interestingly, Ca^{2+} influx data shows that total Ca^{2+} permeability to depolarization does not decrease with down-regulation of $Ca_V1.2$. Here we provide new data that Ca^{2+} permeability of the osteoblast treated with $1,25(OH)_2D_3$ is maintained through the up-regulation of other VSCCs, including the T-type VSCC.

M277

Accelerated Bone Turnover in Mice Overexpressing Cathepsin K Results in Retarded Bone Growth and Increased BMD of the Diaphyseal Cortical Bone. R. Kiviranta,* J. Morko,* H. Karra,* E. Vuorio, J. Rantakokko.* Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland.

Cathepsin K is a lysosomal cysteine proteinase that is abundantly expressed in osteoclasts. Several recent reports indicate that cathepsin K is essential for normal osteoclastic bone resorption. Pycnodysostosis is an inherited osteopetrotic disease that is linked to mutations in cathepsin K gene. Similarly cathepsin K-deficient mice develop osteopetrosis due to inability of the osteoclasts to degrade organic bone matrix. We have recently described that overexpression of cathepsin K in mice leads to accelerated turnover of metaphyseal cancellous bone and further to metaphyseal osteopenia. Purpose of this study was to further characterize the bone phenotype of these transgenic mice. For the present study, UTU17.2 mouse line overexpressing cathepsin K gene was chosen. Both male and female mice homozygous, heterozygous and negative for the transgene locus were studied at the age of 12 weeks. The whole left hind limbs were collected for pQCT measurements. The limbs were subsequently cleaned of soft tissues for radiography and biomechanical testing. X-rays were used for measurement of bone dimensions with MCID camera and image analysis software. The femurs were used for cantilever bending and the tibias for 3-point bending. The right tibias were decalcified and embedded in paraffin for histology. The right femurs were embedded in methylmethacrylate for histomorphometry. Serum samples were prepared for ELISA analysis of type I collagen cross-links and osteocalcin. Measurements of the x-rays revealed that both the tibias and the femurs of homozygous and heterozygous mice were shorter than in their control littermates indicating retarded bone growth in both sexes. pQCT measurements clearly showed that trabecular BMD was statistically significantly lower in homozygous females. In males the effect was seen in metaphyseal cortex were the cortical BMD, BMC and cortical thickness were significantly lower than in their control littermates. Surprisingly, analysis of diaphyseal scans revealed that total BMD was increased in both males and females homozygous for the transgene. The cortices were thickened, more pronouncedly in females. Three point bending test of the diaphyseal region of the tibias revealed that the ultimate failure load was somewhat higher in the homozygous females (p=0.153). Overexpression of cathepsin K results in growth retardation of long bones, possibly due to the premature degradation of the cartilaginous and/or calcifying spiculae. Accelerated turnover of metaphyseal bone leads to compensatory reaction in diaphyseal region via an unknown mechanism.

M278

Enhanced Bone Resorption in Osteoclasts Overexpressing Cathepsin K. J. Morko,*[1] M. Mulari,*[2] K. Ivaska,*[2] E. Vuorio,[1] K. Väänänen,[2] T. Laitala-Leinonen.*[2] [1]Department of Medical Biochemistry and Molecular Biology, Institute of Biomedicine, University of Turku, Turku, Finland, [2]Department of Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland.

Cathepsin K is a lysosomal cysteine proteinase, which is expressed abundantly in osteoclasts. This enzyme is capable of degrading the extracellular matrix proteins of bone, including type I collagen, osteopontin and osteonectin. In addition to resorption lacunae, cathepsin K has been detected in intracellular vesicles, granules and vacuoles throughout the cytoplasm. In humans, mutations in the cathepsin K gene cause pycnodysostosis, an autosomal recessive disease characterized by an osteopetrotic phenotype. Cathepsin K knock-out mice develop osteopetrosis of the long bones and vertebrae due to insufficient degradation of the bone matrix. Inhibition of cathepsin K activity by selective or general cysteine proteinase inhibitors and by antisense oligonucleotides has been shown to reduce bone resorption. We have recently shown that overexpression of cathepsin K in transgenic mice leads to accelerated turnover of metaphyseal trabecular bone. The present study was designed to describe the bone resorption capacity of osteoclasts derived from these transgenic mice. Two-day old mice heterozygous and homozygous for the transgene locus and nontransgenic controls were used as a source of osteoclasts, which were cultured on bovine cortical bone slices. Cathepsin K transcript levels were studied by Northern hybridization. The localization of cathepsin K, gelatinase B (matrix metalloproteinase 9), tartrate-resistant acid phosphatase and type I collagen were studied by immunofluorescence confocal microscopy. The number of osteoclasts and the number and area of resorption pits were determined. Resorption pits were analyzed by field emission scanning electron microscopy. The levels of degradation products of bone proteins were measured in the culture medium. Increased levels of cathepsin K mRNA were seen by Northern hybridization in osteoclasts from transgenic mice. Immunohistochemistry showed a dramatic increase in cathepsin K staining both in osteoclasts and in the resorption pits. Bone slice analysis demonstrated enhanced osteoclastic bone resorption. This was further supported by the findings that the amounts of bone protein degradation products were increased in culture medium. The current data demonstrates that excessive cathepsin K expression alone leads to enhanced bone resorption by osteoclasts. In transgenic mice this enhanced bone resorption resulted in high turnover osteopenia of metaphyseal trabecular bone.

Disclosures: Leiras Oy,2.

M279

Tartrate-resistant Acid Phosphatase Destroys Bone Degradation Products in Transcytotic Vesicles of Osteoclasts and Foreign Compounds in the Antigen Presentation Route of Macrophages. J. M. Halleen, S. Räisänen,* S. L. Alatalo,* M. Mulari,* V. Parikka,* J. Vääräniemi,* K. Väänänen. Department of Anatomy, University of Turku, Turku, Finland.

Bone-resorbing osteoclasts and activated macrophages, two phagocytosing cell types originating from the same hematopoietic stem cells, express high amounts of tartrate-resistant acid phosphatase (TRAP), an enzyme with unknown biological function. In addition to its acid phosphatase activity, TRAP is capable of producing highly destructive reactive oxygen species (ROS). We show here that the ROS generated by TRAP are able to destroy type I collagen, the main protein in bone matrix. A polyclonal antibody generated using highly purified human osteoclastic TRAP as antigen was used for intracellular localization of TRAP in osteoclasts and macrophages. In resorbing osteoclasts, TRAP was localized in transcytotic vesicles transporting endocytosed bone matrix degradation products from the resorption lacuna to the functional secretory domain in the basolateral membrane. An analogous transport route is involved in the antigen presentation process of macrophages, where antigenic peptides are bound to MHC II molecules and transported to cell membrane for T-cell recognizition. To study the function of TRAP in macrophages, we generated a murine macrophage-like cell line RAW-264 overexpressing TRAP. This cell line produced significantly elevated levels of ROS compared to its parental cell line, and it had an increased capacity of bacterial killing. Double-staining experiments in alveolar macrophages revealed colocalization of TRAP with MHC II and with phagocytosed Staphylococ-

cus aureus. These results suggest that ROS generated by TRAP destroy bone degradation products in transcytotic vesicles of osteoclasts and foreign compounds in the antigen presentation route of macrophages. Thus, TRAP may have an important biological function in both osteoclastic bone resorption and in the defense mechanism of macrophages.

M280

Longitudinal pQCT Analysis of Cathepsin K Null Mice Reveals Increased Bone Mass and Strength Indices. S. J. Hoffman, M. W. Lark, M. Gowen, G. B. Stroup. MusculoSkeletal Diseases, GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, USA.

Cathepsin K (EC 3.4.22.38) has been shown to play an important role in bone matrix degradation during osteoclastic bone resorption. In the current study, longitudinal analysis by pQCT of both wild-type (Cat K +/+) and cathepsin K null (Cat K -/-) mice is examined to study the long term effects of cathepsin K deletion on bone mass and strength.Analysis by pQCT was performed at the proximal metaphysis and the distal tibia. Monthly scans were performed from one month of age to six months of age and then at 7.5, 10, and 12 months of age. Body weights and tibial lengths were also measured.Cat K +/+ mice had significantly higher body weights throughout the study than Cat K -/- mice. Tibial lengths, as determined from the distance between the proximal and distal tibio-fibular junctions, were significantly longer in the Cat K +/+ mice for the duration of the study (excluding 12 months of age).At the proximal metaphysis, total cross-sectional area tended to be higher in the Cat K -/-mice ($p<0.05$ at 2 months of age). Total bone mineral content (BMC) for both groups increased until approximately 4 months of age and then plateaued. BMC was significantly greater in the Cat K -/- mice throughout the study. There was a 66% difference between the two groups at 1 month of age which declined to 31% at 12 months of age. Total bone mineral density (BMD) showed a very similar trend with the Cat K -/- having higher BMD at all timepoints.At a region of predominantly cortical bone, the distal tibia, the total cross-sectional area was significantly greater in the Cat K null mice (except at one month of age). Cross-sectional area in both strains increased slightly over the study and the Cat K null mice were consisently greater (7-12%). Total BMC was greater in the Cat K -/- mice with a consistent difference of ~11% throughout the study (from 3 months to 12 months of age). No difference in total BMD was observed showing that the bone at the distal tibia of both groups was of similar quality. Periosteal circumference was greater in the Cat K null mice with no difference in endosteal circumference between the groups. Bone strength index (BSI) increased throughout the study for both groups of mice. BSI was significantly greater in the Cat K -/- mice except at 6 months of age.In conclusion, cathepsin K null mice, while smaller in size and bone length, have much greater bone mass at both sites of the tibia. This increase in bone mass correlates with an increase in a surrogate marker of bone strength. This study provides further evidence that cathepsin K plays an important role in bone matrix degradation during osteoclastic bone resorption and may be a viable target for the treatment of osteoporosis.

Disclosures: GlaxoSmithKline Pharmaceuticals,3.

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Inhibition of Cathepsin K Results in Dose-dependent Inhibition of Bone Resorption in Medically Ovariectomized Non-human Primates. G. B. Stroup,* M. Lark,* D. F. Veber,* A. Bhattacharyya,* S. Blake,* L. C. Dare,* S. J. Hoffman,* J. A. Vasko-Moser,* K. Erhard,* R. Marquis,* Y. Ru,* M. Gowen.* GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, USA.

Cathepsin K (EC 3.4.22.38) has been shown to play an important role in bone matrix degradation during osteoclastic bone resorption. In the current studies, we test the ability of a novel compound to inhibit cathepsin K activity in vitro. We then evaluate the anti-resorptive efficacy in vivo, using estrogen deficient cynomolgus monkeys with elevated bone turnover. The compound used is a potent cathepsin K inhibitor (Ki = 0.11nM) that is selective versus cathepsins -L (Ki = 5.7nM) and -S (Ki = 2.5nM). This molecule also inhibits cathepsin K activity in a cytochemical assay using human osteoclasts (IC50=60nM). For in vivo assessment, cynomolgus macaques were rendered medically ovariectomized (ovx) by depot administration of a gonadotropin releasing hormone agonist (GnRHa). Five weeks after the first GnRHa administration, estrogen deficiency and elevated bone turnover were verified. The treatments were carried out in a crossover design. A period of at least 7 days between experiments was used to allow suitable washout time for the compound. The treatments consisted of a single administration of vehicle (70% PEG 400 aqueous, 1.4 ml/kg) or test compound at 1.2, 4.0 or 12.0 mg/kg in vehicle by subcutaneous injection. Serum markers of bone turnover were measured in timed samples taken out to 24h post-dose. A significant reduction in serum C-telopeptide of type I collagen (CTx) was observed at the two higher doses. Total reductions in CTx over 24h were 28%, 54% and 62% for the low, mid and high doses, respectively. A similar reduction was observed in serum N-telopeptide of type I collagen (NTx). Total reductions in NTx over 24h were 31%, 47% and 55% for the low, mid and high doses, respectively. There was no effect of this compound on osteocalcin. These data show that a potent inhibitor of cathepsin K can substantially reduce markers of bone resorption in vivo in a dose-dependent manner. This provides further evidence that inhibition of cathepsin K is a viable strategy for the treatment of osteoporosis.

Disclosures: GlaxoSmtihKline Pharmaceuticals,3.

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Bone Induction by Genetically Manipulated Stem Cells Derived from Human Fat. J. L. Dragoo,* J. Y. Choi,* P. Benahaim,* J. R. Lieberman. Orthopaedic Surgery, UCLA, Los Angeles, CA, USA.

Human liposuction aspirates contain pluripotent processed lipo-aspirate cells (PLAs), which have mesenchymal capacity. The purpose of this study was to determine if PLA cells can be transduced with the BMP-2 gene and undergo osteogenic differentiation, at a rate comparable to bone marrow aspirate cells (BMAs) and human osteoblasts, and induce in-vivo bone formation.Cells were placed in osteogenic media containing DMEM-10 with 50mM ascorbic acid-2-phosphate and 10mM beta-glycerol phosphate. Recombinant BMP-2 (rhBMP-2) or an adenovirus carrying the BMP-2 gene (adBMP-2) was added to appropriate groups. In-vitro osteogenic differentiation was assessed at 4 and 7 days using functional staining, RT-PCR, spectrophotometry, western blotting and ELISA. Bone induction was assessed by implanting transduced cells on matrices in the hind limbs of SCID and athymic mice.Processed lipo-aspirate cells (PLAs) had significantly more alkaline phosphatase activity (spectphotometric assay and histomorphometry) than bone marrow aspirate cells (BMAs) ($p=0.01$) and positive controls ($p=0.001$) at 4 and 7 days. Matrix calcification (von Kossa) of PLAs (1.6%) and positive controls were superior to BMAs (0.56%) ($p=0.001$). RT-PCR showed type I collagen, osteonectin, osteopontin and bone sialoprotein in all PLA cultures with BMP-2 and the positive control at day 4. Bone sialoprotein and osteocalcin remained negative at day 7 in BMAs. Four day ELISA showed adBMP-2 groups achieved higher levels of BMP-2 production than cells cultured with rhBMP-2 [1 μg/ml]. H&E sections from hydroxyapatite and collagen I matrices seeded with PLA-adBMP-2 cells confirmed bone formation at 6 weeks.Human liposuction aspirates contain pluripotent processed lipo-aspirate cells (PLAs), which can be transduced successfully with an adenovirus containing the BMP-2 gene. These cells posses an osteogenic capacity that is equivalent to human osteoblast controls, and superior to BMA cells, and are able to induce in-vivo bone formation.

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Proteomic Analysis of Differential Protein Expression Induced by ODF in Osteoclastogenesis. E. Lee,* S. Li,* Y. Jin,* S. Lim. Internal Medicine, Medical School of Yonsei University, Seoul, Republic of Korea.

Osteoclasts are highly specified multinucleated bone resorbing cells known as part of the mononuclear phagocyte system. Osteoclast formation from bone marrow and cord blood cells requires the presence of macrophage-colony stimulating factor (M-CSF) and osteoclast differentiation factor (also termed ODF, RANKL, OPGL, and TRANCE). Our aim was to identify and characterize the proteins induced by ODF that are structurally and functionally undefined. In this study, we attempted to investigate the difference, using proteomics technique, of expression pattern between ODF-induced cells and those not induced. Briefly, the cells were isolated from bone marrow of mouse femur and tibiae and human umbilical cord blood, respectively, and then were cultured in complete a-MEM media overnight. Next day, the non-adherent cells were removed, and the left cells were further cultured in the presence of M-CSF. After 2 days, the cells were treated with ODF for 6hr and 24hr, respectively. And the obtained cells were subjected to protein purification and subsequent two-dimensional electrophoresis. The 2D protein pattern of the cells treated with only M-CSF was compared with those treated together with ODF. We found some proteins from human umbilical cord blood cells and mouse bone marrow cells induced by M-CSF with or without ODF. Furthermore, the proteins of interest will be subjected to in-gel digestion with trypsin, and the masses of the resulting peptides will be determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry/ electrospray ionization-mass spectrometry (MALDI-MS/ESI-MS) analysis. These resulting protein targets offer insights into the mechanisms of osteoclastogenesis in human and mouse and further provide opportunities to find out markers to facilitate early diagnosis of osteoporosis.

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Osteoclasts Fail to Produce Inflammatory Cytokines in Response to LPS. K. Itoh,*[1] K. Kobayashi,*[1] N. Udagawa,[1] K. Suda,*[1] X. Li,*[1] N. Okahashi,*[2] T. Nishihara,*[3] N. Takahashi.[1] [1]School of Dentistry, Showa University, Tokyo, Japan, [2]Faculty of Dentistry, Osaka University, Osaka, Japan, [3]Kyushu Dental College, Fukuoka, Japan.

LPS, a major constituent of the Gram-negative bacterial outer membrane, stimulates production of inflammatory cytokines slush as IL-1, TNFα and IL-6 in many types of the target cells. We examined effects of LPS on the production of inflammatory cytokines in osteoclasts in comparison with those of bone marrow macrophages of osteoclast precursors. M-CSF-dependent mouse bone marrow macrophages were prepared as a pure population of osteoclast precursors. Mature osteoclasts were also prepared from co-cultures of mouse osteoblasts and bone marrow cells. RT-PCR analysis showed that both purified osteoclasts and bone marrow macrophages expressed mRNA of Toll-like receptor 4 (TLR4) and CD14, the receptors for LPS. Electrophoretic mobility shift assay showed that LPS activated NF-κB in both osteoclasts and bone marrow macrophages. Like IL-1 and RANKL, LPS enhanced the survival and pit-forming activity of osteoclasts cultured on dentine slices. LPS stimulated IL-1β production in bone marrow macrophages in a dose-dependent manner. In contrast, osteoclasts failed to produce IL-1β in response to LPS. Immunological staining for IL-1β confirmed that mononuclear TRAP-positive cells as well as mature osteoclasts did not produce detectable levels of IL-1β even in the presence of LPS, although the precursor macrophages produced a large amounts of IL-1β in response to LPS. In addition, production of TNFα and IL-6 in osteoclasts was never stimulated by LPS. We have also shown that mature osteoclasts as well as bone marrow macrophages expressed a similar amount of p38 MAP kinase, but phosphorylation of p38 MAP kinase in response to LPS was observed in bone marrow macrophages but not in osteoclasts. Thus, characteristics of osteoclasts are quite different from those of osteoclast precursors in terms of production of inflammatory cytokines in response to LPS. Signals mediated by p38 MAP kinase may be involved in the production of inflammatory cytokines in the target cells of LPS.

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RANKL/RANK/OPG and Cathepsin K in Aseptic Loosening of Total Hip Replacements. J. Mandelin,[1] T. Li,*[1] M. Liljeström,*[1] M. Hukkanen,*[1] S. Santavirta,*[2] Y. T. Konttinen.*[3] [1]Institute of Biomedicine, University of

Helsinki, Helsinki, Finland, [2]Department of Orthopedics and Traumatology, Helsinki University Central Hospital, Helsinki, Finland, [3]Department of Oral Medicine, University of Helsinki, Helsinki, Finland.

The purpose of the study was to analyze the eventual roles of RANKL/RANK/OPG on monocyte maturation into cathepsin K-containing profusion osteoclasts (OC) in aseptic loosening of total hip replacement implants.Methods used included monocyte isolation and stimulation with RANKL and other rh-cytokines/ growth factors (e.g. M-CSF, IL-6) and pseudosynovial fluid; immunohistochemical staining and quantitative RT-PCR of synovial membrane-like periprosthetic interface tissues and femoral neck fracture control synovial samples.RANK protein was found in CD68+ interface tissue macrophage-like cells and multinuclear foreign body giant cells, but not in the control samples. Similarly, RANKL protein was found in the stroma of interface tissue in CD68+, but also in CD68- cells, but not at all in the control samples. OPG protein was found in both interface and control samples, but in another tissue compartment, namely in the vascular endothelium. Quantitative RT-PCR gave similar results, except that RANK and RANKL mRNA copies were also found in control samples in low numbers as follows: interface tissue samples (n=11) contained 6.0 ± 1 copies of RANK mRNA, whereas synovial membrane samples (n=6) contained only 3 ± 1 copies ($p < 0.05$); for the RANKL mRNA the corresponding figures were 18 ± 3 vs. 5 ± 2 ($p < 0.01$). OPG mRNA levels were the same in both groups (41 ± 11 vs. 41 ± 10). All the copy numbers are normalized by 1000 beta-actin copies. Monocytes were cultured in 2 ml of Macrophage-SFM supplemented with 1% penicillin/streptomycin. RANKL alone did not induce cathepsin K expression, but the combination of RANKL, M-CSF and IL-6 was effective in this respect (262 copies/ 1000 PBGD mRNA copies at 4 hours). Pseudosynovial fluid alone induced 79 copies and was able to cause osteoclastic multinuclear cell formation within three days of culture. RANKL and M-CSF had the same effect but only after 7 days of culture.It can be concluded that interface produces both RANKL and RANK, which can interact in the absence of OPG. This interaction, together with help from M-CSF and IL-6, induces both cathepsin K and TRAP positive profusion OCs and mature OCs. Thus, both the novel acid attack and cathepsin K-driven and OC-driven mechanisms can contribute to periprosthetic bone loss and loosening.

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Osteoclast Inhibitory Peptide-1/hSca Mediates Interferon-Gamma Inhibition of Osteoclast Formation. M. Koide, N. Kurihara, H. Maeda, S. V. Reddy. Medicine/Hematology, Univ of TX Health Science Ctr @ SA, San Antonio, TX, USA.

The osteoclast (OCL) is primary bone-resorbing cell. OCL formation and activity is regulated by local factors produced in the bone microenvironment. We previously identified osteoclast inhibitory peptide-1 (OIP-1)/hSca as a novel inhibitor of OCL formation and bone resorption that is produced by OCLs. OIP-1 is structurally similar to the mouse Ly-6 gene family. OIP-1 mRNA is expressed in early human OCL precursors (CFU-GM) and bone marrow cells. OIP-1 mRNA is also expressed in human osteoblasts. Cycle dependent RT-PCR analysis demonstrated that interferon-gamma (IFN-gamma) strongly enhanced OIP-1 mRNA expression in bone marrow cells and CFU-GM. Similarly, IL-1-beta also enhanced OIP-1 mRNA expression in CFU-GM. In contrast, 1,25-dihydroxyvitamin D3 and vitamin A did not significantly change OIP-1 mRNA expression in CFU-GM and bone marrow cells. IFN-gamma did not affect OIP-1 mRNA expression in osteoblasts. IFN-gamma inhibits OCL formation and bone resorption by inducing degradation of tumor necrosis factor receptor associated factor-6 (TRAF-6). This results in inhibition of RANK ligand (RANKL)-induced activation of NF-kappaB and JNK. To determine the role of OIP-1 in IFN-gamma inhibition of OCL formation, we tested the capacity of a neutralizing antibody to OIP-1 to inhibit IFN-gamma's effects on OCL-like cell formation by RAW 264.7 cells. IFN-gamma inhibited RANKL-induced OCL-like cell formation by RAW 264.7 cells. Anti-OIP-1 partially blocked (approximately 50%) IFN-gamma inhibition of OCL differentiation by RAW 264.7 cells. Furthermore, Western blot analysis demonstrated that OIP-1 decreased TRAF-2 expression in RAW 264.7 cells. However, OIP-1 had no effect on TRAF-6 expression in these cells. These data demonstrate that IFN-gamma regulates OIP-1 expression in OCL precursors and that OIP-1 may in part mediate the inhibitory effects of IFN-gamma on OCL formation. These data suggest that OIP-1 may play an important role in IFN-gamma induced suppression of OCL formation and activity by affecting TRAF-2 expression in OCL precursors.

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Eosinophil Chemotactic Factor-L: A Novel Osteoclast Stimulating Factor. Y. Oba,[1] S. J. Choi,[1] G. D. Roodman.[2] [1]Medicine/Hematology, Univ of TX Health Science Ctr @ SA, San Antonio, TX, USA, [2]Medicine, UTHSCSA/VA Medical Center, San Antonio, TX, USA.

The genetic events that control osteoclast (OCL) formation are still unclear. We developed an immortalized OCL precursor cell line that forms mature OCLs in the absence of stromal cells when treated with macrophage colony-stimulating factor, dexamethasone, and RANK ligand. To identify genes that were upregulated in OCLs compared to OCL precursors and may be involved in OCL differentiation, we used the PCR select cDNA subtraction method to identify genes that are highly expressed in mature OCLs compared to OCL precursors. Eosinophil chemotactic factor-L (ECF-L) was one of the genes identified. ECF-L was originally identified as a factor from mouse splenocytes that enhances chemotaxis of eosinophils. Although ECF-L contains a consensus CXC sequence near the NH2 terminus similar to the chemokine family of proteins, the remainder of the ECF-L sequence shows little homology with other chemokines. A GenBank database search revealed that ECF-L is a chitinase family member but lacks enzyme activity. ECF-L is highly expressed in spleen, bone marrow, lung, and heart. To determine the role of ECF-L in OCL formation, we transiently transfected an ECF-L cDNA into 293 cells. Conditioned media from ECF-L-transfected 293 cells increased OCL formation in a dose-dependent manner in mouse bone marrow cultures treated with 10^{-10} M 1,25-(OH)2D3. OCLs formed in cultures treated with ECF-L conditioned media demonstrated a significant increase in pit numbers and resorption area per dentin slice compared to control OCLs ($p < 0.01$). Furthermore, conditioned media from 293 cells transfected with human ECF-L cDNA increased OCL formation in a dose-dependent manner in human bone marrow cultures treated with 10^{-10} M 1,25-(OH)2D3. Addition of an antisense S-oligonucleotide to mECF-L inhibited OCL formation in murine bone marrow cultures treated only with 1,25-(OH)2D3 compared to the sense S-oligonucleotide control. Time course studies demonstrated that ECF-L acted at the later stages of OCL formation. Expression of mECF-L mRNA was detected in monocytes and OCL precursors by in situ hybridization. We then constructed a mECF-L-Fc fusion cassette, expressed ECF-L in 293 cells, and purified recombinant mECF-L. rmECF-L-Fc stimulated murine OCL formation in the presence of 1,25-(OH)2D3. These data demonstrate ECF-L is a previously unknown factor that is a potent mediator of OCL formation, acting at the later stages of OCL differentiation.

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The Role of VEGF and RANKL in Ischemic Necrosis of the Femoral Head. D. D. Hunter,* D. J. Popp,* H. K. W. Kim.* Shriners Hospital for Children, Tampa, FL, USA.

Ischemic necrosis of the femoral head, both in the pediatric and adult hip joint, is a serious condition that can result in femoral head deformity (FHD). Previously, a piglet model of ischemic necrosis has been established and has demonstrated progressive femoral head deformity following induction of ischemia. Vascular endothelial growth factor (VEGF) and receptors (VEGFRs) are considered essential factors for angiogenesis. Receptor activator of NFkB ligand (RANKL) is an important mediator responsible for osteoclast activity. The purpose of the study was to determine VEGF-, VEGFRs-, and RANKL- immunoreactivity (IR) in acute and late stages of ischemic necrosis of the femoral head.Twenty-four male piglets were used. A suture ligature was placed tightly around the femoral neck to disrupt blood supply to the femoral head. Two to eight weeks after the induction of ischemia, piglets were sacrificed and the femoral heads were removed. The femoral heads were radiographed, fixed, embedded, and sectioned. The sections were prepared for VEGF, VEGFRs, and RANKL immunohistochemistry. Sections were stained with H&E or TRAP. In control femoral heads, VEGF- IR was present in the hypertrophic zone of the cartilage. In the bone marrow, VEGF-IR was observed in osteoclasts and endothelial cells. VEGFRs showed a similar pattern of IR in cartilage and bone. RANKL-IR was also present in the hypertrophic zone of the cartilage and in osteoclasts of the bone marrow, but not in the endothelial cells. Two weeks following the induction of ischemia, cell death was observed in the deeper layers of the cartilage and marrow. In the cartilage, VEGF-, VEGFRs-, and RANKL- IR were no longer present in the hypertrophic zone but observed more superficially in the proliferative region. In the marrow, VEGF-, VEGFRs-, and RANKL- IR were negative except for the residual VEGF- and VEGFRs- IR observed in the osteoclasts and endothelial cells. Fibrovascular tissue containing endothelial cells, mesenchymal cells, and osteoclasts were observed invading the necrotic marrow at later time periods and showed high VEGF-, VEGFRs- and RANKL -IR. In the cartilage, VEGF-, VEGFRs-, and RANKL- IR varied depending on the presence or absence of the invading fibrovascular tissue. In conclusion, VEGF, VEGFRs, and RANKL appear to play an important role in repair and remodeling of the ischemic femoral head. Our in vivo model demonstrates that expression of these proteins is associated with fibrovascular tissue invasion of the necrotic femoral head and resorption of the necrotic bone. Better understanding of the factors that modulate revascularization and bone resorption may lead to development of treatment strategies which can prevent femoral head deformity and stimulate the repair process.

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Divergent Apoptotic Mechanisms in TGF-β Induced MOCLs. A. Gingery,[1] A. K. Shaw,[1] J. Holy,*[2] M. J. Oursler.[3] [1]Biochemistry and Molecular Biology, University of Minnesota, Duluth, MN, USA, [2]Anatomy and Cell Biology, University of Minnesota, Duluth, MN, USA, [3]Biology, Microbiology and Immunology, Biochemistry and Molecular Biology, University of Minnesota, Duluth, MN, USA.

Research has shown that transforming growth factor-β (TGF-β) can either stimulate or repress mouse osteoclast-like cell (mOCL) differentiation and survival. We have shown that mOCLs that are induced and maintained in TGF-β at maturity exhibit decreased apoptosis as compared to mOCLs withdrawn from TGF-β. This suggests that TGF-β is a survival factor for TGF-β induced mOCLs. To further define these differences in TGF-β induced mOCL responses, we have evaluated the role of caspases in apoptosis. MOCLs were differentiated in the presence of 0.002 ng/mL TGF-β_1. At maturity, the mOCLs were purified and maintained or withdrawn from TGF-β treatment for 90 minutes in the presence of caspase inhibitors. MOCLs were identified as TRAP positive multinucleated cells. Apoptosis was detected by staining for chromatin condensation. First, initiator caspase inhibitors were evaluated. YVAD (caspase 1 and 4 inhibitor) inhibited apoptosis in mOCLs that are either maintained or withdrawn from TGF-β treatment. LEHD (inhibitor of caspases 4 and 9) and IETD (caspase 8 inhibitor) had no significant effect on apoptosis. We conclude that caspase 1 is a likely initiator caspase involved in apoptosis of both TGF-β maintained and withdrawn mOCLs. Next, effector caspase involvement in apoptosis was evaluated. DEVD (caspase 3-like inhibitor) significantly decreased apoptosis in TGF-β maintained but not withdrawn mOCLs. In contrast, VEID (caspase 6 inhibitor) significantly reduced apoptosis in the mOCLs withdrawn from TGF-β treatment but not from mOCLs maintained in TGF-β. We conclude that different effector caspases are involved in driving apoptosis when mOCLs apoptose in the presence or absence of TGF-β. Specifically, caspase 3-like caspases are involved in driving apoptosis in TGF-β maintained mOCLS and caspase 6 drives the apoptosis in TGF-β withdrawn mOCLs. In summary both treatments of mOCLs result in the activation of caspase 1. However the apoptotic program diverges into two different pathways depending on whether TGF-β is present or not. Among other targets, caspase 3 has been implicated in the proteolytic activation or deactivation of signaling proteins whereas caspase 6 disrupts structural proteins such as nuclear lamins. We conclude that significantly different apoptotic molecular mechanisms are activated depending on whether TGF-β is present during apoptosis of TGF-β induced mOCLs.

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RANKL Expressed by Mature Osteoclasts Is Involved in Their Own Survival and Fusion. T. Nagai,*[1] N. Udagawa,[1] K. Itoh,*[1] M. Mogi,*[2] Y. Murase,*[3] T. Nishihara,*[3] A. Togari,*[2] S. Wada,*[4] S. Katayama,*[4] N. Takahashi.*[1] [1]School of Dentistry, Showa University, Tokyo, Japan, [2]School of Dentistry, Aichi-Gakuin University, Aichi, Japan, [3]Kyushu Dental College, Fukuoka, Japan, [4]Saitama Medical School, Saitama, Japan.

Receptor activator of nuclear factor-kB ligand (RANKL) and osteoprotegerin (OPG) produced by osteoblasts/stromal cells are involved in osteoclast differentiation and fusion as a positive regulator and a negative regulator, respectively. It has been reported that RANKL mRNA and protein are expressed in osteoclasts as well as in osteoblasts and lymphoid cells. However, physiological roles of RANKL derived from osteoclasts have not been investigated. In the present study, roles of RANKL expressed by mature osteoclasts were examined using osteoclast preparations obtained from the co-culture of mouse primary osteoblasts and bone marrow cells. When osteoblasts were removed from the crude osteoclast preparation, osteoclasts spontaneously died due to apoptosis. Addition of OPG to the purified osteoclast preparation accelerated the spontaneous apoptosis of osteoclasts. Expression of RANKL mRNA in osteoclasts as well as in primary osteoblasts was detected by an RT-PCR system. Bone marrow-derived macrophages of osteoclast precursors also expressed a significant level of RANKL mRNA. A soluble form of RANKL was detected by ELISA in the conditioned medium of osteoclast cultures and of bone marrow-derived macrophage cultures. In contrast, osteoblasts and stromal cells (MC3T3-G2/PA6, ST2) did not produce a detectable amount of soluble RANKL even in the presence of bone-resorbing factors. TRAP-positive and calcitonin-receptor-positive mononuclear osteoclasts were purified from co-cultures. When mononuclear osteoclasts were treated with or without TGF-β for 48 h, TGF-β strongly stimulated the survival and fusion of mononuclear osteoclasts even in the absence of adding exogenous RANKL. Addition of OPG to the culture of mononuclear osteoclasts almost completely inhibited their survival and fusion induced by TGF- β . These results suggest that RANKL expressed by osteoclasts is involved in their own survival and fusion. It is also proposed that there is a big difference between expression forms of RANKL by osteoclasts/macrophages and by osteoblasts/stromal cells.

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Tumor Necrosis Factor Mediates the Stimulatory Effect of β_2-Microglobulin on Osteoclast Formation. C. Menaa,[1] C. Marshall,[2] S. M. Sprague.[3] [1]Medicine, Evanston Northwestern Healthcare, Evanston, IL, USA, [2]Research Institute, Evanston Northwestern Healthcare, Evanston, IL, USA, [3]Medicine, Northwestern University, Evanston Northwestern Healthcare, Evanston, IL, USA.

End stage renal disease (ESRD) is associated with an erosive, destructive arthropathy characterized by amyloid fibril formation and cystic bone lesions. The mechanism leading to the bone loss is not clearly defined but appears to be related to β_2-microglobulin (β_2M) deposition. Evidence supporting that β_2M is associated with bone destruction includes: 1) β_2M is highly expressed not just in ESRD, but also in other bone diseases associated with increased bone resorption such as multiple myeloma and rheumatoid arthritis; 2) β_2M can induce the expression of cytokines known to regulate osteoclast (OC) formation such as, IL-1, IL-6 and TNF-α; and 3) β_2M induces calcium release *in vivo* and *in vitro*, which can be inhibited by calcitonin. To determine whether β_2M causes bone destruction by directly stimulating OC activity, the effect of β_2M on OC formation and activation was determined. The number of TRAP positive multinucleated cells were determined from mouse bone marrow cells cultured with increasing concentrations of β_2M (10^{-7} - 10^{-5} M) for 6-7 days. These cells satisfied major criteria of OC including, β-integrin and calcitonin receptor expression and the capacity to form pits on dentine slices. The effect was dose-dependent and did not require the RANK/RANKL pathway since osteoprotegerin was unable to block the OC formation induced by β_2M. To examine the possible role of osteoblast/stromal cells in mediating β_2M induced OC formation, CFU-GM (purified bone marrow OC precursors) or a murine monocytic cell line, (Raw cells 264.7) were incubated with β_2M. The effect of β_2M was observed even in the absence of accessory cells. β_2M up-regulated TNF-α and IL-1 expression in a dose dependent manner. In neutralizing antibody experiments, TNF-α antibody was able to block the stimulatory effect of β_2M on OC formation. In conclusion, these data demonstrate, for the first time, that β_2M stimulates OC formation. This effect is direct and requires the induction of TNF-α expression and secretion by OC precursors, which in turn induces differentiation of these precursors into mature OC in an autocrine/paracrine fashion. Thus, these findings further support the role of β_2M in causing bone destruction in ESRD patients and other diseases where β_2M is also highly expressed.

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Two Indispensable Tyrosine Residues in the Cytoplasmic Domain of the M-CSF Receptor Mediate Osteoclast Formation and Function. S. Takeshita, X. Feng, S. L. Teitelbaum, F. P. Ross. Pathology, Washington University, St. Louis, MO, USA.

The cytoplasmic domain of the M-CSF receptor, c-Fms, contains six auto-phosphorylated tyrosine (Y) residues, but the role of each in osteoclastogenesis (OCg) and bone resorption is totally unknown. To explore the structural basis of c-Fms signaling in authentic OCs and their precursors, which express the endogenous receptor, we constructed a chimeric cDNA, which encodes the extracellular domain of the erythropoietin receptor linked to the transmembrane and cytoplasmic domains of c-Fms (EpoR/c-Fms). Using a pMX-puro-based Phoenix retroviral transduction system, this construct was used to generate virus for infection of M-CSF dependent bone marrow macrophages (BMMs). Overall transduction efficiency in this system is >95%. BMMs expressing wild-type chimeric receptor proliferate and differentiate into OCs following exposure to Epo and RANKL as efficiently as BMMs treated with M-CSF and RANKL. We next assessed the role of point mutations in which each of the six Y residues is changed to phenylalanine (F), individually or in selected combinations. While mutation of Y559F and Y807F inhibits proliferation of transduced BMMs by 82% and 95% respectively, all other single mutations (Y697F, Y706F, Y721F and Y921F) have no effect. Importantly, the double mutant, Y559F/Y807, completely abolishes BMM proliferation. We next asked which c-Fms Y residues regulate OC differentiation and function. Thus, pre-OCs were prepared by treatment of BMMs (transduced with virus expressing one or more Y to F mutations in the c-Fms cytoplasmic tail) with M-CSF and RANKL for 2 days, during which time cells receive signals from endogenous c-Fms. When these committed pre-OCs are exposed to RANKL plus Epo, to activate the chimeric receptor, the mutations Y559F or Y807F diminish OC formation by 83% and 85% respectively, and when combined completely arrest OCg. Addressing function, OCs bearing Y559F fail to generate resorption pits in dentin, while those expressing Y807F were able to degrade the matrix to a limited degree. Reflecting their capacity to support OCg, mature cells expressing the four remaining Y to F mutants are completely competent to resorb bone. Thus, we have described, for the first time, components of c-Fms which signal in primary BMMs and OCs. Both Y559 and Y807 play a role in the proliferation and differentiation of OC precursors. In contrast, while Y559 is required for the resorptive function of OCs, Y807 may be dispensable.

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High Extracellular Calcium Alone Stimulates Osteoclast Formation but Inhibits in the Presence of 1,25-$(OH)_2$VitD$_3$. S. N. Kim,* J. H. Baek, Y. H. Kim,* M. M. Shin,* G. S. Kim. Dept. of Pharmacology and Dental Therapeutics, College of Dentistry, Seoul National University, Seoul, Republic of Korea.

It has been suggested that high ambient calcium at resorptive site might have an important role in regulation of bone remodeling although its precise mode of action remains unclear. The present study was performed to examine the effect of the high extracellular calcium on the osteoclast formation employing cocultures of mouse bone marrow cells with mouse calvarial osteoblastic cells. Concentration of calcium in media (1.8 mM) was adjusted to 5, 7, or 10 mM respectively by addition of $CaCl_2$. Osteoclast formation was measured with the number of TRAP-positive multinuclear cells and the expression of osteoclast phenotype markers was identified by RT-PCR. High extracellular calcium (5 to 10 mM) significantly enhanced osteoclast formation in dose-dependent manner. According to the increase in osteoclast formation, the expression of osteoclast phenotype markers (calcitonin receptor, vitronectin receptor, cathepsin K, MMP-9, carbonic anhydrase II) was increased. However, when osteoclast formation was induced by 10 nM 1,25-$(OH)_2$vitD$_3$, increase in extracellular calcium significantly inhibited 1,25-$(OH)_2$vitD$_3$-induced osteoclast formation in dose-dependent manner. This inhibitory effect of high calcium was also observed when osteoclast formation was induced by treatment with M-CSF and soluble RANKL in coculture. Treatment of calvarial osteoblasts with high calcium medium stimulated the expression of RANKL mRNA up to 96 h, while OPG expression was only transiently increased at 3 h. However, in the presence of 1,25-$(OH)_2$vitD$_3$, high calcium did not significantly change 1,25-$(OH)_2$vitD$_3$-induced RANKL expression while transiently increased OPG expression at 48 h. High calcium medium stimulated IL-6 expression both in the presence and in the absence of 1,25-$(OH)_2$vitD$_3$. These results suggest that high extracellular calcium may enhance osteoclast formation through the up-regulation of RANKL expression in osteoblasts but its mode of modulating activity may be different depending on the presence of other hormones or cytokines.

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Expression of CD44 and Osteopontin are Required for Normal Osteoclastogenesis. K. Suzuki,[1] H. Amano,[1] S. R. Rittling,[2] D. T. Denhardt,*[2] S. Yamada,*[1] J. Sodek.*[3] [1]Pharmacology, Showa University, Tokyo, Japan, [2]Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ, USA, [3]CIHR Group in Periodontal Physiology, University of Toronto, Toronto, ON, Canada.

The CD44 receptor is expressed in variant forms that mediate cell adhesion, migration and signaling activities in immune cells and transformed cells as well as bone cells. Osteopontin (OPN) is a phosphorylated glycoprotein, expressed as a secreted protein in a variety of tissues, which can mediate cell attachment and signaling, through an RGD motif that recognizes several integrins. OPN is also a putative ligand for CD44 through which it can generate both chemotactic and signaling activities in cells of the macrophage/monocyte lineage. In previous studies we have shown that CD44 and OPN are co-localized in cell processes of osteoclasts, and that osteoclasts derived from mice with a targeted disruption of either the CD44 or the OPN genes have impaired resorptive activities. To investigate the basis of the impaired resorption in CD44- and OPN-null osteoclasts, we have studied the role of CD44 and OPN in osteoclastogenesis. Osteoclasts were generated from wild-type (WT) as well as CD44- and OPN-null mouse bone marrow-derived cells by culturing in the presence of CSF-1 and RANKL. The expression of CD44 and OPN in these cells was studied by double-immunofluorescence labelling while cell migration was measured using a modified Boyden chamber assay and the resorptive activity of the osteoclasts analyzed on Osteologic^{TM} discs. Analysis of these cells after 3 days revealed extensive cell processes in the WT cells that stained for actin, CD44, intracellular OPN (iOPN), as well as the β1 and β3 integrins, whereas fewer processes were formed by the CD44- and OPN-null cells, which displayed a strongly impaired migration. After 6 days in culture, WT cells generated multinuclear TRAP-positive osteoclasts in which large actin rings were formed, and CD44 and iOPN staining was evident in filopodia and lamellipodia. In contrast, in the CD44- and OPN-null cell cultures most of the TRAP-positive cells were mononuclear and the actin rings were small. In the additional presence of TNFα (50ng/ml), the formation of cell processes, cell fusion and the resorptive activity of osteoclasts, derived from WT mice, was increased markedly. In comparison, only a modest effect of TNFα was observed in the CD44- and OPN-null cells. These studies indicate that both CD44 and OPN are required for the formation of cell processes involved in cell migration and for fusion of osteoclast precursors to form functional osteoclasts. Moreover, the co-distribution of CD44 and iOPN in cell processes suggests an interaction between these proteins that impacts on cytoskeletal organization.

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Flt3+ Macrophage Precursors Commit Sequentially to Osteoclasts, Dendritic Cells and Microglia. P. Jurdic,[1] C. Servet-Delprat,*[2] S. Arnaud,*[3] S. Nataf,*[4] M. Grasset,*[3] C. Domenget,*[1] O. Destaing,*[1] A. Rivollier,*[2] M. Belin,*[4] C. Rabourdin-Combe,*[2] G. Mouchiroud.*[3] [1]ENS, Lyon, France, [2]INSERM U503, Lyon, France, [3]UMR CNRS 5534, Villeurbanne, France, [4]INSERM U433, Lyon, France.

Macrophages, osteoclasts, dendritic cells (DC), and brain-located microglia are all highly specialized cells that belong to the monocytic pathway. Monocytic precursors require M-CSF to form macrophages but, in addition they need RANKL to differentiate into osteoclasts; in presence of GM-CSF plus IL-4, or TNFa, they produce DC, whereas differentiation toward microglia is not well defined yet. Our aim was to identify culture conditions to amplify such a monocytic precursor compartment from mouse bone marrow in order to obtain large amounts of precursors and to better characterize the commitment toward macrophages, osteoclasts, DC and microglia. Since, in vivo, DC expansion is obtained with flt3-ligand (FL), we have tested the capacity of this cytokine to stimulate growth of monocytic precursors. FL sustains intense proliferation of a compartment of hemopoietic precursors whose monocytic phenotype has been characterized by Facs analysis. We have shown that these precursors exhibit constitutive potential towards macrophage differentiation, but time-dependent potential towards osteoclast, DC, and microglia. Indeed, when FL-induced precursors are grown in presence of M-CSF alone they give rise to macrophages; addition of both M-CSF and RANKL on cells maintained 6 days in presence of FL (day 6-FL) stimulates formation of TRAP positive osteoclasts able to resorb dentin slices. With time in culture in presence of FL, monocytic precursors lost their ability to differentiate into osteoclasts but maintained their macrophage potential, and acquired new potential: day 8-FL induced precursors could differentiate into functional DC in the presence of GM-CSF plus TNFa, while addition of glia-conditionned medium in culture of precursors maintained 11 days in presence of FL induced formation of typical microglial cells. Our results provide the first evidence that osteoclasts, macrophages, DC and microglia derive from identical bone marrow precursors through specific windows of commitment. Moreover, precise characterization of interconversion from one of these cell types toward one of the others will allow us to discuss the plasticity of such monocyte-derived cells.This culture model will be very useful in the bone field since it amplifies a highly enriched population of bone marrow-derived osteoclast precursors. Moreover, it provides a powerful tool to analyze the first steps of the commitment of monocytic precursors, toward related cell types involved in different specialized physiological activities.

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IL-1βIncreases Osteoclast Differentiation in the Co-cultures of Bone Marrow Cells and Calvarial Osteoblasts from Adult Rats. M. Kiriu,* H. Kaneki, H. Ide. School of Pharmaceutical Sciences, Toho University, Funabashi, Japan.

Recently, it has been shown that osteoblasts play an important role in the regulation of osteoclast differentiation by using MC3T3-E1 cells and osteoblast-like cells from newborn rat and mouse calvariae. Therefore, the age-related changes in mechanisms for osteoclasts development by the direct contact of osteoblasts to bone marrow cells (BMC) are yet unclear. The present study was undertaken to establishment of co-culture system using osteoblasts and BMC from adult rats, and we also examined the effect of IL-1 on the induction of the formation of TRAP-positive multinucleated cells (MNC). Osteoblasts (ROB) were enzymatically isolated from the calvaria of 25- to 35-week-old female rats. BMC were corrected from the femur of 5-week-old female rats and were co-cultured with ROB in α-MEM containing 10% FBS in 48 well dish for 8 days and then they were stained for TRAP activity. MNC with three or more nuclei were counted as osteoclasts. TRAP-positive MNC were not formed in the cultures of BMC regardless the presence of IL-1. By contrast, the formation of MNC was observed in the co-cultures of BMC and ROB, and IL-1 increased the number of TRAP-positive MNC in a dose-dependent manner. The stimulatory effect of IL-1 on the formation of TRAP-positive MNC in co-cultures of BMC and ROB were inhibited by a selective cyclooxygenase-2 inhibitor, NS-398, and PGE2 increased the number of TRAP-positive MNC in a dose-dependent manner. To investigate whether the direct interaction between BMC and ROB is required for the formation of osteoclasts, we used cellinsert with membrane filters at bottom. No TRAP-positive MNC were formed regardless the presence of IL-1. The present study suggests that IL-1 induces a MNC formation factor on the surface of osteoblasts through PGE2 synthesis, and the MNC formation factor activates the differentiation of BMC to osteoclasts through cell-to-cell interaction.

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Inhibition of Osteoclasts Differentiation on Bone Mineral-like Apatite Crystals and Its Reversal With IL-1α . S. J. Park, S. H. Kim, J. S. Ko, H. M. Kim. Oral Anatomy, College of Dentistry and Intellectual Biointerface Engineering Center, Seoul National University, Seoul, Republic of Korea.

Functional osteoclasts differentiate on the calcified matrix such as bone, calcified cartilage, and tooth from precursors which are known macrophage/monocyte moved through blood vessels and connective tissue from bone marrow hematopoietic tissue. Although cells showing the activity of tartrate resistant acid phosphatase (TRAP) were found throughout the body, osteoclasts immunoreactive to anti-calcitonin receptors antibodies were found only on the mineral surfaces in our preliminary study. This implys that mineral apatite environment may have a role in the final differentiation of osteoclasts. In the present study, differentiation of osteoclasts on the bone mineral-like apatite crystals was studied by generating osteoclasts from mouse bone marrow cells with macrophage colony stimulating factor (rhM-CSF), tumor growth factor-β (rhTGF-b1), and osteoclast differentiation factor (rhODF) on synthetic apatite crystals. Thin film of poorly crystalline apatite crystals similar to bone crystals in physicochemical properties was prepared on the floor of polystylene culture dishes to give cells a bone mineral-like environment. TRAP activity was shown in 100 and 90.2±4.1 % cells cultured on the surface of culture dishes and the substrate of apatite crystals respectively for 10 days. Number of total cells was about 2.4 times lower on the mineral surface than the culture dishes in the end of culture. Large effect of apatite surface was revealed on the formation of multinucleated cells (MNC). Under the influence of cytokines of which concentrations were 10 ng, 1 ng, 70 ng per ml for rhM-CSF, rhTGF-β1, and rhODF respectively, MNC formation decreased on the apatite surface to a great extent. Number of MNC formed on mineral surface was only 23.7 % of the culture dishes. Interestingly, decrease in MNC formation on the apatite crystals was reversed by adding interleukin-1a (IL-1α) to the culture media generating osteoclasts. 0.1 ng/ml of IL-1a was sufficient to markedly increase MNC formation on the crystal surface by 9.4 times than cultures in the absence of IL-1a. On the other hand, there was a slight increase in MNC formation, about by 1.3 times, on the surface of culture dishes by treatment with IL-1α. Furthermore, IL- α generated more MNC on the mineral surface than cultures on culture dishes by 1.96 times. These results indicate that surface of bone mineral-like apatite crystals is a condition limiting a final osteoclast differentiation and this modulation can be reversed with a trace amount of IL-1α.

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Human Osteoclast Precursors Proliferate with IL-6, IL-1 and Kit-ligand, Produce TNFα, and Express TRAP when Attached to Mineral, but Do Not Multinucleate without Exogenous RANKL. R. M. McKeon, L. Cao, K. M. Buddie,* R. Bu, H. C. Blair. Departments of Pathology and Cell Biology & Physiology, University of Pittsburgh, Pittsburgh, PA, USA.

Osteoblasts produce the monocyte proliferation/differentiation factor CSF-1 (the fms ligand) and the osteoclast differentiation factor RANKL. These cytokines promote efficient osteoclastic differentiation in vitro but do not support significant proliferation of precursor cells. Since PTH-stimulated osteoblasts are known to produce large quantities of additional proliferation and growth factors, the role for which is unknown, we studied growth of peripheral blood monocytes produced by aphersis and microbead selection using recombinant human growth factors identified in bone that have been related to increases in osteoclast number and activity. We found that promonocytes proliferate in media containing kit-ligand, IL-1β, and IL-6. The kit-ligand stimulates pathways closely related to fms, but is more prominently elevated in PTH stimulated bone. Interleukin 6, with or without receptor subunits, is critical in maintaining cells in nondifferentiated, proliferative stages. Interleukin 1 is elevated in hyper-resorptive states. Long term cultures demonstrated nonadherent blast-like cells dividing in 3-4 days. A fraction of plated cells formed adherent, foreign body-like cells that expressed α-naphthyl acetate esterase during this time, and these cells were required for efficient proliferation of the nonadherent fraction. No colonies of fibroblast-like cells were recovered in any cell culture. Following separation of nonadherent cells and plating in MEMα with 10 ng/ml hrCSF-1, essentially 100% of these nonadherent, proliferating cells attached, expressed α-naphthyl acetate esterase, and ceased proliferation within 4 d. Surprisingly, some cells attached to devitalized mineralized bone substrate, expressed TRAP strongly, and secreted acid, but did not multinucleate. Western analysis of the growth medium showed no detectable RANKL, but autocrine TNFα, a member of the TNF superfamily implicated in partial support of osteoclast development, was secreted at ~20 ng/ml. In cells grown on glass or plastic TRAP expression was weak or negative, however. These results support the hypothesis that secondary growth factors produced by PTH-stimulated osteoblasts promote proliferation of osteoclast precursors, and further support studies showing that TNFα is important to osteoclast differentiation but is insufficient for full maturation in the absence of RANKL. The culture system described also, for the first time, provides a mechanism for study of nontransformed human osteoclast precursors grown in vitro.

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SDF-1 Increases Recruitment of Osteoclast Precursors by Upregulation of Matrix Metalloproteinase-9 (MMP-9) Activity. X. Yu, P. Collin-Osdoby, P. Osdoby. Biology, Washington University, St. Louis, MO, USA.

Recruitment of osteoclasts (OC) and OC precursors (pre-OC) to resorption sites in bone is essential for normal bone modeling and remodeling. Various soluble and matrix-derived signals may influence pre-OC recruitment into and within the bone marrow microenvironment under normal or pathological conditions, but the exact mechanism(s) involved are not yet well defined. Because chemokines are the key signals that regulate recruitment and activation of many hematopoietic cell types, they may exert similar important regulatory actions on pre-OC and OC. To investigate a potential role for CXC chemokines in pre-OC recruitment and migration, we examined CXCR chemokine receptor expression and functional responses in the pre-OC murine macrophage cell line RAW 264.7 (RAW) which differentiates into TRAP+ multinucleated bone pit-resorptive OC (RAW-OC) following 6 days of culture with recombinant mouse RANKL. CXCR mRNA expression was analyzed by multiprobe RNase protection assays (RPA). Whereas RAW and RAW-OC did not exhibit mRNA for CXCR-2 or -5, both expressed CXCR-4 mRNA. CXCR-4 mRNA levels were highest in RAW and downregulated during their differentiation into RAW-OC. This suggests that SDF-1, the unique ligand for CXCR4, may act primarily on pre-OC. SDF-1 is reported to stimulate hematopoietic cell homing to the bone marrow and increase MMP activities involved in cell migration. We therefore examined SDF-1 effects on MMP activity in RAW and RAW-OC. Gelatin zymography revealed MMP-9 cell-associated and secreted activities associated with both RAW and RAW-OC. MMP-9 activity was greater in RAW-OC (~3-fold) than in RAW. SDF-1 (10-10 to 3´10-8 M, 16 h) significantly and dose-dependently increased MMP-9 activity in RAW (up to 2.7-fold over control, $p<0.0001$), while it had no significant effect on the already high MMP-9 activity of RAW-OC. The functional consequences of SDF-1 induced MMP-9 activity in RAW were explored via a trans-migration assay using collagen coated transwells (a modified Boyden chamber). SDF-1 (10-8 M) significantly stimulated trans-migration of RAW through collagen coated transwells (~2-fold, $p<0.001$). An MMP inhibitor, GM6001, reduced both basal and SDF-1 stimulated collagen trans-migration of RAW ($p<0.01$), indicating that both collagen trans-migration processes are MMP-dependent. We conclude that: 1) OC and pre-OC express the SDF-1 receptor, CXCR-4, 2) CXCR-4 is downregulated in OC development, and 3) SDF-1 promotes pre-OC recruitment via increasing MMP-9 activity. Thus, SDF-1 is likely to be an important local factor involved in the homing and targeting of pre-

OC to bone resorption sites.

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Profound Stimulation of Bone Resorption by Hypoxia. T. R. Arnett,[1] D. C. Gibbons,*[1] A. Hoebertz,[1] M. Rosendaal,*[1] S. Meghji.*[2] [1]Department of Anatomy and Developmental Biology, University College London, London, United Kingdom, [2]Eastman Dental Institute, University College London, London, United Kingdom.

In atmospheric air, arterial and venous blood, oxygen tension (PO2) is 160, ~95 and ~40 mmHg, respectively (20%, 12% & 5%). Interstitially, PO2 is <40 mmHg, whereas in hypoxic environments such as the poorly vascularised yellow fatty marrow associated with aging, or in inflamed tissue, tumors and fracture sites, PO2 may be ~10-20 mmHg. These pathophysiologic states are associated with increased bone resorption. We therefore investigated the effects of hypoxia on osteoclastic resorption. Two systems were used. First, mouse marrow cells, free of stromal cells, were cultured 7d on ivory discs in MEM with 10 ng/ml RANKL, 30 ng/ml M-CSF and 10% serum in flasks containing 20%, 12.5%, 5% or 2% O2 atmospheres (all with 5% CO2, balance N2); 8 replicates / treatment were used. Culture medium was acidified to pH 7.0 for the final 2d to activate resorption by osteoclasts (OC); PO2, PCO2 and pH were monitored by blood gas analyzer. In 12.5%, 5% & 2% O2, TRAP-positive OC formation was increased 2.3, 2.6 & 3.2-fold, respectively, compared with 20% O2. However, OC formed in 20% O2 were small (1-2 nuclei), whereas those generated in 2% and 5% O2 were large, with multiple nuclei. The mean number of discrete resorption pits formed on each disc showed 2.0, 3.5 & 5.1-fold increases, but the total plan area resorbed was stimulated 4.0, 9.0 & 21.2-fold for 12.5%, 5% & 2% O2, respectively ($p<0.001$ for 5% and 2% O2 effects on resorption area). Pits formed in 2% O2 cultures were additionally about twice as deep (~9 uM) as those in 20% O2. Thus, hypoxia causes accelerated formation of large OC, with up to ~40-fold increases in resorption volume after 7d. Second, we found that OC-mediated Ca2+ release from 3d cultures of neonatal mouse calvaria was 5.4-fold greater in 2% O2 than in 20% O2 (5 replicates / treatment; $p<0.001$). The effect of 2% O2 was equivalent to the maximum stimulation elicited by PGE2. Hypoxia-stimulated resorption was completely blocked by 0.1 uM indomethacin, and was strongly inhibited by an IL-1 receptor antagonist protein. In calvarial but not marrow cultures, hypoxia was associated with significant culture medium acidification, consistent with increased anaerobic metabolism, and sufficient to account for some, but not all of the increased resorption. At constant pH, the stimulation of OC function in hypoxia may be mediated via increased production of angiogenic cytokines such as TNFalpha, IL-1 and VEGF, as well as by PGs; the role these factors, as well as that of RANK/RANKL is presently under investigation. Our experiments reveal a new control mechanism for bone resorption of major importance.

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Dynamic Changes in Gene Expression Patterns During RANKL-Induced Osteoclast Formation. A. C. Bendixen,[1] N. K. Shevde,[1] D. E. Murphy,*[1] R. Sladek,*[2] T. J. Hudson,*[2] J. W. Pike.[1] [1]Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA, [2]Human Genome Centre, McGill University, Montreal, PQ, Canada.

RANKL is a newly discovered TNF-like factor that stimulates osteoclast (OC) formation. Recently, several RANKL signaling pathways including the MAPKs, PI3K/AKT and NF-kB have been identified. Despite this, the gene targets of these pathways and the changes in gene expression that occur during OC differentiation have yet to be defined. To elucidate these events, we have used DNA microarrays to track the global alterations in gene expression that result in murine RAW264.7 cells following stimulation with RANKL and to evaluate the effects of OC inhibitors such as IL-4. Since treatment of RAW264.7 cells with RANKL (2 nM) leads to OC formation, first evident at 48 hrs and maximal within 96 hr, we initially examined RNA from cells cultured for 96 hr in the absence or presence of RANKL (2 nM). Dramatic changes in gene expression were observed: the expression of over 400 genes was either enhanced or suppressed at least 2.5-fold in response to RANKL, some by as much as 200-fold. All the classical genes associated with the OC phenotype were identified in the induced category, including TRAP, MMP-9, cathepsin K, CAII, and CTR. To examine OC formation in more detail, we prepared RNA from cells treated with RANKL for up to 120 hours and determined the changes in gene expression using the microarrays. RANKL induced rapid (1 hr) yet transient changes in the expression of over a dozen transcription factors of the immediate early gene class, including c-Jun, c-Fos, EGR1, and EGR2. Within 1 to 4 hr, RANKL also stimulated massive expression of inflammatory genes including IL-1a, IL-1b, TNFa and IL-6. In a slightly delayed action, RANKL also induced cytokine inhibitory factors such as SOCS-3. At 12 hrs, the induction of several sets of transcription factors by RANKL is evident, coincident with strong suppression of factors involved in the maintenance of the macrophage phenotype. Within 24 hr and prior to OC formation, transcripts for osteoclast marker genes such as TRAP, MMP9, and cathepsin K begin to accumulate. These transcripts increase rapidly over the next 72 hours concomitant with OC formation. Coincident with the appearance of these OC markers is a dramatic downregulation of genes such as Mac-1, F4/80, C3R and scavenger receptors inherent to macrophage function. Inhibitors such as IL-4 dramatically alter these expression patterns thus suppressing OC formation. Our studies begin to define the dynamic changes in gene expression that occur in response to RANKL during OC formation, and provide mechanistic clues as to the identity of regulatory factors that may play critical roles in the process of OC formation.

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The Role of the PU.1 Transcription Factor in Osteoclastogenesis. G. L. Barnes,[1] S. Marecki,*[2] T. A. Einhorn,[1] M. J. Fenton,*[2] L. C. Gerstenfeld.[1] [1]Department of Orthopaedic Surgery, Boston University Medical Center, Boston, MA, USA, [2]Department of Medicine, Boston University Medical Center, Boston, MA, USA.

The transcription factor PU.1, a member of the ETS family of factors, has been demonstrated to play an important role in hematopoietic cell differentiation. PU.1 knockout animals display severely impaired lymphoid and myeloid lineage development including an abnormally large population of immature myeloid progenitors but a complete absence of both mature macrophages and osteoclasts. We have developed a unique in vitro embryonic stem cell (ES cell) differentiation system that supports osteoclast differentiation in response to sRANKL, TNFa, or LPS treatment. Using this system we have analyzed the role of the PU.1 transcription factor in osteoclastogenesis and osteoclast function. Specifically, we have utilized a system in which PU.1 knockout embryonic stem cells have been "rescued" by reintroducing either the wild type PU.1 under the control of the PU.1 promoter or alternatively defined mutations in the PU.1 transcription factor. The results of this study clearly demonstrate that PU.1 deficient ES cells do not support osteoclast differentiation when induced by RANKL, TNFa, or LPS alone or in combination. Additional experiments demonstrate that those PU.1 mutant ES cells that support macrophage differentiation also appear able to support osteoclast differentiation. However, our data indicate quantitative and qualitative differences in osteoclastogenesis between individual mutant lines when compared to the wild type cells. Analysis of the cytokine expression profiles of the mutant PU.1 ES cells also demonstrate unique differences in several important cytokines with known functional roles in osteoclastogenesis, specifically M-CSF and IL-11. Together these data demonstrate an important regulatory role for the PU.1 transcription factor in osteoclast formation and function.

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Transcription from the Tartrate-Resistant Acid Phosphatase Promoter Is Negatively Regulated by the Myc Oncoprotein. K. M. Daumer,*[1] E. J. Taparowsky,*[2] D. J. Hall,*[1] M. J. Steinbeck.*[1] [1]Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA, USA, [2]Biological Sciences, Purdue University, West Lafayette, IN, USA.

We have previously reported an inverse relationship between the levels of *myc* and tartrate-resistant acid phosphatase (TRAP) mRNA in a clonal pre-osteoclast cell line (HD-11EM) before and after $1\alpha,25(OH)_2D_3$-induced differentiation. TRAP, a characteristic marker of osteoclast differentiation, is an enzyme that plays an active role in the process of bone resorption. Despite the importance of TRAP in osteoclast biology, factors involved in its transcriptional regulation have not been clearly defined. The present study investigated the regulation of TRAP transcription in response to Myc using several different cell types, which included HD-11EM chicken pre-osteoclasts, $P388D_1$ murine macrophages, C3H10T1/2 murine embryonic fibroblasts, and 293 human embryonic kidney cells. The different cell types were chosen based on their varying expression levels of endogenous Myc and, except for HD-11EM cells, their lack of expression of TRAP. These cells were co-transfected with a series of nested TRAP promoter deletion constructs and a backbone control plasmid or expression plasmids containing v-Myc, c-Myc, or an inactive v-Myc protein construct (delta84/NLS) using ExGen 500 (MBI Fermentas, Amherst, NY). At 48 hours post-transfection, whole cell extracts were prepared and luciferase activity was determined and normalized to protein. Our results showed that down-regulation of endogenous Myc by $1\alpha,25(OH)_2D_3$-treatment of HD-11EM cells increased the level of transcription from the full-length TRAP promoter construct. In addition, ectopic v-Myc or c-Myc protein expression negatively regulated transcription from the TRAP promoter constructs in $P388D_1$ and C3H10T1/2 cells, by 90±5% and 50±8%, respectively. In contrast, no effect on transcription from the TRAP promoter was observed in 293 cells in response to ectopic v-Myc or c-Myc expression. The Myc-responsive element within the TRAP promoter was localized to a region between -881 and -463 bp (relative to the ATG). This region contains a putative Myc-inhibitory binding site located immediately 5' of the transcription start site at -588 bp. The present report provides the first evidence of a cell type specific regulation of TRAP at the transcriptional level by Myc; a transcription factor normally expressed at relatively high levels in pre-osteoclasts and other myelomonocytic cells. This data suggests that Myc plays an active role in negatively regulating the transcription of a mature osteoclast gene.

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Inducible Expression of a Dominant Negative Mutant of IkappaBalpha Blocks RANKL-mediated Osteoclast Differentiation of RAW264.7 Cells. B. G. Darnay,*[1] N. K. Shevde,[2] B. Lamothe,[1] K. Du,[1] B. B. Aggarwal,[1] J. W. Pike.[2] [1]Bioimmunotherapy, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA, [2]Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA.

Knockout mouse models of RANKL, RANK, and OPG, a secreted, soluble receptor that binds RANKL, have demonstrated an essential role of these molecules in osteoclastogenesis. Although osteoclasts are present, TRAF6-, c-Src-, c-Fos-, and p50/p52 (NF-kB subunits)-deficient mice develop osteopetrosis due to a defect in bone resorption activity. RANK activates the transcription factor NF-kB, which regulates the expression of a large number of genes that play essential roles in immune and inflammatory responses. We demonstrated the interaction of TRAF2, TRAF5, and TRAF6 with RANK and that RANK could activate both the NF-kB and JNK pathways. Subsequently, we identified a novel TRAF6-binding motif in RANK that is distinct from the TRAF2- and TRAF5-binding domains. The TRAF6 binding domain in RANK was sufficient for activation of NF-kB, suggesting that TRAF2 and TRAF5 are not necessary for NF-kB activation. RANK is expressed on the cell surface of RAW264.7, a mouse macrophage, and upon stimulation by RANKL differentiate into multi-nucleated, TRAP+ osteoclasts. NF-kB remains in the cytoplasm by its interaction with an inhibitory subunit known as IkBa. Upon stimulation

by RANKL, IkBa is phosphorylated and degraded, allowing for the translocation of NF-kB to the nucleus, where it causes gene transcription. We sought to further understand the role of NF-kB in RANKL-mediated osteoclast differentiation. We generated RAW264.7 cell lines stably expressing an inducible mutant form of IkBa, which lacks two critical serine residues required for its phosphorylation and degradation. In the presence of the mutant IkBa, RANKL-meidated NF-kB activation was suppressed as indicated by gel mobility shift assays. The expression of the mutant IkBa did not affect RANKL-mediated activation of JNK or ERK indicating specific inhibition of the NF-kB pathway. Furthermore, expression of the mutant IkBa caused suppression of RANKL-mediated osteoclast differentiation as indicated by the decrease in multi-nucleated, TRAP+ osteoclast numbers. These data reflect the necessary requirement of NF-kB in the differentiation of RAW264.7 cells into osteoclasts by RANKL and may suggest that TRAF6 is the key molecule in RANKL-mediated osteoclast differentiation.

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Osteoclast Formation in Osteoclast Precursor Cell Line RAW264.7 Is Not Correlated to OPG, RANK or RANKL Expression, But Is Influenced by Culture Conditions. R. J. Arends,* E. Veen - van den Berk,* M. Beuningen - de Vaan,* A. G. H. Ederveen. Pharmacology, N.V. Organon, Oss, The Netherlands.

Osteoporosis occurs in women and men as the result of age and sex steroid status. Steroid hormone deficiency leads to an imbalance between bone formation and bone resorption, in part the result of an increased osteoclast activity. Estrogens (and androgens) have been shown to limit osteoclast formation from myelomonocytic cell precursors. An in vitro assay was built to profile selective estrogen receptor analogues on inhibition of osteoclast formation. Treatment of RAW264.7 cells (osteoclast precursor cell line) with human receptor activator of NF-kappa-B-ligand (RANKL) induced formation of multinucleated (>3 nuclei) tartrate resistant acid phosphatase-positive (TRAP+) osteoclasts after 4 days of culture. Optimal osteoclast formation and TRAP activity was dependent on culture media used e.g. (a) DMEM complete + foetal calf serum (FCS), (b) aMEM + FCS, (c) aMEM + charcoal-treated FCS, or (d) aMEM + bovine serum. RAW 264.7 cells cultured in medium 'a' or 'd' showed low induction of TRAP activity (stimulation factor less than 1.5-fold) after stimulation with RANKL. More than 3-fold induction of TRAP activity was found in cells cultured in medium 'b'. Stimulation factor was similar in cell lysates and culture media. Quantitative- PCR was performed to detect RANK, RANKL and osteoprotegerin (OPG) mRNA expression in RAW264.7 cells cultured in media 'a-d'. Highest RANK mRNA levels were measured in cells cultured in medium 'c', lowest in medium 'a'. No correlation was found between osteoclast formation in vitro and RANK mRNA expression. To our surprise, RAW264.7 cells expressed both OPG and RANKL mRNA, essential components for osteoclast formation, which were so far only ascribed to stromal cells and osteoblasts. Similar expression levels for RANKL and OPG were measured; however, large variations were detected depending on culture conditions. Under culture condition 'a' relative expression of OPG and RANKL was 7-fold higher compared to condition 'd'. No correlation was found between osteoclast formation in vitro and OPG or RANKL mRNA expression. The role of RANKL and OPG transcripts in RAW264.7 cells is unknown. This study suggests that profiling of selective estrogen receptor analogues by measuring the effects on osteoclast formation and TRAP activity in RAW264.7 cells in vitro may be influenced by culture conditions.

Disclosures: N.V. Organon,3.

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Proton-translocating Subunit of a Vacuolar Type H+-ATPase from Osteoclasts Enhances pH Recovery from Acid-load. H. Sakai,[1] H. Mori,*[2] H. Morihata,*[2] J. Kawawaki,[3] A. Shioi,[4] M. Kuno.[2] [1]Departments of Orthopedic Surgery, Osaka City University, Osaka, Japan, [2]Physiology, Osaka City University, Osaka, Japan, [3]Central Laboratory, Osaka City University, Osaka, Japan, [4]Cardiovascular Medicine, Osaka City University, Osaka, Japan.

Osteoclasts secrete massive protons into the resorption pit of bone via a vacuolar type H^+-ATPase (V-ATPase) located in the raffled membrane. The V-ATPase is composed of at least 13 subunits, and subunit c is the principal molecule for H^+ translocation. To understand regulatory mechanisms of H^+-secretion and intracellular pH (pHi) of osteoclasts via the V-ATPase, we examined the pHi recovery process from intracellular acidosis in COS7 cells transfected with cDNA encoding subunit c gene. Total RNA was extracted from murine osteoclasts generated in the co-culture with ST2 cells in the presence of vitamin D. The PCR product of subunit c was subcloned into pcDNA3.1 expression vector and used to transfect COS7 cells by the Ca^{2+}-phosphate co-precipitation method. Transformed cells were identified by co-expression of EGFP: overexpression of subunit c mRNA was confirmed by single cell RT-PCR. Confocal microscopy revealed that the chimeric subunit c-EGFP protein was localized to the membrane compartment. Measurements with a pH-sensitive dye, BCECF, showed that pHi of non-transfected COS7 cells was 7.45 ± 0.18 (n = 4) after incubating with 40 mM NH_4Cl for 15 min at 37ºC, and decreased to 6.53 ± 0.43 (n = 4) by washings of NH_4Cl. The pHi of non-transfected cells recovered only slightly in the Na^+-free high-K^+ medium. Changes in pHi in the acid-loaded transformed cells were detected using EGFP fluorescence that was sensitive to pH. In the Na^+-free high-K^+ medium, pHi gradually recovered from the acutely-induced acidosis in cells transfected with subunit c cDNA, but not in cells transfected only with the plasmid carrying EGFP cDNA, indicating that overexpression of subunit c enhanced the pHi recovey process independently of Na^+. As the pHi recovery was inhibited by replacing K^+ with $NMDG^+$, depolarization was likely to be required for the increase in pHi. An addition of DCCD (100 μM), an inhibitor for subunit c, blocked the pHi recovery, thus subunit c seemed to mediate the changes in pHi. These results suggest that subunit c of the V-ATPase induces or potentiates pHi recovery from intracellular acidosis under the conditions where cells are depolarized.

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The Kinetics and Biochemical Features of Bisphosphonate (Alendronate)Induced Apoptosis. M. Muzylak,* G. Stenbeck,* S. A. Nesbitt, M. A. Horton. Bone and Mineral Centre, Department of Medicine, The Rayne Institute, University College London, London, United Kingdom.

Bisphosphonates are synthetic pyrophosphate analogues that are used intreatment of metabolic bone diseases to inhibit excessive bone resorptionand hypercalcaemia. The exact mechanisms of action of anti-resorptivebisphosphonate drugs remains unclear, although they may inhibit boneresorption by mechanisms that can lead to osteoclast apoptosis. Themolecular pathways that lead to osteoclast apoptosis following Alendronate(ALN) treatment remain to be fully elucidated. We have used isolatedrabbit and human osteoclasts (OCs) plated on dentine slices to examine byconfocal microscopy the effect of ALN on the kinetics and biochemicalfeatures of bisphosphonate-induced apoptosis.Exposure to ALN (dose range from 10 to 500uM) caused time and dosedependent decrease in the number of TRAP-positive human and rabbit OCs (44%and 90% reduction). As early as 2 hours after ALN treatment activation ofryanodine receptor is observed and mitochondria exhibit profound changes instructure and function as evidenced by the formation of the permeabilitytransition pore complex (PTPC). Damaged mitochondria liberated at least twodifferent factors: apoptosis-inducing factor (AIF) and cytochrome c. Thesefactors proteolytically activate caspase-3 as shown by detection ofantibody-defined activated caspase-3 epitopes. Apoptosis-Detection-Systemconfirmed that DNA fragmentation started 8 hours after treatment with 100uMALN. Overexpression of p53 oncoprotein in the nucleus was seen. Ras-Rafcomplex accumulated in the cytoplasm preventing the activation of MAPK kinaseand the phosphorylation of transcription factors (c-fos). Additionally, weobserved alteration in the actin and tubulin cytoskeletal organisation ofosteoclasts, affecting protein and membrane trafficking. Plasma membranechanges were also observed with reduced staining of lipid raft-associatedCD44, CD45 and vitronectin receptor integrins. The movement of phosphatidylserine (PS) fromthe inner to the outer surface of the plasma membrane, confirmed by AnnexinV-FITC staining, supports removal of apoptotic cells and bodies byneighbouring cells.In conclusion, these observations suggest that ALN treatment inducesprofound changes in mitochondrial structure, activation of caspase-3,accumulation of Ras-Raf GTP-binding proteins and modification of plasmamembrane receptor expression.These events play a role in the process bywhich aminobisphosphonates inhibit osteoclastic bone resorption.

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A Novel Inhibitor of Rab Prenylation Causes Basolateral Membrane Changes in Osteoclasts and Reduces Their Resorptive Activity. F. P. Coxon,[1] M. Muzylak,*[2] M. H. Helfrich,[1] D. Marshall,*[3] M. A. Horton,[2] B. Larijani,*[4] S. A. Nesbitt,[2] M. C. Seabra,*[4] F. H. Ebetino,[5] M. J. Rogers.[1] [1]Medicine & Therapeutics, University of Aberdeen, Aberdeen, United Kingdom, [2]University College London, London, United Kingdom, [3]Medical Microbiology, University of Aberdeen, Aberdeen, United Kingdom, [4]Cell & Molecular Biology, Imperial College, London, United Kingdom, [5]P&G Pharmaceuticals, Cincinnnati, OH, USA.

Rab proteins are a large family of small GTP-binding proteins involved in vesicular trafficking. Membrane localisation,essential for the function of these proteins, is dependent on prenylation, which involves the attachment of hydrophobic geranylgeranyl groups to their C-terminus. This process is catalysed by the enzyme Rab geranylgeranyl transferase (Rab GGTase). We have recently found that NE10790, a phosphonocarboxylate analogue of the bisphosphonate risedronate (RIS), inhibits recombinant Rab GGTase *in vitro* without affecting FPP synthase, farnesyl transferase or GGTase I. This results in the selective inhibition of prenylation of Rab proteins in intact cells, demonstrated by loss of incorporation of [^{14}C]mevalonate into Rabs in osteoclasts, and by loss of prenylation of immunoprecipitated Rab6, but not Ras or Rap1. Since the functions of Rab proteins in osteoclasts remain unclear, we have studied the effect of NE10790 on osteoclast function and morphology *in vitro*. NE10790 dose-dependently inhibited bone resorption by rabbit osteoclasts *in vitro*, reducing both pit area (IC_{50} ~900μM) and pit depth. However, unlike RIS, anti-resorptive concentrations of NE10790 had little effect on the number of osteoclasts with F-actin rings. Marked effects were also seen in the basolateral membrane, away from the resorption surface, in osteoclasts treated with 1mM NE10790. Confocal microscopy, after staining for F-actin and $\alpha_v\beta_3$ or paxillin, showed that 20-55% of $\alpha_v\beta_3$-positive osteoclasts on dentine discs contained large vacuoles and exhibited single or multiple large, dome-shaped membrane protrusions. Scanning EM confirmed that approximately 20% of NE10790-treated osteoclasts had dome-shaped basolateral surfaces that were devoid of membrane ruffles, features rarely seen in untreated or RIS-treated osteoclasts. Unlike RIS-treated osteoclasts, these domed cells remained spread and did not show signs of membrane retraction. Transmission EM of NE10790-treated osteoclasts confirmed the presence of large vacuoles beneath these dome-shaped structures. These data indicate that prenylated Rab proteins are essential for bone resorption. The changes at the osteoclast basolateral membrane likely reflect disruption of membrane trafficking, in which Rabs are intimately involved.

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Inhibition of Osteoclast Formation by Statins. A. P. Baumann,* W. Grasser, S. Petras,* H. J. Harwood,* D. D. Thompson, V. Paralkar.* Pfizer Global Research and Development, Groton, CT, USA.

HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl coenzyme A reductase) inhibitor (statin) treatment is frontline therapy for lowering lipid levels in patients with hyperlipidemia. Recently it has been reported in animal studies that statins have bone anabolic activity. Although the activity of statins has not yet been characterized in osteoporotic women without cardiovascular complications, retrospective data on women taking statins to reduce cholesterol levels has shown a decrease in fracture risk. However, this decreased risk is not always supported by an increase in bone density. In order to understand at the cellular level the role of statins on bone we studied the effect of statins in cultured rat osteoclasts. In this study, osteoclasts were generated from bone marrow of ovariectomized

(OVX) female S-D rats. Bone marrow cultures were grown in the presence of RANK (receptor activator of NF-kB) and recombinant human M-CSF for three days (complete media). On the third day lovastatin was added to the cells in complete media and the cells were cultured for three more days followed by TRAP (tartrate resistant acid phosphatase) staining. After counting the number of multinucleated TRAP positive cells, it was observed that osteoclasts as defined by the above parameters (TRAP positive multinucleated cells) were significantly decreased in a dose dependent manner upon treatment with lovastatin. We also show that statins decrease the HMG-CoA reductase activity (assessed in intact cells by incorporation of 14C actetate into sterols) in rat bone marrow cells in a dose dependent manner although the IC50 observed in bone marrow cells was right shifted as compared with liver cells.In conclusion, our data indicate that lovastatin inhibits osteoclast development and the observed bone effects of statins at least partially could be due to their effects on osteoclasts.

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A Mini-Model to Test Inhibitors of Bone Resorption In Vivo. A. M. Heegaard, A. W. Vestergaard,* J. M. Delaissé. OsteoPro A/S, Herlev, Denmark.

Experimental models for screening of anti-resorptive compounds in vivo often demand large quantities of the test compound. Here we present a novel small-scale in vivo model to test inhibitors of bone resorption. Mouse pups were labelled in utero with [^{3}H]tetracycline at day 18 of gestation. The two-day old pups were then treated with subcutaneous injections for 4 days of either an anti-resorptive agent or vehicle. At the end of treatment the pups were sacrificed and the remaining [^{3}H]tetracycline was extracted from the femora and measured in a scintillation counter. To evaluate the maximum possible loss of [^{3}H]tetracycline during the 4 day period, experiments were performed where half the litter was sacrificed at day 2 postpartum and the rest of the pups on day 6. Comparison of the amount of extracted [^{3}H]tetracycline from these two days showed that approximately 35% of [^{3}H]tetracycline is removed from the bones of untreated mice between day 2 and 6. Tetracyclines have been reported to inhibit bone resorption, and to test if the tetracycline labelling on its own could affect resorption in this model, pups were treated with 10, 30 and 50-fold excess of unlabelled tetracycline. There was no inhibition of resorption with 10 and 30-fold excess tetracycline suggesting that the effect of the [^{3}H]tetracycline is negligible. An anti-resorptive effect was only observed when the mice were treated with more than 50-fold excess of unlabelled tetracycline. A range of well-established anti-resorptive agents worked well in this mouse resorption model. Treatment with the bisphosphonates pamidronate or clodronate resulted in 27% to 37% more [^{3}H]tetracycline retained in the bones compared to controls. This corresponds to near maximal inhibition and was highly reproducible. There was also a significant anti-resorptive response to inhibitors of cysteine proteinases (E64) and matrix metalloproteinases (RP59794, GM6001). Femora that were isolated from mice treated with E64, RP59794 or GM6001 contained 16% to 20% more [^{3}H]tetracycline than controls. The amount of compound used in a single experiment was in the range of 6-24 mg. In conclusion, [^{3}H]tetracycline labelled neonatal mice is a promising small-scale model for the initial in vivo evaluation of new anti-resorptive agents.

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Design of Novel Bone-Targeting Chemical Groups and Their Exploitation in the Discovery of Anti-Resorptive Src Tyrosine Kinase Inhibitors. D. Dalgarno,[1] S. Pradeepan,*[1] R. Yuan,*[1] J. Bogus,*[1] S. Wardwell,*[1] L. Xing,[2] M. Ram,*[1] S. Liou,*[1] J. Keats,*[1] S. Narula,*[1] C. Metcalf,*[1] Y. Wang,*[1] T. Keenan,*[1] R. Sundaramoorthi,*[1] W. Shakespeare,*[1] C. Sebesta,*[3] R. Bohacek,*[1] M. R. van Schravendijk,*[1] B. Boyce,[2] J. Iuliucci,[1] M. Weigele,*[1] T. Sawyer.[1] [1]ARIAD Pharmaceuticals, Cambridge, MA, USA, [2]Univ. Rochester Med Ctr, Rochester, NY, USA, [3]Primedica, Worcester, MA, USA.

We recently discovered that citrate binds to the phosphotyrosine binding pocket of the Src SH2 domain through multiple well-characterized interactions. Citrate is also known to bind bone, possessing an affinity for the hydroxyapatite component of bone. This insight led to the design of Src inhibitors incorporating bone-targeting chemical groups. We describe here the design and bone-targeting properties of a series of novel chemical groups which we have incorporated into Src tyrosine kinase inhibitors to confer significant *in vitro* and *in vivo* activities related to the inhibition of bone resorption. The bone-binding affinities of bone-targeting groups were measured relative to known bone-targeted molecules such as bisphosphonates and tetracycline using a hydroxyapatite (HA) chromatographic method. This HA method has proven a versatile tool for identifying a series of bone-targeting groups that may be incorporated into therapeutic agents for osteoporosis or other bone diseases. Src tyrosine kinase inhibitors designed to incorporate bone-targeting groups were screened using *in vitro* Src kinase and osteoclast pit assays followed by a short-term parathyroid hormone (PTH) induced hypercalcemia assay in mice. The bone-targeted Src tyrosine kinase inhibitor AP23236 shows potent Src kinase inhibitory activity (IC_{50}=19.5nM), and was determined to possess significant *in vivo* efficacy in the mouse hypercalcemia assay. A ^{14}C-radiolabeled analog of AP23236 was used to evaluate the tissue distribution of this compound in rats. Approximately 10-15% of the dose was associated with bone 24 hrs post-dose. A sustained targeting of the compound to bone was demonstrated by the fact that about 6-7% of the dose was associated with the skeleton at 7 days post-dosing. Optimization of this lead compound has led to the progressive discovery of more potent and efficacious compounds, AP23317, AP23381 and AP23451. AP23451 is the most active bone-targeted Src kinase inhibitor tested to date in the mouse hypercalcemia assay. Single daily doses of 10, 3 and 1 mg/kg/day inhibited PTH-induced hypercalcemia by 120%, 88% and 56% respectively. Exploitation of the discovery of novel bone-targeting chemical groups has successfully led to the development of novel small-molecule inhibitors of Src tyrosine kinase as anti-resorptive agents.

