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1001

PTHrP Haploinsufficiency Reduces Bone Volume in Postnatal PTH Deficient Mice. D. Miao[1], J. Li*[1], Y. Xue*[1], H. Su*[2], A. C. Karaplis[2], D. Goltzman[1]. [1]Department of Medicine, Calcium Research Lab, McGill University, Montreal, PQ, Canada, [2]Medicine, SMBD-Jewish General Hospital, McGill University, Montreal, PQ, Canada.

We previously reported that neonatal mice with targeted disruption of the PTH gene ($PTH^{-/-}$) have decreased trabecular bone volume in long bones due to decreased osteoblast numbers in the primary spongiosa. At 4 months of age, however, $PTH^{-/-}$ mice have increased trabecular bone volume. Haploinsufficient $PTHrP^{+/-}$ mice are normal at birth but at 4 months have decreased trabecular bone volume. To determine whether PTHrP plays any role in maintaining higher bone mass in postnatal $PTH^{-/-}$ mice, we generated mice which are homozygous for the PTH null allele and heterozygous for the PTHrP null allele ($PTH^{-/-}$; $PTHrP^{+/-}$) and compared these to wild type and to $PTH^{-/-}$ mice. At 4 months of age, $PTH^{-/-}$; $PTHrP^{+/-}$ mice displayed similar biochemical alterations as $PTH^{-/-}$ mice, i.e. they had undetectable PTH levels and were hypocalcemic, hyperphosphatemic, had enlarged parathyroid glands and decreased serum 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) levels. Trabecular bone volumes of tibiae, femora and vertebrae were increased by 148%, 156% and 159%, respectively, in $PTH^{-/-}$ mice compared to their wild littermates, but were decreased in $PTH^{-/-}$; $PTHrP^{+/-}$ mice by 37%, 36% and 36%, respectively, compared to their wild type littermates, and by 58%, 59% and 60%, respectively, compared to $PTH^{-/-}$ mice. Alkaline phosphatase positive osteoblast numbers and bone formation rates (assessed by double-calcein labeling) were decreased in $PTH^{-/-}$ mice, and even more dramatically decreased in $PTH^{-/-}$; $PTHrP^{+/-}$ mice compared to their wild type littermates. The number and size of TRAP positive osteoclasts were also diminished in $PTH^{-/-}$ mice, and more prominently reduced in $PTH^{-/-}$; $PTHrP^{+/-}$ mice compared to their wild type littermates. This reduction was associated with a decrease in RANKL positive osteoblasts as demonstrated by immunohistochemical staining. Bone levels of PTHrP mRNA as determined by RT-PCR and immunohistochemical levels of PTHrP in osteoblasts were enhanced in $PTH^{-/-}$ mice, but not in $PTH^{-/-}$; $PTHrP^{+/-}$ mice compared to their wild type littermates. These studies show that PTHrP haploinsufficiency can reduce further the decreased bone turnover in PTH deficient animals and that the increased postnatal trabecular bone volume observed in PTH deficient animals is dependent on the action of PTHrP. PTHrP therefore is an important mediator of postnatal bone growth.

Disclosures: ***D. Miao****, None.*

1002

Relief of Inhibition by Twist Proteins Determines the Onset of Osteoblast Differentiation. P. Bialek*[1], K. Britt[1], M. Schrock*[1], D. Sosic*[2], K. Yu*[3], D. M. Ornitz*[3], E. N. Olson*[4], G. Karsenty[1]. [1]Moleuclar and Human Genetics, Baylor College of Medicine, Houston, TX, USA, [2]Molecular Biology, U T Southwestern Medical Center, Dallas, TX, USA, [3]Molecular Biology and Pharmacology, Washington University Medical School, St Louis, MO, USA, [4]Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA.

The transcription factor *Runx2* is necessary and sufficient for osteoblast differentiation. That *Runx2* expression precedes osteoblast differentiation by three to four days suggests that its function may be transiently inhibited. The craniosynostosis of *Twist*+/- patients (Saethre-Chotzen syndrome) and mice led us to investigate whether Twist inhibits Runx2 function. Here we show that, in the developing skull, *Twist* is expressed at higher levels than *Runx2* in *Runx2*-expressing cells before osteoblast differentiation occurs. *Twist* inhibits osteoblast gene expression and Runx2 transactivation function without affecting *Runx2*'s expression. Compound heterozygotes for *Twist* and *Runx2* inactivations have none of the skull abnormalities observed in *Runx2*+/- or *Twist*+/- mice revealing a genetic interaction between *Twist* and *Runx2*. Transgenic mice expressing *Twist* in osteoblast progenitors mimic the *Runx2*-null phenotype despite normal *Runx2* expression. Twenty residues in Twist's C-terminus form a unique antiosteogenic domain, the Twist box, that interacts with Runx2's DNA-binding domain to inhibit its DNA-binding function. These findings reveal that osteoblast differentiation only begins when an inhibition is relieved and provide a molecular explanation for Saethre-Chotzen syndrome. *Twist*'s broad expression during development suggests that this may be a general mechanism to control *Runx*-dependent cell differentiation programs.

Disclosures: ***P. Bialek****, None.*

1003

GADD34/PP1c Recruited by Smad7 Dephosphorylates TGFβ Type I Receptor. W. Shi[1], C. Sun*[1], B. He*[2], W. Xiong[1], X. Shi[1], D. Yao*[1], X. Cao[1]. [1]Pathology, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Microbiology and Immunology, University of Illinois at Chicago, Chicago, IL, USA.

Transforming growth factor β (TGF-β) superfamily, including bone morphogenetic proteins (BMPs), exerts extensive effects on a broad spectrum of cellular processes such as cell growth, differentiation, mobility and apoptosis, and is therefore crucial for development and maintenance of many different tissues, including bone. The cascade of phosphorylation from receptors to effectors is a well-characterized, pivotal event in TGF-β signaling. Smad7, induced by TGF-β, binds to type I receptor (TβRI) and functions as a receptor kinase antagonist that inhibits TβRI Ser/Thr kinase activity as part of a negative feedback mechanism. However, the possible inhibitory mechanism by dephosphorylation in the TGF-β signaling pathway is not known. Screening a human chondrocyte cDNA library in yeast two-hybrid system with Smad7 as bait, we found that Smad7 interacted with the growth arrest and DNA damage protein, GADD34, a regulatory subunit of the protein phosphatase 1 (PP1) holoenzyme. Immunoprecipitation (IP) experiments demonstrated that Smad7 mediated the formation of a complex with GADD34 and PP1 on TGF-β type I receptor (TβRI). The complex was shown to dephosphorylate TβRI both *in vitro* and *in vivo*. Blockage of Smad7 expression by siRNA inhibited association of the GADD34/PP1c complex with TβRI. Overexpression of either dominant-negative Smad7 or GADD34, absent of their binding domain, inhibited Smad7-mediated dephosphorylation of TβRI. Moreover, SARA, as a Smad anchor for receptor activation, also acts as anchor for PP1c via its PP1c-binding motif. Most importantly, dominant-negative SARA, absent of its PP1c-binding motif, inhibited involvement of PP1c in this proposed complex and dephosphorylation of TβRI, indicating that the availability of PP1c to this complex for TβRI dephosphorylation was controlled by SARA. Furthermore, transcriptional response assay and FACS-DNA profiling analysis demonstrated that Smad7/GADD34/PP1c complex inhibited TGF-β-induced gene transactivation and anti-proliferative effect in Mv1Lu cells, respectively, while PP1 inhibitors rescued the effects of TGF-β. These results indicate that Smad7 acts as an adaptor protein in formation of the PP1 holoenzyme that targets TβRI for dephosphorylation. Thus, we have characterized a novel TβRI dephosphorylation mechanism of the first phosphatase identified in the TGF-β/BMP signaling pathway. Dephosphorylation of TβRI mediated by Smad7 is an effective mechanism for governing negative feedback in TGF-β signaling. This complex could potentially be used as a drug target to modulate TGF-β signaling for bone and cancer diseases.

Disclosures: ***W. Shi****, None.*

1004

Evidence that JNK/c-Jun Signaling Promotes Osteoclastogenesis using Transgenic Mice Overexpressing Dominant-Negative c-Jun Selectively in Osteoclasts. F. Ikeda[1], T. Matsubara*[1], K. Hata*[1], T. Tsurukai[2], T. Watanabe*[3], T. Kukita[3], S. V. Reddy[4], K. Yoshioka*[5], R. Nishimura[1], T. Yoneda[1]. [1]Dept Biochem, Osaka Univ Grad Sch Dent, Osaka, Japan, [2]Kaken Phram Co., Inc, Kyoto, Japan, [3]Sec Oral Mol Biol, Facult Dent Kyushu Univ, Fukuoka, Japan, [4]Div Hematol Oncol, Univ Pittsburgh Cancer Inst, Pittsburgh, PA, USA, [5]Cancer Res Inst, Kanazawa Univ, Kanazawa, Japan.

RANKL is a central cytokine that promotes osteoclastogenesis. Upon RANKL binding to RANK, cytoplasmic molecules involving JNK, c-Jun, c-Fos and NF-κB are activated, suggesting that these molecules relay RANKL signaling. This notion is supported by genetic studies which demonstrated that c-Fos and NF-κB knockout mice exhibited osteopetrosis due to impaired osteoclastogenesis. On the other hand, the role of JNK and its substrate c-Jun, which forms the AP-1 complex with c-Fos, still needs to be elucidated. In the present study, we investigated the role of JNK/c-Jun signaling in osteoclast differentiation by generating transgenic mice (DN-c-Jun Tg) carrying DN-c-Jun driven by the TRAP gene promoter. The DN-c-Jun Tg manifested severe phenotype of osteopetrosis including defect of tooth eruption, smaller body size and increased radiodensity in long bones. Histological examination showed that bone marrow cavity was markedly reduced due to reduced trabecular bone resorption and the number of osteoclasts was markedly diminished. To examine whether the osteopetrosis was due to the abnormality of osteoclast development, we determined multinucleated TRAP-positive osteoclast-like cell (OC) formation in spleen cells isolated from DN-c-Jun Tg or wild-type littermate mice. Number of OC formed in spleen cells of DN-c-Jun Tg was significantly reduced compared with that of wild type mice, indicating that the osteopetrosis seen in DN-c-Jun Tg is due to impaired osteoclastogenesis. We then examined the relationship between RANKL and JNK/c-Jun activation in vitro. Soluble RANKL (sRANKL) activated JNK and c-Jun in the BMMø osteoclast progenitor cells and the RAW264 mouse monocytic cells. Consistent with the in vivo results, OC formation in the presence of sRANKL and M-CSF in mouse bone marrow cultures was significantly inhibited by a specific inhibitor of JNK, SP600125. Furthermore, overexpression of dominant-negative (DN)-JNK1 or DN-c-Jun using adenovirus system suppressed OC formation and increased apoptosis. In conclusion, these results collectively suggest that activation of JNK/c-Jun signaling pathway is essential to osteoclastogenesis that regulated by RANKL both *in vivo* and *in vitro*. Thus, our data provide the new insight into the molecular mechanisms by which RANKL regulates the osteoclast differentiation.

Disclosures: ***F. Ikeda****, None.*

1005

The Role of the PERK eIF2alpha Kinase in Regulating Skeletal Growth and Development. D. R. Cavener, A. Frank*, A. Gabai*. Biology, Penn State University, University Park, PA, USA.

The orchestration of skeletal growth is regulated by a complex repertoire of differentiation factors and hormonal signals that control cell proliferation and secretion of the extracellular matrix (ECM). Inasmuch as the ECM comprises the major organic fraction of bone and cartilage, the growth and development of the skeletal system is more dependent upon protein secretion than any other major tissue in the body. Recently, a new eIF2alpha kinase denoted PERK was discovered that is highly expressed in secretory tissues, predominately in bone tissue and the endocrine and exocrine pancreas. We have generated a knockout mutation of the mouse *Perk* gene (Zhang, et al., Mol. Cell. Biol 22:3864-74, 2002); these mice display severe multiple skeletal dysplasias, postnatal growth retardation, and loss of endocrine and exocrine pancreatic functions similar to defects seen in human Wolcott-Rallison syndrome (WRS). The skeletal dysplasias exhibited by *Perk-/-* mice include severe osteopenia, reduced cortical bone, delayed mineralization, and a marked deficiency of ECM in the hypertrophic region of the growth plate. The long bones of *Perk-/-* mice display reduced trabecular bone (24% normal in tibias), reduced BMD (2.3 fold compared to controls, femur) and reduced growth plates. Bone collagen-I in *Perk-/-* mice is reduced approximately 2-fold, whereas intracellular procollagen-I in osteoblasts is increased 8-fold. Ultrastructural analysis of osteoblasts indicated that procollagen-I processing and secretion are impaired in the endoplasmic reticulum. These defects are similar to that seen

in osteogenesis imperfecta associated with mutations in the procollagen gene that result in abnormal procollagen retention in the ER. In addition to the cortical defects, histomorphic analyses of long bone growth plates in *Perk-/-* mice identified an array of defects including a reduction in the height of the hypertrophic and proliferative zones. The reduction in the hypertrophic zone is due to a 28% reduction of the number of cells in the longitudinal axis. In addition, an abrupt transition from the proliferative to the hypertrophic zone in the *Perk-/-* growth plates was seen, as indicated by a deficiency in chondrocytes exhibiting intermediate width-height ratios. *Perk-/-* mice display a 3.1-fold reduction in the apparent volume of ECM in the hypertrophic zone. In *Perk-/-* mice, the parallel ECM tracts are diminished in number and width giving rise to abnormally thin trabeculae. We are examining the cell-autonomous nature of these defects by generating tissue-specific knockout mutations of *Perk* in the skeletal system using the Cre/loxP system.

Disclosures: ***D.R. Cavener****, None.*

1006

Fra-2: A Novel Regulator of Bone Remodeling. A. Hoebertz*[1], R. Eferl*[1], F. Karreth*[1], A. F. Schilling[2], M. Priemel[2], M. Amling[2], E. F. Wagner[1]. [1]Research Institute of Molecular Pathology, Vienna, Austria, [2]Hamburg University School of Medicine, Hamburg, Germany.

The three Fos proteins c-Fos, FosB, Fra-1 have been shown to play crucial roles in bone biology, but little is known about Fra-2, the fourth Fos protein. To study its role in bone development and remodeling, we generated both Fra-2 knock-out mice, mice carrying a conditional "floxed" Fra-2 allele, and mice overexpressing Fra-2.