Disclosures: ARIAD Pharmaceuticals,3.

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Osteoclasts from TRAP Deficient Mice Accumulate Cytoplasmic Vesicles. K. Hollberg,*[1] K. Hultenby,*[1] A. R. Hayman,[2] T. M. Cox.*[3] [1]Dep Pathology, IMPI, Karolinska Institutet, Stockholm, Sweden, [2]Div Mol Cell Biology, School of Clinical Veterinary Science, Bristol, United Kingdom, [3]Dep Medicine, University of Cambridge, Cambridge, United Kingdom.

The enzyme tartrate-resistant acid phosphatase (TRAP) is expressed at high levels in the bone resorbing osteoclasts (OC). Attachment of the osteoclast to the mineralized bone surface and development of specialised cell surface areas facing bone; i.e the clear zone and the ruffled border, is critical for bone resorption. Resorption is confined to the ruffled border where TRAP and proteolytic enzymes are secreted via transport vesicles into the resorption vacuole. The bone matrix phosphoprotein, osteopontin (OPN), has been shown to bind to osteoclast cell surface integrins via an RGD motif, thereby mediating attachment of osteoclasts to bone in vivo. Studies have shown that TRAP from rat OC partially dephosphorylates the bone matrix phosphoproteins, osteopontin and bone sialoprotein; after modification these proteins are no longer permissive for OC attachment.The TRAP knockout mice are phenotypically characterized by osteopetrosis and increased volume density of osteoclasts. This indicates normal differentiation of OC but a defect in OC resorptive activity. TRAP deficient OCs display an increased total area with decreased relative volume density of clear zones but increased relative volume density of ruffled borders in young mice. We have used quantitative electron microscopy histomorphometry to analyze variations in cytoplasmic vesicles near to the ruffled border in TRAP deficient OC compared to wild-type, with the aim of correlation to functional deficiency in resorption capacity in TRAP deficient OCs. The result demonstrates an increase in total number of vesicles per OC (61%) in TRAP deficient mice compared to wild-type mice. The number of vesicles per OC with a diameter in the range 50-199 nm and 200-499 nm was significantly increased by 35 and 92%, respectively. However, the number of large vesicles (diameter >500nm) was not changed in the TRAP deficient OC.In summary, the shift in the normal distribution of ruffled border membranes and clear zones in TRAP deficient OCs was associated with accumulation of small and medium-sized vesicles. This could be the consequence of malfunctioning intracellular traffic and/or signalling, possibly resulting in resorptive dysfunction of the OC.

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Statins Inhibit Protein Prenylation in Osteoclasts In Vivo. J. C. Frith,[1] K. J. Armour,[1] J. H. M. Feyen,[2] M. J. Rogers.[1] [1]Medicine and Therapeutics, University of Aberdeen, Aberdeen, United Kingdom, [2]Bristol-Myers Squibb, Hopewell, NJ, USA.

Recent studies have suggested that statins have anabolic effects on bone in vitro and in vivo. However, since statins also have direct inhibitory effects on osteoclastic resorption in vitro it remains unclear whether statins affect osteoblasts or osteoclasts in vivo. Statins inhibit HMG CoA reductase, thereby preventing cholesterol synthesis and preventing the prenylation of small GTPases such as Rap1A. To help determine the site of action of statins, we examined their ability to inhibit protein prenylation in osteoclasts and osteoblasts in vivo. Three- to four-day old rabbits were injected subcutaneously with 0.05mg/kg cerivastatin (CER), 0.3mg/kg CER, 40mg/kg pravastatin (PRA), 10mg/kg alendronate (ALN) or an equivalent volume of PBS. After 24 hours the rabbits were euthanised and the osteoclasts isolated from the minced long bones by immunomagnetic bead separation with an anti-vitronectin receptor (VNR) antibody. This approach removed >98% TRAP-positive multinucleated cells from the bone marrow suspension. Western blot analysis of lysates from the purified osteoclasts (VNR-positive cells) and VNR-negative cells was carried out using an antibody that specifically hybridises to the unprenylated form of Rap1A (the level of unprenylated Rap1A thus indicating the degree of inhibition of protein prenylation). Treatment with CER in vivo caused a dose-dependent accumulation of unprenylated Rap1A in both VNR-positive and VNR-negative cells, demonstrating that CER inhibits protein prenylation in osteoclasts and other (uncharacterised) bone marrow cells in vivo. By contrast, injection of ALN caused accumulation of unprenylated Rap1A only in the VNR-positive osteoclast fraction, whereas PRA (which can only be internalised by hepatocytes in vivo) or PBS had no effect on protein prenylation in either cell fraction. To determine whether statins inhibit protein prenylation in osteoblasts in vivo, 2-day old C57Bl6 mice were injected twice over the calvaria with 10mg/kg mevastatin (MEV), 0.3mg/kg CER or an equivalent volume of PBS. After 24 hours the mice were euthanised, the calvaria removed and the osteoblasts isolated by collagenase digestion. Treatment with MEV or CER in vivo had no effect on the level of unprenylated Rap1A in the isolated osteoblasts. These results demonstrate that subcutaneous administration of CER inhibits protein prenylation in osteoclasts in vivo. By contrast, protein prenylation in calvarial osteoblasts was not inhibited by locally-injected statin at a concentration that has apparent, localised anabolic effects on bone. Statins may therefore be anti-resorptive rather than truly anabolic in vivo.

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A Novel Method for Characterization of Bone Derived Human Osteoclasts. J. del Pino-Montes,[1] E. Benito,*[2] M. L. Sanchez,*[3] J. J. Calvo,*[2] P. Menendez,*[3] M. A. Garcia-Marcos,*[4] P. Osdoby,[5] A. Orfao.*[3] [1]Medicina, Universidad de Salamanca, Salamanca, Spain, [2]Fisiologia y Farmacologia, Universidad de Salamanca, Salamanca, Spain, [3]Servicio General de Citometria, Universidad de Salamanca, Salamanca, Spain, [4]Servicio de Hematologia, Universidad de Salamanca, Salamanca, Spain, [5]Washington University, St Louis, MO, USA.

Osteoclasts are cells of hematopoietic origin whose major function relates to bone resorption. Different bone diseases show a change in the number and function of osteoclasts which also represent the target for different therapeutic agents. However, there are a lot of methodologic drawbacks for the study of individual osteoclasts, related to the difficulties to isolate and characterize these cells from in vivo sources. Recently, a new method for the isolation of human osteoclasts using the specific monoclonal antibody 121F has

been described. The aim of the present study was to show the feasibility of using flow cytometry for the identification and characterization of human mature osteoclasts directly obtained from bone tissues, based on the use of immunophenotypic techniques. A total of nine bone femoral heads obtained as discarded surgical material from osteoporotic and/or fracture patients undergoing hip replacement were included in the present study. In order to check for the nature of 121F+ cells by flow cytometry we used laser scanning cytometry for the simultaneous analysis of the immunophenotype and the DNA cell content of osteoclast-like cell enriched bone samples. Results obtained were compared with conventional morphological and cytochemical (TRAP reactivity) studies. Our resuts showed that the percentage of cells which showed both cytochemical (TRAP+) and immunophenotypic (121F+) osteoclast-associated characteristics was very similar (12.5 ± 6.2 vs 14.7 ± 11.7; p=0.46). Using laser scanning cytometry, it was observed that 121F+ cells had a greater size (p=0.04), a higher DNA cell content (p=0.04) and greater numbers of nuclei per cell (p=0.04), than the 121F- cells present in the same sample. In bone tissue only osteoclast-like cells display these characteristics. The possibility of analyzing bone-derived samples by flow cytometry using the 121F monoclonal antibody reagent, would therefore open new perspectives on the study of human osteoclasts in health and disease, due to the relatively high speed and simplicity of this analytical approach.

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Phosphatidylinositol Trisphosphate Mediated Regulation of Podosome Organization by the Gelsolin-Associated Signalling Complex. M. A. Chellaiah,[1] R. S. Biswas,*[1] U. M. Alvarez,*[2] K. A. Hruska.[2] [1]OCBS, University of Maryland, Baltimore, MD, USA, [2]Internal Medicine, Washington University, St.Louis, MO, USA.

Podosomes are adhesion structures in osteoclasts, structurally related to focal adhesions mediating cell motility during bone resorption. Podosomes have been shown to contain many of the same proteins found in focal adhesions, such as the $\alpha_v\beta_3$ integrin, F-actin, vinculin, talin, gelsolin, fimbrin, and α-actinin. Direct relationships have been demonstrated between the phosphoinositides, PtdIns 3,4 P2, PtdIns 4,5 P2, and PtdIns 3,4,5 P3 (PIP3) associated with gelsolin and actin polymerization. Osteoclasts generated from gelsolin null mice lack podosomes and fail to respond to $\alpha_v\beta_3$ activation. Previous studies have defined unique biochemical properties of gelsolin related to PIP3 in osteoclast podosomes. The increase in PIP3 levels associated with gelsolin resulting from liganding of the $\alpha_v\beta_3$ integrin is unique to osteoclasts, and here we demonstrate PIP3/gelsolin function in mediating organization of the podosome signaling complex. Overlay assays demonstrated strong PIP3 PI 3-kinase interation based on the SH2 domains of PI 3-kinase. Furthermore, lipid extraction of lysates from activated osteoclasts eliminated interaction between gelsolin, c-src, PI 3-kinase and FAK despite equal amounts of geloslin in both lipid extracted and unextracted experiments. Moreover, the tryosine phosphatase PTP-PEST was also found associated with gelsolin in osteoclast podosomes. Stimulation of $\alpha_v\beta_3$ regulated phosphorylation of PTP-PEST, and the phosphatase regulated tyrosine phosphorylation status of signaling proteins associated with gelsolin in podosomes. We conclude that gelsolin plays a key role in recruitment of signaling proteins to the plasma membrane through phospholipid-protein interactions and regulation of their phosphorylation status through its association with PTP-PEST. Since both gelsolin deficiency and PI 3-kinase inhibition impair bone resorption, we conclude that PIP3 based protein interactions are critical for osteoclast function.

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ERK Regulates the Formation of Ruffled Borders in Osteoclasts. H. Nakamura,* T. Tsuji,* A. Hirata,* T. Yamamoto. Oral Morphology, Okayama Univ. Graduate Sch. of Med. and Dent., Okayama, Japan.

Extracellular signal-regulated kinase (ERK) of mitogen-activated protein kinase (MAPK) has been reported to participate in the survival and apoptosis of osteoclasts. However, the signal transduction mechanism in this phenomenon remains unknown. Recent reports suggest that ERK also play an important role in the cell motility through the phosphorylation of myosin light chain kinase (MLCK). We assessed immunohistochemistry to investigate the localization of ERK and fine ultrastructure after the administration of PD98059, MAPK/ERK kinase (MEK) inhibitor, to clarify the signal transduction of ERK in osteoclasts. Western blotting analysis, using cell lysate from rat incisor alveolar bone containing numerous osteoclasts, showed that osteoclasts expressed MEK2 and ERK2. Intense immunoreactivity for panERK was detected in a band-like pattern on the contact region between osteoclasts and bone surfaces. Immunogold method revealed that ERK was mainly localized in the cytoplasm of clear zone. However, little labeling was detected in nuclei of osteoclasts. PD98059 administration experiment in calvarial organ culture exhibited that numerous vacuoles were accumulated in the cytoplasm of osteoclasts after 1 h. Ruffled border was scarcely seen in osteoclasts in spite of attaching bone surface with clear zone. After 3 h, many osteoclasts were detached from bone surfaces and lost their cell-polarity. Some osteoclasts showed apoptosis with nuclear condensation. These results indicate that ERK in osteoclasts may be involved in the formation of ruffled border and the maintenance of cell-polarity, in addition to the survival, by regulating the actin/myosin interaction through MLCK.

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Estrogen Inhibition of Parathyroid Hormone-Stimulated Osteoclast Formation and Attachment In Vitro: Involvement of Both PKA and PKC. B. Liu,[1] P. Wu,*[1] R. Bringhurst,[2] J. Wang.*[1] [1]Oral Pathology and Oral Diagnosis, Dental School, National Taiwan University, Taipei, Taiwan Republic of China, [2]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

Estrogens modulate the catabolic effects of parathyroid hormone (PTH) on bone *in vivo* and *in vitro*. PTH-stimulated cAMP accumulation in osteoblasts is thought to be linked to increased osteoclastic activity, but the precise mechanism is still unknown. In cocultures of clonal marrow stromal cells (MS1) and normal mouse spleen cells, the formation of osteoclast-like cells, *i.e.* tartrate-resistant acid phosphatase (TRAP)- and calcitonin receptor-positive multinucleated cells (MNCs) with bone resorbing activity, is stimulated by 1,25-$(OH)_2D_3$ (10^{-8}M) and rat parathyroid hormone (rPTH)-(1-34) (10^{-7} M). These osteoclast-like cells were functional, as evidenced by their attachment to dentine slices and production of resorption pits. Osteoclastogenesis stimulated by PTH, but not by 1,25-$(OH)_2D_3$, was suppressed by 17β-estradiol (17β-E2; 10^{-10} M to 10^{-8} M), whereas 17α-E2 (10^{-8} M) had no effect. Exposure to 10^{-8} M 17β-E2, but not 17α-E2, also significantly decreased the PTH-induced attachment of osteoclast-like cells to dentin slices. 17β-E2 significantly inhibited osteoclast-like cell formation induced by 8-Br (10^{-4} M), by TPA (10^{-8} M), or by rPTH-(1-34) (10^{-7} M) in combination with either Rp-cAMPS (10^{-4} M) or H-7 (10^{-5} M). 17β-E2 suppressed the partial stimulation of TRAP-positive MNCs formation induced by [Arg^2]human (h)PTH-(1-34) (10^{-7} M) or hPTH-(3-34) (10^{-7} M) but not that caused by 10^{-7} M hPTH (53-84). We conclude that estrogens suppress rPTH-(1-34)-stimulated osteoclast-like cell formation by blocking both the cAMP-dependent protein kinase A (PKA) pathway and the phospholipase C-coupled calcium/protein kinase C (PKC) pathway. Estrogen may regulate osteoclastic bone resorption not only by inhibiting formation of osteoclasts but also by blocking their attachment to bone.

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Facilitative Glucose Transporter GLUT2 Is Present in Osteoclasts. K. I. Larsen,[1] M. L. Falany,*[1] W. Wang,*[2] J. P. Williams.[2] [1]Pathology, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Internal Medicine, University of Kentucky, Lexington, KY, USA.

Bone resorption by osteoclasts is energy intensive and glucose concentration-dependent. The goal of this study was to identify glucose transporters in osteoclasts and identify mechanisms regulating this process. We demonstrate that osteoclasts utilize the facilitative transporter GLUT2 and that p38 MAP-kinase (p38) is important in regulating glucose uptake. The distribution of GLUT2 is restricted to pancreatic islets and liver cells; the glucose responsive tissues. These cells also express glucokinase (hexokinase IV), the rate limiting enzyme in glucose uptake. Glucokinase phosphorylates glucose upon entering the cell. We demonstrate by confocal immunofluorescence microscopy that avian osteoclasts express both GLUT2 and glucokinase. Immunocytochemical localization of GLUT2 in 18.5 day mouse embryos demonstrated that GLUT2 is present in both osteoblasts and osteoclasts. This strongly suggests that both bone cells utilize the glucose sensing mechanism. We focused our studies on glucose dependent regulation of osteoclast activity, while little remains known about glucose metabolism in osteoblasts. Multiple signals are altered in osteoclasts in response to increasing glucose concentrations including upregulation of the A subunit of the H^+-ATPase, increases in bone resorption and the activation of the stress activated p38 MAP-kinase. Studies using the specific inhibitor of p38, SB203580, have implicated p38 in regulating glucose transport in other cells. We have previously reported that SB203580 inhibited glucose stimulated increases in bone resorption without addressing the mechanisms involved. We tested the effects of SB203580 on glucose transport in osteoclasts measured by [^{3}H]-2-deoxyglucose uptake. 10 μM SB203580 had maximal and sustained effects on glucose transport in 10 minutes. Cells were washed, lysed, and [^{3}H]-2-deoxyglucose uptake quantified by liquid scintillation spectrometry. SB203580 inhibited the V_{max} of glucose transport by ~50% with no appreciable effect on the K_m. This data indicates that p38 MAP-kinase is an important mediator of glucose transport in osteoclasts. These results suggest that SB203580-dependent inhibition of glucose transport could alter, directly or indirectly, multiple downstream signaling processes. We conclude that GLUT2 is present with glucokinase in osteoclasts and that p38 MAP-kinase plays an important role in regulating glucose metabolism, and hence energy production, in these cells.

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TNF-alpha Mediated Apoptosis in Osteoclasts Is Driven by Caspases. A. K. Shaw,[1] M. J. Oursler.[2] [1]Biochemistry and Molecular Biology, University of Minnesota Duluth, Duluth, MN, USA, [2]Biology, University of Minnesota Duluth, Duluth, MN, USA.

Tumor necrosis factor alpha can induce both apoptosis and survival in cells derived from the hematopoietic line. Recently, TNF-α has been documented to induce differentiation of osteoclast precursors and apoptosis of mature osteoclasts. In order to understand the molecular mechanisms by which these opposing effects arise, we have examined the TNF-α apoptotic pathway in osteoclasts. We have previously reported that mature mouse osteoclast-like cells (mOCLs) generated in cocultures with ST2 stromal cells are induced to apoptose within 90 minutes of TNF-α treatment following purification to remove ST2 support cells. This effect is not dependent on protein synthesis since TNF-α-induced apoptosis occurs in the presence of cycloheximide. Caspases are proteases that are cleaved and activated in many apoptotic programs. We have treated purified mOCLs with caspase inhibitors to ascertain the role of caspases in TNF-α-mediated osteoclast apoptosis. The initiator caspase inhibitor VAD-FMK inhibited TNF-α mediated apoptosis, suggesting that the apoptotic program involves initiator caspase activation of downstream caspases. Apoptosis was not prevented by inhibition of caspase 1-like activity, indicating that this initiator caspase does not play a role in TNF-α mediated apoptosis. However, an inhibitor to the initiator caspase 8 prevented apoptosis, suggesting that caspase 8 may be a primary initiator caspase in this cascade. Inhibition of effector caspase 6 and 3-like activity blocked TNF-α induced apoptosis. Together, these results suggest that the initiator caspase 8 is a primary factor in driving apoptosis in osteoclasts and downstream effects of caspase 8 activation include activation of effector caspases 3 and 6.

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Distinct Roles for p130Cas and c-Cbl in Osteoclast Spreading Induced by Adhesion or M-CSF. I. Nakamura,[1] G. A. Rodan,[2] L. T. Duong.[2] [1]Dept of Orthopaedics and Bone Biology, Merck Res Labs & Univ of Tokyo, Tokyo, Japan, [2]Dept of Bone Biology & Osteoporosis, Merck Res Labs, west point, PA, USA.

Both $p130^{Cas}$(Cas) and c-Cbl have been reported to play critical roles in osteoclast function as downstream targets of c-Src kinase. Src-deficient prefusion-osteoclast-like cells (pOCs) attach but do not spread on vitronectin (VN)-coated surfaces, while M-CSF induces spreading and migration in these Src(-) cells. We therefore examined the effects of adhesion and/or M-CSF on tyrosine phosphorylation of Cas and c-Cbl in pOCs attached or in suspension. pOC attachment to VN induces cell spreading and increases tyrosine phosphorylation of Cas and to a lesser extent of c-Cbl. M-CSF causes further spreading and increases tyrosine phosphorylation of both Cas and c-Cbl, suggesting cooperation between $\alpha_v\beta_3$ (major attachment integrin) and *c-fms* in the cytoskeletal organization of osteoclasts. However, in pOCs in suspension M-CSF induces tyrosine phosphorylation of c-Cbl, but not Cas, confirming the role of c-Cbl as a down stream effector of *c-fms*, and suggesting that M-CSF induction of Cas phosphorylation is dependent on ligand engagement of $\alpha_v\beta_3$. These results suggest that in osteoclasts tyrosine phosphorylation of Cas is fully dependent on the outside-in signaling mediated by ligand engagement of $\alpha_v\beta_3$ and c-Src, whereas M-CSF-induced tyrosine phosphorylation of c-Cbl is independent of this signaling pathway. Taken together, Cas and c-Cbl appear to play distinct roles in the signal transduction pathway for osteoclast function.

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Pivotal Role of Mitochondria in Regulating Osteoclast Apoptosis. T. Akiyama,* Y. Kadono,* A. Yamamoto, T. Ogata,* I. Nakamura, H. Oda, K. Nakamura, S. Tanaka. Department of Orthopaedic Surgery, The University of Tokyo, Tokyo, Japan.

Osteoclasts are terminally differentiated cells with a very short life span, and once differentiated, they undergo apoptosis, or programmed cell death. However, the molecular events leading to osteoclast apoptosis still remains elusive. Recently, it was found that in apoptosis triggered by many stimuli, mitochondria play a pivotal role in coordinating caspase activation through the release of cytochrome *c*. In apoptotic cells, cytochrome *c* leaves its normal mitochondrial localization and accumulates in the cytoplasm, where it binds to the adaptor molecule, Apaf-1, rendering it competent to bind and activate the initiator caspase, procaspase 9. Caspase 9, in turn, activates the downstream effector caspases, inducing a caspase cascade. When osteoclast-like cells (OCLs) formed in mouse co-culture system were purified by removing osteoblastic cells, OCLs caused apoptotic cell death within 24 hours. During apoptosis the mitochondrial membrane potentilal (δYm), as measured by the retention of potential-sensitive fluorescent dyes, was decreased, and the cytochrome *c* release from mitochondria was observed by immunohistochemical staining by anti-cytochrome *c* antibody. To elucidate the role of mitochondrial membrane depolarization and cytochrome *c* release in osteoclast apoptosis, we constructed adenovirus vectors carrying *bcl-2* or *bcl-xL* gene. Overexpression of Bcl-2 or Bcl-xL protein clearly inhibited δYm decrease and cytochrome *c* release from mitochondria in OCLs, and efficiently prevented their apoptosis. When intact mitochondria isolated from mouse liver were incubated with cytosol fraction of OCLs, rapid release of cytochrome *c* was observed within 30 min, while cytosol from macrophages did not have such activity, indicating that OCLs accumulate proapoptotic molecule(s) in their cytosol. We found that during the differentiation of OCLs induced by M-CSF and RANKL, the expression of proapoptotic molecules Bid, Bad and Bax was increased, and activation of caspase-9 and caspase-3 was observed. The specific inhibitor of caspase-9 or caspase-3 efficiently suppressed OCL apoptosis. These results suggest that mitochondrial membrane depolarization and cytochrome *c* release, which may be caused by proapoptotic molecules such as Bid, Bad and Bax, lead to caspase-9 activation in OCLs and induce their apoptosis. Anti-apoptotic molecules such as Bcl-2 and Bcl-xL can prevent their apoptosis by preventing depolarization of mitochondrial membrane and cytochrome *c* release.

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RANK Ligand Prevents Bisphosphonate-Induced Osteoclast Apoptosis In Vitro. H. L. Benford,* M. J. Rogers. Bone Research Group, Medicine & Therapeutics, University of Aberdeen, Aberdeen, United Kingdom.

Bisphosphonates can cause osteoclast apoptosis, an effect that may contribute to their ability to inhibit bone resorption. Alendronate (ALN) causes osteoclast apoptosis by inhibiting FPP synthase and causing the loss of prenylated small GTPases, whereas clodronate (CLO) causes apoptosis due to the intracellular accumulation of the cytotoxic metabolite $AppCCl_2p$. Several studies have shown that RANKL promotes osteoclast survival in vitro. We therefore examined whether RANKL could also suppress apoptosis of osteoclasts induced by bisphosphonates, and whether this affected the ability of bisphosphonates to inhibit bone resorption in vitro. Osteoclasts were isolated from rabbit long bones and seeded either into multi-well plates and purified by pronase/EDTA digestion, or seeded onto ivory discs. Cultures were then treated for 48 hours with 10-100μM ALN or CLO, in the absence or presence of 50ng/ml soluble, recombinant RANKL. Bone resorption was quantitated by reflected light microscopy, and the number of adherent osteoclasts and the proportion of apoptotic osteoclasts (identified by staining with DAPI) were counted. RANKL alone did not significantly stimulate osteoclastic resorption or alter the number of osteoclasts or apoptotic osteoclasts in cultures in the absence of bisphosphonates. Furthermore, RANKL did not alter the ability of alendronate to inhibit protein prenylation (measured by the accumulation of unprenylated Rap1A). However, in osteoclasts the inhibitory effect of 10-100μM ALN and 50-100μM CLO on bone resorption was reduced by the presence of RANKL. This effect was more pronounced with CLO than ALN. Treatment with 100μM ALN or CLO reduced the number of adherent osteoclasts in culture dishes to ~52% and ~57% of control cultures respectively. This was increased to ~77% and ~85% of control cultures in the presence of RANKL ($p<0.01$). Approximately 17% of adherent osteoclasts in culture dishes were apoptotic after treatment with ALN or CLO; this was reduced to ~9% in the presence of RANKL ($p<0.001$). These observations demonstrate that RANKL prevents osteoclast apoptosis induced by bisphosphonates. Since CLO and ALN cause apoptosis by different mechanisms, RANKL presumably prevents apoptosis via a signalling pathway downstream of RANK (such as activation of Akt) that converges at a stage of the apoptotic pathway common to both bisphosphonates. Furthermore, since RANKL was more effective at reducing the anti-resorptive effect of CLO compared to ALN, this suggests that CLO may inhibit resorption primarily by causing osteoclast apoptosis, whereas ALN (by preventing protein prenylation) has more subtle effects on osteoclasts that inhibit resorptive function.

Disclosures: Merck,2.

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Estrogen Modulates RANK Expression and RANK-Mediated Signaling in the Rabbit Osteoclast. J. Shyu,[1] Y. Lin,*[1] J. J. Wang,*[1] C. Lin,*[2] C. Shih.[1] [1]Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan Republic of China, [2]Microbiology and Immunology, National Yang-Ming Universtiy, Taipei, Taiwan Republic of China.

The discovery of new members of the tumor necrosis factor (TNF) receptor ligand family has elucidated the precise mechanism by which osteoblasts/stromal cells regulate osteoclast differentiation and function. Osteoblasts/stromal cells express OPGL (ligand for OPG) as a membrane-associated factor. Osteoclast precursors, which possess RANK (receptor activator of NF-kB), recognize OPGL through cell-to-cell interaction with osteoblasts/stromal cells, and differentiate into osteoclasts. Mature osteoclasts also express RANK, and their bone-resorbing activity is also induced by OPGL which osteoblasts/stromal cells possess. Thus OPGL, RANK, and OPG are three key molecules, which regulate osteoclast recruitment and function. However, the RANK-mediated signaling in osteoclasts is poorly understood. The major mechanism of the rapid phase of bone loss in postmenopausal osteoporotic women is estrogen deficiency. The exact mechanism of action of estrogen on bone is still unclear. As an initial attempt to address these issues, we examined the effect of various calcitrophic agents on the RANK-mediated signaling in the rabbit authentic osteoclast cell and the RANK expression in the osteoclast-like cell co-cultured with osteoblasts/stromal cells. Western blot analysis revealed that estrogen but not 1a,25(OH)2D3 induced a dose- and time-dependent change of RANK tyrosine phosphorylation. The associations between RANK and TNF-associated factor 6 protein were also increased by estrogen as detected by co-immunoprecipitation. The expression of RANK in the osteoclast-like cell was up regulated by the 1a,25(OH)2D3. Furthermore, confocal microscopy analysis indicated that estrogen treatment caused redistribution of RANK and TNF-associated factor 6 from cell periphery to central portion of the cytosol. In conclusion, the current study provided evidences that estrogen modulates RANK expression and RANK-mediated signaling in the rabbit osteoclast.

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Cyclosporines and FK506 Inhibit Osteoclastogenesis and Osteoclast Survival: Role of Down-Regulation of RANK/RANKL Signaling. K. Igarashi, C. Menaa, J. T. Woo, S. M. Sprague, P. H. Stern. Northwestern University Medical School, Chicago, IL, USA.

Cyclosporine A (CsA) and tacrolimus (FK506), inhibitors of the protein phosphatase calcineurin, are the predominant immunosuppressive agents used to prevent transplant rejection. One side effect of this treatment is osteopenia. The bone loss is paradoxical, since in bone organ cultures, CsA and cyclosporine G (CsG) inhibit resorption. The direct effects of cyclosporines and FK506 on osteoclast differentiation (osteoclastogenesis) and on osteoclast survival were determined. For osteoclastogenesis, two models were used, M-CSF-dependent non-adherent cells from mouse marrow cultures, which represent cells of the monocytic lineage, and the murine monocyte precursor cell line RAW 264.7. Cells were treated with M-CSF and RANKL to stimulate osteoclastogenesis. Tartrate-resistant acid phosphatase (TRAP) staining was used to visualize mononuclear and multinucleated cells. TRAP activity in the cultures was also quantified. Activated c-Jun N-terminal kinase, an essential pathway for the RANKL osteoclastogenic effect was determined in RAW 264.7 cell lysates after immunoprecipitation with its substrate, c-Jun fusion protein. CsA and CsG were potent inhibitors of osteoclastogenesis. Treatment of the marrow cells or RAW 264.7 cells with 0.1 μg/ml of either compound for 4 days produced significant inhibition, with further inhibition at higher concentrations. CsA treatment during the last 2 days was as effective as 4-day treatment. FK506 was 20-50X more potent than CsA. Cyclosporine H was a weaker inhibitor; 1 μg/ml was required for significant inhibition. Effects of osteoclast survival were evaluated in marrow cultures, which were first treated with M-CSF and RANKL for 4 days to allow osteoclast formation, and then treated for one day with the inhibitors. Multinucleated osteoclasts and TRAP activity were markedly decreased by CsA and CsG, but less by FK506. The general caspase inhibitor, z-VAD, significantly inhibited the effect of CsA, whereas the effect of FK506 was not significantly inhibited. The results suggest that CsA, but not FK506, decrease osteoclast survival. Treatment of the RAW 264.7 cells for 3 or 24 hr with FK506 or CsA inhibited RANK/RANKL-stimulated Jun kinase activity. The findings indicate that cyclosporines and FK506 have inhibitory effects on osteoclastogenesis that are mediated, at least in part, by repressing RANK/RANKL signaling. The inhibitory effects on osteoclasts may mediate the effects of cyclosporines and FK506 on bone resorption in organ culture. The inhibitory effects must be overridden by other mechanisms to elicit the bone loss associated with the clinical use of these agents to prevent transplant rejection.

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Dietary Calcium Intake Assessment in 5990 Women in Tuscany, Italy. L. Bernini,*[1] P. Bandini,*[1] L. Papini,*[1] V. Vismara,*[1] W. Bencivelli,*[2] M. Mazzantini,*[2] O. Di Munno.*[2 1]Geriatric Unit, S. Miniato Hospital, S.Miniato, Italy, [2]Internal Medicine, University of Pisa, Pisa, Italy.

Calcium represents one of the main factors in the bone metabolism regulation. It is well documented that low calcium intake (CaI) plays an important role in both postmenopausal and senile osteoporosis. Aim of our study was the assessment of dietary CaI in a population of 5990 consecutive women (mean age 57.5 years, range 20-80) referred to the Center for Bone Densitometry of the S. Miniato Hospital (Pisa, Tuscany) for their fist bone assessment, from January 1993 to July 2000. Women were asked by a questionnaire about the habitual intake of cheese, milk, yogurt, type of water and vegetables. We also evaluated body mass index (BMI), age and duration of the menopause (MNP), number of deliveries, and bone mineral density assessed at the distal third of the forearm by single photon absorptiometry (Osteometer DT 100). Mean daily CaI was 703 ± 335 mg; In women over 65 years the value was 665 ± 317 mg, significantly lower ($p<0.005$) than that found in women between 56-65 years (695 ± 327), 46-55 years (722 ± 335) and 20-45 years (751 ± 424). Furthermore in 4064 women (67,8%) mean daily CaI was <800 mg. The table shows age, BMI and osteoporosis (OP) defined as T-score <-2.5 SD, according to different ranges of daily CaI.In conclusion these data show that in 5990 women living in Tuscany, Italy, the mean daily CaI is 703 mg which is below the recommended dietary allowance. The lower daily CaI was found among the older women (>65 years) who are at higher risk for osteoporosis.

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The Adequate Intake (AI) for Calcium During Pregnancy and Lactation. H. Ishida,[1] K. Uenishi,[1] A. Kamei,*[1] M. Shiraki,[2] H. Fukuoka,[3] T. Hosoi,[4] H. Orimo.[4 1]Kagawa Nutrition University, Saitama, Japan, [2]Research Institute and Practice for Involutional Diseases, Nagano, Japan, [3]University of Tokyo, Tokyo, Japan, [4]Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan.

Recommended dietary allowance (RDA) for calcium in non-pregnant women is 600mg/day in Japan. Additional 300mg/day of calcium is recommended for pregnant women and 500mg/day is added during lactation period. On the other hand, the mean calcium intake for women 15 through 49 years of age is 515mg/day on the National Dietary Survey (1999). However, it is not fully understood how the dietary calcium level and food consumption patterns in pregnancy and lactation are.This study was designed to focus on the dietary calcium intake and the food sources of calcium during pregnancy and lactation periods. The subjects were healthy 10 Japanese women aged from 23 to 40. The mean body weight before pregnancy was 50.7kg (ranging from 43 to 60 kg). Dietary assessment was conducted in the 18th, 27th and 34th week in pregnancy, and in the 5th, 13th and 24th week after the delivery. Duplicate portion analysis combined with weighed food records was used for assessing calcium intake and food consumption patterns. The total calcium content in the diet was measured by atomic absorption spectrometry. Energy and other nutrients intake level were calculated by using food composition table. As a result, the average daily calcium intake was 745mg/day (ranging from 561 to 1163 mg) in pregnancy, and 764 mg/day (ranging from 391 to 1136 mg) in lactation. 50 percent of calcium in their diet was supplied from milk and milk products. Green vegetables were the second most important food source of calcium. The birth weight of infants was normal, and the mean maternal bone mineral density of L2-4 measured after the delivery was within normal level. For these reason, we think that these calcium intake levels (745 or 764 mg/day, respectively) may be suitable as the adequate intake (AI) of calcium during pregnancy and lactation, though our subject size was small. The present RDA for calcium during pregnancy and lactation may be overestimated.

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Dietary Silicon and Bone Mineral Density: The Framingham Study. K. L. Tucker,*[1] D. P. Kiel,[2] J. J. Powell,*[3] N. Qiao,*[1] M. T. Hannan,[2] R. Jugdaohsingh.*[3] [1]Tufts University, Boston, MA, USA, [2]Harvard Medical School, Boston, MA, USA, [3]St Thomas Hospital, London, United Kingdom.

Silicon has been shown to affect the organic matrix of bone and cartilage, and a reduction in matrix components has been documented with Si deficiency. Si is also a major ion of osteogenic cells, particularly in the mitochondria. Connective tissues, particularly bone, contain Si at higher levels than muscle, mainly as an integral component of glycosaminoglycans and their protein complexes. We hypothesized that Si intake would be associated with BMD in the offspring cohort of the original population-based Framingham Study. In 1995-99, Framingham offspring participants (30-87 y) had BMD measured with Lunar DPX-L at hip (femoral neck, trochanter, Wards area) and lumbar spine. Usual dietary intake was assessed at the 5th and 6th (1991-99) exams with a semi-quantitative food frequency questionnaire. We added Si values for a standard serving of each of the 278 food items that contribute to the food frequency questionnaire. Si values were obtained from the literature for solid foods, and by analysis at St Thomas Hospital, for fluids. For combination foods, recipes were calculated based on the Si content of ingredients. We regressed BMD at the 6th exam on the log of average Si intake from the food frequency at the 5th and 6th exams, adjusting average dietary intake of energy, calcium, and vit D (exams 5 & 6), and age, height, BMI, physical activity, Ca and vit D supplements, season of bone measurement and for women, current estrogen use (exam 6). Because alcohol is a major source of Si and has previously been associated with BMD, we repeated analyses adjusting for alcohol, and for non-alcohol users. Mean Si intake was 26 mg/day for women and 33 mg/day for men and was significantly inversely associated with age. Si intake was significantly related to BMD at all 3 hip sites for men (N=1189; $p<0.01$), and approached significance at the spine ($p<0.1$). Significance remained after adjustment for alcohol, but was not significant among the subset (n=219) of non-drinkers. In contrast, none of the sites was significant for the total group of women (N=1546), but among non-drinking women (n=390) Si intake was significant at the troc ($p<0.05$), and approached significance at the spine ($p=0.1$). Coefficients for the difference in BMD associated with a log unit difference in Si intake ranged from 0.027 g/cm2 (spine) to 0.040 (troc) in men. Among non-drinking women they were 0.044 (troc) and 0.079 (spine). These results suggest that Si intake is associated with BMD in men, and this appears to be independent of alcohol. Further investigation of the mechanisms involved and the reasons for the apparently different associations with BMD by sex are needed.

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Protein Intake - Effect on Bone Mineral Density and the Rate of Bone Loss in Elderly Women. P. B. Rapuri,[1] J. C. Gallagher,[1] K. L. Ryschon.*[2 1]Bone Metabolism Unit, Creighton University, Omaha, NE, USA, [2]Ryschon Health and Technology Services, Valentine, NE, USA.

The importance of dietary protein as a risk factor for osteoporosis has received very little attention. In the present study, we examined the effect of dietary protein on the bone mineral density (BMD) at baseline and also on the rate of bone loss over 3 years in postmenopausal elderly women recruited for an osteoporotic intervention trial. The study population included 444 postmenopausal elderly women (aged 65-77 years) for the baseline analysis. Ninety six women assigned to the placebo group constituted the study population for the longitudinal analysis. The baseline BMD data and the percent change in BMD over 3 years were compared between groups divided into quartiles of protein intake. The analysis was performed using a General Linear Model after adjusting for smoking, alcohol intake, calcium intake, caffeine intake, baseline BMD (only for longitudinal analysis) and other significantly correlated covariates. Low protein intake was related to lower BMD and greater bone loss at most of the skeletal sites measured. At baseline, women in the lowest protein quartile had significantly ($P<0.05$) lower BMD at the femoral neck, total body and total femur compared to that of women in the third and fourth quartiles of protein intake (approximately 3-4%). Even at spine and mid-radius, similar trends were observed. The rate of bone loss at femoral neck (-5.05±2.37 vs 0.012±1.59) over three year period was also marginally higher ($P<0.06$) in lowest quartile of protein intake compared to that of highest quartile of protein intake. The rate of bone loss at other skeletal sites showed a similar trend. The results presented suggest that dietary protein plays an important role in the etiology of osteoporosis and optimum protein intake is necessary to maintain good bone health.

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Dietary and Lifestyle Determinants of Bone Loss from the Proximal Femur in Men and Women in their 7th and 8th Decades. S. Kaptoge,*[1] N. Dalzell,*[1] A. Welch,*[1] K. T. Khaw,*[2] S. Bingham,*[3] J. Reeve.[1 1]Strangeways Research Laboratory, University of Cambridge, Cambridge, United Kingdom, [2]Clinical Gerontology, University of Cambridge, Cambridge, United Kingdom, [3]MRC Dunn Nutrition Unit, Cambridge, United Kingdom.

Almost all investigations of the dietary determinants of bone loss and fracture have used food frequency questionnaires, despite the serious limitations of this approach for quantitating nutrient and food intakes. We aimed to identify dietary determinants of rapid bone loss in older men and women with a view to devising triallable dietary interventions for preventing hip fracture. We recruited 1469 men and women participants in a diet and cancer prospective population-based cohort study to a study of femoral neck bone loss, since bone loss at this site has similar determinants to hip fracture in SOF and other cohort studies. Each participant recorded their food intake using a validated 7 day food diary on three occasions 18 months apart and had their total hip bone density (BMD, g/cm-2) measured on a Hologic 1000W twice in the same season at intervals of 2 - 5 years. The diet diaries were analysed to generate quantitative data on intakes of foods, food groups and nutrients and the BMD data was used to derive bone loss rates (%/year). The European Prospective Osteoporosis Study (EPOS) questionnaire was administered to gather other risk factor data. Subjects with a diagnosis of osteoporosis, on anti-osteoporosis treatment or on glucocorticoids were excluded (n = 767 in analysis).Overall, rates of bone loss in men (-0.14% pa +/-1.4SD) and women (-0.33% pa +/-1.3) were lower than presented in previous studies. Principal components proved unhelpful in simplifying the structure of statistical models, so a combination of least squares regression and partial least squares regression was used. In both genders, the rate of weight gain/loss was the most consistent determinant of bone loss. Historical measures of high physical activity predicted faster bone loss, while measures related to current activity (FEV1 in men, stairclimbing in women) were associated with bone conservation.These models accounted for 8.4% (women) and 4.6% (men) of the variance in the data. In women, inclusion of foods or food groups did not consistently improve the predictability of bone loss. In men there were moderate effects ($P<0.03$) of margarine and vegetable-based products, both negative and of nuts, seeds and whole-grain cereals (positive). We are continuing longer-term BMD data collection to improve the precision of outcome estimation; but so far no major effect of variation in diet has been identified in this population.

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Bone Mineral Density and Content in Young Women: Relations to Physical Activity and Calcium Consumption. S. Moyer,[1] L. Wallace,[1] P. Cussen,*[2] J. Chalmers,*[2] S. Ridings-Hesser,*[2] J. Ballard.[1 1]The University of Texas at Tyler, Tyler, TX, USA, [2]The University of Texas Health Center at Tyler, Tyler, TX, USA.

This cross-sectional study assessed the effect of current and lifetime behaviors on bone mineral density (BMD) and content (BMC) in 50 premenopausal Caucasian women (mean age=22.3±3.03 yrs, mean BMI=23.69±5.45). BMD and BMC for the whole body, lumbar spine (L2-L4), and femur (neck, Ward's triangle, trochanter, total hip) were measured with Hologic QDR-2000 DEXA. Subjects completed a valid and reliable oral questionnaire recalling information pertaining to lifetime and current weight-bearing activity and calcium intake (dietary and supplemental) and perceived self-efficacy (i.e., confidence) related to these behaviors. Spearman's rank or Pearson product moment correlations were used to explore simple relationships between the variables. Scores were ranked and divided

into quartiles for current weight-bearing activity and calcium intake. The quartiles were labeled as little/no, low moderate, high moderate, or high. ANOVAs with Tukey HSD Post Hoc Tests were calculated between the quartiles on all BMD and BMC values. Statistical significance was determined at p<.05. Results revealed: 1) total lifetime weight-bearing activity was positively correlated with spine BMC, spine BMD, total hip BMC, and total body BMC, 2) childhood (5-12 years) and adolescent (13-18 years) weight-bearing activity were positively correlated with current activity, 3) no significant difference at any site as function of lifetime calcium intake, 4) childhood and adolescent milk intakes were positively correlated, 5) adolescent milk intake was positively correlated with current calcium intake, 6) significant differences at Femoral Neck, Trochanter, and Total Hip with high levels of current weight-bearing activity producing higher BMD values, and 7) significantly higher self-efficacy in those reporting greater levels of current activity and calcium intake. On the basis of these data, it was concluded that weight-bearing physical activity predominately affected BMD and BMD. Children and parents should be made aware of the positive lifetime benefits of a physically active lifestyle and adequate calcium consumption.

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Prevalence of Hypovitaminosis D and Vitamin D Seasonal Variation in South Florida. S. Levis,[1] A. A. Gomez,*[2] C. Jimenez,*[2] L. S. Veras,*[2] B. W. Hollis,[3] S. Lai,*[4] B. A. Roos.[1] [1]State of Florida Teaching Nursing Home, GRECC, VAMC, University of Miami School of Medicine, Miami, FL, USA, [2]JMH/UM School of Medicine/VAMC, Miami, FL, USA, [3]Medical University of SC, Charleston, SC, USA, [4]Johns Hopkins University, Baltimore, MD, USA.

A 30% prevalence of hypovitaminosis D, a known risk factor for osteoporosis and fractures, has been found in northern latitudes and in fully clothed individuals with low sun exposure living in latitudes closer to the equator. We investigated the prevalence and seasonal variation of 25(OH) vitamin D (25D) in a population expected to have high sun exposure throughout the year. 212 ambulating subjects (77 men and 135 women) attending an outpatient clinic at a county hospital in Miami, FL, were evaluated at the end of winter (March 2000).The prevalence of hypovitaminosis D (< 15 ng/ml) was higher in women (22%) than in men (11%) and in African-Americans (29%) and non-Hispanic Whites (25%) than in White Hispanics (13%). To determine the seasonal variation, 99 subjects were evaluated again at the end of summer (September 2000). In both men and women, 25D winter levels were significantly lower than summer levels, but parathyroid hormone (PTH) levels were not higher in winter. Men and women living in this region experience a mild seasonal variation in 25(OH) vitamin D, although not sufficient to cause a significant rise in PTH levels. For men, mean 25D levels were 24.90 ± 7.50 standard deviations (SD) in winter and 31.00 ± 8.74 in summer (p = 0.003); mean PTH levels were 45.01 ± 22.66 pg/ml in winter and 36.48 ± 13.74 in summer (p = 0.58). In women, mean 25D levels were 21.91 ± 7.71 in winter and 24.96 ± 8.09 in summer (p = 0.02); mean PTH levels were 46.13 ± 29.36 in winter and 47.01 ± 28.93 in summer (p = 0.85). Our data show a significant increase of hypovitaminosis D in ambulating subjects in South Florida. This prevalence is higher in women, a group already at higher risk of developing osteoporosis, and in African-Americans, in whom this prevalence is similar to that reported in northern latitudes. This finding could represent a risk factor for developing osteoporosis in African-Americans and non-Hispanic Whites.

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Impact of Sodium Intake and Dietary Patterns on Biochemical Markers of Bone and Calcium Metabolism. P. Lin,*[1] F. Ginty,*[2] L. Appel,*[3] L. Svetkey,*[1] A. Bohannon,*[4] D. Barclay,*[5] R. Gannon,*[5] M. Aickin.*[6] [1]Duke Univ Med Ctr, Durham, NC, USA, [2]MRC Human Nutr Res, Cambridge, United Kingdom, [3]Johns Hopkins Univ., Baltimore, MD, USA, [4]Proctor and Gamble, Cincinnati, OH, USA, [5]Nestle Res Ctr, Lausanne, Switzerland, [6]Ctr for Health Res, Portland, OR, USA.

Research into the influence of diet on bone health has mainly focused on calcium, there has been little investigation of other dietary factors or multiple factors using a whole diet approach. A high sodium intake and acid load has been suggested to negatively affect calcium and bone metabolism . The DASH (Dietary Approaches to Stop Hypertension) diet emphasizes fruits, vegetables, and low-fat dairy foods, and is reduced in red meats and may create a lower acid load. The primary aim of this study was to examine the impact of two dietary patterns (the DASH diet and a control diet) and three levels of sodium intakes on biological markers of bone formation (serum osteocalcin), and bone resorption (serum C-terminal telopeptide of type I collagen (CTx). Changes in PTH, cyclic AMP and urinary calcium levels were also examined. This study was ancillary to the Dietary Approaches to Stop Hypertension-Sodium (DASH-Sodium) trial and was conducted at two of the four clinical sites (n=187). The three sodium levels were 50, 100 and 150 mmol Na/d/2100 kcal, respectively. All participants consumed the control diet at the 150 mmol Na/d level for two weeks and were then randomly assigned to eat either the DASH diet (n=91) or the control diet (n=96) at three sodium levels, each of which was consumed for 30 days in random order.Compared to the control diet, DASH diet reduced both serum osteocalcin and CTx at all three sodium levels (p<0.0001). This reduction was true for all participants regardless of age, race, gender and hypertension status. On the contrary, sodium intake did not significantly affect either of the bone markers in either the control diet or the DASH diet groups. Neither PTH nor cAMP was consistently affected by dietary pattern or sodium intake. These results indicate that irrespective of sodium intake, the DASH dietary pattern had a significant positive impact on bone metabolism in several population subgroups. More research is needed to investigate the long-term clinical impact of the DASH diet on bone health and its mechanism.

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Comparison of Two Measures of Weight Bearing Physical Activity to Bone Mass in Adults. M. T. Hannan,[1] R. R. McLean,[1] L. A. Cupples,*[2] K. Coyle,*[3] D. P. Kiel.[1] [1]Hebrew Rehab Ctr & Harvard Med Sch, Boston, MA, USA, [2]BU Sch Public Health, Boston, MA, USA, [3]Jefferson Med College, Phila., PA, USA.

Physical activity has a large impact upon bone health, yet no standardized method exists to measure it in large studies. Our goal was to compare self reported physical activity, which may not capture weight-bearing activities sufficiently, to weight bearing activity measured directly with an ambulatory monitor, as they both relate to bone mass. We examined these relations in a cross-sectional study of adults. Subjects were recruited from both the Framingham Offspring Study (n=79) and Harvard Cooperative Program on Aging research subject registry (n=28). We obtained 2 ultrasound measures of calcaneal bone mass using the Sahara bone sonometer (Hologic): broadband ultrasound attenuation (BUA) and speed of sound (SOS). Higher values indicate greater bone mass. Self-reported physical activity was assessed by the Physical Activity Scale for Elderly (PASE) questionnaire. PASE is validated for adults ages 50+ and produces a score based on activities over past 7 days. Weight bearing activity was measured as the number of steps over 3 days via the Ambulatory Monitoring System (AMS), an accelerometer worn on top of a shoe. Correlation analyses examined the relation between bone mass and PASE as well as AMS. Linear regression tested relation of bone mass for PASE and AMS controlling for age, sex, weight and height. Separate models were also considered for each sex. Mean age was 62 ± 11 years (range 36-86) for the 107 subjects (47 F, 60 M); mean PASE 139 ± 86, mean AMS 7990 steps ± 3783; mean ultrasound BUA 76 dB/MHz ± 19 and mean SOS 1556 m/second ± 33. The PASE and AMS were correlated (r=0.21, p=0.027). Ultrasound measures were weakly correlated to PASE (r=0.11, p=.26) but more directly correlated to AMS count (r=.21, p=0.03). Regression models continued to show a significant relation between bone mass and AMS (p=.01), but showed no relation between bone mass and PASE (p=.66). AMS contributed 6% to R^2 in regression models, whereas PASE did not explain any variance in models. Results were similar for sex-specific analyses.In sum, AMS activity provides a more direct measure of weight-bearing activity than PASE score. AMS also is a better indicator of bone mass than the PASE. Indeed, in our study, PASE was not related to bone mass. Collecting valid physical activity data in large, observational studies is a daunting task. Nevertheless, despite the slightly more difficult and burdensome approach in using activity monitors, this study underscores the superior specificity of devices that are able to non-invasively measure actual numbers of weight-bearing steps of physical activity.

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Vitamin D Insufficiency Is Common and Under-Diagnosed among African American Patients. S. Shewakramani,* D. Rakita,* V. Tangpricha,* M. F. Holick.* Vitamin D, Skin & Bone Research Laboratory, Boston University School of Medicine, Boston, MA, USA.

Traditionally African-American patients were considered a low-risk group for developing osteoporosis. These patients might not be screened for common causes of osteoporosis such as vitamin D insufficiency by the primary care physicians. African-Americans have decreased effectiveness of cutaneous vitamin D production due to their darker pigmented skin. We conducted a study to examine how frequently levels of 25-hydroxyvitamin D (25(OH)D) were determined by primary care physicians in African American patients with and without osteoporosis.We reviewed the medical records of 116 inner-city African American women who had undergone bone mineral density (BMD) within the last ten years. This population consisted of patients with osteoporosis, osteopenia and normal BMD. We recorded 25(OH)D levels and specialty of the physician ordering the test. A majority of osteoporotic, osteopenic, and normal patients (75.0%, 68.8%, and 63.2%, respectively) were vitamin D deficient (25(OH)D<20ng/ml). Overall, 69.9% of all patients in this study were vitamin D deficient. Only 38.6% of all 25(OH)D tests were ordered by primary care physicians. Specialist physicians ordered the remainder of the 25(OH)D tests. Limited information about parathyroid hormone levels was available to determine the incidence of secondary hyperparathyroidism.We conclude that vitamin D insufficiency is present in the majority of African-American patients being referred for bone mineral density testing and that primary care physicians infrequently order 25(OH)D levels in this high risk patient population.

Disclosures: Proctor and Gamble,2.

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Case Control Study for Risk Assessment of Fall in Aged Patients Using Questionnaire. J. Hashimoto,[1] M. Fujii,*[2] Y. Fukumoto,*[3] H. Yoshikawa.[1] [1]Orthopaedic Surgery, Osaka University Medical School, Osaka, Japan, [2]Dept of Orthopaedic Surgery, Glacia Hospital, Osaka, Japan, [3]Dept of Internal Medicine, Glacia Hospital, Osaka, Japan.

Hip fracture is a major public health problem because of its high frequency in older age, the resulting functional deficiencies and the high costs of treatment. Low bone mass is a contributory risk factor for hip fracture, but increasing risk of falls or poor neuromuscular protective response are also important factors. Therefore the efforts for prevention of hip fracture include prevention of falls. Several tests of functional or mental assessment have been reported to assess the risk of falls, but they require the diagnostic skill, specialized equipment, walking space, or much time. Hence the risk of falls is not always assessed especially for outpatients, while bone mass is generally evaluated to assess the risk of fracture. In this study we examined the usefulness of simple questionnaire for assessment of the risk of fall. 376 outpatients visiting our osteoporosis clinic were examined about risk factor of osteoporosis and fall with questionnaire. 11 patients with the neurological disease that cause the disturbance of gait ability were excluded and remaining 365 patients (average±SD: 61.4±8.99) were studied. We used questionnaire obtaining information on fall history in past a year, dietary habits (history of diet restriction, habitual skipping of meal, dairy-product consumption, milk consumption, alcohol intake), physical activity (daily

walking amount, gymnastic exercise), functional status of gait (decrease of gait speed, use of walking aid), postural change (loss of height, progressing kyphosis), pain in gait (low back pain, gonalgia) and current medication. On the data obtained from cases with fall and controls, logistic regression test was performed with SPSS 6.1J statistical software (SPSS Inc., Chicago, IL, USA). 78 cases of fall were included in the analysis of 365 people. Logistic regression model revealed that the risk of fall was not related to dietary habits, physical activity and current medication but was associated with decrease of gait speed, height loss, progressing kyphosis, fracture history, back pain and gonalgia. The relative risk of fall increased as follows: self consciousness of decreased gait speed 2.15 (1.23-3.75 p=0.007), self consciousness of height loss 1.86 (95% CI 1.07-3.21 p=0.026), self consciousness of progressing kyphosis 2.55 (95% CI 1.51-4.29 p=0.0003), gonalgia 2.77 (1.42-5.4 p=0.0022), often back pain 1.88 (0.99-3.56 p=0.05). Our result showed that simple questionnaire could provide the useful information for the risk of fall.

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Low Dietary Vitamin K Intakes Are Associated with Low Bone Mineral Density in Women: The Framingham Study. K. E. Broe,*[1] R. R. McLean,[1] S. L. Booth,*[2] D. R. Gagnon,*[3] K. L. Tucker,*[2] M. T. Hannan,[4] L. A. Cupples,*[3] D. P. Kiel.[4] [1]Hebrew Rehab Ctr, Boston, MA, USA, [2]USDA HNRCA, Tufts University, Boston, MA, USA, [3]Dept of Epidemiology, BU Sch Public Health, Boston, MA, USA, [4]Hebrew Rehab Ctr & Harvard Med Sch, Boston, MA, USA.

Low dietary vitamin K (Vit K) intakes are associated with increased risk of hip fracture in elders. We examined the role of dietary Vit K in age-related bone loss among younger adults by performing a cross-sectional analysis of dietary Vit K intakes in relation to bone mineral density (BMD) and heel ultrasound in 2,652 men and women (mean age=59 yrs, range 29-86) in the Framingham Offspring Study. Between 1996 and 2000 dietary Vit K intake and additional nutrients (total energy, dietary calcium and vitamin D, calcium and vitamin D supplement use, caffeine) were assessed by a Willett semi-quantitative food frequency questionnaire, validated for Vit K. Subjects currently taking anticoagulants and those with total energy intakes 4000 kcal were excluded. Total dietary Vit K was defined as the sum of Vit K intake from both food and supplements. Hip (neck, trochanter, and Ward's area) and spine BMDs (L2-L4) were measured using a Lunar DPX-L scanner and heel ultrasound (SOS, BUA, QUI), using the Hologic Sahara. Additional covariates included age, body mass index, smoking status, alcohol intake, physical activity score, and in women, menopause status and current estrogen use. Sex-specific linear regression models were used to examine BMD and ultrasound measures as a function of Vit K (both as a continuous variable and as quartiles of intake). Mean total dietary Vit K was 153 mcg/day in the 1,136 men and 171 mcg/day in the 1,516 women. Significant positive associations between total Vit K as a continuous variable and BMD were seen in women at the femoral neck (p=0.02), Ward's area (p=0.04) and spine (p=0.02) while the trochanter site (p=0.09) was borderline. Women in the lowest quartile of total dietary Vit K (mean=69 mcg/day) had significantly lower BMD than those in the highest quartile (mean=310 mcg/day). The adjusted means of femoral neck BMD across quartiles of Vit K in women were 0.857, 0.877, 0.882, and 0.887 g/cm^2 (p-trend: p=0.004). Similar differences were seen in women at the other BMD sites (p-trend: trochanter p=0.01, Ward's p=0.006, spine p=0.003), and remained after adjusting for potential confounders. No significant associations were found in men. Also, no significant associations were seen between Vit K intakes and ultrasound measures in either men or women. In this population low dietary Vit K intakes were associated with low BMD in women but not in men. Reasons for the different results in men and women are not clear and will require additional research.

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Lebanese Patients With Hip Fractures Are Relatively Young, but Have Osteoporosis. G. A. El-Hajj Fuleihan,[1] M. Badra,*[2] A. Tayim,*[2] M. Salamoun,*[2] N. Afeiche,*[2] O. Baddoura,*[2] S. Boulos,*[2] R. Haidar,*[2] S. Lakkis,*[2] R. Musharrafieh,*[2] A. Nsouli,*[2] A. Taha.*[2] [1]Medicine/Endocrinology, American University of Beirut Medical Center, Beirut, Lebanon, [2]American University of Beirut Medical Center, Beirut, Lebanon.

Osteoporosis is a major public health problem to-date, with hip fractures incurring the highest morbidity and mortality. There are clear geographic variations in bone mineral density (BMD) and fracture incidence worldwide, and the toll of the problem is projected to be higher in the Middle East compared to the Western world. However there is very little data, if any, on hip fracture patients from this part of the world. The purpose of this study is to test the hypothesis that as compared to their western counterparts:

1. Lebanese patients with hip fractures have the same mean BMD.

2. Lebanese patients sustain their hip fractures at a younger age.

49 consecutive patients with hip fractures admitted to a tertiary referral center had their BMD on the contralateral hip measured within one week of fracture occurrence. A Lunar DPX-L densitometer was used for the first 35 subjects and a Hologic 4500 A for the last 14. A cross-calibration formula based on 72 subjects measured on both machines allowed calculation of mean total hip BMD and total hip T-scores using the manufacturer's database, expressed in terms of Lunar DPX-L measurements. Osteoporosis was defined as a T-score<-2.5 using the manufacturer's database. Numbers expressed as mean ± SD.

	Males, N=18	**Females, N=31**
N Lunar/N Hologic	10/8	25/6
Age yrs	74± 8	74± 9
BMI Kg/M^2	25± 4	28± 4
BMD Total hip Lunar gm/cm^2	0.73±0.13	0.72± 0.11
BMD FN Lunar gm/cm^2	0.66± 0.07	0.65± 0.1
FN T-score Lunar	-3.1± 0.6	-2.7± 0.8
BMD FN Hologic	0.55± 0.19	0.59± 0.09
FN T-score Hologic	-2.8± 1.4	-2.4± 0.8

In summary, Lebanese subjects fracture at the same mean BMD as that of western subjects when matching for gender and densitometer type (1-3), but at a younger age. These findings may have major implications on the epidemiology of osteoporotic fractures in Lebanon.

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M338

Risk Factors for Distal Forearm Fracture in Women: Results From the European Prospective Osteoporosis Study. T. W. O'Neill,[1] S. R. Pye,*[1] A. A. Ismail,*[1] M. Lunt,*[1] J. Reeve,[2] A. J. Silman,*[1] EPOS Study Group.[1] [1]ARC Epidemiology Unit, University of Manchester, Manchester, United Kingdom, [2]Institute of Public Health, Cambridge, United Kingdom.

Distal forearm fracture is one of the most frequent osteoporotic fractures in women, however, there are few data from prospective studies concerning the determinants of this fracture. The aim of this analysis was to characterise the lifestyle and hormonal risk factors for distal forearm fracture in European women.Women aged 50-79 years were recruited from population registers in 32 European centres (European Prospective Osteoporosis Study - EPOS). Subjects were invited to attend for an interviewer administered questionnaire which included questions about various lifestyle and hormonal factors. Subjects were followed up using a postal questionnaire to ascertain the occurrence of incident fractures. Self-reported fractures were confirmed, where possible, by radiograph, attending physician or interview. The relationship between baseline predictors and future risk of distal forearm fracture was assessed using a cox-proportional hazards model.6978 women (mean age 63.1 years) were included in this analysis. During a median follow-up time of 3.0 years, 152 women sustained a distal forearm fracture. After age adjustment, frequent walking (> 1 hour per day) was associated with an increased risk of fracture (hazard ratio [HR]=1.7; 95% Confidence Interval [CI] 1.2, 2.3). Other lifestyle factors including consumption of milk and other calcium containing foods, cigarette smoking, and alcohol consumption were unrelated to future fracture risk. An older age at menarche (>= 15 years) was associated with an increased risk of fracture [HR=1.4; CI 1.0, 2.0] though menopausal age appeared unrelated.Frequent walking is linked with an increased risk of distal forearm fracture. Diet and other adverse lifestyle factors beyond the age of 50 years appear to have little impact on future fracture risk.

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Comparison between Qualitative and Quantitative Methods for the Detection of Incident Vertebral Fractures. T. Blenk,* G. Armbrecht,* W. Gowin, D. Felsenberg. Center of Muscle and Bone Research, University Hospital Benjamin Franklin, Free University Berlin, Berlin, Germany.

A comparison between quantitative methods with different thresholds (ratio and/or height reduction of vertebral bodies) and a qualitative evaluation for the detection of incident osteoporotic vertebral fractures was performed. Spinal radiographs of a vertebral fracture population (1733 cases) with four follow up visits over four years were analyzed. A qualitative evaluation including a differential diagnosis of incident osteoporotic fractures and morphometric measurements were performed. The incidence rate of each method and sensitivity, specificity, and kappa score compared to the qualitative method were calculated (see table).

Incidence rates, sensitivity, specificity, and kappa score of selected methods

method	**incidence rate**	**sensitivity**	**specificity**	**kappa score**
qualitative	224 (12.93%)			
4mm ah	204 (11.77%)	0.90	0.99	0.88
20% rr	196 (11.31%)	0.89	0.99	0.89
4mm ah and 20% rr	184 (10.62%)	0.87	1.00	0.90
3mm ah and 15% rh	198 (11.43%)	0.83	0.98	0.82
3mm ah and either 20% rh or 20% rr	190 (10.96%)	0.90	0.99	0.92
4mm ah and either 20% rh or 20% rr	186 (10.73%)	0.88	1.00	0.91

In the table "rr" means relative ratio reduction, "ah" absolute height reduction, and "rh" relative height reduction. For example, 4mm ah and 20% rr means a new fracture is defined as >= 4mm absolute height reduction and >= 20% relative height reduction.In the data presented only new fractures were considered. Most methods analyzed yield comparable results. Even the simple method of only a relative ratio reduction of 20% is a reasonable method for the detection of incident osteoporotic fractures compared with the qualitative evaluation. The advantage of only a relative ratio reduction is the independence of film object and film focus distances. The best results were obtained by the method 3mm ah and either 20% rh or 20% rr. If increasing fractures are considered as well a combination of relative ratio reduction of 20% with an absolute height reduction of 4mm is necessary. The best method for the detection of new and increasing osteoporotic fractures is 4mm ah and either 20% rh or 20% rr.

M340

Fracture Prevalence and 1 Year Incidence in the Young Elderly Female Population. J. Thomas,* S. A. Steel, S. M. Doherty. Centre for Metabolic Bone Disease, Hull Royal Infirmary, Hull, United Kingdom.

This is part of a continuing study comparing the logistical feasibility of undertaking diagnosis, risk assessment and treatment of osteoporosis among the young elderly female population in a hospital and a primary care based setting. We report on the prevalence and 1 year incidence of fracture in the study group which comprises 418 females aged 70 to 75 years . Urinary DPD, Vitamin D, Calcium, Alkaline Phosphatase and Broad Band Ultrasound Attenuation (BUA) of the heel was assessed in all patients. Bone densitometry by Dual Energy Xray Absorptiometry was performed in 149 subjects who attended the hospital as part of the study. Overall 129 (41%) of subjects reported one or more previous fractures 48 (37.2%) of which were considered as low trauma fractures. The relative risk for subjects with prior fracture being defined osteoporotic was 2.37 at spine and 2.33 at hip.During the first year of follow-up, 10 subjects suffered a fracture, all of which were low trauma. 7 had upper limb fractures (4 Colles) and none had fractures neck of femur. 7 of these subjects had reported no previous fracture. Of the 6 who had undergone DXA, only 1 was defined osteoporotic. BUA of the heel in 9 subjects (1 failed reading) was low (<60 dBMHz-1). The majority (7) had an adequate dietary intake of calcium, 4 had a family history of osteoporosis. 5 subjects in the incident fracture group had increased urinary DPD, 1 of whom also had low vitamin D, and 1 patient had a marginally low serum calcium with normal vitamin D and DPD.At this stage, the incident fracture at 1 year is too low to statistically examine predictive ability of risk factors. The study is ongoing to determine the power of the parameters assessed to predict further fractures and the impact of intervention on fragility fractures.

M341

Typical Shape of Vertebral Osteoporotic Fractures. G. Armbrecht,* T. Blenk,* W. Gowin, D. Felsenberg. Center of Muscle and Bone Research, University Hospital Benjamin Franklin, Free University Berlin, Berlin, Germany.

The aim of the study is to describe the typical shape and location of osteoporotic fractures .A sample of 83 randomly selected cases with 144 osteoporotic vertebral fractures in t-spine and l-spine were evaluated. 68% of all fractured vertebrae have an anterior height reduction (less than 80% of the expected height), 87% a medial height reduction, and 1% a posterior height reduction. Four typical shapes of osteoporotic fracture could be distinguished: wedged (23%), concave (71%), biconcave (5%) and crushed (1%). These four types are associated with different risks for further osteoporotic vertebral fractures.Wedge fractures are located in the mid t-spine. In 50% of the wedge fractures the medial height is also reduced but less than the anterior height. No fracture line is visible in wedge fractures.The concave fractures are located in the thoracic-lumbar junction and in the lumbar spine mostly. In 65% of the concave and biconcave fractures the anterior height is also reduced but less than the medial height. 82% of the concave fractures have an upper endplate fracture, 12% a lower endplate fracture and 6% an upper and lower endplate fracture (biconcave fractures). In 90% of the concave and biconcave fractures a fracture line is visible projected onto the vertebral body (see figure 1). In semiquantitative and quantitative methods the end of the fracture line should be used for the height assessment. Therefore the projected area of the vertebral body is sometimes not reduced in case of concave fractures and severity of the osteoporotic fracture could be underestimated.Crush fractures show always a decrease of all verbral heights.

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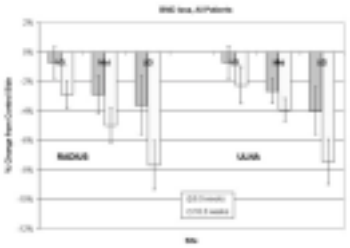
Osteopenia Following Forearm Disuse With and Without Fracture. J. A. Spadaro, W. H. Short.* Orthopedic Surgery, Upstate Medical University, Syracuse, NY, USA.

The purpose of this work is to characterize unilateral forearm osteopenia in a group of patients following treatment in a cast. 30 patients (mean age 47 yrs., 21-80 yrs.)., half undergoing carpal surgery and half with uncomplicated Colles' fracture, were treated with casts for approximately 6 weeks. Bone densitometry (DXA) was used to characterize the bone mineral density (BMD) in the radius and ulna of both forearms before surgery (surgical group only), after the cast was removed (~7 weeks) and 2 months later (~ 16 weeks) after the start of treatment Standard forearm BMD scans were performed in the midshaft (1/3), distal metaphysis (MID) and ultradistal (UD) regions, using either the QDR-1000W or 4500A densitometers (Hologic, Inc.). Data was expressed as % change from contralateral (unaffected) side to enable inclusion of fracture with surgery cases. Data referenced to baseline correlated well with that referenced to contralateral (R=0.87, radius UD). Three subjects were eliminated from the analysis because of severe pre-existing osteopenia with asymmetry, or missing data.Results showed that BMD was lower on average at all sites at both time periods ($p < 0.001 – 0.05$) except for the midshaft sites at 7 weeks. Loss over the entire group at 16 weeks was about twice that at 7 weeks, reaching 7.5-8% (UD) ($p < 0.001$-0.02), except at ulna MID site.. This loss pattern was similar between the surgical and fracture subgroups with the exception of the radius UD in the fracture group. Patients whose dominant forearm was treated tended to have greater BMD loss at most sites than non-dominant treated forearms, but statistically significantly so only for the radius UD site in this analysis. Linear regression analyses showed that there was a small but significant decrease in UD and MID ulna bone loss at 16 weeks with increasing global BMD level (R= 0.4, $p<0.02$), and an increasing global BMD loss with increasing age notably in the non-dominant treated arms (radius and ulna, R= 0.48).These observations confirm previous observations of a forearm bone loss in fracture patients that lags the cessation of immobilization, with parallel effects in ulna and radius, and suggest that hand dominance and increasing age accelerates disuse osteopenia in the forearm. Aided by a grant from the Orthopedic Research and Education Foundation.



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Bone Mineral Density Measurements and Proximal Femur Geometry Parameters to Differentiate Vertebral, Femoral Neck and Trochanteric Fractures. N. Malavolta,[1] M. Frigato,*[1] L. Lisi,*[1] R. Mule',*[1] S. Gnudi.*[2] [1]Medicina Interna, Azienda Ospedaliera di Bologna, Bologna, Italy, [2]Medicina Interna, Istituto Ortopedico Rizzoli, Bologna, Italy.

Vertebral fractures (VF) and hip fractures have been reported to be characteristic respectively for type 1 and type 2 osteoporosis. Nevertheless, among hip fractures differences have been found between subjects with femoral neck fracture (FNF) from those with trochanteric fracture (TF) concerning bone mineral density (BMD) body weight, age at occurrence and, more recently, with regards to proximal femur geometry (PFG). The aim of this study was to evaluate whether different BMD and PFG values, generally associated to the fracture risk, can separate type 1 osteoporosis from the two types of hip fracture of type 2 osteoporosis. 250 healthy and 260 osteoporotic age-matched women were studied. In the osteoporotic group 100 women had VF, 90 women FNF and 70 women TF. With the step wise discriminant analysis the BMD measurement significantly separated each group of fracture from healthy people, while PFG separates only FNF and TF from healthy subjects. These two fractures were also significantly separated from VF by the PFG neck shaft angle (narrow in VF) and shaft diameter (wider in VF), while the other measured parameters (hip axis length and neck diameter) were not included in the model. BMD of the trochanter (lower in TF) alone is enough, among BMD measurement, to effectively separate VF from TF. Conversely, spine BMD (lower in VF) and femoral neck BMD (higher in VF) separates FNF from VF. This suggests that the same PFG parameters significantly separate FNF and TF from VF. Neck shaft angle in FNF is the closest correlated with group separation. BMD behaves differently according to the type of femoral fracture. BMD is lower in TF than VF at each measurement site. This indicates that a general bone loss is a common mechanism to these two types of fracture. VF and FNF are separated by different patterns of bone loss, being prevalent in the vertebral body (mainly cancellous bone) in the former and at the femoral neck (mainly cortical) in the latter. The different association of the fractures to the kind of bone loss might indicate a different pathogenesis between the two types of fracture.

M344

Body Height Loss Relates to Incident Vertebral Fractures in Patients as a Group but Not as Individuals. G. Jiang,*[1] N. A. Barrington,*[2] R. Eastell.[1] [1]Bone Metabolism Group, Clinical Sciences Centre, Sheffield University, Sheffield, United Kingdom, [2]Diagnostic Image Department, Northern General Hospital, Sheffield, United Kingdom.

Vertebral fractures are associated with height loss when individuals are considered as a group; however, it is unclear whether height loss can be used as a guide to further investigations in individuals. We studied 372 elderly women ages 50 to 75 years, a randomly drawn sample from three general practices. 236 of them returned five years later. We identified incident vertebral fractures by visual reading of lateral spinal radiographs (T4 to L5). We found that height loss was greater in the women with fracture (mean, SD, 1.4, 5.8) than in those without (0.2, 1.0, P=0.0003). The sensitivity for identifying incident fractures was low. Using 2 cm as the criterion, all patients who had multiple fractures and one patient who had a moderate wedge fracture at L1 were identified. Patients who had a single fracture were missed. These included minor or moderate wedge fracture in the middle or lower thoracic spine, moderate or severe compression fracture in the upper or middle thoracic spine, and concave fracture in the lumbar spine. We conclude that 2-cm height loss is a useful criterion for identifying multiple fractures, but not for identifying a single vertebral fracture.

Height loss	Sensitivity (%)	Specificity (%)	Positive predicted value (%)
≥1cm	64 (9/14)	66 (147/222)	11
≥2cm	43 (6/14)	94 (209/222)	32
≥3cm	21 (3/14)	97 (216/222)	33
≥4cm	21 (3/14)	99 (219/222)	50

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Patients With Forearm Fracture Should be Diagnosed for Osteoporosis. E. Waern,*[1] O. Johnell,[2] H. Jutberger,*[1] J. Karlsson,*[3] C. Nyman,*[1] D. Mellström.[1] [1]Geriatric Medicin, University of Göteborg, Göteborg, Sweden, [2]Orthopaedics, University of Malmö, Malmö, Sweden, [3]Orthopaedics, University of Göteborg, Göteborg, Sweden.

Distal forearm fracture is the most common fracture in perimenopausal women. The incidence rises sharply around age 55 years in women. The increase in distal forearm fracture rate in this period of life has been attributed to a reduction in bone strength caused by the accelerated phase of bone loss at the menopause. Forearm fractures have been found to be an early indicator of osteoporosis and new fractures and women who have experienced a distal forearm fracture have reduced bone density especially in the forearm compared to controls. The aim of this project was to try a model for taking care of fracture patients and find those in need of drug therapy for osteoporosis. All patients presenting with a fracture of the distal forearm at the orthopaedic clinic at Sahlgrenska University Hospital/Östra, Göteborg, over a two year period, were offered to participate in this project. BMD was measured in calcaneus on a Calscan DXA-T at the outpatient clinic (5-7 days after fracture)in order to find those with BMD less than -1SD T-score in calcaneus. 15% had normal BMD and 85% had a BMD less than -1 SD. All these patients were measured by DXA, using Hologic QDR 4500A, in lumbar spine, proximal femur, total body and in some patients, non-fractured ultra distal radius (54 women, 4 men). 192 subjects (176 women, 16 men), mean age 65,2 years (range 22-90) were measured in all sites. 75% of the women were osteoporotic (T-score<-2,5SD) in calcaneus and 59% in spine, hip or total body. Mean Z-score in spine was -0.4SD, in hip -0.3 SD and in total body -0.9 SD. All patients were clinical assessed. BMD in calcaneus correlated to BMD in hip and total body (r=0.58) and to lumbar spine respectively (r=0.47). Hip BMD correlated to spine BMD (r=0.58) and total body (r=0.68). The guidelines in Sweden recommend drug treatment to a fracture patient at a BMD of T-score -2SD. 73% of these women with a fracture of the distal forearm had a BMD of -2SD or less and should be offered treatment to avoid a new fracture.

M346

Vertebral Fractures Identified by IVA in Postmenopausal Women. S. M. Nattrass,[1] L. A. Jones,*[2] T. L. Kelly,[2] E. von Stetten,[2] K. E. Wilson.*[2] [1]PacMed Clinics, Seattle, WA, USA, [2]Hologic, Inc., Bedford, MA, USA.

Two primary risk factors for osteoporotic fracture are low axial BMD and an existing vertebral fracture. However, many vertebral fractures do not come to clinical attention, resulting in under-recognition of those in need of osteoporosis therapies. In part, this is due to the cost, radiation dose, and inconvenience of conventional spine x-rays, especially for asymptomatic patients. As a result, patients with a prevalent vertebral fracture classified as "normal" or "osteopenic" by BMD are mis-classified in terms of fracture risk. This study is a follow-up to one reported previously that had fewer patients, included perimenopausal women, and a broader age range. Using a convenient, low-dose spine imaging technique, this study evaluated the percentage of postmenopausal women in a clinical setting with a high fracture risk based upon the presence of a vertebral fracture, but a low or moderate fracture risk based upon DXA T-score alone. Women over age 50 and at least one year postmenopausal obtained BMD measurements of the AP spine, femoral neck, and total hip, along with AP and lateral spine images using a Delphi QDR Series bone densitometer with Instant Vertebral Assessment (IVA). IVA uses a single-energy scan to obtain a rapid (10 s), low-dose (7 mR) image of the spine (typically T4-L4). Fractures identified on IVA images were graded as mild, moderate, or severe using the Genant semi-quantitative method. Patients were classified by WHO criteria using the lowest BMD T-score. A total of 122 women were evaluated with a mean age of 63.2 ± 9.6 years; 35 were classified as normal (age 56.6 ± 4.7 years), 65 as osteopenic (age 64.7 ± 9.7 years) and 22 as osteoporotic (age 69.0 ± 9.7 years). Vertebral fractures (VFx) were identified in 35 patients.

WHO Classification	Mild VFx	Moderate or Severe VFx	Moderate, Severe, or Multiple VFx	Any VFx
Normal (n=35)	11%	6%	11%	17%
Osteopenia (n=65)	14%	17%	20%	31%
Osteoporosis (n=22)	27%	14%	23%	41%

In conclusion, 26% of women in this study would have been mis-classified as having a low or moderate fracture risk using only the lowest BMD T-score. Some of these women may have had elevated BMD due to osteoarthritis, osteophytes, etc. Therefore, the use of IVA identified patients who could significantly benefit from therapies that reduce fracture risk who would have been overlooked by measuring BMD alone.

Disclosures: Hologic, Inc.,2.

M347

Stress Fracture Occurrence Is Not Related to Variables of Calcium Homeostasis, Bone Turnover, or IGF-1 in Elite Military Cadets. F. Cosman,[1] J. Nieves,[1] M. Zion,*[1] J. Ruffing,*[1] J. Uhorchak,*[2] S. Gordon,*[1] R. Lindsay.[1] [1]Clinical Research Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Keller Army Hospital, West Point, NY, USA.

The etiology and pathophysiology of stress fractures in young adults may differ from that of other types of fractures including those related to osteoporosis. In some populations, low levels of vitamin D, and IGF-1 and high levels of PTH and bone turnover indices increase the risk of fracture occurrence. Our objective was to determine whether a relationship could be found between blood levels of these indices and stress fracture in a population of Caucasian military cadets from an ongoing study of the class entering the USMA at West Point in 1998 (initial n=758.) Over 2.5 years, stress fracture occurrence was diagnosed by standard orthopedic procedures (bony tenderness and swelling with bone, cortical or periosteal abnormality found on x-ray or increased uptake on scan). A total of 64 cadets had one or more confirmed stress fractures (25 females and 39 males) and they were matched for gender and age ± 6 months with cadets who had no bony injury. 2 non-fractured cadets were compared with every 1 fracture case for this nested case/control study. Blood samples were obtained upon cadet entry to USMA and serum samples were stored in separate aliquots and frozen at -70°. Intact PTH, BSAP and IGF-1 were measured by IRMA, 25(0H)D by radioceptor assay, and NTx by Elisa. Mean levels of PTH, 25(0H)D and IGF-1 were all in the normal range with no gender or fracture case-related differences. Levels of bone turnover, particularly bone formation, were higher in males than females but, again, there were no differences between fracture cases and controls. These results indicate that stress fracture incidence in military cadets does not appear to be related to prospective measurement of PTH, 250HD, IGF-1, or bone turnover indices.

M348

Fractures with Chronic Spinal Cord Injury: Epidemiology, Morphology, and Healing Outcomes. B. J. Kiratli, I. Perkash,* G. D. O'Mara,* G. E. Sims.* VA Palo Alto Health Care System, Palo Alto, CA, USA.

Rapid bone loss occurs following spinal cord injury (SCI) resulting in up to 35% decrease of skeletal mass in paralyzed regions by 4 years post-injury. Fracture risk is assumed to be elevated but there have been few epidemiologic studies regarding occurrence and outcomes of fractures in this population. Our goal was to evaluate incidence and prevalence, anatomic location and morphology, risk factors, related secondary conditions and quality of life issues, and healing of lower extremity fractures in individuals with SCI and paralysis. A retrospective cohort study was conducted to identify and characterize post-SCI fractures. Chart review was completed on 833 patients with SCI seen in our Center since 1972 and demographic and medical data were collected. Serial radiographic review was performed for those with fracture history and a hierarchical classification system used to characterize fracture type, morphology, and complexity. A total of 323 long bone fractures were found in 170 patients, thus overall prevalence is 39% with a mean of 1.9 fractures per person. The majority, 88%, occurred in the lower extremity, nearly evenly distributed in the femur and tibia. These fractures occurred at a mean duration of 15.8 years (range: 0.5 - 54 years) post-SCI. Most fractures resulted from minimal or no identifiable trauma: 40% from normal daily activities (dressing, stretching, transfers or range of motion); 36% from falls from the wheelchair; and 12% from unknown causes. Fractures were concentrated around the knee, and the majority were not complex or multi-fragmentary. Analyzed by bone segment, more fractures occurred after the first decade in the distal femur and proximal tibia sites, while the rate was steady in other regions. Incidence rates, determined by 5-year periods, were unchanged over time (ranging from 4-8%/year). Most fractures were treated conservatively (ie, non-surgically), and 90% achieved bony union with good alignment in over 50%. There was little difference in healing rate or outcome by bone segment or by fracture complexity. Callus formation began as early as 3 weeks, bridging bone was present by 4-6 weeks, and remodeling was observed by 12-15 weeks. In 50-60% of fractures, added assistance was required until the fracture healed. While lower extremity fractures are experienced by many persons with chronic SCI, healing is rapid and uncomplicated and achieved without surgical intervention in most cases. Fracture imposes little long-term effect on quality of life, but there may be significant short-term effects due to limited mobility and decreased independence. Preventive efforts should focus on fall prevention and modification of force generation during daily activities.

M349

Osteoporosis in Institutionalized Male Veterans: Common but Underrecognized. M. E. Elliott,[1] P. J. Drinka,*[2] P. Krause,*[2] J. E. Mahoney,*[3] N. C. Binkley.[3] [1]School of Pharmacy, University of Wisconsin, Madison, WI, USA, [2]Wisconsin Veterans Home, King, WI, USA, [3]Department of Medicine, University of Wisconsin Medical School, Madison, WI, USA.

Twenty-five to 30% of hip fractures occur in men. Institutionalized men have a 5- to 11-fold greater fracture risk than community-dwellers, but osteoporosis prevalence in this group is poorly defined. The goals of this project were to determine: osteoporosis prevalence in a convenience sample of institutionalized male veterans using peripheral BMD (bone mineral density) measurement; if osteoporosis was previously documented; and if urinary N-telopeptide (NTx) concentrations were stable over time. Men were recruited at the Wisconsin Veterans' Home, King, WI. Bilateral calcaneal and distal radial BMD were measured by dual-energy X-ray absorptiometry (Peripheral Instantaneous X-Ray Imager, GE Lunar Corporation, Madison, WI). Prior osteoporosis documentation was sought in medical records. For 12 randomly chosen subjects, morning urinary NTx was measured at one-month intervals for four months.One hundred and three veterans (approximately 25% of residents) were enrolled. Subjects' age range was 51-95, mean 76. BMD T-scores ranged from +1.6 (heel), -0.4 (forearm) to -4.9 (heel), -6.0 (forearm). Of 99 men with calcaneal BMD measurements, twenty (20%, 95% confidence interval [CI] 12-28%) were osteoporotic (T-score <-2.5). Of 95 men with forearm BMD measurements, 59 (61%, confidence interval [CI] 51-71%) were osteoporotic. Forearm and calcaneal BMD were correlated ($r = 0.678$, $p < 0.001$). Additionally, BMD of the left and right radius and calcaneus were highly correlated ($r = 0.880$, $p < 0.001$ and $r = 0.931$, $p < 0.001$ respectively). Prior documentation of osteoporosis existed for only five men; one out of 20 (5%) with calcaneal osteoporosis and four of 59 (7%) with radial osteoporosis. NTx was stable over four months (mean 29.3 nM NTx/mM creatinine, coefficient of variation 20% [range 13-30%]).In conclusion, osteoporosis was prevalent but poorly documented in this sample of institutionalized veterans. The broad range of T-scores among subjects suggests that peripheral BMD measurement may be useful for clinical fracture risk stratification. Correlation between heel and forearm BMD and between left and right sides suggests that measurement at a single site may be practical. As NTx is stable over time, it may prove useful for monitoring of therapy in these individuals.This study suggests that peripheral BMD and urinary NTx can be valuable tools for improving recognition and management of osteoporosis in institutionalized men.

Disclosures: Novartis,2; Merck,2,5,8; Aventis,2.

M350

Evaluation of Osteopenia and Osteoporosis in Five Groups of Men, Aged 65-93 Years. C. Herron,*[1] L. Harrington,*[1] K. Mobbs,*[1] P. Cussen,*[2] J. Chalmers,*[2] S. Ridings-Hesser,*[2] D. Holiday,*[2] J. Ballard.[1] [1]The University of Texas at Tyler, Tyler, TX, USA, [2]The University of Texas Health Center at Tyler, Tyler, TX, USA.

The purpose of this study was to evaluate in a cross-sectional sample of 51 adult males, aged 65-93 years, changes in BMD (g/cm2) Lean Tissue (g), and Fat Tissue (g), and to assess the number of men, according to WHO T-Scores, who were classified with osteopenia (OSPA) or osteoporosis (OSPS) at either the forearm, spine, femur, or total body. Subjects were placed by age into 5 groups (65-69, n=10; 70-74, n=14; 75-79, n=12; 80-84, n=9; 85+, n=6). Lean and fat tissues were measured by anthropometry and with Whole Body DEXA. BMD at regional sites and for whole body were measured with Hologic QDR 2000 Bone Densitometer. Body weight (kg), standing and sitting heights (cm), hand grip strength (kg), and eight skinfolds (mm) (Jackson et al., 1980) were measured along with eight body girths (cm) (Lohman et al., 1991). ANOVAs were used to determine significant differences across age groups in bone, lean, and fat tissues. Significance was determined at p<.05. Results revealed: 1) No significant difference between groups for standing or sitting heights but a decrease in mean body weight between the youngest and oldest groups (87.4+/-9.8 kg to 72.2 +/-10.1 kg), 2) No significant group differences for skinfolds or fat tissue, 3) Decreased hand grip strength as well as muscle girths at extremities: upper (relaxed and contracted bicep, contracted forearm) and lower (thigh and calf) with decreased values as the age of the subjects increased, 4) Decreased lean tissue in whole body and in both arms but not in legs with youngest group having more tissue than oldest group, 5) Decreased BMD in both arms and at 1/3 and Mid Radius with youngest group having higher BMD values than oldest group but no differences in spine or femur, 6) Percent loss across 15 years (65-69 vs. 80-84 years) at Total Wrist, Total AP Spine, Total Lat Spine, and Total Femur: -4.59%, -2.34%, -9.26%, and –6.26% respectively, and 7) Number of men who had OSPA or OSPS at Total Wrist, AP Spine, and Total Femur were respectively: 65-69, OSPA=4, 3, 3 OSPS=1, 0, 0; 70-74, OSPA=3, 5, 8 OSPS=2, 0, 0; 75-79, OSPA=2, 3 ,4 OSPS=4, 2, 1; 80-84, OSPA=3, 2, 7 OSPS=3, 2, 0; and 85+, OSPA=2, 0, 1 OSPS=3, 2, 1. T-Scores not available for Lat Spine. On the basis of these data, it was concluded that body weight, muscle girth, lean tissue, hand grip strength, and BMD at 1/3 and Mid Radius and in Right and Left Arms decreased as the age of the subjects increased. The total number of men who had OSPA and OSPS respectively at wrist were 14 and 13; at AP Spine were 13 and 4; at Total Femur were 23 and 2. Men should be made aware that as they age they become at risk for bone loss and possible non-traumatic fractures.

M351

The Effect of Lifestyle Factors upon Bone Mineral Density in 51 Elderly Males, Aged 65-93 Years. K. Mobbs,*[1] C. Herron,*[1] L. Harrington,*[1] P. Cussen,*[2] J. Chalmers,*[2] S. Ridings-Hesser,*[2] D. Holiday,*[2] J. Ballard.[1] [1]The University of Texas at Tyler, Tyler, TX, USA, [2]The University of Texas Health Center at Tyler, Tyler, USA.

This study assessed the effect of lifestyle factors on forearm, spinal, and femoral BMD in elderly males. Standing and sitting heights, body weight, skinfolds, body girths, and hand grip strengths were evaluated to describe the physical characteristics of the men in this study. Subjects completed an oral questionnaire recalling information pertaining to physical activity (occupational, recreational and activities of daily living), dietary and supplemental calcium, sun exposure (Vitamin D3), fracture history, and use of alcohol and tobacco. BMD was measured with Hologic QDR-2000 DEXA. From the questionnaires, scores were obtained, ranked, and divided into quartiles for physical activity, calcium intake, and sun exposure. The quartiles were labelled as little/no, low moderate, high moderate, or heavy. ANOVAs with Tukey Post Hoc Tests were calculated between the quartiles on forearm, spinal, and femoral BMD values. Additionally, alcohol and tobacco behaviors were reported for the last 10 years, and subjects were categorized as users and non-users. Fractures at any site across the life span were reported, and subjects were divided into those with and without fractures. T-tests were used on BMD values for the two-category variables. Statistical significance was determined at p<.05. Results revealed: 1) significant differences at Total Wrist, Femoral Neck, Inter Trochanter, and Total Hip with moderate levels of occupational physical activity producing higher BMD values, 2) significant differences at Lateral Spine and Inter Trochanter with the lower levels of recreational activities and those of daily living producing higher BMD values, 3) significant differences at Femoral Neck and Ward's Triangle with high levels of dietary calcium producing the greatest BMD values but no differences in BMD due to supplemental calcium, 4) significant differences at Lateral Spine with moderate levels of sun exposure resulting in higher BMD values, 5) significantly higher BMD values in non-users of alcohol than in users at Femoral Neck, Trochanter, Ward's Triangle, and Total Hip, 6) no significant difference at any site between tobacco users and non-users, and 7) significantly higher BMD values at Femoral Neck, Trochanter, and Total Hip in those who never had a fracture over those who had a history of fracture. On the basis of these data, it was concluded that lifestyle factors predominately affected femoral BMD. Men should be made aware of the importance of these factors, their effect on bone density, and the subsequent danger of hip fracture with its associated increased mortality rate.

M352

Studies on BMD of Lumbar Spine and Femoral Neck Among the Male Ankylosing Spondylitis AS Patients in Khuzestan Province-Iran. K. Mowla. Head of Rheumatology Dep., Ahwaz University of Medical Sciences, Ahwaz, Iran (Islamic Republic of).

Objective: One of important factor causes fracture among AS patients is the reduction of BMD of lumbar spine and femoral neck.Material and method: For study of BMD of lumbar spine and femoral neck 51 AS male patients between 20-50 years were selected. The mean ages patients was 34.4 years.For the control group 59 males with mean ages of 34 years were selected. Results: In the present study, 51 AS patients showed low BMD of femoral neck compared to 59 cases of control group (p<0.001). While BMD of lumbar spine among 51 AS patients did not showed significant compared to the control group (P>0.05). Conclusion: The reduction of BMD of femoral neck showed significant among the AS male patients .

M353

Relationships Between Body Composition and BMD in 51 Caucasian Males, Aged 65-93 Years. L. Harrington,[1] K. Mobbs,[1] C. Herron,[1] P. Cussen,*[2] J. Chalmers,*[2] S. Ridings-Hesser,*[2] D. Holiday,*[2] J. Ballard.[1] [1]The University of Texas at Tyler, Tyler, TX, USA, [2]The University of Texas Health Center at Tyler, Tyler, TX, USA.

This study evaluated relationships between body composition variables and BMD of forearm, spine, femur and total body in 51 elderly males, aged 65-93 years. This was done to determine if associations existed between specific types of body composition (total body weight, lean weight, or fat weight) and BMD. Body composition was assessed with anthropometry (ANTHR) and Whole Body DEXA. ANTHR consisted of standing and sitting heights, body weight, 8 skinfolds, 8 body girths, and both hand grip strengths. Lean Tissue and Fat Tissue were assessed from Whole Body Scan on the Hologic QDR-2000 DEXA. This equipment also measured regional BMD values in forearm (radial and ulnar 1/3, mid, and ultra distal sites), spine (Total and Mean AP and Lateral sites), and femur (Femoral Neck, Inter Trochanter, Trochanter, and Total Hip sites) along with Total Body BMD. Zero-order correlation coefficients were obtained between Body Composition Variables and BMD values at regional and Whole Body Bone sites. Statistical significance was determined at p<.05. Results revealed: 1) from ANTHR, mean standing height and body weight significantly and positively correlated with BMD values at all sites in forearm, spine, femur and Total Body indicating that as body size increased so did BMD values, 2) from DEXA Scans, Lean Tissue significantly and positively correlated with BMD values at all bone sites, while Fat Tissue was not consistently related at spinal or femoral sites nor related at any forearm site, indicating that increases in Lean Tissue rather than Fat Tissue was most highly associated with increased BMD values, 3) similarly with ANTHR measurements, body girths (indirect measurement of muscle) showed more statistical significance with BMD values than did skinfold thicknesses (subcutaneous fat sites) suggesting that increased muscle rather than increased fat was most highly associated with increased BMD values. On the basis of these data, it was concluded that greater amounts of lean tissue resulting in more total body weight rather than a greater amount of body fat was associated with increased BMD values at forearm, spine, femur and total body. Elderly men should be encouraged to remain physically active in order to maintain adequate muscle because of its positive association with increased BMD values.

M354

Knowledge and Attitudes Towards Male Osteoporosis. J. M. Harke, D. C. Krueger, T. N. Kawahara,* N. C. Binkley. Institute on Aging, University of Wisconsin, Madison, WI, USA.

Given increased attention to male osteoporosis in the bone research community, we sought to determine if this recognition has been conveyed to physicians. To this end we mailed a survey to 5646 Wisconsin primary care, medical subspecialty (MSS), orthopedic and urology physicians. The survey consisted of 14 statements covering general knowledge, diagnosis, treatment and attitudes towards male osteoporosis. Physicians were asked to be neutral towards, agree, or disagree with these statements.Twenty-six percent (1484) of physicians responded, with 69% being family physicians (FP) or internists (IM). Of all physicians responding, 63% recognized that osteoporosis is not rare in men and 71% were aware that men have higher mortality after hip fracture than women. More than 80% felt that low-trauma fracture or corticosteroid therapy initiation were indications for bone mass measurement. Treatment initiation at a T-score of –2.5 was accepted (80%), but physicians were less sure that treatment of osteopenia was indicated. FP were more likely than IM to be neutral towards measuring bone mineral density (BMD) when initiating corticosteroids (p<.05) and treating men with low BMD (p<.01). Most physicians agreed that both alendronate and calcitonin were effective osteoporosis treatments for men. The majority of physicians believed that male patients viewed osteoporosis to be a woman's disease and 43% thought men would not be interested in osteoporosis prevention. In conclusion, this group of physicians appear to: 1) recognize osteoporosis as an important disease in men; 2) accept corticosteroid therapy and prior low-trauma fracture as indications for bone mass measurement in men; and 3) apply WHO diagnostic criteria and NOF treatment guidelines to men. However, ~ 20% of primary care physicians consider osteoporosis to be rare in men. In addition, most physicians believe that osteoporosis prevention measures are not well received by men. In this regard, limited data suggest these impressions about men's attitudes toward osteoporosis are correct, but improved understanding of men's perceptions about this disease would be beneficial. As such, a survey of 175 men presenting for primary care is being conducted; these data will be presented. A need for continuing physician and public education about male osteoporosis likely exists.

M355

Parental and Individual Histories of Fracture Are Associated With Osteoporosis in Men. E. A. Krall,[1] J. J. Anderson,*[1] D. R. Miller,*[2] A. Rourke,*[2] J. Chan,*[2] S. E. Rich.*[1] [1]Boston University, Boston, MA, USA, [2]Massachusetts Veterans Epidemiology Resource & Information Center, Boston, MA, USA.

Knowledge of family history of osteoporosis or previous fractures is a useful screening tool for identifying women at increased risk of the disease, but few studies have examined these risk factors in men. The purpose of this study was to examine the associations of parental and individual fracture histories with presence of osteoporosis at the hip in middle aged and elderly men. The subjects are participants in the VA Longitudinal Osteoporosis Research (VALOR) study and range in age from 50 to 91 years (mean ±SD=70±8). Forty-eight percent of the cohort consists of men who are VA patients. Femoral neck and total

femur bone mineral density (BMD) of each hip were measured with dual energy x-ray absorptiometry (model DPX-IQ, Lunar Corp., Madison, WI). The averages of right and left sides were computed. Osteoporosis was defined as BMD more than 2.5 SD below the mean value of males age 20-29 years. Family history and individual history of fracture after age 40 were obtained from an interviewer-administered questionnaire. Parental history was positive if either parent ever had osteoporosis or a hip fracture. Positive individual history was defined as a fracture at any site, regardless of the level of trauma, after age 40. Complete data on family history and femur BMD were available for 585 men. Eleven percent of men were categorized as osteoporotic at the femoral neck and 6% at the total hip. Thirteen percent of men had a positive parental history, and 24% had a positive individual fracture history. For men with a positive parental history, the odds ratio (OR) of osteoporosis at the femoral neck (adjusted for age, body mass index, and VA patient status) was 3.5 (95% confidence interval, CI=1.8 to 6.8). For men with a positive individual fracture history, the adjusted odds of osteoporosis at the femoral neck were 2.4 (95% CI=1.3 to 4.2). The adjusted odds of osteoporosis at the femoral neck increased to 7.8 (95% CI=2.4 to 25.2) if both parental and individual histories were positive relative to men with both negative family and individual histories. The sensitivity and specificity for a report of either positive history were 59% and 69% respectively. Only individual fracture history was associated with osteoporosis at the total femur (adjusted OR=2.6, 95% CI=1.9 to 9.2). Similar results were found when osteoporosis was defined by age-matched normal BMD values. These results indicate that parental history of osteoporosis or hip fracture and individual history of any fracture after age 40 may be useful components of risk assessment and screening tools for osteoporosis in men.

M356

Effect of Cigarette Smoking and Alcohol Consumption on Bone Mineral Density in Men - Data from the Canadian Multicentre Osteoporosis Study (CaMos). W. P. Olszynski,[1] C. O. Polischuk,*[2] D. T. Drinkwater,*[2] K. S. Davison,*[2] T. M. Murray,[3] J. P. Brown.[4] [1]Saskatoon Centre of CaMos, Saskatoon, SK, Canada, [2]Kinesiology, U. Saskatchewan, Saskatoon, SK, Canada, [3]U. Toronto, Toronto, ON, Canada, [4]U. Laval, Quebec, PQ, Canada.

To determine whether there were differences in bone mineral density (BMD) between smokers, non-smokers (n=2568), alcohol drinkers and non-drinkers (n=2583), men, aged 25-96 y, were assessed at the lumbar spine (LS) and femoral neck (FN) using dual energy X-ray absorptiometry. Smoking status was determined from the lifetime equivalent packs of cigarettes smoked. Smokers were divided into NON (0 to < 6 mo ever smoked), LO (>6 mo smoked, 0 to 5000), MOD (5000 to 10000), HI (10000 to 20000), and VHI (20000+ packs) groups. Drinkers were determined from servings of alcohol consumed per day in the last 12 months. Drinkers were divided into NON (0), LO (>0 to 0.5), MOD (0.5 to 1.0), HI (1.0 to 2.0), and VHI (2.0+ servings) groups. WT and AGE-adjusted values (to means of 81.7 kg and 59.3 y) for BMD (g/cm^2) at the LS and FN for smokers and alcohol drinkers were:

	Smoking Status		Alcohol Status	
Group	**LS**	**FN**	**LS**	**FN**
NON (873,649)[1]	1.050 ± .148[2]	0.820 ± .118	1.025 ± .153	0.798 ± .127
LO (520, 916)	1.052 ± .160	0.820 ± .114	1.039 ± .151	0.810 ± .121
MOD (414, 510)	1.022 ± .163*	0.804 ± .122*	1.035 ± .158	0.816 ± .113^{Ý}
HI (493, 317)	1.022 ± .155*	0.796 ± .111*	1.059 ± .160^{Ý}	0.827 ± .125^{Ý}
VHI (268, 191)	1.040 ± .164	0.803 ± .115*	1.060 ± .166^{Ý}	0.813 ± .111

[1]n, by group for smokers, drinkers; [2]Mean ± SD, adjusted for WT and AGE; *different from NON-smokers, ^{Ý}different from NON-drinkers, p<0.05 Adjusted LS and FN BMD values for MOD and HI smokers were less than that of NON but not VHI-smokers. In contrast, LS and FN BMD values were greater in drinkers (LO to HI) than NON-drinkers. There was a low (r=0.16, p<0.01) correlation between smokers and drinkers (n=1289); 14.7% of LO-smokers were LO-drinkers, whereas 16% of HI and VHI smokers were also HI and VHI drinkers. Regression analyses revealed that WT accounted for 7.5% and 10.7% of the explained variance for LS and FN BMD, respectively; and AGE another 0.4% and 5.8%. Alcohol consumption accounted for only 0.3% of the variance for LS BMD, whereas smoking made no additional contribution to LS or FN BMD. When men with medical conditions or taking medications known to affect BMD were excluded, similar trends were found. We conclude that, in men, BMD appears to be negatively affected by smoking whereas alcohol consumption may have a protective effect.

M357

Tobago Bone Health Study: Epidemiologic Study of High Bone Mineral Density on a Population Level. J. A. Cauley,*[1] J. M. Zmuda,[1] A. Patrick,*[2] V. W. Wheeler,*[2] C. Bunker.*[1] [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]Tobago Regional Hospital, Tobago, Trinidad and Tobago.

Population-based studies of individuals with high bone mineral density (BMD) could lead to the identification of factors, both genetic and environmental, which contribute to their high BMD. The Caribbean Tobago-Bone Health Study was initiated in 2000 as part of a prostate cancer screening study. This population of African descent shares considerable genetic heritage with African Americans (AA). Both populations are of West African descent. However, genetic admixture is low in the Tobago population compared to that in the AA population, estimated at 25%. To date, total hip BMD was measured by DXA (Hologic QDR 4500W, Bedford, MA) in 1195 men, age range 40 to 91 years. All of the men reported 100% African descent. We compared the mean BMD across age groups in Tobago men with published data on white and AA men from the third National Health and Nutrition Examination Survey (NHANES III). (Figure) No data are reported for AA men age 80+, so we limited our comparisons to 1164 Tobago men age 40 to 79. At every age, total hip BMD and femoral neck BMD was 10-12% higher among Afro-Caribbean men compared to AA men. The absolute difference in hip BMD between Tobago men and AA men was approximately one full standard deviation (SD) difference. Compared to Caucasian men, the total hip and femoral neck BMD among the Afro-Caribbean men was 17-20% higher with absolute differences >1 SD. The greater BMD among Tobago men could not be explained by differences in body weight, since the average body weight among the Tobago men was about 4-6 kg lower thean US men. BMD declined across age groups in Tobago and US white and black men. The decline in total hip BMD from age 40-49 to 70-79 years was 7.3%, Afro-Caribbean; 9.2%, AA and 9.8%, white US men.This population of Afro-Caribbean men may represent a population with the highest BMD known to date. Identification of the factors that contribute to their high BMD may extend to other populations and lead to preventive strategies.

M358

Clinical Findings of Osteoporosis in Relationship to Bone Mineral Density. K. Abendroth,[1] A. Defer,*[2] R. Poetzschner.*[3] [1]Regional Expert Group for Osteoporosis(REGO), Jena, Germany, [2]REGO, Dresden, Germany, [3]REGO, Gera, Germany.

One clinical or x-ray examination findings alone cannot determine the diagnosis of an osteoporosis. Therefore a combination of symptoms and findings is used to the diagnostics. The validity of the different symptoms has to be checked in connection with the bone density**.** QCT technology was used for the determination of the bone mineral density (BMD) at the lumbar spine with all patients. The standardization of the QCT measuring results carried out to in Germany valid limits for the spongy bone of the vertebral bodies: Normal BMD > 120 mg/cm^3, osteopenia 120-80 mg/cm^3 and osteoporosis < 80 mg/cm^3.For the task 684 women, mean age 67 years (32-95 years) from our medical practices were examined. After BMD 75% of these women had an osteoporosis, 18% an osteopenia and 6% a normal bone density. The following 5 clinical symptoms[C] and 4 x-ray signs [X] were analyzed in relationship with BMD. After every symptom its frequencies (%) in the complete population [mean value = M], with osteoporosis patients [O], with patients with osteopenia [P] and with patients with normal bone density [N] and last the differences between osteoporosis and normal density [D] as % points follow in brackets. Clinical results were: C1.-Body height removal - pathologic >= 3 cm [M-60%, O-69, P-34, N-25, D = 44 %-points]. C2.- Body-Mass-Index - pathologic >22 [M-4%, O- 4, P- 3, N-0, D = 4 %-points]. C3.- Humpback, Kyphosis [M-64%, O-71, P-46, N-39, D = 32 %-points]. C4.- Identified back pain [M-74%, O-77, P-70, N-59, D = 18 %-points]. C5.- Shortening with “Fir-Tree” sign [M-54%, O-61, P-33, N-20, D = 41 %-points]. X-ray results were: X1.- Pencilling of the vertebral bodies [M-63%, O-70, P-52, N-18, D = 52 %-points]. X2.- Emphasis of the vertical trabecular structure [M-73%, O-81, P-55, N-20, D = 61 %-points]. X3.- Deformation of a vertebral body = with < 20% height reduction [M-55%, O-65, P-29, N-16, D = 49 %-points]. X4.- Fracture of a vertebral body = > 20% height reduction [MV-26%, O-33, P- 5, N-7, D = 26 %-points]. In conclusion, the sensitivity of the different symptoms is various, the accuracy is low usually. Almost every symptom happens both at the densitometric osteoporosis and at normal bone density. The diagnostic accuracy doesn't improve the combination of symptoms and sign either. It seems that at these uncertainties the BMD could determine the diagnosis of an osteoporosis into combination with the fracture history and fracture signs in the practice. Is the extensive diagnostics procedure of the osteoporosis today still necessary? Yes, all of them of clinical information are necessary for the therapy decision.

M359

Osteoporosis Risk Factors in the Medical Practice. A. Defer,*[1] K. Hammer,*[2] A. Linzmayer,*[3] R. Poetzschner,*[4] K. Abendroth.[5] [1]Regional Experts Group for Osteoporosis(REGO), Dresden, Germany, [2]Quality Team Osteoporosis (QT), Zwickau, Germany, [3]QT, Saalfeld-Ilmenau, Germany, [4]REGO, Gera, Germany, [5]REGO, Jena, Germany.

The practical meaning of the osteoporosis risk factors is unclear. Our task was to test a better assessment. The frequency of osteoporosis risk factors was analyzed in comparison with the result of a densitometrical measurement of the spine. The risk factors were to be restricted by help of a patients questionnaire. The determination of the bone mineral density (BMD) was carried out uniformly at the lumbar vertebra bodies by means of QCT technology. The standardization of the QCT measurement results are based on the in Germany valid limiting values: Normal BMD(only spongy bone) >120 mg/cm^3, osteopenia 120-80 mg/cm^3 and osteoporosis less than 80 mg/cm^3. Only female patients from our medical practices were questioned and examined correspondingly to this. The analysis was carried out on 684 women, mean age 67 years (32-95 years). The osteoporosis risk questionnaire consisted of 15 complexes. The results of the analysis as frequency in % follow the questions in brackets in the order - mean average value[M], osteoporosis[O,], osteopenia [P,], normal BMD [N,]: 1.- Back pain [M= 87%, O= 87, P= 86, N= 89], 2.- Bone fracture after the age of 35 [M= 44%, O= 51, P= 28, N= 18], 3.- Distal forearm fracture after the age of 35 [M= 22%, O= 25, P= 14, N= 9], 4.- Low mobility = <1 hours outdoors of the flat [M= 8%, O= 9, P= 4, N= 3], 5.- Falls in the last 3 years [M= 39%, O= 42, P= 40, N= 45], 6.- No dairy products in the diet [M= 14%, O= 15, P= 8, N= 9], 7.- Drinking alcohol = more than 4 glasses per week [M= 6%, O= 5, P= 7, N= 7], 8.- Smoking = more than 5 cigarettes per day [M= 2%, O= 2, P= 2, N= 3], 9.- Late menarche = later the age of 14 [M= 62%, O= 64, P= 58, N= 48], 10.- Early menopause = before the age of 44 [M= 26%, O= 25, P= 29, N= 32], 11.- Childlessness [M= 8%, O= 7, P= 7, N= 16], 12.- Family history of osteoporosis = hip fracture, widow hump of the sister, mother or grandmother [M= 30%, O= 28, P= 34, N= 36], 13.- Intake of Cortisone = more than 6 months [M= 11%, O= 10, P= 14, N= 14], 14.- Disease of thyroid gland in the patients history [M= 26%, O= 25, P= 31, N= 23], 15.- Too little sunlight for the skin in the last years [M= 42%, O= 46, P= 38, N= 30]. Only some of the 15 risk factors were helpful at the distinction between osteoporosis, osteopenia and normal bone density. In conclusion, clearest differences between osteoporosis and normal bone density were with 32% points: fractures after the 35th year of life, with 16% points: too little sunlight for the skin,and with 6% points: no diary products in the diet.These show a relatively high specifity. Back pains and falls don't help in the distinction of osteoporosis, osteopenia and normal bone density.

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Relationship of Risk Factors, Clinical and X-ray Findings of Osteoporosis with BMD Results of Different QCT and pQCT measurements. R. Poetzschner,*[1] K. Abendroth.[2] [1]Regional Experts Group for Osteoporosis(REGO), Gera, Germany, [2]REGO, Jena, Germany.

For the assessment of BMD results from vertebral and peripheral locations its important to know their relationship to risk factors, clinical and x-ray findings of osteoporosis.We examined 203 patients (23 male, 180 female, mean age 65 years)of an orthopedic ambulance, suspected of having osteoporosis. Risk factors investigated included back pain,fractures > 35 year, falls in the last 3 year, milk intolerance, use of alcoholics > 4 glasses weekly,more than 5 cigarettes daily, menopause < 44 year, and family history of osteoporosis (humpback, fractures).The physical examination included height and weight measurement, BMI, observation of humpback and located back pain. The x-ray findings of thoracic and lumbar spine judge by spine fractures and deformities. BMD measurement of all patients by QCT, usually vertebral body L 1-4 (spongy bone) and by pQCT (Stratec XCT 900) in the region of non dominant ultra distal radius, evaluated with area/threshold algorithm. The following BMD ranges, valid limits in Germany for spongy bone, used for diagnostic assessment: QCT: Normal BMD > 120 mg/cm³, osteopenia 120-80 mg/cm³ and osteoporosis < 80 mg/cm³. pQCT: Normal BMD (spongy bone > 120 mg/cm³, osteopenia 120-85 mg/cm³, osteoporosis < 85 mg/cm³; total BMD: Normal > 280 mg/cm³, osteopenia 280-250mg/cm³, osteoporosis < 250 mg/cm³. We value and compare the proportional incidence of the risk factors, the clinical and radiological diagnosis with results of spongiosa BMD in QCT and pQCT measurement and also with the total density and with combined density assessment from spongy and total bone density in pQCT. There are no significant relationship between the risk factors back pain, fall tendency, milk intolerance, alcohol and nicotine, early menopause and family history of osteoporosis with all groups of BMD of both methods of measurement. Fractures (Fx) correlates significantly with BMD: QCT to Fx: Normal BMD (N) 24, osteopenia (P) 25, osteoporosis (O) 71 %-points; pQCT- spongy bone BMD to Fx: N-27, P-48, O-60 %-points; pQCT-total BMD to Fx: N-26, P-27, O-61 %-points; pQCT-spongy bone + total BMD to Fx: N-21, P-23, O-71 %-points; Colles fractures (CFx) correlates also significantly with QCT-BMD: N-15, P-14, O-33 %-points, pQCT-spongy bone BMD to CFx: N-10, P-16, O-40 %-points; pQCT total BMD to CFx: N-7, P-23, O-32 %-points and pQCT-spongy bone + total BMD to CFx: N-12, P-14, O-33 %-points. The correlation between the clinical diagnosis and BMD of both methods and also the correlation between the radiological diagnosis with the BMD of both methods is significant. In conclusion, BMD results of QCT and pQCT-spongy + total bone density are comparatively in their sensitivity.

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Bone Mineral Density in Lupus Patients and Healthy Controls. R. Ramsey-Goldman,[1] A. Bongu,*[1] C. Langman,[2] S. Spies,*[1] S. Manzi.[3] [1]Northwestern University, Chicago, IL, USA, [2]Northwestern University & Children's Memorial Hospital, Chicago, IL, USA, [3]University of Pittsburgh, Pittsburgh, PA, USA.

Women with lupus are at risk for low bone mineral density (BMD), but this is usually not reported in the context of concurrent controls. The goal of this study was to measure BMD at the hip, spine, and wrist and to record risk factors by questionnaire in women with lupus matched to healthy, unrelated women by age (+/-4 years), race, and menopause status. The mean age of the 113 pairs of cases and controls was 41.1 and 41.3 years, respectively, and the mean lupus disease duration in cases was 8.8 years. Forty-two pairs of women were menopausal; 71 pairs still menstruating. The mean age at menopause for lupus women was 41.9 years and for control women was 44.9 years (p=0.05). Only women with lupus reported taking corticosteroids. Lupus women compared with control women had a higher mean daily calcium intake 1242 mg vs. 998 mg (p=0.003) and were more likely to smoke 42% vs 31% (p=0.09). In contrast, control women compared with lupus women were more likely to have used oral contraceptives, 56% vs. 38% (p=007). There were no differences between lupus and healthy women in history of alcohol or caffeine intake, BMI, or estrogen use for replacement.

Unadjusted Mean Hip, Spine, & Wrist BMD in Lupus and Control Women; Mean BMD Difference within Pairs

	Lupus Women	Control Women	Mean Difference (95% CI)
Site	**BMD (g/cm2)**	**BMD (g/cm2)**	**BMD (g/cm2)**
Hip	0.897	0.917	-0.020 (-0.054, 0.014)
Spine	0.989	1.023	-0.03 (-0.066, -0.002)
Wrist	0.673	0.683	-0.010 (-0.026, 0.004)

Mean BMD at all sites was lower in women with lupus compared with controls, and the mean difference in spine BMD within pairs was significantly decreased in lupus compared with control women. When stratified by menopause status, the mean difference in spine BMD within pairs was -0.035 g/cm2 (95% CI -0.074, 0.005) in menstruating women and -0.034 g/cm2 (95% CI -0.090, 0.023) in menopausal women. The mean difference in wrist BMD within pairs was -0.036 g/cm2 (95% CI -0.062, -0.010) in menopausal subjects and was 0.004 g/cm2 (95% CI -0.015, 0.022) in menstruating subjects. Differences in potentially modifiable risk factors (smoking, calcium intake, medication use -oral contraceptives and corticosteroids) may partially explain why lupus women have lower BMD compared with controls. Strategies to change these risk factors should be considered in order to minimize the occurrence of low BMD, especially in young women with lupus

Disclosures: Merck,2,5; P&G,2.

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A Brief Questionnaire for Screening Women with Osteoporosis. F. Silveri,[1] C. Morbidelli,[2] M. Sfrappini,[3] M. Pozone,[4] R. Ciaschini,[5] A. Tarone,[6] S. Muti,[1] F. Salaffi,[1] C. Francucci.[7] [1]Department of Rheumatology, University of Ancona, Jesi, Italy, [2]Department of Medicine INRCA, Hospital of Ancona, Ancona, Italy, [3]Department of Geriatric, Hospital of S. Benedetto del Tronto, Italy, [4]Department of Geriatric, Hospital of L'Aquila, Italy, [5]Department of Geriatric, Hospital of Fano, Italy, [6]Department of Medicine, Hospital of Rimini, Italy, [7]Department of Endocrinology, University of Ancona, Ancona, Italy.

The aim of the study was to evaluate the discriminant validity of a brief questionnaire for screening women with osteoporosis. The screening was performed by a questionnaire that was used by Albrand in French women from Lyon area (Osteoporosis International 8; P278, 1998). The questionnaire values seven variables: age, years since menopause, weight, history of fragility fractures after 45 years, estrogen and glucocorticoid therapy, disorders associated with osteoporosis (hypothyroidism, intestinal malabsorption, hyperparathyroidism, Cushing's syndrome, and chronic renal failure). The score of this questionnaire range from 1 to 19. We studied 568 consecutive ambulatory Caucasian postmenopausal women (45-80 years old) from middle Italy (Adriatic coast). All patients were valued by questionnaire and bone mineral density (DXA; Hologic QDR 4500) at the lumbar spine and proximal femur. The area under receiver operating characteristic (ROC) curve was employed to evaluate the screening method's performance. For screening test the area under the curve is 0.814 ± 0.02 (SE) (95% CI 0.785 to 0.842), and an optimal cut-off point of 3 comes close to maximizing both sensitivity (73.4%) an specificity (75.9%). A strong relation was found between questionnaire score and proximal femur BMD ($p < 0.001$) and lumbar spine BMD ($p < 0.001$). In conclusion, in our preliminary data, this brief questionnaire can be used to identify postmenopausal women who are at risk of osteoporosis.

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A Self-Administered Bone Mineral Density Risk Instrument for Pre-and Perimenopausal Women. K. E. Bainbridge,* M. F. Sowers. Epidemiology Department, University of Michigan, Ann Arbor, MI, USA.

As part of the state of Michigan's osteoporosis prevention efforts, a self-administered risk instrument was developed for pre- and perimenopausal women. This six-item instrument classifies women between the ages of 20 and 50 into risk groups for low and high bone mineral density (BMD) according to easily assessed risk factors. We followed 614 women, 24-44 years of age at baseline, for six years beginning in 1992/93. BMD measurements of the lumbar spine (L_{2-4}) and the femoral neck were obtained annually using dual x-ray absorptiometry (DXA) from 1992/93 until 1995/96 and again in 1998/99. Anthropometric measurements, dietary information, history of chronic illness, use of medication, family history of osteoporosis as well as physical activity, alcohol use, smoking behavior, and reproductive factors were also assessed through interviews or self-administered questionnaires. Premenopausal women were identified as those who self-reported having at least 9 menstrual bleeds per year or if fewer menstrual bleeds could be accounted for by pregnancy, lactation, or the use of a hormonal preparation. Otherwise, women who self-reported having fewer than 9 menstrual bleeds per year were classified as perimenopausal. Linear mixed models were developed to identify independent risk factors for BMD at a given time while simultaneously identifying risk factors for BMD change. Generalized estimating equations were used to identify six risk factors for either high (femoral neck z-score ≥ 2 and ≤ 3.5) or low (femoral neck z-score ≤ -2) BMD among a randomly selected half of the data. The six important risk factors were body weight, alcohol use, family history of osteoporosis, irregular menses, bilateral oophorectomy, and reproductive cancer. A six-item risk instrument was developed and comprised of six 'yes or no' questions. Each item is weighted according to the strength of the odds ratio for the corresponding risk factor, and a woman's risk score is the sum of the weights for each answer. The instrument was validated by calculating risk scores for each woman, using the other half of the data. ROC curves were plotted to identify cutpoints for classifying women into risk groups. For the femoral neck, the instrument has 33% sensitivity and 64% specificity for identifying women with low BMD (z-score ≤ -2) and 70% sensitivity and 54% specificity for identifying women with high BMD (z-score ≥ 2 and ≤ 3.5). For the lumbar spine, the instrument has 67% sensitivity and 66% specificity for identifying women with low BMD (z-score ≤ -1.5) and 56% sensitivity and 54% specificity for identifying women with high BMD (z-score ≥ 2). This instrument will be a useful tool to identify women who are not likely to be of high BMD.

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Individualized Information on Osteoporosis Risk Influences Health-Related Behaviour of Peri- and Postmenopausal Women: Follow-up of the Swiss Osteoporosis Campaign. A. W. E. Popp,[1] S. Risi,*[1] M. Wegmueller,*[1] M. Krieg,[2] K. Lippuner.[1] [1]Osteoporosis Unit, University Hospital, Berne, Switzerland, [2]Medecine Interne, CHUV, Lausanne, Switzerland.

Little is known about the impact of osteoporosis campaigns on health behaviour of visitors.We prospectively followed 585 peri- and postmenopausal women who had visited the Swiss osteoporosis information campaign in spring 1998. Based on forearm BMD (pDXA) and the presence of risk factors, visitors had been individually informed on their personal osteoporosis risk. They had been told to be at high risk (HR) if forearm BMD t-score was <-1SD and if they had at least one additional risk factor, i.e. low body mass index (BMI), early menopause, family history of fractures, previous low trauma fracture, smoking, lack of dairy products, lack of physical activity, corticosteroid use or chronic diarrhea. Otherwise, they had been told to be at normal risk (NR). To estimate the long-term impact of that information on health behaviour in NR and HR visitors, respectively, we sent out a 30 months follow-up questionnaire asking whether changes in nutritional habits or physical activity had been induced by the campaign. It was also asked whether the results of the risk-assessment had later on been discussed with the family physician and if the knowledge

of the personal risk had led to start or stop use of anti-osteoporotic drugs. The follow-up questionnaire was completed by 366 women (168 HR, 198 NR). Age (mean±SEM) was similar in both groups, i.e. 58.3±0.8 ys (HR) vs. 57.7±0.6 ys (NR, p=0.6). HR had lower mean BMI (22.7±0.3 kg/m2), lower forearm bone density t-score (-2.0±0.1 SD) and z-score (-0.8±0.1 SD) as compared with NR (24.5±0.3 kg/m2, -0.8±0.1 SD, 0.4±0.1 SD; p<0.001 for all comparisons by unpaired t-test). The number of women in each group who changed their health behaviour with respect to osteoporosis after the campaign are given below.

	High Risk n=168/ (%)	Normal Risk n=198/(%)	P (chi-square)
Modification of nutrition	70 (41.6)	31 (15.6)	<0.001
Increase of physical activity	65 (38.7)	31 (15.6)	<0.001
Consulting of family doctor	140 (83.3)	108 (54.5)	<0.001
Start with anti-osteoporotic drug	48 (28.6)	-7 (-3.5)	<0.001

The campaign was rated as "useful" by 350 (95.6%) women (163 HR, 187 NR) who also indicated interest in receiving further information on bone health.We conclude that individualized information on osteoporosis risk leads to sustained beneficial modification of health behaviour in both normal and high risk women, with a higher impact in the latter.

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The Prevalence of Celiac Disease in an Osteoporotic Population. W. F. Stenson,*[1] R. D. Newberry,*[1] R. Lorenz,*[2] R. Civitelli.[3] [1]Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA, [2]Department of Pathology, Washington University School of Medicine, St. Louis, MO, USA, [3]Bone and Mineral Metabolism, Washington University School of Medicine, St. Louis, MO, USA.

The purpose of this study was to compare the prevalence of celiac disease in osteoporotic and non-osteoporotic populations. We screened a group of 84 osteoporotic volunteers (T-2.5) for celiac disease. Anti-gliadin IgA, antigliadin IgG, antiendomysial and anti-tissue transglutaminase antibodies were assessed. Volunteers with either a positive anti-endomysial antibody or a positive tissue transglutaminase antibody underwent endoscopic biopsy of the duodenum to confirm the diagnosis of celiac disease,Of the 84 volunteers with osteoporosis 4 had both a positive anti-endomysial antibody and a positive anti-tissue transglutaminase. Endoscopic biopsies confirmed the diagnosis of celiac disease. Two of these 4 volunteers had the lowest T-scores among the 224 volunteers tested (T<-4.37, and T<-4.48).Among the 140 volunteers without osteoporosis only one had a positive antiendomysial antibody and a positive tissue transglutaminase antibody. This volunteer was osteopenic with a T-score of -1.78. Endoscopic biopsy confirmed the diagnosis of celiac disease.There were a total of 5 volunteers with positive antiendomysial antibodies, all five were also positive for antigliadin IgG, antigliadin IgA and anti-tissue transglutaminase antibodies. There were, however, a large number of volunteers who were positive for antigliadin IgG or antigliadin IgA antibodies who were negative for antiendomysial and anti-transglutaminase antibodies. These patients were distributed in both the osteoporotic and non-osteoporotic groups.This study leads to the following conclusions: 1) The prevalence of celiac disease in the osteoporotic population was 4.7%. This is at least 14-fold greater than the reported prevalence of celiac disease in the United States population. If these numbers are confirmed in a larger study it would be reasonable to recommend that all patients with osteoporosis be screened for celiac disease. 2) Screening with anti-tissue transglutaminase antibody or antiendomysial antibody is more specific than screening with antigliadin antibodies.

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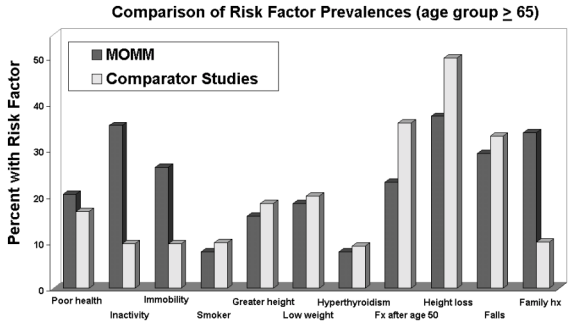
The Impact of Co-morbid Conditions on Bone Mineral Density and Vertebral Deformities in Community Dwelling Men and Women Across Canada. **J.** D. Adachi,[1] G. Ioannidis,[1] C. Berger,*[2] L. Pickard,[1] J. Prior,[3] D. A. Hanley,[4] W. P. Olszynski,[5] T. Murray,[6] T. Anastassiades,[7] J. P. Brown,[8] S. Kirkland,[9] C. Joyce,[10] L. Joseph,*[2] A. Papaioannou,[1] S. Poliquin,[2] A. Tenenhouse.[2] [1]McMaster University, Hamilton, Canada, [2]McGill University, Montreal, Canada, [3]University of British Columbia, Vancouver, Canada, [4]Calgary University, Calgary, Canada, [5]University of Saskatchewan, Saskatoon, Canada, [6]University of Toronto, Toronto, Canada, [7]Queen's University, Kingston, Canada, [8]Laval University, Ste-Foy, Canada, [9]Dalhousie University, Halifax, Canada, [10]Memorial University, St.John's, Canada.

Using data obtained from the Canadian Multicentre Osteoporosis Study (CaMos) we conducted a cross-sectional cohort study in 5566 women and 2187 men 50 years of age and older to determine the impact of co-morbid conditions on bone mineral density (BMD) and vertebral fractures. At study entry, anthropometric, therapeutic drug use, lifestyle factors, fracture history and co-morbid conditions data were collected using the CaMos questionnaire. Comorbidities included hypertension, heart attack, rheumatoid arthritis, thyroid disease, diabetes, kidney stones, breast cancer, inflammatory bowel disease, neuromuscular disease, Paget's disease, and chronic obstructive pulmonary disease. We performed multivariate regression analyses modeled for lumbar spine (LS), femoral neck (FN), and trochanter (TR) BMD. These analyses were conducted for women and men separately at each skeletal site. Coefficient parameter estimates and 95% confidence intervals (CI) were determined. Results indicated that hypertension was associated with higher BMD measurements at the LS (0.26; 95% CI: 0.01, 0.04) and FN (0.01; 95% CI: 0.001, 0.018) in women, and at the LS (0.02; 95% CI: 0.001, 0.047) in men as compared with those without the condition. In hypertensive men, this corresponded to a lower number of vertebral deformities (-0.14; 95% CI: -0.28, -0.01). Diabetes was associated with higher BMD measurements at the LS (0.06; 95% CI: 0.04, 0.08), FN (0.03; 95% CI: 0.01, 0.04) and TR (0.03; 95% CI: 0.01, 0.05) in women; and at the FN (0.03; 95% CI: 0.004, 0.050) in men. Furthermore, diabetic men had a lower number of vertebral deformities (-0.20; 95% CI:-0.39, -0.01). In addition, men with kidney stones had lower BMD values at the FN (-0.03; 95% CI:-0.05, -0.01) and TR (-0.04; 95% CI: -0.06, -0.01). In conclusion, various co-morbid conditions were associated with BMD and vertebral deformities. Men with nephrolithiasis should be assessed for the presence of osteoporosis.

M367

Population-Based Prevalence of Clinical Risk Factors from the Maximizing Osteoporosis Management in Manitoba (MOMM) Project. W. D. Leslie,[1] C. Metge,[1] B. Kvern,*[1] W. A. Anderson,*[2] L. J. Manness,*[3] K. Yuen.[2] [1]University of Manitoba, Winnipeg, Canada, [2]Manitoba Clinic, Winnipeg, Canada, [3]Merck Frosst Canada & Co., Winnipeg, Canada.

MOMM is a population-based initiative that aims to determine whether the management of patients at increased risk of osteoporosis can be optimized through a patient health management strategy (JBMR 2000;15:S560). As part of a larger initiative, MOMM sought to determine the prevalence of clinical risk factors (CRFs) associated with hip fractures in Manitoba women age 50 and over. The specific CRFs included 11 factors that were previously studied in a multidimensional model of hip fracture risk (JBMR 2000;15:S416): poor general health, inactivity, immobility, current smoking, greater height, height loss, low body weight, hyperthyroidism, fractures after age 50, falls, and family history of osteoporotic fracture. A stratified random sampling of 40,000 Manitoba women 50 and over identified from the Manitoba Health registry were sent an osteoporosis risk-assessment survey. Sample size was based on a Monte Carlo-estimated prevalence for increased risk of hip fracture (total CRF score ≥ 3) of 62% in Manitoba women 50 and over. A total of 8,681 risk-assessment surveys responses were received (response rate 22%). After age 50 approximately 53% of women reported a total CRF score ≥ 3. Most CRFs became more prevalent with increasing age though no age-correlation was seen with height loss or falls and there was a reduction in smoking prevalence with age. Total CRF score increased progressively with age (r=0.31, slope 0.6 per decade). Mean RFS was 2.0 (SD 1.5) for the 50-54 age group and increased linearly to 6.7 (SD 2.3) after age 90. There was minimal collinearity among the CRFs assessed (redundancy r^2<0.2 for all factors). Relative independence in the CRFs justifies their combined use in mathematical models of fracture risk. Although the prevalence of CRFs for hip fracture in Manitoba largely parallels estimates from other comparator populations (NEJM 332:767, 1995; Bone 19:407, 1996; Bone 23 Suppl 5:S392, 1998; Bone 23 Suppl 5:S472, 1998; Osteoporos Int 8 Suppl 4:S1, 1998), regional differences were found (more immobility, inactivity and positive family history). This supports the notion that population-specific information may be necessary to develop strategies for preventing and treating osteoporosis.



Disclosures: Merck Frosst Canada & Co.,2.

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Population-Based Prevalence of Medical Risk Factors for Osteoporosis from the Maximizing Osteoporosis Management in Manitoba (MOMM) Project. C. Metge,[1] W. D. Leslie,[1] B. Kvern,[1] W. A. Anderson,[2] L. J. Manness,[3] K. Yuen.[2] [1]University of Manitoba, Winnipeg, MB, Canada, [2]Manitoba Clinic, Winnipeg, MB, Canada, [3]Merck Frosst Canada & Co., Winnipeg, MB, Canada.

MOMM is a population-based initiative that aims to determine whether the management of patients at increased risk of osteoporosis can be optimized through a patient health management strategy (JBMR 2000;15:S560). This large prospective study is being conducted in Manitoba, a Canadian province with a single-payer health care system and a well-developed administrative database for tracking patient health care utilization. The overall goal of the project is prevent osteoporosis sequelae like hip fracture and optimize osteoporosis care on a province wide basis. As part of a larger initiative, MOMM determined the prevalence of medical risk factors within the population of Manitoba women aged 50 and over. The specific risk factors investigated included: systemic corticosteroid therapy (>3 months), premature menopause (prior to age 45), abnormal premenopausal menstrual cycles (5 or more missed periods/yr.), natural premature menopause (prior to age 45), and surgical menopause (before and after age 45). A stratified random sampling of 40,000 Manitoba women 50 years of age and over was identified from the Manitoba Health registry and sent a risk-assessment survey. A total of 8,681 responses were received (response rate 22%). After age 50 approximately 41% of women reported at least one medical risk factor. The percentages of women with medical risk factors in the two age groups examined (50-64 and ≥ 65) are similar (42% and 39%). Premature menopause due to surgery wa much more common in the younger age group ($p<10^{-8}$), while the rates of natural menopause and later surgical menopause were similar. Overall 29% of women reported a surgical menopause. As expected, the number of physician-confirmed cases of osteoporosis (as reported by the respondent) rose from 10% in the 50-54 age group to 23% in the ≥ 65 age group ($p<10^{-8}$)with an overall mean identification rate of 16%. Based on the high prevalence of surgical menopause in Manitoba this medical risk factor needs to be given

special consideration when developing strategies for addressing osteoporosis.

Disclosures: Merck Frosst Canada & Co.,2.

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Impact of Adjuvant Chemotherapy on the Bone Mineral Density of Postmenopausal Women with Early Breast Cancer. N. C. Greep,[1] A. E. Giuliano,*[2] N. Hansen,*[2] T. Taketani,*[1] F. R. Singer.[1] [1]Skeletal Biology, John Wayne Cancer Institute, Santa Monica, CA, USA, [2]Breast Clinical Research Center, John Wayne Cancer Institute, Santa Monica, CA, USA.

Adjuvant chemotherapy (adj chemo) can precipitate premature menopause in premenopausal patients with breast cancer and place them at risk for bone loss. The impact of adj chemo on the bone mineral density (BMD) of postmenopausal patients with breast cancer is unknown, but we speculated that it might have an adverse impact. We did a retrospective study of the BMD of all breast cancer patients who had been seen at our center from 2/1993 to 2/2001 and who had had their BMD measured by DEXA on our hospital's Hologic QDR 2000 sometime after their initial treatment for breast cancer. We selected for analysis all patients who were postmenopausal at the time of their diagnosis, had early breast cancer (Stage 0-II), and had no other cause for metabolic bone disease. We compared the BMD of all eligible patients who received adj chemo (N=31) to the BMD of all eligible patients who did not receive adj chemo (controls, N=90). Both groups had experienced menopause at a similar age (48yrs), had a similar time interval between their cancer diagnosis and their first hip BMD (2.9yrs), and had a predominance of ductal histopathology (90%). They also had a similar prevalence of recognized risk factors for osteoporosis (such as a family history of osteoporosis, Caucasian race, smoking, and alcohol consumption) and past use of medications known to affect BMD (such as thyroid, tamoxifen and bisphosphonates). Adj chemo consisted of cytoxan combined with either methotrexate and 5-fluorourcil or with adriamycin. Patients who received adj chemo tended to be younger at the time of their initial breast cancer diagnosis (57.1 vs. 62.7 yrs, $p < .05$) and have a higher stage of disease (distribution of stage 0 (in situ),I,and II was 0%,16.7%, and 83.3% for chemo patients vs. 24.4%, 60.0%, and 15.6% for controls). Patients who received adj chemo had lower BMD assessed by Z-scores compared to patients who had not received adj chemo (femoral neck: -0.58 vs. -0.10, $p< 0.05$; total hip: -0.69 vs. -0.04, $p< 0.05$; Ward's triangle: -0.04 vs. 0.27, p=ns; AP spine -0.03 vs. 0.47, p=ns; lateral spine -0.30 vs. 0.46, $p <0.05$). We conclude that adj chemo for early breast cancer in postmenopausal women may adversely impact BMD. A prospective study of BMD before and after adj chemo would clarify whether the lower BMD observed in our patients treated with adj chemo was a consequence of chemotherapy or a systemic manifestation of these patients' more advanced, although apparently still localized, breast cancer.

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Developing a Comprehensive Public Health Model for Osteoporosis Education: New York State Osteoporosis Prevention & Education (NYSOPEP) Regional Center. S. Y. Liu, L. Robbins, J. Andariese,* J. M. Lane. Hospital for Special Surgery, New York, NY, USA.

Osteoporosis affects over 2 million people in NYS over the age of 50. To address this growing public health concern, the New York State Osteoporosis Prevention and Education Program (NYSOPEP) was established in 1997. Five NYSOPEP regional centers were created and funded from 1998 to 2002 - Helen Hayes Hospital, Buffalo, Syracuse, Rochester and the Hospital for Special Surgery. Each of these centers has the common goal of creating a public health model that focuses on osteoporosis prevention through education programs. As the NYC regional center, the Hospital for Special Surgery chose to reflect the city's diverse populations by focusing on adolescents, non-traditional populations (Latina and Chinese) & premenopausal women. Osteoporosis health needs were assessed through key informants, community boards and focus groups.Assessment identified three crucial factors in developing a successful program: •Osteoporosis education is needed at multiple levels-medical, community and individual •Open channels of communication with all members involved are necessary •Collaborations with community-based organizations are vital to create innovative and appropriate programs as well as necessary to expand the scope of our services Intervention strategies were developed on different levels: •primary prevention to reduce disease occurrence •secondary prevention to encourage early disease diagnosis and treatment •tertiary prevention to limit disease effects through self-management Successful programs at HSS include Be a Bone Builder Girl Scouts Patch Program, Healthy Girls 2000 (a peer developed curriculum), community awareness lectures, medical education symposiums, lectures to community-based health care providers, support groups and exercise classes. These programs are evidence-based, needs driven and outcomes directed. Standard outcome measures were used to evaluate the effectiveness of interventions. Over 1500 children and adolescents completed our programs promoting bone health. Over 2000 healthcare professionals attended our professional programs and over 1500 attended our community awareness programs. We have utilized traditional and nontraditional media sources to increase awareness -professional bulletins, consumer newsletters, press releases, poster presentations and a billboard. As a regional NYSOPEP center, HSS has taken the initiative in promoting osteoporosis awareness through our collaborations with public and private organizations. We will continue to assess the efficacy of these programs by measuring knowledge and behavioral changes in order to impact public health in New York State.

Disclosures: Hospital for Special Surgery,3.

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Prednisolone Decreases Quantity and Quality of Bone in Two Mouse Models for Glucocorticoid Induced Osteoporosis. D. Banffer,*[1] M. Karperien,[1] J. H. Waarsing,*[2] H. Weinans,*[2] N. A. T. Hamdy,[1] S. E. Papapoulos.[1] [1]Metabolic diseases and Endocrinology, Leiden University Medical Centre, Leiden, The Netherlands, [2]Orthopedics, Erasmus University Rotterdam, Rotterdam, The Netherlands.

In glucocorticoid-induced osteoporosis bone loss is predominantly induced by inhibition of osteoblastogenesis and an increase in osteoblast and osteocyte apoptosis. However, not only the quantity of bone but also its quality may be affected by GC use. The aim of our study was to assess the effects of GC on trabecular and cortical bone in an established murine model for GC-induced osteoporosis. Prednisolone was administered for 28 days to two different strains of mice: Swiss Webster and Swiss NMRI. A Hologic QDR-4500 was used to assess the overall quantity of bone as measured by bone mineral density (BMD) at various sites at the start and end of the experiment. MicroCT scans using a Skyscan 1072 were used for a three dimensional (3D) reconstruction of the right femur and fifth lumbar vertebra after sacrificing the animals. Nine month old male Swiss Webster mice and 4 month old male Swiss NMRI mice were given 2.1 mg/kg/day prednisolone s.c. using a slow release pellet. BMD decreased significantly in the prednisolone group, most prominently at the lumbar spine and right femur sites. Swiss Webster mice lost overall more bone than Swiss NMRI mice (-13 % vs -10 % and -30 % vs -7 % compared to controls at the lumbar spine and femur respectively). In contrast to Swiss NMRI mice, Swiss Webster mice significantly lost weight over the duration of the experiment. In prednisolone-treated mice, preliminary microCT 3D reconstruction of the lumbar vertebrae demonstrated a clear decrease in trabecular thickness and number of connecting trabeculae. Cortical thickness was also decreased. Mice treated with prednisolone demonstrated an overall decrease in femoral bone volume compared to controls, likely to be due to a decrease in trabecular as well as cortical bone. The mechanical strength of the femora, as measured by the polar moment of inertia, was also decreased, further substantiating the observed structural changes on microCT. The femora of prednisolone-treated mice were shorter than those of controls. Our data suggest that both Swiss Webster and Swiss NMRI mice may be valid models for glucocorticoid-induced bone loss. Based on microCT data, it seems that glucocorticoids are able to alter the quality of cortical as well as trabecular bone. These findings may hold significant implications in the assessment of the risk of fracture in glucocorticoid-treated patients.

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Prevalence of Non-vertebral Fractures in Women Over 50yr on Corticosteroids. I. W. Chantler,*[1] J. S. Rees,*[2] T. Moylan,*[2] M. W. J. Davie.[1] [1]Charles Salt Centre, RJ&AH Orthopaedic Hospital, Oswestry, United Kingdom, [2]Shropshire Health Authority, Shrewsbury, United Kingdom.

Corticosteroid therapy (CST) is associated with an increased incidence of vertebral and hip fracture, but the prevalence of other fractures and possible associations of disease with fracture are less well evaluated. We investigated the prevalence of non-vertebral fracture in women over 50yr exposed to CST. Women over 50yr who had received CST between 1/4/97 and 31/3/98 were retrieved from General Practitioner records covering 62000 women. One in three records of women taking CST were identified at random and data extracted for age, disease, non-vertebral fracture over age 50yr (whether on CST at the time or not), total dose of steroid taken during that year, duration of steroid exposure, use of bone sparing drugs (including calcium) and whether bone density had been measured. 644 women, all on Prednisolone, were recorded in detail. Patients were divided into those taking 2000mg (Gp3) in the period of data collection. In Gp1, there were 319 patients aged (69 ± 11yr) with 15.4% fracture prevalence; in Gp2 166 patients (72 ± 10yr) and 22.3%; and Gp3 159 patients (71 ± 10yr) and 21.4%.13% of patients had been taking CST for 5yr (71.3 ± 11yr). Asthma was the most frequent cause of treatment in women <70yr, and rheumatoid arthritis (RA) and polymyalgia rheumatica (PMR) in older women: these 3 diseases accounted for 82.5% of cases. There was no effect of steroid dose on fracture prevalence, but non-wrist fractures increased significantly after 5yr on CST (Chi sq $p<0.05$) with all doses combined. Hip fractures were more common in RA and PMR (Chi sq $p<0.01$) compared with all other diseases. Fracture prevalence increased with age as expected, but use of prophylaxis for osteoporosis declined with age from 52% at 50-59yr to 47% at 70-79yr (Chi sq for trend $p<0.01$). Use of HRT and bisphosphonates fell from 49% to 33% (Chi sq $p<0.01$). Patients with RA and asthma were less likely to receive prophylaxis than patients with other conditions (Chi sq $p<0.005$), but those on the higher doses of steroids were more likely to be taking treatment (Chi sq for trend $p<0.01$). 19% of patients had had a bone density scan at spine, hip or wrist, of which 60% were osteoporotic (WHO criteria). Asthma is the most frequent reason for CST. Most patients have taken CST for between 1-5yr. Duration of treatment is important for non-wrist fractures. RA and PMR patients are especially at risk of hip fracture, and whilst fracture prevalence increases with age, prophylaxis against osteoporosis is less common with age.

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QUS and DXA in the Assessment of Corticosteroid-Induced Osteporosis. C. Cepollaro,* S. Gonnelli, A. Montagnani,* D. Bruni,* C. Pondrelli,* B. Rossi,* M. Mangeri,* M. Breschi,* C. Gennari. Institute of Internal Medicine, University of Siena, Siena, Italy.

Osteoporosis is one of the major complications of corticosteroid therapy. Many studies have demonstrated that patients treated with corticosteroids present a decrease in lumbar spine and femoral neck bone mineral density (BMD). Few data are present in literature on the usefulness of quantitative ultrasound (QUS), a technique that could theoretically provide information on bone structure, in the management of corticosteroid-induced osteoporosis. The aim of the present study was to investigate the usefulness of QUS at calcaneus and phalanxes, compared to DXA, for detecting bone status in patients taking corticosteroids. We studied 162 patients (mean age 57.6±12; 106 women and 56 men) in chronic treatment with corticosteroids and 162 sex and age-matched controls. The patients were being treated with oral prednisone or equivalent at a dose of >7.5 mg/day for at least 1 year. In all subjects we measured bone mineral density at lumbar spine (BMD-LS) and at femoral subregions (femoral neck: BMD-FN, total hip: BMD-T, trochanter: BMD-TR, intertrochanter: BMD-ITR, Ward's triangle: BMD-W) by DXA (QDR 4500, Hologic, USA), and ultrasound parameters at calcaneus: speed of sound (SOS), broadband ultrasound attenuation (BUA) and Stiffness, by Achilles plus (Lunar Corp.), and at phalanxes:

amplitude dependent speed of sound (AD-SoS), by DBM Sonic (Igea). All densitometric and ultrasonographyc parameters, expressed as T scores, were significantly lower (P<0.05 for SOS, P<0.001 for the others) in patients treated with corticosteroids with respect to controls. In corticosteroid-treated patients, the T-scores were -2.4 for BMD-LS, -2,3 for BMD-FN, -1,5 for BMD-TR, BMD- ITR and BMD-T, -2,6 for BMD-W, -1,6 for SOS, -2,4 for BUA, -2,6 for Stiffness and -2,4 for AD-SoS. Moreover, the analysis of ROC curves showed that both DXA and QUS parameters are able to discriminate between subjects treated or not with corticosteroids. In particular, among DXA parameters, BMD-FN and BMD-LS and, among QUS parameters, AD-SoS, were the best in discriminating the two groups. In conclusion QUS at the heel and at phalanxes could be considered a useful tool in the management of glucocorticoid-induced osteoporosis.

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Increase in Corticosteroid Dosage Increases Risk of Development of Vertebral Deformities and Symptomatic Vertebral Fractures in Patients with Rheumatoid Arthritis. R. N. J. de Nijs,*[1] J. W. G. Jacobs,*[1] R. F. J. Laan,*[2] W. F. Lems,*[3] H. C. van Paassen,*[4] J. W. J. Bijlsma.[1] [1]Rheumatology and Clinical Immunology, University Medical Center, Utrecht, The Netherlands, [2]Rheumatology, University Medical Center, Nijmegen, The Netherlands, [3]Rheumatology, Free University Hospital, Amsterdam, The Netherlands, [4]Rheumatology, Sint Franciscus Gasthuis, Rotterdam, The Netherlands.

This study was performed to determine whether the prevalence of vertebral deformities in patients with rheumatoid arthritis (RA) treated with corticosteroids (Cs) is higher than in RA patients without Cs therapy and if there is an influence of current daily and cumulative Cs dosage. In this multicenter cross-sectional study, 205 patients with RA receiving Cs on a daily basis and 205 patients with RA who did not receive Cs, matched for gender and age, were included. Vertebral deformities were scored according to the Kleerekoper method. Univariate odds ratios (OR) were calculated, using logistic regression, to describe the crude relationship between Cs use (yes or no), current daily Cs dosage, cumulative Cs dosage and the prevalence of vertebral deformities and symptomatic vertebral fractures. Bivariate logistic models were used to adjust for incomparabilities between the RA patients on Cs and those not on Cs. Variables that changed the crude OR importantly in bivariate analyses were taken together for multivariate adjustment. Vertebral deformities were found in 52 (25%) patients on Cs versus 26 (13%) patients not on Cs. Sixteen (8%) patients in the group on Cs had experienced in the past clinical manifestations of an acute vertebral fracture versus only 3 (1.5%) in those not on Cs. Cs use tended to increase the risk of developing a vertebral deformity (adjusted OR 1.56 (95% CI 0.81 to 2.99) and symptomatic vertebral fracture (adjusted OR 1.42 (95% CI 0.24 to 8.32). Each one-miligram increase in current daily Cs dosage increased the risk of developing a vertebral deformity (adjusted OR 1.05 (95% CI 0.98 to 1.13) and symptomatic vertebral fracture (adjusted OR 1.05 (95% CI 0.89 to 1.24). The cumulative Cs dosage however did not increase the risk of developing a vertebral deformity nor symptomatic vertebral fracture (crude OR 1.00). Our data indicate that there is a higher prevalence of vertebral deformities and clinical manifestations of vertebral fractures in patients on Cs than in those not on Cs. Cs use and in particular every one-milligram increase of the current daily Cs dosage, but not the cumulative dosage of Cs, were a risk factor for developing a vertebral deformity and symptomatic vertebral fracture in patients with RA.

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Characterisation of the Effects and Mechanism of Action of the Glucocorticoid Dexamethasone on Mouse Bone. P. L. Salmon,[1] J. A. Humphreys,*[1] F. McLaughlin,*[2] J. MacKintosh,*[2] G. Brown,*[2] S. Farrow.*[2] [1]Harwell, Didcot, United Kingdom, [2]Cell Biology, GlaxoSmithKline Medicines Research Centre, Stevenage, United Kingdom.

Glucocorticoid administration to human patients can induce significant bone volume loss. We employed dynamic histomorphometry together with computer simulation of bone turnover, to elucidate the mode of action of glucocorticoid on bone using a mouse model. Thirty BALB/C mice were divided into six groups of five mice. A control group of 5 mice, and two further groups of 5 mice received vehicle, low and high dose of dexamethasone ("dex") respectively, by daily i.p. injection, for two or three weeks. At 2 and 10 days prior to sacrifice, calcein was given by i.p. injection to all groups. Trabecular bone was studied at the distal femur and lumbar vertebra. Cortical tibial diaphysis was studied in cross section.Dynamic parameters for trabecular bone showed a sharp decrease in bone turnover rate in response to dex administration, by up to an order of magnitude. This decrease was substantial and significant after two weeks dosing, and showed no essential further change after three weeks dosing. In fact, in the low dose group the decrease in dynamic parameters after 2 weeks was greater than after 3 weeks. In the cortical cross-sections, bone turnover decreased by 70-75% at the endosteal surface. The degree of decrease was essentially the same after two or three weeks of dosing with dex. At the periosteal surface, turnover decreased so sharply as to almost cease completely, after both two and three weeks of dex treatment. These data suggest that the decrease in turnover during 3 weeks of dex administration was not linear but exponential-like.There are three ways in which bone turnover can be slowed down, (1) by directly decreasing the formation and resorption activity by osteoblasts and osteoclasts across the whole turnover cycle within each bone modelling unit (BMU), (2) by decreasing the "activation rate" of new BMUs being recruited every day, and (3) slowing down and elongating the BMU cycle. In computer simulations of turnover, decreasing turnover by mechanisms 1 and 2, caused bone volume to increase. Only the third mechanism, extending the duration of the resorption-formation cycle, caused a decrease in bone volume. This may be the mechanism of action of dex on bone, as also suggested in a histomorphometric study of ewes (Chavassieux et al. 1997). It is of note that change of bone volume due to altered duration of the turnover cycle is slower than change by other mechanisms.ReferencesChavassieux P, Buffet A, Vergnaud P, Garnero P, Meunier PJ (1997) Short-term effects of corticosteroids on trabecular bone remodeling in old ewes. Bone 20 (5): 451-455.

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Low Prevalence of Osteoporosis and Fractures and no Significant Bone Loss After One Year Follow-up in Well Controlled Crohn's Disease. R. A. van Hogezand,*[1] D. Banffer,*[2] A. M. Zwinderman,*[3] E. V. McCloskey,[4] S. E. Papapoulos,[2] C. B. H. Lamers,*[1] N. A. T. Hamdy.[2] [1]Gastroenterology, Leiden University Medical Center, Leiden, The Netherlands, [2]Endocrinology & Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands, [3]Statistics, Leiden University Medical Center, Leiden, The Netherlands, [4]WHO Collaborating Center for Metabolic Bone Diseases, Sheffield, United Kingdom.

In Crohn's disease, the pathophysiology of skeletal disturbances is multifactorial including the use of glucocorticoids (GC) often given to secure disease remission. In a previous cross-sectional study of 146 unselected consecutive patients with Crohn's disease, we demonstrated that the prevalence of osteoporosis was low (15% and 19% respectively at the lumbar spine and hip sites) despite previous or current use of GC, providing that disease activity was reasonably well controlled. We also showed that the main risk factor for developing osteoporosis was ileum resection. The aim of this study was to establish the prevalence of vertebral and non-vertebral fractures in this population and to evaluate the extent of bone loss and incidence of new non-vertebral fractures after one year follow-up. Lateral X-rays of the thoracic and lumbar spine were evaluated in Sheffield, UK, using the McCloskey technique (132 evaluable X-rays). Non-vertebral fractures were documented by direct questioning. At baseline, 15 patients (10%) had sustained a non-vertebral fracture in which inappropriate trauma was involved. 8 patients (6%) had 9 documented vertebral fractures. BMD was repeated one year after the original evaluation and the incidence of new non-vertebral fractures recorded. The only pharmacological intervention allowed, other than necessary GC dose changes, was adequate supplementation with calcium and vitamin D. Three patients with severe osteoporosis requiring treatment with bisphosphonates were excluded and 13 patients were lost to follow-up. 62 patients were still using GC at one year. There was no significant change in mean BMD at the lumbar spine or hip sites regardless of use of GC. Our data suggest that, in contrast to their deleterious effects in other disease entities, glucocorticoids do not appear to result in significant adverse skeletal effects when optimally used in the management of patients with active Crohn's disease. The beneficial effects of using glucocorticoids to control disease activity may outweigh the apparently more important deleterious effects of disease activity on the skeleton in this inflammatory bowel disease.

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Amelioration of Low-Turnover Osteoporosis in the GnRH-Deficient Hypogonadal Hyperleptinaemic (*hpg*) Male Mouse by Estrogen. X. B. Wu,*[1] G. Rajendren,*[1] L. Sun,[1] K. Jepsen,[2] M. Schaffler,[2] D. Kimmel,[3] H. C. Blair,[4] M. Zaidi,[1] E. Abe.[1] [1]The Mount Sinai Bone Program and the Bronx VA GRECC, Mount Sinai Medical Center, NY, USA, [2]Department of Orthopedics, Mount Sinai Medical Center, NY, USA, [3]Merck Research Laboratories, PA, USA, [4]Department of Patology, University of Pittsburgh, PA, USA.

Hypothamic hypogonadism in the *hpg* mouse arising from a truncation deletion of the gonadotropin-releasing hormone (GnRH) gene, the human equivalent of Kallman's syndrome, results in a complete loss of sex steroids, obesity and infertility. We report here that the male *hpg* mouse has a severe form of low turnover osteoporosis and ~50% higher circulating levels of leptin. Although body weight was not different from its wild type littermate, the *hpg* mouse showed significant decrements in the wet weight of liver, kidney, testes and seminal vesicles. Wet bone weight, bone length, bone mineral density (*Piximus*), bone strength (maximum load and stiffness) were all decreased in the *hpg* mouse compared with wild type littermates. Histostaining revealed decreased cortical thickness and a ~60% reduction in osteoclast surface. To characterize the cellular defect further, we examined osteoblast and osteoclast formation in bone marrow cell cultures. There were significant, ~50 %, decreases in both alkaline phosphatase-positive colony forming units (CFU-Fs) and TRAP-positive osteoclasts in 3 week- and 3 month-old *hpg* mice compared with the respective wild type littermates. Taken together, the results suggest dramatic decreases in both osteoblastic bone formation and osteoclastic bone resorption resulting in low turnover osteoporosis. We next administered β-estradiol (20 μg, twice a week, for 1 month) to 4 to 6 month-old male *hpg* mice and their wild type littermates. Estradiol expectedly decreased fat weight, but had no effect on the wet weight of the liver, kidney, testes or seminal vesicles. Serum leptin levels decreased to normal and there was a significant increase in bone mass and length. In addition, there was a dramatic reversal to baseline of the suppressed TRAP-positive osteoclast formation in bone marrow cultures. The decrement in osteoblast formation in the *hpg* mouse was nevertheless not reversed upon estrogen treatment. The amelioration of osteoporosis and reversal of the resorptive phase of remodeling to normal by estrogen in a male *hpg* mouse highlights the importance of estrogen in bone remodeling that is independent of the organism's sex. The *hpg* mouse is therefore a unique model to study the recently described non-sex-specific effects of sex steroids on bone. The mouse should also allow us to characterize potentially critical interactions between estrogen and leptin.

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Sex Hormones and Mineral Metabolism in Male Osteoporosis. I. M. Frieling,* H. P. Kruse,* H. P. Kruse.* Nephrology/Osteology, Medical Clinic, University Hospital Hamburg, Hamburg, Germany.

The interest on male Osteoporosis is increasing during the last years. Like in female osteoporosis primary osteoporosis with and without different known risk factors and secondary osteoporosis is found. Different risk factors like hypercalciuria and low ß-estradiol levels are newly discussed in male osteoporosis. Our primary endpoint of this study is to find out, which laboratory data are helpful in diagnosis of male osteoporosis.We examined in this prospective study all male patients newly referred to our outpatient clinic with a suspected or diagnosed osteoporosis from 34 to 81 years, mean 53.1 years. We didn't differen-

tiate between already treated and not treated patients. All patients received a risk factor questionnaire, which was developed in our department. Laboratory routine tests were performed, also the examination of TSH, testosteron, ß-estradiol, phosphate clearance, calcium in 24 hour urine, 25-OH-vitamin D, 1,25-(OH)2-vitamin D, bone specific alkaline phosphatase, deoxypyridin crosslinks and PTH. Densitometry control on lumbar-spine and hip with Hologic QDR 1000+ was done. X-ray of vertebra was performed, when no x-ray was present.Mean DXA values of spine were decreased with 0.725 g/cm², although in 15% no osteoporosis was confirmed. Mean alkaline phosphatase was normal with 176 U/l and bone specific alkaline phosphatase with 15.7 µg/l. Deoxypyridin crosslinks were elevated in 55%, with an increased mean of 6.7 nmol/mmol creatinine and a negative significant correlation to 25-OH-vitamin D levels (r = -0.58). Mean phosphate clearance was elevated to 25.5 ml/min (normal range 5.5-16 ml/min). Testosteron levels were measured in lower normal range (mean 4.5 µg/l) and showed a significant correlation to ß-estradiol levels (r = 0.58). Beta-estradiol levels were decreased in 60% (normal range: 13.5 - 59.5 ng/l), but there was no correlation to DXA.The results of this study confirm the assumption, that low ß-estradiol levels have an important rule in the pathogenesis of male osteoporosis, although no correlation to DXA was found. A part of high turnover osteoporosis is due to vitamin D deficiency. The examination of ß-estradiol and 25-(OH)-vitamin D is helpful to the physician in the diagnosis of male osteoporosis.

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Intestinal Calcium Absorption Assessed by Stable Strontium Test in Elderly Men. S. Gonnelli,[1] L. Gennari,*[2] A. Montagnani,*[1] M. Campagna,*[1] M. Franci,*[1] B. Lucani,*[1] D. Merlotti,*[1] C. Cepollaro,*[1] C. Gennari.*[1] [1]Institute of Internal Medicine, University of Siena, Siena, Italy, [2]Institute of Internal Medicine, University of Florence, Florence, Italy.

The mechanisms leading to bone loss in men are still poorly understood. In particular, the role of calcium malabsorption in male osteoporosis remains controversial. Recently, stable strontium (Sr) has been proposed as an alternative to dual tracer procedure for the assessment of intestinal calcium absorption. The aim of this study was to assess the determinants of intestinal Sr absorption in elderly men. We studied a cohort of 91 consecutive men (age range: 51 - 85 yrs) who attended our clinic for osteoporotic risk evaluation. In all subjects we performed a Sr absorption test using an oral load of 2.5 mmol, without additional calcium or meal. Plasma samples were analyzed for Sr by graphite furnace atomic spectrophotometry and both fractional absorption (Fc240) and the area under the concentration time curve (AUC 0-300) were evaluated. The reproducibility was assessed in 15 healthy males by repeating the test with an interval of 10 days; the coefficients of variation were 7.8% for AUC0-300 and 11.2% for Fc240. In all we measured serum total testosterone (TT), estradiol (E2), 25OHD, insulin-like growth factor 1 (IGF-1), IGF binding protein 3 (IGFBP3), PTH and bone alkaline phosphatase. Bone mineral density at lumbar spine (BMD-LS) and at femur (BMD-F) by DXA and dietary calcium intake by a validated self-reporting questionnaire were also assessed. On the basis of BMD-F, 31 men were classified as osteoporotics, 25 as osteopenics and 35 as normals. Both Fc240 and AUC0-300 negatively correlated with age (r=-0.31 and -0.3 respectively) and with calcium intake (r=-0.45 and -0.31 respectively). A weak, but significant correlation (r=0.28) was found between 25OHD and AUC0-300. AUC0-300 showed positive, but not significant correlations with BMD-LS (r=0.19), BMD-F (r=0.23), E2 (r=0.20) and TT (r=0.24). No significant correlations were found between IGF-1, IGFBP3 and Sr absorption. An inverse relationship was found between TT and BMD-F (r=-0.28). E2 was significantly lower in osteoporotics and showed positive, but not significant, correlations with BMD-LS and BMD-F. In conclusion, because of its reproducibility, AUC0-300 is better than Fc240 for the evaluation of Sr absorption. In our study intestinal Sr absorption decreases in men with advancing age and seems more related to calcium intake and 25OHD than to TT, E2 or IGF-1 serum levels.

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Heel Ultrasound Discriminates Osteoporotic Males with or without Vertebral Fractures. E. Toth,*[1] S. Meszaros,*[1] E. Hosszu,*[2] E. V. McCloskey,[3] C. Horvath.[1] [1]1st Department of Medicine, Semmelweis University, Budapest, Hungary, [2]2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary, [3]WHO Collaborating Centre for Metabolic Bone Diseases, Sheffield University, Sheffield, United Kingdom.

In females a growing body of data suggest that quantitative ultrasonometry (QUS) of the bone reflects not only density but also non-mass properties. To test this hypothesis in male osteoporosis 130 men (age 56.7+/-9.1 ys) were studied after dividing into three groups (osteoporosis n=54, osteopenia n=36 and age-matched controls n=40). BMD was measured at lumbar spine and hip (DPX-L, Lunar) and at radius (NK-364, Gamma). QUS was done at the calcaneus by DTU-ONE (Osteometer). The numbers of vertebral fractures (VF) detected by Minne's method were found as follows: 184 in osteoporosis, 68 in osteopenia and 62 in the control group.BUA was lower in groups with low BMD (40.3+/-8.4 and 47.0+/-6.8 vs 51.3+/-5.6 dB/MHz, p<0.0001), while SOS did not differ. In the separate groups there were no differences neither in BMD nor in BUA between the fractured and non-fractured patients. A decrease of SOS in patients with VF was found in all three groups compared to results obtained in patients without fractures. The relative risk of VF was found as 1.14 for BUA and 1.51 for SOS and these results were not changed by adjustement for age and BMI. After adjusting for BMD only the risk for SOS but not for BUA remained high.Conclusion: in men the heel SOS seems to reflect bone properties related to vertebral fractures, independently of density measured at spine or at any site. BUA seems to be a surrogate of BMD in males.

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Bone Mass, Strength and Turnover Markers in a Rat Model of Male Osteoporosis. V. Shen, S. Tellefson,* M. Heggem,* M. Bailey,* T. Bailey,* R. Leininger,* P. Hara,* S. Bain. Skeletech, Inc., Bothell, WA, USA.

Male osteoporosis is becoming a significant public health issue. While ovariectomized (OVX) animals have been shown to be very useful in the study of female postmenopausal osteoporosis, an animal model for male osteoporosis has not been fully established. In order to further characterize the usefulness of such a model in the study of male osteoporosis, we examined changes in bone mass, strength and turnover markers in the orchidectomized (ORX) male rat. Experimentally, three-month old male Sprague Dawley rats were either sham-operated or ORX. One sham and one ORX group were given vehicle (ORX + Veh) and one group of ORX was given dihydrotestosterone (ORX + DHT), s.c. for five weeks. At the end of the five-week period, serum and bones were collected for analyses. Three bone turnover markers, urinary deoxypyridinoline and serum osteocalcin and pyridinoline, showed significant increases in ORX + Veh animals and DHT treatment prevented these increases. pQCT scanning of the excised femurs displayed a significant decrease of the BMD at both distal and midshaft femur in ORX + Veh animals, representing significant loss of cancellous and cortical bone, respectively. Treatment of ORX animals with DHT partially protected the bone loss. In addition, DXA scan of the vertebral column showed significant bone loss following ORX while DHT therapy completely prevented this bone loss. Finally, mechanical testing of the lumbar vertebral body, and femoral neck and midshaft demonstrated significant deterioration of bone strength in the ORX + Veh treated rats. In conclusion, ORX induces a rapid increase of bone turnover and causes a generalized loss of bone mass and strength at all sites examined. Most notably, ORX decreases both cancellous as well as cortical bone mass, a finding not typically seen in the OVX rat model but seen in bone loss in males. These results provide further support for the ORX rat as a useful model of male osteoporosis.

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Plasma Lipid Levels Are Associated with Bone Mineral Density and the Presence of Vertebral Fractures in Postmenopausal Women. T. Yamaguchi, T. Sugimoto, S. Yano,* M. Yamauchi,* H. Sowa,* K. Chihara.* Third Division, Department of Medicine, Kobe University School of Medicine, Kobe, Japan.

Many clinical studies show that osteoporosis is associated with atherosclerosis and cardiovascular death. Although both high plasma levels of low density lipoprotein cholesterol (LDL-C) and low plasma levels of high density lipoprotein cholesterol (HDL-C) are known to be risk factors for atherosclerosis, it is unclear whether such lipid derangements are also associated with the pathogenesis of osteoporosis. In this study, we evaluated the relationships between plasma levels of total C, LDL-C, HDL-C, or triglyceride (TG) versus bone mineral density (BMD) at the lumbar spine, femoral neck, radius, or total body as well as the presence of vertebral fractures in 214 Japanese postmenopausal women (age range, 47-86 years, mean 62.7). Multiple regression analysis was performed between BMD at each skeletal site versus each lipid level adjusted for age, body mass index (BMI), and %fat. Plasma LDL-C levels were significantly and inversely correlated with the absolute and age-adjusted (Z score) values of both 1/3 radial (1/3R) and ultradistal radial (UDR) BMD (p<0.01), and with the absolute values of lumbar (L) BMD (p<0.05). In contrast, plasma HDL-C levels were significantly and positively correlated with the absolute values and Z scores of 1/3R-BMD (p<0.05 and p<0.01, respectively) as well as the Z scores of UDR-BMD (P<0.01), and tended to be positively correlated with the absolute values of both UDR-BMD (p=0.051) and L-BMD (p=0.059). On the other hand, plasma TG levels were significantly lower in women with vertebral fractures than in those without fractures (97.0•36.5 vs. 126.4•65.8 mg/dl, mean•SD, p<0.05). When multivariate logistic regression analysis was performed with the presence of vertebral fractures as a dependent variable and each lipid level adjusted for age, BMI, and %fat as independent variables, TG alone was selected as an index affecting the presence of vertebral fractures (Odds ratio: 0.51, 95% confidential interval: 0.29-0.89 per SD increase, p<0.05). Our study showed that plasma LDL-C and HDL-C levels were inversely and positively correlated with both R- and L-BMD values, respectively, while low plasma TG levels were associated with the presence of vertebral fractures in postmenopausal women. Thus, plasma lipids might affect bone mass and bone fragility, and might be the common factors underlying the pathogenesis of both osteoporosis and atherosclerosis.

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Osteoporosis Associated With Idiopathic Hypercalciuria: A Peculiar Form of Metabolic Bone Disease. S. Giannini, M. Nobile,* M. G. Lodetti,* L. Dalle Carbonare,* F. Silva Netto,* S. Sella,* L. Sartori, G. Crepaldi. of Medical and Surgical Sciences, Clinica Medica I, University of Padova, Padova, Italy.

Hypercalciuria, in particular fasting hypercalciuria, is an important risk factor for bone demineralisation in patients with recurrent calcium nephrolithiasis. While several studies have focused on the prevalence of low bone density in patients with nephrolithiasis and hypercalciuria, few data are available on the prevalence of hypercalciuria in osteoporotic patients.In order to investigate this phenomenon, we retrospectively examined the clinical records of 914 patients admitted to our outpatient Clinic between 1997 and the end of 1998 for their first examination for a suspect metabolic bone disease. Of the 914 subjects, 509 post-menopausal women (age range 40-88 yrs.) were selected who had a primary reduction in bone density of at least 2 standard deviations at spine and/or femur. Creatinine, calcium, phosphate, PTH, alkaline phosphatase and its bone isoenzyme were evaluated on blood samples; calcium, phosphorus and creatinine were assessed on 24-hour urine samples. Bone density, evaluated both at the spine and femur, was expressed as T score or Z score. Hypercalciuria, defined as 24-hour urine calcium excretion greater than 4 mg/kg body weight and/or greater than 250 mg in females and 300 mg in male patients, was reported in 18.6% of the selected population. This percentage rose to 22.3% when only patients who were not treated specifically for osteoporosis were considered (n = 328). An inverse correlation between years since menopause and T score both at the spine (p< 0.05) and femur (p < 0.001) and between age and femoral T score (p < 0.001) emerged in the group with osteoporosis/no hypercalciuria (OP-NHC n = 255). Multiple regression analysis confirmed the importance of years since menopause and age in determining bone loss in this group. Conversely, in the group of patients with osteoporosis and hypercalciuria (OP-HC n = 73), urine calcium excretion was important in determining the decrease in bone mass at the spine and it was even more important when the most severe forms of bone loss (T-spine < -3 DS, r = - 0, 43, p < 0.05) were considered.Moreover, hypercalciuria was the strongest predictor of bone mass (R2 0.50) in the subgroup of patients with vertebral osteoporo-

sis.The results of this study confirm that the association between hypercalciuria and osteoporosis is frequent and peculiar. To establish the type of the metabolic defect then leading to calcium losses and to treat it correctly could be crucial to the success of the therapy of osteoporosis in these patients.

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BMD as Measured by DXA Does Not Predict Incident Breast Cancer Cases in Early Postmenopausal Women. A. Stewart,*[1] J. Harvie,*[2] F. Gilbert,*[3] D. Torgerson,*[4] D. M. Reid.[1] [1]Medicine and Therapeutics, University of Aberdeen, Aberdeen, United Kingdom, [2]Dept. of Rheumatology, University of Aberdeen, Aberdeen, United Kingdom, [3]Dept of Radiology, University of Aberdeen, Aberdeen, United Kingdom, [4]University of York, York, United Kingdom.