Transgenic mice overexpressing Fra-2 (Fra-2 Tg mice) showed initially no overt phenotype. However, histomorphometrical analysis of 3 month-old Fra-2 Tg mice revealed that these mice had a 2 times increase in bone volume and an increased bone formation rate, whereas numbers of osteoclasts and osteoblasts were unchanged. This indicates that enhanced bone matrix production by osteoblasts leads to the increase in bone mass. *In vitro*, primary Fra-2 Tg calvarial osteoblasts and bone marrow osteoclasts displayed both enhanced differentiation potential.

Fra-2 knock-out (-/-) mice die postnatally between day 1-5, are growth retarded and display severe osteoporosis. Analysis by bone histomorphometry showed that bone volume is reduced by 50% and both number and size of osteoclasts were dramatically increased, whereas the absolute number of osteoblasts was unchanged. However, *in situ* hybridization revealed a dramatic decrease in mature, osteocalcin-expressing osteoblasts in Fra-2 -/- long bones, suggesting that decreased bone formation by osteoblasts is also contributing to the bone loss *in vivo*, in addition to enhanced bone resorption. To study the mechanism leading to the bone loss and "giant" osteoclast appearance, we performed *in vitro* cultures of osteoclasts derived either from Fra-2 -/- fetal liver cells or from Fra-2 deficient bone marrow, and primary calvarial osteoblasts. Primary Fra-2 -/- osteoblasts showed a severe differentiation defect, as assessed by bone nodule formation. Unexpectedly, primary osteoclast cultures showed a defect in differentiation and fusion; the numbers, but also the size, of TRAP-positive osteoclasts were smaller compared to controls. However, this defect could be rescued by addition of TGFbeta to the cultures. Reciprocal co-cultures of Fra-2 -/- osteoclasts and wildtype or Fra-2 -/- osteoblasts could not rescue the osteoclast differentiation defect, indicating that other systemic or paracrine signals must be responsible for the "giant" osteoclasts *in vivo*. This loss-of-function approach supports our data from the Fra-2 transgenic model.

In conclusion, we provide first evidence that Fra-2 plays important roles in both osteoclast and osteoblast differentiation and that loss or overexpression of Fra-2 result in imbalanced bone remodeling leading to severe bone diseases.

Disclosures: ***A. Hoebertz****, None.*

1007

The Effects of PTH, Alendronate Alone or in Combination on Bone Mass and Turnover: 12 Month Results of the PATH Trial. D. M. Black[1], K. Ensrud[2], S. Greenspan[3], J. Bilezikian[4], J. McGowan[5], T. Lang[6], P. Garnero[7], C. Rosen[8]. [1]Dept of Epidemiology & Biostatistics, University of California, San Francisco, CA, USA, [2]Dept of Medicine, University of Minnesota, Minneapolis, MN, USA, [3]Dept of Medicine, University of Pittsburgh, Pittsburgh, PA, USA, [4]Dept of Medicine, Columbia University, New York, NY, USA, [5]National Institute of Health, Bethesda, MD, USA, [6]Dept of Radiology, University of California, San Francisco, CA, USA, [7]Synarc, Lyon, France, [8]Maine Ctr for Osteo. Res & Education, St. Josephs Hosp., Bangor, ME, USA.

Parathyroid hormone (PTH) increases bone strength, in part, by stimulating bone formation while antiresorptives work, in part, by reducing bone resorption. Their use together might be expected to lead to synergistic effects in bone by simultaneously increasing formation while reducing resorption. The PaTH study was designed to test the effects of PTH or alendronate (ALN) alone or concurrently administered on bone mass and bone turnover. We randomized 238 PM women (not currently using bisphosphonates) with low hip or spine BMD (T-score <-2.5 or <-2.0 with a risk factor) to a daily regimen of: 100 µg rhPTH (1-84) (NPS); ALN 10 mg (Merck); or PTH and ALN together. The single therapy arms had placebo ALN or PTH. BMD at the spine and hip was assessed by DXA and QCT. Bone turnover markers were measured in fasting blood samples.

Mean 1 year % change in bone mass/marker (p-value vs. PTH alone)

	PTH	PTH + ALN	ALN
DXA Spine BMD	+6.2%	+6.1% (0.8)	+4.6% (0.09)
QCT trabecular spine BMD	+23.8%	+11.3% (0.001)	+7.6% (0.001)
DXA Femoral neck BMD	+0.8%	+1.8% (0.09)	+2.0% (0.05)
QCT fem neck cortical BMD	-2.4%	-0.1% (0.002)	+1.2% (<.001)
QCT fem neck cortical volume	+3.4%	-0.6% (0.03)	+0.9% (0.25)
Formation (P1NP)	+146%	-16% (<.001)	-74% (<.001)
Resorption (serum CTX)	+104%	-14% (<.001)	-73% (<.001)

In the spine, DXA BMD increases were similar for PTH alone and in combination, and somewhat greater (ns) than those for ALN. However, for trabecular spine BMD, there were larger increases with PTH compared to either PTH+ALN or ALN. At the femoral neck, DXA BMD increases were greater for ALN than PTH alone and the combination was similar to ALN. However, cortical volume at the hip increased with PTH alone compared to PTH + ALN (or ALN). Large increases in both formation and resorption were seen with PTH while PTH + ALN resulted in slight decreases in both formation and resorption. In summary, changes in hip BMD suggest an advantage of adding ALN to PTH. However, changes in vertebral trabecular BMD, hip cortical volume and bone markers, suggest that concurrent use of ALN may blunt the anabolic effect of PTH. Our data shows no evidence of a synergism between PTH and ALN when used concurrently. Longer term and larger studies are needed to determine if and how antiresorptives should be optimally combined with PTH.

Disclosures: ***D.M. Black****, Merck 2, 8.*

1008

Novel and Selective Small Molecule Stimulators of Osteoprotegerin Expression Inhibit Bone Resorption. R. J. S. Galvin[1], J. E. Onyia[1], Y. L. Ma[1], D. L. Halladay*[1], R. R. Miles*[1], X. Yang*[1], T. Fuson*[1], R. L. Cain*[1], Q. Q. Zeng*[1], S. Chandrasekhar[1], R. Emkey*[1], Y. Xu*[1], K. Thirunavukkarasu[1], H. U. Bryant[1], T. J. Martin[2]. [1]Gene Regulation, Lilly Research Labs, Indianapolis, IN, USA, [2]St. Vincent's Institute of Medical Research, Fitzroy, Australia.

Treatment with osteoprotegerin (OPG) inhibits osteoclastogenesis and bone resorption in numerous animal models for diseases associated with increased osteoclast formation and activity. Since OPG is produced in bone and acts as a paracrine factor, a high-throughput screen (approximately 387,000 compounds) was conducted to identify small molecule stimulators of OPG expression. The compounds were screened in UMR 106 osteosarcoma cells stably transfected with the human OPG promoter (-5917 to +19) fused with β-galactosidase (βgal)-reporter gene (OPG-βgal). Three structurally unrelated molecules (LY359454, LY119863, and LY42584) were identified which selectively increased OPG transcription (OPG-βgal activity following 24 h of treatment). These compounds did not induce βgal activity in UMR 106 cells harboring the SV40 promoter-βgal. In SaOS cells, these compounds increased OPG mRNA levels within 24h, but the time course for onset of OPG activation was compound dependent. Additionally, these compounds increased OPG protein production and release from SaOS cells (2-5 fold following 48 h of treatment), as determined by ELISA. The benzamide derivative, LY359454, was further analyzed and 4 structurally related molecules (LY481736, LY377332, LY54224, LY368571) were identified as OPG inducers. In this class of compounds, LY481736 was the most potent (EC_{50}=4.6 µM in the OPG-βgal assay and EC_{50} =0.25 µM for OPG protein release from SaOS cells) and was examined in functional assays. *In vitro*, LY481736 inhibited osteoclast formation in $1,25(OH)_2D_3$-treated co-cultures of bone marrow and BALC cells (IC_{50}=0.02 µM). Parathyroid hormone (PTH)- induced bone resorption, in a calvarial organ culture model, was also inhibited in a concentration-dependent manner by LY481736. *In vivo*, LY481736 reduced basal bone resorption and completely blocked resorptive activity (serum calcium, osteoclast number) in rats infused subcutaneously with rhPTH (1-38). Furthermore, LY481736 reduced bone tumor burden (number and size following 3 and 4 weeks of treatment) in a rat mammary carcinoma (13762)- induced-bone metastasis model. Finally, periarticular bone loss was reduced by LY481736 in a rat adjuvant arthritis model. These results provide proof of the concept that low molecular weight compounds can enhance OPG production in a manner that could be therapeutically beneficial for the treatment of bone diseases associated with increased bone resorption.

Disclosures: ***R.J.S. Galvin****, Eli Lilly and Company 1, 3.*

1009

Increased Plasma Homocysteine Concentrations Are Strongly Associated with Increased Risk of Hip Fracture in Elderly Men and Women: The Framingham Study. R. R. McLean[1], P. F. Jacques*[2], J. Selhub*[2], E. J. Samelson[1], K. E. Broe[1], M. T. Hannan[3], L. A. Cupples*[4], D. P. Kiel[3]. [1]Hebrew Rehab Ctr for Aged, Boston, MA, USA, [2]USDA HNRC, Tufts Univ, Boston, MA, USA, [3]Hebrew Rehab Ctr for Aged & Harv Med Sch Div on Aging, Boston, MA, USA, [4]Biostat Dept, BU Sch of Public Health, Boston, MA, USA.

Increased prevalence of osteoporosis among individuals with homocystinuria suggests high serum homocysteine levels may weaken bone by interfering with collagen cross-linking. Thus, individuals with elevated plasma total homocysteine (tHcy) concentration may be at increased risk of osteoporotic fracture. To our knowledge, no studies have investigated the relation between tHcy level and hip fracture (HFx). We examined the association between tHcy concentration and risk of HFx in men and women in the Framingham Study original cohort. Non-fasting blood samples were drawn from 825 men and 1174 women (mean age 70 yr, range 59-91) between 1979 and 1982 and stored at -20ºC. tHcy (µmol/L) was measured by high-performance liquid chromatography with fluorescence detection in 1997. Incident

HFxs were ascertained from time of blood draw through June 1998. Sex-specific age-adjusted incidence rates of HFx were calculated for quartiles (Q1=low, Q4=high) of tHcy. Cox proportional hazards regression was used to calculate hazard ratios (HR) to estimate relative risk of HFx across quartiles (Q1 referent), adjusting for baseline measures: age (yr), height (in), weight (lbs), smoking (cig/d), alcohol consumption (oz/wk), caffeine intake (>2 cup/d: y/n), high school education (y/n) and current estrogen use (y/n) in women. Mean tHcy was 13.4±9.1 and 12.1±5.3 μmol/L for men and women, respectively. Mean follow-up time was 11.1 yr for men and 12.2 yr for women, with 41 and 146 HFx occurring for each gender, respectively. Age-adjusted incidence rates (1000 person-yrs^{-1}) for HFx from Q1-Q4 were 1.96, 3.24, 4.43 and 8.14 for men and 9.42, 7.01, 9.58 and 16.57 for women.

Range of tHcy and Hazard Ratios for Hip Fracture per Quartile of tHcy

	Men		Women	
Quartile	Range (μmol/L)	HR (95% CI)	Range (μmol/L)	HR (95% CI)
Q1 (low)	5.8-9.8	1.00	4.1-8.9	1.00
Q2	9.8-12.0	1.67 (0.54-5.14)	8.9-11.1	0.78 (0.45-1.33)
Q3	12.1-15.0	2.07 (0.70-6.09)	11.2-13.7	1.07 (0.64-1.78)
Q4 (high)	15.0-219.8	3.84 (1.38-10.7)	13.8-59.3	1.92 (1.18-3.10)

Men and women in the highest quartile had a significantly elevated risk of HFx: almost four-fold in men and nearly double in women. These findings suggest that tHcy concentration, easily modifiable through dietary intervention, is an important risk factor for HFx in older populations. Thus, folic acid fortification of grain products may potentially decrease fracture rates in the U.S.

Disclosures: ***R.R. McLean****, None.*

1010

Overweight Protects Against Vertebral Fracture but Increases Risk for Estrogen-Related Cancers: The PERF Study. Y. Z. Bagger, L. B. Tankó, P. Alexandersen, B. J. Riis, C. Christiansen. Center for Clinical and Basic Research, Ballerup, Denmark.

Vertebral bone loss and related fracture are almost exclusively related to estrogen deficiency accompanying the menopause. Body fat mass is an important resource of endogen estrogens in the elderly and overweight tend to be associated with higher bone mineral density (BMD). However, overweight may also increase the risk for estrogen-related cancers. In the present study, we investigated longitudinal associations between baseline body weight and changes in body weight and the incidence of vertebral fractures or the risk for estrogen related cancers.

We followed a group of 2552 healthy postmenopausal Danish women for 7 years. BMD at the spine, hip or forearm were assessed both at baseline and the follow-up using DEXA. Vertebral fractures at the follow-up visit were assessed on digitalized lateral X-rays of the thoracic and lumbar spine. A more than 20% reduction of the vertebral height was considered as a fracture. Non-vertebral fractures were recorded by questionnaire. Cut-off values for the quartiles of baseline weight were 54.3, 62.6, 69.2 and 82.2 kg. Cut-off values for the quartiles of yearly changes in weight were -0.7, -0.1, 0.3 or 1.1 kg/year. Information on the new incidence of breast cancer, uterine cancer and ovarian cancer was collected at the follow-up.

The mean age at baseline was 62.7 years old (years since menopause 14.5 years). In this population, 38.5% of the women had experienced either a vertebral or a non-vertebral fracture. Age-corrected baseline measures of BMD at the spine, hip or forearm were significantly different between the lowest 75th highest quartile of body weight. When comparing women belonging to the highest quartile of body weight (>75th) who showed the largest changes in body weight (>75th, n=181) with those belonging to the lowest body weight (75th quartile) increased the risk for estrogen related cancer to 2.1 compared to the lowest (< 25th) quartile (OR: 2.1, 95% CI, 0.99-4.53, p=0.05).

These results indicate that body fat mass provides an important long-term protection against vertebral fracture, but may increase the risk for breast and genital cancer, emphasizing the potential benefits and risks of being overweight in the elderly age.