There have been 2 previous reports in older women that BMD predicts breast cancer cases, with those with high BMD being at greater risk. We have examined a cohort of women aged 45-54 years prospectively. At baseline and follow-up we enquired whether the women had ever been diagnosed as having breast cancer. At baseline (1990-4) spine and hip BMD (neck BMD) was measured by dual energy x-ray absorptiometry (DXA). At follow-up 114 women of the 3883 who returned indicated a history of breast cancer. Mean follow-up interval was 6.4 years (SD 0.9). Several women were deceased at follow-up and from their medical records it was found that 2 further women had suffered from breast cancer. Further details were gathered from a review of the hospital records including date of diagnosis, type of breast cancer, and where stated the estrogen receptor status. Of these 116 women, 46 had an incident breast cancer since the baseline BMD scan, 57 had prevalent breast cancer at baseline and 13 had benign or unconfirmed diagnosis. Baseline BMD of the women with incident breast cancer (BC group) did not differ significantly from those without (C group) (Spine BMD: BC 1.02 +/- 0.14, C 1.05 +/- 0.16, p = 0.153; neck BMD: BC 0.84 +/- 0.10, C 0.88 +/- 0.12, p = 0.051). The majority of the BC group had estrogen positive results (80%), and comparing estrogen positive and negative cases separately did not show any significant results. Analysis by quartiles of BMD did not show any significance (p = 0.67 for spine BMD, p = 0.18 for neck BMD). However if we examined only those who were postmenopausal and never taken HRT we find significant results (Spine BMD: p = 0.002; Neck BMD: p = 0.035). When in situ cases were excluded the relationship remained significant (Spine BMD p = 0.003; Neck BMD p = 0.045). Relative risks (RR) for 1SD change were also calculated using Cox Regression, however significant results were only found for Neck BMD (RR = 1.48, CI 1.08-2.04). Interestingly none of the women with incident breast cancer indicated another family member with the disease. In conclusion, our study does not show a relationship between spine or hip BMD and breast cancer incidence. Although not significant the trend is in the opposite direction to what has been previously found, i.e. those with lower BMD were found to be at greater risk. The previous studies have included older women and older methods of assessing appendicular BMD. Therefore using axial BMD in a younger group of patients no relationship was found between BMD and breast cancer risk.

M385

Skeletal Effects of Cyclosporin A Are Gender-Related in Rats. R. G. Erben,[1] K. S. Brunner,*[1] L. C. Hofbauer,[2] M. Goldberg.*[1] [1]Institute of Animal Physiology, University of Munich, Munich, Germany, [2]Division of Gastroenterology and Endocrinology, Zentrum fuer Innere Medizin, Philipps University, Marburg, Germany.

A common and serious side effect of organ transplantation is osteoporosis, and the immunosuppressive drug cyclosporin A (CsA) is thought to be involved in the pathogenesis of post-transplantation osteoporosis. To evaluate further the skeletal effects of CsA, we treated aged male and female sham-operated (SHAM) and gonadectomized Fischer 344 rats with low doses of CsA for 4 months. CsA did not influence estradiol or testosterone levels in male or female SHAM or gonadectomized animals. Also, CsA did not alter sex hormone levels in orchiectomized (ORX) and ovariectomized (OVX) rats supplemented with physiological doses of testosterone or estradiol. However, endogenous estradiol in SHAM female rats and especially exogenous administration of 17beta-estradiol in OVX rats markedly diminished blood levels of CsA by increasing hepatic CsA metabolism. Following CsA treatment, urinary excretion of calcium and deoxypyridinoline increased dose-dependently with a maximum at 2 months in male SHAM and ORX rats, while renal calcium excretion and urinary deoxypyridinoline in female SHAM and OVX rats remained unchanged or even decreased. Cancellous bone area in the proximal tibia and the first lumbar vertebra was nonsignificantly decreased in male SHAM and ORX rats after the 4-month treatment period. In contrast, CsA treatment increased tibial and vertebral cancellous bone area in female SHAM and OVX rats. Similarly, CsA dose-dependently decreased bone mineral density and cortical thickness of the tibial shaft measured by peripheral quantitative computed tomography in male SHAM and ORX rats, but had no effect in female SHAM or OVX rats. We conclude that CsA is antiresorptive and bone-sparing in aged female rats but increases bone resorption and reduces bone mass in aged male rats. However, even in male rats, long-term CsA treatment at clinically relevant doses increased bone resorption only transiently, and did not result in pronounced cancellous bone loss. The gender-specific skeletal effects of CsA were not modulated by sex hormones or gonadectomy. Although the mechanism for the gender-specific skeletal effects of CsA is still obscure, our findings may have important implications for clinical therapy with CsA.

M386

Possible Involvement of High Glucose and TNF Alpha in Diabetic Osteopenia. S. Wada, S. Suda,* S. Kitahama,* S. Yasuda,* T. Nagai,* M. Iitaka,* S. Katayama.* Fourth Department of Internal Medicine, Saitama Medical School, Iruma-gun, Japan.

Recent epidemiological surveys have revealed that diabetes is a risk factor for fractures. This mechanism would be associated with various factors accompanied by diabetes (e.g., insulin deficiency or resistance and/or continuous hyperglycemia). We, therefore, studied the effects of high glucose and TNF alpha on osteoblasts (OBs) and osteoclasts (OCs); TNF alpha has been shown to be a key factor responsible for insulin resistance. The high glucose concentrations (60 mM) did not affect alkaline phosphatase (ALP) activity in mouse primary OBs, whereas treatment with TNF alpha significantly decreased ALP activity. Flow cytometric analysis using fluorescein labelled Annexin V revealed that treatment with TNF alpha increased the number of apoptotic OBs but not OC progenitors. When mouse OBs and bone marrow cells were cultured in the presence of PGE2 and 1,25(OH)2D3, OCs were formed under high glucose concentrations (5.6-60 mM), while TNF alpha attenuated PGE2- and 1,25(OH)2D3-stimulated OC formation in the co-cultures. Since this effect was inhibited by antibody against TNF alpha receptor (p55), it seemed that TNF alpha acted on the type 1 receptor. When mature OCs were studied on bone resorbing function, it was found that bone resorption was inhibited by exposure to high glucose (15-60 mM). TNF alpha also inhibited bone resorbing capacity. The effects of decreased bone resorption were, at least partly, due to the deranged actin ring formation of OCs. Although OCs formation and function were modified, treatment with high glucose and TNF alpha did not influence the expression of RANKL and OPG in OBs nor the expression of RANK in OC progenitors. These results indicate that the functions of OBs and OCs could be modified by the factors associated with diabetes. This study suggests that multiple factors or cytokines produced in the microenviroment of bone could affect the cellular function of OBs and OCs, which would be related, to some extent, to low turnover of bone observed in diabetes.

M387

An Age Related Reduction of Musculoskeletal Response to Change in Body Weight and Prevention by OSA 117M. S. A. F. Peel,*[1] C. E. Webber,[2] C. Bier,*[1] Y. Gu,*[1] C. S. Tam.*[1] [1]Osteopharm Inc, Oakville, ON, Canada, [2]McMaster University, Hamilton, ON, Canada.

Evidence indicates that there is a positive relationship between bone mass, as reflected by total bone mineral content (BMC), and body weight (BW). A change in this relationship could result in a reduction in BMC and may be a contributing factor to the development of osteoporosis. This study investigated an age-related change in BMC/BW and lean body mass (LBM)/BW ratios and the effect of a synthetic peptide OSA 117M on these ratios. OSA 117M has previously been reported to increase bone mineral content in rats subjected to exercise [1].Seven month old female Sprague Dawley rats were divided into a control and test group, (n=8 per group). The test group was treated with 300nmoles/Kg/day of OSA 117M, 5 days per week, while the control group received no treatment. All rats were housed in tall cages to promote physical activity and allowed unlimited access to food and water. Each month for 8 months the rats were weighed and BMC and LBM estimated by DEXA. The control group showed a significant and steady decline in both BMC/BW and LBM/BW over the eight months, reaching -3.52±0.56% (mean±SE; P<0.05) and -10.87±2.39% (P<0.001) respectively, by the end of the experiment. The rats treated with OSA117M showed no significant changes in the BMC/BW (-0.43±0.64%) or LBM/BW (1.40±1.36%) ratios over the test period.In conclusion aging appears to alter the BMC/BW and LBM/BW ratios while treatment with OSA 117M results in the maintenance of these ratios. The changes in BMC/BW and LBM/BW with age suggests an alteration in the musculoskeletal response to physical loading and this may contribute to the development of osteoporosis. [1] Tam et al. (1998) Bone 23 (supplement): SA367

Disclosures: Osteopharm Inc,3.

M388

Histomorphometric Analysis of Bone Biopsies in Patients with Cystic Fibrosis.S. L. Elkin,*[1] S. Bord,[2] N. J. Garrahan,*[3] M. E. Hodson,*[4] J. E. Compston.*[2] [1]Department of Cystic Fibrosis, National Heart and Lung Institute, Royal Brompton Hospital, London, United Kingdom, [2]Medicine, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom, [3]Department of Pathology, University of Wales College of Medicine, Cardiff, United Kingdom, [4]Department of Cystic Fibrosis, National Heart and Lung Institute, Royal Bompston Hospital, London, United Kingdom.

Low bone mineral density (BMD) is well documented in children and adults with cystic fibrosis (CF) and results both from failure to achieve peak bone mass and increased rates of bone loss in adult life. The mechanism of bone loss in these patients has not been defined nor has it been established whether defective mineralisation is an important contributory factor. The aim of the present study was to investigate bone turnover and remodelling in patients with CF, using histomorphometric analysis of iliac crest biopsies.Trans-iliac biopsies were performed after double tetracycline labelling in 21 CF patients, mean age 31.0 (range 18-40 years), 12 male, with femoral neck and/or lumbar spine BMD Z scores of less than -1.5. Samples were embedded in LR white medium resin and 8µm undecalcified sections were stained by the von Kossa and toluidine blue techniques. Histomorphometric measurements were made by image analysis using an in house system. Biopsies from 18 healthy subjects, 10 male aged 20 to 44 years (mean 32.6) served as controls. Data were log transformed and differences between groups analysed using Student's unpaired t test.The mean cancellous bone volume (TBV) was significantly lower in CF patients compared to the controls (p=0.003). Bone formation rate at tissue level and mean wall width were also significantly lower in the patient group (p=0.0002 and p<0.0001 respectively). The mineral apposition rate was reduced in comparison to the controls p=0.02) with a small increase in mineralization lag time (p=0.003). One patient had osteomalacia as defined by classical histomorphometric criteria. The eroded perimeter was increased in CF patients (5.2% vs 1.4% in controls) but indices of resorption cavity size were not increased.These results demonstrate that bone loss in patients with CF is predominantly due to a reduction in bone formation without a demonstrable increase in active bone resorption. In this group of patients, osteomalacia was seen in only one individual. The cause of osteoblast dysfunction in these patients remains to be established.

M389

A Possible Role of Osteocalcin Carboxylation in Compensation Mechanism in Bone. T. Sugiyama, A. Yamaguchi. Department of Oral Pathology, Nagasaki University School of Dentistry, Nagasaki, Japan.

Recently, it has been reported that vitamin K treatment prevents fractures without increasing bone mass, and that low carboxylation of serum osteocalcin (OC) predicts high fracture risk. We also found that carboxylation of serum OC in healthy children was positively correlated to the Z score for ultrasound velocity of tibia. Furthermore, vitamin K treatment has been reported to improve bone strength through modification of hydroxyapatite crystals without affecting bone mass or bone microarchitecture in rat with diabetes, suggesting that carboxylated OC increases bone strength by improving bone material property. In contrast, use of warfarin, an inhibitor of carboxylation of OC, has been reported to be not associated with the occurrence of hip fracture. To resolve these contradictory results, we investigated the effect of 6-month jumping exercise on bone in a premenarcheal girl with X-linked hypophosphatemic rickets (XLH) and healthy women. First, we observed the marked in rease of lumbar bone mineral content (BMC) in the girl with XLH, with an increase of serum intact osteocalcin and a decrease of urinary deoxypyridinoline. Although high-impact exercise induces the increase of BMC at weight-bearing bones in premenarcheal normal girls, the degree of increased BMC in the girl with XLH was much greater than in normal girls. Abnormal mineralization in rickets indicates a poor bone material property, and the markedly increased BMC could be interpreted as the compensated result to maintain bone strain on the basis of the "mechanostat" concept that skeletal bone possesses a homeostatic mechanism to maintain bone strain generated by mechanical stimuli. The compensation mechanism could resolve the difference of bone mineral density (BMD) in patients with XLH, i.e., lumbar BMD is high while radius BMD is low. Second, we found in a prospective controlled study that pre- and postmenopausal women had different BMD responses to high-impact exercise, and that the baseline value of urinary carboxyglutamate (Gla) was inversely correlated to the change of whole body BMD after high-impact exercise in the premenopausal women, suggesting that high-impact exercise-induced bone gain is greater as bone material property deteriorates. Thus, it is likely that impairment of bone material property is compensated by increase of bone mass. By applying the compensation mechanism, the effect of warfarin on bone could be interpreted as follows. Warfarin treatment induces the marked decrease of carboxylated OC, and the bone strain level increases by impairment of bone material property and reaches above the modeling threshold. Thus, the compensation mechanism works and fracture risk is not increased at weight-bearing bones.

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Comparison of Circadian Variation of Urinary N-terminal Telopeptide of Type I Collagen (NTX) in Healthy Premenopausal Women, Blind Subjects and Permanent Hypoparathyroid Patients. S. Di Gregorio,*[1] S. N. Zeni,[1] M. B. Oliveri,[1] A. Wittich,*[1] C. Casco,[1] J. Somoza,*[1] E. Cutrera,*[2] C. A. Mautalen,[1] D. Cardinali.*[2] [1]División Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Diurnal rhythm bone turnover has been reported in several bone metabolic markers; however its etiology is not known. This study was performed to clarify the influence of PTH or luminous stimulus on the circadian variation of the bone resorption marker NTX.Six healthy women (aged: 31-45) (G1), six blind subjects (congenital amaurotic people) (G2) and six hypoparathyroid patients (undetectable PTH)(G3) were recruited for the study. Twenty-four hours urine was collected in six separate samples: between 8 and 12 a.m,12 am and 4 p.m, 4 and 8 p.m., 8 and 12 p.m., 0 and 4am, 4 and 8 a.m. NTX was measured by ELISA (Osteomark) and expressed as absolute amount in nMECO per 4 h corrected by creatinine excretion (mM). Results (mean±SE).(*) p<0.0001 compared to G2 and G3. (#) p<0.01 minimal significant valueSimilar circadian patterns exhibiting a peak value between 4 and 8am and the lowest value between 8am and 4 pm in G2 and between 4 and 8pm in G1 and G3 were observed, although concentration of NTX excretion was significantly higher in healthy women than in hypoparathyroid patients or blind subjects. These results suggest that hypoparathyroid patients present a diurnal rhythm of bone resorption that is partly independent of PTH. The rhythm in bone resorption seems to be endogenous, as shown by its persistence in blind people. Conversely, healthy pre-menopausal women have higher levels of NTX excretion, because of the PTH-dependent and light-dependent components of bone resorption. According to these findings the circadian rhythms of NTX excretion seem to be relatively unaffected by PTH and light. However, the magnitude of its response is clearly affected by the two factors considered in this abstract.

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Quantitative Ultrasound of Phalanges and Bone Mineral Density in Early and Moderate Stage of Chronic Renal Failure. J. Przedlacki,*[1] W. Pluskiewicz,*[2] J. Trebicka,*[1] B. Drozdzowska,*[2] J. Matuszkiewicz-Rowinska,*[1] K. Ostrowski.*[1] [1]Medical University of Warsaw, Warsaw, Poland, [2]Silesian Medical Academy, Zabrze, Poland.

Quantitative ultrasound of phalanges (QUS) informs on bone mineralisation and elasticity when DXA on bone mineralisation only. Decreased QUS results were found in our previous study in patients with end-stage renal failure (ESRF) treated with dialysis. The aim of our current study was to evaluate QUS results and compare them to DXA in early and moderate stage of chronic renal failure (CRF). Fifty-one patients (31 males and 20 females) aged 55.4+/-10.8 years (29-71), with serum creatinine 3.1+/-1.4 mg% (1.5-6.0) and CRF lasting for 52.7+/-49.9 months (2-216) were examined. QUS examination (amplitude-dependent speed of sound) was performed with the use of DBM Sonic 1200 (Igea, Italy) device, at the distal metaphysis of the proximal phalanges of II-V fingers of the left hand. DXA of lumbar spine, proximal femoral bone, radial bone and total body was performed with the use of Lunar DPX-L. QUS was 2010+/-83 m/s (1819-2171) in men and 1939+/-73 m/s (1850-2136) in women. The results were normal (Z-score>-1 SD) in 44 patients (28 males and 16 females) and decreased, expressed as Z-score below -2SD in 3 patients (3 females). There was positive correlation between QUS results and DXA (expressed in g/cm2) of: lumbar spine (r=0.3771, p<0.01), femoral neck (r=0.4536, p<0.001), Ward triangle (r=0.2862, p<0.05), trochanter (r=0.3591, p<0.01), ultradistal radius (r=0.5385, p<0.001), radial shaft (r=0.4751, p<0.001) and total body (r=0.5297, p<0.001). There was correlation between QUS and age (r=-0.6048, p<0.001) in men and between QUS and age (r=-0.7435, p<0.001), serum calcium (r=0.6820, p<0.005) and alkaline phosphatase activity (r=-0.6505, p<0.005) in women. There was no correlation between QUS and duration of CRF, serum creatinine, phosphorus, i-PTH and ionized-calcium in blood in males and females. QUS results in men were significantly lower (p<0.01) than in males with ESRF treated with dialysis (comparable in age). QUS results in females were not statistically different from dialysed females, although dialysed women were significantly younger (p<0.001). We conclude that decreased QUS results of phalanges are rarely seen in early and moderate stage of CRF than in dialysed patients and that there is a positive correlation between QUS and DXA results of all typical sites of examination in these patients. It is difficult to conclude if QUS and DXA methods can be taken as a complementary or an alternative techniques in patients in early and moderate stage of CRF.

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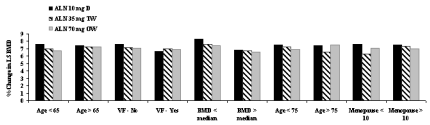
Once-Weekly Alendronate: Two Year Subgroup Efficacy Analyses in Postmenopausal Women With Osteoporosis. H. Bone,[1] D. Felsenberg,[2] M. Greenwald,[3] T. Schnitzer,*[4] A. Kaur,*[5] M. Cudny,*[5] C. Peverly,*[5] J. Orloff,[5] A. Santora.[5] [1]Michigan Bone and Mineral Clinic PC, Detroit, MI, USA, [2]Freie Universitat Berlin Klinikum, Berlin, Germany, [3]Desert Medical Advances, Palm Desert, CA, USA, [4]Northwestern Univ., Chicago, IL, USA, [5]Merck Research Laboratories, Rahway, NJ, USA.

In a multicenter study of 1258 postmenopausal women with osteoporosis, treatment with alendronate (ALN; manufactured by Merck & Co., Inc.) 70 mg once weekly (OW) was shown to be therapeutically equivalent to treatment with ALN 10 mg once daily (D) (Schnitzer et al, Aging 2000;12:1-12). We further examined the efficacy of treatment with ALN 70 mg OW (n=519), ALN 35 mg twice weekly (TW) (n=369), and ALN 10 mg D (n=370) by way of a subgroup analysis of two-year spine and hip BMD including a one-year study extension. The following prespecified patient subgroups were analyzed for percent change from baseline in lumbar spine (LS) and total hip BMD at month 24: baseline age greater or less than 65 or 75 years; vertebral fracture (VF); LS BMD greater or less than median; and years since menopause greater or less than 10 years. The treatment response was consistent across subgroups for LS BMD. In all ALN treatment groups, patients without baseline VF had a slightly but not statistically significantly greater response to treatment than those with VF, as did those with lower vs. higher baseline LS BMD. Additional analyses showed consistent treatment responses across subgroups for total hip BMD (data not shown).In summary, for all ALN treatment groups (once weekly, twice weekly and once daily), percent change in LS and hip BMD was consistent across all examined baseline demographic characteristics.



Disclosures: Merck & Co., Inc.,2.

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The Efficacy of Alendronate in Postmenopausal Osteoporotic Women. M. Bernad,*[1] T. Del Campo,*[2] M. Gonzalez,*[3] J. Fernandez,*[1] M. Garces,*[4] E. Martin-Mola,*[1] M. Martinez.[5] [1]Rheumatology, HU La Paz, Madrid, Spain, [2]Research, HU La Paz, Madrid, Spain, [3]Biochemistry, Gomez Ulla Hospital, Madrid, Spain, [4]Jimenez Diaz Fundation, Madrid, Spain, [5]Biochemistry, HU La Paz, Madrid, Spain.

OBJECTIVE: To evaluate the efficacy of Alendronate in postmenopausical osteoporotic women.METHODS: In a prospective study, we included 210 women with postmenopausical osteoporotic. 98 received alendronate 10 mg daily, they were permitted to take calcium or vitamine D supplementation. The subjects were assessed at baseline, 12 and 24 months for bone mineral density (BMD) measurements and every 3 months for blood and urinary biochemistries and markers of bone turnover.RESULTS: We evaluated 98 patients with a mean aged of 59.07 (SD: 6.68), 13 (13.2%) have dropped out because of adverse effects, the most common was gastrointestinal events. 17 (17.34%) had spine fractures, 12 colles´s fracture and one hip fracture; three patients had new ones during this time (1 spine, 1 scaphoid and one colles´s fracture). 60 subjects have completed one year of treatment and 24 have completed two years. The results were analyzed by Student´s t-test, patients treated with alendronate for 1 year had significantly greater improvement in BMD at spine, the mean BMD at baseline was 0.373 vs 0.773 at one year (p<0.05) and at hip 0.665 vs 0.6435 at baseline (p<0.05), the mean BMD of patients who completed two years at spine was 0.745 (p =0.826) and at hip was 0.646 (p=0.712).CONCLUSION: Patients treated with alendronate for one year had significantly greater improvement in BMD at both the spine and hip, there were no differents between those treated for 24 M.

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Can Digital BMD and Multi-Site Speed of Sound Conduction Be Used to Monitor Therapy with Alendronate in Postmenopausal Osteoporosis? W. M. Drake,*[1] J. P. Brown,[2] C. Banville,*[2] D. L. Kendler.[1] [1]Osteoporosis Research Centre, Vancouver, BC, Canada, [2]Centre de Recherche du CHUL, Université Laval, Ste-Foy, Quebec, PQ, Canada.

Response to therapy with bisphosphonates in women with postmenopausal osteoporosis (PMO) is conventionally monitored using central site (hip and spine) bone mineral density (BMD), but more convenient alternatives are desirable. Digital BMD and multi-site speed of sound (SOS) distinguish, cross-sectionally, women with prevalent vertebral fracture from those without; and both show promise for use as screening tools for PMO. However, their clinical utility in the monitoring of patients on therapy is unknown. During a randomized parallel group study comparing the efficacy and tolerability of once weekly (80 mg vs 160 mg) oral alendronate in the treatment of PMO, 81 women (mean age 69.1 ± 5.2 SD) had BMD measurements of total hip (TH) and lumbar spine (LS) (L1-4, Hologic); and of the middle phalanx of the middle digit of the non-dominant hand (accuDEXA) at baseline and after six and twelve months (m) of therapy with alendronate. At the same timepoints, speed of sound (SOS) through bone was measured at four sites (distal 1/3 radius [RAD], proximal phalanx of the third finger [PLX], mid shaft of the tibia [TIB] and fifth metatarsal [MTR], using the Sunlight Omnisense Ultrasound Bone Sonometer (Sunlight Medical Ltd., Rehovot, Israel). Data from both patient groups were pooled for this analysis. Values of TH and LS DEXA, accuDEXA and SOS at 0, 6 and 12 m were compared using Student's t test and significance accepted at P<0.05. Mean TH BMD at baseline was 0.713 g/cm2 ± 0.077 (SD) and increased by 1.7% ± 2.3% and 2.4% ± 2.2% at 6m and 12m respectively (P both <0.0001). Mean LS BMD at baseline was 0.718 ± 0.073 g/cm2 and increased by 3.91 ± 3.56% and 6.12% ± 3.50 % at 6m and 12m respectively (both P<0.0001). There was no significant change from baseline in mean BMD by accuDEXA at either 6m or 12m. Correlation coefficients between accuDEXA and LS were 0.17 (baseline), 0.23 (6m) and 0.15 (12m) (all P=NS). Correlation coefficients between accuDEXA and TH were 0.09 (baseline), 0.03 (6m) and 0.02 (12m) (all P=NS). There was no significant change from baseline in mean SOS at any site at either 6m or 12m. These data suggest that the response to alendronate therapy over this time period cannot be measured by accuDEXA or Sunlight SOS at the sites studied.

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Effects of Long-Term Treatment with Minodronate on Bone Loss and Mechanical Strength in Ovariectomized Rats. H. Mori,* M. Tanaka,* Y. Ochi,* N. Kawada,* H. Yamada,* K. Kawabata,* T. Obata.* Ono Pharmaceutical Co., Ltd., Osaka, Japan.

Present study examined the effects of 12-month treatment with an aminobisphosphonate, minodronate (YM529/ONO-5920) on bone loss and mechanical strength in ovariectomized (OVX) rats. Seventy-five Fischer F344 rats, 14 weeks of age, were assigned into five groups (one sham group and four OVX groups) each consiting of 15 animals. In OVX groups, rats were orally administered either a vehicle or three doses of minodronate (6, 30 or 150 μg/kg) once a day for 12 months beginning the day after ovariectomy. Bone mineral density (BMD) of lumbar vertebrae was measured by dual x-ray absorptiometry every three months under anesthesia. Twelve months after ovariectomy, rats were sacrificed and their BMD (lumber vertebrae, femur and tibia) and mechanical strength (lumber vertebral body, femoral shaft and femoral neck) were measured. In the vehicle treated group, lumbar vertebrae BMD at 3, 6, 9 and 12 month post-ovariectomy was significantly reduced (9.2, 11.5, 14.8 and 19.7 %, respectively) as compared with the sham group. Minodronate treatment significantly ameliorated the reduction in lumbar vertebrae BMD at all the time points in a dose dependent manner. Particularly, at 12 months post-ovariectomy, the increase in lumber vertebrae BMD was 15.1, 23.3 and 30.8 %, at 6, 30 and 150 μg/kg, respectively as compared with the vehicle group. Moreover, the treatment dose-dependently increased BMD of femur and tibia with a potency order of tibia < femur < lumber vertebrae. In mechanical strength studies, minodronate significantly increased both the ultimate compressive load of the fifth lumbar vertebral body and the ultimate bending load of femoral shaft, although its effects on the ultimate bending load of the femoral neck were not clear. Biochemical markers and bone histomorhometry will also be discussed. We conclude that oral minodronate treatment prevents loss of BMD and mechanical strength in OVX rats.

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Comparing the Efficiency of Bisphosphonates in the Treatment of Osteoporosis Using a New Graphic Analysis of Numbers Needed to Treat. P. Dagenais,*[1] T. Niyonsenga.*[2] [1]Medicine, University of Montreal, Montreal, PQ, Canada, [2]Faculty of Medicine, University of Sherbrooke, Sherbrooke, PQ, Canada.

Comparing the efficiency of bisphosphonates, alendronate and risedronate, in the treatment of post-menopausal osteoporosis (OP) is difficult as these drugs have been studied in patient groups with different absolute risk levels for fractures. Comparison of studies with respect to number needed to treat (NNT) is also affected by absolute risk levels. Using the available data from different vertebral OP fracture trials (FIT I, FIT II, FOSIT, VERT MN, VERT NA), we evaluated the relationship between the percentage of vertebral fracture in the placebo groups and the corresponding NNTs measured. The correlation (Spearman's coefficiant) between the risk of fracture and NNTs was very high for both bisphosphonates (alendronate: r = -0.84, p = 0.002; risedronate: r = -1.0, p <= 0.001). Different models (linear, quadratic, inversed and exponential)were fitted to the data and, observed and predicted curves were compared. Inverse model gave the best fit for both bisphosphonates. We created a linear graphic representation of the relationship between NNTs and risk levels of fracture in patients treated with each bisphosphonate. This graphic allowed us to compare and extrapolate NNTs for different levels of fracture risk. At a 3-year cumulative fracture risk level of 19%, both medications had the same NNTs. When the fracture risk was higher than 19%, risedronate had better NNTs than what was extrapolated for alendronate. On the other hand, alendronate seems more useful in patient with lower risk of fracture (< 19%) than projected for risedronate. Although these results may reflect a true clinical difference in the action of these bisphosphonates, further data on efficacy of those drugs and action mechanisms in different stages of OP is needed before any firm conclusion can be reached.

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Alendronate in the Treatment of Secondary Osteoporosis in Childhood. M. Bayer,*[1] J. J. Stepan,[2] S. Kutilek.[1] [1]Dept. of Pediatrics, Charles University, Prague, Czech Republic, [2]Internal Medicine, Charles University, Prague, Czech Republic.

Secondary osteoporosis due to osteogenesis imperfecta or administration of glucocorticoids is encountered in children. Various therapeutic procedures, including calcitonin or vitamin D application were of little benefit in those patients. Only recently, satisfactory therapy with the use of bisphosphonates has been proposed and occassionaly performed in children with osteoporosis.We evaluated the efficacy of orally applied alendronate (10 mg daily) administered for one year (mean 12.4 months +/- 0.9 months, range 11-13 months) in 5 children. Out of those patients, four children had osteogenesis imperfecta - OI (OI type Ia - n=2; OI type Ib - n = 1; and OI type IV - n = 1) and one child had steroid-induced osteoporosis due to the prednisone treatment of relapsing nephrotic syndrome. The patients´ mean age at the onset of alendronate therapy was 11.6 years +/- 1.1 year (SD), range 10-13 years. The Z-score of L1-L4 DXA at the onset of therapy was less than 3 in all enrolled patients, which was significantly lower when compared to the reference values (p < 0.0003). After one year of alendronate therapy we have noticed significant increase in spinal bone density (p < 0.04), yet these values were still significantly lower when compared to the reference ones (p < 0.005). Alendronate was well tolerated, there were no signs of gastrointestinal discomfort in our patients. Further application of alendronate and prospective follow-up of these children is necessary.

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Respiratory Distress Due to Pamidronate in a Case with Osteogenesis Imperfecta Type II. I. Fujiwara, E. Ogawa,* H. Chiba,* T. Sakai,* K. Iinuma.* Pediatrics, Tohoku University School of Medicine, Sendai, Japan.

Bisphosphonates have been suggested to be effective in patients with osteogenesis imperfecta (OI) because they increase bone mineral density and decrease occurrences of fracture. Flu-like symptoms are occasionally seen after first administration of nitrogen-containing bisphosphonates such as pamidronate, while no severe adverse events have been reported. Here we report a patient with OI type II who manifested respiratory distress after pamidronate treatment and needed mechanical ventilation. The patient was born by caesarian section because OI was suspected prenatally after 38 weeks of pregnancy. She weighed 1948g and had blue sclerae, and X-ray showed multiple fractures with deformed extremities and bell-shaped thorax, thus diagnosed OI type II. She showed tachypnea and retraction, but could be treated with oxygen therapy only. Pamidronate was given intravenously at 44, 75, 110 days of age, and 3 - 4 days after pamidronate, respiratory distress was observed each time and she needed nasal directional positive airway pressure or mechanical ventilation. For the first two episodes, fever and high CRP were preceded to the dyspnea, so pulmonary infection could not be ruled out. On the other hand, at the third time, fever and positive CRP were not seen until five days after pamidronate. We suspected that pamidronate could be the cause of the three episodes of dyspnea though direct evidences were not obtained. The authors recommend that treatment with bisphosphonates for OI patients should be carefully considered when they have primary respiratory problems.

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Bisphosphonate Dosing Preferences of Patients with Osteopenia/ Osteoporosis. A. Berarducci.* Nursing, ARNP, University of South Florida, Sarasota, FL, USA.

The purpose of this descriptive study was to determine bisphosphonate dosing preferences of patients with osteopenia and osteoporosis confirmed by dual-energy x-ray absorptiometry. The sample included (N = 73) 70 females and three males with central bone mineral density (BMD) confirmed osteoporosis or osteopenia with significant fracture risk. Age range of the sample was 44 to 89 years. Baseline lumbar spine BMD T-scores (compared to young adult mean) ranged from + 0.2 to –4.1. Baseline hip BMD T-scores ranged from – 0.2 to – 3.2. The sample included 21 patients currently being treated with alendronate and 52 newly diagnosed patients. Newly diagnosed patients were informed of indications of bisphosphonates, dosing regimes, and potential side effects per manufacturer prescribing information. Of the newly diagnosed patients (n = 52), 41 opted to be treated with risedronate based on flex dosing options, previous history of peptic ulcer disease or gastrointestinal reflux disorder, and side effect profile. Eleven of the newly diagnosed chose alendronate due to length of time in the market and having heard of it. Thirteen (13) patients currently being treated with alendronate opted to change to 70 mg. once-weekly dosing (OAW). Eight (8) others were tolerating alendronate daily and opted to stay on the daily regiment. The most commonly cited reason for choosing daily dosing was fear of forgetting to take medicine. Of those who chose once-weekly dosing, the most commonly reported reason was “not liking to take pills” and “upset stomach with any medicines”. The overwhelming majority of patients (82%, n = 60) preferred daily. These findings indicate that OAW bisphosphonate dosing may not be the choice of many patients if the patient is fully informed of the options and drug profiles.

Disclosures: Merck & Co., Inc.,2; Procter & Gamble Pharmaceuticals,2,8.

M400

Evolution of Bone Mineral Density and Bone Turnover in Osteoporotic Women Shifting from Pamidronate to Alendronate. A. M. E. Peretz,[1] V. Siderova,*[1] J. Body,[2] J. Dumon,*[2] C. Fellemans,*[3] M. Fuss,*[4] S. Rozenberg,[3]

P. Bergmann.[5] [1]Rheumatology, CHU Brugmann, Brussels, Belgium, [2]Laboratoire d'étude du métabolisme osseux, Institut Bordet, Brussels, Belgium, [3]Gynecology, CHU Saint Pierre, Brussels, Belgium, [4]Endocrinology, CHU Brugmann, Brussels, Belgium, [5]Nuclear Medicine, CHU Brugmann, Brussels, Belgium.

Different bisphosphonates have been shown to increase bone mineral density (BMD) and reduce the risk of fracture in osteoporotic patients. It is unclear how shifting from a treatment with one bisphosphonate to another will influence the evolution of BMD and bone turnover. In the present study, we followed BMD (DXA, Hologic QDR1000), bone alkaline phosphatase (BAP, Ostase, Hybritech), and urinary collagen cross links (Pyr, D Pyr, HPLC, Biorad) in 36 patients treated with IV pamidronate (60 mg/3 months) since at least 2 years and who were shifted to oral alendronate (10 mg/day, n= 15) or left on IV pamidronate (n=21) for 2 years. The results (% of baseline) are summarized in the table:

Parameters	**1 year**		**2 years**	
	Alendronate	**Pamidronate**	**Alendronate**	**Pamidronate**
LS BMD	102.1	101.2	103.8*	104.1
T Hip BMD	99.8	103.2*	104.3*	103.6*
BAP	96	109	113	107
Pyr	100	94	91.6	101.5
D Pyr	72.7	90	72.7	120

LS: lumbar spine, Thip: total hip, * p<0.05 (sign test). BMD increased significantly and similarly in both groups. There was no significant change in the biological parameters of bone turnover in both groups. The increase of BMD in osteoporotic patients with bisphosphonates was that expected after 2 years of treatment and did not differ according to the molecule and treatment scheme used. Bone turnover was similarly unaffected by the change in bisphosphonate treatment

M401

Two Year Results of Once-Weekly Administration of Alendronate 70 mg for the Treatment of Postmenopausal Osteoporosis. R. Rizzoli,[1] C. Roux,[2] S. Greenspan,[3] H. Bone,[4] T. Schnitzer,*[5] N. Watts,[6] S. Adami,[7] B. Uebelhart,[1] C. Peverly,*[8] A. Kaur,*[8] M. Wu,*[8] J. Orloff,[8] A. Santora.[8] [1]Hopital Cantonal, Geneva, Switzerland, [2]Hopital Cochin, Paris, France, [3]University of Pittsburgh, Pittsburgh, PA, USA, [4]Michigan Bone Clinic, Detroit, MI, USA, [5]Northwestern U., Chicago, IL, USA, [6]Emory University, Atlanta, GA, USA, [7]University of Verona, Verona, Italy, [8]Merck Research Labs, Rahway, NJ, USA.

The therapeutic equivalence of alendronate (ALN) 70 mg once weekly (OW) (provided by Merck and Co., Inc., Whitehouse Station, NJ, USA), ALN 35 mg twice weekly (TW), and ALN 10 mg once daily (OD) in the treatment of postmenopausal osteoporosis for one year has been reported previously (Schnitzer et al, Aging 12:1-12, 2000). We now present two-year BMD results from a one-year extension. We compared the efficacy of treatment with OW ALN 70 mg (n=519), TW ALN 35 mg (n=369), and OD ALN 10 mg (n=370) over 2 years in a double-blind, multicenter study of postmenopausal women (age 40 to 90) with osteoporosis (BMD of either the lumbar spine or femoral neck >= 2.5 SDs below peak mean, or prior vertebral or hip fracture). The primary efficacy endpoint was change in lumbar spine BMD. Secondary endpoints included changes in BMD at the hip and total body.

	ALN 10 mg OD	**ALN 35 mg TW**	**ALN 70 mg OW**
SPINE	7.4 (6.9, 7.8)	7.0 (6.6, 7.5)	6.8 (6.4, 7.3)
TOTAL HIP	4.3 (3.9, 4.7)	4.3 (3.9, 4.7)	4.1 (3.8, 4.5)
FEMORAL NECK	3.5 (3.1, 4.0)	3.5 (3.1, 4.0)	3.3 (2.9, 3.7)
HIP TROCHANTER	6.1 (5.6, 6.7)	6.2 (5.6, 6.8)	5.9 (5.4, 6.4)
TOTAL BODY	1.6 (1.2, 1.9)	1.6 (1.3, 2.0)	1.8 (1.5, 2.1)

Mean (95% CI) BMD increases at the lumbar spine, total hip, femoral neck, hip trochanter, and total body sites for each treatment regimen after two years were very similar (see table). These data confirm that once-weekly ALN 70 mg is therapeutically equivalent to once-daily ALN 10 mg in patients with postmenopausal osteoporosis. In addition, the two-year results demonstrate that once-weekly ALN 70 mg is generally safe and well tolerated as ALN 10 mg once daily

Disclosures: Merck & Co., Inc.,2.

M402

Efficacy, Safety and Tolerability of Once Weekly (80 mg vs. 160 mg) Oral Alendronate in Postmenopausal Osteoporosis. J. P. Brown,[1] C. Banville,*[1] S. Jean,*[1] W. M. Drake,*[2] D. L. Kendler.[2] [1]CHUL Research Center, Ste-Foy, PQ, Canada, [2]University of British Columbia, Vancouver, BC, Canada.

Over the last ten years oral bisphosphonates progressively became the gold standard for treatment of postmenopausal osteoporosis. Recently, 70 mg of oral alendronate weekly was shown to have a similar effect on bone mineral density com-pared with 10 mg daily. In the present study we explore the efficacy, safety and tolerability of higher once weekly doses of oral alendronate in postmenopausal women with osteoporosis.In this 2-yr randomized parallel group study, 81 postmeno-pausal women (mean age 69.1 ± 5.2 SD) with lumbar spine T-score -3 or <-3). The investigators were blinded to treatment groups. All women received calcium carbonate 500 mg twice daily. BMD of the lumbar spine (LS), femoral neck (FN) and total hip (TH) were measured by DXA (Hologic) at baseline, 6, 12, 18 and 24 months. We are reporting the results from the first year of treatment. Adverse events were collected at each visit. BMD results at 6 and 12 months are shown in the table below as mean % changes (± SEM) from baseline.

Site	**Weekly Dose**	**6 months**	**12 months**	**p-value***
LS	80 mg	+ 3.5% (0.6)	+ 5.5% (0.5)	p = 0.025
	160 mg	+ 4.0% (0.5)	+ 6.7% (0.6)	p = 0.019
FN	80 mg	- 4.0% (1.1)	+ 0.6% (1.1)	NS
	160 mg	+ 1.5% (0.5)	+ 3.2% (0.5)	NS
TH	80 mg	+ 1.3% (0.4)	+ 2.2% (0.3)	NS
	160 mg	+ 2.1% (0.4)	+ 2.7% (0.4)	NS

*p-value compared to baseline; NS = not statistically significant.Using an analysis of repeated measurements (SUDAAN software; Research Triangle Park, Durham, NC), we tested the statistical significance of treatment effect (80 mg vs. 160 mg) over time (one year) for each site and each center. The Satterwaite p values were 0.214 for the LS, 0.035 for the FN and 0.001 for TH. There was no significant effect of time for the FN and TH. The effect of center on TH was significant. The following adverse events possibly related to study drug, were reported (80 mg/160 mg) at least once over one year: nausea (16.2%/30%), diarrhea (16.2%/25%), arthralgia (13.5%/25%), abdominal pain (8.1%/20%), vomiting (10.8%/15%), dyspepsia (10.8%/17.5%), and constipation (10.8%/7.5%). All were of mild severity without significant impact on daily activities and resulted in discontinuation only in one subject.We conclude that oral alendronate 80 and 160 mg once weekly, are safe and well tolerated, and increase BMD significantly and similarly over one year at the lumbar spine

Disclosures: Merck Frosst Canada Inc.,2,8.

M403

Concurrent Therapy of Osteoporosis Using Etidronate and Value Change of Bone Metabolite Markers. I. Nakajima,*[1] H. Kohno,*[2] Y. Hamada.*[2] [1]Orthpedics, Yamanashi Medical University, Yamanasi, Japan, [2]Orthopedics, Yamanashi Medical University, Yamanashi, Japan.

Several pharmaceutical agents exist that have more or less proven positive effects on osteoporosis. Among these drugs, alphacalcidol (D3) and menatetrenon (K2) are commonly used in Japan. However , the windows of efficacy for these two drugs are quite narrow. Therefore we investigate the efficacy of concurrent treatments using etidronate in increasing bone mineral density (BMD) and preventing development of fragility fracture. And there has been little available data regarding bone metabolite markar (especially, urinary N-telopeptide of type I collagen (NTX), urinary deoxypyridinoline (D-Pyr)) associate with concurrent therapy using etidronate. To evaluate the metabolite marker changes and the effects on the BMD of concurrent treatment, following four groups were assigned to 400 female patients : A) established osteoporosis who is no history of treatment start D3 (1microgram/day) continuing 6 months and three months after starting , etidronate (200mg/day for 2 weeks) was given, B) also no history of treatment start K2 (45mg/day) continuing 6 months and three months after starting, etidronate (200mg/day for 2 weeks) was given, C) alredy continuing D3 with etidronate (200mg/day for 2 weeks followed by 10 weeks intermission) was given two cycles, D) alredy continuing K2 with etidronate (200mg/day for 2 weeks followed by 10 weeks intermission) was given two cycles. Thoracic and lumber spine radiographs and biochemical bone markers were taken at the time of beginning and every three months interval. After using etidronate, lumber BMD is getting higher ranging 1-4 percents. But after administration of etidronate, NTX and D-Pyr significantlly decreased through three months demonstrated highly gain in BMD. And there were no significant differences the suppression of bone absorption between two agents comparing NTX and D-Pyr. There were no significant differences in the rate of development of new vertebral fractures among four groups, also.

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Two-Year Gastrointestinal Safety and Tolerability of Alendronate 70mg Once Weekly. R. J. Genco,*[1] T. Van Dyke,*[2] A. Leung,*[3] L. Meng,*[3] S. Ritter,*[3] S. Grossman,*[3] G. Salzmann,*[3] A. Lombardi.*[3] [1]Department of Oral Biology, State University of New York, Buffalo, NY, USA, [2]Boston University, Boston, MA, USA, [3]Merck Research Laboratories, Rahway, NJ, USA.

Alendronate (ALN) 70mg once weekly (OW) has been shown to be therapeutically equivalent to ALN 10mg daily (D) in the treatment of osteoporosis (Schnitzer, et al. Aging 2000;12:1-12). We reviewed the safety and tolerability of oral ALN 70mg OW (manufactured by Merck & Co., Inc.) in a two-year placebo-controlled, randomized, double-blind trial in women and men with periodontal disease (average age 50 years). Overall and upper gastrointestinal (GI) adverse experience (AE) results are summarized below. In this two-year study, ALN 70mg OW was generally well tolerated with an overall AE profile that was comparable to placebo (PBO). The incidence of upper GI AEs was similar between treatment groups. In addition, the incidence of specific categories of upper GI events, including esophageal and gastroduodenal irritation, was comparable between groups. These long-term clinical data are consistent with the observations made during a 10-week double-blind endoscopy study in which 70mg ALN OW had effects on the esophageal,

gastric, and duodenal mucosae similar to those of PBO.

	PBO OW (N=168)	70mg OW (N=167)
Number (%) of patients:		
with one or more AE	151 (89.9)	147 (88.0)
with one or more upper GI AE	29 (17.3)	26 (15.6)
withdrawn due to an upper GI AE	2 (1.2)	1 (0.6)
with one or more serious upper GI AE	1 (0.6)	1 (0.6)
with esophageal irritation	1 (0.6)	2 (1.2)
with gastric or duodenal irritation	1 (0.6)	2 (1.2)
with gastric or duodenal PUBs	1 (0.6)	0

PUB: perforation, ulcer, or bleeding

Disclosures: Merck & Co., Inc.,2.

M405

Alendronate Consistently Reduced the Risk of Vertebral Fracture in Different Populations Under Age 65. S. Quandt,[1] M. Hochberg,*[2] D. Thompson,[3] U. Liberman,*[4] A. Santora.[3] [1]Wake Forest University, Winston-Salem, NC, USA, [2]University of Maryland, Baltimore, MD, USA, [3]Merck Research Labs, Rahway, NJ, USA, [4]University of Tel-Aviv, Tel-Aviv, Israel.

Patients with vertebral fractures are at an increased risk for future fracture, hospitalization, and mortality. Thus it is important to minimize the risk of these fractures at any age. We asked the question - Is there evidence of the consistency of the effect of alendronate in reducing the risk of vertebral fractures across different populations under age 65? To determine this, in all alendronate studies we pre-defined subgroups on the basis of age at baseline: < 65 vs 65 or older. We pooled data from the three studies in which morphometric vertebral fractures were collected: FIT I which enrolled women (T < -1.6) with vertebral fractures, FIT II which enrolled women (T < -1.6) without existing vertebral fracture and the Phase III studies (T < -2.0) (Liberman). We performed a meta-analysis to examine the consistency across different populations.

	N	# with fractures	N	# with fractures
FIT I	154	14	162	6
FIT II	692	13	709	2
Phase III	190	9	132	6
Total	1036	36	1003	14

The meta-analysis showed an overall risk reduction of 57% (RR = 0.43, 95% CI 0.25 to 0.74) p = 0.002. This was comparable to the effect seen in women over 65: an overall risk reduction of 44% (RR = 0.56, 95% CI 0.45 to 0.69) p = 0.001. We conclude that women under age 65 regardless of vertebral fracture status can benefit from alendronate therapy.

M406

Compliance With Alendronate Treatment in an Osteoporosis Clinic. C. Lombas,* C. Hakim,* J. R. Zanchetta. IDIM and USAL University School of Medicine, Buenos Aires, Argentina.

In asymptomatic chronic conditions, noncompliance is clearly one of the most significant problems facing medical practice today. We present here data on compliance, collected among patients under alendronate treatment in our osteoporosis clinic. Alendronate treatment 10 mg QD was prescribed to 401 postmenopausal women mean age: 60.5 years (R: 35-85); mean years since menopause: 15.5 (R: 1-49). BMD was measured at baseline at lumbar spine and/or femoral neck. According to WHO criteria, osteopenia (T-score -2.5) was found in 27% and osteoporosis (T-score < -2.5) in 73%. Fifty two patients (13%) never started treatment: 25 (48%) due to lack of acceptance of treatment, 6 (11.5%) due to other physician's advice, 5 (9.6%) due to fear after reading the insert, 5 (9.6%) because of complicated way of taking the drug, 3 (5.7%) due to other health problems, 3 (5.7%) due to cost of therapy, 2 (3.8%) due to dissatisfaction with the assisting physician , 1 (2%) because of belief that treatment is not necessary , 1 (2%) due to relatives' advice , 1 (2%) refusal to take many drugs. Among 349 patients who started treatment, 182 (45.4%) are still under treatment.One hundred sixty seven patients (41.6%) discontinued treatment. Causes for dropout were: gastrointestinal (GI) disorder that appeared in 45 patients (12.9%) , physician's advice in 41 (11.7%), complicated way of taking the drug in 15 (4.3%), cost of therapy in 13 (3.7%), lack of acceptance of treatment in 13 (3.7%), no clear reason in 13 (3.7%), other health problems in 6 (1.7%) , belief treatment is not necessary in 5 (1.4%) , refusal to being treated with many medications in 5 (1.4%), own decision due to family problems in 4 (1.1%); fear after reading insert in 3 (0.85%), belief the drug is not effective in 2 (0.6%), skin rash in 1 (0.28%), dissatisfaction with phyisician in 1 (0.28%).At 3 months the probability of continuing was 67% , at 6 months 58%, at 12 months 49%, and at 24 months it was 30% . The following variables did not affect risk of discontinuation: age, number of concomitant medication, T-score of lumbar spine and femoral neck and presence of vertebral or non-vertebral fractures.Despite full information on treatment characteristics was provided to patients by the assisting physician, a high proportion of patients (13%) never started treatment. As it is expected in chronic asymptomatic diseases, compliance rate is low. However, being aware of the reasons for discontinuation allows physicians to look carefully into them in order to improve patients' compliance.

Disclosures: Merck,2,8.

M407

Skeletal Benefits Are Maintained Through 7 Years of Alendronate Treatment. R. Weinstein,[1] P. J. Meunier,[2] P. Geusens,[3] H. Bone,[4] R. P. Tonino,[5] P. D. Ross,[6] A. Santora.[6] [1]Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Hopital Edouard Herriot, Lyon, France, [3]Limburg University, Diepenbeek, Belgium, [4]Michigan Bone and Mineral Clinic, Detroit, MI, USA, [5]Fletcher Allen Health Care, Burlington, VT, USA, [6]Merck & Co., Inc., Rahway, NJ, USA.

The results of alendronate (ALN) treatment for osteoporosis have been reported for yrs 1-3 of the Phase III trials [1], and yrs 6-7 of the extension [2]. Biochemical markers of bone turnover decreased from relatively high baseline levels to well within the premenopausal normal range, and spine BMD continued to increase through yr 7. Comparison of active treatment with placebo during yrs 1-3 demonstrated a substantial reduction of fracture rates. Was this fracture risk reduction maintained? The published vertebral fracture (VFx) rates for the two periods are not directly comparable, because the methods of ascertainment were different: VFx were a secondary efficacy endpoint during yrs 1-3 and were diagnosed by standardized morphometric assessment [1]. Only VFx meeting rigorous prespecified morphometric criteria by centralized analysis were counted. In contrast, during yrs 6-7 of the extension, new VFx (including asymptomatic deformities) were identified on spinal radiographs by the local investigators and reported as adverse experiences (AEs); neither central confirmation nor morphometric analysis was required [2]. Therefore, we re-examined the incidence of VFx among the 235 women (mean age at baseline, 63 yr) who completed continuous ALN treatment (5 or 10 mg daily; source: Merck & Co., Inc.) for 7 yrs in extended Phase III trials. In order to employ consistent methodology, we retrieved data on VFx reported as AEs for this group during yrs 1-3. The incidence of new VFx AEs in yrs 6-7 (3.3%/yr) was no greater than that in yrs 1-3 (3.8%/yr). The mean rate of height loss for yrs 6-7 (1.2 mm/yr) was similar to that for yrs 1-3 (1.0 mm/yr). The incidence of non-vertebral fractures (3.4 cases per 100 patient-yrs, PY) during yrs 6-7 was very similar to that for yrs 1-3 (3.1/100 PY) for the patients who participated in the extension. This difference was no more than might be expected due to the age-related increase in fracture risk independent of osteoporosis. Neither stress fractures nor fracture malunion emerged as adverse experiences in these studies through yrs 6-7. We conclude that not only were increased BMD and stable reductions in turnover to within the premenopausal range maintained during ALN treatment for 7 yrs, but also that the data available indicate that fracture risk reduction was maintained. 1. Liberman UA, et al. N Engl J Med 333:1437-43 1995.2. Tonino RP, et al. J Clin Endocrinol Metab 85:3109-15, 2000.

M408

Etidronate and Vitamin D versus Etidronate Alone for the Treatment of Senile Osteoporosis in Japanese Women 65 Years of Age and Over. J. Takada,[1] M. Kawamura,*[2] M. Yoshimoto,*[2] K. Imoto,*[2] K. Ishii,*[2] T. Matsuyama,[1] K. Iba,[3] S. Ishii.*[1] [1]Dept. Orthop. Surg., Sapporo Medical University, Sapporo, Japan, [2]Dept. Orthop. Surg., Takikawa Municipal Hospital, Takikawa, Japan, [3]Dept. Orthop. Surg., Kushiro Red Cross Hospital, Kushiro, Japan.

The purpose of this study is to investigate the effect of intermittent, cyclic etidronate and vitamin D versus etidronate alone on the bone mineral density (BMD) of senile osteoporosis in Japanese women 65 years of age and over. The subjects with low BMD (below 80 % of young mean adult) were randomized to two groups. Group I (+ VD) was treated with etidronate 200 mg/day for 14 days followed by suspension for 70 - 84 days, plus 0.75 - 1.0 ug of 1-a-(OH)D3 and 500 mg of calcium for every day. Group II (no VD) was treated with the same procedure that of Group I except for 1-a-(OH)D3. These two groups were not different in age, body mass index, period of menopause, BMD at baseline, and urinary N-telopeptide : creatinine ratio. BMD at lumbar spine was measured by DXA. In group I(+ VD) and group II(no VD), the mean BMD increased by 2.82 %, 2.18 % after 6 months (no significant) and 4.89%, 3.12 % after 12 months from baseline ($p<0.05$), respectively. These changes of BMD after 12 months in two groups was significant. We conclude that the increase of BMD at lumbar spine in etidronate and vitamin D therapy for 12 months is superior to that of etidronate alone in Japanese women 65 years of age and over.

M409

Effect of Clodronate-Raloxifene Combined Therapy in Bone Mass in Women with Post-menopausal Osteoporosis. M. Muratore,*[1] G. Isaia,*[2] L. Cosentino,*[1] F. Calcagnile,*[1] E. Quarta,*[1] G. Santacesaria.*[1] [1]Rheumatology, Galateo Hospital, S. Cesario di Lecce LE, Italy, [2]Internal Medicine, S. Giovanni Battista Hospital, Torino, Italy.

Several clinical trials have shown the ability of some molecules to increase bone mass density (BMD) and reduce bone turnover markers and relative fracture risks. Various are the mechanisms of action of these molecules and different the receptor sites on which they act.The lumbar and femoral BMD increase brought about by Raloxifene (RLX) in women with postmenopausal osteoporosis, shown by the MORE study, induced the Authors to test the drug in combination with another molecule having different antiresorption action on bone, and determine whether the combined treatment would lead to a BMD increase greater than that obtained with Raloxifene alone. Clodronate (CLD) was chosen on account of the possibility of cyclic administration by intramuscular route.We consecutively selected 38 women aged 56 to 68 years (mean 62 years) in menopause since not less than 5 years (8 +/- 3 years) and with BMD less than -2.5 DS, with no other pathology.The patients were divided into two treatment groups: RLX 60 mg/die (20) and RLX 60 mg/die + CLD 100 mg every 10 days (18); all the patients also received 1 g of Ca supplemented with 800 IU of Vit D3.Lumbar and femoral BMD was evaluated at T0 and T12 using a DEXA Norland XR36 instrument, and the bone turnover markets, N and C-telopeptide (NTx and CTx) normalized towards creatinine, alkaline phosphatase and osteocalcine at T0, T6 and T12.At T12 compared to T0, the BMD had increased to a significant extent at the two skeletal sites examined, in both groups. The BMD increase in the RLX+CLD group was significantly greater than that in the control group under RLX alone treatment; in both groups a bone turnover reduction was observed (see table).Treatment with Raloxifene+Clodronate was more effective in increasing BMD than Raloxifene alone with no negative influence on compliance and side effect incidence.

M410

See Friday Plenary number F363.

M411

Prevention of Bone Loss by Combined Treatment with Risedronate and 1α,25-Dihydroxyvitamin D3 in Ovariectomized Rats. R. G. Erben,[1] L. Mosekilde,[2] J. S. Thomsen,*[2] K. Weber,*[1] K. Stahr,*[1] S. Y. Smith,[3] R. J. Phipps.[4] [1]Ludwig Maximilians University, Munich, Germany, [2]University of Arhus, Arhus, Denmark, [3]ClinTrials BioResearch, Senneville, Canada, [4]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Bisphosphonates inhibit bone loss through inhibition of osteoclast-mediated bone resorption. At low doses, vitamin D metabolites can prevent bone loss by an antiresorptive effect, while at high doses they also stimulate osteoblast activity and show an anabolic effect. Therefore, combined therapy with bisphosphonates and vitamin D analogs might be expected to be more effective than either treatment alone. The purpose of this study was to compare the efficacy of risedronate and of the naturally occurring vitamin D hormone 1α,25-dihydroxyvitamin D_3 (calcitriol) alone and in combination, for the prevention of ovariectomy-induced bone loss in rats. Female 4-month-old Sprague-Dawley rats were used for this study. Rats were bilaterally ovariectomized (OVX), and groups (nine groups, 10/group) received vehicle, risedronate (0.1 or 0.5 mg/kg/day), or calcitriol (0.05 or 0.1 µg/kg/day), alone and in all combinations. Both compounds were administered orally via gavage, starting on the day after surgery. Animals were food-fasted for at least 4 h pre- and 2 h post-dosing with risedronate or risedronate vehicle. In addition, one group of rats was sham operated and another served as baseline controls. Ovariectomy induced expected bone loss in vertebra and long bones, including decreased bone mineral density and loss of cancellous bone. This estrogen deficiency-induced bone loss was prevented dose-dependently by both risedronate and calcitriol given alone. Combination treatment with risedronate and calcitriol however increased bone mineral density, cancellous bone area, and bone strength in long bones and vertebrae more than did risedronate alone. Calcitriol enhanced the suppressive effect of risedronate on osteoclast number, and partially counteracted the suppressive effect of risedronate on bone formation and on the histomorphometric indices of osteoblast team performance. Importantly, risedronate did not reduce the anabolic effect of calcitriol, and at the high dose it normalized the hypercalcemia in calcitriol-treated OVX rats. Therefore, this study in OVX rats suggests that combined therapy with risedronate and vitamin D analogs may offer advantages over the treatment with bisphosphonates or vitamin D analogs alone.

M412

Effects of LY333334 [Recombinant Human Parathyroid Hormone (1-34)] and Alendronate Sodium on Markers of Bone Metabolism in Postmenopausal Women with Osteoporosis. J. J. Body,[1] G. A. Gaich,[2] W. H. Scheele,*[2] P. D. Miller,[3] P. M. Kulkarni,*[2] A. B. Hodsman.[4] [1]Inst. J. Bordet, Univ. Libre de Bruxelles, Brussels, Belgium, [2]Eli Lilly and Company, Indianapolis, IN, USA, [3]Colorado Center for Bone Research, Lakewood, CO, USA, [4]St. Joseph's Health Care, London, ON, Canada.

Currently available treatments for osteoporosis, such as bisphosphonates, reduce bone resorption, leading to moderate increases in bone mineral density (BMD). Parathyroid hormone, given once daily, stimulates new bone formation and increases bone mass substantially. Markers of bone turnover were measured in a randomized, double-blind, placebo-controlled clinical trial comparing the effects of 40 µg of recombinant human parathyroid hormone (1-34) [rhPTH(1-34)] (PTH40, n=73) and 10 mg of alendronate sodium (ALN10, n=73) in postmenopausal women with osteoporosis. The median treatment period was 14 months. Markers of bone formation (serum bone specific alkaline phosphatase (BAP), serum procollagen I C-terminal propeptide (PICP)) and resorption (urinary cross-linked N-telopeptides (NTx) and urinary free deoxypyridinoline cross-links (fDPD) were measured at baseline, 1, 3, 6, and 12 months. The results are expressed as median percent change from baseline. The differences between groups were significant at each study visit for each marker (P<0.001). ALN10 reduced the markers of bone turnover by up to 50%. In contrast, PTH40 rapidly stimulated bone formation, with maximum increase in PICP at 1 month. BAP more than doubled by 6 months and the markers of bone resorption also increased, but more slowly than those of bone formation. Bone mass increased significantly more in the PTH40 group, compared with ALN10, and the difference in spine BMD was statistically significant at 3 months (P<0.001). The bone forming agent, rhPTH(1-34), stimulated new bone formation and bone remodeling, resulting in greater and more rapid increases in BMD than the antiresorptive agent, alendronate. The distinctly different pattern of change in bone markers illustrates the different mechanism of action whereby rhPTH(1-34) can increase BMD more than alendronate.

Bone marker (median % change from baseline)	1 month	3 month	6 month	12 month
PICP-PTH40	50	45	25	-2
PICP-ALN10	-11	-38	-31	-37
BAP-PTH40	40	50	102	49
BAP-ALN10	-4	-41	-44	-51
NTx-PTH40	30	85	125	159
NTx-ALN10	-54	-59	-60	-41
f-DPD-PTH40	16	25	68	66
f-DPD-ALN10	-20	-23	-24	-15

Disclosures: Eli Lilly andCompany,5; Merck,5.

M413

Acute Effects of Salmon Calcitonin on Bone Resorption. V. Zikan,* J. J. Stepan. 3rd Dept. of Internal Medicine, Charles University Faculty of Medicine, Prague, Czech Republic.

The aim of the present study was to determine the time course and extent of suppression of osteoclastic bone resorption after an administration of different doses of salmon calcitonin (sCT). Effects of subcutaneous and nasal sCT on bone resorption were assessed by using plasma type 1 collagen cross–linked C–telopeptide (CTX). In addition, effects of subcutaneously administered sCT on biochemical markers of bone formation, aminoterminal propeptide of type I procollagen (PINP) and osteocalcin were evaluated. In six healthy young women, after overnight fasting, single subcutaneous doses of 2, 10 and 50 IU as well as a nasal dose of 200 IU of sCT were administered consecutively with a one-week washout period between each dose. The subjects were kept fasting for the next 9 hours. During the control period no drug was given. Blood samples were drawn at 0700 a.m. (baseline) and throughout the 9-hour period. The plasma CTX concentrations showed a dose dependent decrease over a dosage range of 2, 10 and 50 IU after subcutaneously administered sCT ($p < 0.05$). No significant difference was observed between the area under the curves for plasma CTX concentrations following intranasal and subcutaneous administration of 200 and 2 IU of sCT, respectively. The diurnal rhythm of plasma CTX was diminished during the fasting control period. Plasma PINP did not change significantly even after the administration of 50 IU sCT subcutaneously. In conclusion, both intranasally and subcutaneously administered sCT significantly decreased bone resorption in healthy young women as assessed by plasma CTX. Irrespective of dose and route of administration, sCT resulted in a significant reduction in plasma CTX concentrations by 50-60 % as early as in 1 hour in comparison with the baseline ($p < 0.05$). The increase in the sCT dose prolonged suppression of osteoclastic bone resorption. This effect, however, was modified by an increase in serum PTH in response to a decrease in serum calcium concentrations. Synthesis of types I collagen remained unaffected by the dose of 50 IU of subcutaneously administered sCT when the short-term effects were considered.

M414

Salmon Calcitonin Nasal Spray (SCNS) Is Effective and Safe in Older Osteoporotic Women - Results from the PROOF Study. S. L. Silverman,[1] C. Chesnut,[2] D. Baylink,[3] A. Gimona,*[4] K. Andriano,*[5] L. Mindeholm.*[4] [1]UCLA, Los Angeles, CA, USA, [2]University of Washington, Seattle, WA, USA, [3]Pettis Veterans Hospital, Loma Linda, CA, USA, [4]Novartis Pharma, Basle, Switzerland, [5]Novartis Pharma, East Hanover, NJ, USA.

Optimal pharmacologic treatment of postmenopausal (pm) OP in the elderly patient is increasingly contingent upon not only efficacy in terms of fracture reduction but also upon the therapy's safety and tolerability. We hypothesized that SCNS would be a particularly effective and safe therapy to prevent vertebral fractures (VFx) in women above 75 years of age and utilized a post hoc stratification of the PROOF data to test this hypothesis. The PROOF (Prevent Recurrence of Osteoporotic Fractures) study is a 5 year, double-blind, randomized, placebo-controlled study on prevention of VFx. In 1255 postmenopausal women with established OP (mean age 68, range 44-94) randomized to placebo or SCNS 100IU,200IU,400 IU/day plus 1g Calcium and 400 IU Vit D, a 33% reduction in the relative risk of new VFx using life table methods (p=0.03, 95%CI= 0.03 to 0.53) was found in patients taking 200 IU as compared to placebo. In women >75 years in PROOF (n=105) taking 200 IU (n=58) as compared to placebo (n=47), a 62% relative reduction in the number of new VFx using categorical data (p=0.03, 95%CI = 0.1 to 0.84) was found. The effect in the younger population <= 75 years (n=452) taking 200 IU (n=229) as compared to placebo (n=223) was similar to that observed in the overall population with a 31% relative reduction in the number of new Vfx using categorical data (p=.121, 95%CI= -0.1 to 0.56). In these elderly patients there was a 20.6% reduction (p=.046) in serum CTX in the 200 IU group as compared to placebo at the end of the study and a 1.55% increase relative to baseline at 5 years in lumbar spine BMD. No unexpected adverse events were observed in these elderly patients treated with 200 IU SCNS. The drug was very well tolerated. Rhinitis was the only adverse effect reported more frequently in the active group (37.5%) compared to placebo (17.3%, p=.02) and it was mostly mild. No headaches were reported in the SCNS 200 IU group compared to 5.8% in the placebo group (p=0.052). In summary SCNS is an effective and safe therapy for elderly patients with osteoporosis. In PROOF there was a significant 62% relative reduction in the number of new vertebral fractures in patients over age 75. SCNS was very well tolerated, an important consideration for the elderly, for whom tolerance to chronic osteoporosis medication may be of particular concern.

Disclosures: Novartis,2,3,5,8.

M415

The Effect of Fortical™ Nasal Spray and Miacalcin™ Nasal Spray on Serum and Urinary Bone Turnover Markers. J. Gilligan,[1] N. Mehta,*[1] A. Sturmer,*[1] S. Philip,*[1] A. Malootian,*[1] E. Leary,*[2] T. Aggoune,*[2] T. Carlson.*[2] [1]Unigene Labs, Fairfield, NJ, USA, [2]PBI, Seattle, WA, USA.

Serum and urine bone turnover markers (BTM) were measured in a 12-week, multicenter, double-blind, multiple-dose, parallel study comparing two nasal calcitonin preparations, Miacalcin Nasal Spray (Novartis) and Fortical Nasal Spray (Unigene). The study protocol included 134 postmenopausal osteoporotic women (T score at least -2.5 SD below the mean for premenopausal women) age 45 years or older with demonstrable accelerated rates of bone turnover. Enrolled patients were randomized to receive daily 200 IU doses of either formulation with all participants receiving calcium (1200 mg) and vitamin-D (400 IU) supplementation. Two serum markers of bone resorption were monitored, serum C-telopeptide (sCTX, Roche Diagnostics) and serum N-telopeptide (sNTx, Ostex International) and one urine marker deoxypyridinoline (Pyrilinks-D, Metra Biosystems/Quidel). Two bone formation markers were monitored, bone specific alkaline phosphatase (BSAP,

Metra Biosystems/Quidel), and osteocalcin (OC, Roche Diagnostics). Intact PTH was also measured (Roche Diagnostics). Serum samples were collected in the fasted state between 8 a.m. and 10 a.m., urine samples were second morning voids that were corrected for creatinine levels. To identify patients with accelerated rates of bone turnover two baseline samples were collected a week apart and sCTx, the primary clinical endpoint, measured in serum. Postmenopausal women with both baseline sCTx levels above 0.24 ng/ml were enrolled in the study. Serum and urine samples were collected at 4, 8 and 12 weeks following initiation of nasal calcitonin treatment. Bone turnover as measured by all markers was significantly decreased after nasal calcitonin treatment. At 12 weeks, analysis of 104 patients revealed s-CTx decreased by 43%, sNTx by 18%, urinary DPD by 13%, BSAP by 9%, and OC by 19%. In summary, the current study indicated that nasal calcitonin (Fortical and Miacalcin) caused significant suppression of bone turnover, as demonstrated by serum and urine BTM. The 40% decrease of s-CTx obtained within the first month of treatment demonstrates that nasally administered calcitonin when accompanied by calcium and Vitamin-D supplementation induces a rapid, significant, and persistent decrease in bone resorption.

Disclosures: Unigene Laboratories,1,3.

M416

The Importance of the Dietary Calcium/Phosphorus Ratio for Bone Mineralization and Bone Formation in Vitamin D Receptor Knock-out Mice. R. Masuyama,*[1] S. Tanaka,*[2] Y. Nakaya,*[1] H. Tsurukammi,*[2] T. Nakamura,*[2] M. Uehara,*[1] K. Suzuki.*[1] [1]Department of Nutritional Science, Tokyo University of Agriculture, Tokyo, Japan, [2]Department of Orthopaedic Surgery, University of Occupational and Environmental Health, Kitakyushu, Japan.

The role of the balance in the amounts of calcium (Ca) and phosphorus (P) intakes on bone formation and mineralization has not been explored in detail. In this study to clarify the significance of dietary Ca / P ratio on bone, we compared the effects of diets containing different ratios of Ca and P contents on trabecular bone formation in VDR(-/-) and their littermate. At the age of 4 weeks, VDR(-/-) mice and their littermate VDR(+/+) mice were assigned to five groups depending on the dietary Ca/P ratios fed during the feeding experiment for 4 weeks: Group 1 fed with the diet containing 0.5% Ca, 0.5% P, Group 2 with the diet containing 0.5% Ca, 1.0% P, Group 3, containing 1.0% Ca, 0.5% P, Group 4, containing 1.0% Ca, 1.0% P, or Group 5, containing 0.5% Ca, 0.25% P. Groups 1 – 5 respectively consisted of mice with two genotypes. Metabolic studies for calcium balance were conducted and bone histomorphometry were performed after calcein labelings. At the age of 8 weeks, the experiments were terminated and the right tibiae were harvested. Intestinal Ca absorption: In Groups 1, 2 and 4, Ca absorption in VDR(-/-) mice significantly reduced compared to VDR(+/+) mice. In Groups 3 and 5, although the values in VDR(-/-) mice were also reduced, but the difference from VDR(+/+) was not marked as in other 3 Groups. Histomorphometry: Primary spongiosa. In Groups 1, 2 and 4, the values of trabecular bone volume (BV/TV) and oteoid surface (OS/BS) in VDR(-/-) mice were significantly increased compared to the wild type mice. In Groups 3 and 5, however, these values did not significantly differ between VDR(+/+) and VDR(-/-) mice. Secondary spongiosa. In Groups 1 and 2, mineralization front was not observed, thus secondary spongiosa was not identified in VDR(-/-) mice. In Groups 3, 4 and 5, mineraliztion front was clearly observed in VDR(-/-) and VDR(+/+) mice as well. The values of BV/TV and bone formation rate (BFR/BS) did not significantly differ between the two genotype mice. In Group 4, the value of OS/BS in VDR(-/-) mice was significantly larger than the value in VDR(+/+) mice. In Groups 3 and 5, however, no significant difference were found in OS/BS values between VDR(-/-) and VDR(+/+) mice. These results suggest that the sufficient amount of Ca is the principle factor for normal bone mineralization as observed in Groups 3,4 and 5. However, since OS/BS values in Group 4 did not normalize in VDR(-/-) mice, the ratio of dietary Ca and P seems to be important to regulate the balance between osteoid formation and mineralization.

M417

Comparative Effects of Soy Isoflavones, Soy Protein and 17beta-Estradiol on Calcium and Bone Metabolism in Adult Ovariectomized Rats – I. Analysis of Calcium Balance, Bone Densitometry and Mechanical Strength. D. J. Cai,[1] J. Glasier,*[1] C. Turner,[2] C. M. Weaver.[1] [1]Foods and Nutrition, Purdue University, West Lafayette, IN, USA, [2]Indiana University, Indianapolis, IN, USA.

Several animal and short-term human studies indicate that isoflavones may have estrogenic actions on bone metabolism. However, none of the animal studies have explored the effects on calcium mechanism, a pre-requisite to perturbations in bone. This study was designed to determine if consumption of isoflavones as soy protein or supplements will prevent bone loss similar to estrogen, and have effects on calcium and bone metabolism in an adult animal model. Unmated 6 mo. old ovariectomized (OVX) and sham operated female Sprague-Dawley rats were randomly assigned to 9 groups (16 rats/group) and pair-fed for 8 weeks. Estrogen (E2) was administered via subcutaneous implants. Diets consisted of soy protein or/and casein with or without isoflavones (Iso). Total Ca balance and ^{45}Ca retention were measured for the last 2 days of the feeding period following an oral gavage administration of ^{45}Ca. BMD of the left femur was analyzed. Mechanical strength was determined at midshaft and femoral neck of the left femur by a 3-point bending test. Estrogen reduced bone loss significantly in the trabecular region of the left femur, but not in cortical bone as indicated by BMD (Table). Isoflavones given as soy protein or supplements did not prevent trabecular bone loss. Combining isoflavone with estrogen significantly lowered BMD in the distal region of the left femur. None of the treatments affected either total Ca balance or ^{45}Ca absorption significantly. However, soy protein showed significant effects on reducing urinary loss of Ca in animals irrespective of isoflavone level, perhaps due to lower amount of sulfur containing amino acids in soy protein. We conclude that estrogen, but not isoflavones at the levels tested, prevented bone loss after hormone deficiency.

Treatment	Base protein	Iso (mg/g diet)	Urinary Ca (mg)	Femoral neck BMD (mg/cm2)	Distal end BMD(mg/cm2)	Femoral neck Breaking Force (N)
Sham	Casein	0	5.3 ± 2.6 a	0.25±0.02 b	0.19±0.01a	100 ± 11
OVX	Casein	0	4.8 ± 2.4 ab	0.24±0.02 c	0.16±0.01c	91 ± 13
OVX	Casein	0.3	3.8 ± 1.5 bc	0.24±0.02 c	0.16±0.01c	90 ± 10
OVX	Casein	0.8	3.5 ± 1.1 bc	0.23±0.03 c	0.16±0.02c	93 ± 16
OVX	Soy	0	2.9 ± 1.4 c	0.23±0.01 c	0.16±0.01c	94 ± 14
OVX	Casein/Soy	0.2	3.5 ± 1.3 bc	0.24±0.02 c	0.16±0.01c	95 ± 16
OVX	Soy	0.4	3.1 ± 0.9 c	0.24±0.01 c	0.16±0.01c	94 ± 18
OVX + E2	Casein	0	5.3 ± 2.9 a	0.26±0.01 a	0.20±0.02a	102 ± 16
OVX + E2	Casein	0.3	6.0 ± 3.4 a	0.25±0.02 b	0.18±0.01b	97 ± 16

Values are means ± SD. Means with different letters are significantly different ($P < 0.05$).

M418

Effects of Menatetrenone, A Vitamin K Analog, on Prevention of Vertebral Fracture in Corticosteroid-Induced Osteoporosis. I. Tanaka,[1] H. Oshima.[2] [1]Internal Medicine, Fujita Health University School of Medicine, Toyoake, Japan, [2]Laboratory Medicine, Fujita Health University School of Medicine, Toyoake, Japan.

Menatetrenone, which is an analog of vitamin K and a co-factor for calcium deposition at bone formation, has been approved for treatment of primary osteoporosis in Japan. In this study, we accessed therapeutic effectiveness of menatetrenone on corticosteroid-induced osteoporosis.Patients with collagen diseases except for rheumatoid arthritis and treated with corticosteroids were enrolled in this non-randomized study and followed prospectively by measuring bone mineral densities (BMD) of the lumbar spine with DXA and taking radiographs of the thoracic and lumbar spines every year. In total, 136 patients were followed for 2 years. At a base line, means of age and BMD were 47 years old and 0.876 g/cm2, respectively. The mean prednisolone dosage for 2 years of the follow-up period was 10.4 mg/day. New fractures in this study were defined as an appearance of new fractures or an increase in the number of fractures on radiographs of the thoracic and lumbar spine after 2 years. Subjects were analyzed in four groups based on therapeutic agents used for osteoporosis (group M; menatetrenone, group E; etidronate, group D; active vitamin D3, group N; non-treated including calcium only). Demographic data including age, gender, basal BMD, and corticosteroid dosage were not different between the groups. Statistical analysis used was a logistic regression with SAS (6.02).As a result, independent risk factors for the appearance of new fractures were increases in corticosteroid dosage ($p=0.001$) and age ($p<0.001$), a decrease in BMD ($p=0.008$), existed fractures ($p=0.006$), and the therapeutic agents (menatetrenone or etidronate, $p<0.001$). New fractures of the spine were revealed in 31 patients (31%) in the group N (n=81), whereas 2 patients (13%) showed new fractures in the group M (n=15, OR; 0.190, 95%CI; 0.045-0.803 vs group N after the adjustment with age, corticosteroid dosage, BMD, existed fractures), and 2 (11%) in the group E (n=18, OR; 0.137, 95%CI; 0.029-0.646 vs group N after the adjustment), and 9 (41%) in the group D (n=22).In conclusion, these results suggested that a vitamin K analog, menatetrenone, is an effective therapeutic agent to prevent vertebral fractures in corticosteroid-induced osteoporosis.

M419

The Effects of Boron (Boric Acid) Supplementation on Bone Histomorphometry and Bone Metabolism in Aged Female F-344 Rats. M. T. Gallardo-Williams,*[1] R. R. Maronpot,*[2] C. H. Turner,[3] C. S. Johnson,[4] M. W. Harris,*[1] M. J. Jayo,[4] R. E. Chapin.[5] [1]Laboratory of Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA, [2]Laboratory of Experimental Pathology, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA, [3]Department of Orthopaedic Surgery, Indiana University, Indianapolis, IN, USA, [4]Pathology Associates, a Charles River Company, Advance, NC, USA, [5]Laboratory of Toxicology, National Institute of Environmental Health Sciences, DuPont Pharmaceuticals Co., Newark, DE, USA.

Introduction: Optimal bone health may be achieved by supplemental dietary boron. The effects of supplemental dietary boron on bone strength have been evaluated previously in intact male F-344 rats (Chapin RE, et al. Biol Trace Elem Res. 1998; 66:395-9). Male rats that received dietary boron had increased vertebral resistance to a crushing force. The present study was designed to evaluate the effects of supplemental dietary boron, with or without 17-beta-estradiol (E_2), in ovariectomized 13-month-old female F-344 rats.**Design:** Post-ovariectomy, the rats were divided into four treatment groups: 1) OVX-only (control); 2) E_2 treated; 3) Boron treated (sub-toxic dose of 8.7mg/kg/day); and 4) E_2 + Boron treated. Treatment lasted 40 days, Boron was administered via gavage, and E_2 via subcutaneous timed-release pellets (0.36mg). **Results:** Serum estrogen levels were below detectable levels in the control and Boron treated groups. Mean serum levels of estradiol were 56 ± 14 and 85 ± 48 pg/ml in the E_2 and E_2 + Boron groups, respectively. Serum biomarker results indicated that minerals, as well as osteocalcin, were dependent on the hormonal status of the animals, but not on boron supplementation. Serum IGF-I was increased due to ovariectomy, suppressed by E_2, and not affected by Boron treatment. By *ex vivo* bone pQCT, bone mineral density (BMD) of the L5 vertebra and proximal femur were significantly highest in both of the E_2 treated groups, and there were no differences in BMD between the control and Boron treated groups. By histomorphometry in the proximal tibial

metaphysis, osteoblastic, osteoid, and eroded surfaces were significantly suppressed by E_2 treatment, but not by Boron treatment. By biomechanical testing of femur and vertebra, E_2 treatment provided some bone strength that was not significantly different from control, and with no signification contribution from Boron treatment. **Conclusion:** Compared to male F-344 rats, supplemental dietary boron does not appear to have a beneficial effect on bone status in ovariectomized female F-344 rats and appears not to affect estrogen replacement therapy.

M420

Knowledge of Osteoporosis Risk factors and Diagnosis of Osteoporosis Improves Dietary Intakes of Calcium and Vitamin D. S. J. Whiting, T. Fehr,* Y. Suzuki,* P. D. Chilibeck,* C. M. Arnold. University of Saskatchewan, Saskatoon, SK, Canada.

Postmenopausal women with osteoporosis (PMO) recruited into a study of land vs. aquatic exercise were questioned about dietary calcium (Ca) and vitamin D (VD) intakes. It was hypothesized that the diagnosis of osteoporosis would provide knowledge about appropriate dietary Ca and VD intakes such that: 1) their intakes would meet Adequate Intake (AI) recommendations of 1200 mg Ca and 10 ug VD, and 2) their intakes would exceed that of a cohort of postmenopausal women without osteoporosis (PMNO). Dietary and supplemental intakes of 59 PMNO (age 57 ± 7 y) and 59 PMO (age 69 ± 6 y) were determined by food frequency questionnaire. PMO had higher food, supplement and total intakes of Ca and VD (Ca, mg: 968 ± 431, 632 ± 449, 1600 ± 644; VD, ug: 6.2 ± 2.8, 7.2 ± 6.2, 13.3 ± 6.7, respectively) than the PMNO (Ca, mg: 813 ± 413, 350 ± 468,1163 ± 577; VD, ug: 5.1 ± 3.4, 3.9 ± 6.5, 9.0 ± 8.1, respectively). Food intakes of Ca and VD were similar between the 2 groups, however, twice as many PMO took calcium or vitamin D supplements than PMNO, resulting in higher total intakes of Ca and VD. There were fewer PMO below the AI for Ca (25 %) compared to PMNO (55 %) and more above the upper tolerable level (UL) for Ca of 2.5 g (9 % vs 2 %, respectively). A Knowledge and Behavior Questionaire (KBQ) was given to a subgroup of 33 PMO. The KBQ showed an increase in knowledge of general risk factors and the role of vitamin D in osteoporosis after diagnosis with the disease, however, knowledge of calcium started high and did not change with diagnosis. Overall, the KBQ results demonstrated an increase in self-reported appropriate dietary behaviors with increased knowledge about osteoporosis. While diagnosis of osteoporosis triggered higher intakes of Ca and VD in most subjects, there remained a substantial portion of the PMO group not following dietary recommendations.

M421

Effects of Soy Isoflavones on Calcium Kinetics in Postmenopausal Women. L. A. Spence,*[1] E. R. Lipscomb,*[1] J. Cadogan,[2] B. R. Martin,*[1] M. Peacock,[3] M. Wastney,[4] C. M. Weaver.[1] [1]Foods & Nutrition, Purdue University, West Lafayette, IN, USA, [2]University of Sheffield, Sheffield, United Kingdom, [3]Indiana University, Indianapolis, IN, USA, [4]Metabolic Modeling Services, Dalesford, New Zealand.

Several lines of evidence suggest that soy isoflavones and related compounds act as estrogen agonists and have beneficial skeletal effects. However, little is known about the metabolic effects of isoflavones, and specifically, their effects on calcium handling by the body. The aim of this study was to test the effects of soybean isoflavones on calcium kinetics in postmenopausal women. Fifteen postmenopausal women were studied under three different one-month controlled dietary interventions in a randomized, crossover design: soy protein enriched with isoflavones, soy protein void of isoflavones, and a casein-whey control. Dietary calcium was 1106 mg/d and dietary isoflavones were ± 65 mg/d. After a 7-day adaptation to the diet, 10 uCi of ^{45}Ca was administered orally. One week later, 10 uCi of ^{45}Ca was administered intravenously. A series of blood samples were obtained following the radioisotopic tracer administration along with a 21-day collection of urine and feces. Total calcium was measured by atomic absorption spectrophotometry and ^{45}Ca was measured by Beta-scintillation counting. Calcium kinetics using compartmental modeling was analyzed by WinSAAM to calculate rates of calcium absorption, bone deposition, bone resorption, and pool sizes. The most striking finding was that soy protein reduced urinary calcium by 33% regardless of isoflavone levels compared to milk protein. Soy protein may be reducing urinary calcium through its lower content of sulfur containing amino acids.

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Comparative Effects of Animal and Legume Proteins on Urinary Calcium, Urinary Sulfate, and Urinary Net Acid Excretion and Kidney Function in Postmenopausal Women. E. R. Lipscomb,*[1] L. A. Spence,*[1] J. Cadogan,*[2] B. R. Martin,*[1] M. Peacock,[3] C. M. Weaver.[1] [1]Foods and Nutrition, Purdue University, West Lafayette, IN, USA, [2]University of Sheffield, Sheffield, United Kingdom, [3]Indiana University, Indianapolis, IN, USA.

Metabolism of sulfur-containing amino acids generates sulfate which increases urine acidity and urinary calcium excretion. Various studies have shown that the variability in urinary calcium excretion is related to differences in the sulfur-containing amino acids of protein diets. Furthermore, there is evidence of a strong correlation between urinary sulfate and both total renal acid excretion and urinary calcium excretion in elderly men and postmenopausal women fed meat protein simulated diets. However, few studies have compared the effects of animal protein (milk) and legume protein (soy) in a controlled metabolic study. The purpose of this study was to determine whether soy protein has a protective effect on bone relative to milk protein in postmenopausal women. Fifteen Caucasian postmenopausal women participated in two one-month controlled dietary interventions in a randomized, crossover design. The protein isolates supplied ~40g protein/d or ~50% of daily protein in the diet. Dietary calcium was 895 mg/d. Urinary calcium was measured by atomic absorption spectrometry. Urinary sulfate was measured by a turbidimetric technique. Urinary net acid excretion was measured by titration. Kidney function was calculated by: U/S*V*(1.73/BSA) where U=urine creatinine(dL), S=serum creatinine(dL), V=urine volume (mL/min), BSA=body surface area (m^2). Dietary protein significantly affected urinary calcium excretion ($P<0.01$). The mean urinary calcium excretions were 79±13mg and 123±13mg from the soy and milk protein diets, respectively, suggesting a protective effect of soy protein on bone. In addition, dietary protein significantly affected urinary net acid and urinary sulfate excretion ($P<0.05$). Mean urinary net acid and sulfate excretions were 31±4Meq/L and 3.6±0.03Meq/L for the soy diet and 40±4Meq/L and 4.5±0.03Meq/L for the milk protein diet, suggesting lower sulfur-containing amino acid content and lower acid generation of soy relative to milk protein. Kidney function as determined by creatinine clearance tended ($P<0.1$) to increase on the soy protein diet. The ~20% reduction in urinary acid and sulfate excretion reflects the ~30% less sulfur-containing amino acid content of soy vs. milk protein powders. The impact on reducing calcium loss was greater at 35%. The data suggest that soy protein may possess non-estrogenic bone-protective effects as indicated by reduced urinary calcium, urinary sulfate, and urinary net acid excretion relative to milk protein.

Disclosures: National Institute for Aging,2.

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Increasing Dietary Calcium Intake in Children: Preliminary Findings of a Behavior-Modification Nutrition-Education Intervention Program. A. V. Owen,*[1] L. J. Stark,*[2] M. C. Nelson,*[1] V. A. Stallings,[1] B. S. Zemel.[1] [1]Division of GI & Nutrition, The Children's Hospital of Philadelphia, Philadelphia, PA, USA, [2]Division of Psychology, Children's Hospital Medical Center, Cincinnati, OH, USA.

Increased calcium intake using calcium supplements has been shown to improve bone mineral density, but sustained, increased calcium intake is difficult to achieve. As part of a larger pediatric bone health study targeting healthy children, ages 7-10 years, an intervention program has been created to improve dietary calcium intake though behavior modification and nutrition education (BM-NE). Children were excluded on the basis of obesity, chronic illness, and use of medications known to effect growth, bone density, or dietary intake. In this program, parents and children separately participated in 5 structured sessions that taught the families how to gradually increase calcium with dietary modifications. The target calcium intake was 1500 mg/day. These dietary changes were supported with behavior-modification techniques that helped families make changes in the children's lifestyles. Individualized weekly calcium goals were incorporated into the program and each session focused on a single meal including breakfast, lunch, dinner, and snack. During the classes, dietary information was provided on sources of calcium, both natural and fortified, and participants were taught how to calculate the calcium content of individual meals. Calcium intake was tracked during the session using weighed diet records that were recorded by the families. A registered dietitian reviewed all records for accuracy. The children also tracked their daily progress using sticker charts, and rewards were given to each child when they met the weekly calcium goal. Currently, 31 children have been randomly assigned to this program from the larger study. Of the 31 children, 16 have completed the program, and another 15 are either in the current session or will attend a future session. Complete data has been collected on 13 children. Initial dietary calcium intake averaged 742 mg/day. Upon completion of the program, the average calcium intake was 1605 mg/day, an average increase of 863 mg/day. These results show initial support for the success of the BM-NE program as a method for increasing dietary calcium.

M424

Flaxseed Has No Effect on Bone Markers of Postmenopausal Women but Improves Lipid Profiles. E. A. Lucas,*[1] L. Hammond,*[1] R. A. Wild,*[2] L. Chandler,*[2] D. A. Khalil,[1] B. P. Daggy,[1] B. J. Stoecker,[1] B. H. Arjmandi.[1] [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Obstetrics and Gynecology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

Flaxseed is a rich source of precursors to the lignans: enterodiol and enterolactone. Lignans are structurally similar to selective estrogen receptor modulators, which are reported to have beneficial effects on bone. Flaxseed is also a rich source of polyunsaturated fatty acids, especially a-linolenic acid, which may decrease the rate of bone resorption through inhibiting biosynthesis of prostaglandins. Hence, we examined the effects of flaxseed consumption on biomarkers of bone turnover in postmenopausal women. Fifty-eight postmenopausal women not on hormone replacement therapy were randomly assigned to consume 1000 mg of calcium, 400 IU vitamin D, and 40 g of either ground flaxseed or wheat-based comparative control daily for three months in a double-blind randomized study. Fasting blood samples and 24-hour urine samples were collected at the beginning and at the end of the study. Specimens were analyzed for serum lipid profiles and serum and urinary biomarkers of bone formation and bone resorption. Contrary to our expectations, flaxseed did not affect the assessed bone biomarkers; however, flaxseed significantly lowered serum total cholesterol (6%) and triglycerides (12%) without reducing HDL-cholesterol, while the comparative control regimen had no such effects. These findings suggest that flaxseed may reduce the risk of cardiovascular disease in postmenopausal women. However, the effects of flaxseed on bone biomarkers are not apparent and may require a longer supplementation duration. The effects of flaxseed on the hormonal metabolism of these women are being investigated. Supported by NIH grant No. R03-AG16487-01

M425

Dried Plums Improve Indices of Bone Formation in Postmenopausal Women. D. A. Khalil,[1] E. A. Lucas,*[1] A. Georgis,*[1] B. J. Stoecker,[1] C. Hardin,*[1] M. E. Payton,*[2] R. A. Wild,*[3] B. H. Arjmandi.[1] [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Statistics, Oklahoma State University, Stillwater, OK, USA, [3]Obstetrics and Gynecology, University of Oklahoma Health Sciences Center, Stillwater, OK, USA.

Our recent findings in a rat model of osteoporosis suggest that dried plums are highly

effective in preventing and reversing ovarian hormone-deficiency-associated bone loss. Dried plums are rich in polyphenolic compounds and other nutrients that contain high antioxidant properties which may prevent bone loss that is in part due to the rise in oxygen-derived free radical formation. The objective of this study was to examine whether the addition of dried plums to the diets of postmenopausal women influences the rates of bone turnover. For this, postmenopausal women not on hormone replacement therapy were randomly assigned to consume either 100g of dried plums (12 dried plums) or 75g of dried apples (an equivalent amount of calories, fat, and fiber) daily for three months. Serum and urinary biochemical markers of bone status were assessed before and after treatment. Only dried plums significantly increased serum levels of insulin-like growth factor-I (IGF-I) and bone-specific alkaline phosphatase activity, a specific marker of bone formation, in comparison with baseline values. Serum and urinary markers of bone resorption, however, were not affected by either treatment. These results suggest that dried plums may exert positive effects on bone in postmenopausal women. Longer duration studies are needed to confirm the beneficial effects of dried plum consumption on bone mineral density and the skeletal health of postmenopausal women.Supported by a grant from California Dried Plum Board

M426

Soy Protein With Its Isoflavones Improves Bone Markers in Women Particularly Those With Low Estrogen Status. B. H. Arjmandi,[1] D. A. Khalil,[1] E. A. Lucas,*[1] S. Juma,[1] M. E. Payton,*[2] M. E. Munson,*[1] A. B. Arquitt,*[1] R. A. Wild.*[3] [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Statistics, Oklahoma State University, Stillwater, OK, USA, [3]Obstetrics and Gynecology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

This study investigated the effects of daily consumption of 40 g soy protein containing 90 mg isoflavones or 40 g of milk-based protein for three months in a double-blind randomized study on bone biomarkers in 42 women. The effects were further examined in women with adequate estrogen status (n = 10 with average serum estradiol level of 93 ± 17 pg/mL, and n = 12 with average serum estradiol level of 123 ± 27 pg/mL for soy protein and milk-based protein, respectively) and estrogen-deficient women (n = 10 with average serum estradiol level of 13 ± 2 pg/mL, and n = 10 with average serum estradiol level of 17 ± 4 pg/mL for soy protein and milk-based protein, respectively). Soy protein and not milk-based protein supplementation resulted in an average 25% reduction ($P<0.08$) in urinary excretion of deoxypyridinoline in women; however, a significant ($P<0.05$) reduction (33%) was only observed in estrogen-deficient women. Overall, both protein supplements resulted in significant increases in serum insulin-like growth factor-I (IGF-I), a factor associated with bone formation. However, soy protein but not milk-based protein resulted in a significant ($P<0.05$) increase in serum IGF-I in estrogen-deficient women. Urinary calcium excretion was significantly ($P<0.05$) increased in women with adequate estrogen status after supplementation with milk-based protein but not with soy protein although both supplements provided equal amounts of calcium (1400 mg/d) and vitamin D (200 IU/d). No significant changes due to treatment were observed in serum activities of alkaline phosphatase and tartrate-resistant acid phosphatase. We conclude that estrogen-deficient women may benefit from soy protein supplementation more than those with adequate estrogen status. Longer duration studies are needed to confirm the effects of soy protein on bone mineral density of women with or without adequate estrogen status.(Supported by grants from Oklahoma Center for the Advancement of Science and Technology and Protein Technologies International)

M427

Effects of Isoflavones, Vitamin E, and Their Combination on Bone in an Aged Rat Model of Osteopenia. B. H. Arjmandi,[1] M. P. Akhter,[2] D. Chakkalakal,[3] D. A. Khalil,[1] E. A. Lucas,*[1] S. Juma,[1] M. El-Osta,*[1] L. Devareddy,*[1] B. J. Stoecker.[1] [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [3]Biomedical Engineering Center, Creighton University, Omaha, NE, USA.

The findings of animal studies indicate that soy or its isoflavones prevent loss of bone in ovarian hormone deficiency. Recent reports also suggest that vitamin E positively influence bone in different animal species. The dose-dependent effects of soy isoflavones (3 doses: 0, 500, and 1000 mg/kg diet), vitamin E (75, 300, 525, 750), or their combinations on bone mineral density (BMD), bone mineral content (BMC), and biomechanical properties in aged ovariectomized osteopenic rats. Twelve-month old female Sprague-Dawley rats were divided into 13 treatment groups (n =14); one group was sham, and the remaining groups were ovariectomized (ovx). Animals in all groups were placed on a semi-purified diet for 120 days to allow significant bone loss to occur as confirmed by BMD assessment using DXA. Thereafter, animals were placed on dietary treatments for a period of 100 days. As expected, ovariectomy significantly reduced BMD of the 4th lumbar, tibia and femur. The combination of isoflavones and vitamin E at 1000 and 525 mg/kg diet, respectively was able to bring BMC of the femur and BMD of the tibia to that of the sham animals. This combination also resulted in higher BMD of the 4th lumbar vertebra when compared to all the other treatment groups. However, the ovx-induced reduction in biomechanical properties of the femur were improved with the highest dose of vitamin E (750 mg/kg diet) alone. These findings suggest that while BMD and BMC benefit from the combination of vitamin E and isoflavones, only vitamin E improves bone strength in the rat model of established osteopenia. Supported by USDA NRI grant No. 99-35200-7606

M428

Vitamin E Improves Bone Repair in Ovariectomized Rats in a Dose-Dependent Manner. D. Chakkalakal,*[1] J. R. Novak,[2] E. D. Fritz,*[2] M. P. Akhter,[3] E. A. Lucas,*[4] D. A. Khalil,[4] S. Juma,[4] M. El-Osta,*[4] B. J. Stoecker,[4] B. H. Arjmandi.[4] [1]Biomedical Engineering Center, Creighton University, Omaha, NE, USA, [2]VA Medical Center, Creighton University, Omaha, NE, USA, [3]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [4]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA.

In a previous study we demonstrated that a moderately high dose of vitamin E in the diet improves biomechanical and biochemical properties of bone in old mice. Other recent studies suggest that vitamin E may also have beneficial effects in the early stages of fracture healing. Ovarian hormone deficiency has been reported to compromise the fracture healing in rats. The present study was designed to investigate the effect of dietary vitamin E on the outcome of bone repair in ovariectomized rats. Fifty-six, 12-month old female Sprague-Dawley rats were ovariectomized (ovx) and eight rats had sham surgery (sham) to serve as positive control. For a period of 120 days, all rats were maintained on a semi-purified casein-based diet (AIN 93M). Thereafter, the bone repair model was created in both fibulae of each rat by osteotomy. Immediately after surgery, the 56 ovx rats were divided into four equal groups and fed 75, 300, 525, and 750 mg vitamin E per kg diet, respectively. The standard AIN93M diet contains 75 mg and hence the first of these groups was regarded as the negative control. One-hundred days after osteotomy, rats were sacrificed to evaluate the outcome of bone repair by bend testing. We found that the 525 mg dose increased the bending strength by 9.4% ($P=0.03$) over the negative control. But it was still 6.5% less than the positive control. The other two doses of vitamin E were ineffective. The 525 mg dose also increased the bending rigidity of the repaired fibula by 20%, albeit not significantly, over the negative control group but it was 18% less than the positive control. We have previously found that the mRNA levels of insulin-like growth factor-I and osteocalcin in two-year old mice were increased by vitamin E suggesting stimulation of new bone formation. Further studies are needed to determine if this occurs in the bone repair model as well. Supported by USDA NRI grant No. 99-35200-7606

M429

Correlation Between Lumbar and Femoral Changes After Pharmacological Treatment in Postmenopausal Osteoporosis. M. Di Stefano, G. Isaia. Department of Internal Medicine, University of Torino (Italy), Torino, Italy.

Aim of our study was to evaluate the correlation between lumbar and femoral BMD changes induced by pharmacological treatment in osteoporotic women.We studied 80 women (age range 52-71 years) with postmenopausal osteoporosis and we examined on the same day both lumbar and femoral bone mineral density (BMD) by DXA technique (Hologic QDR 4500) at baseline and after 12 and 24 months of regular treatment with various drugs (raloxifene, clodronate, calcium plus vitamin D) affecting phosphocalcium metabolism.After 24 months, we observed a mean increase of 1.61% of lumbar BMD ($p<0.005$), while femoral BMD did not show any significant increase vs basal values.Moreover, the results obtained (expressed as rate of change for year of treatment from baseline, both in terms of absolute and percentage values) at lumbar (L2-L4) and at femoral (total, neck, trochanter, intertrocanteric and Ward's triangle) levels were correlated by using Pearson's r correlation coefficient.We observed the better correlation among rate of change of lumbar BMD and rate of change of total femoral BMD ($r=0.299$, $p<0.05$ at 12 months, $r=0.36$, $p< 0.05$ at 24 months), while Ward's triangle showed the worst correlation (p=n.s.). We also observed highly statistically significant correlation between rate of change/year among the femoral subregions, mainly after 24 months of treatment (see Table) in respect of after 12 months.Our data suggest that pharmacologic treatment of osteoporosis can play a different effect on BMD at lumbar and at femoral level; total BMD can be considered the best femoral site for DXA follow up and a duration of 24 months of treatment allows us a more omogeneous evaluation than 12 months about femoral BMD change.

M430

Serum Tartrate-Resistant Acid Phosphatase 5b Is a Useful Marker for Monitoring Alendronate Therapy. J. M. Halleen,[1] S. L. Alatalo,*[1] K. K. Ivaska,*[1] S. Cheng,[2] K. Uusi-Rasi,*[3] A. Nenonen,*[3] P. Kannus,*[3] H. Sievänen,*[3] K. Väänänen.[1] [1]Department of Anatomy, University of Turku, Turku, Finland, [2]University of Jyväskylä, Jyväskylä, Finland, [3]The UKK Institute, Tampere, Finland.

Recent studies indicate that serum tartrate-resistant acid phosphatase isoform 5b (TRAP 5b) is a specific and sensitive marker of bone resorption. The purpose of this study was to evaluate the usefulness of serum TRAP 5b to monitor alendronate therapy in early postmenopausal women. The subjects of this one-year double-blinded intervention trial were 164 healthy postmenopausal women who were within 5 years after onset of menopause and with no previous use of medication related to bone metabolism. The subjects were randomly assigned into two groups, one receiving 5 mg alendronate daily (n = 82), and the other receiving placebo (n = 82). All subjects received a daily supplement of calcium and vitamin D. Serum TRAP 5b was assessed using a commercial immunoassay (BoneTRAP, SBA-Sciences, Oulu, Finland) at baseline and at 3, 6 and 12 months after start of treatment. Total serum osteocalcin was measured as a marker of bone formation at the same time points using an in-house two-site immunoassay. Bone mineral density (BMD) of the lumbar spine was measured with dual-energy X-ray absorptiometry (Norland XR-26, Norland Inc., Fort Atkinson, WI, USA) at baseline and at the end of the study. The BoneTRAP assay had an inter-assay variation of 2.1%, an intra-assay variation of 1.9% and a least significant change (LSC) of 33.6%. We found that serum TRAP 5b decreased 10.3% in the placebo-group, and 40.0% in the alendronate-group after 3 months intervention, and stayed in these levels at all later time points. The decrease was significantly higher ($p < 0.0001$) in the alendronate-group, and alendronate treatment changed the serum TRAP 5b values of these postmenopausal women to normal premenopausal level. The decrease was more than LSC in 5.2% of the individuals in the placebo-group and 75.0% of the individuals in the alendronate-group. Serum osteocalcin decreased 30% after 3 months alendronate therapy, and an additional 10% within the next 3 months, suggesting that the balance between bone resorption and bone formation was reached after 6 months intervention. BMD increased significantly in the alendronate-group, and the change in BMD at 12 months correlated significantly with the change in TRAP 5b at 3 months ($r = -0.40$, $p < 0.0001$). These results

show that serum TRAP 5b is a useful marker for monitoring alendronate therapy.

Disclosures: SBA Sciences,7.

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Significantly Improved Rate of Treatment of Osteoporosis in Patients Following Hip Fractures. M. J. Gardner,*[1] K. Flik,*[1] P. A. Mooar,*[1] J. M. Lane.[2] [1]Medical College of Pennsylvania and Hahnemann University School of Medicine, Philadelphia, PA, USA, [2]Hospital for Special Surgery, New York, NY, USA.

Osteoporosis is a common disease characterized by decreased bone mass and increased fracture risk in postmenopausal women and the elderly. Vertebral fractures and hip fractures are the most common consequences of osteoporosis, which unfortunately present late in the course of the disease. When a patient is admitted to the hospital with a fragility fracture, a unique opportunity for diagnosis and treatment presents itself. Fortunately, several medications have proven effective in lowering the risk for future fractures.A retrospective cohort study was performed using patient databases from two university medical centers and one university affiliated community hospital with orthopaedic residents. Using codes for femoral neck fractures, 300 randomly selected patient charts were analyzed. This consisted of 100 patients from each center, which included 25 patients from each year between 1997 and 2000. Similarly, the database was sorted by the ICD-9 code for both thoracic and lumbar vertebral fracture and 25 patients from one of the centers were randomly included in the study. Patient charts were obtained and reviewed, confirming that these fractures were not the result of high-energy trauma. Admitting diagnosis, admission medications, procedures during hospital stay, and discharge medications were then extracted and analyzed.In the hip fracture group. of the 75 patients in each group from 1997 to 2000, 11%, 13%, 24% and 29%, respectively, were discharged with prescriptions for some medication targeting osteopenia, either supplemental calcium or an antiosteoporotic medication (estrogens, calcitonin, bisphosphonates, or raloxifene). A trended Chi-square analysis of this increase revealed a p value of <0.0001, indicating that this improvement in treatment is unlikely due to chance alone. In the vertebral fracture group, 13 patients (52%) received medication on discharge. Of all 325 patients in the study, 71 (21.8%) received a prescription on discharge. However, only 28 of these patients (8.6%) received a medication to actively prevent bone resorption and treat osteoporosis. In addition, no patient underwent a bone density scan while in the hospital. Elderly patients and postmenopausal women who are admitted to the hospital and diagnosed with either a vertebral compression fracture or femoral neck fracture have been under treated for osteoporosis. However, over the last four years there has been a statistically significant increase in the rate of treatment. With continuing educational efforts the rate of osteoporosis treatment is likely to increase further in the future.

M432

The Cost-Effectiveness of Calcium and Vitamin D or Hormone Replacement Therapy Versus no Intervention in the Prevention of Hip Fractures in Canadian Postmenopausal Women. E. A. Papadimitropoulos,[1] R. Goeree,*[2] P. C. Coyte,*[3] R. G. Josse,[4] C. E. Greenwood.*[5] [1]Research and Development, Eli Lilly Canada Inc., Scarborough, ON, Canada, [2]Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, ON, Canada, [3]Department of Health Admin and the Institute for Policy Analysis, University of Toronto, Toronto, ON, Canada, [4]Division of Endocrinology, St. Michael's Hospital, Toronto, ON, Canada, [5]Department of Nutrional Sciences, University of Toronto, Toronto, ON, Canada.

As average life expectancy increases, the incidence of hip and other fractures in postmenopausal women (PMV)also increases. The number of hip fractures (HF) and health care resources required to manage them are expected to quadruple in the next 40 years, under a No Intervention (NI)scenario, as the Canadian population ages. Consequently, implementation of effective preventative interventions are necessary in PMW to avoid future HFs. Supplementation with Calcium and Vitamin D (CaVD) may result in fewer HFs, lower healthcare cost, and life years gained (LYG) compared to NI, if therapy is initiated in PMW at either age 50 (CaVD50), or age 65 (CaVD65), and continued until age 90 or death. An economic evaluation was conducted to determine the incremental cost-effectiveness of CaVD or hormone replacement therapy (HRT) versus NI, using a Markov model. Canadian specific probabilities and cost of events, needed for the model, were generated by manipulation of medical databases and literature reviews; efficacy of CaVD in osteoporosis prevention was evaluated via the conduct of a meta-analysis. The forty-year model was run under the base case assumptions (CaVD and HRT had 20%, and 50% reduction in HF risk respectively, versus NI) and 3% rate of discounting. CaVD65 strategy was considered weakly dominant (resulted in fewer HFs and lower cost than NI) under the base case assumptions. HRT strategy initiated at age 65 was dominant over all strategies evaluated. HRT initiated at age 50 was cost-effective ($10,969/LYG;fewer HFs, but cost more than NI), while CaVD50 was not($94,843/LYG). The model results were sensitive to the cost of CaVD, HRT, long-term care, hip fracture repair, and to treatment efficacy assumptions, however use of acceptable cost ranges for these paramenters did not alter the rank-ordering of the interventions. In conclusion, CaVD65 is a cost-effective alternative for those women who choose not to take HRT for the prevention of HFs.

Disclosures: Papadimitropoulos,1,3.

M433

Predictive Value of Bone Markers in the Treatment of Japanese Postmenopausal Osteoporotic Women with Hormone Replacement Therapy, Etidronate or 1alpha-hydroxy-vitamin D3. O. Chaki, I. Gorai, K. Mochizuki,* H. Yoshikata,* Y. Arata,* R. Kikuchi, F. Hirahara.* Gynecology, Yokohama City Univsity, Yokohama, Japan.

There are some cases which are contraindicated to or discouraged to hormone replacement therapy (HRT) . In this study, we assessed the predictive value of biochemical indices in treatment of postmenopausal osteoporosis with HRT, etidronate or 1alpha-hydroxy vitamin D3 (1alpha-(OH)-D3) .Forty five postmenopausal Japanese women aged 44-77 years (mean age, 62.4+/-7.5 years) were enrolled into this study. Fifteen women (mean age, 61.6+/-7.3 years) were treated with HRT (conjugated estrogens 0.625mg/day, medroxyprogesterone acetate 2.5mg/day or conjugated estrogens 0.625mg/day alone ; HRT group) ,15 women (mean age, 64.2+/-8.1 years) with Etidronate (200mg/day for 2 weeks every 12 weeks ; E group) , 15 women (mean age, 63.1+/-7.6 years) with 1a-(OH)-D3 (1mg/day ; D group). Lumbar spine bone mineral density (L-BMD) was determined at the start of the study (0 month) and every 6 months thereafter and biochemical indices, serum alkaline phosphatase (ALP), intact osteocalsin (IOC), N-peptide of type I procollagen (PINP), urinary deoxypyridinoline (DPD) and crosslinked N-telopeptide of type I collagen (NTx) were measured at 0,1,3,6 and 12months. BMD measurement was performed using dual energy X-ray absorptiometry (DXA, QDR2000). Baseline L-BMD and biochemical indices did not show any substantial differences in among each group. There were significant differences in the percent changes of BMD (HRT group;2.5+/-2.3%,E group; 4.0+/-1.4%,D group; -1.5+/-2.3%, HRTor E group vs. D group, $p < 0.05$ at 6 months, HRT group;4.9+/-2.7%,E group; 6.6+/-1.5%,D group; -2.1+/-1.7%, HRT group vs. E or D group, $p < 0.05$, E group vs. D group, $p< 0.05$ at 12 months,)NTx and DPD significantly decreased at 3 months in HRT group as well as E group from baseline ($p < 0.05$). The magnitude of decrease was grater in E group than HRT group.(NTx: HRT;-35.6+/-12.1%,E;-39.2+/-10.6%, DPD: HRT;-9.7+/-4.1%,E;-21.3+/-15.4%). Whereas, in D group, there were no significant changes of biochemical markers in this study. In conclusion, 1) The bone spearing effect of etidronate for treatment of postmenopausal osteoporosis appeared earlier than that of HRT. 2) Biochemical markers of bone metabolism were not clinically useful in the prediction of future BMD in 1alpha-(OH)-D3 treated women.

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Raloxiene, But Not Alendronate, Increases Intestinal Calcium Absorption in Elderly Women with Established Osteoporosis. J. R. Zanchetta,[1] J. San Martin,*[2] E. E. Fradinger,*[1] A. Marino,*[1] A. Mango,*[1] L. Rubio,*[1] C. E. Bogado.*[1] [1]IDIM and USAL University School of Medicine, Buenos Aires, Argentina, [2]Eli Lilly Research Laboratories, Eli Lilly, Indianapolis, IN, USA.

It is well established that intestinal calcium absorption declines with age and this decrease may be implicated in the pathogenesis of bone loss and fractures in elderly women. On the other hand, the effects of commonly prescribed antiresorptive agents on calcium absorption have not been fully elucidated.The effect of raloxifene and alendronate on intestinal calcium absorption was assessed in 50 elderly women aged 65-83 years, with established osteoporosis, defined as the presence of at least 2 mild or 1 moderate vertebral fracture. All patients received calcium (1 g/day) and Vit. D (400 IU/day) for at least 1 year before entering and continued during the study. After giving written informed consent patients were assigned to receive raloxifene 60 mg/day (n=23), alendronate 10 mg/day (n=22) or no treatment (n=5). There were no differences between groups in age, years since menopause, body weight or calcium intake. Intestinal calcium absorption, serum calcium, phosphate, iPTH, 25 (OH) vit D, 1,25 (OH)2 vit D, Cross Laps, osteocalcin and bone specific alkaline phosphatase were measured at baseline and after 3 months of treatment. Intestinal calcium absorption was measured by a single isotope, low carrier oral radiocalcium absorption test which is independent of body weight, as described by Nordin et al (Journal of Nuclear Medicine 1998, 39: 108-113). Results are expressed as mean ± SEM. Changes from baseline were assessed by paired t test and differences between groups by simple one-way ANOVA.After 3 months of treatment, fractional calcium absorption increased significantly only in the raloxifene group (baseline= 0.6472 ± 0.036, 3 months=0.7805 ± 0.0490, p=0.0066). 1,25(OH)2 vit D did not change in any group. Serum CrossLaps, a marker of bone resorption, decreased significantly in both raloxifene and alendronate groups by 29.4 and 52.2% respectively. Among markers of bone formation, osteocalcin decreased significantly by 18.5% in the alendronate group and by 15.3% in the raloxifene group. Bone specific alkaline phosphatase decreased significantly by 28.2% in the alendronate group, but not in the raloxifene group. Bone markers did not change in the control group. There were no differences between groups in serum calcium, phosphate, iPTH or 25(OH) vitamin D at baseline or after treatment.Our results suggest that raloxifene increases intestinal calcium absorption efficiency in calcium and vitamin D repleted elderly osteoporotic women and this effect is independent of changes in serum levels of 1,25 (OH)2 vitamin D.

Disclosures: Eli Lilly,2,8; Merck,2,8.

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Consequences of Drop-Outs in Clinical Trials:Biased Estimation of Event Rates and Rate Ratios. M. Vaeth,*[1] D. Thompson.[2] [1]Department of Biostatistics, University of Aarhus, Aarhus, Denmark, [2]Merck Research Labs, Rahway, NJ, USA.

It is well-known that drop-outs in a clinical trial will lead to less precise estimation of event rates and the rate ratio used for treatment comparison; as a consequence excessive drop-outs may invalidate the sample size calculation for the trial. Increased uncertainty in estimation and reduced statistical power are, however, not the only effects of drop-out. Perhaps more seriously, drop-outs may also introduce bias in the estimation of the event rates and rate ratios and this is not commonly appreciated. Based on a simple model for a clinical follow-up study with time-to-event as the primary endpoint situations in which drop-

out may introduce bias in summary rates and rate ratios are characterized. The problem occurs if the true event rate is time-varying or if the patient population is heterogeneous and the bias becomes larger as the drop-out frequency increases. Outcome-dependent drop-out is particularly problematic, but bias may be present also with outcome-independent drop-out. In the latter case an increasing (decreasing) event rate will lead to a negative (positive) bias in summary rates. Bias in the rate ratio will also be present, but the direction of the bias depends on whether or not the drop-out frequencies are the same in the two treatment groups.

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Survey of Calcium, Vitamin D and Prescription Medication Use In Men and Women with Recent Fractures. A. Pro-Risquez,*[1] S. Harris,[2] E. Ross,*[1] S. Rudicel,*[1] B. Barnewolt,*[1] B. Dawson-Hughes.*[2] [1]New England Medical Center, Boston, MA, USA, [2]Calcium and Bone Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center at Tufts University, Boston, MA, USA.

In adults, an osteoporotic fracture is a well-recognized risk factor for further fractures. Since osteoporosis is a common cause of fractures in the elderly, it is extremely important that patients with an acute fracture be evaluated for this condition, and that it be recognized and treated before another fracture occurs. To gain information on the extent to which adult fracture patients, age 51 and older, are currently being evaluated and treated for osteoporosis, we conducted a study in 68 patients, 44 female and 24 male, mean age 66.8±10.5 years, treated at a Boston hospital over the past 10 months for an acute fracture. Medical history and diet questionnaires were administered. At the time of enrollment, 69.1% of patients had not discussed osteoporosis with their doctors and 66.2% had not had a bone density test. Only 20.6% had a current prescription for a drug effective in osteoporosis treatment, including supplemental estrogen in women. Of the 68 patients, 69.1% had not been advised to take calcium or vitamin D supplements, 64.7% did not take calcium supplements, and 91.2% did not take vitamin D supplements (excluding multivitamins). In conclusion, our preliminary results are consistent with previous studies, and show that, among older men and women who sustain an acute fracture, few had been evaluated for osteoporosis. Any patient who fails to receive proper evaluation or treatment represents a failure of the system. It is important to document how patients are currently being evaluated and to make changes in practice patterns in order to best prevent and treat this widespread and debilitating disease.

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A Comparison of Number Needed to Treat for Selected Therapies. H. Dimai,*[1] S. Sieghart,*[2] K. Klaushofer.*[3] [1]Dept. of Internal Medicine, University Hospital, Graz, Austria, [2]Kaiserin-Elisabeth Hospital, Vienna, Austria, [3]Ludwig Boltzman Institute of Osteology, Vienna, Austria.

The number needed to treat (NNT) is useful in describing to the physician the amount of effort that must be expended to prevent one fracture. As a consequence when used appropriately it allows comparison with the amount of effort that must be expended to prevent the same or other elements in patients with other disorders. The classic definition of NNT (the inverse of the absolute risk reduction) does not permit the user to make comparisons among different therapies in different populations. Our objective was to compare NNTs for raloxifene (RLX), risedronate (RIS), alendronate (ALN) and calcitonin (CAL) using the same reference populations. We used an alternative definition of NTT which depends explicitly on the population risk and the relative risk. We obtained the relative risks for selected therapies from clinical trials. In addition, we computed the risk of vertebral fractures in each of the placebo groups. Using the baseline risk for a given study, we used the relative risk from studies conducted in similar populations to compute the respective NNTs. As expected NNTs for each therapy varied with the baseline risk .

		Placebo	**NNT for 3 years**			
Study	VFX FRX status	Incidence	RLX	RIS	ALN	CAL
FIT I	VFX	15	22	20	13	20
FIT II	No VFX (T < -2.5)	3	67	NA	67	NA
VERT NA	Existing VFX	14	24	22	14	22
VERT MN	Existing VFX	26	13	10	7	12
MORE I	Existing VFX	21.1	16	14	9	14
MORE II	No VFX (T < -2.5)	4.5	45	NA	44	NA
PROOF	Existing VFX	26.0	13	10	7	12

In conclusion, the NNT differs among the treatments with bisphosphonates being the lowest.

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Effect of Cenestin® (Synthetic Conjugated Estrogens, A) on Biochemical Markers of Bone Turnover in Non-Hispanic Caucasian and Mexican-American Postmenopausal Women. S. A. Ayres, R. E. Stevens, K. V. Phelps.* Clinical Affairs, Duramed Pharmaceuticals, Inc., Cincinnati, OH, USA.

The prevalence of osteoporotic fracture is greater in non-Hispanic Caucasian (NHC) women than Mexican-American (MA) women. Limited data is available comparing bone turnover as determined by biochemical markers in MA versus NHC women. The present study was designed to evaluate the effect of Cenestin, a synthetic conjugated estrogens product on bone turnover in early postmenopausal women. A post-hoc comparison examined the effect of ethnicity on biochemical markers of bone turnover. Twenty-two MA women (mean age 55.1 yrs) and thirteen NHC women (mean age 56.6 yrs) participated in a 3-month randomized placebo-controlled trial. The two ethnic groups were not significantly different in time since last menses, BMI and baseline biochemical markers of bone turnover. Biochemical markers: serum osteocalcin (OC) measured by automated immunofluorescent assay (Kryptor, Cis), serum bone alkaline phosphatase by ELISA (BAP, Metra), serum N-terminal propeptide of type I collagen (PINP) by RIA (Orion), urinary N-telopeptide of type I collagen (U-NTX) by automated ELISA (Vitros Eci, Ortho-Clinical Diagnostics) and serum C-telopeptide of type I collagen (S-CTX) by automated electrochemiluminescence immunoassay (Elecsys, Roche) were evaluated at baseline (3 measurements at day -2, day -1 and day 0), and days 30, 60 and 90. Overall, Cenestin in contrast to the placebo group, normalized bone turnover to the premenopausal level. Further analysis determined that the magnitude of treatment effect observed with Cenestin was independent of ethnicity. The responses after treatment between the two groups were not significantly different. Cenestin was effective in reducing bone turnover in menopausal women irrespective of ethnicity.

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Randomized Trial of the Effectiveness of Hormone Replacement Therapy, Etidronate, Calcitonin, Vitamin D, and Vitamin K in Women with Postmenopausal Osteoporosis. Y. Ishida, H. Soh,* S. Tsuchida,* S. Kawahara,* H. Murata.* Orthopedic Surgery and Department of Obstetrics and Gynecology, Tsushimi Hospital and Yamaguchi University School of Medicine, Yamaguchi, Japan.

Currently, several pharmacologic agents exist that have more or less proven positive effects on osteoporosis. This study was conducted to assess the effectiveness of various pharmacologic therapies on bone mineral density (BMD), biochemical bone markers, and new fracture incidence in postmenopausal women. Postmenopausal women (n=250) with established osteoporosis were randomly allocated into six groups: control (no treatment); hormone replacement therapy (HRT, conjugated estrogen 0.625 mg/day plus medroxyprogesterone 2.5 mg/day); etidronate (200 mgx14d q3m); eel calcitonin (CT, 20 IU/week); vitamin D3 (alfacalcidol 1 microg/day); and vitamin K2 (45mg/day). BMD of distal 1/3 radius was measured by dual energy X-ray absorptiometry, along with biochemical markers of bone turnover [serum bone specific alkaline phosphatase (B-AP), serum osteocalcin (OC), urinary N-telopeptide of type I collagen (NTX), and urinary deoxypyridinoline (D-Pyr)] at baseline and after 3, 6, 9, 12, 15, 18, and 24 months of treatment. All new symptomatic non-vertebral, radiographically defined fractures were recorded, and thoracic and lumbar spine radiographs were taken every 3 months to assess new vertebral fractures. Over 2 years, the new vertebral fracture risk in HRT, etidronate, CT, vitamin D3, and vitamin K2 groups was reduced by 80%, 47%, 67%, 64%, and 65% versus control, respectively. The control group showed a significant decrease from baseline in BMD over 2 years (-2.3%, $p < 0.01$). Over 2 years, subjects in the HRT group experienced a significant increase in BMD from baseline (2.6%, $p < 0.001$). Other treatments, etidronate, CT, vitamin D3, and vitamin K2, experienced -0.3%, +1.9%, -2.0%, and +0.3% changes in BMD from baseline, respectively. Logistic regression analysis revealed that changes in BMD at 3 months predicted changes in BMD over 2 years in all treatment groups (odds ratio, 2.39; p=0.01). Changes in NTX and D-Pyr at 3 months significantly predicted changes in BMD over 2 years only in HRT and etidronate groups. In conclusion, HRT is the most effective treatment to reduce bone turnover, increase BMD, and produce a rapid and clinically important reduction in the risk of bone fracture, in postmenopausal women. The data suggest that the clinical utility of BMD change at 3 months to predict future change in BMD in response to pharmacologic treatments in postmenopausal osteoporosis.

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Comparative Effects of Soy Isoflavones, Soy Protein and 17beta-Estradiol on Trabecular and Cortical Bone in Adult Ovariectomized Rats – II. A Histomorphometric Analysis. D. J. Cai,[1] D. M. Cullen,[2] A. C. Peyton,*[2] C. M. Weaver.[1] [1]Foods and Nutrition, Purdue University, West Lafayette, IN, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Several animal and short-term human studies indicated that isoflavones may have estrogenic actions on bone metabolism. However, none of the animal studies have explored the effects of isoflavones on bone histomorphometry. Nor, have the effects of isoflavones on bone been compared to estrogen replacement therapy. This study was designed to use histomorphometric analysis to determine (1) if isoflavone consumption will prevent bone loss similar to estrogen replacement therapy; and (2) whether base diet protein from soy or casein will influence the isoflavone effects on bone metabolism in an adult animal model. Unmated 6 mo. old ovariectomized (OVX) and sham operated female Sprague-Dawley rats were randomly assigned to 9 groups (16 rats/group) and pair-fed for 8 weeks. Estrogen was administered via subcutaneous implants (20-35 pg/L plasma). Diets consisted of casein and/or soy protein with or without isoflavones(Iso) added. Calcein was injected 10 and 3 days before the sacrifice. The proximal and midshaft tibia were processed for histomorphometry. Measurements for trabecular (t), periosteal (p) and endocortical (e) bone included: bone volume (BV/TV), mineralizing surface (MS), and bone formation rate (BFR=MS*MAR) (Table 1). Data were analyzed by ANOVA and Student Newman-Kuels. After ovariectomy, estrogen prevented bone loss in the proximal tibia and suppressed formation on trabecular and cortical surfaces. Isoflavones given as soy protein or supplements did not prevent bone loss or suppress formation. Combining isoflavones with estrogen had no additional benefits. There were no differences in response to isoflavones due to protein source. If increased formation represents increased bone remodeling and bone loss, then we conclude that estrogen, but not isoflavones at the levels tested, suppressed remodeling after ovariectomy.

Treatment	**Base diet**	**Iso (mg/g diet)**	**BV/TV (%)**	**tMS (%)**	**pMS (%)**	**eMS (%)**
Sham	Casein	0	18 ± 4 a	5 ± 2 a	13 ± 9 a	7 ± 6 a

OVX	Casein	0	4 ± 3 b	17 ± 8 b	33 ± 17 b	18 ± 12 b
OVX	Casein	0.3	6 ± 4 b	17 ± 8 b	29 ± 16 b	15 ± 11 b
OVX	Casein	0.8	6 ± 4 b	17 ± 7 b	37 ± 23 b	18 ± 12 b
OVX	Soy	0	7 ± 5 b	15 ± 8 b	39 ± 22 b	17 ± 11 b
OVX	Casein/Soy	0.2	6 ± 3 b	15 ± 6 b	34 ± 20 b	19 ± 14 b
OVX	Soy	0.4	6 ± 2 b	19 ± 7 b	35 ± 19 b	13 ± 11 b
OVX + E2	Casein	0	20 ± 4 a	2 ± 1 a	4 ± 5 a	5 ± 3 a
OVX + E2	Casein	0.3	19 ± 4 a	2 ± 1 a	9 ± 12 a	6 ± 7 a

Values are means ± SD. Means with different letters are significantly different ($P < 0.05$).

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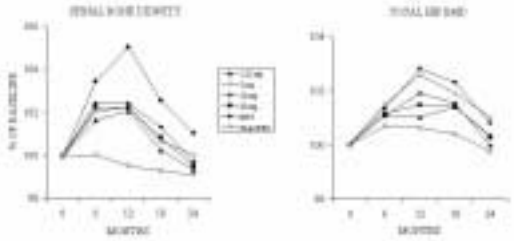
Bone Mineral Density Treatment and After Withdrawal of Levormeloxifene - A SERM. A. Moellgaard, L. Warming,* B. J. Riis,* C. Christiansen. Center for Clinical and Basic Research, Ballerup, Denmark.

BMD was assessed during 12 months of treatment with and 12 months of withdrawal of Levormeloxifene (L). 301 women between 45 and 65 years old were randomized to 12 months of treatment and 233 women completed 12 months of follow-up. The doses were 1.25, 5, 10 or 20mg L per day, HRT (1 mg of 17beta-E2 and 0.5 mg of NETA per day) or placebo. During the treatment phase, spinal BMD increased by approximately 2% in all the L groups (no dose response), 5% in the HRT group and decreased by 1% in the placebo group. In the total hip the changes were smaller, but followed the same pattern. After withdrawal of treatment BMD approached baseline levels, but the HRT group still had about 2 % and 1 % more BMD in the spine and hip, respectively, than the placebo group. It is concluded that L given in doses of 1.25 mg and maybe less prevents the postmenopausal bone loss. The effect is neutralized after 12 months of treatment withdrawal. The development of Levormeloxifene was discontinued in phase III due to gynecologic adverse events. Estradiol combined with NETA showed a double effect that was still present after 12 months withdrawal.



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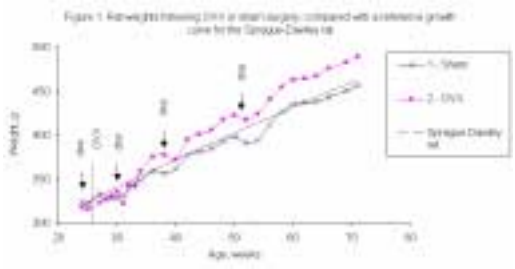
Influence of Experimental Procedures on the Growth Divergence of Ovariectomised and Sham-Operated Rats. P. L. Salmon,[1] C. G. Collier,[1] J. E. Hart,*[1] K. Halonen.*[2] [1]Harwell, Didcot, United Kingdom, [2]Hormos Medical Ltd, Turku, Finland.

Growth curves of female rats diverge after ovariectomy and sham operation: it is customary to observe a relative increase in weight in OVX rats compared to shams. This is due to a disturbance of central growth control by estrogen deprivation (Hart 1990). The extent and timescale of this divergence differ widely between studies. It is important to understand the growth curves of experimental animals so as to exclude any abnormal processes that would compromise the adequacy of a study. A review of the numerous rat OVX studies in the literature is highly informative on the influences of experimental design on relative growth curves of OVXed and sham operated rats. Three main factors influence the timescale and magnitude of this post-OVX weight divergence: age of rats at OVX, pair feeding, and the procedures to which the rats are subject, particularly those involving anaesthesia. The first two, age at OVX and pair-feeding, have a minor effect, only slightly modifying the weight divergence. However, procedures involving anaesthesia have a stronger effect, substantially delaying the onset of and reducing the magnitude of relative post-OVX weight gain - sometimes negating it altogether. Tanizawa *et al.* (2000) showed that following surgical OVX, inflammation delayed the onset of increased bone turnover and bone loss in rats. It can be concluded from this review that, if prompt effects of OVX on bone are to be investigated by a rat OVX study, then anaesthetic procedures should be reduced to a minimum. It is also shown from data from a study by our laboratory, that despite transient weight setbacks after anaesthesia (for DXA), the growth curve of sham operated rats closely tracks the published mean growth curve of the rat strain (Sprague-Dawley), recovering after each anaesthetic procedure to return to parity with the reference growth curve (see figure 1). **References** Hart JE (1990) Endocrine pathology of estrogens: species differences. *Pharmac. Ther.* **47**: 203-218. Tanizawa T, Yamaguchi A, Uchiyama Y, Miyaura C, Ikeda T, Ejiri T, Nagai Y, Yamato H, Murayama H, Sato M, Nakamura T (2000) Reduction in bone formation and elevated bone resorption in ovariectomised rats with special reference to acute inflammation. *Bone* **26** (1): 43-53.



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Prevention of Osteopenia in the Ovariectomized Rat by Phytoestrogens. M. Horcajada-Molteni,*[1] B. Chanteranne,*[1] M. Davicco,*[1] P. Lebecque,*[1] J. Barlet,*[1] P. Pastoureau,[2] V. Coxam.[1] [1]INRA, Theix, France, [2]Laboratoires Servier, Suresnes, France.

In Europe, low incidence of osteoporosis correlates with high levels of fruits and vegetables consumption (the Mediterranean diet). Consequently, polyphenols, very concentrated in such foods, could be involved. We thus investigated the effect of 2 different polyphenols on bone loss in OVX rats. A comparison with a soy isoflavones-rich diet was established. In that purpose, ten controls rats were sham-operated (SH) while 40 were OVX and then fed either a standard diet (OVX), or were supplemented for 3 months with isoflavones (OVXI) (genistein : 159, daidzein : 156, glycitine : 33 mg/g, 0.25% in the diet (Archer Daniels Midland Company, USA), lignans (10% flaxseed in the diet i.e. 0.20% lignans) (OVXL) or rutine, a flavonol (0.25%, OVXR).None phytoestrogen exhibited any uterotrophic effect, the uterine weight being decreased in OVXI, OVXR and OVXL as well. With regards to bone parameters, the decrease in bone mineral density (BMD, g/cm2) demonstrated in OVX (0.2137±0.002 vs 0.2378±0.002 in SH) was prevented by isoflavones (0.2227±0.003) or rutine (0.2341±0.005), while lignans did not elicit any significant effect. However, those 3 diets improved bone strength, as shown by a higher femoral failure load (N) (OVXI : 130±5; OVXL : 150±6; OVXR : 147±5 vs 128±2 in SH and 106±5 in OVX). With lignans, the discrepancy between BMD and bone strength could be explained by protection of bone architecture. In any case, the osteoprotective effect could result from a slower resorption (low deoxypyridinoline values in animals receiving polyphenols), rather than by an increased bone formation.In conclusion, lignans could be an interesting alternative to soy isoflavones in Occidental countries because they are quite ubiquitous in the plant kingdom. Furthermore, different mechanisms could be involved : rutine, a strong antioxidant, but a weak phytoestrogen was very efficient in preventing bone loss in the OVX rats.

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Effects of Triphasic Norgestimate/Ethinyl Estradiol (NGM/EE) on Biochemical Markers of Bone Metabolism in Women with Osteopenia Secondary to Hypothalamic Amenorrhea. S. K. Grinspoon,[1] M. P. Warren,*[2] K. K. Miller,*[1] A. J. Friedman,*[3] W. H. Olson,*[3] T. B. Oei.*[3] [1]Neuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [2]Department of OB/GYN and Medicine, Columbia University, New York, NY, USA, [3]Ortho-McNeil Pharmaceutical, Raritan, NJ, USA.

Hypothalamic amenorrhea (HA) is a disorder associated with osteopenia due to estrogen deficiency. The effects of estrogen on bone in young women with HA have not been established. This double-blind, placebo-controlled, multicenter study evaluated the effects of NGM/EE (ORTHO TRI-CYCLEN®, Ortho-McNeil Pharmaceutical, Raritan, NJ), an oral contraceptive containing 180-250 mcg NGM and 35 mcg EE (21 active days), on biochemical markers of bone resorption (deoxypyrodinoline [DPYR], N-telopeptide [NTX]) and formation (bone specific alkaline phosphatase [BSAP], osteocalcin, procollagen of Type I peptide [PICP]) in osteopenic women with HA. 45 patients (17-40 yrs) with amenorrhea (no menses in the past 3 months and ≤2 menses in the past 12 months), body mass index ranging from 16-24 kg/m^2, and AP lumbar spine ≤0.937 g/cm^2 (T score ≤ -1.0) received NGM/EE or placebo for three consecutive 28-day cycles. Urinary and fasting serum biochemical bone markers were measured at baseline and final visit. Results are shown below.

	NGM/EE				Placebo				
Parameter	**N[a]**	**Baseline[b]**	**Change[b]**	**% Change[b]**	**N[a]**	**Baseline[b]**	**Change[b]**	**%Change[b]**	**P value[c]**
DPYR (nmol/mmol creatinine)	20	6.8 (3.2)	-0.9 (2.8)	-7.6 (33.1)	20	6.7 (2.2)	-0.5 (1.5)	-6.3 (22.4)	NS
NTX (nmol BCE/mmol creatinine)	21	50.0 (20.2)	-9.5 (20.6)	-16.8 (31.8)	20	49.4 (28.6)	1.2 (23.8)	13.0 (57.0)	0.02
BSAP (ng/mL)	20	14.1 (5.8)	-4.6 (3.0)	-32.0 (15.8)	19	12.3 (4.7)	0.4 (30.5)	1.3 (21.3)	<0.0001
Osteocalcin (ng/mL)	21	13.0 (5.8)	-5.4 (3.4)	-41.8 (24.4)	19	14.3 (6.3)	-2.9 (3.7)	-23.7 (26.2)	0.047
PICP (ng/mL)	21	144.1 (69.1)	-39.4 (45.1)	-21.5 (21.5)	19	116.4 (40.0)	-0.2 (30.0	1.1 (30.2)	0.018

[a]Intent-to-treat: subjects who took study-drug and had on-study evaluations [b]Mean(SD) [c]Based on ANCOVA 14 (63.6%) NGM/EE patients and 5 (25.0%) placebo patients experienced adverse events, none serious. Bone turnover decreased in response to NGM/EE versus placebo. This study demonstrates that this oral contraceptive may have beneficial

effects on bone in osteopenic young women with HA.

Disclosures: Ortho-McNeil Pharmaceutical,3.

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Tibolone Treatment in Ovariectomized Macaques. C. J. Lees,[1] A. G. H. Ederveen,[2] T. B. Clarkson.*[1] [1]Wake Forest University School of Medicine, Winston-Salem, NC, USA, [2]Organon NV, Oss, The Netherlands.

The purpose of this study was to examine the effects of two doses of tibolone on the cardiovascular, reproductive and skeletal systems in ovariectomized cynomolgus monkeys and compare these results with macaques treated with conjugated equine estrogens (CEE) with and without medroxyprogesterone (MPA). This report covers the skeletal response to these treatments.144 adult, ovariectomized female cynomolgus monkeys were divided into 5 groups: 1) Placebo (OVX, n=30), 2) Premarin (CEE, 0.042 mg/kg, n=28), 3) CEE with medroxyprogesterone (CEE [0.042 mg/kg]+MPA [0.167 mg/kg], n=28), 4) tibolone (0.2 mg/kg, n=31) and tibolone (0.05 mg/kg, n=27). Treatments were mixed in the food and given daily for24 months. Total alkaline phosphatase (ALP, U/L) was determined at baseline and 24 months after OVX. Lumbar vertebrae 1-5 bone density was determined just prior to necropsy. Lumbar vertebra 2 and right femur were collected at necropsy for strength testing. All treatments suppressed ALP levels when compared to OVX. When covaried for baseline body weight, tibolone treated monkeys had significantly higher bone mineral content and density compared to OVX. Additionally, bone strength was increased in mid-shaft femurs from tibolone treated macaques. Neither CEE nor CEE + MPA treatment affected bone density or bone strength when compared to OVX. No statistical differences were found between treatment groups in lumbar vertebra strength.Tibolone treatment was better than CEE or CEE + MPA in preventing OVX-induced decreases in bone density and in increasing bone strength in the mid-shaft femur. Tibolone was equivalent to CEE and CEE + MPA in decreasing bone turnover, as reflected in decreased ALP levels.

Disclosures: Organon NV, Oss, The Netherlands,2.

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Adherence to HRT after Screening for Osteoporosis Is Independent of Coexistent Climacteric Symptoms. D. W. Purdie, S. A. Steel, P. Albertazzi.* Centre for Metabolic Bone Disease, Hull Royal Infirmary, Hull, United Kingdom.

Long term adherence to hormone replacement therapy (HRT) is difficult to achieve. Published data indicate that over 40% of women with low bone mineral density (BMD) stop taking HRT within 8 months of commencement and this is an argument often deployed against the adoption of universal densitometric screening of postmenopausal women for osteoporosis.As part of an evaluation of the technical and logistical feasibility of densitometric screening of peri- and post-menopausal females, 1462 women aged 50 to 54 years were offered osteoprotective HRT from an agreed menu of regimens, on the basis of low BMD (T score spine <-1.31 or hip <-1.37). Patients returned for 2 year and for 5 year re-assessments. Information on prevalent climacteric symptoms was available in 1293 (88.4%) of the 1462 women studied.A total of 832 (64%) of women offered treatment also reported climacteric symptoms while 461 (36%) were asymptomatic. The latter group thus based their decisions on initial acceptance of, and subsequent adherence to, treatment solely on the basis of the bone density results. In the symptomatic group 631 (76%) accepted the offer of HRT compared to 338 (73%) among the asymptomatic women (Chi-sq test 1.01, p=0.32). Similarly, treatment adherence at 2 years was 408 (65%) and 231 (68%) in the symptomatic and asymptomatic groups respectively (Chi-sq test 1.19, p=0.28) while at five years, adherence had fallen slightly to 58% in both groups ; 319 (symptomatic) and 162 (asymptomatic). Thus two thirds of 'at-risk' women were adherent to treatment at two years and over half were still adherent after five years from bone densitometry screening. These adherence rates are high compared to published data.In conclusion, we report that densitometric screening for osteoporosis positively induces long term adherence to HRT, and that this effect is independent of climacteric symptoms coexisting at the initiation of treatment.

Disclosures: Eli Lilly and Co,5; Wyeth Ayerst,5; Proctor and Gamble,5.

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Periostin Expression Levels Are Suppressed in Bone and Uterus by Estrogen Receptor Agonists. P. V. Nantermet,*[1] D. A. Towler,*[2] S. Harada,[1] A. Schmidt,[1] G. Rodan,[1] J. Ray.[1] [1]Bone Biology & Osteoporosis, Merck&Co, West Point, PA, USA, [2]Internal medicine, Washington University in St Louis, St Louis, MO, USA.

Estrogen replacement therapy is widely used in the prevention of osteoporosis in postmenopausal women. While 17-beta-estradiol (E2) provides the benefit of reducing bone resorption, potential adverse effects include uterine hypertrophy and an increased risk of breast cancer. Selective Estrogen Receptor Modulators (SERMs) that are estrogen receptor agonists for bone but antagonists in the breast and uterus offer the potential to prevent bone loss without adverse effects in the uterus. We have evaluated the in vivo effects of E2 and SERMs on the expression of periostin, a cell adhesion protein secreted by periosteal osteoblasts, in ovariectomized (OVX) rat tibia and uterus, using quantitative real-time PCR. OVX rats have a marked increase in periostin RNA levels compared to sham-OVX animals, suggesting that estrogen negatively regulates periostin expression. In agreement with this observation, E2, as well as the SERMs raloxifene and lasofoxifene, reduce periostin expression 2-fold in bone of OVX rats. This reduction could either be a novel primary effect of ER ligands on periosteal osteoblasts or reflect E2 effects on bone turnover. E2 administration to OVX rats leads to uterine hypertrophy. We also found that periostin is expressed in the uterus and its RNA levels are down regulated 24 hours after E2 treatment. Raloxifene and lasofoxifene had similar effects to E2 in uterine expression of periostin. Thus, periostin is expressed not only in periosteal osteoblasts but also in the uterus. In both tissues, expression levels are negatively regulated by E2 and selected SERMs.

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Correlation Study on Effects of Levonorgestrel-Releasing Subdermal Contraceptive Implants on Quantitative Ultrasound of Calcaneus and Bone Mineral Density of Femur and Spine. D. Xiao,*[1] Y. Z. Li,[1] Y. Xue,[2] J. Li,[2] L. Zhu,[2] J. W. Jiang,[1] S. J. Gu.[1] [1]Beijing Obstetrics and Gynecology Hospital Affiliated to Capital Medical University, Beijing, China, [2]Beijing Research Institute of Traumatology & Orthopaedics and Beijing Ji Shui Tan Hospital, Beijing, China.

A prospective, randomized clinical trial observed the effects of Norplant ® long-term contraceptive implants and domestic implants similar to Norplant on quantitative ultrasound (QUS) of calcaneus and on bone mineral density (BMD) of both proximal femur and lumbar (L2-4) measured by dual-energy X-ray absorptiometry (DXA) in female acceptors for one year. Thirty-eight normal women of child-bearing age (25-40 years) were divided into two groups, 18 in the Norplant implant group, and 20 in the domestic implant group. Speed of Sound (SOS) of calcaneus and Stiffness (STI) in the Norplant implant group 12 months after implant insertion were significantly increased (P<0.05). BMD and bone mineral content (BMC) of proximal femoral trochanter in the domestic implant group were also significantly increased 12 months after implantation (P<0.01, P<0.05). The increase of lumbar 2-4 BMD in the subgroup of women at age >34 in the domestic implant group was significant different after implant insertion (P<0.05). There were moderate positive linear correlations among Broadband Ultrasound Attenuation (BUA) of calcaneus and STI and BMD of femoral trochanter in the Norplant implant group before and 12 months after implant insertion, correlation coefficients being r=0.4861(P<0.05) respectively and r=0.6136 (P<0.01), and correlation coefficients 12 months after insertion being r=0.7036(P<0.01) and r=0.6733(P<0.01) respectively. There was positive correlation between BUA of calcaneus and BMD of lumber 2-4 in the subgroup of women at age <34 in the Norplant implant group before and 12 months after implant insertion (r=0.6144, r=0.8211 P<0.05). The effects of levonorgestrel-releasing subdermal contraceptive implants on QUS increase of calcaneus and BMD increase of both femur and spine in premenopausal women were observed. There was moderate positive correlation between calcaneal QUS and femoral BMD measured by DXA in premenopausal women. The correlation was strengthened after the contraceptive implant insertion up to a certain extent. However, more studies about this are needed.

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HRT/ERT Alters Estrogen Catabolism and Catabolic Products May Predict Response to Therapy. R. C. Villareal,[1] R. Leelawattana,*[2] T. Klug,*[3] R. Civitelli.[1] [1]Medicine, Washington University School of Medicine, St. Louis, MO, USA, [2]Medicine, Washington University, St. Louis, MO, USA, [3]Immuna Care Corporation, Bethelehem, PA, USA.

We have previously demonstrated that estrogen catabolism is a determinant of bone density after menopause. Increased hydroxylation to the inactive estrogen metabolites, 2-hydroxyestrogen (2OHE1) and 2-methoxyestrogen (2MeOE1), is associated with low bone mineral density (BMD), while increased hydroxylation to active metabolites, 16a-hydroxyestrogen (16aOHE1) and estriol (E3) is associated with higher BMD. In this study, we tested the hypothesis that hormone/estrogen replacement therapy (HRT/ERT) alters estrogen metabolism by increasing 16aOHE1 and E3, and that these changes may predict response to HRT/ERT. Urinary estrogen metabolites were measured from the urine of 311 postmenopausal women using ESTRAMET immunoassay kit (Immuna Care Corp., Bethlehem, PA, U.S.A.). One hundred fifty-one of these women were on HRT with conjugated equine estrogen and medroxyprogesterone acetate (Premarin and Provera), or ERT with conjugated equine estrogen alone (Premarin), and 71 women had more than one BMD measured by DEXA while on HRT/ERT before measurement of urinary estrogen metabolites. Annual rates of change in BMD were calculated for these women. Controlling for age, years since menopause, body mass index, waist to hip ratio, and smoking, we found that urinary 2OHE1 was significantly higher in HRT/ERT-treated compared to untreated women (14.88+0.798 vs 4.82+787, P=<0.001). Although there were no significant differences in other urinary metabolites including 16aOHE1, 2MeOE1, and E3, the 2OHE1/16aOHE1 ratio was higher in HRT/ERT treated than untreated women (10.23+0.965 vs 3.75+0.948, P=<0.001). Among women on HRT/ERT, women in the highest quartile of 2OHE1/16aOHE1 ratio (>6.9) had greater increments in BMD of the total femur and femoral neck compared to those in the lower quartiles. In conclusion, administration of conjugated equine estrogens alter estrogen catabolism favoring the inactive 2-hydroxyl pathway and an elevated 2OHE1/16aOHE1 ratio may predict a good response to HRT/ERT.

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Effects of Intermittent Administration of Parathyroid Hormone on Tibial Cancellous Bone Loss in Tail-Suspended and Sciatic Neurectomized Young Rats. I. Moriyama,[1] J. Iwamoto,[2] K. Matsuzaki,[1] T. Takeda,[2] Y. Toyama.[1] [1]Orthopaedic, Keio University, Tokyo, Japan, [2]Sports Clinic, Keio University, Tokyo, Japan.

The aim of this study was to determine whether intermittent administration of parathyroid hormone (PTH) could prevent cancellous bone loss in young rats with tail-suspension plus and sciatic neurectomy. Fifty-six 6-week-old male Wistar rats were randomly divided into 7 groups of 8 animals each: age-matched controls, tail-suspension (TS), bilateral sciatic neurectomy (NX), TS + NX, TS + PTH administration (TS+P), NX + P, and TS+NX +P. Human PTH (1-34)(80ug/kg) or vehicle was injected subcutaneously every day. On day 15, the left proximal tibial metaphysis were processed for static and dynamic bone histomorphometric analyses. The right limb gastrocnemius muscle of each animal was removed and weighed immediately. Both TS and NX significantly reduced the weight of the gastrocnemius muscle (by 19% and 55%, respectively), and resulted in the significant reductions of cancellous bone volume (BV/TV, by 91% and 46%, respectively) and bone formation rate (BFR/BS, by 60% and 36%, respectively). PTH administration to TS and NX rats significantly increased BV/TV to a level significantly higher than that of age-matched controls as a result of significant increase in BFR/BS. TS+NX accelerated the

reductions of the weight of gastrocnemius muscle, BV/TV, and BFR/BS (69%, 94%, and 91%, respectively), and resulting in a severe cancellous bone loss with a marked decrease in bone formation. However, PTH administration to TS+NX rats could restore BV/TV to a level not sifnificantly different from that of age-matched controls as a result of a significant increase in BFR/BS. Osteoclast number (N.Oc/BS) and eroded surface (ES/BS) did not significantly altered either by TS, NX, and TS+NX, or by PTH administration for them. These findings suggest that although TS+NX markedly decrease bone formation and result in a severe cancellous bone loss, intermittent administration of PTH may have the potential to completely prevent cancellous bone loss induced by TS+NX as a result of an increase in bone formation. We conclude that intermittent administration of PTH appeared to be useful for the prevention of osteoporosis induced by the decrease in mechanical stress i.e. body weight loading and/or muscle force.

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Cortical Bone Is More Responsive to Intermittent PTH (1-34) Administration than Cancellous Bone in Mice With or Without Estrogen Depletion. H. Zhou, A. Iida-Klein, S. S. Lu, M. Ducayen-Knowles,* D. W. Dempster, R. Lindsay. Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA.

Parathyroid hormone (PTH) exerts anabolic action on the skeleton of rats and humans. However, the effects of PTH on mice have not been well characterized. Furthermore, the effects of estrogen depletion in mouse skeleton have yet to be determined. Given the advantages of murine models for molecular biology studies, we have examined the effects of PTH on the tibiae of mice, with or without estrogen depletion. Twelve-week-old C57BL/J6 mice were sacrificed (n=6) and the rest were ovx or sham operated at baseline. Ovx and sham groups (n =5) were sacrificed after 2 and 4 weeks. After 4 weeks, PTH (40µg/kg/d, 5d/week, s.c)was given to the ovx and sham groups, and vehicle to the controls for 3 to 7 weeks (n=5/group). Calcein and demeclocycline were given s.c. 9 and 3 days prior to sacrifice. Histomorphometry was performed on the cancellous and cortical bone of proximal tibiae and cortical bone of tibial diaphysis. There were no age-related changes of cancellous and cortical bone structure from 12 to 23-weeks of age. Four and 11 weeks of estrogen deficiency decreased cancellous bone volume and trabecular number with increases in osteoclast and osteoblast perimeters and mineral apposition rate (MAR). Seven to 11 weeks of estrogen deficiency increased endocortical osteoid perimeter, MAR and bone formation rate (BFR) with no change in periosteal BFR and cortical width in the proximal tibia. In the tibial diaphysis, ovx enhanced endocortical resorption at 2 to 4 weeks. In the intact mice, PTH enhanced proximal tibial trabecular width at 7 weeks and cortical width at 3 to 7 weeks with increases of endosteal osteoclast, osteoblast, osteoid and mineralized perimeters (Md.Pm), MAR and BFR. Periostreal bone formation was unchanged. In the diaphysis, PTH decreased medullary area and increased cortical width at 7 weeks due to increases in periosteal Md.Pm, MAR and BFR at 3 to 7 weeks, and endocortical Md.Pm and BFR at 7 weeks. PTH did not restore ovx-induced cancellous bone loss but increased cortical width in the proximal tibia at 7-weeks. In the diaphysis, PTH increased periosteal Md.Pm, MAR and BFR and mitigated the ovx-induced endocortical resorption at 3 weeks, but not at 7 weeks; this resulted in a trend towards increase in cortical width, cross sectional area and cortical area. We conclude that the ovx mouse represents a good model of estrogen-deficiency bone loss. The beneficial effect of intermittent PTH administration is more pronounced in cortical than cancellous bone of tibia in the mice in agreement with recent observations in humans.

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Twice-Weekly Injection of a Sustained-Duration PTH Construct Increases Bone Density in Osteopenic Ovariectomized Rats. P. J. Kostenuik,[1] S. Morony,[1] K. S. Warmington,*[1] M. V. Porkess,*[1] Z. Geng,[1] S. Adamu,[1] V. Shen,[2] J. Delaney,*[3] T. Boone,*[3] D. L. Lacey.[1] [1]Pharmacology/Pathology, Amgen, Inc., Thousand Oaks, CA, USA, [2]Skeletech, Inc., Bothell, WA, USA, [3]Process Science, Amgen, Inc., Thousand Oaks, CA, USA.

A PTH construct that increases bone mass with infrequent SC injections could be an attractive option for the treatment of osteoporosis. Towards this goal, we recombinantly manufactured a sustained-duration PTH construct (SD-PTH) with a sustained half-life compared to PTH-(1-34). We tested the efficacy of SD-PTH, alone and in combination with the bisphosphonate APD, in aged ovariectomized (OVX) rats. SD rats were OVXd (n=54) or sham-operated (n=13) at 4 months of age. Eleven months later, DEXA analysis revealed significant osteopenia in the lumbar vertebra (LV) and femur of OVX rats compared to shams. OVX rats were then treated 2X/week (SC) for 4 weeks with vehicle (PBS), or with SD-PTH (1.5 mg/kg), or with the bisphosphonate APD (0.75 mg/kg), or with SD-PTH + APD (n=12-14/group). Shams were treated with vehicle. SD-PTH alone, but not APD alone, significantly increased femoral BMD within 3 weeks. SD-PTH + APD significantly increased femoral BMD within 2 weeks. In the LV, APD alone and SD-PTH alone caused modest and non-significant increases in BMD. SD-PTH + APD significantly increased LV BMD within 3 weeks compared to vehicle-treated rats. SD-PTH treatment was associated with mild hypercalcemia which resolved after 10 days. This hypercalcemic response was associated with a similarly transient increase in serum TRAP, a marker of osteoclast activity. Co-treatment with APD blocked the SD-PTH-induced increases in serum calcium and TRAP. SD-PTH also significantly increased serum osteocalcin, and APD co-treatment blunted this response. Mechanical testing of LV4 revealed significant decreases in stiffness, max load, ultimate strength and elastic modulus in OVX rats vs. shams. SD-PTH treatment partially reversed these deficits in mechanical strength, suggesting that bone formed during SD-PTH treatment is mechanically competent. In summary, SD-PTH as a monotherapy caused significant increases in BMD which were associated with mild and transient hypercalcemia. The coadministration of the bisphosphonate APD prevented this hypercalcemia and caused earlier increases in BMD compared to APD or SD-PTH alone. These data suggest that SD-PTH might represent an attractive therapeutic option for the treatment of osteoprorosis. The therapeutic index of SD-PTH therapy might be significantly increased with the co-administration of an antiresorptive agent such as a bisphosphonate or osteoprotegerin.

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BIM-44058, A Novel PTHrP Analog, Restores In Vivo Spinal Bone Mineral Density in Old Ovariectomized Osteopenic Cynomolgus Monkeys. J. Legrand,[1] C. Fisch,[2] P. Guillaumat,[2] S. de Jouffrey,*[2] J. Z. Dong,*[3] C. W. Woon,*[3] J. Claude,*[4] M. D. Culler.*[3] [1]Beaufour-Ipsen, Paris, France, [2]CIT, EVREUX, France, [3]Biomeasure Inc., Milford, MA, USA, [4]Faculty of Pharmacy, Paris, France.

BIM-44058, a novel analog of human PTH-related protein (1-34) ($hPTHrP_{1-34}$), was evaluated for bone anabolic activity in old ovariectomized, osteopenic cynomolgus monkeys. Old female monkeys from Mauritius, weighing an average of 4.2 $\pm$ 0.1 kg, were either ovariectomized (OVX) or sham-operated (SHAM), and were maintained for 9 months to allow development of osteopenia in the OVX animals. The OVX, osteopenic animals were treated daily by subcutaneous injection with either vehicle (saline) (n=7) or BIM-44058 at the doses of 0.1 µg/kg/day for 6 months (n=7), 1 µg/kg/day for 10 months (n=10) or 10 µg/kg/day for 10 months (n=8). BMD was measured by dual energy X-ray absorptiometry and blood and urine were sampled for determination of biochemical markers of bone metabolism (alkaline phosphatase, osteocalcin, urinary deoxypyridinoline) both before surgery and every 3 months thereafter. The mean body weight of the animals increased 17% over the 21 months of the study. The lumbar vertebral BMD of SHAM animals decreased very slightly at 3 months post-surgery but returned to pre-surgery levels and remained unchanged through the end of the study. The BMD of OVX animals decreased rapidly, reaching circa -10% by 9 months after surgery, and then remained relatively stable. BIM-44058, at 10 µg/kg/day, fully restored, and at 1 µg/kg/day, restored 97% lumbar vertebral BMD of OVX, osteopenic animals within 6 months. The BMD levels of these animals remained close to the SHAM values through the remainder of the study. Biochemical markers of bone metabolism were increased in OVX animals 3 months after surgery, and remained higher than in SHAM animals but relatively stable through the end of the study; thus, reflecting higher bone turnover. BIM-44058 treatment induced a second increase in these biochemical markers; however, alkaline phosphatase and osteocalcin, both markers of bone formation, were increased following the initiation of BIM-44058 treatment, while deoxypyridinoline, a marker of bone resorption, was increased only during the last 3 months of treatment. These results demonstrate that BIM-44058 effectively restores BMD in old, osteopenic monkeys, and suggest that BIM-44058 treatment initially induces a selective increase in bone formation.

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BIM-44058, A Novel PTHrP Analog, Does Not Increase Total Plasma Calcium in Cynomolgus Monkeys at an Effective Pharmacological Dose. J. Legrand,[1] P. Guillaumat,[2] R. Forster,[2] J. Z. Dong,*[3] C. W. Woon,*[3] J. Claude,*[4] M. D. Culler.*[3] [1]Beaufour-Ipsen, Paris, France, [2]CIT, EVREUX, France, [3]Biomeasure Inc., Milford, MA, USA, [4]Faculty of Pharmacy, Paris, France.

BIM-44058, a novel analog of human parathyroid hormone (hPTH)-related protein (1-34) ($PTHrP_{1-34}$), has less calcium mobilizing potential than $hPTH_{1-34}$ at high doses in rats. The purpose of the present study was to determine if BIM-44058 has less potential to induce hypercalcemia than $hPTH_{1-34}$ in primates. Four male and four female, young, cynomolgus monkeys were utilized for this study. In order to establish the control baseline for the parameters of total plasma calcium and inorganic phosphorus, blood samples were collected from each animal on 4 separate days at 15 min before and 0.5, 1, 2, 4, 8, 12, and 18 hours after an injection of physiological saline. To compare the effects of $hPTH_{1-34}$ and BIM-44058, one male and one female monkey each received daily subcutaneous injections of either 0.75 µg/kg $hPTH_{1-34}$, or 0.75, 7.0 or 17.5 µg/kg BIM-44058 for three days. Blood samples were collected at the same time intervals as utilized in the baseline study following the injections on treatment days 1 and 3. No differences were observed in the response of male and female animals in either total plasma calcium or inorganic phosphorus. At 2 and 4 hours after the injection of 0.75 µg/kg $hPTH_{1-34}$, total plasma calcium was increased up to or slightly above the upper baseline range. Plasma phosphorus levels remained within the baseline boundaries. At the equivalent dose, BIM-44058 had no effect on total plasma calcium levels. Slight increases in total plasma calcium levels were observed only with the higher doses of 7.0 and 17.5 µg/kg BIM-44058. As with $hPTH_{1-34}$, the maximal increase in calcium levels was reached 2 to 4 hours after administration and remained within or slightly above the upper baseline range. At subsequent time points following BIM-44058 administration, total calcium levels were decreased below the baseline range. Plasma inorganic phosphorus levels were slightly increased at nearly all time points after administration of BIM-44058, but remained within the baseline range. The 0.75 µg/kg dose of BIM-44058 that caused no change in total calcium levels is close to the dose of 1 µg/kg/day that has been demonstrated to effectively restore bone mineral density in a model of ovariectomy-induced established osteopenia in this same species. Thus, the results from this study demonstrate that BIM-44058 does not cause hypercalcemia at the effective bone anabolic dose in primates. These findings confirm that BIM-44058 has a higher safety margin than $hPTH_{1-34}$ when used in an intermittent administration regimen.

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BIM-44058, A Novel PTHrP Analog, Increases Bone Formation But Not Bone Resorption Histomorphometric Parameters in Old Ovariectomized Osteopenic Cynomolgus Monkeys. J. Legrand,[1] A. Bécret,[2] C. Fisch,[2] M. Attia,*[2] S. de Jouffrey,*[2] J. Z. Dong,*[3] C. W. Woon,*[3] J. Claude,*[4] M. D. Culler.*[3] [1]Beaufour-Ipsen, Paris, France, [2]CIT, Evreux, France, [3]Biomeasure Inc, Milford, MA, USA, [4]Faculty of Pharmacy, Paris, France.

PTH_{1-34} effectively stimulates bone formation; however, concomitant stimulation of bone resorption, especially in cortical regions, remains an issue. The effects of BIM-44058, a novel analog of $PTHrP_{1-34,}$ were evaluated on bone histomorphometric parameters in old, ovariectomized (OVX), osteopenic, cynomolgus monkeys. Old female monkeys (approx. 4 kg) were either OVX or sham-operated (SHAM), and maintained for 9 months to allow development of osteopenia in the OVX animals. The OVX, osteopenic animals were then treated by daily subcutaneous injection of either saline (n=7) or BIM-44058 (n=8) at a dose

of 10 µg/kg. Biopsies of the iliac crest and rib were obtained following dual calcein labeling at 6 months of treatment. The bone samples were cut, undecalcified, and either stained with Goldner stain or examined under fluorescence to quantify both static and dynamic endpoints of bone remodeling using validated image analysis software. As expected, both resorption (ES/BS, Ct.Po.) and formation (ObS/BS, OS/BS, MS/BS, MAR and BFR) parameters were higher in the vehicle-treated, OVX group as compared with the SHAM group. BIM-44058 increased formation parameters as compared with the vehicle-treated OVX controls; however, resorption markers were decreased and were similar to those observed in SHAM controls (Table: mean ± SEM (number evaluated); *: parameter quantified at iliac crest).

Group	**SHAM**	**OVX / Vehicle**	**OVX / BIM-44058**
Dose (µg/kg)	**-**	**0**	**10**
ObS/BS (%)*	5.86 ± 0.76 (12)	5.62 ± 2.00 (6)	16.08 ± 3.03 (8)
OS/BS (%)*	16.70 ± 2.17 (12)	27.70 ± 4.57 (6)	44.77 ± 3.90 (8)
MS/BS (%)*	11.51 ± 2.45 (12)	22.20 ± 4.19 (6)	40.20 ± 4.49 (8)
MAR (µm/day)*	0.56 ± 0.08 (12)	0.83 ± 0.04 (6)	1.00 ± 0.11 (8)
BFR (μm^2/µm/day)*	8.03 ± 2.27 (12)	18.68 ± 4.13 (6)	41.12 ± 6.15 (8)
ES/BS (%)*	4.86 ± 0.66 (12)	6.62 ± 1.24 (6)	6.49 ± 0.93 (8)
Ct. Po. (%)*	1.23 ± 0.23 (13)	2.39 ± 0.69 (7)	0.94 ± 0.31 (8)
Ct. Po. (rib) (%)	2.41 ± 0.67 (13)	6.46 ± 1.00 (7)	4.50 ± 1.01 (8)

These results demonstrate that treatment with BIM-44058 for 6 months at the dose of 10 µg/kg increases bone formation at trabecular sites, but decreases bone resorption at both trabecular and cortical sites in old, OVX, osteopenic, cynomolgus monkeys.

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Effects of Combined Treatment of Hindlimb Unloaded Rats with PTH and Tamoxifen. G. L. Evans,*[1] A. M. Kennedy,*[1] E. Morey-Holton,[2] R. T. Turner.[1] [1]Orthopedic Research, Mayo Clinic, Rochester, MN, USA, [2]NASA Ames Research Center, Moffett Field, CA, USA.

Hindlimb unloading (HUL), an earth-based model for spaceflight, results in cancellous bone loss in rats. This bone loss is due to a combination of decreased bone formation (BFR) and increased bone resorption. Parathyroid hormone (PTH) has been shown to have limited beneficial effects in this model; the hormone maintained cancellous bone volume (BV/TV) by increasing trabecular thickness (Tb.Th). Treatment was ineffective in preventing the decrease in trabecular number (Tb.N). The purpose of the present study was to determine the effects of combining the selective estrogen receptor modulator tamoxifen (TAM), a known inhibitor of bone resorption, with PTH. Six-month-old male Fisher 344 rats were HUL for 14d. The treatment groups were as follows: Control (vehicle s.c. injection and placebo pellet), TAM (10 mg/pellet), PTH (s.c. injection 80 µg/kg), combination (10 mg pellet and s.c. injection 80 µg/kg), placebo (s.c. injection vehicle and 10 mg/placebo pellet). Fluorochrome labels were administered 0, 6, and 12 days prior to sacrifice. Static and dynamic histomorphometry were evaluated in the tibia. The cortical static measurements resulted in no significant difference between groups. The cortical dynamic measurements showed a significant decrease between the TAM treated rats vs the placebo rats. HUL resulted in the expected decreases in BV/TV and BFR. PTH increased BFR and Tb.Th compared to vehicle, whereas TAM decreased BFR and increased Tb.N. Combination treatment of HUL rats preserved normal BV/TV, Tb.Sp and Tb.N and increased Tb.Th. These results demonstrated that combining an anabolic agent with an antiresorbing agent is effective in maintaining Tb.N as well as Tb.Th in HUL rats.

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Intermittent hPTH Administration Prevents the Reduction of Trabecular Bone Volume Due to Skeletal Unloading. S. Tanaka,* A. Sakai,* H. Tsurukami,* S. Ikeda,* T. Sakata,* S. Uchida,* T. Nakamura. Department of Orthopaedic Surgery, University of Occupational and Environmental Health, Kitakyushu, Japan.

To clarify the effects of intermittent hPTH(1-34) administration on trabecular bone turnover and bone marrow cell development in skeletal unloaded limbs, we performed experiments with tail-suspended mice.Eighty C57BL/6J male mice, 8 weeks of age, were assigned to 4 weight-matched groups (Groups 1, 2, 3 and 4; n = 20 each). Mice of Groups 3 and 4 were tail suspended. Mice were given subcutaneous injections of hPTH five times a week at the respective dose of 0 (vehicle) for Groups 1 and 3, 40 micro gram /kg body weigh for Groups 2 and 4. Bilateral tibia were harvested at 8 days and 15 days after the start of the experiment. We performed histomorphometric analyses on the proximal metaphyses and bone mallow cell cultures. Bone histiomorphometry: At 8days, the value of trabecular bone volume (BV/TV) and bone formation rate (BFR/BS) of Group 3 was significantly reduced compared to that of Group 1. But, the values of Group 4 was larger than that of Group 3, maintaining at the similar level as that of Group 1. At 15 days, the value of BV/TV of Group 3 was significantly reduced. BV/TV value of Group 4 reduced to the level of Group 3. While BFR/BS values of Group 4 maintained larger compared to Group 3, the value of Oc.N/BS significantly increased compared to the values of Groups 2 and 3.Bone marrow cell: At 8 days. The number of ALP positive CFU-f colonies of Group 3 reduced, and that of Group 2 increased compared to Group 1. In Group 4, the value also increased to Group 1. The number of TRAP positive cell developed from bone marrow cell culture of Group 3 did not differ, and that of Group 2 increased compared to that of Group 1. In Group 4, however, the value further increased compared to that of Group 2.These data clearly indicated that the bone mass increasing effect of intermittent hPTH administration reduced during the unloading of the skeleton. Intermittent hPTH injections increased osteogenic and osteoclastogenic activities in bone marrow cells, consequently leading to bone mass increase in the ground condition. But, during unloading, the increase in osteoclastogenic activity seemed to further increase with passage of time, leading to alleviate the bone mass increasing action of the agent.

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Oral Delivery of PTH Analogs by a Solid Dosage Formulation. N. Mehta,* W. Stern,* A. Sturmer,* A. Bolat,* J. Chen,* J. Gilligan. Unigene Laboratories Inc, Fairfield, NJ, USA.

Oral delivery of peptide hormones is a convenient regimen for chronic administration and is known to increase patient compliance. We have developed a proprietary formulation in enteric-coated capsules that allows for the absorption of peptide hormones from the duodenum into the bloodstream. Depending on the size, charge, and structure of the peptide hormone, we have shown previously that bioavailabilities as high as 10% can be obtained in dog and human studies. Using salmon calcitonin as a model peptide, previous results have demonstrated that there is a linear correlation between the bioavailabilities in dog and man using this oral capsule technology. We have formulated PTH(1-34) and a PTH analog of equivalent bioactivity, PTH131A, using this technology for enteric-coated capsules. The bioavailability and pharmacokinetic parameters following oral administration of these peptides in dogs was evaluated. Plasma PTH(1-34) was quantified by RIA using a kit from Peninsula Laboratories. Plasma PTH131A was measured by an internally developed competitive ELISA with a sensitivity of 100 pg/ml.Administration of an oral capsule containing PTH(1-34) to each of eight dogs gave a mean Cmax of 379 pg/ml ± 152 SEM per mg of peptide. The shorter PTH analog (PTH131A) gave a mean Cmax of 2155 ± 456 SEM per mg. The significantly higher bioavailability of the PTH131A analog makes it a suitable candidate for the development of an oral PTH formulation as an anabolic agent for the treatment of osteoporosis and related bone disorders. An acute serum pharmacokinetic profile was observed following oral administration, and this is consistent with the need for pulsatile dosing regimens for PTH-related compounds in order to observe an anabolic effect.

Disclosures: Unigene Laboratories Inc,1.

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Anabolic Effects of Various Forms of PTH on the Femur of Young, Male Albino Rats. L. E. Borella,* W. Zhao,* R. Murrills, M. Tarby, F. Bex. Bone Metabolism, Wyeth-Ayerst Research, Radnor, PA, USA.

It is well known that intermittent injections of human parathyroid hormone (1-34) to humans and experimental animals produces a strong anabolic effect on the skeleton. Among the laboratory animals, the ovariectomized rat is the most commonly used species for assessment of PTH-induced bone activity. Its use is targeted towards reversing bone loss due to estrogen deficiency. The present investigation focuses on the bone anabolic activity profile of PTH in the intact, young growing male rat under normal conditions of bone development. Male albino Sprague Dawley rats weighing 40 g were injected SC once daily for 14 days with hPTH(1-34) and other PTH analogues at doses ranging from 15 micrograms/kg/day to 300 micrograms/kg/day. Femurs were analyzed by pQCT and by total femoral ash weight(850F for 18 h). At doses from 45 to 300 micrograms/kg, hPTH(1-34), hPTH(1-31) and hPTHrp caused statistically significant increases in ash weight of up to 15% with little differences between the active doses. The lowest dose of all three PTH analogues, 15 micrograms/kg, was inactive. In contrast, in the same regimen, hPTH(3-34), hPTH(2-34)and hPTH(2-38) at doses up to 750 micrograms/kg were inactive. Total, trabecular and cortical BMD (pQCT)of femurs of young rats treated with a daily dose of 100 micrograms/kg hPTH(1-34) for 14 days were significantly ($p<0.05$)increased by 27%, 31% and 4%, respectively. Histomorphometry of the same bones showed a marked 31% ($p<0.05$) increase in cortical thickness by hPTH(1-34). Mineral apposition rate (MAR) in the periosteum was significantly increased by 38% ($p<0.05$), but less in the endosteum (18 % NS) with respect to the vehicle-treated control group. The present results indicate that the young growing male rat represents a sensitive model for the bone anabolic effects of PTH.