Disclosures: ***Y.Z. Bagger****, None.*

1011

A Missense Mutation in FGFR1 Causes a Novel Syndrome: Craniofacial Dysplasia with Hypophosphatemia (CFDH). K. E. White[1], J. M. Cabral*[2], W. E. Evans*[1], S. Ichikawa*[1], S. I. Davis*[1], M. J. Econs[1]. [1]Medicine, Indiana Univ. School of Medicine, Indianapolis, IN, USA, [2]Endocrinology, Cleveland Clinic Florida, Weston, FL, USA.

Craniofacial dysplasia with hypophosphatemia (CFDH) is a novel renal phosphate wasting disorder with a previously undetermined molecular etiology. Here we report a CFDH kindred distinguished by short limb dwarfism (proband stature=40 in.) and brachydactyly, as well as craniofacial deformities, including craniosynostosis, prominent superorbital ridge, and depressed nasal bridge. X-ray analyses revealed shortened humeri, and insufficiency fractures. The skeletal manisfestations of the disorder cosegregated with hypophosphatemia (1.0 mg/dl) secondary to severe renal phosphate wasting (TmP/GFR=0.9 mg/dl), inappropriately low calcitriol concentrations (10 pg/ml or undetectable), normocalcemia, and elevated alkaline phosphatase concentrations. The kindred displayed male to male transmission, indicating an autosomal dominant mode of inheritance. In light of the fact that mutations in FGF23 result in autosomal dominant hypophosphatemic rickets (ADHR) we tested genomic DNA for mutations in the known FGF receptors (FGFR1-5). Direct DNA sequencing of FGFR1 exons revealed that the proband and his father had heterozygous A1115G substitutions in exon 10. This change resulted in replacement of Tyr 372 with a Cys (Y372C) in the extracellular portion of FGFR1. RFLP analysis demonstrated that the A1115G mutation was not present in 880 control alleles. The Y372C allele was transcribed in a CFDH patient's lymphocytes and the mutation creates a seventh, and unpaired extracellular Cys, which may lead to inappropriate coupling and activation of mutant FGFR1. Y372 is conserved between human and mouse FGFR1/Fgfr1, and resides at the site where homologous mutations in FGFR2 and FGFR3 cause neonatal lethal syndromes. We did not detect mutations in FGFR1-5 for index cases of tumoral calcinosis, hypophosphatemic bone disease (HBD), or several isolated adult-onset hypophosphatemias. In summary, CFDH is caused by a single FGFR1 defect that results both in renal phosphate wasting and in impaired vitamin D regulation. ADHR, tumor induced osteomalacia, and CFDH patients share strikingly similar metabolic bone and biochemical disease profiles, thus we hypothesize that FGF-23 signals through FGFR1 to regulate kidney phosphate reabsorption and $1,25(OH)_2$vitamin D_3 production. We conclude that in addition to its known roles in skull and extremity patterning, FGFR1 has essential functions in long bone development, phosphate homeostasis, and vitamin D metabolism.

Disclosures: ***K.E. White****, None.*

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Serotonin (5-HT) Influences Bone Mass, Size and Strength, but Is not Involved in Mechanotransduction. S. J. Warden*[1], A. G. Robling[2], M. M. Bliziotes[3], 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, [3]Oregon Health and Science University/Portland VAMC, Portland, OR, USA.

There is mounting evidence for the central regulation of bone via neural pathways, with possible involvement of neurotransmitters. One transmitter showing potential in modulating bone is serotonin (5-HT). Osteoblasts and osteocytes contain 5-HT transporters (5-HTT), and functional receptors for 5-HT have been identified on osteoblastic cells within the periosteum. 5-HT potentiates the PTH-induced increase in AP-1 activity in UMR cells and modulates the response of osteoblasts to mechanical stimuli. This study compared 5-HTT knockout mice (5-HTT-/-) to wild-type controls (5-HTT+/+) to investigate the impact of 5-HT on bone mass, size and strength, and investigated whether 5-HT is involved in mechanotransduction. The 5-HTT regulates the duration of 5-HT activity with its deletion allowing prolonged 5-HT transmission. 5-HTT-/- mice had lower bone mass compared to 5-HTT+/+ mice. Whole body and femoral BMC were 12.9% and 13.8% lower by DXA, and femoral and tibial midshaft BMC were 14.8% and 14.7% lower by pQCT. At the distal femur 5-HTT-/- mice had 10.9% lower vBMD and 15.4% lower BMC, and at the proximal tibia BMC was 14.4% lower. 5-HTT-/- mice had smaller bones than 5-HTT+/+ mice with femoral and tibial midshaft cross-sectional area being 14.7% and 16.7% lower, and tibial midshaft cortical area being 9.1% lower. The polar second moment of area in 5-HTT-/- mice was 11.9% and 16.9% lower in the femoral and tibial midshaft. Consistent with the lower bone mass and smaller bones, femurs from 5-HTT-/- mice failed in three-point bending at 12.5% lower force and 16.5% lower energy, whereas tibias failed at 20.7% lower force and 20.9% lower energy. Thus, 5-HTT-/- mice had a consistent skeletal phenotype of lower bone mass, size and strength. One possible mechanism for this phenotype is a reduction in skeletal mechanical responsiveness in 5-HTT-/- mice. However, exposing ulnas of 5-HTT-/- and 5-HTT+/+ mice to an identical mechanical stimulus, no influence of genotype was observed on histomorphometric measures of bone formation. Thus, 5-HT does not appear to play a major role in mechanotransduction. Alternative explanations for the observed skeletal phenotype include a direct effect of 5-HT on skeletal biology or indirect effects like genotype differences in physical activity levels. Overall, the results show that deletion of the 5-HTT in mice generates significant skeletal effects, raising questions regarding the potential skeletal effects of widely-used selective 5-HT reuptake inhibitors.

Disclosures: ***S.J. Warden****, None.*

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Osteoblast and Osteoclast Isolated and Cultured *In Vitro* from Wild Type and TIEG Null Mice Show Defects in Differentiation. M. Subramaniam[1], G. Gorny*[2], S. A. Johnsen*[1], K. Rasmussen*[1], M. Oursler[2], T. C. Spelsberg[1]. [1]Biochem & Mol Biol, Mayo Clinic, Rochester, MN, USA, [2]Biology, Univ of Minnesota, Duluth, MN, USA.