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BIM-44058, a Novel Analog of PTHrP With Enhanced Bone Building Activity, but Decreased Calcium-Mobilization Potential. M. D. Culler,*[1] J. Dong,*[1] Y. Shen,*[1] J. E. Taylor,*[1] L. Carlile,*[1] T. Sullivan,*[1] I. Batista,*[1] P. Bonin,*[1] M. Carlson,*[1] J. Lauer,*[1] A. Savola,*[1] P. Kasprzyk,*[1] B. A. Morgan,*[1] C. Fisch,*[2] A. Bécret,*[2] J. J. Legrand,*[3] C. W. Woon.*[1] [1]Biomeasure, Inc., Milford, MA, USA, [2]CIT, Evreux, France, [3]Beaufour-Ipsen, Paris, France.

PTH_{1-34} effectively stimulates bone formation; however, concomitant stimulation of bone resorption, especially in cortical regions, and induction of transient hypercalcemia, remain areas of concern. We have identified a novel analog of parathyroid hormone-related protein (1-34) ($PTHrP_{1-34}$), BIM-44058, that retains the potent anabolic activity of PTH, but has reduced calcium-mobilizing potential. In HEK293 cells transfected with the human PTH-1 receptor, BIM-44058 stimulates cAMP accumulation in a dose-related manner with an ED_{50} 2.4x lower (0.17 ± 0.06 nM) than that of PTH_{1-34} (0.40 ± 0.16 nM) and 2.8x lower than it's parent compound, $PTHrP_{1-34}$ (0.48 ± 0.13 nM). Like $PTHrP_{1-34}$, BIM-44058 has minimal activity at the human PTH-2 receptor. The *in vivo* calcium mobilizing potential of BIM-44058 and PTH_{1-34} were compared in rats that were maintained on a calcium-free diet for 4 days and were then parathyroidectomized immediately before injection of the test compound. Measurement of total plasma calcium 6 hours later revealed that both PTH_{1-34} and BIM-44058 stimulated dose-related increases in plasma calcium as compared with vehicle-treated controls; however, at higher doses the calcium mobilizing activity of BIM-44058 plateaued while increasing doses of PTH_{1-34} continued to increase plasma calcium. These results suggest that BIM-44058 may have a wider safety margin than PTH_{1-34} in terms of inducing hypercalcemia. To compare the anabolic activity of BIM-44058 with that

of PTH_{1-34}, the two compounds were administered by daily subcutaneous injection for 4 weeks into ovariectomized, osteopenic rats. Both peptides produced dose-related increases in BMD; however, in keeping with it's higher receptor activating activity, BIM-44058 fully restored femoral BMD to the level of vehicle-treated, sham-operated controls at a dose 2x less than required for PTH_{1-34}. Histological analysis of the femurs revealed high-quality, mature bone growth in response to BIM-44058. These results demonstrate that BIM-44058 is a potent bone anabolic agent with reduced potential for hypercalcemia. These qualities may render BIM-44058 more amenable than PTH_{1-34} to non-parenteral delivery.

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Correlation of the Effects of hPTH on Established Osteopenia in Female Swiss Webster Mice as Measured by pQCT, MicroCT and Histology. Y. Kharode, L. Borella, R. Murrills, V. Dell,* J. Marzolf,* F. Bex. Bone Metabolism, Wyeth-Ayerst Research, Radnor, PA, USA.

Ovariectomy (ovx) induces significant and rapid bone loss in rodents that can be reversed by treatment with human parathyroid hormone 1-34 (hPTH). To further investigate the effects of hPTH in female Swiss Webster mice with established osteopenia, we compared the results of skeletal measurements by pQCT, µCT and histology on the distal femur. Eight week old Swiss Webster mice (n=36) were allowed to develop osteopenia for three months. A group of age matched sham ovx animals (n=24) served as controls. 12 ovx and 12 sham mice were sacrificed at the end of the three month post-ovx period and femoral density measurements were performed. Following confirmation of significant osteopenia as judged by total and trabecular density measurements using pQCT in the post-ovx mice, two groups of 12 ovx mice were administered either 2 mg hPTH /day, S.C. or vehicle for 20 days. Post-treatment pQCT measurements revealed that the ovx vehicle group had significantly lower total and trabecular femoral density than the sham group (502+/-20 mg/cm^3 vs. 651+/-17 mg/cm^3 and 210+/-16 mg/cm^3 vs. 436+/-26 mg/cm^3,respectively). Values for total and trabecular density in ovx mice treated with hPTH were significantly greater than those of the ovx-vehicle group (588+/-26 mg/cm^3 and 314+/-37 mg/cm^3 respectively). Histological assessment revealed that the percent bone mineral area of the distal femoral metaphysis of the ovx-vehicle group was significantly reduced compared to that of the sham group (2.78+/- 0.50 and 9.45+/-1.13, respectively). Treatment with hPTH significantly increased the percent bone mineral area (6.32 +/- 1.26) over that of the ovx vehicle controls. µCT was performed on the distal femur in the same region as the histological analysis. Bone volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and connectivity density (CD) were significantly higher while trabecular spacing (Tb.Sp) was significantly lower in the sham group compared to the ovx-vehicle group. In hPTH treated ovx mice, a significant increase over the ovx-vehicle was observed for BV/TV (0.178+/-0.02 vs. 0.123+/-0.008 for ovx-vehicle) and Tb.Th (0.064+/-0.001 vs. 0.053+/-0.002 for ovx-vehicle). A very highly significant correlation (R) was observed between the skeletal analytical techniques: pQCT and histology R=0.885; pQCT and µCT (BV/TV) R=0.8674; µCT (BV/TV) and histology R=0.9131. We conclude that in Swiss Webster mice with established osteopenia hPTH significantly increases bone density, bone volume and trabecular thickness in the distal femur and that a very high correlation is observed between pQCT, µCT and histological measurements of these effects.

Disclosures: Wyeth-Ayerst Research,1,3.

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The Impact of Incident Vertebral and Non-Vertebral Fractures on Health Related Quality of Life (HRQOL) in Established Postmenopausal Osteoporosis: Results from the rhPTH(1-34) Randomized, Placebo-Controlled Trial in Postmenopausal Women. M. E. Minshall,*[1] S. L. Silverman,[2] W. Shen,*[1] S. Xie.*[1] [1]Eli Lilly and Company, Indianapolis, IN, USA, [2]UCLA, WLA-VAMC, OMC, Beverly Hills, CA, USA.

We previously reported that women who experience osteoporotic vertebral fractures have decreased health-related quality of life (HRQOL) as measured by the Osteoporosis Assessment Questionnaire (Arth Rheum, In Press) compared to women without fractures. We now report a study on the impact of both incident vertebral and non-vertebral fractures on HRQOL using OPAQ for a subset (n=365) of English-speaking postmenopausal women (mean age=70.7) with established postmenopausal osteoporosis (>=1 prevalent vertebral fracture[s]), who participated in a double-blind, randomized, placebo-controlled trial of the efficacy for daily subcutaneous administration of rhPTH(1-34) on the risk of osteoporotic fracture. Women were randomly assigned to 1 of 3 study arms: placebo, 20 µg or 40 µg of rhPTH(1-34) by daily self-injection. All patients received daily calcium (1000 mg) and vitamin D (400-1200 U) supplements with a median length of follow up at 21 months. Lateral spinal radiographs were assessed centrally for incident vertebral fractures using a semi-quantitative (SQ) grading score (JBMR 1993;8:1137). Incident non-vertebral fractures were ascertained by patient self-report at subsequent study visits. HRQOL was assessed at baseline and annually until study termination. Of the 365 women in the HRQOL sub-study, 53 experienced an incident vertebral or non-vertebral fracture during the study period. Compared to women without incident fractures, women with incident fractures had worse HRQOL from baseline to endpoint in the symptom dimension (p=0.008) and back pain domain (p=0.023). When we compared the proportion of patients who had significant loss in HRQOL (as defined by a change in HRQOL of greater than 1 SD from baseline) between patients who suffered an incident fracture and those who did not fracture, we found statistically significant increases in the proportions of patients with symptoms (p=0.032), back pain (p=0.004), and decreased emotional status (p=0.007) with a trend towards an increase in the proportion of patients with decreased physical function (p=0.053). In conclusion, our results confirm and extend our previous findings of the association between incident vertebral fracture and decreased HRQOL and show that in a clinical trial, incident fractures (including both vertebral and non-vertebral) in patients with established postmenopausal osteoporosis are associated with significant decreases in HRQOL.

Disclosures: Michael E. Minshall, MPH,1,3; Stuart L. Silverman, MD,5; Wei Shen, PhD,1,3; Sunny Xie, MS,5.

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Evaluation Of Osteoporosis Quality Circle- A 1.5 Year Assessment. W. H. Kneer,*[1] C. Wüster,*[2] P. Hadji.[3] [1]Orthopaedic Practice, Stockach, Germany, [2]HAG, Heidelberg, Germany, [3]Zentrum f. Frauenheilkunde u. Geburtshilfe, Philipps-Universität Marburg, Marburg, Germany.

Background: In May 2000, an international quality circle on osteoporosis was introduced following the suggestions of the Oxfordshire Medical Audit Advisory Group. The 10 participants are specialised in orthopaedic surgery (4), gynaecology (2), radiology (1), endocrinology (1), rheumatology (1) and general medicine (1). The quality circle (REKO-Südbaden) fully realises four criteria and meets the requirements of the highest stage of development (full audit). After one and 1.5 years a self-assessment was performed.Results: Success of work was considered positive (4,36 with a scouring from 1-5, 1 meaning "not right at all" to 5 meaning "completely right"). Surreys regarding efficiency of the participants show that the usefulness of work was assessed high (4,51 with a scouring from 1-5, 1 meaning "not at all" to 5 "fully fulfilled"). Highly significant is the increase of competence (4,56) and the cooperation with other colleagues (from 2,6 increased to 3,9).Conclusion: Two-stage auditing of an osteolgical quality circle according exact guidelines leads to significant improvement of diagnostic and therapeutic methods. Self assessment shows increase of medical behaviour (+1,0), the profession in general (+0,8), as well as motivation of employees (0,84).

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The Reliability and Validity of the Turkish Version of Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO). H. Kocyigit,*[1] S. Gulseren,*[2] A. Erol,*[2] N. Hizli,*[3] A. Memis.*[1] [1]Physical Medicine & Rehabilitation, Izmir Ataturk Training Hospital, Izmir, Turkey, [2]Psychiatry, Izmir Atatürk Training Hospital, Izmir, Turkey, [3]Rheumatology, Izmir Atatürk Training Hospital, Izmir, Turkey.

The purpose of this study was to investigate the reliability and validity of the Turkish version of Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO). The patient group included 30 females (ages between 55 and 80 yrs.) with vertebral fractures due to osteoporosis. The control group consisted of 30 healthy female volunteers which their ages matched with the patients. All of the participants were evaluated using both QUALEFFO and SF-36. In the reliability studies, the Cronbach alpha coefficients of each domain of QUALEFFO was found to be between 0.7494 and 0.8836. Receiver operating characteristic (ROC) curves were constructed to evaluate the capacity to discriminate between patients with vertebral fractures and controls. A cutoff value of the total QUALEFFO score was also calculated. Convergent and discriminant validity rates of domains were found to be between 71% and 100%, and between 78% and 100%, respectively. In conclusion, the Turkish version of QUALEFFO was found reliable and valid in the evaluation of patients with vertebral fractures due to osteoporosis. Our study also suggests that the patients with vertebral fractures due to osteoporosis have impaired quality of life.

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Gamma Test of *Choices* for Better Bone Health™. S. L. Silverman,[1] D. T. Gold,[2] B. Miller.[3] [1]OMC Clin Res Ctr, Beverly Hills, CA, USA, [2]Duke University, Durham, NC, USA, [3]Procter and Gamble, Cincinnati, OH, USA.

Choices for Better Bone Health™ is an osteoporosis patient education program which uses a self-management approach. ***Choices*** consists of five (5) two to three hour sessions given over a 5-10 week period. The course includes lectures, discussions, group & individual activities. The course is led by two facilitators with experience in teaching and osteoporosis: an allied health professional and a patient with personal experience of osteoporosis. The maximum number of participants in a group is 20. ***Choices*** provides patients with osteoporosis important information about osteoporosis and its physical and psychosocial consequences. ***Choices*** teaches people with osteoporosis how to develop and follow a plan for better bone health based on adherence to four bone health behaviors (taking enough calcium and vitamin D, taking an osteoporosis medicine as recommended, managing the back pain or discomfort related to osteoporosis, and exercising correctly). We have previously shown in a beta test that postmenopausal women with established osteoporosis who participate in ***Choices*** have improved self efficacy for each of these bone health behaviors. In the gamma test we will determine if postmenopausal women who participate in ***Choices*** will have improved adherence to bone health behaviors over a 12 month period compared to postmenopausal women who receive usual care for osteoporosis. Both groups will receive an NOF membership. To test this hypothesis, we will randomize 200-1000 postmenopausal women with osteoporosis from at least ten different Regional Osteoporosis Boards of the Alliance for Better Bone Health who have had a prescription for risedronate within the last six months to one of two groups: a treatment group which will receive ***Choices*** within 3 weeks after informed consent and a control group which will receive ***Choices*** educational materials 12 months later. Members of both groups will receive NOF memberships. Questionnaires on adherence to each of the four bone health behaviors as well as data on self-efficacy for each of these behaviors will be obtained at baseline, 3,6,9, and 12 months. We will report 3 month data. In conclusion, the gamma test of ***Choices*** for Better Bone Health™ presents a unique opportunity to assess the impact of a patient education program using a self management approach on the health behaviors of postmenopausal women with osteoporosis.

Disclosures: Procter and Gamble,3,5,8.

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The Impact of Treatment Interventions on Health Related Quality of Life (HRQL) in Post-Menopausal Women Registered in the Canadian Database of Osteoporosis and Osteopenia (CANDOO) Patients. D. A.

Hanley,[1] R. J. Sebaldt,[2] G. Ioannidis,[2] A. Petrie,[2] W. P. Olszynski,[3] J. P. Brown,[4] R. G. Josse,[5] C. H. Goldsmith,*[2] A. Papaioannou,[2] J. D. Adachi.[2] [1]Calgary University, Calgary, Canada, [2]McMaster University, Hamilton, Canada, [3]University of Saskatchewan, Saskatoon, Canada, [4]Laval University, Ste-Foy, Canada, [5]University of Toronto, Toronto, Canada.

There are a great deal of data available on the impact of interventions in terms of treatment-induced changes in bone mass and fracture risk in osteoporotic patients. However, therapeutic regimen should also be evaluated based on their impact on HRQL. HRQL was examined in 1860 postmenopausal women 50 years of age and older who were registered in CANDOO. This database is a prospective registry of patients designed to capture a comprehensive set of osteoporosis-related clinical data during the course of routine specialist care. The mini-Osteoporosis Quality of Life Questionnaire (mini-OQLQ) was used to measure HRQL. This instrument includes 10 items divided into 5 domains (physical functioning, emotional functioning, activities of daily living, symptoms and leisure). Each item is associated with a seven-point scale in which a rating of 1 represents the worst possible function and a rating of 7 represents the best possible function. The total score for the instrument can vary from 10 to 70, while the domain scores can vary from 2 to 14. We conducted multiple regression analyses using 20 patient variables. Medications included in the analyses were bisphosphonates (etidronate or alendronate), estrogen, and others (calcitonin or fluoride). Other patient factors used in the regression models were incident vertebral fracture status, prevalent vertebral and non-vertebral fracture status, co-morbid conditions, age, dietary calcium intake, hours spent exercising, and bone mineral density of the lumbar spine and femoral neck.Adjusted results indicated that patients who received bisphosphonate therapy had higher HRQL scores as compared with those who were not taking bisphosphonates in the total score (1.4; 95% confidence intervals (CI): 0.1, 2.8) and the symptoms (0.6; 95% CI: 0.3, 0.8), activities of daily living (0.4; 95% CI: 0.1, 0.7), and leisure (0.3; 95% CI: 0.01, 0.6) domains of the mini-OQLQ. No significant differences in HRQL were observed between patients treated with estrogen, or other medications and those who were not taking these medications. In conclusion, quality of life was higher in post-menopausal women treated with bisphosphonates as compared with non-users. Further research will be needed to determine the clinical relevance of these HRQL changes.

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Conservative and Preventive Approach for Alveolar Bone and Tooth Loss with Clodronate. M. Muratore,*[1] F. Muratore,*[2] G. Santacesaria,*[1] E. Quarta,*[1] F. Festa.*[2] [1]Rheumatology, Galateo Hospital, S. Cesario di Lecce LE, Italy, [2]Applied Science of Oral and Dental Diseases, G. D'Annunzio University, Chieti, Italy.

Periodontitis, a chronic inflammatory process, is a primary cause of alveolar bone loss and tooth loss. The mechanism by which the bone loss occurs is not completely clear. Several microorganisms induce the peridontal disease, with alveolar bone destruction, activating T cells CD4(+) and stimulate the poduction of osteoprotegerin ligand (OPG-L). The OPG-L is a key modulator of osteoclasogenesis and osteoblast activation. The Authors suppose that the subministration of a strong and selective inhibitor of osteoclastic bone resorption currently used for treatment and prevention of osteoporosis, such as bisphosphonate, can be effective when the bone loss has to be reduced.From a randomized group of 30 peridontal with grade II mobility subjects, were selected 2 groups of 15. Both groups had an x-ray evaluation of the periodontal deasese and a BMD performed with Norland XR 36 (Norland, USA) to measure spine and hip bone density. All subjects used paracetamol 4g/ day as needed and an initial periodontal therapy with scaling and rooth planing, repeatd every 3 months. Group A: single dose of Clodronate 100 mg i.m. weekly per 1 year. Group B: controls. For both groups was evaluated tooth mobility and tooth loss. After 1 year, the Group A showed a significant decrease of tooth loss and decrease of mobility: 12 out of 15 showed no mobility and no tooth loss. The Group B showed reduced mobility in 8 cases and tooth loss in 4 sobjects. The BMD performed at 1 year, showed a hip recovery of 2.8% and spine recovery of 3.3% compared with control group. The clinical evaluation of periodontal disease showed an improved status of the periodontal health in both groups.The results show how to improve the efficacy of the conventional periodontal therapy with the use of the bisphosphonate and is underlined the clinical significance of the conservative and preventive use of a single dose of Clodronate 100 mg weekly i.m. in the progression of the alveolar bone and tooth loss.

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Follow Up of Bone Mineral Density in Patients Treated With Long Term Parenteral Nutrition:Contributing Factors on Lumbar Spine and Femoral Neck. M. E. Cohen-Solal,[1] C. Baudoin,*[1] K. Vahedi,*[2] P. Crenn,*[2] M. C. de Vernejoul,[1] B. Messing.*[2] [1]INSERM U349, Paris, France, [2]Gastroenterology, Hopital Lariboisière, Paris, France.

Patients with chronic intestinal failure (CIF) require long term parenteral nutrition (LTPN). It has been suggested that parenteral nutrition might be responsible for bone loss. However, the impact of this treatment on bone mineral density has not been properly evaluated in the recent years. Moreover, several factors may influence the bone density such as the age at the onset of the disease and the presence of corticosteroid treatment. Therefore we followed 53 subjects (mean age 37±17 yrs) with a CIF diagnosed at various age (range 20-60, tertile 30 and 45 yrs) and with a duration of LTPN of 4 (0-20) years (median, range). Bone mineral density adjusted on age (Z-score) was measured by DPX at the lumbar spine (LS) or at the femoral neck (FN) using Lunar densitometer. Follow up for BMD was 2 years (range 0-8) with 2 to 8 DPX per patient (n= 163 DPX). We used a mixed-effects model to determine the factors which could change the intercept and slope of individual linear regression of Z-score (S-PLUS software). 64 % of patients had osteoporosis at baseline (T-score <-2.5 SD). At the beginning of LTPN, LS Z-score was lower ($p = 0.02$) in patients with corticosteroid therapy: -2.37±1.53, no corticosteroid therapy: -1.06±1.79. Z-score was negatively correlated to the duration of the disease prior to LTPN. FN Z-score at baseline increased with age at which the disease occurred (0.3 every 10 years, $p<0.001$) and with BMI (0.06 every kg/cm2, $p = 0.03$). Both effects were additive. During the course of LTPN, the individual evolution of Z-score at the LS was positively linked to the age at which the disease occurred ($p<0.001$). The estimated Z-score changes within 4-year follow-up were: over 45 years from -1.54 to –1.09; between 30 and 45 years from –2.12 to –1.88, and before 30 years from –2.66 to –2.78. At the FN, Z-score did not change significantly. In conclusion, this study did show that subjects whose CIF was diagnosed late in life had a higher Z-score at the beginning of LTPN. Moreover during the course of LTPN, at LS, they had an increase in Z-score. Corticosteroids have a negative impact on LS bone and BMI a positive impact on the FN.

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Densitometry and Markers of Bone Metabolism Can Distinguish the Patients with Osteogenesis Imperfecta from the Other Patients with Connective Tissue Diseases. V. Vyskocil,*[1] J. Varvarovska,*[2] K. Koudela,*[1] B. Kreuzberg.*[3] [1]Department of Orthopaedic Surgery, Plzen, Czech Republic, [2]Department of Pediatry, Plzen, Czech Republic, [3]Department of Radiology, Plzen, Czech Republic.

The authors followed 240 patients with congenital connective tissue diseases from 1996 to 2000 (Osteogenesis imperfecta /OI/, Marfan syndrome /MS/, Ehlers-Danlos syndrome / MS/). The study population consists of 50 patients with OI, 67 children with ED and 122 patients with MS /based on 120 characteristic signs/.Patients with OI were treated first with calcitonin, calcium and vitamin D, later on with bisphosphonates. In the other 2 groups (ED, MS) the patients with decreased bone density were treated with calcium and vitamin D. The authors stated that in MS osteopenia would improve at the age of 15 - 18 years even without any therapy. On the contrary the patients with ED or OI having decreased bone density need to be treated.The patients with OI (18.5% of the study population) had mean Z-score - 1.89 (80.4% of BMD normal values according to the age) and following changes in markers of bone resorption and formation during the treatment were found : PTH was increased from 28.1 to 43.1 (not significant increase), osteocalcin decreased from 73.5 to 65.0, also PICP had no significant change from 133.8 to 136.2. The important changes were found in crosslinks - from 915.8 to 628.4. Mean change in hip was 2.5%, in neck hip 6.1% and spine 5.9%, forearm had no significant change 0.8%. Patients with ED syndrome were 24.8% of the total study population, PTH decreased from 34.4 to 24.8 during the treatment, osteocalcin, PICP and crosslinks were not changed significantly. On the contrary the patients with MS had decreased PTH, OC and also crosslinks. Osteocalcin values were much higher in ED syndrom than in MS. The patients with OI had significantly lower BMD and the remaining groups and as well as the response to the first therapy with calcium and vitamin D (before the calcitonin and bisphosphonates introduction) was lower than in ED or MS. Different response to the therapy could be the method for differential diagnosis of difficult cases. The best response was observed in patients with MS, lower increase of bone density was in patients with ED syndrome and the lowest in OI patients (before the bisphosphonates introduction).The authors assume that the combination of different diagnostic methods is useful and could differentiate among those 3 groups of patients. Molecular genetics is the method of choice in unclear cases.

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Secondary Dentin Formation and Root Canal Occulsion in Middle-aged to Elderly Men. A. J. Kahn,[1] S. Woo,*[1] H. Goodis,*[2] R. Garcia,*[3] L. Yu,*[4] S. Majumdar.[4] [1]Growth and Development, University of California, San Francisco, CA, USA, [2]Preventive and Restorative Dental Sciences, University of California, San Francisco, CA, USA, [3]Health Policy and Health Policy Research, Boston University, Boston, MA, USA, [4]Radiology, University of California, San Francisco, CA, USA.

The purposes of the present study were to test the hypothesis that secondary dentin formation (SDF) leading to root canal occlusion occurs in a continuous fashion throughout life and to assess whether SDF is affected by or associated with select oral and systemic factors. X-rays taken at 3 year intervals of caries-free mandibular first bicuspids (premolars) were attained from 72 middle-aged to elderly men enrolled in the Boston VA Dental Longitudinal Study and Normative Aging Study. The films were scanned and the dimensions of the root canal measured from the digitized images using computer-based, morphometrics software. Three ratios were calculated from these measurements to normalize and quantify the extent of SDF/canal occlusion: canal height to tooth height, root canal width to root width at the cervico-enamel junction (CEJ) and at increments down the root surface. At 1/2and 3/4 distances down the root surface, the canal was scored as being non-occluded or occluded. In addition, data were obtained on periodontal health, cervical and occlusal wear, and a history of smoking and heart disease for each patient. Statistical analysis of canal closure was performed using a random effects model and $p<0.05$ was considered to be significant. Even though there was substantial individual-to-individual variation, SDF (canal calcification) as a function of age was found to be highly significant by all measures ($p<.0001$ to $p<.0003$). Furthermore, a statistically valid association was also observed between heart disease and SDF using measurements of the CEJ ratio and 3/4 distance ratio ($p<.014$ and $p<.05$). On the other hand, no linkage was observed between SDF and smoking, periodontal disease, occlusal wear and cervical wear. These results confirm earlier reports indicating that, on average, the degree of SDF/canal occlusion increases with age but this increase is not true for all individuals. Some subjects show no evidence of change with age. In addition, the data also indicate that there may be a significant association between SDF and heart disease; a finding suggestive of what has been previously reported for periodontal disease (a source of chronic infection) and cardiovascular disease. Finally, our results suggest that neither smoking nor local factors such as periodontal disease accelerate the rate or increase the degree of SDF.

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Importance of Subtracting Placebo Phase in the Pharmacokinetic Analysis of Endogenous Minerals. H. J. Heller,[1] C. E. Reilly,*[2] J. R. Poindexter,*[1] B. Adams-Huet.*[3] [1]Center for Mineral Metabolism, UT Southw**estern Medical**

Center, Dallas, TX, USA, [2]Department of Urology, UT Southwestern Medical Center, Dallas, TX, USA, [3]Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA.

Pharmacokinetic parameters of a drug are assessed from the kinetics of serum levels of the drug over time. If the drug is naturally found in the blood or food, these factors may interfere with the serum response curve of the preparation. We hypothesized that subtraction of the placebo phase affects pharmacokinetic parameters of phosphate, a food component that is known to have a wide circadian fluctuation in plasma.In a randomized crossover design, this study evaluated the single-dose bioavailability of a slow-release form of neutral potassium phosphate (SR KPO_4, 30 mmol phosphate in a wax matrix as UroPhos-K), immediate-release neutral potassium phosphate (IR KPO_4, 30 mmol phosphate) and placebo. After stabilization on a constant metabolic diet, each of the 13 normal volunteers took the dose with food at 8 a.m. Lunch was given at 2 p.m. and supper at 8 p.m. Samples of blood were collected hourly from 8 a.m. to 2 p.m., every 3 hours from 2 p.m. to 11 p.m., and again at 8 a.m. the following morning. Pharmacokinetic parameters were derived for each of the 3 phases per individual. The results below represent the results as the median (25th percentile, 75th percentile) derived by two methods: change from baseline before and after subtraction of the placebo phase.

Pharmacokinetic Parameters for Plasma P	**Change From Baseline**		**Change From Baseline After Subtraction of Placebo**	
Phosphate Type	**SR KPO4**	**IR KPO4**	**SR KPO4**	**IR KPO4**
Delta AUC, mg/dl/24-h	10.1 (7.8,15.5)	7.1 (0.1,9.1)*	6.5 (2.0,12.3)	4.1 (-1.3,5.9)*
Cmax, mg/dl	1.4 (1.1,1.5)	1.2 (0.9,1.8)	1.4 (1.2,2.1)	1.9 (1.4,2.3)*
Tmax, h	5 (4,6)	4 (3,4)**	3(2,4)	2 (2,3)*
T1/2, h	7.6 (6.4,8.5)	6.6 (5.6,6.9)***	7.2 (5.5,10.9)	4.9 (4.5,6.7)**

*p<0.10; **p<0.05, ***p<0.01 compared to the SR phase by Wilcoxon Signed-Rank test The pharmacokinetic parameters differed greatly between the two analyses. After subtracting the placebo phase, we found that all of the parameters and the level of significance changed greatly. This subtraction eliminated the effects of phosphorus meal content and the circadian variation of phosphorus to generate classic dose-response curves. We conclude that subtraction of the placebo phase may better represent the true pharmacokinetic effect of an endogenous mineral

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The Effects of Residronate on GCF Cytokines in the Treatment of Periodontal Disease. P. M. Loomer, M. Khan,* G. A. C. Armitage,* N. E. Lane. University of California, San Francisco, CA, USA.

Chronic periodontitis is a common inflammatory disease of bacterial etiology that results in the loss of alveolar bone supporting the dentition. Clinical studies have shown that in periodontitis, the levels of specific inflammatory bone active cytokines (IL-1b, IL-6 and TNF-a) in the gingival crevicular fluid (GCF) surrounding the teeth are increased. Animal studies have demonstrated that bisphosphonates can reduce both local and systemic concentrations of these cytokines. The overall aim of this clinical study is to determine if bisphosphonate administration, when used in conjunction with conventional periodontal therapy, results in significantly greater new bone formation and clinical attachment, in comparison to conventional therapy alone, in patients with moderate to severe chronic periodontitis. In this portion of the study, the effects of treatment on GCF cytokine levels of IL-1b, IL-6 and TNF-a were examined. A total of 58 subjects (18 male, 40 female) with a mean age of 44.1 ± 10.5 years (range: 24 – 67) were enrolled in the study. The mean clinical attachment loss prior to therapy was 3.5 ± 1.3 mm. All subjects received at the start of the study conventional periodontal therapy, which consisted of oral hygiene instruction and scaling and root planing. Subjects either received 5 mg Residronate (Procter and Gamble, Norwich, NY) daily. or placebo. Both treatment and placebo groups also received 400 IU vitamin D and 630 mg calcium daily. All subjects were subsequently seen every 3 months for periodontal maintenance therapy. GCF cytokine levels were measured using ELISA kits (R & D Systems, Minneapolis, MN). Cytokine levels were measured prior to therapy, and annually thereafter. The results indicated that there was no correlation between site specific CGF cytokine level (IL-1b, IL-6 and TNF-a) and clinical attachment (P> 0.05) at the subjects' initial evaluation prior to therapy. However, a significant correlation was observed between IL-1b level in GCF and clinical signs of inflammation (bleeding upon probing, Gingival Index) (P< 0.05) The effects of bisphosphonate and conventional periodontal therapy will be reported after one-year reevaluation examination of the subjects.

M473

Cadmium-Induced Bone Loss Is Independent of Estrogen. A. K. Wilson,[1] T. Flores,*[2] M. Bruzik,*[1] M. H. Bhattacharyya.[2] [1]Biological Sciences, Benedictine University, Lisle, IL, USA, [2]Biosciences Division, Argonne National Laboratory, Argonne, IL, USA.

Etiology of Itai-Itai disease suggests that cadmium caused greater boneloss in females who had increased bone turnover due to multiple cycles ofpregnancy and lactation. The degree of bone loss in males was not asextensive as in females. To investigate the direct effect of cadmiumexposure on males, mice were adjusted to a low calcium diet and exposed to cadmium using a single gavage dose of 200 ug CdCl2. Fecal calcium, a direct measure of calcium released from bone in this animal model, was measured by atomic absorption spectrophotometry (Tox.Appl.Pharm.,1997). These mice were also deficient in metallothionein, a metal binding protein that sequesters cadmium to the kidney for long term storage. Male mice demonstrated a similar response to an acute cadmium exposure as did female mice. Fecal calcium levels increased within 24 hours of exposure and returned to baseline levels by 72 hours indicating a transient calcium release from bone. The overall extent of skeletal calcium release was approximately 2 mg over 72 hours, similar to that of females. A second cadmium exposure caused slightly less calcium excretion, approximately 1.4 mg in both males and females. Ovariectomized female mice exposed to the same gavage dose of cadmium did not demonstrate an increase in fecal calcium excretion above normal females as expected. These data taken together suggest that the mechanism of acute cadmium-induced bone resorption is not dependent on estrogen status.

M474

Effect of Parathyroidectomy on Bone Mineral Density in Patients with Renal Hyperparathyroidism: a Longitudinal Comparative Study. S. Yano,*[1] T. Sugimoto,[1] T. Tsukamoto,*[1] T. Yamaguchi,[1] M. Kanatani,[1] H. Kaji,[2] S. Hattori,*[2] A. Kobayashi,*[3] K. Chihara.*[1] [1]Third Division, Department of Medicine, Kobe University School of Medicine, Kobe, Japan, [2]Hattori Hospital, Miki city, Japan, [3]Kobayashi Clinic, Kobe, Japan.

No longitudinal studies are available in which bone mineral density (BMD) changes are compared between groups with and without parathyroidectomy (PTx) in hemodialysis patients with severe secondary hyperparathyroidism (2HPT). In this longitudinal study, we compared BMD changes between groups with and without PTx, and further investigated effects of continuous excess as well as abrupt loss of endogenous PTH action on BMD in patients under maintenance hemodialysis. Subjects are 31 Japanese patients with serum intact PTH level >400 pg/ml. Total parathyroidectomy with autotransplantation was performed in 15 patients; PTx(+). Intensive medical therapy, including active vitamin D pulse therapy without PTx or PEIT, was continued in the remaining 16 patients; PTx(-). BMD was measured using DXA (QDR2000) at lumbar L2-4 and 1/3R (distal 1/3 region of radius) within 1m before, 1m, 3m, 6m, 1y, 2y and 3y after PTx in PTx(+) group, and at least 2 times at 12m intervals in PTx(-) group. Serum corrected Ca and intact PTH levels were 10.7 vs 9.9 mg/dl and 1629 vs 623 pg/ml in PTx(+) vs (-), respectively. Basal Z-score at L2-4 was significantly lower in PTx(+) group, compared with PTx(-) group (-0.59 vs +0.73), while markedly low Z-scores were found in both groups at 1/3R (-3.07 vs -2.57). Significant decrease was found in not L2-4 but 1/3R Z-score during 1y follow-up in PTx(-) group. In contrast, in PTx(+) group, marked increase was observed in L2-4 Z-score 1m-2y after PTx, and Z-score at 1/3R also showed an increasing tendency after PTx. Compared with PTx(-) group, a %change of BMD /y was significantly higher at L2-4 (+15.6 vs -1.0 %) as well as at 1/3R (+6.4 vs -3.3 %). The %change of BMD /y at both sites was positively correlated with intact PTH level just before PTx with strong correlations [r=0.638 (L2-4), r=0.886 (1/3R)], and were inversely correlated with Z-score at the corresponding site with weak correlations. In conclusion, BMD was markedly increased at lumbar spine within 1 year after PTx, and a significant increase in BMD was also observed at distal radius, compared with PTx(-) group. An abrupt loss of long-term PTH excess would be responsible for an increase in bone mass. Intact PTH levels just before PTx could be useful for the prediction of these BMD changes after successful PTx.

M475

Bone/Lean Mass Relationships in Peritoneally-Dialyzed and Hemodialyzed Men and Women. A. L. Negri,*[1] G. R. Cointry,*[2] D. Salica,[3] J. R. Zanchetta,[4] J. L. Ferretti.[5] [1]Nephrology, Instituto de investigaciones Metabolicas, Buenos Aires, Argentina, [2]Bone Metabolism, CEMFoC Natl Univ Rosario, Rosario, Argentina, [3]Healing, Cordoba, Argentina, [4]Instituto de Investigaciones Metabólicas, Buenos Aires, Argentina, [5]CEMFoC Natl Univ of Rosario, Rosario, Argentina.

We had shown that the whole-body mineral content (BMC), either crude or statistically adjusted to a common 18-Kg fat mass (FA-BMC) was linearly correlated to lean mass (LM) showing the same slopes but different intercepts in decreasing order for pre-menopausal (preMP) women > men > postmenopausal (post-MP) women > boys and girls. On regarding LM as linearly proportional to muscle mass, this would indicate that bone mass is determined by the mechanical usage of the skeleton the same way in the species (bone "mechanostat" theory) but the proportionality of that relationship is normally affected by nonmechanical factors related to gender and reproductive status. This study aims to compare the whole-body BMC or FA-BMC and LM (DEXA, Norland XR-26) in stable chronic peritoneal dialysis (CAPD) and hemodialysis (HD) men (n=37, age 51.5 ± 12.5 yr) and pre-(24, age 36.2 ± 10.6 yr) and post-MP women (47, age 55 ± 10.8yr) in which a different metabolic interference with the mechanical control of bone mass can be proposed to furthur affect the bone/muscle relationship. Data were compared with those from 600 sex and age-matched controls.The dialyzed patients had a lower bone mass than the mean values shown for the corresponding sex and ages. The linear correlations between the BMC (y) and LM (x) showed that both CAPD and HD patients plotted significantly lower than their respective controls. The curves for men and pre-MP women were similar in slope but showed significantly lower intercepts than those of their controls. Distinctly, post-MP women showed a significant tendency to have lower BMC per unit of LM than their controls in proportion with the reduction observed in LM, reaching very low BMC values in the extreme cases.Results suggest that 1. dialyzed men and pre-MP women follow the normal biomechanical laws concerning the control of bone mass by muscle mass; 2.however, the metabolic interference from either CAPD or HD reduces the proportionality of the consequent BMC/LM relationship and 3. this situation is further affected by menopause, after whichthe skeletal mass control seems to be impared in proportion with the reduction in mechanical usage resulting from the deterioration of muscle mass. This would confirm the hypothesis that metabolic (nonmechanical) factors induced by disease and its treatment would have affected the bone "mechanostat" setpoint over the natural endocrine influences in these patients.

M476

Soybean and Soyfood Consumption Increases Urinary Calcium and Oxalate Excretion. L. Massey, L. Grentz.* Human Nutrition and Dietetics, Washington State University, Spokane, WA, USA.

Soybeans and commonly consumed soyfoods, such as tofu, soy beverage and tex-

tured vegetable protein (TVP), contain over 110 umol (10 mg) oxalate per serving, primarily in the form of calcium oxalate. Calcium oxalate is highly insoluble and it was previously assumed that the oxalate could not be absorbed, until its intact absorption was shown in rats by Hayes et al in 1999.. Human absorption of oxalate from soyfoods is unstudied, so the potential of their consumption to increase urinary oxalate, and therefore risk of calcium oxalate stone formation, is unknown. This study examined oxalate absorption from soybeans and soyfoods by measuring changes in urinary oxalate excretion. Eight healthy individuals 23 to 59 years of age with no prior history of kidney stones participated in eight oxalate load (OL) tests comprised of two soybean lines, five soyfoods and a 8.3 mmol sodium oxalate solution. Each OL test was separated by one week for eight consecutive weeks. For two days prior to each OL test, ten oxalate rich foods (spinach, rhubarb, beets, beans, chocolate, tea, wheat bran, nuts, parsley, and strawberries) were avoided to reduce baseline oxalate excretion. Similarly, dairy product consumption was limited to two servings daily. After twelve hour fasting, a pre-load urine was collected, then the OL consumed. All post-load urine was collected for eight hours. Pre-load and post-load urines were analyzed for calcium and oxalate and expressed as a ratio to creatinine. After correction for basal Ca excretion, the increase in 8 hour post-load urinary Ca excretion ranged from 0.70 ± 0.40 mmol (28.0 ± 16.0 mg) from the magnesium precipitated tofu to 1.29 ± 1.13 mmol (51.8 ± 45.3 mg) Ca from the Ca precipitated tofu load. Ca absorption from the soybean and soyfoods ranged from 7.6 ± 4.3 to 13.1 ± 10.9%. After correction for pre-load baseline excretion, increases in urinary oxalate excretion ranged from 19.6 ± 23.3 to 124 ± 156 mmol (1.7 ± 2.1 to 10.9 ± 13.8 mg) for the two soybean lines and five soyfoods during the eight hours after ingestion of each OL. Absorption ranged from 2.1 ± 2.1% from high oxalate soybean line L95-1409 to 5.4 ± 4.2% from soynuts. Individual increases varied widely, from zero to 481 υmol. Since normal urinary oxalate excretion is defined as 110 to 440 µmol (10 to 39 mg) per day, the inclusion of soybeans and soyfoods in the diet is capable of increasing urinary oxalate excretion to 450 µmol (40 mg) or more per day, a concentration defined as hyperoxaluria. Thus, frequent consumption of soybeans and soyfoods may be a risk factor for calcium-oxalate kidney stone formation in susceptible individuals, such as those with a prior history of calcium stones or high normal oxalate levels.

M477

Bone Mineral Density at Various Sites for Prediction of Vertebral Fractures in Hemodialysis Patients. K. Atsumi,[1] K. Kushida,[1] S. Okamoto,[1] H. Aoshima.[2] [1]Orthopaedic Surgery, Hamamatsu University School of Medicine, Hamamatsu, Japan, [2]Iwata City Hospital, Iwata, Japan.

To determine the best measurement for predicting vertebral fractures in hemodialysis (HD) patients, we assessed bone mineral density (BMD) at several sites. Seventy-eight female HD patients and age-matched 272 healthy women as the reference population were assessed vertebral fractures with lateral radiographs, and the BMD were measured at the lumbar spine, femoral neck, ultra distal radius, 1/3 distal radius and calcaneus by dual-energy X-ray absorptiometry. Twenty-six patients (33.3%) had vertebral fractures and healthy women did not. The value of BMD results were expressed as T-scores (the standard deviation of the BMD of the young adult in the reference population). The mean T-scores for the ultra distal radius, 1/3 distal radius and calcaneus BMD were significantly lower than those of lumbar spine ($p<0.001$) and femoral neck BMD ($p<0.001$). The rates of patients with osteopenia (T-score worse than –1) of the ultra distal radius (90.9%), 1/3 distal radius (91.0%) and calcaneus (88.5%) were significantly higher than those of lumbar spine (66.2%) ($p<0.001$) and femoral neck BMD (85.7%) ($p<0.001$). To compare the predictive value of vertebral fractures at various sites, we analyzed the area under receiver operating characteristic (ROC) curves for each site and assessed the statistical significance of difference between these areas. The areas under the ROC curve for the ultra distal radius, 1/3 distal radius and calcaneus BMD were significantly higher than those for lumbar spine ($p<0.001$) and femoral neck BMD ($p<0.001$). We conclude that low BMD in the radius and calcaneus are better predictors of vertebral fracture than those in the lumbar spine and femoral neck in HD patients.

M478

Risk of Osteoporosis in Long-Term Hemodialysis Patients. C. V. Albanese,[1] R. Passariello.*[2] [1]University "La Sapienza, Rome, Italy, [2]Institute of Radiology, Rome, Italy.

High turnover bone disease due to alteration of PTH and Vitamin D is the central feature of renal osteodistrophy see in long-term hemodialysis patients. The aim of this study was to measured bone mineral density (BMD) by DXA and the relationship among BMD, age and biochemical parameters of bone metabolism. We enrolled 47 male patients that were divided in two subgrups: groups A with age under 60 years and group B with age over 60 years (mean age 44+/-10 years and 68+/-5 years respectively). BMD were measured at lumbar spine (L2-L4) and hip (neck, trochanteric region and Ward's triangle) using a DXA method (Hologic QDR 2000plus). We measured biochemical markers of bone turnover such as osteocalcin (BGP), alkaline phosphatase (FA), calcium (CA), phosphorous (P) and serum intact PTH. Body mass index (BMI) was also calculated in all patients. There were no significant differences for BMI, L2-L4, PTH, FA, CA and P, between the two groups. We found in group B a significant reduction ($p<0.001$) of BMD at all sites examined (Neck:0.640+/-0.12 vs. 0.760+/-0.12; Trock: 0.580+/-0.13 vs. 0.640+/-0.15; Ward: 0.450+/-0.16 vs. 0.590+/-0.16). Osteoporosis was diagnosed in the 25% of group B and osteopenia in 15% of the group A, using WHO classification. A negative correlation was found between femoral BMD and age (Neck: $r= 0.46$, $p<0.01$; Ward: $r= 0.43$, $p<0.01$), PTH (Neck: $r= 0.35$, $p<0.05$), and FA (neck: $r=0.42$, $p<0.01$; Ward: $r=0.36$, $p<0.05$). These results indicate that long-term male hemodialytic patients are at high risk of osteoporosis in elderly age and we consider that measurement of BMD by DXA at femoral sites may be useful to select the patients at risk of bone mass decreases. On the contrary the parameters of bone turnover investigated were not able to discriminate patients at risk with respect to DXA measurements.

M479

Microarray Analysis of mRNA Gene Expression Altered by Low Phosphate Diet in the Hypothalamic-Pituitary Complex of the Rat. M. H. Meyer,[1] B. Louie,*[2] S. E. Mulroney,[2] M. Levi,*[3] R. A. Meyer.[4] [1]Department of Orthopaedic Surgery, Carolinas Medical Center, Charlotte, NC, USA, [2]Georgetown Univ. Sch. Med., Washington, DC, USA, [3]Univ. Texas Health Sci. Ctr., Dallas, TX, USA, [4]Carolinas Medical Center, Charlotte, NC, USA.

The source and the identity of the phosphate-regulating hormone(s) have proven elusive. Mulroney et al. (Faseb J. 14: A659, 2000) have reported that infusion of phosphate into the third ventricle of the brains of P-deprived rats resulted in the down-regulation of the type II sodium-phosphate cotransporter (NaPi-2) protein in both the hypothalamus and the kidney. If the hypothalamus were the source of factor(s) regulating phosphate homeostasis, low phosphate diets should alter their mRNA expression level. Thus, microarray technology was used to search for hypothalamic and pituitary genes sensitive to change in phosphate levels. Male Wistar rats (264 ± 4 g) were fed a normal (n = 3) or low phosphate (n = 4) diet for two days. The pituitary and hypothalamus were combined and rapidly frozen. Total RNA was extracted, aliquots were pooled to give a control and a low-P-diet sample, and the pools were processed to cRNA. Each cRNA pool was hybridized to an Affymetrix Rat U34A DNA microarray. 4,855 genes were scored as absent, and 3,944 were expressed. Of these, 17 were scored as up-regulated 2 fold or more and 33 down-regulated 2 fold or more in the low P sample. Known pituitary hormones were scored as present in the control and unchanged in the low P sample (IBMS, June 2001, Abstract P471). In this study, primers were constructed for the altered genes, and mRNA expression measured by reverse transcription - polymerase chain reaction (RT-PCR) in the mRNA from the hypothalamic/pituitary samples collected from individual animals. Of the 24 genes evaluated, 13 did not change significantly upon assay by RT-PCR and were considered false positives. However, eleven genes, mostly EST sequences, were up-regulated significantly in the rats fed the low phosphate diet. One up-regulated gene was NaPi-2β. Seven other up-regulated genes were (or were EST sequences resembling) metabolic genes indicating a change in cell activity. Three up-regulated genes had no homology to known sequences. In conclusion, low phosphate diet results in a change in the mRNA expression of specific genes in the hypothalamic/pituitary complex of rats. Future studies will be needed to elucidate the role of these genes in phosphate homeostasis.

M480

Lower Serum Crosslaps in Male Cardiac Transplant Recipients Treated Without Prednisolone. G. Hoefle,*[1] H. Holzmueller,*[1] G. Gouya,*[1] K. Hergan,*[2] M. Hubmann,*[3] G. Tautermann,*[1] H. Drexel.*[1] [1]Internal Medicine and Vorarlberg Institute of Vascular Investigation and Treatment (VIVIT), LKH Feldkirch, Feldkirch, Austria, [2]Department of Radiology, LKH Feldkirch, Feldkirch, Austria, [3]Central Medical Laboratory, Feldkirch, Austria.

Osteoporosis and increased incidence of vertebral fractures are a common problem after cardiac transplantation (CTX). Important factors contributing to bone loss are: use of corticosteroids and Cyclosporin A (CyA), secondary hyperparathyroidism due to renal impairment, hypogonadism and relative immobilisation. The highest bone loss occurs in the first year after CTX.We performed a cross-sectional analysis of male heart transplant recipients in a late post-transplantation period (4.2 ± 2.6 years after CTX, n=21). Group A received an immunosuppressive therapy with CyA and Mycophenolat-Mofetil (without corticosteroids)and Group B had a triple therapy including corticosteroids (Azathioprin, CyA and Prednisolone). We analysed bone mineral density status by osteodensitometry (DXA) as well as the bone turnover markers serum crosslaps and bone specific alkaline phosphatase.Osteopenia (lumbar and/or femoral neck DXA T-Score < -1 SD) was common in both groups (44,4 % in Group A and 50 % in Group B) and many patients had osteoporosis as defined by lumbar and/or femoral neck DXA T-Score < -2,5 SD (30 % and 33,3 % in group A and B, respectively). Vertebral fractures (vfx) were present in 4 of 9 patients (12 vfx) in group A and 5 of 12 patients (14 vfx) in group B. Mean PTH and creatinine levels were only moderately elevated. Serum crosslaps were significantly lower in male cardiac transplant recipients on double immunosuppressive regimen (i.e. corticosteroid free)compared with patients on triple therapy (428,3 ± 109,4 vs. 661,7 ± 337,0 pg/ml, p=0,031).Osteoporosis is frequent in male cardiac transplant recipients and constitutes a major problem in the late post-transplantation period. A significant proportion of patients can be treated with a double immunosuppressive regimen (CyA and Mycophenolat-mofetil, no Prednisolone) without reduction of efficacy. Regarding osteoporosis we did not find a clinically relevant difference between the two groups. However, the higher serum crosslaps levels of group B may indicate a higher long-term risk of bone deterioration in a corticosteroid containing immunosuppressive regimen. Further studies should clarify the role of serum crosslaps as a sensitive marker of corticosteroid induced bone turnover changes.

M481

Delayed Bone Age After Pediatric Heart Transplantation. A. Cohen,*[1] V. Addesso,*[1] B. Softness,*[2] L. Addonizio,*[2] J. Lamour,*[2] E. Shane.[1] [1]Medicine, Columbia University, New York, NY, USA, [2]Pediatrics, Columbia University, New York, NY, USA.

Measurement of bone age (BA) is used to assess skeletal growth and development in children who have chronic illnesses or who are taking medications that may impact negatively on skeletal maturation. Delayed BA has been reported in children at the time of renal, liver and bone marrow TX. Some reports suggest that there is greater potential for improvement in children transplanted at an early age. However, there are no published data on skeletal maturation at the time of or after pediatric heart TX. In 1989, routine BA assessment was added to the yearly TX evaluation of all pediatric heart transplant recipients at this institution. To date, 296 BA determinations have been performed prospectively in 90 patients (56 males) transplanted between 1984 and 1998, at ages ranging from 4 months to 20 years. Of these, 37 were below age 7 (2.7 +/- 2.1 yrs; +/- SD) and 53 were above age 7 (13.1 +/- 3.2 yrs) at time of TX. Pre-TX BA was available in a small subset of

19 patients; Pre-TX BA was more delayed compared to chronological age (CA) in patients above age 7 (-20 +/- 26 months; n=4) than in patients below age 7 (-3 +/- 8 months; n=15), although the difference was not significant. In 6 patients (32%), all above age 7, Pre-TX BA was > 2 years below CA. Post-TX BAs were performed at times ranging from 4 months to 12 years after TX. Repeated measures ANOVA was performed in another subset of 22 patients with sequential BAs performed at one, two and three years post-TX. Patients below age 7 at TX (n=10) demonstrated a significant worsening of BA delay (Yr.1, -10 +/- 12 months, Yr.2, -14 +/- 14 months, and Yr.3, -19 +/- 14 months; p = 0.005). For patients above age 7 (n=12), the delay in BA remained stable (Yr.1, -15 +/- 19 months, Yr.2, -17 +/ - 24 months, and Yr.3, -13 +/- 26 months; p = NS). To further evaluate the apparent increase in BA delay in the younger patients with increasing time post-TX, we performed regression analysis of the first BA performed in each patient against time post-TX. The results revealed a direct association between the delay in BA and time post-TX both in patients transplanted before (r = +0.474; p=0.002) and after (r= +0.297; p =0.036) age 7. In summary, bone age is delayed in a substantial proportion of pediatric heart transplant candidates at the time of transplantation. The delay in BA appears to worsen with increasing time after TX. Maintenance glucocorticoid doses are low (average, 2 mg/day) in pediatric heart TX recipients. Thus the etiology of delayed BA is unlikely to be due to a glucocorticoid effect. As both cyclosporine A and tacrolimus have adverse skeletal effects in adult TX recipients, their potential role in the delayed BA observed in pediatric TX recipients warrants further investigation.

M482

Renal Transplant Recipients Lose Lumbar BMD within 6 Months. T. R. Mikuls,* B. A. Julian, M. Elkins,* A. S. Mudano,* R. Lopez,* K. G. Saag. University of Alabama at Birmingham, Birmingham, AL, USA.

Purpose: Prior investigations suggest significant osteoporosis occurs rapidly among renal transplant recipients. However, the effective use of new steroid-sparing immunosuppressive regimens has lowered glucocorticoid use among transplant patients and it is unknown what effect this therapeutic trend will have on bone disese. We characterized bone health and determined predictors of BMD change in an inception cohort of new renal transplant recipients at a tertiary care center with a large transplant program.Methods: Unselected newly transplanted inpatients (n = 31) were identified on the UAB renal transplant service and were comprehensively evaluated for metabolic bone disease a median of 17 days (range 9 to 33) post-transplant. A follow-up evaluation was conducted a median of 5.6 months (range 4.8 to 8.0) later. BMD was measured on a Hologic QDR 4500 with NHANES reference database. Descriptive characteristics and determinants of change in BMD were determined using non-parametric statistics.Results: Renal transplant recipients (58% cadaveric allografts) had a median age of 44.6 yr (range 20.3 to 69.1 yr), 61% men, 74% Caucasian/23% African American. At the femoral neck, most had osteopenia (55%) and few were osteoporotic (3%), while the majority (77%) had normal BMD at the spine (16% osteopenia). At follow-up, subjects had lost a median of 1.3% BMD at the lumbar spine ($p < 0.05$) but did not experience significant declines at the femoral neck. Ten (32%) subjects lost greater than 3% lumbar BMD over the six-month period. These subjects trended towards higher cumulative glucocorticoid use (median cummulative dose 4097 vs. 3730 mg, $p = 0.07$) and lower baseline vitamin 1,25 D3 levels (19.5 vs. 34.0 pg/dl, $p = 0.13$). Significant decreases in iPTH and vitamin D were also noted post-transplant.Conclusions: Significant loss in lumbar BMD occured in approximately one third of a small prospective cohort of renal transplant recipients within six months of transplantation. Lumbar bone loss appeared to be mediated most by glucocorticoid dose and lower levels of vitamin D.

M483

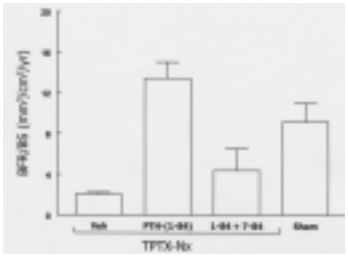
Differential Effects of PTH Peptides on OPG and RANK-L mRNA Expression in Human OHS Osteosarcoma Cells. A Possible Pathway of Osteoblast Dependent Bone Resorption. L. S. Stilgren,*[1] S. Reppe,*[2] B. Abrahamsen,[1] E. P. Rettmer,*[1] K. Brixen,[1] R. Jemtland,*[2] O. K. Olstad,*[2] V. T. Gautvik,*[2] H. Beck-Nielsen,*[1] K. M. Gautvik.[2] [1]Endocrinology, Odense University Hospital, Odense, Denmark, [2]Medical Biochemistry, Oslo University, Oslo, Norway.

Osteoprotegerin (OPG) is a recently described member of the tumor necrosis factor family and is known to induce osteclastic differentiation from hemopoietic precursors and activation of mature osteoclasts. The osteogenic human OHS cell line exhibits characteristics of a relatively well differentiated osteoblastic phenotype, including high expression of alkaline phosphatase and PTH-stimulated adenylyl cyclase activity (Olstad et al., unpublished). In the present study we aimed to study the effect of PTH peptides (PTH 1-84, PTH 1-34, PTH 3-84) on OPG and RANK-L mRNA expression in OHS cells and further examine whether these responses correlate with PTH-induced alterations in the levels of mRNAs for the transcription factor Sox4 and bone morphogenetic protein-4 (BMP4) mRNA.Cultured OHS cells were treated with full length PTH (2x10-9 M) or analogues and the cells were harvested at 0, 4,12, 24 and 48 hours. OPG and RANK-L mRNA levels were quantified using competitiv RT-PCR assay and artificial cDNA standards, normalized to β-actin mRNA to control for loading. Sox4 and BMP4 mRNAs were quantitated using Northern analysis.RANK-L mRNA expression was enhanced 10 to 12 fold in OHS cells treated with PTH 1-84 for 12 hours, and levels were increased over control throughout 48 hours. OPG mRNA decreased transiently to about 60% relative to untreated controls by 24 hours in PTH-stimulated OHS cells and levels were indistinguishable from control at 48 hours of treatment. BMP4 mRNA was down-regulated by PTH by 24 hours and this effect was reproduced by cAMP agonist 8-Br-cAMP with a similar time-course, whereas the phorbolic ester TPA was without effect, indicating a protein kinase A-dependent mechanism. Studies on the effect of PTH 1-34 and PTH 3-84 and other bone active substances are ongoing. Our results indicate that PTH 1-84 upregulates RANK-L mRNA and decreases OPG mRNA expression in human OHS cells add to previous reports in other bone cell systems. The time-dependent regulation of central mediators of osteoblast (BMP4, SOX4) and osteoclast (OPG, RANK-L) activity response to PTH, suggest a possible pathway of osteoblast dependent bone resorption involving these molecular components.

M484

PTH-(7-84) Antagonizes the Effects of PTH-(1-84) on Bone Turnover in Rats. M. C. Faugere, M. C. Langub, H. H. Malluche. Nephrology, Bone and Mineral Metabolism, University of Kentucky, Lexington, KY, USA.

PTH-(7-84) was shown to antagonize the effects of PTH-(1-84) on rise in serum calcium in thyroparathyroidectomized (TPTX) rats. To determine whether PTH-(7-84) antagonizes also the effects of PTH-1-84) on bone turnover, 30 rats were TPTX and nephrectomized (Nx) and 6 rats were sham-operated (Sham). TPTX-Nx rats were divided in 3 groups (n = 6 each) and allocated to receive vehicle (V), hPTH-(1-84) at a dose of 216 ng/kg.hr alone or in combination with hPTH-(7-84) at a dose of 4,000 ng/kg.hr. Sham rats received V. Doses were administered s.c. via Alzet minipumps for 2 weeks. At sacrifice, blood was collected and femurs excised for histomorphometry after calcein labeling. All TPTX-Nx rats had higher serum creatinine compared to sham rats (0.89 ± 0.04 vs. 0.53 ± 0.03 mg/dl, $p<0.05$). Serum calcium levels were lower in TPTX-Nx/V than in Sham (7.68 ± 0.26 vs. 10.44 mg/dl, $p<0.05$). Hypocalcemia was corrected in rats given PTH-(1-84) alone (10.16 mg/dl) whereas addition of PTH-(7-84) blunted this response (8.28 ± 0.27 mg/dl). Parameters of bone turnover were strongly suppressed in TPTX-Nx/V (Fig.). PTH-(1-84) restored bone turnover to sham levels whereas PTH-(7-84) blunted this response (Fig.).



PTH-(7-84) does not only antagonize the calcemic effect of PTH-(1-84) but also its effects on bone dynamics

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PTH Regulates NBRE-Containing Promoters Through Induction of Nuclear Orphan Receptors Nurr1 and Nur77. S. Tetradis,[1] O. Bezouglaia,*[1] J. M. Nervina.[2] [1]Diagnostic and Surgical Sciences, UCLA School of Dentistry, Los Angeles, CA, USA, [2]Orthodontics, UCLA School of Dentistry, Los Angeles, CA, USA.

NGFI-B nuclear orphan receptors are important regulators of the hypothalamic-pituitary-adrenal axis and TCR-mediated T-cell apoptosis. We have shown that PTH rapidly and transiently regulates the expression of the Nurr1 and Nur77 members of the NGFI-B-family in osteoblastic cells mainly through activation of the cAMP/PKA pathway. NGFI-B members regulate transcription by binding as monomers, homodimers or RXR-heterodimers on the promoters of target genes.We present evidence that induced Nurr1 and Nur77 regulate the activity of promoters that contain the consensus NGFI-B binding response element (NBRE). For all our experiments calvarial-derived primary mouse osteoblasts were used. Cells were grown to confluence and were treated with 10 nM PTH. Nuclear proteins were extracted and electrophoretic mobility shift assays (EMSAs) were performed. PTH treatment induced strong binding of nuclear extracts to a consensus NBRE oligo probe while the vehicle treated group showed no binding. Binding activity was present at 30 minutes and reached maximum at two hours. A 50-fold excess of unlabeled probe strongly inhibited binding. Nuclear extracts did not bind to an oligonucleotide probe that contained a single base pair mutation in the core NBRE sequence. Nuclear extract binding was strongly supershifted by Nurr1 and to a lesser extent by Nur77 antibodies. To study promoter activity, luciferase reporter constructs were made that contained three consensus (WT) or three mutant NBRE sites, spaced 10 bp apart, upstream of a 40 bp basal thymidine kinase promoter. Transient co-transfections of the WT reporter constructs with increasing concentrations of Nurr1 or Nur77 overexpressing vectors showed a dose dependent increase in luciferase activity. PTH increased luciferase activity two-fold in osteoblastic cells transfected with the WT NBRE reporter construct. However, PTH had no effect on the luciferase activity in cells transfected with the NBRE mutant reporter construct.In conclusion, our data suggest that PTH-induced Nurr1 and Nur77 nuclear orphan receptors bind NBRE cis-acting elements and enhance promoter activity in osteoblastic cells. Nurr1 and Nur77 might help regulate downstream PTH osteoblastic target genes, thus presenting an interesting cross talk between PTH and nuclear receptor signaling.

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Calcium Mediates Basal and PTH-Activated Phospholipase D in UMR-106 Osteoblastic Cells. A. T. K. Singh, S. A. Foster,* P. H. Stern. Northwestern University Medical School, Chicago, IL, USA.

Phospholipase D (PLD) plays a key signaling role in numerous cellular processes including cytoskeletal organization, vesicle trafficking, cell proliferation and differentiation. PLD hydrolysis of phosphatidylcholine (PC) generates phosphatidic acid, a source of diacylglycerol (DAG), and is stimulated by various hormones and other agonists that activate cell surface receptors. Regulators of PLD include protein kinase C (PKC), calcium, small G proteins, tyrosine kinases and phosphoinositides. We previously reported (Singh et al. 1999) that parathyroid hormone (PTH) stimulates PLD activity in UMR-106 osteoblastic cells. This effect of PTH was not mediated by PKC, since stimulation of PLD by PTH was not prevented when PKC was down-regulated. The current studies investigated the role of calcium in the activation of PLD in the UMR-106 cells. We examined effects of modulating calcium on (a) PTH stimulated PC hydrolysis and (b) on PTH-stimulated transphosphatidylation of ethanol, a reaction catalyzed by PLD. For PC hydrolysis, cells were labelled with 0.25 μCi/ml ^{3}H-choline chloride. After 30 min treatment, radioactivity in the medium was determined by liquid scintillation spectrometry. For transphosphatidy-

lation, cells were labelled with 1 μCi/ml ^{3}H-palmitic acid. Treatments were for 30 min in the presence of 1% absolute ethanol. Lipids were extracted and separated on a silica gel thin layer chromatogram with chloroform:methanol:acetic acid (65/15/4). Radioactivity was measured in bands corresponding to phosphatidylethanol. Ionomycin (1μM) or 2X calcium (3.6 mM) increased basal PLD activity, as assessed by PC hydrolysis and transphosphatidylation. The stimulatory effects of maximal concentrations of PTH or of phorbol-12,13-dibutyrate (PDBu) were not further enhanced in the presence of 2X calcium. EGTA (2.5 mM), BAPTA (30 μM), nifedipine (10 μM) and dantrolene (50 μM) inhibited the effects of PTH and PDBu on PC hydrolysis and transphosphatidylation, showing that both intracellular and extracellular calcium are important for basal and stimulated PLD activity. Sneddon et al. (2000) reported that in distal convoluted tubule cells, the MEK inhibitor PD98059 prevented the PTH-stimulated increase in intracellular calcium. In our studies, PD 98059 (50 μM) and the more selective MEK inhibitor U0126 (10μM) attenuated the effects of PTH, PDBu, and ionomycin on PC hydrolysis and PLD in UMR-106 cells. However, acute effects of PTH and ionomycin on calcium transients in UMR-106 cells were not inhibited by the MEK inhibitors. MAPK may be involved in the maintenance of calcium-dependent activation of PLD and in subsequent downstream signaling events and cellular responses in osteoblasts.

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PTH(1-14) Analogs Conformationally Constrained with α-aminoisobutyric acid Exhibit Full Potency on the P1R. N. Shimizu,* J. Guo, T. J. Gardella. Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

Solution-phase NMR and X-ray crystallographic studies indicate that the N-terminal portion of PTH(1-34) is α-helical; but the ligand conformation required for PTH-1 receptor activation is unknown. We investigated whether or not substitutions of Aib (α-aminoisobutyric acid), a sterically hindered amino acid analog known to nucleate α-helical conformation in oligopeptides, would improve potency in PTH(1-14) analogs. Individual Aib substitutions were introduced at each position in [Ala^{3,12},Gln^{10},Har^{11},Trp^{14},]PTH(1-14)amide {[M]PTH(1-14)} and effects on function were evaluated in hP1R-expressing cells. In LLC-PK1-derived HKRK-B28 cells, the EC_{50} for cAMP-stimulation by [M]PTH(1-14) was 110±30 nM and that of PTH(1-34) was 3.1±1.0 nM. Relative to the potency of [M]PTH(1-14), the analog, [Aib^1,M]PTH(1-14) and [Aib^3,M]PTH(1-14) exhibited 10-fold improvements in cAMP-stimulating potency (EC_{50}s ~ 10 nM each). The disubstituted peptide, [Aib^{1,3},M]PTH(1-14), exhibited an EC_{50} for cAMP production of 1.2±0.2 nM, and was thus 2.6-fold more potent than PTH(1-34). In COS-7 cells expressing hP1R-delNt, a truncated receptor that lacks most of the N-terminal domain and thus responds poorly to PTH(1-34) (EC_{50} for cAMP production = 680±110 nM), [Aib^{1,3},M]PTH(1-14) was remarkably fully potent (EC_{50} =0.7±0.2 nM). [Aib^{1,3},M]PTH(1-14) stimulated inositol polyphosphate production in COS-7 cells expressing intact hP1R with a potency comparable to that of PTH(1-34) (EC_{50} s = 90±1 nM and 20±1 nM, respectively). In addition, like PTH(1-34), [Aib^{1,3},M]PTH(1-14) inhibited differentiation of growth plate chondrocytes in an *ex vivo* model of endochondoral bone formation. The results demonstrate that a peptide as short as 14 amino acids designed to be conformationally constrained can fully activate the P1R. The data support the notion that the N-terminal portion of PTH is α-helical when bound to the receptor, and they provide new information that should be useful in designing new PTH-1 receptor agonists.

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Functional Evidence that the Amino Acid Sidechains at Positions 6 and 10 of PTH Interact when the Ligand Is Bound to the PTH-1 Receptor. N. Shimizu,* B. D. Petroni,* T. J. Gardella.* Massachusetts General Hospital, Boston, MA, USA.

Recent solution-phase NMR and X-ray crystallographic studies of PTH(1-34) indicate that the N-terminal portion of the ligand is α-helical; but the conformation of the hormone when it is bound to the receptor is unknown. In the helical models, the highly conserved residues of glutamine(Q)-6 and asparagine(N)-10 are in an i, i+4 configuration, such that their sidechains could interact. We utilized a functional approach to explore the possibility that an interaction between Q6 and N10 occurs when the ligand is bound to the receptor. The analog [Ala3,Gln10,Arg11]rPTH(1-11)amide {PTH(1-11)} stimulated cAMP formation in the LLC-PK1-derived cell line HKRK-B7 with a potency (EC_{50}) of 4.7±0.4 μM, [Ala10]PTH(1-11) was slightly weaker (EC_{50} = 14±3 μM), and the potency of [Ala6]PTH(1-11) was markedly reduced (EC_{50} = 570±120 μM). Combining the substitutions improved potency, relative to that of [Ala6]PTH(1-11), as the EC_{50} observed for [Ala6,10]PTH(1-11) was 130±20 μM. Thus, the Ala-10 substitution partially rescued the signaling defect imposed by the Ala-6. A similar rescue effect was observed in PTH(1-14) analogs. Alanine substitutions at positions 6 and/or 10 of [Tyr34]hPTH(1-34)amide {(PTH(1-34)} had no effect on cAMP-stimulating potency in COS-7 cells expressing hP1R-WT (EC_{50}s ~ 0.3 nM), however, in COS-7 cells expressing hP1R-delNt, which lacks the large amino-terminal extracellular domain, [Ala6]PTH(1-34) at 10 μM stimulated only a 3-fold increase in cAMP levels, relative to the basal cAMP levels, whereas PTH(1-34), [Ala10]PTH(1-34) and [Ala6,Ala10]PTH(1-34), each stimulated a 30-fold increase in cAMP formation with this receptor. These data indicate that the sidechains of Gln-6 and Asn-10 of PTH functionally interact and they also support the model that the N-terminal domain of PTH is α-helical when bound to the activation domain of the receptor.

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In vivo Expression of a Constitutively Active PTH/PTHrP Receptor (PPR) in Odontoblasts (ODs) Alters OD and Ameloblast (AB) Function and Maturation. L. M. Calvi, H. I. Shin, M. C. Knight,* J. M. Saxton,* H. M. Kronenberg, E. Schipani. Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

PTHrP is an important autocrine/paracrine attenuator of programmed differentiation in many organ systems and has been implicated in mesenchymal/epithelial interactions. In tooth development, expression of PTHrP mRNA is restricted to the epithelial layer. PPR mRNA is instead detected in the dental papilla, suggesting that PTHrP and the PPR may modulate epithelial-mesenchymal interactions.We have previously described transgenic mice in which a constitutively active PPR is targeted to osteoblasts by the 2.3 kb fragment of the a1(I) collagen promoter. These transgenics have a vivid postnatal bone and tooth phenotype. While tooth eruption was normal, mutant crowns had an abnormal appearance, were friable and susceptible to cavities. By in situ hybridization, transgene mRNA expression was seen at birth in the dental papilla and, at 1 week postnatally, in ODs. There was no transgene expression in ameloblasts (ABs). We therefore studied transgenic molars and incisors histologically and by in situ hybridization to explore the role of PPR activation in ODs. At birth, tooth morphology in the transgenic mice was normal. By 1 week of age, there was widening of the dental papilla, disorganization of the OD layer and decreased thickness of the dentin layer, which had a disordered rather than lamellar pattern. Expression of OD markers was unchanged in mutant animals. The number of cusps was abnormally increased, the AB layer was disorganized, and thickness of the enamel layer was decreased. In normal mice, ameloblastin and amelogenin (a gift of M.F.Young), markers of AB differentiation, were homogeneously expressed by ABs at 1 week, while amelogenin expression decreased in molars at 2 weeks, consistent with normal AB maturation. In contrast, both ameloblastin and amelogenin were expressed heterogeneously by mutant ABs at 1 week postnatally, and diffuse amelogenin expression persisted throughout the AB layer at 2 weeks postnatally. Further, in teeth from the mutant animals, cells in the AB layer incorporated BrdU, and were thus proliferative, while there were no proliferating cells in the AB layer of normal littermates. Preliminary data by electron microscopy confirmed these morphologic findings.These findings suggest that activation of the PPR in ODs alters their organization and dentin formation. In addition, even though the transgene was not expressed in ABs, both AB differentiation and enamel formation were abnormal. These data describe a useful model to study how this novel action of the PPR modulates mesenchymal/epithelial interactions in tooth morphogenesis and development.

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PTHrP Inhibits Streptozotocin-Induced Beta Cell Death In Vivo. A. Cebrian, K. Takane, D. Sipula, A. Garcia-Ocaña, A. F. Stewart, R. C. Vasavada. University of Pittsburgh, Pittsburgh, PA, USA.

Targeted overexpression of PTHrP in pancreatic beta cells using the rat insulin II promoter (RIP) results in a 3- to 4-fold increase in islet mass in RIP-PTHrP transgenic mice. Surprisingly, the increase in islet mass is neither due to an increase in beta cell replication rate nor islet cell size, but is likely due to improved beta cell survival. To directly examine whether PTHrP prolongs islet cell survival, the beta cell toxin, streptozotocin (STZ) was used to induce beta cell death in these mice.Pancreata harvested from normal and transgenic adult mice, 12 hours after an intraperitoneal injection of STZ (100mg/Kg body weight) were examined for cell death by TUNEL and propidium iodide (PI) staining. There was a marked increase in beta cell death by TUNEL in normal mice treated with STZ (11.2±3.1%, n=14) vs RIP-PTHrP mice (1.8±0.3%, n=15) (p=0.01). Similarly when quantitated by PI staining beta cell death was 15.4±4.2% (n=5) in normal mice vs 2.4±0.7% (n=5) in transgenic mice (p=0.035). To ensure that the reduced beta cell death observed in transgenic mice is not merely a reflection of the increased islet mass observed in adult RIP-PTHrP mice, similar experiments were carried out in one-week-old mice, in which islet mass is identical in normal and transgenic animals. Again, STZ-induced beta cell death was significantly higher in normal one-week-old mice (6.5±0.7%, n=20) compared to transgenic mice (3.5±0.7%, n=11) (p=. 005). These data unequivocally indicate that PTHrP protects beta cells from STZ-induced cell death in vivo, and that the protection is not merely a function of increased islet mass.To determine if PTHrP exerts its protective effect through regulation of the Bcl-2 family, mRNA levels of Bcl-2 family members were measured by semi-quantitative RT-PCR. Whereas Bcl-XL, Bax and Bad mRNA levels were similar in normal and transgenic islets, Bcl-2 mRNA levels increased 2.7-fold in transgenic islets (p=. 003). Expression of Bcl-2 at the protein level is under investigation.To examine the effect of PTHrP on STZ-induced cell death in vitro, survival of isolated, STZ treated, normal and transgenic mouse islets was quantitated by the MTT assay. Preliminarily, RIP-PTHrP islets were not resistant to the cytotoxic effects of STZ (1mM for 6 hrs) in vitro, with 67.6±4.9% cell survival in transgenic islets vs 79.9±6.4% in normal islets (n=4). Thus, the protective effect of PTHrP observed in vivo may be difficult to study in vitro.In conclusion, PTHrP protects beta cells against STZ-induced cell death in RIP-PTHrP mice in vivo. This is in keeping with the pro-survival effect of PTHrP in other cell-types. It is possible that this protective effect of PTHrP may be mediated through increased Bcl-2 expression.

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PTHrP Activates RANKL Expression in the Alveolar Bone to Accommodate Normal Tooth Development. K. Mekaapiruk,*[1] N. Suda,[1] Y. Kitahara,*[1] O. Baba,*[2] V. E. Hammond,*[3] F. Beck,*[3] Y. Takano,*[2] T. Terashima,*[2] T. Kuroda.*[1] [1]Maxillofacial Orthognathics, Tokyo Medical and Dental University, Tokyo, Japan, [2]Biostructural Science, Tokyo Medical and Dental University, Tokyo, Japan, [3]Howard Florey Institute of Experimental Physiology and Medicine, Melbourne, Australia.

Parathyroid hormone-related protein (PTHrP) is expressed from epithelial cells of tooth germs and suggested to play an important role in both tooth development and eruption. We have reported that the tooth germs are destructed and ruffled border formation is impaired in the osteoclasts located on the alveolar bone surface of homozygous PTHrP-knockout mice. However, the physiological importance of PTHrP on osteoclast formation during tooth and alveolar bone development, and involvement of RANK-RANKL in this process is not yet known. To clarify these issues, osteoclast formation and RANKL expression in the alveolar bone were examined using PTHrP-knockout mice. The mandibular explants at E14 isolated from wild-type (+/+) and homozygous PTHrP-knockout mice (-/-) were subjected to serum-free organ culture system, according to the modified Trowell's method. Before culture, the tooth germs of +/+ and -/- were in the cap stage with clearly defined alignment of alveolar bone. After 10 days of culture, the tooth germs normally developed

to the late bell stage in +/+ cultures. There was no significant difference in the number of TRAP-positive cells in the explants before and after the culture. In contrast, the tooth germs were compressed or penetrated by the alveolar bone and the number of TRAP-positive cells significantly decreased in the -/- cultures. Exogenous PTHrP reduced the severity of this compression or penetration. The result of in situ hybridization showed that RANKL was expressed by osteoblastic cells in the inner aspects of alveolar bone in +/+, and significantly down-regulated in the corresponding regions of -/-. Interestingly, this down-regulation was not seen in sites distant from the tooth germs. Finally, when tooth germs were isolated and cultured independent of the surrounding alveolar bone, the histological features did not show any differences in both explants, suggesting PTHrP is not essential for tooth development proper but the observed tooth destruction in -/- was the secondary effect of abnormally developed alveolar bone. These observations suggest that PTHrP is a pivotal paracrine factor regulating alveolar bone development and essential for normal tooth development, via activation of RANKL expression.

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Alterations in Renal Hemodynamics in Transgenic Mice With Smooth Muscle-Specific Overexpression of the PTH/PTHrP Receptor. W. T. Noonan,*[1] J. Qian,[2] W. D. Stuart,*[2] T. L. Clemens,*[2] J. N. Lorenz.*[1] [1]Department of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA, [2]Department of Medicine, University of Cincinnati, Cincinnati, OH, USA.

PTH-related protein (PTHrP) and the PTH/PTHrP-type 1 receptor (PTHrP-R) are expressed in the renal vasculature and have been postulated to participate in the control of renal vascular tone. We have previously developed transgenic mice that overexpress the PTHrP-R in smooth muscle. The goal of this study was to evaluate the effect of PTHrP-R overexpression on renal function at baseline and during saline volume expansion (SVE). We hypothesized that SVE would cause an induction of PTHrP and the renal responses to this protein would be altered in transgenic mice. Catheters were placed in a femoral artery and vein, and the glomerular filtration rate (GFR) and effective renal plasma flow (EPRF) were measured with FITC-inulin and PAH, respectively. Baseline mean arterial pressure (MAP), body and kidney weights were not significantly different in transgenic and control mice. MAP decreased significantly ($p<0.05$) in both groups during SVE compared to baseline, and this decrease was significantly greater in transgenics. This suggests that PTHrP is released from renal vasculature in response to SVE, and that the peptide causes a greater dilation in mice overexpressing the PTH/PTHrP receptor. The GFR decreased in controls during SVE, indicating a possible post-glomerular dilation. Interestingly, no difference in GFR was observed in transgenics during SVE suggesting a possible pre- and post-glomerular dilation. SVE caused an slightly greater increase in ERPF in transgenics compared to controls. While a decrease in sodium excretion would be expected in transgenics during SVE, no remarkable differences in electrolyte excretion were observed. In conclusion, the renal and hemodynamic effects observed in mice overexpressing the PTH/PTHrP receptor in the renal vasculature suggest that PTHrP plays a role the regulation of renal function.

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Identification of a Nuclear Localization Sequence in the Human-Specific, Carboxy-Terminal Region of PTHrP1-173. R. H. Hastings,[1] J. Y. Wu,[1] D. W. Burton,[2] L. J. Deftos.[2] [1]Anesthesiology, UC San Diego and Veterans Affairs Medical Center, San Diego, CA, USA, [2]Endocrinology, UC San Diego and Veterans Affairs Medical Center, San Diego, CA, USA.

PTH-related peptide (PTHrP) is present in a wide variety of tissues and has both endocrine and intracrine mechanisms of action. At the nuclear level, PTHrP is believed to regulate cell proliferation, differentiation, and apoptosis. The human PTHrP mRNAs encode three isoforms of 139, 141, and 173 amino acids through alternative splicing. Several studies have documented that the 139 and 141 isoforms localize to the nucleus through a bipartite nuclear localization sequence (NLS) in the 87-107 region. The human-specific 140-173 region also contains a bipartite multibasic motif, but few studies have focused on the 1-173 isoform. In this report, we performed transfection studies to further investigate the NLS activity of the PTHrP140-173 region that we previously studied in human cartilage cells (Endocrinology 14:4613, 2000). We studied the trafficking of transfected PTHrP1-173 and various missense and truncated PTHrP mutants, including PTHrP1-139, in COS-1 cells. Media, cytoplasmic and nuclear compartments were separated 48 hours after transfection (n = 4 separate studies). The subcellular fractions were >95 % pure and PTHrP levels measured by three region-specific immunoassays confirmed the fidelity of the plasmids. The level of nuclear PTHrP was increased in the PTHrP1-173 transfected cells vs. PTHrP1-139 (33, and 8 pg/mg DNA , respectively). Missense mutations in the 147-150 (KKKK) and 154-155 (RR) basic motifs reduced the nuclear PTHrP levels by 2 and 13-fold, respectively. Missense mutations in the 87-107 region (88-91 and 96-98) decreased the nuclear PTHrP 2.5-fold and 1.8-fold, as was expected. Exogenously added PTHrP1-34, 38-64, 107-139, and 140-173 peptides had no effect on the nuclear levels of PTHrP in PTHrP1-173 transfected cells, indicating that receptor-mediated or adsorptive transport of secreted PTHrP was not contributing significantly to the total nuclear PTHrP measured. A PTHrP1-173-GFP (green fluorescent protein) chimeric protein expressed after transfection into COS-1 cells demonstrated greater nucleolar localization than a PTHrP1-108-GFP chimeric. Thus, PTHrP140-173 appears to constitute an NLS dependent on two clusters of basic residues. This region may interact with the 87-107 NLS in mediating intracrine effects of PTHrP.

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PGE2 is a Mediator in TPA-Induced PTHrP Production in Synovial Fibroblasts of Patients With RA. A. Suematsu,*[1] H. Sakamoto,*[2] T. Horiuchi,[3] S. Yamamoto,[3] T. Nishikawa,*[4] Y. Koshihara.[5] [1]Department of Nutrition, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan, [2]Sakamoto Clinic, Kagoshima, Japan, [3]Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan, [4]Toranomon Hospital, Tokyo, Japan, [5]Tokyo Metropolitan Institute of Gerontorogy, Tokyo, Japan.

We have found that phorbol 12-mysistate 13-acetate (TPA) promoted parathyroid hormone related peptide (PTHrP) production in synovial fibroblasts of patients with rheumatoid arthritis (RA). In this study, we clarify the mechanism of TPA-induced PTHrP production in RA synovial fibroblasts.These fibroblasts were established in culture from articular tissue of RA as described previously (Yoshida, 1998). These cells were plated into 24 wells, and cultured with a-MEM containing 20% horse serum until confluence. The cells were treated with TPA in the presence or absence of NS-398, a cylooxygenase-2 (COX-2) inhibitor for 24 hrs. PTHrP and prostaglandin E2 (PGE2) released into the medium, which were measured by IRMA and ELISA, respectively. Fuethermore the cells were treated with PGE2 receptor agonists in the presence or absence of cholera toxin (CTX), pertussis toxin (PTX) or PKI amide (14-22) , a protein kinase A inhibitor, for 24 hrs. The expression of PTHrP and COX-2 was confirmed by RT-PCR.1) TPA-induced PTHrP production was significantly suppressed by the addition of NS-398. The suppression was recovered by the addition of PGE2 receptor agonists. 2) TPA induced PGE2 production. 3) TPA-induced PTHrP production was produced through PGE2 and mostly mediated by EP2/EP4 PGE2 receptors. 4) CTX that stimulate adenylyl cyclase increased PTHrP production, whereas PTX that inhibit adenylyl cyclase inhibited PTHrP production. PKI amide inhibited PTHrP production. In conclusion, these results clearly indicate that TPA-induced PTHrP production in RA synovial fibroblasts is carried out by an increase of intracellular cAMP level, which is mediated via EP4 PGE2 receptor.

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Parathyroid Hormone-related Protein Regulates Apoptosis in Pancreatic Adenocarcinoma. M. Bouvet,* D. W. Burton, S. R. Nardin,* A. R. Moossa,* L. J. Deftos. University of California San Diego and the San Diego VA Medical Center, San Diego, CA, USA.

We have recently demonstrated that PTHrP is produced and secreted by human pancreatic adenocarcinoma cells and has growth regulating properties in this cancer. To date, the exact mechanism of PTHrP's growth regulation in pancreatic cancer is unknown, although we and others have demonstrated that PTHrP can regulate apoptosis in other cell types and cancers. Therefore, we treated the human pancreatic adenocarcinoma cell line AsPC-1 with varying concentrations of PTHrP 1-34 peptide. Growth of AsPC-1 cells was stimulated in a dose-dependent manner by PTHrP 1-34. By 48 hours, statistically significant differences in cell number were seen at all concentrations of PTHrP compared with control (by Student's t test). AsPC-1 cells were then incubated with 10 nM PTHrP 1-34 peptide and challenged with 25mM hydrogen peroxide in growth medium. Apoptosis was evaluated by TUNEL staining. The staining was evaluated microscopically by counting 10 fields of >20 cells. AsPC-1 cells treated with 10 nM PTHrP1-34 peptide demonstrated less TUNEL positive cells after hydrogen peroxide induced apoptosis compared to control. The P values determined by Student's t-test were significant ($P< 0.05$) compared to control. Therefore, PTHrP1-34 treatment protected AsPC-1 cells against hydrogen peroxide induced apoptosis. These results indicate that PTHrP may regulate the growth of pancreatic cancer in part by regulation of apoptosis.