TGFβ inducible early gene (TIEG) is a member of the Krüppel-like transcription factor family. We have recently demonstrated that TIEG overexpression enhances TGFβ induced Smad signaling by down-regulating negative feedback through the inhibitory Smad7. In order to understand the role of TIEG in bone development, we generated TIEG knockout mice (-/-). TIEG null mice appeared to be normal with normal breeding. Calvarial osteoblasts (OB) were isolated from 3 day old -/- and +/+ pups, cultured these cells *in vitro*, and performed RT-PCR for alkaline phosphatase, Cbfa-1, osteocalcin and osterix gene expression. The -/- OB cells displayed a reduced expression of important OB differentiation markers. The osteoblast cells were then differentiated *in vitro* with BMP-2 for 18 days. The OB from +/+ calvaria displayed several nodules in culture when stained with Alizarin red, whereas the OB from -/- calvaria showed no nodule formation. These -/- calvarial OB appear to have a defect in mediating the signals to differentiate in culture. To characterize the osteoclasts (OC) from +/+ and -/- mice, we isolated OC precursor cells from bone marrow and characterized the interactions between OC precursors and calvarial OB from both -/- and +/+ mice. Bone marrow containing OC precursors were cultured with calvarial OB or ST2 stromal support cells in presence of vitamin D and dexamethazone to generate OC-like cells. Cultures of -/- OC precursors with either ST2 or +/+ OB cells significantly decreased OC differentiation compared with +/+ marrow cells cultured with either support cell type. These data support that there is a defect in OC precursors in -/- mice. Cultures of +/+ OC precursors were cultured with -/- OB also resulted in significantly lower differentiation. Examination of gene expression by real time PCR of the calvarial OB revealed decreased RANKL and increased OPG expression in the -/- calvarial OB compared with +/+ calvarial OB cells. These data suggest that the decreased ability of -/- calvarial cells to support OC differentiation is due to a

decrease in ratio of RANKL to OPG in these cells. To examine TGFβ responses in OC precursors during differentiation, we cultured spleen cells with RANKL and M-CSF during differentiation with and without TGFβ . In +/+ cultures, there was a TGFβ dose-dependent increase in the number of OC, as expected. Interestingly, the -/- OC cells showed no impact of TGFβ on OC differentiation. These data support that TGFβ stimulation of OC differentiation is mediated by TIEG gene expression. We conclude from these data that TIEG expression is critical for OB and OC differentiation *in vivo*.

Disclosures: ***M. Subramaniam****, None.*

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Receptor Tyrosine Kinase Orphan Receptor 2 (Ror2) Modulates Wnt Signaling Pathways in Osteoblastic Cells. J. Billiard, R. Moran*, D. Way*, P. Bodine. Women's Health Research Institute, Wyeth, Collegeville, PA, USA.

Ror2 is an orphan receptor tyrosine kinase with no known ligand or association with a signaling pathway. Mutations in the Ror2 gene cause severe skeletal defects in humans and mice. Ror2 contains an extracellular cystein-rich domain that has been shown to mediate Wnt binding to other proteins. Using GeneChip and TaqMan analyses of human conditionally immortalized cell lines in discrete stages of osteoblast differentiation, we show that Ror2 is downregulated during human osteoblast differentiation. We also demonstrate that osteoblastic cells stably overexpressing a Wnt antagonist, secreted frizzled-related protein 1 (SFRP1), have significantly less Ror2 than cells expressing the control vector; whereas SFRP1-null mice have 3.5 times more Ror2 than the wild-types.

We used luciferase reporter assays to test if Ror2 modulates Wnt signaling in osteoblasts. Luciferase expression driven by Wnt-responsive TCF binding elements was stimulated over 20-fold after co-transfection of U2OS human osteosarcoma cells with Wnt3. In contrast, co-transfection with Ror2 dose-dependently inhibited Wnt3-induced activation, while Ror2 alone did not affect promoter activity. When the same reporter was activated by Wnt1, Ror2 potentiated Wnt1 activity. SFRP1 suppressed Wnt3 and Wnt1 activity even in presence of Ror2. Thus, Ror2 has opposing effects on Wnt1 and Wnt3 signaling.

We next asked if Ror2 binds Wnts. COS7 cells were transfected with flag-tagged Ror2 and HA-tagged Wnt1 or 3. Anti-flag agarose precipitated both Ror2 and Wnt1 and 3 as shown by western immunoblotting with anti-flag and anti-HA antibodies. The Ror2-Wnt interactions were specific since no Wnts were precipitated out of the extracts not containing Ror2.

To address participation of the Ror2 functional domains in modulating Wnt signaling, we generated Ror2 lacking the cytoplasmic domain. This construct inhibited Wnt3 activity, but had no effect on Wnt1 activation. Based on these results, we believe that Ror2 inhibits Wnt3 signaling by binding Wnt3 and sequestering it away from the frizzled receptor. On the other hand, potentiation of Wnt1 requires the intracellular domain of Ror2, and may involve activation of the Ror2 tyrosine kinase.

In summary, our studies have identified Ror2 as a new regulator of Wnt signaling with selective activities, antagonizing Wnt3 and enhancing Wnt1 mediated transcription in osteoblasts. The downregulation of Ror2 during osteoblast differentiation and its inverse expression pattern with SFRP1 suggest that Ror2 may regulate osteoblast survival and differentiation.

Disclosures: ***J. Billiard****, Wyeth Research 3.*

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MINT (Msx2-Interacting Nuclear Target) Promotes Runx2-Dependent Transcription via Runx2 Activation Domain 3 (AD3). O. L. Sierra, A. P. Loewy*, N. Charlton-Kachigian*, S. Cheng, D. A. Towler. Bone & Mineral Diseases, Washington University, St Louis, MO, USA.

Msx2 suppresses expression of the late osteoblast phenotypic marker osteocalcin (OC) by inhibiting the assembly of nuclear protein-DNA complexes on the OCFRE, the rat OC FGF2 responsive element (nucleotides –154 to –113). This FGF2-activated promoter region is recognized by the osteoblast transactivator, Runx2, and by Ku antigen and MINT – components of the osteoblast nuclear matrix. Msx2 selectively inhibits Ku and Runx2 association with the OC promoter. However, the role and regulation of MINT (Msx2-interacting nuclear target) in transcription driven by the OCFRE is poorly characterized. Therefore, we studied the effect of MINT on transcription driven by the OCFRE (OCFRE-LUC) in transient co-transfection studies using CV-1 fibroblasts. In one-hybrid analyses, we identify that the major FGF2 – stimulated trans-activation functions reside in the Runx2 component of the OCFRE DNA-protein complex; no intrinsic transactivation functions reside in the nuclear matrix constituents. By generating and analyzing a series of systematically altered Runx2 variants, we show that FGF2 stimulation is dependent upon Runx2 activation domains AD1 and AD2. By contrast, Runx2 AD3 is not required for basal or FGF2-stimulated activity. We next examined the effect of MINT on OCFRE – dependent transcription. Under basal conditions co-expression of MINT has no effect on OCFRE – dependent transcription. By contrast, MINT stimulates Runx2-dependent transcription 4-fold; upregulation is completely dependent upon activated FGFR2 signaling. Runx2 structure-function analyses reveal that MINT-dependent transcription requires Runx2 AD3; although equivalent to full length Runx2 (1-528) under basal and FGF2-stimulated conditions, Runx2(1-528; Δ 307-376) that lacks AD3 cannot functionally interact with MINT to augment OCFRE-dependent transcription. As expected, Msx2 completely abrogates all transcription directed by the OCFRE. In pull-down analyses, Msx2 physically interacts with both MINT and Runx2; interactions with Runx2 require residues 118-256 (Runt domain) and 312-392 (AD3). Taken together, these data indicate that the nuclear matrix protein MINT regulates Runx2-dependent transcription, triggered by FGFR2 activation. Msx2 inhibits OC transcription via protein-protein interactions with the nuclear matrix complex assembled by the OCFRE. Since MINT is homologous to Drosophilan *spen* -- genetically identified as participating in growth factor signaling and homeoprotein-dependent cell specification -- this nuclear matrix protein may biochemically integrate growth factor signaling with stage-specific osteoblast gene expression.

Disclosures: ***O.L. Sierra****, None.*

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Transcription-Independent Inhibition of Caspases by C/EBPβ in Osteocytes: An Anti-Apoptotic Signaling Cascade Uniquely Activated by Bisphosphonates. L. I. Plotkin, J. I. Aguirre, S. C. Manolagas, T. Bellido. Endocrinology, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. Arkansas for Med. Sci., Little Rock, AR, USA.

The anti-fracture efficacy of bisphosphonates (BPs) may be due in part to preservation of the osteocyte network resulting from prevention of osteocyte apoptosis. This effect of BPs involves opening of connexin (cx) 43 hemichannels and requires phosphorylation of the extracellular signal regulated kinases (ERKs). However, it does not require gene transcription nor the activation of ERK-dependent transcription factors, and it is abolished by restricting ERKs to the nucleus, suggesting that the nuclear pool of ERKs is not involved. We report that, in contrast to ERK activation by growth factors and sex steroids, which results in nuclear ERK accumulation, exposure of MLO-Y4 osteocytic cells to BPs from 2 to 60 min did not induce nuclear accumulation of ERK2 fused to green fluorescent protein. Moreover, BPs prevented apoptosis in cells expressing an ERK2 mutant with impaired nuclear translocation as effectively as in cells expressing wild type ERK2; whereas they were ineffective in cells expressing an ERK2 mutant that constitutively localizes in the nucleus. Taken together, these findings indicate that cytoplasmic ERK substrates mediate the effect of BPs. Previous results have demonstrated that one of these substrates is C/EBPβ, in which phosphorylation of ^{217}Thr by ERKs creates a functional XGluXAsp sequence through which C/EBPβ, binds to and prevents procaspase activation leading to cell survival – independently from its function as a transcription factor. Consistent with the dispensability of nuclear functions of ERKs for the effect of BPs, we found that cells expressing a transcriptionally inactive C/EBPβ mutant were protected from apoptosis by BPs as effectively as cells expressing wild type C/EBPβ. On the other hand, cells expressing a caspase binding deficient mutant in which ^{217}Thr was replaced by Ala were not protected. Notably, cells expressing a C/EBPβ mutant in which the caspase inhibitory site was mimicked by replacing ^{217}Thr by Glu were resistant to apoptosis, even in the absence of BP treatment. Lastly, and consistent with the requirement of cx43 hemichannel opening for survival, BPs induced C/EBPβ phosphorylation in MLO-Y4 osteocytic cells and in embryonic fibroblasts derived from wild type mice that express cx43, but not in embryonic fibroblasts derived from cx43 deficient mice. We conclude that the cx43/ERK pathway activated by BPs promotes osteocyte survival through a novel, transcription-independent mechanism that involves the creation of a functional caspase inhibitory domain in C/EBPβ.

Disclosures: ***L.I. Plotkin****, None.*

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Osteoblastic and Stromal Cell Activation of the PTH/PTHrP Receptor Alters the Bone Marrow Microenvironment and Increases Osteoblastic Expression of the Notch Ligand Jagged1. L. M. Calvi[1], J. Weber*[1], G. B. Adams*[2], J. Harvey*[1], E. Schipani[2], J. E. Puzas[1], H. M. Kronenberg[2], D. T. Scadden*[2], L. A. Milner*[1]. [1]University of Rochester School of Medicine, Rochester, NY, USA, [2]Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

In the bone marrow, hematopoietic stem cells (HSC) are found in close proximity to osteoblastic cells. We recently showed that mice expressing a constitutively active PTH/PTHrP receptor in osteoblastic cells (col1-caPPR) have an increased frequency of HSC. This effect could be reproduced by treating normal stromal cells with PTH (1-34) or by injecting normal mice with PTH. To understand how activation of the PPR in osteoblastic cells could improve their ability to support hematopoiesis, we studied *in vivo* and *in vitro* models of PTH excess. Col1-caPPR mice have a striking increase in trabecular bone and osteoblastic cells, as we previously reported. Consistent with this *in vivo* finding, there was a substantial increase in alkaline phosphatase positive cells in primary stromal cell cultures from col1-caPPR mice compared to normal littermates. This was in part due to a doubling in osteoprogenitor numbers in transgenic vs normal littermates. *In vitro* treatment of normal primary stromal cells with PTH(1-34) also resulted in an increase in alkaline phosphatase positive cells compared to vehicle treated cells. In addition to the increased number of osteoblastic cells, the metaphyseal areas of the long bones of col1-caPPR mice compared to littermates showed a striking increase in sinusoids and PECAM positive endothelial cells. Therefore, osteoblastic PPR activation significantly alters the cellular composition of the bone marrow microenvironment.

We next explored the involvement of the Notch receptors and their ligands Jagged1 and Delta1 in mediating the PTH induced enhanced osteoblastic support of HSCs, since this cell-cell interaction pathway plays a fundamental role in HSC self-renewal and vascular development . By immunohistochemistry, we observed a dramatic increase in osteoblastic Jagged1 protein levels in long bones from col1-caPPR mice compared to normal littermates, while Delta1 protein level was unchanged. Interestingly, the Notch3 protein, which is thought to be important for vascular smooth muscle and endothelial cell homeostasis and survival, was increased in the endothelial cells along the metaphyseal trabecular bone in the transgenic mice.

In conclusion, activation of the PPR in osteoblastic cells increases protein levels of the Notch ligand Jagged1, and alters dramatically the cellular composition of the bone marrow microenvironment. These changes may play an important role in the PTH induced enhanced osteoblastic support of HSC.

Disclosures: ***L.M. Calvi****, None.*

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Notch 1 Constitutive Activation Induces a Shift from Osteoblastogenesis to Adipogenesis in Marrow Stromal Cells. E. Gazzerro, M. Sciaudone*, L. Priest*, A. M. Delany, E. Canalis. Department of Research, Saint Francis Hospital and Medical Center, Hartford, CT, USA.

Pluripotential mesenchymal cells from bone marrow stroma can differentiate into osteoblasts, adipocytes, chondrocytes and myoblasts. Glucocorticoids induce differentiation of stromal cells toward the adipocytic pathway at the expense of osteoblastogenesis. Notch receptors are a family of single pass transmembrane proteins activated by their membrane-bound ligands, Delta/Jagged**.** Unstimulated osteoblasts express modest levels of Notch 1 and 2, and their transcripts are increased by cortisol. Activation of the Notch signaling pathway regulates the expression of transcription factors involved in terminal differentiation, and is essential for adipocyte formation. To investigate the effects of Notch 1 constitutive activation on the differentiation of marrow stromal cells, we used a retroviral system to overexpress Notch 1 intracellular domain (NIC) in a murine stromal cell line (ST-2). NIC overexpression was confirmed by Northern blot analysis, and by the increased activity of a HES-5 promoter construct, known to be induced by Notch. Compared to ST-2 cells transduced with vector alone, overespression of NIC abolished the ability of bone morphogenetic protein 2 to stimulate their osteoblastic differentiation. These cells had decreased type I collagen and osteocalcin mRNA and did not form mineralized nodules. Further, ST-2 NIC presented decreased levels of deltafosB and delta2deltafosB, transcription factors that favor osteosblastogenesis at the expense of adipogenesis. In ST-2, cortisol precludes osteoblast differentiation and induces the expression of the adipocytic markers. NIC overexpression enhanced the positive effect of cortisol on adipocytic makers and accelerated the formation of mature fat cells. In conclusion, Notch 1 constitutive activation in marrow stromal cells causes a shift from osteoblastogenesis to adipogenesis, an action similar to that observed with glucocorticoid excess. Notch 1 receptor could play a role in enhancing cortisol effects on marrow stromal cell differentiation.