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Regulatory Interactions of PTHrP and Interleukin-8 (IL-8) in Breast Cancer. W. Jen, S. Tu,* D. W. Burton, A. Gujral, L. J. Deftos. Dept. of Medicine, University of California, San Diego and SD VA Medical Center, San Diego, CA, USA.

Parathyroid hormone-related protein (PTHrP) was initially identified as a mediator of humoral hypercalcemia of malignancy (HHM) in breast cancer. Expression of PTHrP in breast cancer cells may directly regulate the development of the malignant tumor cell itself, and it may also regulate the metastatic potential of these maglinant cells by interacting with various growth factors, cytokines and their receptors. We investigated PTHrP gene expression, and interleukin (IL)-6 and IL-8 production in four immortalized human breast cancer cell lines. We subsequently focused on IL-8, an angiogenic cytokine, and the breast cancer cell line MDA-MB-435S by generating stable clones through transfection with either vector (pCi-neo), wild-type PTHrP1-173, or COOH-terminus truncation mutant PTHrP1-87. Compared to vector alone, both PTHrP1-173 and PTHrP1-87 stable clones demonstrated approximately a three-fold increase in the PTHrP expression, as documented by region specific immunoassays that can specifically identify each PTHrP species. In the cell proliferation assay, using the MTT method, we showed that PTHrP1-87 clone and PTHrP1-173 clone caused a three-fold increase and no change, respectively, in proliferation rate when compared to the vector-only clone. To elucidate the molecular pathway of PTHrP interacting with IL-8, we conducted IL-8 promoter analysis. Observing that PTHrP positively regulates the IL-8 promoter, leading to an increase of IL-8 expression, we further dissected the IL-8 promoter using the Stratagene PathDetect approach. These studies showed that PTHrP induced the production of AP1, NF-kB, Serum Response Factor (SRF), cAMP Response Element (CRE), MEK and MEKK, ranging from approximately two-fold to five-fold increase. Some of these factors, such as AP1 and NF-kB, can exert regulatory effects through their respective binding elements on the IL-8 promoter. We extended these observations through suppression subtractive hybridization studies with our PTHrP-transfected stable cell lines, which also identified novel regulatory partners for this oncoprotein. In summary, our studies demonstrate that PTHrP stimulates the expression of IL-8 in breast cancer cells through several molecular pathways, extending our corresponding observations in prostate cancer. The increase expression of this angiogenic cytokine may contribute to the complex effects of PTHrP on breast cancer pathogenesis and progression. These observations add to the growing body of evidence for an angiogenic regulatory role of PTHrP in breast cancer metastases.

M497

PTH/PTHrP Receptor Transcript Expression Levels are Modulated During the Murine Hair Cycle. Y. Cho,* J. A. Jimenez,* J. Foley. Anatomy and Cell Biology, Indiana University School of Medicine, Bloomington, IN, USA.

In murine skin, PTHrP mRNA and protein expression are largely restricted to epithelial components of the developing and adult hair follicle. Overexpression of PTHrP reduces the length of anagen, whereas a peptide antagonist of the PTH/PTHrP receptor appears to extend this phase of the hair cycle. To better understand PTHrP signaling in the hair follicle, PTH/PTHrP receptor expression was evaluated by in situ hybridization and RNase protection in a series of murine skin samples ranging from E-14 to two years. PTH/PTHrP receptor transcripts were diffusely expressed at high levels in the mesenchyme of the E-14 to E-16 samples, but were primarily expressed in the upper third of the developing dermis in late prenatal and early postnatal samples. At one to two weeks of age, the majority of PTH/PTHrP receptor transcripts was observed in the upper dermis and fibroblasts lining the bulge region of anagen hair follicles. Using wax stripping to synchronize the hair cycle in mice 6-8 weeks of age, high levels of PTH/PTHrP receptor transcripts were observed in the early to middle anagen phase of the cycle, whereas the highest levels of PTHrP mRNA were observed in late anagen and catagen phases. Taken together, these findings indicate that that the PTH/PTHrP receptor is expressed in close proximity to the major source of PTHrP in murine skin and that both ligand and receptor expression are modulated during the murine hair cycle.

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Purification and Characterization of Solubilized Human PTH/PTHrP Receptors (PTH1R). M. Shimada,* X. Chen, T. Cvrk,* H. Hilfiker,* M. Parfenova,* G. V. Segre. Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

To apply physical approaches to understanding the relationship between function and structural features of the PTH1R requires the availability of functional, highly purified receptors. Studies of such receptors define its precise topography within the plasma membrane, sites important for interactions with ligands and transducers, and allosteric changes that occur upon binding of agonists and antagonists. PTH1R, containing a nine amino-acid sequence of rhodopsin at its C-terminus, was transiently expressed in COS7 cells and solubilized with dodecyl maltoside. It was then bound to Sepharose-immobilized monoclonal antibody, 1D4, and eluted by peptide competition at pH 7.5. Sequence analysis revealed a homogenous preparation. The initial residue was Y23, reflecting processing of the signal peptide. Approximately 30 ug of solubilized PTH1R were recovered from 5 mg of crude membranes; recovery of PTH1R after affinity chromatography was 60%. Next, we examined ligand binding using two radioligands, ^{125}I-[Nle^{8,21},Y^{34}]-rPTH(1-34)amide [PTH(1-34)] and ^{125}I-[I^{5},W^{23},Y^{36}]-rPTHrP(5-36)amide [PTHrP(5-36)]. 0.3 ug of solubilized PTH1R were bound to immobilized 1D4, and incubated with radioligands for various periods of time. Receptors were eluted as described above and bound radioligand was counted. Binding of both radioligands to solubilized PTH1R reached equilibrium between 48~72-hour at 4°C; total binding was 4% with only 0.3% non-specific binding. Dissociation studies of both radioligands were performed by adding cold PTH(1-34) or PTHrP(5-36) to solubilized PTH1R after 72-hour incubation. Dissociation curve had multiple components. Half times of rapid and slow phase were less than 30 min and several hours, respectively. In competitive binding assays using ^{125}I-PTH(1-34), the affinities of PTH(1-34), PTHrP(5-36) and [A^{3,12},Nle^{8},Q^{10},Har^{11},W^{14},R^{19},Y^{21}]-rPTH(1-21)amide [PTH(1-21)] were 13, 19 and 7000 nM, respectively. With ^{125}I-PTHrP(5-36), the affinities of PTH(1-34), PTHrP(5-36) and PTH(1-21) were 18, 155 nM and 100 uM. Thus, studies with both PTH(1-34) and PTHrP(5-36) confirm the importance of the N-terminal extracellular domain for binding the C-terminal portion of PTH(1-34). Competition with PTH(1-21) was at least 15-fold less efficient with ^{125}I-PTHrP(5-36) than ^{125}I-PTH(1-34). These properties are consistent with those reported for PTH1R in membranes (Hoare, JBC, 2001) and support a two-domain binding model. Importantly, the close similarity between binding properties of membrane-embedded and purified, solubilized receptors suggests that the latter are suitable for detailed physical/biochemical studies.

M499

Conformational Changes of the Parathyroid Hormone (PTH)/PTH-related Protein (PTHrP) Receptor Induced by Agonists Modified in Position 1 Influence Receptor Interaction with Beta-Arrestins and Desensitization of Cyclic AMP Signaling. S. L. Ferrari,[1] L. Monticelli,*[2] M. Rosenblatt,[1] M. Chorev,[1] D. F. Mierke,*[2] A. Bisello.[3] [1]Div. of Bone and Mineral Metabolism, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA, [2]Molecular Pharmacology, Biology & Medicine, Brown University, Providence, RI, USA, [3]Div. of Endocrinology and Metabolism, University of Pittsburgh, Pittsburgh, PA, USA.

Agonists binding to the PTH/PTHrP receptor (PTH1Rc) induces both signal transduction (resulting in increases in cAMP and intracellular calcium, iCa++) and interactions with cytoplasmic beta-arrestin2 (b-arr2), resulting in receptor desensitization and endocytosis. Interestingly, these mechanisms appear to be largely dependent on the structure of residue 1 in the agonist. In order to determine PTH1Rc conformational changes required for receptor coupling to b-arr2, we have now analyzed a number of PTH- and PTHrP-derived ligands modified in position 1.The cAMP- and iCa++-stimulating activities of PTH-(1-34), PTH-(1-31) and PTHrP-(1-36), and a series of residue 1-modified ligands [Bpa1-PTHrP-(1-36), PTHrP-(2-36), Bpa1-PTH-(1-34), PTH-(2-34) and desamino-PTH-(1-34)] were characterized in HEK 293 cells stably expressing the human PTH1Rc. Their effects on b-arr2 mobilization and receptor internalization was determined by fluorescence microscopy in both HEK 293 and human (SaOS-2) osteosarcoma cells expressing either b-arr2-GFP or PTH1Rc-GFP. Receptor conformational changes in response to various ligands were analyzed by extensive molecular dynamics utilizing an experimentally-based model of PTH–PTH1Rc complex as a template.While all ligands acutely stimulated cAMP accumulation (EC50 1-10 nM), none of the analogs modified on residue 1, except Bpa1-PTH-(1-34), stimulated iCa++. In contrast to PTH-(1-34), PTH-(1-31) and PTHrP-(1-36), residue 1-modified agonists all failed to mobilize b-arr2 and internalize PTH1Rc, irrespective of their signaling selectivity. In addition, PTHrP-derived agonists, Bpa1-PTHrP-(1-36) and PTHrP-(2-36), caused a sustained and prolonged cAMP accumulation compared to PTH-derived ligands. Molecular modelling indicated that a significant alteration of the arrangement of receptor transmembrane domain (TM) 5 and TM6 occurred with Bpa1-PTHrP-(1-36), and not with Bpa1-PTH-(1-34) or PTH-(1-34), leading to an altered conformation of the PTH1Rc third intracellular loop.These results suggest some conformational changes in the experimentally-based molecular model of the ligand--PTH1Rc complex that may influence coupling to b-arr2, internalization and desensitization of cAMP signaling in response to PTH and PTHrP.

M500

Transient Expression of GFP-PTH/PTHrP Receptor Constructs in LLC-PK1 (Clone 46) Cells. E. Patterson,*[1] P. H. Watson,[2] L. E. Canaff,*[3] G. N. Hendy,[3] R. Bringhurst,[4] A. B. Hodsman,[2] L. J. Fraher.[2] [1]Biochemistry, University of Western Ontario, London, ON, Canada, [2]Medicine, University of Western Ontario, London, ON, Canada, [3]Medicine, Calcium Research Laboratory, McGill University, Montreal, PQ, Canada, [4]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

Nuclear localization of the type 1 PTH/PTHrP receptor (PTHR) has been demonstrated in rat liver, kidney, uterus, ovary and gut, as well as the cultured osteoblast-like cell lines, MC3T3-E1, SaOS-2, ROS 17/2.8 and UMR106 using both ICC and indirect immunofluorescence. Residues 446-473 of the mature PTHR protein have been identified as a possible bipartite nuclear localization signal (NLS), and thus may play a role in directing the PTHR to the nucleus. We engineered a series of green fluorescent protein (GFP) labeled PTHR gene constructs and transiently expressed them in cultured LLC-PK1 (clone 46) porcine kidney cells which show no biochemical response to PTH *in vitro*. Constructs included the entire PTHR sequence, or lacked either the putative NLS, or signal sequence, or both. One construct contained only the putative NLS. LLC-PK1 cells were grown to ~70% confluence on glass coverslips before being transfected using Lipofectamine 2000 (Gibco). Cultures were maintained for 24 hr following transfection, then were fixed, counterstained with propidium iodide and observed under fluorescence. As controls, cells were transfected with either empty vector (no fluorescence was observed) or vector containing GFP alone (bright non-specific fluorescence throughout the cell). In cells transfected with the wildtype construct fluorescence was observed throughout the cell, including membranes and nuclei and fluorescent filamentous membrane projections were present. Small, intense, juxtanuclear foci of fluorescence were also commonly observed, similar to previously reported results obtained by ICC in cultured MC3T3-E1 cells in late S-phase. Deletion of the signal sequence did not alter the intracellular and nuclear localization of the construct. However, in cells transfected with constructs lacking the NLS fluorescence was restricted to a perinuclear ring. In cells transfected with the NLS alone fluorescence was scattered throughout the cell with no particular localization to membranes or nuclei. These results suggest that the putative NLS identified in the PTHR protein is most likely functional in the context of the intact receptor. Larger regions surrounding the NLS may be important to regulating the function of the NLS and future experiments will employ constructs in which a greater portion of the C-terminus of the PTHR is retained to determine whether such regulatory regions exist.

M501

Novel Mutations in the Type I PTH/PTHrP-Receptor Causing Blomstrand Lethal Osteochondrodysplasia. M. Karperien,[1] H. Sips,*[1] H. van der Harten,*[2] L. Wijnaendts,*[2] L. Kindblom,*[3] S. E. Papapoulos,[1] C. Lowik.[1] [1]Endocrinology, Leiden University Medical Center, Leiden, The Netherlands, [2]Pathology, Free University Hospital, Amsterdam, The Netherlands, [3]Pathology, Goteborg University, Goteborg, Sweden.

Blomstrand lethal osteochondrodysplasia (BOCD) is caused by homozygous inactivating mutations in the type I PTH/PTHrP-receptor and presents in two forms. Type I is the most severe form. Type II is characterized by a relatively less severe phenotype. BOCD is characterized by dwarfism due to accelerated endochondral bone formation and premature ossification of all skeletal components, a phenotype which is very similar to that observed in PTH/PTHrP-receptor knock out mice. Of the 3 different mutations described thus far, the P132L substitution has been found in 3 affected unrelated families and is associated with BOCD type II. The other 2 mutations are associated with type I and include a splicing defect and a frame-shift mutation resulting in the expression of a truncated receptor.In this study, we have analyzed two other cases of BOCD. The first case has previously been described in literature (Blomstrand et al., 1985, Pediatric Radiol.) and belongs to BOCD type I. Sequence analysis of all coding exons of the type I PTH/PTHrP-receptor identified a homozygous C > T conversion in exon E2 coding for part of the extracellular domain. This mutation changes the CGA codon at position 104 in a premature TGA stopcodon (R104Stp) resulting in complete loss of function of the PTH/PTHrP-receptor. The second case was a stillbirth at 33 weeks and belongs to type II. The diagnosis BOCD was confirmed by stimulating case-derived dermal fibroblast cultures with bPTH(1-34). In sharp contrast to controls, these cultures did not respond with an increase in intracellular cAMP after challenge with bPTH(1-34). However, sequence analysis of all codon exons and flanking intron-exon boundaries, did not show any nucleotide alterations. Further examination of the PTH/PTHrP-receptor mRNA expression in dermal fibroblasts demonstrated a slight increase in size of one of the PCR amplicons spanning the receptor's transmembrane domain. Sequence analysis identified an insertion of 27 nucleotides between exon M4 and EL2 due to an extension of exon M4. This insertion introduced a premature stopcodon directly after G350. The splicing defect was not caused by nucleotide alterations in the exon-intron boundary of exon M4, but was most likely the result of a mutation located further downstream in the intron. In conclusion, we have identified two novel inactivating mutations in the type I PTH/PTHrP-receptor, providing further support for a causative role of this receptor in this disorder.

M502

Identification of Regions Within the Type 3 Zebrafish PTH-Receptor That Prevent Activation of the Phospholipase C Signaling Pathway. K. B. Jonsson,* D. A. Rubin, H. Jüppner. Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

The recently cloned zebrafish type 3 PTH-receptor (zPTH3R) fails to show PTH- and PTHrP-dependent accumulation of inositol phosphate (IP), while the type 1 zebrafish PTH/PTHrP receptor (zPTH1R) shows an agonist-dependent activation of this signaling pathway that is indistinguishable from the findings in the mammalian homologs. To explore which region(s) of these closely related receptors are responsible for the distinct signaling properties, we first generated several chimeras between zPTH1R and zPTH3R, and tested these receptor mutants, after transient expression in COS-7 cells, for their ability to stimulate the accumulation of total IP in response to PTHrP(1-36). These studies indicated that intracellular loops 2 and 3 (ICL 2 and ICL3) of the zPTH1R both contain important determinants required for agonist-dependent IP accumulation. We next introduced several cassette mutations into the zPTH3R, i.e. clusters of zPTH3R-specific residues within the intracellular loops and transmembrane regions were replaced with the corresponding zPTH1R-specific residues. Again, the results suggested that ICL2 and ICL3 are important for IP accumulation. Specifically, we found that a receptor mutant in which residues 325, 326 and 328 within ICL3 of the zPTH3R were replaced with the corresponding zPTH1R-specific residues restored agonist-dependent IP accumulation to about 50% of the level observed with cells expressing the zPTH1R. A zPTH3R mutant in which these residues were replaced in combination with three additional amino acid exchanges in ICL2 showed further improvement in signaling through the IP/PLC pathway. Furthermore, COS-7 cells expressing a zPTH3R with a single point mutation, W328R, showed about 15% of the agonist-dependent IP accumulation observed with the zPTH1R. The zPTH3R-specific residues that appear to prevent efficient, agonist-dependent accumulation of IPs were then introduced into the zPTH1R and the rat PTH1R. In either type 1 receptor species, the introduction of these amino acids led to impaired IP formation, indicating that at least two different intracellular regions of the PTH/PTHrP receptor are required activation of the PLC signaling pathway.

M503

Interaction of Tuberoinfundibular Peptide of 39 Residues with the Human PTH-1 Receptor, PTH-2 Receptor, and a Mutant PTH-1 Receptor Expressed in HEK 293 Cells. O. Jakob,* E. M. Endress,* U. Heindel,* B. Allolio, E. Blind. Dept. of Medicine - Endocrinology, University of Wuerzburg, Wuerzburg, Germany.

The physiological role of the PTH-2 receptor in calcium metabolism is unknown. Tuberoinfundibular peptide of 39 residues (TIP39), a polypeptide isolated from bovine hypothalamus [1], is thought to be likely the physiological ligand of the P2R in brain. We studied the interaction of this peptide with the human PTH-1 receptor (P1R), PTH-2 receptor (P2R), and a mutated P1R (P1RCC) in which two key cysteine residues (present only in the third extracellular loop of the PTH2R and possibly important for ligand binding) had been introduced (Ala426Cys; Tyr443Cys). Human embryonic kidney (HEK) 293 cells were stably transfected with the human P1R, P2R, and P1RCC cDNA constructs using the expression vector pCEP4. The binding properties of custom-synthesized bovine TIP39 and of hPTH(1-34) were compared in a radioreceptor assay using [^{125}I]-Nle^{8,21}-Tyr^{34}-rat PTH(1-34)-amid as radioactive ligand. TIP39 did bind to all three receptor-transfected cells. Binding affinity was moderate with the P1R ($IC_{50}\approx10^{-7}$ - 10^{-6}M) and did not change by introducing the two cysteines from the P2R (P1RCC). TIP39 showed higher affinity binding to the P2R ($IC_{50}\approx10^{-8}$M), comparable to the affinity of hPTH(1-34) to all three receptors. To determine the activation of the cyclic AMP pathway, cells were exposed to ligand, and accumulated intracellular cAMP was extracted and measured by standard RIA. In P2R-transfected cells, the cAMP dose-response curve was shifted about 10-fold to the left with TIP39 as a ligand compared to hPTH(1-34). In P1R-transfected cells, only hPTH(1-34) but not TIP39 was able to elicit a cAMP response; this was also the case for P1RCC at doses of up to 10^{-7}M. TIP 39 antagonised the cAMP-stimulating effect of 0.25 nM hPTH(1-34) in P1R-transfected cells with a half-maximal effect with 1 nM. In conclusion, in our model system TIP39 acts on the P2R as a more potent agonist than PTH itself, whereas on the P1R it is acting as an antagonist. Introducing extracellular cysteine residues from the P2R into the P1R did not change these properties. The physiological role of TIP39 in calcium metabolism with regard to these actions remains to be determined. 1. Usdin TB et al. (1999) Nature Neuroscience 2: 941-943.

M504

Influence of Agonist Choice on Parathyroid Hormone Receptor 1 Antagonist Potency. J. Meyer,* A. deRosier,* S. Sankuratri.* Roche Bioscience, Palo Alto, CA, USA.

To determine the effect of agonist choice on the functional inhibition constant K_b value for [Nle^{8,18}D-Trp^{12}Tyr^{34}]bPTH-(7-34)NH_2, a parathyroid hormone receptor 1 (PTHR1) antagonist, parathyroid hormone (PTH) or parathyroid hormone-related (PTHrP) peptides from four species were used as agonists. Functional antagonism against these four peptides were studied in two cell lines, rat derived UMR 106 cells endogenously expressing PTHR1 and HEK 293 cells over-expressing transfected rat PTHR1. In addition, the K_b values against the four agonists were determined by two different methods, short-term assay where stimulated cAMP was measured directly and a long-term cAMP-luciferase gene reporter assay. No difference in K_b values were observed between various agonists either in UMR 106 cells or HEK 293 cells over-expressing PTHR1 when antagonism was determined by direct estimation of cAMP. In the cAMP-luciferase gene reporter assay, however, a highly significant difference was seen in the K_b values depending upon the agonist used. The K_b values varied between 0.49 and 14 µM. The differences observed in K_b values for the PTH receptor antagonist suggest that the choice of agonist does influence the potency of a PTHR antagonist in a long-term assay. Based on the results presented here as well as other reports in the literature, it is suggested that the assay parameters, including the type of assay, should be consistently maintained when reproducing functional inhibition constants for antagonists in different laboratories.

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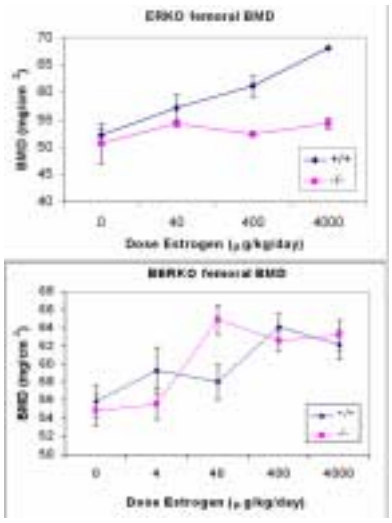
Distinct Effects of Deletion of ERα and ERβ on Responsiveness of the Skeleton to Exogenous Estrogen in Male Mice. K. E. McDougall,*[1] M. J. Perry,[2] J. H. Tobias.[1] [1]Rheumatology, University of Bristol, Bristol, United Kingdom, [2]Anatomy, University of Bristol, Bristol, United Kingdom.

We recently found that 17β-estradiol (E_2) induces a dose-responsive increase in bone mass in intact male mice as assessed by DXA, to an equivalent extent to that previously observed in female animals. Following the report that acquisition of peak bone mass is impaired in male mice lacking the alpha isoform of the estrogen receptor (ERα) but not the beta isoform (ERβ) (Vidal et al., 2000, PNAS 97:5474), we examined whether estrogen's action in increasing bone mass in male mice shows a similar preference for ERα. Separate alpha and beta knockout experiments were carried out. Fourteen-week-old alpha knockout (ERKO) mice (Lubahn et al., 1993 PNAS 90:11162) (2 animals per group), together with age-matched wild-type (WT) littermate controls, were administered vehicle or E_2 40, 400, 4000 µg/kg/day for 28 days. Twelve week-old beta knockout (BERKO) mice (Krege et al., 1998, PNAS 95:15677) (4-7 animals per group), together with age-matched WT littermate controls, were administered vehicle or E_2 4, 40, 400, 4000 µg/kg/day for 28 days. In each case, the subsequent response was quantified by measurement of bone mineral density (BMD) at the femur and tibia using a Lunar PIXI scanner with dedicated mouse software. Unlike WT animals, ERKO mice were unresponsive to E_2 administration as assessed at the whole whole femur, whole tibia, distal femoral metaphysis and proximal tibial metaphysis ($p < 0.02$ by two-way ANOVA). In contrast, in the BERKO experiment, knockout mice showed increased sensitivity to E_2 treatment, since 40µg/kg/day was associated with maximal response in BERKO animals, while 400µg/kg/day was required to elicit maximal response in WT mice ($p < 0.02$ by one-way ANOVA as assessed at all four measurement sites).



Preliminary histomorphometric analysis indicated that increased sensitivity of the skeleton of male BERKO mice to E_2 reflects altered responsiveness of cortical, as opposed to trabecular, bone. We conclude that the estrogen's action in increasing bone mass in male mice is abrogated in ERKO mice and enhanced in BERKO mice. Thus, while ERα is the principle mediator of this response, ERβ also appears to be involved as a negative modulator.

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Evidence that Androgen Receptor Expression in Human Fracture Is More Sensitive to Age than Estrogen Receptor Alpha and Estrogen Receptor Beta. G. S. Batra,*[1] L. J. Hainey,*[1] G. Andrew,*[1] P. T. K. Saunders,*[2] A. J. Freemont,*[1] J. A. Hoyland,[1] I. P. Braidman.[1] [1]Musculoskeletal Research Group, University of Manchester Medical School, Manchester, United Kingdom, [2]MRC Human Reproductive Sciences Unit, Edinburgh, United Kingdom.

Gonadal steroids are important in regulating bone cell activity. Decreased levels of these hormones in ageing men and women are associated with bone loss. The effect of age on expression of their receptors in the skeleton, however, is unclear. We therefore investigated in vivo expression of androgen receptor (AR), estrogen receptor-α (ERα) and ERβ protein in fracture callus, with both chondrogenesis and osteogenesis, from patients aged 3-86 years (n=34; M=19, F=15). Cellular localisation of receptor protein was by indirect immunoperoxidase, with affinity purified polyclonal antibodies against AR (sc-816, Santa Cruz) and ERβ (ERβ40, MRC Edinburgh), and a monoclonal antibody to ERα (sc-8002, Santa Cruz). Western blotting, with human recombinant ERα and ERβ demonstrated specificity of both antibodies. Fracture callus was demineralised in 20% EDTA, formalin-fixed and wax embedded. Retrieval in glycine/EDTA buffer (pH 3.5) by heat and pressure was essential for receptor detection. All immunoreactivity was blocked by pre-incubation of antibody with immunising peptide.Estrogen Receptor α and β: Expression of both receptors was clearly localised to nuclei of osteoblasts, osteocytes and some osteoclasts in males and females. In areas of endochondral ossification, nuclear ERα and ERβ was expressed in small chondrocytes, adjacent mesenchymal cells and vascular endothelium, but not in hypertrophic chondrocytes. In fractures from individuals up to 40-50 years, cells of osteoblast lineage expressed ERα, although ERβ expression declined more rapidly. Osteoclasts, in contrast, were still positive for ERα and ERβ, even when receptor expression was lost in

other cells. In men and women over 70 years of age, most cells were negative.Androgen Receptor: Cellular localisation of AR was similar to that of ERα and ERβ, although small chondrocytes were the most immunoreactive. AR expression was most widespread in fractures from young individuals. In contrast to both ER's, it fell markedly in men and women aged over 20 years.We conclude that AR expression may be associated specifically with skeletal growth in the young, whereas ERα and β expression may be related to skeletal growth and maturation in the adult. Expression of both receptor isoforms in osteoclasts of older men and women suggests these cells may be estrogen-responsive well into later life.

M507

In the Absence of Estrogen Receptor alpha, Estrogen Promotes Bone Resorption via Estrogen Receptor beta in Female Mice. S. H. Windahl,*[1] M. Lindberg,*[2] C. Ohlsson,[2] J. Å. Gustafsson,*[3] G. Andersson.[4] [1]Department of Bioscienses, Karolinska Institutet, Novum, Huddinge, Sweden, [2]Department of Internal Medicine, Division of Endocrinology, Sahlgrenska University Hospital, Göteborg, Sweden, [3]Department of Medical Nutrition, Karolinska Institutet, Novum, Huddinge, Sweden, [4]Division of Pathology, Karolinska Institutet, Huddinge University Hospital, Huddinge, Sweden.

Ovariectomy in rodents results in decreased trabecular bone mineral density (BMD), which can be prevented by estradiol treatment. Recent studies in mice lacking either ER alpha (ERKO), ER beta (BERKO) or both known estrogen receptors (DERKO) show that wild-type (WT) and BERKO mice, but not ERKO and DERKO, respond to estrogen treatment after ovariectomy (ovx) by maintaining trabecular bone mass and volume (Lindberg et al., this meeting). In order to delineate the mechanisms behind these effects, we have used histomorphometry to measure volume and numbers of osteoclasts as well as Real-time PCR to measure the mRNA levels of osteoclast markers. The estrogen treated, ovx WT mice had lower osteoclast number and volume than vehicle treated ovx mice. Interestingly, ovx ERKO mice displayed a significant increase in both osteoclast number (3-fold) and volume (4-fold) in response to estrogen treatment, while these parameters in BERKO and DERKO mice were not affected by estrogen. The mRNA level of tartrate resistant acid phosphatase (TRAP) was 6-fold higher in ovx ERKO mice treated with estrogen compared to vehicle treated ovx ERKO mice, while remaining unaffected by estrogen in all other genotypes. Thus estrogen increases the number and the volume of osteoclasts and TRAP mRNA levels in ovx ERKO but not in BERKO or DERKO mice. This indicates that ERKO mice increase both osteoclast number and activity in response to estradiol treatment. The inability of ERKO mice to maintain trabecular bone mass following estrogen treatment of ovx mice could therefore be due to increased bone resorption. In conclusion, in the absence of estrogen receptor alpha, estrogen promotes bone resorption via estrogen receptor beta in female mice. It thereby implicate an important role of estrogen receptor alfa in the repressive control of osteoclastogenesis stimulated by estrogen receptor beta.

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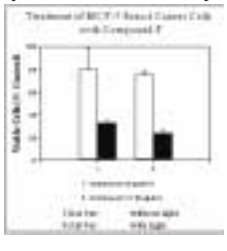
Estrogen Receptor-Mediated Targeted Photodynamic Therapy. N. Swamy,*[1] A. Gacio-Fernandez,*[2] A. Purohit,*[3] C. Fernandez Marcos,*[2] G. B. Jones,*[3] R. Ray.[1] [1]Medicine, Boston University School of Medicine, Boston, MA, USA, [2]Universidad de Santiago de Compostela, Santiago de Compostela, Spain, [3]Northeastern University, Boston, MA, USA.

An efficient therapy for cancer calls for selective localization of a toxin to cancer cells with resultant cell-kill. For example, immunotherapy involves targeting of unique antigens on cancer cells by antibodies that are chemically coupled to drugs or toxins. However, immunotoxins and other delivery systems often deliver the drug or toxin to the plasma membrane of the cancer cells instead of the nucleus, where most damage is desired. In the current study we targeted nuclear estrogen receptor (ER) in hormone-sensitive breast cancer cells with synthetic conjugates of estradiol and tamoxifen with porphyrins. Porphyrins are photoactivable toxins that are used for photodynamic therapy of cancer (PDT). We demonstrated that the conjugates, that bound specifically to ER, accumulated in a dose-dependent manner into ER +ve MCF-7 breast cancer cells at a much higher concentration and than into ER -ve Hs578t breast cancer cells. Furthermore, several of these conjugates selectively killed MCF-7 cells in the presence of light (to photo-activate the toxin) than in the dark. A representative example is shown in the accompanying figure.



M509

Target Related Affinity Profiling (TRAP) in the Identification of Small Molecules that Selectively Bind to Estrogen Receptor Alpha, Estrogen Receptor Beta and their Sequence Variants (SERMs). H. Villar,*[1] C. Hartnett,*[2] M. Sommer,*[2] R. R. Denton,*[2] W. D. Henner,*[1] R. Gomez,*[1] F. Meng,*[1] P. Lin,*[1] G. L. Brown.[1] [1]Telik, Inc., South San Francisco, CA, USA, [2]Genaissance Pharmaceuticals, New Haven, CT, USA.

The discoveries of estrogen receptor beta (ESRbeta) and of single nucleotide polymorphisms (SNPs) for both ESRalpha and ESRbeta have provided new potential targets for selective estrogen receptor modulators (SERMs). SERMs with selectivity for a particular ESR subtype or genotype may produce a more favorable therapeutic profile. Purpose: This study was designed to determine if Telik's TRAP chemogenomic technology and Genaissance's genomic profiling techniques could identify novel compounds that selectively inhibit binding to specific ESR subtypes. TRAP uses protein affinity fingerprinting to rapidly and efficiently identify pharmaceutically active molecules. Methods: A total of 185 TRAP-selected compounds were screened for inhibition of estradiol binding to ESRalpha, ESRalpha and four ESR SNPs in a plate-based assay. Results: 14 active molecules (Ki < 2 mM) in at least three structurally distinct non-steroidal classes were identified among the 185 compounds screened. 6/14 active molecules have Kis < 400 nM. Inhibition is highly selective for ESR as compared to a panel of non-ESR receptors and active molecules include examples of chemicals that are non-selective among ESR subtypes as well as chemicals highly selective (> 10-fold) for either ESRalpha or ESRbeta. Active molecules specific for particular SNPs of ESRalpha or ESRbeta have also been obtained. Conclusions: Telik's chemogenomic TRAP can efficiently identify new classes of molecules active in ESR binding. Since TRAP requires the screening of only a very limited number of chemicals it is particularly well-suited for discovery of drugs targeting the very large number of new potential targets identified through genomics. These selective estrogen binding compounds are useful in the development of new SERMs for treatment of metabolic bone diseases and hormone sensitive cancers.

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A Novel Intracellular Estradiol Binding Protein in Estrogen-Resistant New World Primates Cells Is a Member of Heat Shock Protein-27 (hsp27) Family. H. Chen,[1] B. Hu,[2] G. Huang,*[1] A. G. Trainor,*[3] J. S. Adams.[1] [1]Endocrinology/Medicine, Cedars-Sinai Burns and Allen Research Institute, UCLA School of Medicine, Los Angeles, CA, USA, [2]Pathology, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA, USA, [3]Pathology, Wisconsin Regional Primate Research Center University of Wisconsin, Madison, WI, USA.

New World primates exhibit a compensated form of gonadal steroid hormone resistance. Resistance results from overexpression of dominant-negative-acting estrogen response element binding protein in estrogen target cells. We have previously characterized a similar vitamin D-resistant state in New World primates that is compensated in an autocrine mode by the overexpression of hsp70-related, intracellular vitamin D binding proteins. We hypothesized that overexpression of an intracellular estradiol binding protein (IEBP) may be a similar form of compensation for the estrogen-resistant state in these monkeys. Using 17ß-estradiol affinity chromatography, we achieved a 3000-fold enrichment in specific estradiol binding activity from post-nuclear extracts of the hormone-resistant New World primate cell line B95-8. N-terminal sequencing data from purified protein indicated that the IEBP retained nearly 100% sequence identity with human hsp27. Employing anti-human hsp27-specific antibody in Western blot analyses of primate tissues, we found IEBP was expressed in liver>mammary gland>>kidney=skin. Tissue-specific expression of hsp27 was 2-3-fold higher in female New World primates than in females status-post oophorectomy or in male monkeys of the same species. Analysis of postnuclear extracts of cell lines derived from different primate suborders showed expression of hsp27 protein to be 2-3 fold greater in New World than in Old World primate cell lines and to be only weakly responsive to heat shock. In summary, we have purified a novel, intracellular estradiol binding protein or IEBP with high homology to human hsp27. This IEBP is 1] overexpressed in estrogen-resistant New World primate cells but easily identifiable in human (i.e. Old World primate) cells, 2] preferentially expressed in premenopausal females, and 3] present in relatively high levels in estrogen-sensitive mammary tissue. If similar in function to its hsp70-related vitamin D-binding counterparts, hsp27(s) will act to chaperone 17ß-estradiol and possibly other gonadal steroids to specific intracellular destinations for enhanced action and/or metabolism.

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Prenatal Glucocorticoid Exposure Affect Bone Tissue in Female Rats. D. A. K. Swolin-Eide,*[1] J. Dahlgren,[1] C. Nilsson,[2] K. Albertsson-Wikland,[3] C. Ohlsson.[1] [1]Research Center for Endocrinology and Metabolism, Gothenburg, Sweden, [2]Sahlgrenska University Hospital, Goteborg, Sweden, [3]Pediatric Growth Research Center, Goteborg, Sweden.

Glucocorticoids are well known to exert effects on skeletal growth and on adult bone metabolism. Prenatal events appear to program hormonal homeostasis and are able to play important roles in the pathogenesis of diseases at adult age in both animals and humans. Different sorts of stress or hormonal influences during a defined developmental stage or at a certain time during pregnancy may result in persisting or transients changes. The aim of the this study was to examine whether or not exposure to Dexamethasone (Dex) during fetal life had any effects on bone tissue in female rat offsprings. Pregnant Wistar rats were injected on day 9, 11 and 13 of gestation i.m. with either 100 mg/kg Dex or vehicle. Female offsprings from the two groups were then investigated. No difference in weight or length was observed at birth. Dex offsprings showed at 3-4 weeks of age a transient period with increased weight and body length. Female offsprings were sacrificed at either 6 or 12 weeks of age. No difference was observed in any group in vertebrae heights, tibial lengths or femur lengths, compared to controls. Detailed analysis of trabecular and cortical bones were performed at 6 weeks and at 12 weeks of age. Areal Bone Mineral Density (Areal BMD; BMC/cm^2) and Bone Mineral Content (BMC) were measured with DEXA. Areal BMD and BMC were unchanged in the vertebrae, tibia and femur. Peripheral Quantitative Computerized Tomography (pQCT) was performed on femura to determine the cortical bone mineral and the metaphyseal trabecular bone mineral density. The cortical thickness was reduced while the cortical periosteal and endosteal circumferences were increased in the female Dex treated rats. No effect was seen on trabecular bone. Baseline hormone levels were measured; at 5 weeks of age for IGF-I and corticosterone and at 8 weeks of age for leptin, testosterone, progesterone and estradiol. Only leptin had significant elevated levels in the Dex treated group. Taken together, our results suggest that female offsprings to glucocorticoid treated pregnant rats show affected bone tissue.

M512

Strain-Dependent Effects of Glucocorticoids on Bone in Mice. Q. Zeng, R. L. Cain,* J. Hoover,* A. Harvey,* H. Bryant, M. Sato, Y. L. Ma. Eli Lilly, Indianapolis, IN, USA.

Chronic exposure to excessive doses of glucocorticoids leads to bone loss and subsequent spontaneous fractures in humans. There is no widely accepted animal model to mimic glucocorticoid-induced bone loss so far. Mice have the advantage of small size, thus, requiring small amounts of test compound. However, previous studies showed that mice have a strain-dependent response to many compounds active on bone, including estrogen and PTH. The purpose of this study was to examine the effects of glucocorticoids on bone in mice. Male retired breeder mice, aged 6-7 months old from various strains (Balb/C, C3H/HeN, DBA/2, C57BL/6, ICR and Swiss-Webster) were treated with prednisone acetate at 0, 1, and 5 mg/kg/day po for 6 weeks. Prednisone treatment lowered body weight in most strains but only DBA mice reached the statistical significance (-16%). Body weight was increased in prednisone-treated C3H/HeN mice. Longitudinal whole body p-DEXA scanning showed that a significant decrease in whole body contents (BMC) but not in bone mineral density (BMD) in DBA mice. There were no BMD and BMC changes in all other strains when compared to vehicle controls. Osteocalcin decreased by 20 to 65% in most animals except for ICR mice. Histomorphometry analyses in Swiss-Webster mice found that dose-dependent decreases of mineralizing and osteoid surfaces, mineral appositional rate and bone formation rates (-83~-309%) were seen after 1 week treatment. No bone formation rate could be calculated in high dose group due to completely absent double label at this time point. Osteoclast surface increased 197 ~ 240% from vehicle control. However, all the dynamic changes were returned to near the control levels after 4 weeks treatment. No trabecular area change was found among the groups by end of the study. Our data suggest that prednisone produces deteriorative effects in mouse skeleton, and that strain differences need to be considered when selecting mice to analyze glucocorticoid effects on the skeleton.

M513

The Effects of Age on the Responses of Mouse Bone Marrow Stromal Cells to Dexamethasone. T. L. Chen,[1] A. Szizygiel,*[2] R. Rebong,*[2] N. Antonio.*[2] [1]Natural Sciences, College of Notre Dame, Belmont, CA, USA, [2]College of Notre Dame, Belmont, CA, USA.

Primary cultures of bone marrow stromal cells (BMSC) from young (4 months) and old (24 months) C57BL/6 male mice were used to study how age modulates the effects of dexamethasone (DEX) on osteoblast growth and differentiation. The formation of total (CFU-F) and alkaline phosphatase positive (CFU-ALP+) colonies and the specific activity of ALP were assessed. BMSC were removed from long bones, dispersed into single cell suspensions and grown in culture. For studying colony formation, cells were plated onto 6-well culture plates. DEX at varying concentrations was added immediately after plating and again every 2 days during the medium change. After 11-12 days in culture, cultures were fixed and first stained for CFU-ALP+ then CFU-F. The images of stained cultures were scanned into a computer and the number and colony area were measured by using Colcount (software). For studying ALP activity, BMSC were plated onto 24-well plates and the same treatment regimen was followed. At the end of each experiment, cells were lysed with 10 mM Tris buffer containing 0.1 % Triton X-100 and ALP assays performed by the formation of p-nitrophenol (p-NP) from p-NP phosphate. The activity was measured by unit which was defined as µmol p-NP/mg protein/min. When young cells were plated at a density of $2x10^6$ cells/well, an average of 50 CFU-F with an area averaging 120 mm^2 were formed in which 60% were ALP+. DEX reduced the number in both types of colonies equally well reaching 50% of the control at the maximal inhibition concentration of 100 nM DEX. At this concentration of DEX, the CFU-F area was reduced to 60% and the ALP+ area to 25%. The responses of the old cells were similar to the young ones except that higher plating density of $3x10^6$ cells/well was required for them to form the same number of CFU-F and CFU-ALP+ as for the young. The effects of DEX on ALP activity were similar in young and old cells which are highly dependent on plating density. In sparsely plated ($1x10^6$ cells/well) cultures, DEX stimulated the enzyme activity up to 3 fold maximally. In contrast, the enzyme activity was dramatically reduced to 30% of the control in densely plated ($3x10^6$ cells/well) cultures. In conclusion, the responses of BMSC from young and old mice to DEX on colony formation and ALP activity are similar. DEX inhibited the number and colony area in CFU-F and CFU-ALP with more profound effect on the colony area. ALP activity can be stimulated or inhibited by DEX depending on the cell plating density.

M514

Corticosteroid and Hormone Replacement Therapy Use in Patients with Rheumatoid Arthritis: Their Effect on Bone Mineral Density and Fractures. A. Berard,*[1] J. Adachi,[2] C. Berger,*[3] L. Joseph.*[3] [1]Epidemiology, Albert Einstein College of Medicine, Bronx, NY, USA, [2]Rheumatology, McMaster University, Hamilton, Canada, [3]McGill University, Montreal, Canada.

Background: Hormone replacement therapy (HRT) is an effective treatment that prevents bone mass loss and fractures in post-menopausal women. However, its efficacy in preventing osteoporosis associated with systemic inflammatory disorders such as rheumatoid arthritis (RA) is not well documented. Objective: Calculate bone mineral density and the number of patients with prevalent fractures associated with the use of corticosteroid and HRT, separately or concomitantly, in a cohort of RA patients.Methods: Data were obtained from the Canadian Multicentre Osteoporosis Study (CaMOS). CaMOS randomly recruited 9423 subjects over the age of 18 between 6/1/1995 and 10/1/1996. Data were collected by questionnaire. All CaMOS subjects with a history of RA formed the RA subcohort. Patients were excluded if they were men or pre-menopausal women without a history of hysterectomy or with at least one ovary remaining. All patients who reported taking oral corticosteroids during 1995-1996 were classified as exposed to corticosteroids. All patients who reported taking HRT during 1995 - 1996 were classified as exposed to HRT. Baseline characteristics included age, age at menopause, parity, family history of osteoporosis, history of hysterectomy, weight, height, number of falls in past month, marital status, education level, geographic region and race. Multivariate linear and logistics regression analyses were done for bone mineral density and fractures, respectively, adjusting for unbalanced distribution in the relevant confounders. Results: 389 post-menopausal women formed the RA cohort. After adjusting for potential confounding variables, subjects on corticosteroids alone had lower BMD measurements than those on HRT alone (0.90 g/cm2 (standard deviation (s.d.)=0.17) vs. 0.99 g/cm2 (s.d.=0.15), p<0.01); subjects on corticosteroids alone also had lower BMD than those on both corticosteroids and HRT (1.00 g/cm2 (s.d.=0.20), p<0.01). In multivariate logistic regression analysis, subjects who reported corticosteroid use were as likely to have fractures than those on HRT alone (OR=1.7, 95%CI= 0.7-4.1) or when compared to those on both corticosteroids and HRT (OR=1.4, 95%CI= 0.6-3.3).Conclusion: The American College of Rheumatology recommends the addition of HRT in RA patients treated with corticosteroid to prevent bone loss that is believed to be the result of corticosteroid therapy. Although BMD is lower in patients taking corticosteroids the number of patients with prevalent fractures were similar between groups.

M515

Serum Vitamin D Metabolites in Community-dwelling Elderly Men and Women: Its Relation to Muscle Strength: Results from the cross-sectional analyses of the baseline measurements of the AIMS-Study*, an ongoing double-blind randomised controlled trial*Alfacalcidol Influence on Muscle Strength. L. Dukas,*[1] H. A. Bischoff,[2] G. Boos,*[1] H. B. Stähelin.*[1] [1]Geriatric University Clinic, Basel, Switzerland, [2]2 Devision of Rheumatology, Immunology and Allergy, the Robert B. Brigham Multipurpose Arthritis Cen, Brigham and Women's Hospital, Boston, MA, USA.

The objective of the AIMS-Study, an ongoing randomised double-blind controlled trial, is to investigate whether supplementation of Alfacalcidol (1(OH)D) in a healthy elderly population is associated with an increase in muscle strength, balance and a reduction in the frequency of falls. The objective of this preliminary cross-sectional analysis of the baseline measurements was to investigate the distribution of the serum 25-hydroxy (25(OH)D) and 1, 25 vitamin D (1,25 (OH)2D) and iPTH concentrations in this population. In addition we compared performance in 4 strength measures in below or above median concentrations of serum vitamin D metabolitesWe included 380 healthy community-dwelling Swiss elderly (192 women and 188 men). Serum 25(OH)D, 1,25 (OH)2D and iPTH concentration were measured by radio immunoassay (Nichols®). For group comparison we used t-test, Wilcoxon rank sum test, Chi-square and ANOVA. The p values are two sidedWomen in this study group were slightly younger than men (74 year respectively 76 year, p 65 pmol/l). Significantly higher performance for grip srength for women and men was found in the above median 25(OH) D serum concentration group ($p < 0.05$ respectively $p < 0.02$). In the above median 1,25(OH)2D serum concentration group the performance was significantly increased for grip strength in both genders ($p < 0.001$ for women and $p < 0.04$ for men) as well as the performance for knee extensor strength and leg extension power in women ($p < 0.04$ resepctively $p < 0.03$) In early fall only about 25% of community-dwelling elderly women and men reach 25(OH)D serum concentrations above 30 ng/ml. Women had significantly higher 1,25(OH)2D serum concentrations than men.

M516

A Central Dinucleotide Within Vitamin D Response Elements Modulates DNA Binding and Transactivation by Vitamin D Receptor in the Response to Natural and Synthetic Ligands: Significance of Cellular Context. G. J. C. van den Bemd,*[1] M. Jhamai,*[1] A. Staal,*[1] A. J. van Wijnen,[2] J. B. Lian,[2] G. S. Stein,[2] H. A. P. Pols,[1] J. P. T. van Leeuwen.[1] [1]Internal Medicine, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands, [2]Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA.

The vitamin D receptor (VDR) regulates gene expression in response to 1,25-dihydroxyvitamin D3 (D3) by interacting with its cognate vitamin D responsive element (VDRE) as a heterodimer with the retinoid X receptor (RXR). Most VDREs are composed of a direct repeat of two half elements separated by 3 basepairs (DR3-type), but there is considerable divergence in the sequences of distinct half elements. Aims: to gain insight into the contribution of these nucleotide variations 1) to the DNA binding of VDR/RXR and transactivation, and 2) the VDR-DNA binding and action induced by the potent synthetic D3 analog KH1060. We performed protein/DNA interaction assays using in vitro synthesized VDR/RXR and osteoblast nuclear extracts with oligonucleotides spanning distinct VDREs from human and rat osteocalcin (OC) genes, and mouse osteopontin (OP) gene. We found that VDR/RXR binds more tightly to the OP-VDRE than to either OC-VDRE. Studies using point-mutants reveal that the internal dinucleotide at positions 3 and 4 of the proximal half element are most important for modulating the strength of VDR/RXR binding. To establish the functional consequences of differences in VDRE binding strength, we monitored ligand-dependent transactivation by the VDR using VDREs (differing only at positions 3 and 4) driven luciferase reporter gene constructs that were transfected into ROS 17/2.8 osteosarcoma cells. Our results show that the central dinucleotides are important for the transactivation potential of VDR/RXR. Furthermore, KH1060 is a more potent stimulator of transcription, and inducer of nuclear extract binding to the VDREs than the natural ligand D3. However, both ligands have similar effects in stimulating VDRE binding of in vitro synthesized VDR/RXR heterodimers. Thus, the extent of D3 and KH1060 dependent binding of VDR/RXR heterodimers is specified by a central dinucleotide in the VDRE, and the potency of D3 analogs is in part controlled by nuclear/cellular context.Our findings are consistent with the concept that the dinucleotide motif may alter the conformation of VDR/RXR heterodimers, their affinity for DNA and the intrinsic transactivation potential, which provides a framework for understanding the different biological responses of cells to distinct D3 analogs.

M517

Identification of Genes Induced by 1,25Dihydroxyvitamin D_3 in Mouse Kidney Using Gene Chip Arrays. X. Peng,*[1] V. M. Aris,*[2] A. Galante,*[2] S. Ghanny,*[2] P. Soteropoulos,*[2] P. Tolias,*[2] S. Christakos.[1] [1]Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School, Newark, NJ, USA, [2]Center for Applied Genomics, PHRI and UMDNJ- New Jersey Medical School, Newark, NJ, USA.

Although 1,25dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the most active metabolite of vitamin D, acts by altering gene expression, relatively few $1,25(OH)_2D_3$ regulated genes are known in the target issues involved in maintaining calcium homeostasis. In this study we used Gene Chip array analysis to identify genes induced in the kidney of $1,25(OH)_2D_3$ deficient mice following systemic injection of $1,25(OH)_2D_3$. Poly (A^+) RNA was prepared from kidneys of $1,25(OH)_2D_3$ deficient mice [mice were placed on a 0.8% strontium diet (which has been reported to produce functional vitamin D deficiency by inhibiting the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$) for 6 days] and deficient mice injected with $1,25(OH)_2D_3$ (48h, 24h and 6h prior to sacrifice; 30 ng $1,25(OH)_2D_3$ per injection). cDNA synthesis was carried out at 37°C for 60 min using Superscript II and cRNA, prepared using T7 RNA polymerase, was purified on an affinity resin, biotin labeled and hybridized to mouse Gene Chip probe array from Affymetrix. Most array hybridization signals did not change significantly upon $1,25(OH)_2D_3$ treatment. As expected the gene expressed at the highest level in the kidneys of mice injected with $1,25(OH)_2D_3$ was 25hydroxyvitamin D_3 24hydroxylase (24(OH)ase ; 43 fold induction compared to the kidneys of vehicle treated mice). 57 other genes, whose hybridization signals were activated by a factor greater than 50% by $1,25(OH)_2D_3$,were noted including CCAATT/enhancer binding protein β (C/EBPβ), an important activator of transcription and FK506 binding protein. We have verified the induction of C/EBPβ by $1,25(OH)_2D_3$ by Northern blot analysis and have noted two putative C/EBPβ binding sites in the rat 24(OH)ase promoter. In addition when COS-7 cells were transfected with the rat 24(OH)ase promoter (-1367/+74) and the vitamin D receptor, we found that the response to $1,25(OH)_2D_3$ (10^{-9}M) could be enhanced 3 fold by cotransfection with C/EBPβ expression vector (5μg). In summary by applying Gene Chip arrays to the study of $1,25(OH)_2D_3$ action we have begun to identify novel $1,25(OH)_2D_3$ target genes and have used this information to provide new insight into the mechanism of $1,25(OH)_2D_3$ transcriptional regulation in the kidney. We are in the process of confirming by Northern analysis the upregulation by $1,25(OH)_2D_3$ of the mRNA expression of other genes identified by Gene Chip array analysis. The use of Gene Chip arrays is a promising new technology that will provide new insight into the diverse functions of $1,25(OH)_2D_3$ in various target tissues.

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Differences in Vitamin D-Mediated Gene Expression and Calcium Transport Between Three Caco-2 Cell Lines. J. C. Fleet,*[1] F. Eksir,*[2] R. J. Wood.*[3] [1]Purdue University, West Lafayette, IN, USA, [2]UNC-Greensboro, Greensboro, NC, USA, [3]HNRCA at Tufts University, Boston, MA, USA.

The parental cell line of Caco-2 cells (P) is heterogeneous and clones from P may have different phenotypes related to vitamin D action and calcium absorption that limit their suitability as experimental models. Here we studied P cells and two well-differentiating clones, BBe and TC7. All cultures were studied at 11 days post-confluence. Vitamin D receptor levels in nuclear extracts (nVDR) were measured by western blot analysis. 24-hydroxylase (24Ohase), calbindin D9K (CaBP), Cat1, and ECaC1 mRNA levels were examined by RT-PCR using gene-specific primers after treatment of cells +/- 10 nM calcitriol for 8 hours (24-Ohase, Cat1, ECaC) or 48 hours (CaBP). Net mucosal-to-serosal calcium transport (CaTx) was examined in cells grown on permeable membrane filter supports (0.4 m) and treated +/- 10 nM calcitriol for 48 hours. nVDR (0.38±0.09 relative value (RV) vs. 1.33±0.32 and 1.00±0.17, $P<0.05$) and vitamin D-inducible 24-Ohase mRNA levels (0.55±0.06 RV vs. 1.04±0.05 and 1.00±0.09, pP). Calcitriol-induced CaBP mRNA (3.53±0.88 relative induction vs. 1.99±0.16 and 1.7±0.13, $p<0.05$) and net CaTx (0.39±0.03 nmol/well/min vs. 0.2±0.02 and 0.13±0.05, $p<005$) were highest in BBe compared to TC7 and P. By comparing the 3 cell lines we found that nVDR level strongly correlated with 24OHase induction but not any other vitamin D-mediated event we measured (e.g. Cat1, ECaC, CaBP mRNA level or net CaTx). In addition, while the relative change in CaBP mRNA was reflective of the induction of net CaTx by calcitriol in the three cell lines, absolute levels did not predict the efficiency of net CaTx. This suggests that CaBP level may not be the rate limiting determinant of calcium transport in these cells.

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Calcium Absorption Is Only Partially Disrupted in Vitamin D Receptor Knockout Mice. Y. Song,*[1] S. Kato,[2] J. C. Fleet.*[1] [1]Interdepartmental Program in Nutrition, Purdue University, West Lafayette, IN, USA, [2]Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan.

Vitamin D receptor (VDR) knockout mice (KO) are hypocalcemic, presumably due to the absence of intestinal calcium absorption (CaTx). Paradoxically, Li et al. (Endocrinology 139:4391) showed that in KO mice a 2% calcium (Ca), 20% lactose recovery (HCa) diet increased intestinal calbindin D9k (CaBP9k) mRNA, an effect normally associated with increased CaTx. Here we examined how CaTx is influenced by the HCa diet and the absence of VDR. Female KO and wild-type (WT) mice were weaned onto either a 0.5% Ca diet (NCa) or the HCa diet. At 60 days of age, CaTx was determined using in situ ligated loops (2 cm duodenum, 10 min, 2 mM Ca, 45Ca). Duodenal scrapings and kidneys from additional mice were examined for CaBP9k and Cat1 mRNA by RT-PCR and CaBP9k and calbindin D28k (CaBP28k) protein by Western blot. CaTx was reduced 65% in KO mice on the NCa diet. HCa diet reduced CaTx by 64% in WT but did not influence CaTx in KO mice. Duodenal CaBP9k mRNA and protein were reduced 54% and 57% in KO mice on the NCa diet. HCa diet reduced CaBP9k mRNA and protein by 79% and 60% in WT and 25% and 42% in KO mice. On the NCa diet, Cat1 mRNA in KO mice was only 7% that seen in WT; HCa diet reduced Cat1 mRNA in both WT (to 20% NCa level) and KO (to 12% NCa level) mice. In kidney, CaBP9k protein was completely eliminated in KO mice regardless of diet. In KO mice fed the NCa diet, CaBP28k protein was reduced 86% compared to WT mice. The HCa diet decreased CaBP9k by 21% and CaBP28k by 46% in WT mice. Surprisingly, CaBPD28k was increased by 66% in KO mice fed the HCa. Our data suggest (1) that control of duodenal CaBP9k and renal CaBP28k occurs in the absence of VDR, (2) neither CaBP9k nor Cat1 mRNA levels predict changes in CaTx in KO, and (3) there may be a significant role for renal Ca excretion in the development of the KO phenotype and its prevention by the HCa diet.

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The 20-epi-1,25-dihydroxyvitamin D_3 Analog KH1060: Deciphering the Elusive Mechanism for the Enhanced Transcriptional Potency of this Vitamin D Receptor Agonist. P. D. Thompson, S. M. Myskowski,* M. A. Galligan,* P. W. Jurutka, R. Chan,* G. K. Whitfield, C. A. Haussler,* M. R. Haussler. Biochemistry & Molecular Biophysics, College of Medicine, University of Arizona, Tucson, AZ, USA.

The nuclear vitamin D receptor (VDR) mediates the transcriptional effects of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) as a heterodimer with the retinoid X receptor (RXR). Heterodimerization, binding to a vitamin D responsive element (VDRE) in the promoter region of a target gene, and the subsequent interaction of the DNA-bound heterodimer with transcriptional coregulators such as steroid receptor coactivator-1 (SRC-1) are all highly $1,25(OH)_2D_3$-dependent processes. We observe that KH1060 and MC1288, both 20-epi synthetic vitamin D analogs, are 100- to 1000-fold more potent than $1,25(OH)_2D_3$ in eliciting a transcriptional response from either mouse osteopontin or rat osteocalcin natural promoters, using ROS 17/2.8 or COS-7 cells as cotransfection recipients. To determine which of the above processes involved in VDR action may be differentially regulated by KH1060, we utilized GST-fusion protein binding assays and ligand-dependent gel mobility shift analysis to probe whether the synthetic ligand elicits a response significantly different from that of $1,25(OH)_2D_3$. The results indicate that KH1060 and $1,25(OH)_2D_3$ exhibit no observable difference in their abilities to promote heterodimerization between VDR and RXR (ED_{50} of approximately $1x10^{-9}$ M for both ligands). In addition, ligand-dependent gel mobility shift experiments, using a 333 amino acid fragment of human SRC-1 encompassing the receptor interaction domain (RID_{hSRC-1}), revealed that KH1060 and $1,25(OH)_2D_3$ were also apparently equivalent in mediating formation of a VDR-RXR-RID_{hSRC-1} complex bound to the mouse osteopontin VDRE (again, ED_{50} ~$1x10^{-9}$ M for both ligands). Whereas previous reports with MC1288 and KH1060 point to enhanced heterodimerization as a proposed mechanism for their increased activity compared to $1,25(OH)_2D_3$, the present results with KH1060 indicate that neither the generation of VDR heterodimerization nor SRC-1 binding are steps at which KH1060 differs significantly from $1,25(OH)_2D_3$. Rather, we put forth the testable hypothesis that modulation of later stages in VDR action, such as the association between VDR and $DRIP_{205}$, or between VDR and TRIP1/SUG, may constitute the mechanism(s) by which KH1060 exerts its superagonist effects on ligand-dependent transactivation of bone-expressed genes by VDR. Molecular characterization of superagonist vitamin D analogs could lead to better therapies for bone and mineral diseases with suppressed intestinal calcium absorption, such as some subtypes of osteoporosis.

M521

Calcitriol-Induced 24-Hydroxylase and hCAT1 Gene Expression Is Dependent Upon Cross Talk Between Genomic and Membrane-Initiated Events in Caco-2 Cells. K. Hance,* J. C. Fleet. Interdepartmental Nutrition Program, Purdue University, West Lafayette, IN, USA.

Calcitriol stimulates intestinal calcium transport through a nuclear vitamin D receptor (nVDR) dependent genomic mechanism. Additional evidence indicates that calcitriol may activate rapid, membrane-initiated signal transduction pathways. We hypothesize that calcitriol-mediated gene expression is dependent upon cross talk between the nVDR and rapid, membrane-initiated signal transduction pathways. To test this hypothesis, the vitamin D-responsive genes 24-hydroxylase (CYP24) and human epithelial calcium transporter hCAT1 were analyzed in human intestinal cell line, Caco-2, treated +/- calcitriol (2 hr., 10 nM) in the presence and absence of chemicals known to inhibit various kinases involved in signal transduction, i.e. the protein kinase C inhibitor H7 (50 uM), the protein kinase A inhibitor Rp-cAMPS (100 uM), the cGMP-dependent protein kinase inhibitor Rp-8-pCPT (5 uM), the tyrosine kinase inhibitor genistein (50 uM) or the MEK inhibitor PD98059 (20 uM). CYP24 mRNA levels were undetectable in the absence of calcitriol and calcitriol treatment elicited a strong expression of the gene. H7, PD98059, and genistein suppressed the calcitriol-induced expression of CYP24 mRNA levels by 100%, 65%, and 50%, respectively. Calcitriol treatment resulted in a 2.4 fold induction in hCAT1 mRNA levels. However, this response was completely inhibited by H7 and PD98059 and it was reduced 77% by genistein treatment. These findings are consistent with our hypothesis that nVDR function is dependent upon a rapid non-genomic pathway. Our data suggest that the cross-talk utilizes the protein kinase C and MAP kinase pathways and that it may depend upon the activation of a tyrosine kinase, possibly src.

M522

Regulation of Calbindin-D9k Expression by 1,25-dihydroxyvitamin D3 and Parathyroid Hormone in Mouse Primary Renal Tubular Cells. L. Cao,* M. J. G. Bolt, M. Wei,* M. D. Sitrin,* Y. C. Li. The University of Chicago, Chicago, IL, USA.

We have shown that calbindin (CaBP)-D9k is highly regulated by 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] in mouse kidneys and is probably a major vitamin D target gene involved in calcium homeostasis. However, studies on the molecular mechanism of CaBP-D9k gene regulation have been hampered by the lack of an appropriate cell culture system. In the present study, we used mouse primary renal tubular cell (PRTC) cultures to investigate the regulation of CaBP-D9k expression by $1,25(OH)_2D_3$. Both CaBP-D9k mRNA and protein levels were highly induced by $1,25(OH)_2D_3$ in a time- and dose-dependent manner

in PRTCs. This induction was inhibited by actinomycin D or cycloheximide, suggesting that new RNA and protein syntheses are required. In contrast, little induction of CaBP-D28k expression was seen in the PRTCs, and no expression of CaBP-D9k was detected in VDR(-/-) PRTCs derived from VDR null mice. Transfection of the VDR(-/-) cells with human VDR restored the induction of CaBP-D9k expression by $1,25(OH)_2D_3$, confirming the requirement of VDR in CaBP-D9k expression. Interestingly, treatment of the PRTCs with $1,25(OH)_2D_3$ also increased VDR protein concentration and phosphorylation, suggesting that enhanced VDR transactivation is involved in the CaBP-D9k up-regulation. Furthermore, co-treatment of the PRTCs with $1,25(OH)_2D_3$ and PTH stimulated CaBP-D9k expression more than $1,25(OH)_2D_3$ alone, suggesting a synergistic effect of PTH on $1,25(OH)_2D_3$ induction of CaBP-D9k. Taken together, these data demonstrate that mouse CaBP-D9k is highly regulated by $1,25(OH)_2D_3$ and PTH in the mouse PRTCs, therefore, the PRTC culture provides a suitable *in vitro* system for further investigating the molecular mechanisms involved in CaBP-D9k gene regulation.

M523

The Distribution and Metabolism of Alfacalcidol in Bone and Bone Marrow Cells after Oral Administration. N. Hayakawa,* S. Higashi,* A. Shiraishi,* T. Masaki,* Y. Uchida,* E. Hoshino.* Product Research Lab., Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

Alfacalcidol, a pro-drug of $1,25(OH)_2D_3$, is clinically proven to reduce bone fracture. There are, however, few studies on its mechanism of action and pharmacokinetics in bone and bone marrow cells. In the present study we report, for the first time, the simultaneous visualization and semi-quantification of changes in alfacalcidol and its metabolites in bone of adult rats using an autoradiographic technique and radio-HPLC analysis. Male rats (8 weeks old, Wistar-Imamichi) were orally administered [22,23-^{3}H]alfacalcidol or [23,24-^{3}H]$1,25(OH)_2D_3$ at a dose of 2 μg/kg. 1, 4, 24 and 72h following administration, the femurs were removed and freeze-sectioned prior to autoradiographic analysis. Autoradiograms demonstrated that, in the alfacalcidol-treated rats, the radioactivity reached a peak after 4h and gradually declined up to 72h in both the distal and middle portions of the femur. On the other hand for the $1,25(OH)_2D_3$-treated rats, the peak of radioactivity was observed as early as 1h with a rapid decline to 24h. The localization of alfacalcidol in the middle portion of the femur was high in the bone marrow area and the periosteum but almost undetectable in the bone tissue. Furthermore, the detailed microautoradiographic analysis showed that the distribution of the radioactivity was predominantly in the lining cells, presumably osteoblastic stromal cells on the trabecular bone and periosteal cells. Next the metabolites of alfacalcidol in the bone marrow were examined using radio-HPLC and compared with those in the plasma. Our data showed that although the total radioactivity in the plasma peaked at 4h, a similar timescale to that observed in the bone marrow, we found that the major metabolite in plasma was $1,25(OH)_2D_3$ whereas unchanged alfacalcidol was detected in the bone marrow of the femur. We suggest that if taken together, alfacalcidol may have potency to distribute to the bone marrow more specifically than $1,25(OH)_2D_3$, and be retained as the unchanged form in the vitamin D receptor-positive cells. Since the localization is consistent with the active site of bone metabolism, it is suggested that alfacalcidol might have a potency to increase bone strength directly without bypassing systemic metabolism to $1,25(OH)_2D_3$. Therefore, the regulatory mechanism of disposition and metabolism of alfacalcidol in bone might be critical in understanding its mode of action, and should be clarified further.

M524

Recombinant Stable Expression of Vitamin D Hydroxylases: New Tools to Examine Vitamin D Analog Metabolism

M. J. Beckman,* A. Bajwa.* Biochemistry, Virginia Commonwealth University, Richmond, VA, USA.

The efficacy of a vitamin D analog being tested for therapeutic potential depends upon numerous factors in the clinical pharmacology category including the mechanism of action, pharmacokinetics and metabolism, and clinical studies. The basis of this study was to perform stable expression of either recombinant human 24-hydroxylase (CYP24) or human 1alpha-hydroxylase (CYP1alpha) and the natural human cofactors (adrenodoxin and NADPH-adrenodoxin reductase) into Spodoptera frugiperda (Sf9) insect cells that would be used as research tools to examine the roles that CYP24- and CYP1alpha-hydroxylation play in the activation, catabolism and clearance of vitamin D analogs. Recombinant plasmid constructs were engineered using an InsectSelect expression vector (pIZT-Zeocin) that expresses a functional complex of human forms of CYP24 or CYP1alpha fused to the two natural cofactors, adrenodoxin and NADPH-adrenodoxin reductase. A separate gene product for green fluorescent protein (GFP) was also expressed from the same vector. Transient transfections into Sf9 cells were carried out to examine that the functional capability of each expressed enzyme system was intact and localized to the mitochondria. After transfecting the cells, the protein expression was monitored for fluorescence of GFP using fluorescence microscopy. Only transfected cells emitted a green fluorescent signal upon illumination, and the fluorescence was used to estimate the transfection efficiency. Once it was demonstrated that functional human CYP24 and CYP1alpha proteins were expressed in Sf9 cells, stable expression cell lines containing each vector construct were created by a method based upon the multiple copy integration principle. Zeocin-resistant Sf9 cells continued to divide at regular intervals to form distinct colonies. Selection for and testing of stably transfected cell lines of Sf9 cells containing high constitutive expression of CYP24 and CYP1alpha activity, respectively, was carried out. The use of these two novel in vitro systems will allow for rapid and non-invasive assessment of the metabolism of vitamin D analogs and generate information about the half-life and potency of specific analogs and intermediates being developed as drug candidates.

M525

VDR-Dependent Inhibition of Osteoblast Apoptosis by Vitamin D Analogs: Evidence for a Novel Mode of Receptor Action. A. M. Vertino,[1] S. Kousteni,[1] T. Bellido,[1] L. Han,[1] A. W. Norman,[2] S. C. Manolagas.[1] [1]Division of Endocrinology & Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Department of Biochemistry, University of California, Riverside, CA, USA.

Based on evidence that HeLa cells transiently-transfected with the wild type (wt) vitamin D receptor (VDR) or the retinoic acid receptor (RXR) were protected from etoposide-induced apoptosis in the presence of $1\alpha,25(OH)_2D_3$, we have examined whether $1\alpha,25(OH)_2D_3$ and related metabolites or synthetic analogs influence apoptosis in calvaria-derived murine osteoblasts which express the classical VDR. Parallel experiments were performed in HeLa cells that were transiently transfected with the wt VDR or the wt RXR along with a vector carrying the green fluorescent protein (GFP) gene containing a nuclear localization sequence, so as to monitor nuclear features of apoptotic cells. Apoptosis was assessed by trypan blue exclusion or by nuclear morphology in cells pretreated for 1h with the various compounds, followed by 6h treatment with 50 -100 μM of the pro-apoptotic agent etoposide. $1\alpha,25(OH)_2D_3$, $24,25(OH)_2D_3$ or $25(OH)D_3$ protected both calvaria and VDR-transfected HeLa cells, but not cells transfected with an empty vector, from apoptosis in a dose-dependent fashion, at concentrations ranging between 10^{-11} to 10^{-7} M. The effect of $1\alpha,25(OH)_2D_3$ was reproduced in HeLa cells transiently-transfected with the RXR instead of the VDR. Moreover, this effect was reproduced in calvaria cells by two 6-s-*cis* locked analogs, $1\alpha,25(OH)_2$-lumisterol (JN) and $1\alpha,25(OH)_2$-7-dehydrocholesterol (JM), which are agonists for the rapid membrane-mediated effects of $1\alpha,25(OH)_2D_3$ and bind poorly to the classical VDR. 1β, $25-(OH)_2D_3$ (HL), which is an antagonist of the membrane-initiated rapid effects of VDR, prevented apoptosis when used alone or in combination with JN or JM. On the other hand, (23S)-25-dehydro-1α-OH-D_3-26,23-lactone (MK), an antagonist of the nuclear actions of the VDR, was ineffective by itself and did block the effects of JN and JM. Finally, the anti-apoptotic effect was exhibited by the 6-s-*trans* locked analogs $1\alpha,25(OH)_2$-tachysterol$_3$ and $1\alpha,25-(OH)_2$-*trans*-isotachysterol, which are weak agonists of both genotropic and nongenotropic actions. These results demonstrate that vitamin D metabolites have anti-apoptotic properties which are mediated by the classical VDR (or the RXR). However, the mechanism of VDR action in these effects is evidently distinct from that implicated in other genotropic or nongenotropic actions, as the range of ligands that can affect it is unique.

M526

Identification of Vitamin D Analogs That Exert Anabolic Effects on Bone With Little Calcemic Activity, Based on Anti AP-1/NF-κB Activity. H. Takasu,[1] Y. Uchiyama,*[1] S. Takeda,*[1] A. Sugita,*[1] T. Kake,*[1] A. Kawase,*[1] K. Morikawa,*[1] N. Kubota,*[1] K. Ikeda,[2] E. Ogata.[3] [1]Fuji Gotemba Res. Lab., Chugai Pharmaceutical Co., Ltd., Shizuoka, Japan, [2]Dept. of Geriatric Res., Natl. Inst. for Longevity Sci., Aichi, Japan, [3]Cancer Inst. Hosp., Japanese Foundation for Cancer Res., Tokyo, Japan.

We have demonstrated that active vitamin D has anabolic (stimulation of bone formation) and anti-catabolic (inhibition of bone resorption) effects on bone (*JBMR 2000*), which makes it a suitable drug for osteoporosis. However, the risk of causing hypercalciuria and hypercalcemia has precluded its world-wide use. On the basis of our previous findings that therapeutic bone effects of active vitamin D can be at least partly dissociated from calcemic activity, we attempted to search for vitamin D analogs that have bone-selective therapeutic effects with little calcemic activity. We have established *in vitro* transcription reporter assay systems, and measured the ability of vitamin D compounds to induce the classic VDRE-dependent transcription (VDRE activity) vs. to inhibit AP-1/NF-κB-mediated transcription (anti-AP-1/NF-κB activity), with the assumption that the former activity may correlate with intestinal calcium absorption/calcemic response and the latter with anabolic/anti-catabolic effects in bone.After screening several hundred compounds, using $1,25D_3$ as the reference, we succeeded in identifying several compounds, including DD-281, that have high anti-AP-1/NF-κB activity (25-30x of $1,25D_3$) with low VDRE activity (1/10 of $1,25D_3$). We also picked up compounds with high anti-AP-1/NF-κB activity (25-30x of $1,25D_3$) but the same VDRE activity as $1,25D_3$, such as DD280. In order to test our concept, we studied these compounds in an ovariectomized (OVX) rat model *in vivo*. Treatment of OVX rats p.o. with DD-281 for 5 weeks increased BMD and bone strength more than sham animals without increasing urinary calcium excretion. DD-280 increased BMD to the same extent as DD-281, but increases in urinary and serum calcium levels were observed. Thus, anti-AP-1/NF-κB activity of vitamin D plays an important role in the therapeutic skeletal effects (inhibition of bone resorption and stimulation of bone formation), while VDRE activity correlates more with intestinal calcium absorption and calcemic effects. In conclusion, new bone-selective vitamin D analogs with little calcium effects that we have identified in the current screening may warrant further investigation at both pre-clinical and clinical levels.

M527

Molecular Mechanism of Differentiation and Apoptosis-Inducing Actions of A-Ring Diastereomers of 1α,25-dihydroxyvitamin D_3 in Human Promyelocytic Leukemia (HL-60) Cells. K. Nakagawa,*[1] K. Ozono,[2] S. Hatakeyama,*[3] N. Kubodera,*[4] T. Okano.[1] [1]Kobe Pharmaceutical University, Kobe, Japan, [2]Osaka Medical Ctr. for Maternal and Child Health, Osaka, Japan, [3]Nagasaki University, Nagasaki, Japan, [4]Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

1α,25-dihydroxyvitamin D_3 [$1\alpha,25-D_3$] has antiproliferative, differentiation and apoptosis-inducing effects on many malignant cells. We have reported the biological activities of several A-ring analogs of $1\alpha,25-D_3$ with respect to regulation of cell differentiation, proliferation and apoptosis in HL-60 cells. It was interesting to note that the analogs could

induce differentiation or apoptosis of HL-60 cells on the basis of the stereochemistry of both hydroxyl groups at positions 1 and 3 of the A-ring. In the present study, using all possible diastereomers of 1α,25-D_3, we further examined their molecular mechanism of differentiation and apoptosis-inducing actions of HL-60 cells in vitro. Cell-surface CD11b antigen and cell-cycle phase distribution were measured by flow cytometry. To examine the regulatory activities of the isomers on HL-60 cell growth, mRNA levels of p21, cyclin C and cyclin E were also measured. Apoptotic cells were detected by DNA fragmentation, caspase-3 activity and mitochondrial membrane potentials. In addition, we examined gene expression of the Bcl-2 family (Bcl-2, Bax-α, Mcl-1) to clarify the molecular mechanism of apoptosis-inducing action. 1β,25-D_3 which lacks vitamin D receptor (VDR) binding potency, was the most potent stimulator of apoptosis. 1α,25-D_3 which strongly binds to VDR, slightly stimulated apoptosis under the concentration of 10^{-10}M but strongly suppressed apoptosis over the concentration of 10^{-9}M. 3-epi-1β,25-D_3 and 3-epi-1α,25-D_3 which are inactive and extremely less active than 1α,25-D_3 with respect to VDR-mediated actions respectively, were weak stimulators of apoptosis. 1α,25-D_3 up-regulated p21 mRNA expression and down-regulated cyclin C and cyclin E mRNA expression after 24h treatment. 1α,25-D_3 up-regulated Mcl-1 mRNA expression after 3h treatment. 3-epi-1β,25-D_3 and 3-epi-1α,25-D_3 were almost inactive and extremely less active than 1α,25-D_3 in regulating the above cell cycle related gene expression. 1β-hydroxyl analogs stimulated Bax-α mRNA expression after 3h treatment, but 1α-hydroxyl analogs did not. From these results, we clearly identified for the first time the structural motifs of vitamin D analogs inducing differentiation and apoptosis of HL-60 cells. The results suggest that cell-cycle related proteins and mitochondrial membrane potential regulating proteins are the major targets for the differentiation and apoptosis initiated by 1α,25-D_3 diastereomers.

M528

Acceleration of Bone Formation by a New Vitamin D Analogue, ED-71: A Comparative Study with 1,25D_3 on Rabbit Leg Lengthening Model. M. Tanaka,*[1] Y. Higuchi,*[1] T. Kake,*[1] Y. Uchiyama,*[1] K. Yamane,[2] S. Yamamoto,[3] H. Kishimoto,[4] K. Yamamoto.[5] [1]Chugai Pharmaceutical Co., Ltd., Shizuoka, Japan, [2]Tottori Prefecture Central Hospital, Tottori, Japan, [3]Kasumi General Hospital, Hyogo, Japan, [4]San-in Rosai Hospital, Tottori, Japan, [5]Orthopaedic Surgery Faculty of Medicine, Tottori, Japan.

The effects of ED-71, a new vitamin D analogue, on the healing process of rabbit leg lengthening model were invenstigated, and were compared with them of 1-alpha,25-dihydroxyvitamin D_3 (1,25D_3). In the right tibiae of rabbits, 10 mm distractions were performed utilizing external fixators (M-100, Orthofix). The animals received subcutaneous injections of ED-71 at dose of 0.1 and 0.2 micro g/kg/week, or 1,25D_3 at dose of 0.3 and 0.6 micro g/kg/week for 5 weeks. The effects of these drugs on healing process of the distraction site was evaluated by a dual energy X-ray absorptiometry and a biomechanical test. Bone mineral density at distraction site was significantly increased in ED-71 treated animals compared with control, but no significant change was observed in 1,25D_3 treated animals. Mechanical properties obtained from compression test of distraction sites were also significantly higher in ED-71 treatment, but no change was observed in 1,25D_3 treated animals, in comparison with control group. Serum calcium content was kept the same level during the experimental period, in contrast to 1,25D_3 treated animals, in which there was significant serum calcium rise in the later phase of the experiment. These results indicate that ED-71 accelerates distraction osteogenesis in the leg lengthening experimental model without hypercalcemia. This unique property might contribute to early release from external fixators. Our results indicate the possibility that ED-71 can be used to accelerate the healing processes required for bone lengthening treatments. This unique property of ED-71 would contribute to the reduction of the risks of complications and also the improvement of the patient's quality of life, due to early release from cumbersome external fixators.

M529

Thyroid Hormone Mediates the Activation of Vitamin D through the Induction of 1-Alpha Hydroxylase in Osteoblast-like Cells. C. H. A. Gouveia,[1] J. Schultz,*[2] G. A. Brent.*[2] [1]Department of Hystology, University of Sao Paulo, Sao Paulo-SP, Brazil, [2]Department of Medicine and Department of Physiology, West LA VA Medical Center and University of California, Los Angeles, CA, USA.

It was previously shown that cholecalciferol (VD3) is metabolized into 25-hydroxycholecalciferol (25-OH-VD3) and to 1 alpha,25(OH)2 cholecalciferol [1,25(OH)2VD3], the hormonal form of vitamin D, in bone cells. It was also shown that the two enzymes responsible for this conversion, 25-hydroxylase and 1-alpha hydroxylase(1alpha-OHase), are expressed in osteoblasts. In the present study, we investigated the effect of triiodothyronine (T3) on the VD3 activation pathway in osteoblast-like cells derived from rat osteosarcoma (ROS 17/2.8 cells). ROS 17/2.8 cells were cultured in serum-free (SF) media and treated with 10-8 M T3 for 24 hours. The mRNA was isolated and the expression of 1alpha-OHase was analyzed by quantitative PCR. We showed that T3 induces 1alpha-OHase mRNA expression for about 2.4 times. In an attempt to show a functional relevance of this effect, we investigated the expression of the osteocalcin (OC) gene, which is directly regulated by 1,25(OH)2VD3. ROS 17/2.8 cells were grown in SF media and treated with 10-11 to 10-7 M of 25-OH-VD3 with or without 10-8 M T3 for 24 hours. 25-OH-VD3 induced OC mRNA expression 2.5-, 4.3- and 17.8-fold at concentrations of 10-11, 10-9 and 10-7 M, respectively, when alone, and 6.3-, 20.1- and 25.2-fold, respectively, when combined with T3. T3 alone also up-regulated OC mRNA expression (~ 3-fold),however it was not able to modify the 1,25(OH)2VD3 induction of OC mRNA expression. The present study suggests that T3 activates the VD3 activation pathway in ROS 17/2.8 cells by increasing the expression of 1alpha- OHase. This effect may partially explain the disturbances of calcium metabolism observed in hyperthyroidism.

M530

The Role of Vitamin D Receptor in Regulation of The Renal 25-Hydroxyvitamin D 1α-Hydroxylase Gene Expression at Prenatal and Postnatal Stages of Mice. N. Tsugawa,*[1] C. Ashiwa,*[1] A. Morishita,*[1] S. Kato,[2] T. Okano.[1] [1]Kobe Pharmaceutical University, Kobe, Japan, [2]University of Tokyo, Tokyo, Japan.

The role of vitamin D receptor (VDR) in regulation of renal 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) is not clearly defined, and the mechanism of action of VDR on CYP27B1 gene expression remains unclear. In the present study, we examined the changes of CYP27B1 mRNA expression and plasma PTH levels at prenatal and postnatal stages of wild type (WT) mice and VDR null mutant (VDRKO) mice. In 16 day-old fetus and 0 day-old neonate, plasma calcium levels of the WT and VDRKO mice were within normal range, however, plasma PTH levels of the VDRKO mice were slightly but significantly higher than those of the WT mice. This finding indicates that VDR is operative in suppression of PTH secretion from the fetal stage. At the fetal stage (16 day), renal CYP27B1 mRNA levels in both the WT and VDRKO mice were extremely low, and no significant difference was observed between both mice. After birth (0 day), renal CYP27B1 mRNA levels rapidly increased in both mice. These results suggest that unknown factor(s) beside VDR and PTH would be involved in the gene expression of renal CYP27B1. Suppressive effect of VDR on CYP27B1 mRNA expression was observed in 1 week-old WT mice, whereas CYP27B1 mRNA levels of the VDRKO mice were approximately 10 times higher than those of the WT mice by 7 weeks old after birth. After weaning, although plasma PTH levels of the VDRKO mice remarkably increased due to plasma Ca levels decreased, CYP27B1 mRNA levels of the VDRKO mice were maintained consistently in high levels from 1 week after birth. To examine whether the plasma PTH levels correlate with the renal CYP27B1 mRNA levels in both the WT and VDRKO mice, the WT mice were continuously infused with PTH at 2-40μg/μl/h doses for 4 days using a mini-osmotic pump. When plasma PTH levels were within 50-500 pg/ml, the CYP27B1 mRNA levels of the PTH-infused WT mice were consistently lower than those of the VDRKO mice. In contrast, when plasma PTH levels were over 1000 pg/ml, the CYP27B1 mRNA levels of the PTH-infused WT mice were almost comparable to those of the VDRKO mice. We conclude that suppressive effect of VDR on CYP27B1 gene expression begin to function at 1 week after birth when plasma PTH levels are within 50-500 pg/ml.

M531

PTH Regulates 24-Hydroxylase mRNA by Altering Its Stability. C. Zierold,* J. A. Mings,* H. F. DeLuca. Biochemistry, University of Wisconsin, Madison, WI, USA.

The upregulation of the 25-hydroxyvitamin D_3-24-hydroxylase by 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is well established, and occurs at the transcriptional level through two vitamin D response elements in the promoter of the gene, while the mechanism of downregulation by parathyroid hormone (PTH) has not yet been elucidated. To study the mechanism of PTH action we used AOK-B50 cells, a porcine kidney cell line with stably transfected opossum PTH receptor, in which both the 24-hydroxylase mRNA and activity are downregulated by PTH. Cells dosed with $1,25(OH)_2D_3$ at O hours, and subsequently at 0, 1, 2, or 4 hours with 100 nM of PTH showed respective levels of 24-hydroxylase mRNA equivalent to 72.6, 65.3, 57.2, and 37.2% of the levels found in cells dosed with $1,25(OH)_2D_3$ only, as determined by Northern analysis. All cells were collected 7 hours after the initial $1,25(OH)_2D_3$ dose. This pattern of expression indicates that PTH does not act by repressing transcription, but rather by making the mRNA for 24-hydroxylase less stable, and susceptible for destruction. PTH requires at least one hour to act, since cells that were dosed with PTH 1 hour or less prior to the end of the experiment did not show any reduction of 24-hydroxylase mRNA by PTH. We also determined that RNA synthesis is required for PTH to act, as actinomycin D blocked the downregulation by PTH of 24-hydroxylase mRNA when administered concomitantly with PTH, but not when administered 2 hours after PTH. It was previously shown that the untranslated regions of genes can alter the stability of genes, thus we analyzed the 5' untranslated region (5'UTR) and the 3' untranslated region (3'UTR) of the rat 24-hydroxylase gene by using reporter gene constructs to identify the sites of PTH action. Using $1,25(OH)_2D_3$-responsive constructs we were not able to effect downregulation by PTH on the reporter gene as on the 24-hydroxylase with either the entire 5'UTR, most of the 3'UTR, or both. Further analysis of the remaining 3'UTR and the translated regions will hopefully reveal the site of action of PTH.

M532

Facilitating Effects of Intracellular Vitamin D Binding Protein 1 (IDBP-1) on Vitamin D-1-Hydroxylation. S. Wu, J. S. Adams. The Burns and Allen Research Institute and Division of Diabetes, Endocrinology and Metabolism, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA, USA.

Modification by hydroxylation is the principal means of vitamin D metabolism. This is especially true for the conversion of substrate 25-hydroxyvitamin D (25-OHD) to 1,25-dihydroxyvitamin D (1,25-(OH)2D), the vitamin D hormone, and to 24,25-dihydroxyvitamin D, the most plentiful 25-OHD catabolite. There are at least three principal mechanisms regulating the hydroxylation reactions: 1] hydroxylase gene expression; 2] co-factor availability; and 3] substrate delivery to the enzyme. Intracellular vitamin D binding proteins (IDBPs) have recently been shown to participate in the latter mode of regulation (Wu, et al. Mol Endocrinol, 14:1387, 2000). IDBP-1 is a member of heat shock protein 70 family. It possesses a high affinity and capacity for internalized 25-OHD and 1,25-(OH)2D. IDBP-1 also facilitates vitamin D-responsive gene transactivation, including 24-hydroxylase expression by promoting delivery of metabolite to the vitamin D receptor (VDR). Preliminary experiments employed primate (COS-7) and human (HKC) kidney cell lines, both endowed with 1- and 24-hydroxylase potential, transiently and stably overexpressing IDBP-1. After incubation with 25-OHD, both IDBP-1-overexpressing cell lines demonstrated enhanced 24-hydroxylase expression that could be blocked with the cytochrome P450 inhibitor. We hypothesized that IDBP-1 promoted the production of 1,25-(OH)2D

and a secondary increase in 24-hydroxylase gene expression by increasing the delivery of substrate 25-OHD to the mitochondrial 1-hydroxylase. To test our hypothesis, COS-7 and HKC cells stably transfected with IDBP-1 were incubated with 200 nM 25-OHD overnight. The conditioned medium was assayed for 1,25(OH)2D produced from 25-OHD. Compared to wild-type cells 25-OHD conversion to 1,25-(OH)2D was increased 2-fold and 3-fold in IDBP-1 transfected COS-7 and HKC cells, respectively. This result indicated that IDBP-1 facilitates 1-hydroxylase activity. To confirm the increase in 1,25-(OH)2D production resulted from an increase in 1-hydroxylase activity and not solely from a reduction in 24-hydroxylase activity in IDBP-1-overexpressing cells, HD-11 chick myelomonocytic cells, rich in 1-hydroxylase but devoid of 24-hydroxylase potential, were stably transfected with IDBP-1 and their 1-hydroxylase potential examined. 1,25-(OH)2D increased a mean of 5.7-fold compared to untransfected cells or to cells transfected with vector alone. We conclude that IDBP-1 promotes the vitamin D-1-hydroxylation reaction by enhancing delivery of substrate to 1-hydroxylase and away from the substrate-competing 24-hydroxylase.

M533

The Mechanism of 1,25-Dihydroxyvitamin D_3 Auto-regulation in Keratinocytes. Z. Xie,[1] S. Munson,*[1] N. Huang,*[1] I. Schuster,*[2] A. Portale,[3] W. Miller,*[3] D. Bikle.[1] [1]Endocrine Unit, VA Medical Center, University of California, San Francisco, CA, USA, [2]Theoretical Biochemistry, University of Vienna, Novartis Research Institute, Vienna, Austria, [3]Department of Pediatrics, University of California, San Francisco, CA, USA.

Vitamin D_3 is synthesized in the skin and then undergoes 25-hydroxylation in the liver, followed by 1α-hydroxylation in the kidney and other tissues such as epidermis, to make the biologically active hormone 1α,25-dihydroxyviatmin D_3 [1α,25$(OH)_2D_3$]. These hydroxylations of vitamin D_3 are carried out by vitamin D-25-hydroxylase (P450c27) and 25-hydroxyvitamin D-1α-hydroxylase (P4501α). The major pathway involved in the inactivation of 25$(OH)D_3$ and 1α,25$(OH)_2D_3$ consists of their 24-hydroxylation to 24,25$(OH)_2D_3$ and 1α,24,25$(OH)_3D_3$, respectively, by 25-hydroxyvitamin D-24-hydroxylase (P450c24). It has generally been postulated that the synthesis of 1α,25$(OH)_2D_3$ from 25$(OH)D_3$ is inhibited by its end product 1α,25$(OH)_2D_3$ through inhibition of P450c1α gene expression just as synthesis of 24,25$(OH)_2D_3$ from 25$(OH)D_3$ or of 1α,24,25$(OH)_3D_3$ from 1α,25$(OH)_2D_3$ is increased by 1α,25$(OH)_2D_3$ through induction of P450c24. However, we observed that in human epidermal keratinocytes 1α,25$(OH)_2D_3$ at a concentration range from 10^{-8} to 10^{-12} failed to reduce the abundance of P450c1α mRNA or protein determined by northern and western analysis, or P450c1α promoter activities assayed by luciferase reporter analysis. In contrast, the P450c24 promoter and the mRNA and protein levels of involucrin were induced by 1α,25$(OH)_2D_3$ in these same experiments. Inhibition of 1α,25$(OH)_2D_3$ catabolism with a selective inhibitor of the P450c24 reversed all feedback inhibition by 1α,25$(OH)_2D_3$ on P450c1α activity. Thus, the apparent reduction in P450c1α activity by 1α,25$(OH)_2D_3$ in keratinocytes is due to increased catabolism of substrate by P450c24. Lack of direct feedback regulation may explain overproduction of 1α,25$(OH)_2D_3$ in diseases such as sarcoidosis in which a compensatory increase in P450c24 does not occur.

M534

Identification and Cloning of a Novel Vitamin D Hydroxylase Which Promotes 1,25-Dihydroxyvitamin D Production in the Macrophage. S. Ren,*[1] S. Wu,[1] J. S. Adams.[2] [1]Burns and Allen Research Institute, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA, [2]Division of Endocrinology, Burns and Allen Research Institute, UCLA/Cedars-Sinai Medical Center, Los Angeles, CA, USA.

Here we report a new pathway for control of 1,25-dihydroxyvitamin D (1,25-(OH)2D) synthesis in the chick macrophage-like cell line HD-11; this cell line has been employed as a model for the macrophage-mediated (i.e. extrarenal), dysregulated overproduction of 1,25-(OH)2D as occurs in human granuloma-forming and malignant lymphoproliferative disease. Previous studies from this laboratory showed that 25-OHD-1-hydroxylase activity is high and 24-hydroxylase activity undetectable in HD-11 cells even when exposed to high levels of substrate 25-OHD and 1,25-(OH)2D to stimulate the 24-hydroxylase gene. It is yet unknown whether the macrophage 1-hydroxylase is the product of the renal 1-hydroxylase or a novel gene. To address this question, we sought to clone the macrophage 1-hydroxylase from LPS-stimulated HD-11 cells. Attempts to clone the macrophage 1-hydroxylase using primers based on human and mouse 1-hydroxylase cDNA sequence failed repeatedly. However, using a series of nested primers based on chick 24-hydroxylase sequence as well as 3' and 5' RACE, a novel 2.5 kb cDNA (including the 3' polyadenylation signal) has been cloned. The cDNA harbors a 2.3 kb 3' end that encodes a protein that possesses a 25-OHD and heme-binding domain that is similar to but distinct from the chick kidney 1- and 24-hydroxylase. The first 5' 200 bp of cDNA bears less than 42% sequence identity with the chick kidney 24- and 1-hydroxylase, suggesting that it is the product of a novel vitamin D hydroxylase gene. Using a 715 bp fragment of this 2.5 kb cDNA sequence as the probe, Northern blot detected a 2.5 kb signal in HD-11 cells. Expression of this mRNA was induced by 1,25-(OH)2D. Stable expression of the antisense construct resulted in a 4.4-fold increase ($p<0.01$) in 1,25-(OH)2D production compared with wild-type HD-11 cells. In summary, we have cloned a novel 1,25(OH)2D- inducible, cytochrome P450-related vitamin D hydroxylase cDNA from macrophages. The new gene product is distinct from the 24- and 1-hydroxylase and functions to increase 1,25-(OH)2D catabolism, decrease 1,25-(OH)2D synthesis or both. We theorize that regulated expression of this gene product, either as a catabolic enzyme or as an endogenous 'decoy' controlling access of substrate to the 1-hydroxylase, regulates 1,25-(OH)2D production by macrophages.

M535

Infliximab, a New anti-TNF Therapy Reduces the Level of 25-OH Vitamin D in Primary Sjögren's Syndrome. V. Gangji,[1] F. Daumerie,*[1] R. Moreno Reyes,*[2] J. Margaux,*[1] T. Appelboom,*[1] S. Steinfeld.*[1] [1]Rheumatology, Hôpital Erasme, Brussels, Belgium, [2]Nuclear medicine, Hôpital Erasme, Brussels, Belgium.

Sjögren's syndrome (SS) is a common chronic inflammatory disease characterized by lymphocytic infiltration of salivary and lacrimal glands and systemic involvement. There are many evidences to support that TNFα plays a critical role in the pathogenesis of SS. Moreover, TNFα was proven to be crucial in the development of inflammatory osteoresorption and in the recruitment and activation of osteoclasts. Infliximab, a chimeric human mouse anti-TNFα monoclonal antibody is capable of neutralizing TNFα activity. TNFα antagonists such as infliximab are also proven to have beneficial effects on the reduction of bone erosions in rheumatoid arthritis. Since rheumatoid arthritis and SS share a central pathogenic role of TNFα, we evaluate the ability of infliximab to prevent bone resorption in SS. Fifteen women and one man with a mean age of 49.6 years suffering from SS received 3 infusions of 3mg/kg of infliximab at week 0, 2, 6 and were followed at week 10 and 14. All patients were receiving 1000 mg of calcium carbonate and 800 U of vitamin D throughout the study. Blood and urinary samples were collected prior to the treatment and at each visit for the measurement of one bone formation marker, osteocalcin and two urinary bone resorption markers, type I collagen cross-linked C-telopeptide and free D-pyridinoline. Intact parathormone (PTH) and 25-OH vitamin D_3 were also measured at the different time points. All patients had a normal bone mineral density at baseline. All markers of bone formation and resorption, PTH and 25-OH vitamin D_3 were within normal limits at baseline and remained so throughout the study. However, 25-OH vitamin D_3 decreased from 26 ± 2.6 to 20 ± 1.7 ng/ml ($p<0.001$) at one and 2 months after the last infusion. Low levels of 25-OH vitamin D_3 can be found in SS and is usually correlated to the activity of the disease due to an activation of hydroxylation of 25-OH vitamin D_3 in macrophages. However, this study demonstrated a good efficacy of infliximab treatment on inflammatory and clinical markers and a decrease of disease activity which should have increased the level of 25-OH vitamin D_3. In conclusion, this study showed that infliximab has no effect on bone formation or resorption markers in SS but lowers significantly the level of 25-OH vitamin D_3 . This effect might have clinical implications since patients with inflammatory disease may already have low level of 25-OH vitamin D_3 before infliximab treatment. Therefore we suggest that 25-OH vitamin D_3 should be measured before treatment in patients receiving infliximab to avoid the deleterious effect of vitamin D deficiency.

ADULT BONE AND MINERAL WORKING GROUP

WG01

Case Report: Spondylo-epiphyseal Dysplasia Tarda. V. Botea, M. Reddy, G. Edelson; Department of Internal Medicine, Sinai-Grace Hospital, Wayne State University, Detroit, Michigan, USA.

Spondyloepiphyseal dysplasia (SED) tarda, an osteochondrodystrophy that occurs in one in a million, is characterized by disproportionate short stature. Radiological features include platyspondyly with hump-shaped central and posterior portions of the vertebral bodies, narrow disc spaces and mild to moderate epiphyseal dysplasia, which may cause secondary osteoarthritis.

A forty-five year old Caucasian male, a retired jockey, presented with a several-year history of right hip pain and bilateral femur pain. The patient denied trauma, fractures or proximal muscle weakness. He has never been on thyroid hormones, anticonvulsants or diuretics. Physical examination revealed a short (4'4"), barrel-chested white male, with no evidence of blue sclera, thyroid abnormality, or bowing of extremities. Investigations revealed normal calcium, phosphorus, alkaline phosphatase, PTH, Vitamin D 25-OH and free testosterone levels, but a low vitamin D 1,25 di-OH cholecalciferol. Bone survey revealed abnormal hump-shaped vertebral configuration, severe arthropathic changes at the right hip joint with small iliac crests, bilateral abnormalities of the distal radial epiphyses and flattening of the metatarsals. Bone mineral density of the lumbar spine and right hip revealed moderate osteopenia with a T-score of –2.

SED has various modes of inheritance – autosomal dominant, autosomal recessive and X-linked recessive. In the most common type, the X-linked form, 'sedlin' mapped to Xp22 is mutated. It causes a mild postnatal defect in endochondral bone formation, most evident in the spine and subsequently in the proximal epiphyses of the femur, usually manifest by the first or second decade of life. Our case is probably a sporadic X-linked variant that remained undiagnosed until the fifth decade of life, making it an unusual case. The molecular basis as well as a specific treatment of this disorder remains to be explored.

WG02

Osteoporosis Categories based on Stiffness Index and Fracture History in Women: the Results of the Population Based ESOPO Study. S.Adami[1], M.Rossini[1], G.Bonomi[2], R.Di Virgilio[3], R.Occhipinti[4], R.Spinazze[5], F.Trotta[6], A.Zanatta[7], R.Giorgino[8]; [1]Verona, [2]Palmanova, [3]Treviso, [4]Belluno, [5]Conegliano, [6]Ferrara, [7]Legnago, [8]Rome, Italy.

A multicenter population based study was conducted in order to relate the prevalence of osteopenia and osteoporosis with personal fracture history in the Italian general elderly male population:. Eighty-three University and Hospital centers located over 18 out of 21 Italian peninsular and insular regions participated to the study. Subjects were randomly invited to participate to the study from 1,827 General Practitioners through their own Patients Directory by a specific randomization protocol. Reasons of decline were accurately recorded. Eleven thousands-eleven (11,011) females aged 40-79 participated to the study: each woman underwent a bone quantitative ultrasound measurement (QUS) on a Lunar Achilles Express device. Personal, maternal and paternal history for clinical vertebral, hip, rib, wrist and other fractures was recorded and low trauma fractures were considered. The table below show the number and proportion of women who reported an history of fractures occurred after the age of 50.

Decade	**50-59**		**60-69**		**70-79**	
Fracture	n	%	n	%	n	%
Vertebral	19	0.6	77	2.5	49	3.9
Hip	4	0.1	32	1	33	2.6
Rib	28	0.9	71	2.3	51	4
Wrist	62	2	197	6.3	130	10.2
Other	143	4.7	410	13.1	214	16.8

For osteopenia (a) and osteoporosis (b) definition arbitrary cut-off were selected corresponding to Stiffness Index T-scores respectively $\leq$-1 and $\geq$-2.5 (a) and <-2.5 (b) based on manufacturer Italian reference data.The table below show the age-standardized proportion (± ES) of subjects with fracture history in the three different categories:

Stiffness Index	**>-1**	**<-1 and $\geq$-2.5**	**<-2.5**
No. of subjects	2,093	3,299	1,656
Site of fractures			
Vertebral	0.97 ± 0.29	1.84 ± 0.27	3.28 ± 0.42***
Hip	0.08 ± 0.06	0.85 ± 0.19	1.91 ± 0.32***
Rib	1.42 ± 0.37	1.94 ± 0.27	3.27 ± 0.44***
Wrist	3.63 ± 0.64	5.55 ± 0.45	7.96 ± 0.67***
Other sites	8.19 ± 0.87	11.25 ± 0.61	15.26 ± 0.92***

The proposed categorization based on Stiffness Index after adjustment for age allows to identify an higher proportion of subjects reporting an history of clinical vertebral fractures (p<0.0001), hip fractures (p<0.0001), rib (p<0.0001), wrist (p<0.0001) and other osteoporotic fractures (p<0.0001) among osteoporotic patients. In particular hip fracture history was about twenty times more frequent in these patients vs. normals. Osteoporotic patients showed an higher frequency of maternal and paternal history of hip fractures (p<0.05) and an higher frequency of maternal history of clinical vertebral fractures (p<0.05). In conclusion this large multicenter observational population based study clearly shows the strong performance of Stiffness Index ultrasound measurement for osteoporosis diagnosis both in young and in elderly female general population.

WG03

Hypercalcemia in a Patient with MEN-1 and Previous Total Parathyroidectomy. S. Dionisi[1], S. Minisola[1], F. Paglia[1], L. Memeo[2], M. Mastropasqua[1], J. Pepe[1], S. De Geronimo[1], N. Raejntroph[1], L. Fitzpatrick[3]. [1]Dipartimento di Scienze Cliniche, [2]Dipartimento di Medicina Sperimentale e Patologia, Università di Roma "La Sapienza", Rome-Italy; [3]Mayo Clinic and Mayo Foundation Rochester, MN, USA.

A 35-yr-old man was admitted with a presumed diagnosis of acute pancreatitis. In 1995 an ectopic parathyroid located in the mediastinum was removed; on histological examination it was found to be parathyroid cancer. One year later a diagnosis of Zollinger-Ellison syndrome was made and a total thyroparathyroidectomy was performed due to a new finding of hypercalcemia. One year before admission the patient had an EGD which revealed diffuse polypoid structures of the gastric fundus, body and antrum and of the duodenum; a total body CT showed a hypodense lesion of 2.5 cm in the anterior mediastinum and a solid mass of 7 cm at the tail level of the pancreas with inner necrotic areas.

Routine laboratories performed on admission showed an elevated calcium level (total Ca = 15.7 mg/dL, Ca^{++} = 2.3 mmol/L); a rapid intact 1-84 PTH assay (N-tact PTH SP, DiaSorin, Stillwater, MN) documented extremely high values of the hormone (1888 pg/mL, nl 10.6-54). Further analyses showed high serum gastrin and chromogranin A (331 ng/mL, nl< 30) levels. The patient was started on fluid therapy, intravenous omeprazole, somatostatin and pamidronate, with reduction of serum calcium levels. Unfortunately, the patient died owing to cardiovascular systemic complications on the third day of the hospitalization.

The autopsy revealed that the lesion in the anterior mediastinum was metastatic parathyroid cancer. The diffuse gastric polypoid masses were mucosal microcarcinoids of the stomach. The pancreatic lesion was positive for chromogranin A and gastrin by immunohistochemistry, and was negative for glucagon, somatostatin, insulin, and VIP. Both the pancreatic and gastric lesions were positive for PTHrP staining.

To our knowledge, this is the first case in which the association of parathyroid cancer, neuroendocrine pancreatic tumour (gastrinoma) with massive carcinoid dissemination of the stomach is reported in the setting of MEN-1. The positive PTHrP staining in both the pancreatic and gastric lesions suggests that this peptide may have contributed to the hypercalcemia in our patient.

WG04

Skeletal Response To Antiresorptive Agents In Androgen Insensitivity Syndrome. M. Honasoge, D.S.Rao, Henry Ford Hospital, Detroit, MI, USA.

Evidence suggests that low bone mineral density (BMD) in patients with androgen insensitivity syndrome (AIS) is due to both insensitivity of the skeleton to androgen and inadequacy of estrogen. Although early gonadectomy and inadequate estrogen therapy is associated with greater bone mineral deficit, lack of skeletal response is noted despite adequate hormone replacement therapy (HRT). In addition bone turnover markers have been reported to be normal in patients with AIS. We report two adult sisters with complete AIS characterized by female external genitalia, absence of uterus and cervix and 46XY karyotype, whose bone turnover markers were elevated despite HRT and who responded more favorably to bisphosphonate therapy. Both patients had osteoporosis at the spine and normal BMD at the hip and proximal radius by NOF criteria (L-Spine T-score -2.38 and -2.86).

Baseline clinical and biochemical characteristics of the patients and response to antiresorptive agents are tabulated below:

	age yrs	**Age in yrs at gonadectomy**	**Ht inches**	**Wt lbs**	**Treatment**
Pt 1	25	19 (dysgenetic gonads+Down's)	61	124	growth hormone Rx (13 -17yrs) HRT (19 - 25 yrs)
Pt 2	20	14½ (testicular tissue removed)	63	133	HRT on and off (14 ½ -20 yrs) Ritalin on and off for ADD

	Patient 1			Patient 2		
	6yrs HRT	**6months**	**Bisphos Rx**	**Baseline**	**5mo HRT**	**Bisphos Rx**
L-Spine	809 mg/cm^2	+1.0%		792 mg/cm^2	-4.7%	
F-Neck	781 mg/cm^2	+2.4%		894 mg/cm^2	-0.8%	
Total Hip	872mg/cm^2	-0.75%		905 mg/cm^2	-2.3%	
1/3 Rad	732 mg/cm^2	NA		654 mg/cm^2	+1.0 %	
UrineNTx	58 bce/cr	89 bce/cr	47 bce/cr	141 bce/cr	168 bce/cr	67 bce/cr

BMD is low in patients with AIS particularly at the trabecular sites and bone resorption markers remain elevated despite HRT, and appear to respond better to bisphosphonate therapy. Conclusion: 1) Lack of adequate skeletal response to HRT may be due to poor compliance and/or skeletal insensitivity to HRT. 2) Patients who cannot tolerate or fail to respond to HRT can be treated with bisphosphonates. 3) Studies on expression and distribution of estrogen receptors in target tissues in patients with AIS and in normal men may contribute to the understanding of the skeletal resistance to HRT in AIS and the role of estrogen in skeletal remodelling in adult men.

WG05

Osteoporosis in Pregnancy is Associated with Inadequate Dietary Calcium Intake and Low Stores of 25-Hydroxy-Vitamin D: Presentation of a Cohort of 8 Cases. M. Pfeifer, W. Pollähne, A.D. Lazarescu, H.W. Minne. Institute of Clinical Osteology "Gustav Pommer" and Clinic "DER FÜRSTENHOF", Bad Pyrmont, Germany.

Acute back pain in the third trimester of pregnancy or during early lactation may be the only clinical symptom of pregnancy-associated osteoporosis. Roentgenograms performed after delivery may reveal severe forms of osteoporosis with multiple vertebral fractures. We present the data of 8 women (mean age 28.5 ± 3.7 years) with a mean follow-up period of 26.8 ± 25.3 months. Calcium intake during pregnancy was assessed by a food-frequency-questionnaire, bone density (BMD) was measured using DEXA (QDR 2000, Hologic), and secondary osteoporosis was ruled out by bone biopsies and intensive laboratory investigations.

Results are presented in the following Table:

Body mass index (kg/m²)	21.6 ± 2.5	
Time to diagnosis postpartal (months)	4.2 ± 3.1	
Calcium intake (mg/day)	543 ± 112	
Loss of height (cm)	5.0 ± 1.9	
Vertebral fractures (No.)	5.2 ± 2.1	
BMD lumbar spine (T-score)	-2.9 ± 1.4	-1.3 ± 1.0#
BMD femoral neck (T-score)	-2.6 ± 1.0	-1.7 ± 1.0#
Serum calcium (mmol/ml), [2.1-2.7]	2.2 ± 0.1	
Serum phosphorus (mmol/ml), [0.7-1.3]	1.0 ± 0.2	
PTH intact (pmol/l), [1.1-6.9]	6.7 ± 1.6	
25-(OH)-Vit. D (nmol/ml), [50-300]	64.4 ± 30.1	
1,25-$(OH)_2$-Vit. D (ng/l), [16-43]	24.2 ± 18.5	
Alkaline Phosphatase (U/l), [40-170]	99.7 ± 35.5	
Urine Desoxy-Pyr (µg/Crea), [20-52]	84.2 ± 26.9	

[normal range]; #: after follow-up period and treatment with 1000 mg calcium and 1000 IU of vitamin D;

Dietary calcium intake was far below recommended levels of 1200 to 1500 mg per day and serum levels for vitamin D were near the lower limit of the normal range. These observations, however, may not explain the pathogenesis of this disease. In addition, rapid estrogen depletion after delivery may result in an exaggerated release of cytokines leading to bone resorption by activated osteoclasts. In conclusion, these cohort of 8 women with osteoporosis associated with pregnancy is not consistent with the thesis that idiopathic osteoporosis occurs in pregnant women by mere chance.

WG06

Normocalcemic Hyperparathyroidism. Response to Oral Calcitriol. G.M.A. Palmieri, B. Fortner*, D. McCommon*, West Clinic, Memphis TN, USA.

Normocalcemic hyperparathyroidism may occur in patients with secondary hyperparathyroidism. We are reporting elevated PTH and normal ionized Ca (i Ca) in 17 women, 13 with osteoporosis, 1 with osteopenia and 3 with other diagnoses. None had history or laboratory findings compatible with renal, gastrointestinal, endocrine or Ca, P and vitamin D metabolism disorders capable of causing secondary hyperparathyroidism. The mean age was 65 y, range 34-84. Serum values (mean ± SD) were: intact PTH 100.0 ± 23.64 pg/ml, (nl 10-65); i Ca 4.87 ± 0.26 mg/dl (nl 4.50-5.30); calcidiol 38.8 ± 20.8 ng/ml (nl 10-55); creatinine 1.03 ± 0.36 mg/dl (nl 0.5-1.4). In order to suppress the elevated PTH, calcitriol, 0.25 µg x 3/day (d) was administered for 7 d. On the 7^{th} d, PTH fell by 66%, ($P < 0.001$) to normal and i Ca rose by 5% ($P < 0.01$). In 4 patients (pts) the i Ca rose just above the upper normal limit of 5.3 mg/dl, in the others it remained within normal limits. Thirteen pts received calcitriol 0.25 µg twice/d for 6.3 months ± 3.41 (mean ± SD). There was no difference between the serum PTH and i Ca obtained on the 7^{th} day of suppression with values obtained at the end of the long term treatment with calcitriol (PTH 34.92 ± 16.4 vs 38.04 ± 15.29; i Ca 5.06 ± 0.21 vs 5.04 ± 0.48). Surprisingly, there was a positive correlation between the pre-treatment values of calcidiol and the magnitude of the PTH reduction during the 7^{th} d suppression test, r + 0.59 ($P<0.02$, Pearson 2 tailed). The correlation between i Ca and the fall of PTH was –0.261, NS. The data suggest that in pts with apparently idiopathic elevation of PTH, oral calcitriol markedly reduces PTH with mild elevation of i Ca. These effects persisted for at least 6 mo. There was a positive correlation between the basal levels of calcidiol and the acute response to calcitriol in patients with adequate calcidiol levels (38.8 ng/ml). This observation suggests an enhancement of calcitriol-induced suppression of PTH by calcidiol.

WG07

Osteoporosis Drugs Usage in the general population: the Results of the Population Based ESOPO Study. G.Isaia[1], A.Angeli[1], C.Cisari[2], M.Cravero[1], M.Ferraris[3], S.Parello[4], R.Pellerito[1], G.Roberti[5], R.Giorgino[6]. [1]Turin, [2]Novara, [3]Vercelli, [4]Asti, [5]Chivasso, [6]Rome, Italy.

A multicenter population based study was conducted in order to relate the prevalence of osteopenia and osteoporosis with osteoporosis drug usage in the Italian general population. Eighty-three University and Hospital centers located over 18 out of 21 Italian peninsular and insular regions participated to the study. Subjects were randomly invited to participate to the study from 1,827 General Practitioners through their own Patients Directory by a specific randomization protocol. Reasons of decline were accurately recorded. A total of 15,992 subjects (11,011 females aged 40-79 and 4,981 males aged 60-79 years) participated to the study: each subject underwent a bone quantitative ultrasound measurement (QUS) on a Lunar Achilles Express device. The current use of osteoporosis drugs (namely bisphosphonates, calcitonin, calcium, vitamin D, or other drugs used for osteoporosis treatment) was recorded in each subject. For osteopenia (a) and osteoporosis (b) definition in females arbitrary cut-off were selected corresponding to Stiffness Index T-scores respectively ≤-1 and ≥-2.5 (a) and <-2.5 (b) based on manufacturer Italian reference data. Due to the lack of reference data for Italian male population, the same above mentioned criteria from female population were applied for males .

The table below shows the age-adjusted proportion (±SE) of subjects among normal, osteopenic and osteoporotic women assuming the above listed drugs (*p<0.05 **p<0.01 ***p<0.001 vs Normal).

FEMALES	Normal	Osteopenia	Osteoporosis
	% ± SE	% ± SE	% ± SE
All OP drugs	9.1 ± 0.7	13.0 ± 0.5	18.3 ± 1.0***
Bisphosphonates	2.9 ± 0.4	5.3 ± 0.4	8.6 ± 0.6***
Calcitonin	0.9 ± 0.3	1.1 ± 0.2	1.8 ± 0.3*
Vitamin D	3.0 ± 0.4	4.4 ± 0.3	7.2 ± 0.7***
Calcium	4.3 ± 0.5	6.6 ± 0.4	8.6 ± 0.7***
Other drugs for OP	1.2 ± 0.2	1.4 ± 0.2	1.2 ± 0.2

The table below shows the age-adjusted proportion (±SE) of subjects among normal, osteopenic and osteoporotic men assuming the above listed drugs (*p<0.05 **p<0.01 ***p<0.001 vs Normal).

MALES	**Normal**	**Osteopenia**	**Osteoporosis**
	% ± SE	% ± SE	% ± SE
All OP drugs	1.5 ± 0.3	2.3 ± 0.4	4.4 ± 0.9***
Bisphosphonates	0.3 ± 0.1	0.7 ± 0.2	1.7 ± 0.6***
Calcitonin	0.2 ± 0.1	0.4 ± 0.2	0.4 ± 0.3
Vitamin D	0.3 ± 0.1	0.4 ± 0.2	2.0 ± 0.6**
Calcium	0.8 ± 0.2	0.9 ± 0.2	2.4 ± 0.7*
Other drugs for OP	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.3

This data clearly shows that fortunately osteoporotic patients are more frequently treated with osteoporosis drugs than normals. The phenomenon is more visible among females, which is probably due to a more accurate awareness among Italian physicians about postmenopausal osteoporosis. However, the study demonstrates that only 18.3% of osteoporotic women and 4.4% of osteoporotic men are currently treated with specific OP drugs. Similar frequencies of bisphosphonates, calcium and vitamin D prescriptions was observed within each gender group, very higher in females than in males. In osteopenic subjects, the frequency of treated is lower than in osteoporotic ones. However, no true differences were observed regarding the use of either bisphosphonates or calcium and vitamin D. This large study confirms that osteoporosis is clearly an undertreated disease: large scale educational programs are needed to increase both patients and physicians awareness of the “silent epidemic” disease.

WG08

Osteitis Fibrosa (OF) in a Patient with Recurrent Osteomalacia (OM): An Unusual Manifestation of Severe Vitamin D Depletion. S.Rangnekar, S.Palnitkar, S-J.Qiu, D. Sudhaker, D.S.Rao, Bone & Mineral Research Laboratory, Henry Ford Hospital, Detroit, MI, USA.

OM due to vitamin D depletion is well known as is OF in severe primary or renal hyperparathyroidism. However histologically documented recurrent OM or a combination of OM and OF in the same patient has not been reported previously. We describe an unusual case of a 50 year old African American woman with OM 7 years after gastric stapling for morbid obesity. She was referred by her gasteroenterologist for possible Paget's disease because of progressive rise in serum alkaline phosphatase (AP) from 101 to 1089 IU/L over a period of 3-5 years. At presentation in 1990 she had diffuse bone pain and severe muscle weakness. Bone scan showed generalized increased uptake consistent with metabolic bone disease and a bone biopsy confirmed OM. She was treated with calcidiol and calcium with complete resolution of clinical symptoms and near normalization of biochemical and bone histomorphometric findings over the next 2 years. She could not continue the therapy because of financial and insurance problems and was without any therapy from 1995-2000. At presentation in 2000 she had recurrent severe bone pain, muscle weakness and abnormal bone scan, but with more severe hyperparathyroidism. Renal function was normal. Relevant sequential data from 1990-2001 are presented in the Table.

Variable	Reference Range	**Before Rx-1990**	After Rx-1992	Return-2001
Calcium	8 –10.4 mg/dl	8.0	9.4	8.3
AP	0 – 120 IU/L	1241	267	654
PTH	12 – 72 pg/ml	653	72	1435
25 OH-D	12 – 60 ng/ml	5	153	<2
O.Th	6 – 16 μm	34	9.3	60
MLT	18 –58 d	α	65	α
Aj.AR	0.15 - 0.57 μm/d	0.0	0.143	0.0
Ocl.S	0.1 – 2.3%	8.716	1.9	3.7
BFR	5 – 40 $\mu m^3/\mu m^2/yr$	0.0	5	0.0

O.Th: Osteoid thickness, MLT: Mineralization Lag Time, Aj.AR: Adjusted mineral apposition rate, Ocl.S: Osteoclast surface, and BFR: Bone Formation Rate.

Repeat bone biopsy showed not only OM but also OF, a feature that has not been seen in any patient with OM at our institution. Severe hyperparathyroidism associated with recurrent OM could be related to the development of autonomous hyperparathyroidism. The latter, if present, will manifest only after resolution of OM. **Conclusions:** OF, a unique feature of severe hyperparathyroidism, has not been reported previously in patients with OM due to vitamin D depletion. Interruption of vitamin D treatment appears to result in a more severe hyperparathyroidism associated with bone histomorphometric changes of OF. Lifelong monitoring is recommended to avoid such complications

WG9

Octreotide Scanning in Hypophosphataemic Osteomalacia. P.L. Selby[1], M Davies[1], B Pal*[3], MC Precott*[2]. Departments of Medicine[1], and Nuclear Medicine[2], Manchester Royal Infirmary and Rheumatology, Withington Hospital[3], Manchester, UK.

Acquired hypophosphataemic osteomalacia is usually the result of a small mesenchymal tumour which produces a phosphaturic substance. In the majority of reported cases these tumours are benign and removal of the tumour results in complete resolution of the osteomalacia. Because of their small size such tumours are often difficult to detect and may easily be missed by conventional imaging techniques. Newer modalities of imaging may enable these tumours to be detected and removed more easily. We report the use of imaging with Octreotide to detect and aid the removal of the causal tumour in a woman with acquired oncogenic osteomalacia.

A 37 year-old Syrian woman who had previously been well presented early in her third pregnancy with symptoms of progressive weakness and non-specific muscular aches and pains. She had skeletal tenderness and a marked proximal myopathy. Investigations revealed hypophosphataemia (0.50 mmol/l) with normal plasma calcium and renal function. Alkaline phosphatase was elevated to twice the upper limit of normal. Serum 25 hydroxyvitamin D levels were normal (21.4 ng/ml) but 1, 25 dihydroxyvitamin D levels were reduced (17 pg/ml). PTH concentrations were normal. A presumptive diagnosis of hypophosphataemic osteomalacia was made and confirmed on bone biopsy. In view of her on going pregnancy no imaging to identify a causal tumour was undertaken and treatment was commenced with phosphate supplements and calcitriol. On this her symptoms gradually improved and by the time of her confinement her pain was almost gone and her mobility had returned to normal.

Following delivery a decision was made to seek an underlying tumour. Whole-body imaging with 111-In Pentatreotide (Octreotide) revealed a small local area of increased uptake in the region of the right maxilla. Computed tomography of this region revealed a 1cm lesion applied to the periosteal margin of the first upper premolar on the right. The appearances were those of an ossifying fibroma. Subsequent exploration of that this region revealed a chondromyxoid fibroma which was removed.

This case illustrates the utility of Octreotide scanning in the identification of tumours causing oncogenic osteomalacia and indicates that this imaging modality may have benefits as the initial screening investigation in such patients.

WG10

Flare-ups of Fibrodysplasia Ossificans Progressiva Associated with Influenza B Infections: A Report of a Family. R. F. Scarlett[1], E. M. Shore[1], J. Patel[2], F. S. Kaplan[1]. University of Pennsylvania School of Medicine, Departments of Orthopaedic Surgery[1] and Pathology[2], Philadelphia, Pennsylvania, USA.

Fibrodysplasia ossificans progressiva (FOP) is a disabling genetic disorder characterized by progressive postnatal ossification of soft connective tissue and congenital malformations of the great toes. FOP flare-ups are marked by warm, painful, fibroproliferative masses in skeletal muscle, fascia, ligaments, and tendons. These lesions mature through a process of endochondral ossification to form mature heterotopic bone, which can bridge and immobilize adjacent joints, rendering movement impossible. FOP flare-ups may be stimulated by blunt soft tissue injury, surgical trauma, intramuscular injections, myotoxic local anesthetics, or may occur spontaneously.

We report a case of a family with a complex FOP history, in which all three sisters (two affected with FOP and one not affected) developed influenza symptoms (fever, cough, sore throat, headache, and myalgias). The oldest sister, age 15, has normal toes and experienced only one episode of heterotopic ossification (HO) prior to the influenza infection. The middle sister, age 10, has juvenile diabetes, but no features of FOP. The youngest sister, age 4, has classic features of FOP with malformed great toes and multiple episodes of HO. The youngest and oldest sisters experienced signs of a FOP flare-up (severe soft tissue swelling, erythema, warmth, and pain) within 6 and 12 hours, respectively, of the onset of influenza symptoms. The older sister's lesion occurred on the neck; however, it regressed slowly over a course of four weeks, and did not form bone. In contrast, the younger sister developed a lesion on the back, and it progressed to form mature heterotopic bone within four weeks. Influenza B virus was isolated from the two sisters with FOP, but not the middle sister, although she too had symptoms of influenza.

Influenza B and other viral infections are known to cause a broad range of myopathies, especially in children. These muscle syndromes range from mild, self-limiting myalgias to life-threatening rhabdomyolysis. The etiology of muscle injury in influenza infection is unclear. Possibilities include direct invasion of muscle by the virus, activation of pro-inflammatory transcription factors, and autoimmune processes.

Mounting anecdotal evidence from patients, families, and physicians has suggested that a relationship exists between viral infections (such as influenza) and FOP flare-ups. However, these are the first cases in which a documented viral infection served as a trigger for a fibrodysplasia ossificans progressiva flare-up.

The impact of this remarkable family on our understanding of FOP may be tremendous. Already, it has served as a catalyst for a larger retrospective study (involving all members of the International Fibrodysplasia Ossificans Progressiva Association) investigating the temporal relationship between influenza infection and FOP flare-ups. The results of this study will have an impact on the recommendations regarding viral immunizations for FOP patients and their families. Also, if a relationship exists, the mechanism by which influenza virus triggers a flare-up will be further investigated. Potentially, this will serve in elucidating the pathophysiology of this enigmatic disease.

PEDIATRIC BONE AND MINERAL WORKING GROUP ABSTRACTS

WG11

Gender and Age Predict Stiffness Index By Quantitative Ultrasound In Early Elementary School Children. C.D. Economos[1], K.Shea[1], W. Wacker[2], E. Naumova[1]. [1] Department of Physiology, Tufts University, Boston, MA, USA, [2] GE Lunar, Madison, WI, USA

The development of osteoporosis is influenced by genetics and a number of lifestyle behaviors that begin during childhood and are likely to track into adulthood. This suggests that preventive efforts should be aggressively focused on children. The "BONES" Project is a multi-faceted intervention designed to investigate bone accretion among early elementary school children. Seven variables were studied to examine with their relationship to bone quality, including age, gender, ethnicity (Caucasian, African American, Hispanic or other), body weight, sports participation (# of sports played per year), sedentary leisure time activities (hours of TV watched and video games played per week) and caffeinated soda consumption. Bone quality was measured by quantitative ultrasound (QU) of the os calcis by a Lunar Achilles and reported as Stiffness Index (SI) calculated from speed of sound (SOS) and broadband ultrasound attenuation (BUA). Body weight was measured on a digital scale and the remaining variables reported by the primary caregiver (parent or guardian). Data were analyzed by generalized linear model (GLM) and results were interpreted as relative risk (RR) with 95% lower and upper confidence intervals (LCI, UCI). Measurements were made on 148 children (71 M & 77 F, mean age = 7.2 yrs, range from 6 to 9 yrs.). On average, children spent 19.3 hours per week engaged in sedentary activities (range from 0-43 hrs) and participated in 2 sports per year (range from1 to 9). Sedentary activity time was negatively associated with sports participation (r=-.173, p=0.03). Fifty one percent of children did not consume caffeinated soda, while the mean soda intake for consumers was 7 ounces per day. Mean body weight was not different by gender (26.9$\pm$6.9 kg for boys and 27.1$\pm$6.9 kg for girls, p= 0.71). However, SI was different by gender (74.6$\pm$17.0 units for boys and 63.6$\pm$10.2 units for girls, p< 0.001). Results of the GLM also demonstrated that boys were 9% higher even after adjustment for all factors. From the same model, SI increased by 4% each year.

Parameter	B	Std. Error	t value	RR	LCI	UCI
(Intercept)	3.96	0.12	33.0			
Gender	0.087	0.011	7.8	1.09	1.07	1.12
Age	0.037	0.017	2.2	1.04	1.00	1.07

All of the remaining variables were not associated with the SI, yet this preliminary data cannot rule out their potential importance. This study may help promote our understanding of health behaviors in young children and how these may potentially affect risk of osteoporosis later in life.

WG12

Can Body Size Related Normative Data Improve the Diagnosis of Osteoporosis in Children? N.J. Crabtree[1], C.M. Boivin*[1], N.J. Shaw[2]. [1]Dept. of Nuclear Medicine, Queen Elizabeth Hospital, Birmingham, United Kingdom. [2]Dept. of Endocrinology, Birmingham Children's Hospital, Birmingham, United Kingdom.

Traditionally, paediatric bone mineral density values have been reported with respect to age without taking in to account the child's body size. We have investigated the significance of body size and composition in the prediction of bone mineral content (BMC) using DXA.

419 healthy children, aged 5-11years, were recruited from local primary schools in the Birmingham area. BMC of the lumbar spine and whole body was measured using paediatric software on a Lunar DPX-L DXA scanner, changing the acquisition mode according to trunk thickness. Total Body BMC and lumbar spine BMC were evaluated using ANOVA and stepwise linear regression. The BMC data was modelled using age, height, weight, lean body mass (LBM), sex as covariates or factors in the model.

For lumbar spine BMC, LBM made the largest contribution, but age, weight, height and sex were all significant. LBM alone could explain 80.5% of the variation and the addition of all the other parameters only increased the explained variation to 83.9%. LBM also explained the majority of the variation for total body (87.7%), weight, height, and ethnic group were still significant but only increased the explained variation by 3.0%. We have used the relationship between BMC and LBM to discriminate between children who have osteoporotic fractures and those whose reduced BMD is due to body size.

We propose that age related normal ranges should no longer be used for prepubertal children and that a body size related normal range is more appropriate.

WG13

Longitudinal 7 Year Changes in Bone Geometry and Density during Growth Hormone Treatment in Pubertal Children using Digital X-ray Radiogrammetry. K. Mohnike,*[1] P. Beye,*[1] S. Pötzsch,*[1] K. Glockmann,*[1] C. Wüster.[2] [1]University Children´s Hospital, Magdeburg, Germany, [2]Novo Nordisk Pharma, Mainz, Germany.

The Pronosco X-posure system estimates bone mineral density (BMD) and bone geometry using digital x-ray radiogrammetry and textural analysis of digitised conventional radiographs of hand and forearm. The system calculates BMD using a weighted average of cortical and bone width measurements at the radius, ulna and second through fourth metacarpal. The output is an absolute BMD estimate called DXR-BMD and parameters of bone geometry in tubular bones. The aim of this study was to measure bone geometry and BMD in growing children. Fifteen children (11 boys, 4 girls) were included in this preliminary analysis. Their mean age at start of analysis was 11.8 years (range 8-17). They all suffered from growth hormone deficiency of various underlying pathologies and were treated with conventional doses of recombinant human growth hormone (Norditropin™, Novo Nordisk, Denmark) during the study. The mean duration was 4.5 years (range 1-7). Conventional x-rays of the hand which were routinely done for bone age determination were used for retrospective analysis of bone parameters using the Pronosco system. There was a parallel and linear increase in BMD, cortical thickness (CT), bone width (BW), volume per area (VPA) and Metacarpal Index (MCI) beginning during Tanner stages 1 and 2. At age 9 to 11 girls increased all five parameters two years earlier than boys as expected, except in one girl with delayed puberty. All patients reached the mean (50th percentile) of the German reference data curve. We conclude that BMD and bone geometry changes can be measured accurately in pubertal children during treatment with growth hormone. These preliminary data show that changes in geometry and BMD are parallel during puberty indicating the importance of bone geometry changes rather than density changes for reaching peak bone mass. Peak bone mass seemed to be normal in those children being treated adequately until final height in this cohort.

WG14

Phytoestrogens, Physical Activity, Vitamin D, Bone Turnover and Short Term Growth in Adolescent Boys. G. Jones, K. Hynes, F. Dalais, V. Parameswaran, T. Greenaway, T. Dwyer. Menzies Centre for Population Health Research, Hobart, Tasmania, Australia.

Physical activity and calcium intake are important determinants of bone health in children. Information on other factors is limited. Recent studies in prepubertal children have suggested a beneficial role for winter sunlight (and hence vitamin D) while studies in adults have suggested that phytoestrogen supplementation may prevent perimenopausal bone loss and that estrogen levels are important in male bone health. The primary aim of this six-week randomised trial was to assess the effect of isoflavone supplementation to the level of the usual Japanese diet on bone turnover markers and short-term growth in adolescent boys. A secondary aim was to assess the effect of sunlight exposure, vitamin D stores, physical activity and winter on bone turnover markers and short-term growth. There were 136 subjects from a single school (mean age 16 years). All subjects received isoflavone or identical placebo. Sun exposure and physical activity were assessed by questionnaire. Vitamin D stores at baseline were assessed by serum 25OHD (Incstar). Bone turnover was assessed by bone specific alkaline phosphatase (ALKPHASE, BSAP) and urinary pyridinoline cross-links (Pyrilinks-D, PYR) at baseline and follow-up. Height and weight change were assessed as were urinary daidzein and genistein. Despite large increases in urinary daidzein and genistein with supplementation (both p<0.001), there was no effect on bone turnover or growth (all p>0.35). Sun exposure was significantly associated with 25OHD and BSAP but not PYR. The mean 25OHD level was low (44nmol/l, 68% <50nmol/l) and significantly associated with BSAP (cutpoint 55nmol/l, p=0.03) and PYR (r=-0.23, p<0.01) but not growth. Both BSAP and PYR increased during the winter 1999 and predicted height but not weight change. Of the physical activity measures, only hours of television watching was significantly negatively associated with short-term height but not weight change (r=-0.26, p=0.003). This association persisted after adjustment for confounders such as Tanner stage and baseline height but decreased after adjustment for 25OHD. In conclusion, phytoestrogen supplementation has little effect on bone turnover and short-term growth in male children while vitamin D stores have an adverse effect on bone turnover markers which, in turn, are related to growth. This association is made more complex by the separate impact of growth and homeostasis on bone turnover in children. Television watching is negatively associated with short-term growth and this appears at least partially mediated by vitamin D stores suggesting that optimising vitamin D stores is important for both bone mineralisation and growth in adolescent boys.

WG15

Key-clinical Features Related to Age of Onset for Mucopolysaccharidoses. D. Rigante, P. Caradonna*, R. Ricci, G. Segni. Department of Pediatrics, *Department of Internal Medicine and Geriatrics, Università Cattolica Sacro Cuore, Rome, Italy.

The Mucopolysaccharidoses (MPS) are an inhomogeneous group of inborn errors in the mucopolysaccharide catabolism. Recent advances in the treatment of MPS have improved their prognosis in the long-term: therapeutic effectiveness, especially for MPS involving central nervous system and skeleton, may rely upon an early diagnosis before the onset of irreversible changes. Mass screening programs have been proposed in some countries by means of urinary mucopolysaccharide analysis, but this technique is not selective in the first months of life. Unfortunately an ideal method of identification suited to pick out pre-symptomatic MPS is not actually feasible and detection depends from early and timely focusing on their clinical features. MPS show a vast array of manifestations which are seldom suspected in the first years of life. However signs or symptoms of MPS may be recognized if carefully searched for. In the following table we list the clinical findings in the infant or in the child suggestive of specific types of MPS which have led to confirm this diagnosis in 38 patients during the period 1978-2001 in our pediatric department of the Università Cattolica Sacro Cuore in Rome.

Type	Pts	M/F	average age at dg.	clinical features suggesting the diagnosis of MPS
MPS I H	4	2/2	1 year and 6 months	coarse facies, skeletal deformities, umbilical hernia, hydrocephalus
MPS I H/S	4	2/2	3 years and 2 months	coarse facies, joint stiffness, muddled language, corneal clouding
MPS II	13	13/0	4 years and 5 months	macrosomia, coarse facies, joint stiffness, umbilical hernia, psychomotor regression
MPS III	11	6/5	6 years and 4 months	hyperactivity, aggressivity, cognitive sparing
MPS IV	5	2/3	2 years and 8 months	severe skeletal dysplasia with waddling gait, short stature
MPS VI	1	0/1	3 years and 7 months	crouched attitude with joint stiffness, corneal clouding, coarse facies

Aim of this report is to emphasyze those clinical signs evocative for MPS and to reinforce the awareness of MPS as diseases needing a precocious recognition in order to make children candidates for current therapies such as enzyme replacement.

WG 16

Iatrogenic Osteopenia in a Young Girl with Idiopathic Pulmonary Gibrosis Controlled through Steroids for a 7-year Period. D. Rigante, A. Stabile, G. Segni, P. Caradonna*. Department of Pediatrics, *Department of Internal Medicine and Geriatrics, Università Cattolica Sacro Cuore, Rome, Italy.

Idiopathic pulmonary fibrosis (IPF) is very rare in the pediatric age and treatment is largely empirical due to the absence of controlled trials. Steroids have been used for their multifaceted antinflammatory properties, but prolonged therapy reduces IGF-1/TGF-b/gonadotropin/sex hormone synthesis or activity, osteoblast number and function and calcium absorbtion, leading to functional bone impairment and insufficient bone quality.

We report a 19-year-old Italian girl inflicted with IPF diagnosed by open lung biopsy at the age of 11 years. The patient has received steroids uninterruptedly at variable doses since diagnosis. Her first menstruation appeared at the age of 14 and ½ years with subsequent normal pubertal development. Her actual height is 159 cm, her weight 44 kg, but her physical activity is very limited because of severely restricted lung volumes. Bone mineral density (BMD) has been measured every two years from time of diagnosis through dual-energy X-ray absorptiometry (DEXA; Hologic QDR-2000) scanning at the lumbar spine and at the proximal femur with these results:

	height	weight	age	prednisone dose	l-BMD	Z-score	f-BMD	Z-score
1994	150	48	12.3	20 mg/day	0.572	-0.82	0.579	-0.66
1996	154	46	14	15 mg/day	0.619	-1.02	0.557	-1.20
1998	158	44.3	16	20 mg on alt. days	0.756	-1.02	0.667	-0.75
2000	158.5	44.5	18.6	15 mg on alt.days	0.748	-1.07	0.668	-0.79

Iatrogenic osteopenia resulting from the long-term use of steroids is a serious problem for a paediatrician, because an important determinant of future fracture risk is the peak bone mass achieved in the second decade of life. Steroid therapy may interphere with this achievement and patients may enter young adulthood with osteopenia and accelerated post-menopausal or involutional osteoporosis. In the reported patient we have supposed a decreased skeletal mineralization particularly at the spinal site, as expression of inadequate mineral acquisition during adolescence. Recently serum and urine calcium/phosphorus were in the normal range, but osteocalcin resulted 13.7 ng/ml (n.v. 3.0-11.0), as expression of increased bone turnover. After the last checking we have prescripted the supplementation therapy with calcium and vitamin D_3 while diphosphonate therapy is debated for this patient. We recommend DEXA evaluation in patients with IPF receiving long-term steroids in consequence of a generalized bone loss risk.

WG17

The use of Medical Foods and of an Inhibitor of 4-hydroxyphenylpyruvate Dioxygenase (NTBC) to Improve Rickets in Tyrosinaemia Type 1. D. Rigante, E Holme°, G. Segni, P. Caradonna^. Department of Pediatrics, ^Department of Internal Medicine and Geriatrics, Università Cattolica Sacro Cuore, Rome, Italy - °Sahlgrenska University Hospital, Gothenburg, Sweden.

Tyrosinemia type 1 (TT1, McKusick 276700) is one example of diet-gene interaction caused by impaired fumarylacetoacetase activity, the enzyme that catalyses the last step of the catabolic pathway of tyrosine. In the presence of a diet containing normal amounts of phenylalanine and tyrosine this abnormal genotype will lead to liver cyrrhosis, often not reflected in routine liver function tests, and Fanconi syndrome. TT1 has an autosomal recessive mode of inheritance and toxic intermediate compounds are the triggers for liver damage progression and generalized renal tubular impairment with hypophosphatemic rickets.

We report a female-child with TT1 diagnosed at 2 years and 6 months of age in consequence of unexpected limping gait, active rickets (wrist and ankle enlargement, rachitic rosary) and high seric succinylacetone (17µmol/L). At the diagnosis her staturo-ponderal growth was at the 25th centile. Then she was started on high-carbohydrate diet with restriction in natural protein intake (phenylalanine and tyrosine at about 12 mg/kg/day) with a tyrosine/phenylalanine free protein substitute (SHS, Xphen, Tyr Maxamaid: 75 mg/day) and NTBC therapy (2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione: 1 mg/kg of body weight), a drug causing a block in hepatic tyrosine degradation, in order to by-pass the formation of succinylacetone.

After 48 months of medical foods and NTBC therapy seric succinylacetone has become negative (<0.10 µmol/L), her deambulation brought back to normal, while rickets have completely disappeared with alkaline phosphatase, 25OH-vitamin D, PTH and IGF-1 normalized.

The signs of renal involvement in TT1 range from mild proximal tubular dysfunction to overt renal failure. Hypophosphatemic rickets is the main clinical sign due to urinary phosphate loss. The objective of nutrition support for TT1 is to provide a biochemical environment allowing normal growth, thus preventing pathophysiologic changes in liver or kidney. Adequate protein cannot be obtained from natural foods without ingesting excess tyrosine and phenylalanine and following the use of NTBC it is necessary to maintain plasma tyrosine levels below 800 µmol/L.

With this described dietetic and therapeutic regimen we have been able to warrant a correct growth in this child, who is now 7 and ½ years old and who has experienced considerable catch-up growth (weight: 24 kg and height: 119.5 cm, both at the 50° centile) with the complete recovery of rickets and no evidence of bone changes or osteopenia.

WG18

Bone Changes in Children with Chronic Liver Disease Following Transplantation. C. Moniz*, L. D'Antiga, H. Abraha*, C. Sangupta, N. Heaton, M. Rela, M. Buxton-Thomas^{+}, G. Mieli-Vergani, A. Dhawan. Departments of *Clinical Biochemistry, Child Health, $^{+}$Nuclear Medicine, Kings College Hospital, London, United Kingdom.

Osteodystrophy is a recognised complication in children with chronic liver disease (CLD) whereas osteomalacia is attributable to vitamin D deficiency, low trauma fractures may have other aetiologies. Since the effect of liver transplantation on bone in children is poorly investigated, we performed a prospective study of bone mineral density (BMD) and bone biochemical markers in 6 children (2 male) median age 8.8 years (range 3.8-16.6 yrs) with chronic liver disease: 3 with biliary atresia 3 and progressive intrahepatic cholestasis 3 at the time of listing for transplant and, 3 and 6 months after liver transplant. Fasting serum 25-OH vitamin D levels, PTH, intact osteocalcin, urinary free deoxypyridinolines (DPyr) adjusted for creatinine and BMD by Lunar DXA (Lunar-DPX) of whole body and spine were measured 3 monthly over one year. Healthy age matched controls were used to compare the results. Post transplant immunosuppression comprised of Neoral, Azathioprin and prednisolone in 5; Tacrolimus and prednisolone in 1. Prednisolone was reduced to a maintenance dose of 0.07 mg/kg/day by the end of 1 month post transplant. Mean results are shown in Table 1. BMD improved by 6 months after liver transplant despite an apparent initial worsening at 3 months. Pre-pubertal children showed a greater % increase in BMD than post-pubertal and demonstrated catch-up growth. Changes in bone markers and PTH and Oc signalled alterations in bone turnover which resulted in overall increases in BMD by 1 year. Pre-transplant PTH was within reference limits for normocalcaemia (32 ± 21 ng/L) although in 4/6 cases PTH was below 50% of the range, despite normal Mg++. At 3 months post-transplant there were 3-4 fold increases in Oc and Dpyr. Liver transplantation resulted in alteration in bone homeostasis with a restoration of depressed osteoclastic and osteoblastic activity and increases in bone density. The mechanism of altered bone metabolism in CLD and the effects of post-operative care and immunosuppression need to be studied to reduce low trauma fractures in the post-operative period.

Table 1

Time (months)	0	3	6	12
Total BMC g/cm^2	0.76	0.79	0.83	0.85
L2-L4 g/cm^2	0.59	0.60	0.68	0.71
z-score	-1.9	-2.5	-1.67	-0.79
D-Pyr nM/mmol creat	22.4	54	63	90
Oc pg/L	17.8	32.4	38.8	40

WG19

Severe Trabecular Osteopenia with Cortical Thickening – a new Bone Dyplasia? T. Cundy, A. King, S. Vogel. University of Auckland, Middlemore Hospital and Starship Children's Hospital Auckland , New Zealand.

A novel bone dysplasia is described in a 15 yr old girl of Pacific Island origin. She was first noticed age 6 to have difficulty running. Investigations at the time revealed short stature (<10th centile), macrocephaly (head circumference >95th centile) with platybasia and wormian bones in the skull. Butterfly vertebrae at T8 and T10, and scoliosis were also noted. A diagnosis of osteogenesis imperfecta was suggested. Over the following 8 yrs her scoliosis deteriorated (necessitating the insertion of a Harrington rod, age 14) and marked acetabular protrusion developed. She was referred for consideration of bisphosphonate therapy.

There was no dentinogenesis imperfecta, her sclerae were white and her hearing normal. The patient had never fractured any bone. There was symmetric short stature (1.405m), but pubertal development was normal. Radiographs showed long bones with normal or thickened cortices and fused epiphyses. Her skin fibroblasts synthesised and secreted normally both type I and III procollagen, and processed these to collagen normally (Collagen Diagnostic Laboratory – Seattle). A transiliac bone biopsy demonstrated thickened cortices (>2 SD above mean), but severe trabecular osteopenia (TV/BV 5%, >3 SD below mean), with little trabecular bone cell activity. Markers of bone turnover were normal, as were parathyroid and vitamin D status, and thyroid, hepatic and renal function.

A search of the literature has failed to find a category into which this case readily falls. The combination of thick cortices and severe trabecular osteopenia is particularly unusual, and distinguishes what may be a newly recognised skeletal dysplasia.

WG20

Sensitivity of Quantitative Ultrasound of Finger Phalanges to Growth Related Skeletal Changes – Results from a European Pediatric Normative Database. R. Barkmann[1], W. Rohrschneider[2], G. Baroncelli[3], R. Lorenc[4], R. Tormo[5], J. Truscott[6], C-C. Glüer[1]. [1]Medizinische Physik, UKK Kiel, Germany, [2]Pädiatrische Klinik der Universität Heidelberg, Germany, [3]Department of Reproductive Medicine and Pediatrics, Pisa, Italy, [4]Children's Mem. Health Institute, Warsaw, Poland, [5]Hospital Materno-Infantil "Vall d'Hebron", Barcelona, Spain, [6]Medical Physics, University of Leeds, United Kingdom.

Quantitative Ultrasound (QUS) of finger phalanges is a radiation-free and sensitive method for the measurement of human bone status and might also be used for the assessment of growth disorders of juvenile bones. To establish a basis for the evaluation of this potential application we started collecting data from six European centers to establish a comprehensive European pediatric reference database and calculated the sensitivity of the method to age-related changes of the QUS parameters.

Six centers in Germany, Italy, Poland, Spain and England are involved in the process of collecting pediatric reference data of QUS of juvenile finger phalanges using the DBM-Sonic 1200 or DBMSonic Bone Profiler (IGEA, Italy). Besides the standard parameter Amplitude Dependent Speed of Sound (AD-SoS) a new parameter, Bone Transmission Time (BTT), is evaluated. BTT reflects the acceleration of the ultrasound wave caused by the presence of solid bone material in the phalanx, not being affected by soft tissue thickness. All centers were included in a quality control procedure using a cross-calibration phantom. In a subset of 1600 children covering an age range between 1 and 17 years we calculated the annual increase of QUS parameters. Precision errors were obtained from 5 measurements on each of 22 children, calculated as root mean square C.V. Monitoring time interval (MTI) and trend assessment interval (TAI) was calculated as described by Glüer et al.

Highly significant positive correlations (R^2=0.66 to 0.82, $p<0.0001$) could be observed between AD-SoS resp. BTT and age for both sexes. Mean annual increase was 0.8% for AD-SoS and 8% for BTT. Precision errors were 0.3% for AD-SoS and 3% for BTT. MTI was 1 year and TAI was 8 months for both parameters.

Age-related changes in QUS parameters can be detected within one year or less in an individual child, which demonstrates the sensitivity of this method to detect changes of bone properties during growth in childhood. Taking into account the lack of ionizing radiation and easy application, QUS on finger phalanges might become a sensitive tool for the measurement of juvenile bone development.

These results should be tested in longitudinal studies, comprising measurements of normal and pathological bone growth, in comparison with the estimation of skeletal age development from hand x-rays.

WG21

Obese Children Have Greater Bone Mass and Strength in Relation to Muscle Area but not Body Weight. L. J. Moyer-Mileur, R. Roberts, S.D Ball. Department of Pediatrics, University of Utah, Salt Lake City, Utah.

Risk of fracture secondary to low bone mineral content (BMC) and bone area by DEXA in relation to body weight measured in overweight and obese children has been suggested. Lean body mass, however, is a stronger predictor of bone mass than body weight and higher levels of BMC and bone area in relation to lean body mass have been reported in obese children. Peripheral quantitative computed tomography (pQCT) is now available for use in pediatric populations. The pQCT technique provides analyses of BMC, bone area, and volumetric bone mineral density (vBMD, mg/cm^3) for total bone and bone compartments, bone strength and muscle area. We obtained cross-sectional data from healthy Caucasian boys (n=105) and girls (n=131) aged 4-19 years to determine whether bone and muscle mass and bone strength measured by pQCT differs in normal weight, overweight, or obese children. Height, weight, pubertal stage, and distal and mid-shaft tibia by pQCT (XCT-2000, Norland) were measured. The distal site measures trabecular bone and the mid-shaft site measures total and cortical bone, bone strength expressed as polar strength strain index in mm^2, and cross-sectional muscle area. Children were grouped by body mass index (BMI, kg/m^2) percentile for age and gender: normal weight (BMI <85th %ile, n=167); overweight (85-94th BMI %ile, n=38), and obese ($\geq$ 95th %ile; n=31). We found overweight and obese children had higher total, cortical, and trabecular BMC and bone area, bone strength, and muscle area adjusted for chronological age and pubertal stage ($p<0.001$) than normal weight children. Values for vBMD did not differ by weight categories. Predicted values for age and pubertal stage adjusted total and cortical BMC and bone area and bone strength relative to body weight did not differ from observed values in any weight category. Observed values for total BMC, cortical and trabecular BMC and bone area, and bone strength relative to muscle area, however, were greater than predicted values in obese children than children with lower BMI ($p\leq0.05$). Our results support the presence of a compensatory mechanism that allows greater accumulation of bone mass and strength in relation to lean mass in obese children. Additional studies are needed to determine the associations between childhood obesity, bone health, and fracture risk.

WG22

Musculoskeletal Analyses of the Forearm in Young Women with Turner Syndrome. S. Bechtold, F. Rauch, V. Noelle, S. Dohnhauser, C.M. Neu, E. Schoenau, H.P. Schwarz.

Turner Syndrome (TS) is associated with multiple skeletal abnormalities. Fracture incidence appears to be increased, but the reasons for this are not entirely clear. In the present study we used peripheral quantitative computed tomography to evaluate bone mass, density, geometry and strength of the radial metaphysis and diaphysis as well as maximum forearm muscle cross-sectional area (CSA) in a group of 21 TS patients. These individuals were between 16 and 25 years of age, had completed growth after having received growth hormone therapy, and all but one were receiving estrogen supplementation. Despite short stature, cross-sectional bone size was normal compared to age-matched healthy controls. However, bone mineral content was decreased, resulting in a low total volumetric bone mineral density. This was due to decreased cortical thickness at both sites of measurement, whereas trabecular volumetric bone mineral density of the metaphysis was normal. Muscular CSA was normal for age. The relationship between muscle CSA and external bone size was similar between TS patients and healthy young women. However TS patients had less bone mineral content and cortical CSA relative to muscle CSA than healthy young women, but similar muscle-bone relationships as healthy prepubertal girls. These findings are compatible with a normal adaptation of external bone size to the mechanical loads imposed by the muscle system and a lack of pubertal effect on the endocortical bone surface despite estrogen supplementation. Bone strength may not be adequate for the relatively high body weight of TS patients, which could contribute to an increased propensity for fractures.

PHYSICAL ACTIVITY WORKING GROUP

WG23

Exercise to Promote Bone Health across the Life Span . C. Snow, Bone Research Laboratory, Oregon State University, Corvallis, OR, USA.

Osteoporosis-related fractures are related to significant morbidity, mortality and economic cost. There are over 340,000 hip fractures annually in this country that carry health care costs of more than $9 billion. Specific exercise reduces risk of osteoporosis-related fractures by increasing peak bone mass, slowing age-related bone loss and reducing fall risk. Changes in these variables reduce the factor of risk, a ratio that reflects the interaction between applied forces (eg. falls) and skeletal fragility (bone mineral density, BMD). If the factor of risk is << 1, fracture is unlikely but if > 1, fracture is probable. **Increasing peak bone mass**: In a randomized controlled trial, we demonstrated that, in 89 prepubescent boys and girls, bone mass at the hip (femoral neck) and spine increased by 4.6% and 3.5%, respectively, from 7 months of jumping at high magnitude and loading rate. Gains in jumpers were maintained after 7 months of detraining, a result that indicates the protocol enhanced bone growth and thus provide a means of increasing bone during skeletal maturation. **Increasing bone mass and reducing fall risk in adults**: In mature premenopausal women, specific exercise increases hip bone mass and reduces fall risk. After 12 months of weighted vest exercise that included jumping, women (30-45 yrs) demonstrated an increase of 2.6% in trochanteric and 1.5% in femoral neck BMD that were significantly greater than controls of similar age, body weight and BMD. This group also gained muscle mass, strength, power and balance. Six months of detraining in the exercisers reversed the gains in bone and muscle strength, power and body composition. **Reducing bone mass and fall risk in postmenopausal women.** In a similar exercise trial, postmenopausal women (>60 yrs) involved in weighted vest exercise for 9 months increased muscle strength, mass power and balance but not change hip bone mass. However, after 5 years of continued exercise, bone loss at the hip was prevented at all regions of the hip (femoral neck BMD = +1.5% vs. –4.43%). These data demonstrate that specific types of exercise increase and/or preserve bone mass across the lifespan and also reduce risk of falling. Altering both the numerator and denominator of the factor of risk should result in a significant reduction in osteoporosis-related fractures.

WG24

Muscle and Bone Interactions: More Questions than Answers? J. Shaw, Department of Exercise and Sport Sciences, University of Utah, Salt Lake City, UT, USA.

Much of the benefit afforded to the skeleton by exercise participation is considered to originate from forces applied by muscular contraction. Lean tissue mass and muscle strength are typically associated with bone mineral density. Some data suggest that the strongest relationships between muscle strength and bone mass are site specific. Others propose that the lean tissue mass itself, and not the force it is capable of producing, explains the relationship between the two tissues. Interestingly, gains in muscle mass and/or muscle strength with exercise intervention rarely predict skeletal adaptation to the intervention, yet exercise participation may attenuate skeletal tissue losses observed with weight loss. Further, the proximal femur and vertebral bodies respond to different types of mechanical loading regimens, and therefore, varying input from skeletal muscle. Non weight-bearing exercise, such as swimming, is associated with low bone mineral density in humans, presumably due to reduced mechanical loading, yet swimming interventions have improved mechanical properties and mass of bone in rodents. Although muscle and bone interactions are readily acknowledged, contradictions about the nature of these interactions exist in the literature. Within the context of exercise participation, the characteristics of muscular contraction and the metabolic demands of the exercise regimen are likely to explain some of the observed contradictions.

WG25

Physical Activity and the Risk of Falls in Older Men and Women: The Longitudinal Aging Study Amsterdam (LASA). P.Lips, S.M.F. Pluijm, V.S. Stel, M. Visser, J.H. Smit, D.J.H. Deeg, Vrije Universiteit, Amsterdam, Holland.

PURPOSE Physical activity has been associated with decrease in the risk of falls. However, the role of physical activity in the prevention of falling remains controversial. In this prospective study we examined the relationship between daily physical activity and falls in older men and women.

METHOD The study population consisted of 1383 participants (705 women and 678 men) of the Longitudinal Aging Study Amsterdam (LASA) who were 65 to 88 years old as of the first of January, 1996. The level of physical activity was measured with the LASA Physical Activity Questionnaire (LAPAQ). The LAPAQ is a detailed, interview-administered questionnaire regarding walking, cycling, sports, gardening, light and heavy household activities of the previous two weeks. For each of these activities, the frequency and duration was assessed. The LAPAQ was validated with a 7-day diary and a pedometer in a subsample of 439 respondents. The LAPAQ was highly correlated with the 7-day diary (r=0.70, p=0.001) and moderately correlated with the pedometer (r=0.53, p=0.001).

During three years of follow-up, fall events were recorded with a ‘fall calendar’. As outcome measures we used the total number of falls, and the number of inside and outside falls. Data were analysed using Generalized Estimating Equation (GEE), adjusted for age, sex, chronic diseases, urinary incontinence, cognition and physical performance.

RESULTS Physical activity was non-linearly, inversely related to the risk of falls. As compared with people in the lowest quintile, people in the highest quintile of energy expenditure (expressed in kcal/d) had an adjusted relative risk for multiple falls of 0.76 (95% CI: 0.59-0.97). Walking outside was also inversely associated with falls; people in the highest quintile, who walked at least 40 minutes per day, had a relative risk of 0.79 (95% CI: 0.61-1.00) as compared with women who never walked. None of the individual activities (yes/no) was associated with multiple falls. However, when we distinguished between inside and outside falls, performing sports activities and cycling appeared to be protective against inside falls (RR: 0.69; 95% CI: 0.55-0.87; RR: 0.75; 95% CI: 0.59-0.96, respectively), but increased the risk of outside falls (RR: 1.19; 95% CI: 1.00-1.41; RR: 1.26; 95% CI: 1.04-1.52, respectively).

CONCLUSION: These prospective data indicate that a high level of total physical activity and walking activity protect against falls among older men and women.

WG26

Physical Activity and Fall Prevention. T. Masud, City Hospital Nottingham, United Kingdom.

The relationship between physical activity (including exercise and fall risk is complex and the evidence has often been conflicting. Although the Study of Osteoporotic Fractures (Cummings SR et al, 1995) suggested that lack of walking for exercise and being on one's feet for 4 hours or less per day were risk factors for hip fracture, the extent to which this association was due to an effect on falls was not clear. Although physical activity in the form of brisk walking is associated with improved balance parameters such as body sway (Brooke-Wavell K et al 1998), a randomised controlled trial in the UK showed that brisk walking in postmenopausal women increased the risk of falls, suggesting that in some circumstances the "opportunity" to fall may increase (Ebrahim S et al, 1997). Many multifactorial studies have included exercise as one of several intervention components and assessing the independent effects of exercise can be difficult. A review of the FICSIT (Frailty and Injuries: Cooperative Studies of Intervention Techniques) trials suggested that those interventions which included some form of exercise reduced falls by 10% (Province MA et al, 1995). A nurse assessment visit and follow-up aimed to increase physical and social activity can significantly reduce falls at one year (Wagner et al, 1994). In contrast, although a program addressing home safety, exercise and behavioral risks led to a decreased odds of falling by 0.85, there was no reduction in falls requiring medical treatment (Hornbrook MC et al 1994). With regard to physical activity as a single intervention, some data suggests Tai Chi as an effective method of reducing fall risk (Wolf SL et al 1996). More recent studies from New Zealand have shown that physiotherapy-taught individualised programs of strengthening exercises in older people can reduce fall rates (Campbell AJ et al 1997). Further work from the same group have shown similar programs delivered by nurses in primary care centers and at home can also reduce fall rates in the over 75 year age group, and have the potential to be cost-effective (Robertson MC et al 2001). In conclusion, up till the last 4 years the data assessing the relationship between physical activity and falls were conflicting. Recent studies however show that targeted individualised exercise and activity programs can reduce fall risk in older populations and may be cost-effective.

ERRATA

2001 Program and Abstracts of the 23rd Annual Meeting of the American Society for Bone and Mineral Research

On the following pages are five reprinted abstracts from the 23rd Annual Meeting of the American Society for Bone and Mineral Research. These abstracts were incorrectly published in the meeting supplement (J Bone Miner Res 2001; S1). The error involved the omission of tables, author affiliations, or the abstract body.

We have provided these reprinted materials to afford our readership the information necessary to correctly cite these abstracts in future literature. To facilitate this we have provided under each abstract the citation with the correct page number from the print version of the supplement.

These omissions resulted from a programming error, and we apologize for this mistake.

Marc K. Drezner, M.D.
Editor-in-Chief

F101

Comparison of Three Bone Ultrasounds for Determining Hip Fracture Odds-Ratios - Results of the Semof Study. M. Krieg*, J. Cornuz, P. Burckhardt and the Semof Study Group*. Internal Medicine, University Hospital, Lausanne, Switzerland.

Background: Bone ultrasound (QUS) is known to discriminate subjects who suffered of fractures and subjects with no fracture history. QUS is predictive of fractures. The Swiss Evaluation of the Methods of Measurement of Osteoporotic Fracture Risk (SEMOF) study is a prospective multicenter study which compares 3 QUS devices for the assessment of hip fracture risk in a population-based sample of elderly Swiss women. The aim of this cross-sectional analysis was to compare the 3 QUS for determining hip fracture odds ratios (OR). Method: Among the 7702 women aged ±75.3 yrs (70 - 87) assessed during the inclusion phase of the SEMOF study. The history of previous hip fractures was obtained with a specific mailed questionnaire. 3696 had no fracture whereas 93 reported a low trauma hip fracture after 50 yrs. All the participants were measured with the 3 QUS: Achilles+ [Stiffness index (SI)], Sahara [Quantitative ultrasound index (QUI)], and DBM sonic 1200 [Amplitude-dependent speed of sound (ADSOS)]. New parameters, such as Speed of sound (SOS), time frame (TF), ultrasound bone profile index (UBPI) were calculated from the DBM data. Results: Mean QUS value (± SD) were adjusted for age and BMI, and ORs were calculated for 1 SD decrease, with 95% CI (***p<0.001, **p<0.01, *p<0.05 compared to non fractured group). Discussion: All the ultrasound parameters could discriminate elderly women with hip fracture from women with no fracture history. In this population of elderly women, discrimination was significantly higher with measurements on the heel than on the phalanges. This could be due to the high prevalence of osteoarthritis of the hands at this age, which could influence the results.

	No fracture	Hip fracture	OR	95% CI
Achilles+ SI(%YA)	72.1 ± 13.4	60.5 ± 11.9***	2.6	2.1 - 3.3
Sahara QUI	78.4 ± 17.6	65.9 ± 11.8***	2.5	1.9 - 3.2
DBM AD-SOS(m/s)	1857 ± 103	1824 ± 104**	1.4	1.1 - 1.7
DBM SOS(m/s)	1940 ± 51	1918 ± 47***	1.6	1.2 - 2.0
DBM TF(us)	1.17 ± 0.21	1.08 ± 0.18***	1.6	1.2 - 2.0
DBM UBPI	0.35 ± 0.16	0.30 ± 0.13*	1.4	1.0 - 1.8

(Continued)

SA288

Accuracy of Physical Examination for Detection of Thoracic Vertebral Fractures. K. Siminoski*[1], R. Warshawski[2], H. Jen[2], K. Lee[3]. [1]Endocrine Centre of Edmonton and Medical Imaging Consultants, Edmonton, AB, Canada, [2]Medical Imaging Consultants, Edmonton, Canada, [3]Endocrine Centre of Edmonton, Edmonton, Canada.

Vertebral fracture is the most common type of broken bone in osteoporosis patients. Little information is available about the accuracy of physical examination in detecting such fractures. We have assessed two simple physical examination maneuvers for their accuracies in determining the presence of thoracic vertebral fractures. The maneuvers were measurement of (1) kyphosis angle (KA) from T4 to T12, quantified with a handheld digital inclinometer (in degrees), and (2) wall-occiput distance (WOD), quantified with a tape measure, as the distance from the wall to the occipital prominence with the patient standing, heels to the wall, looking straight ahead (in cm). A total of 216 women referred for assessment of osteoporosis were studied. The average age was 53 years (range: 18 to 92). Vertebral fracture was defined as a decrease in vertebral height of 20% or more on lateral radiographs (from T4 to T12 for the purposes of this study). One or more thoracic fractures were present in 29% of subjects. Among those with fractures, the average number of fractures was 1.9. KA and WOD were correlated ($r = 0.32$; $p<0.0001$). Both KA and WOD increased with age in those without fractures ($r = 0.29$ and 0.34; $p < 0.001$ for both) The areas under the receiver operating characteristic curves were 0.71 (95% CI: 0.61-0.80) for KA and 0.74 (0.66-0.83) for WOD. Accuracy results are shown in the first table. Likelihood ratios are in the second table. Upper cut-off points were present that produced very high LRs, but there were no lower cut-offs that produced very low LRs.The following applications can be made from this data. KA > 43^o or WOD > 7.0 cm rules in a fracture with a high degree of accuracy. KA < 20^o or WOD = 0 reduces the chance of fracture, but does not reliably rule it out These results show that physical examination of the thoracic spine using simple methods can produce clinically useful accuracy in detecting thoracic vertebral fractures. KA and WOD should be incorporated into the routine physical examination of osteoporosis patients.

(SA288 continued)

KA (o)	Sens	Spec	PPV	NPV
>43	22	99	85	78
>30	63	81	55	86
>20	84	28	30	83
WOD (cm)	Sens	Spec	PPV	NPV
>7	21	99	92	76
>3	38	93	69	79
>0	60	87	65	85

Sens = sensitivity. Spec = specificity. PPV = positive predictive value. NPV = negative predictive value.

Likelihood Ratios			
KA (o)	**LR**	**WOD (cm)**	**LR**
>43	15.1	>7	27.8
21-43	0.9	3-7	2.8
<21	0.6	<3	0.7

Citation: J Bone Miner Res 2001; 16S1:FSA288

SA429

Alfacalcidol in the Prevention of Bone Loss After Heart Transplantation. H. U. Stempfle*[1], C. Werner[1], S. Florian[1], R. Frost[1], W. A. Rambeck[2], R. Gärtner[3]. [1]Cardiology, Medizinische Klinik Innenstadt, Munich, Germany, [2]Tierärztliche Fakultät, Munich, Germany, [3]Endocrinology, Medizinische Klinik Innenstadt, Munich, Germany.

Background: Immunosuppressive therapy induced osteoporosis is a well known complication after heart transplantation (HTx). The aim of this prospective, placebo-controlled study was to assess the effect of alfacalcidol (1 µg) in the prevention of bone loss after HTx. Methods: Study patients (n=56) received a triple-drug immunosuppression including FK506, azathioprine or mycophenolate mofetil and glucocorticoids. Patients were treated with elemental calcium (500 mg/d) and sex hormone replacement in hypogonadismus. 38 patients (mean age: 48±9 yrs.; 5±1 months post HTx) received alfacalcidol and were compared to 18 cardiac transplants with placebo (age: 54±12 yrs.; 5±4 months post HTx) at baseline and 12 months. 16 and 9 patients completed already a 24 months treatment intervall. Bone mineral density (BMD) was measured at the lumbar spine (LS) and at the femoral neck (FN) with DEXA (Lunar Expert, g/T-score %). Fractures were assessed by X-rays of chest, thoracic and lumbar spine. Biochemical markers included gonadal hormones, gonadotropins, urinary and serum para-meters of calcium metabolism, intact PTH, 25OHVitD3 and renal function. Results and Conclusions: Calcium supplemention and sex hormone replacement in hypogonadism proved a sufficient therapy to increase BMD and to prevent fractures after HTx. The additional dose of 1 µg Alfacalcidol demonstrated a significant extra benefit regarding BMD (see table).

	Alfacalcidol		**Placebo**	
	Baseline	**12 mo.**	**Baseline**	**12 mo.**
T-score (%) LS	93±14	97±14	88±11	91±12
T-score (%) FN	93±17	90±16	96±16	94±19
Fractures	4	0	2	0
	12 mo.	24 mo.	12 mo.	24 mo.
T-score (%) LS	95±14	100±14	99±10	98±10
T-score (%) FN	94±20	94±17	88±6	86±6
Fractures		1		0

Citation: J Bone Miner Res 2001; 16S1:SA429

SA527

Heat Shock Proteins Influence Uptake and Subcellular Targeting of Vitamin D. R. F. Chun*[1], C. Segovis[2], S. Wu*[1], H. Chen*[1], M. A. Gacad*[1], J. S. Adams*[1], J. Barsony*[2]. [1]Division of Endocrinology, Cedars-Sinai Medical Center, Los Angeles, CA, USA, [2]Laboratory of Cell Biochemistry and Biology, NIDDK, National Institutes of Health, Bethesda, MD, USA.

Hormonal forms of vitamin D, calcitriol and calcidiol, are targeted to different compartments within the cells to be metabolized and to exert their regulatory effects. Previous studies from our laboratories indicated that calcitriol is targeted to the mitochondria, the endoplasmic reticulum (ER), and the nucleus (J. Biol. Chem. 272:5774-5782, 1997) and that heat shock proteins could play a role in hormone targeting to these compartments (Mol. Endo. 14:1387-1397, 2000). To elucidate the mechanisms of hormone targeting, we generated fluorescent protein chimeras of the 70kD and the 75kD heat-shock proteins (hsc70-GFP and grp75-GFP) and used them together with red fluorescing BODIPY derivatives of calcitriol (red calcitriol and red calcidiol) in advanced live microscopy experiments. Assays of calcidiol binding in extracts from COS-7 cells that transiently express hsc70-GFP and grp75-GFP showed that the tagged proteins retained the ability of the native proteins to bind hormone. Confocal microscopy showed distinct subcellular localization of chimeric proteins in both COS-7 and human kidney cells. Although the majority of hsc70-GFP was in the cytoplasm, to a degree it also accumulated within the nucleus excluding the nucleoli. In the cytoplasm, hsc70-GFP was both in a diffuse pattern and in a perinuclear reticular pattern consistent with ER targeting. The majority of grp75-GFP accumulated in the mitochondria, and to a lesser degree it was also found in the ER and diffusely between organelles. Cellular uptake of red calcitriol was doubled both in the cytoplasm and in the nucleus by the expression of hsc70-GFP. We generated a chimera, MS-hsc70-GFP, by fusing the N-terminal mitochondrial targeting sequences of grp75 to hsc70-GFP. MS-hsc70-GFP exhibited altered subcellular targeting compared to hsc70-GFP and prevented the increase in nuclear uptake of red calcitriol. Expression of grp75-GFP significantly increased the mitochondrial and ER uptake of red calcidiol within 1h and red calcitriol within 4h. A truncation mutant, δMS-grp75-GFP, with the N-terminal mitochondrial targeting sequences deleted from grp75-GFP showed mistargeting and decreased cellular hormone uptake. These experiments demonstrate for the first time the subcellular distribution of these chaperone proteins in living cells and revealed that different members of the heat-shock protein family control the targeting of vitamin D metabolites to the nucleus and to the mitochondria.

Citation: J Bone Miner Res 2001; 16S1:SA527

M056

Differences in Phenotype Shown in Endothelial NOS and Neuronal NOS Knockout Mice. N. Moradi-Bidhendi[1], L. Mancini[1], M. C. O'Shaughnessy[2], P. Forte[1], P. L. Huang[3], L. D. K. Buttery[2], J. M. Polak[2], N. Benjamin[1], I. MacIntyre*[1]. [1]William Harvey Research Institute, London, United Kingdom, [2]Department of Histochemistry, Imperial College School of Medicine, London, United Kingdom, [3]Cardiovascular Research Centre, Harvard Medical School, Boston, MA, USA.

Nitric oxide (NO) has been implicated in the local regulation of bone metabolism. The contribution made by specific NO synthase (NOS) enzymes is unclear. The results obtained using NOS inhibitors have been inconsistent probably arising from their lack of isoform-specific selectivity. We have studied endothelial NOS (eNOS(-/-)) and neuronal NOS (nNOS(-/-)) knockout mice to help clarify this situation and to define more clearly the contribution made by a specific NOS isoform.In our studies we have found that mouse NOS isoform knockout phenotypes differ markedly. eNOS (-/-) knockout mice are hypertensive, transiently osteoporotic and have oestrogen-resistant osteoblasts. nNOS (-/-) mice are hyper-aggressive and their total NO output is only 8% that of intact animals. eNOS (-/-) knockout mice (with intact nNOS) show a normal increase in urinary nitrate with oestrogen demonstrating that oestrogen stimulates nNOS. This finding suggests the possibility that the effect of oestrogen on the central nervous system may be NO-dependent. The differences observed in this study provide clear information on the important roles of both eNOS and nNOS isoforms.

Citation: J Bone Miner Res 2001; 16S1:M056