Disclosures: ***E. Gazzerro****, None.*

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Cbl-Dependent Ubiquitination of Src Proteins Induced by Constitutive FGFR-2 Activation Results in Src Downregulation and Increased Osteoblast Differentiation. K. Kaabeche*[1], J. Lemonnier*[2], J. Caverzasio[2], P. Marie[1]. [1]Biology and Pathology of Bone, INSERM U349, Paris, France, [2]Division of Bone Diseases, Geneva, Switzerland.

We previously showed that constitutive activation of FGFR-2 induced by the activating S252W mutation in Apert syndrome results in increased expression of osteoblast differentiation genes (ALP, COLIA1). Here we examined the role of Src in osteoblast differentiation induced by the activating FGFR-2 mutation. Western blot analysis in immortalised human Apert (Ap) calvaria osteoblastic cells bearing the activating S252W FGFR-2 mutation revealed decreased expression and phosphorylation of Src proteins (Lyn, Fyn) compared to normal age-matched control (Co) calvaria cells, in basal culture conditions and in the presence of FGF-2 (50 ng/ml). In contrast, Src mRNA levels were similar in Ap and Co cells. Biochemical analysis confirmed that Src kinase activity was 50% lower in Ap cells compared to Co cells. Lysophosphatidic acid (LPA, 20 nM, 48 hrs), a non specific activator of Src, decreased ALP mRNA levels and activity in Ap cells. UK 14,304 (20 µM, 48 hrs), a specific Fyn stimulator, also inhibited ALP activity in Ap cells, whereas COLIA1 mRNA levels were unaffected. Transient transfection of Ap cells with Fyn or Lyn vectors reduced ALP activity without change in COLIA1 mRNA levels, further showing a role for Fyn and Lyn in the increased ALP activity induced by FGFR-2 activation in Ap cells. We hypothesized that Fyn or Lyn down-regulation in FGFR-2 mutant osteoblasts may result from increased ubiquitination via the proteasome. Lactacystin (10 µM, 48 hrs), a specific proteasome inhibitor, restored Fyn expression and ALP activity in Ap cells, indicating that the increased ALP activity induced by the mutation results from increased Fyn ubiquitination and proteasome degradation. The proto-oncogene Cbl has recently emerged as a ubiquitin ligase for Src. We examined wether Cbl may be involved in Fyn ubiquitination in Ap osteoblasts. Immunoprecipitation studies showed that Fyn associated with Cbl was decreased in Ap cells compared to Co cells, indicating that Cbl interacts with Fyn to promote Fyn ubiquitination. These data show that 1) constitutive activation of FGFR-2 leads to downregulation of Fyn and Lyn protein levels and kinase activity in mutant osteoblasts; 2) overexpression of ALP in mutant osteoblasts results in part from downregulation of Fyn induced by FGFR-2 activation and 3) Fyn downregulation results from increased Cbl-dependent Fyn ubiquitination. Thus, Cbl-dependent ubiquitination of Src proteins induced by FGFR-2 activation mediates in part the increased osteoblast differentiation induced by the Apert FGFR-2 mutation in human osteoblasts.

Disclosures: ***K. Kaabeche****, None.*

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BMP Signaling through the Smad1 Pathway Is Required for Normal Postnatal Bone Formation. D. Chen[1], M. Qiao*[1], B. Story*[1], M. Zhao[1], Y. Jiang[2], J. Zhao[2], J. Feng[3], Y. Xie*[3], S. Huang*[4], A. Roberts*[4], G. Karsenty[5], G. Mundy[1]. [1]University of Texas Health Science Center, San Antonio, TX, USA, [2]University of California, San Francisco, CA, USA, [3]University of Missouri, Kansas City, MO, USA, [4]NIH, Bethesda, MD, USA, [5]Baylor College of Medicine, Houston, TX, USA.

The physiological role of BMP signaling in bone formation in post-natal life remains undefined. Since null mutations of the Smad1 and Smad5 genes cause perinatal lethality, it is not possible to use these mice to investigate this issue. To determine the role of the BMP signaling pathway, and specifically its dependence on Smad1, in postnatal bone formation, we have used 2 genetic murine models: 1) A tissue-specific, Cre-mediated recombination was achieved in mice to specifically disrupt Smad1 expression in osteoblasts; and 2) transgenic mice were produced in which a truncated dominant-negative BMP receptor (dnBMPR) is targeted to osteoblasts by the 2.3 kb type I collagen promoter. We generated mice for a floxed Smad1 allele, in which exon 2 of the Smad1 gene was flanked by loxP sites. Smad1-loxP (Smad1$^{flox/wt}$) mice were bred with Cre transgenic mice in which Cre recombinase is driven by the 2.3 kb type I collagen promoter (Col1a1-Cre). Col1a1-Cre$^{+/-}$;Smad1$^{flox/wt}$ mice were then bred with Smad1$^{flox/flox}$ mice to generate mice with osteoblasts lacking the Smad1 gene (Col1a1-Cre$^{+/-}$;Smad1$^{flox/flox}$ mice or Smad1 conditional knockout [cko] mice). The Smad1 cko mice are viable and survive into adulthood. Trabecular bone volume, osteoblast numbers and bone formation rates in proximal tibiae were reduced 27, 38 and 44%, respectively in Smad1 cko mice compared with control littermates (Col1a1-Cre$^{+/-}$;Smad1$^{flox/wt}$ and Col1a1-Cre$^{-/-}$;Smad1$^{flox/flox}$ mice). 3D micro CT examination showed that in tibiae and femora of Smad1 cko mice, trabecular volume, number, thickness, and connectivity density were decreased and trabecular separation was increased. The formation of mineralized bone nodules in long-term cultures of primary osteoblasts was decreased in Smad1 cko mice. mRNA expression of Smad1 was detected in osteoblasts in trabecular bone by in situ hybridization and protein expression of Smad1 was detected in primary osteoblasts by Western blot in control mice. In Smad1 cko mice, expression of Smad1 mRNA and protein was undetectable in osteoblasts *in vivo* and *in vitro*. In the dnBMPR transgenic mice, there was an identical phenotype to that observed in the Smad1 cko mice, namely blockage of BMP signaling, decreased trabecular bone volume and decreased bone formation rates. These results demonstrate that Smad1 is necessary for normal postnatal bone formation and suggest that BMPs exert their effects on bone formation through the Smad1 signaling pathway.

Disclosures: ***D. Chen****, OsteoScreen Ltd 3.*

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Subnuclear Targeting Supports Functional Interrelationships between Runx2 and the BMP-2 Regulated Smad 1 Protein. F. Afzal, S. K. Zaidi, A. Javed, A. J. van Wijnen, J. L. Stein*, J. B. Lian, G. S. Stein. Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, MA, USA.

The coordinated activity of Runx2 and BMP/TGFbeta-activated Smads is critical for osteoblast differentiation. The Runx2 C-terminus which is essential for its osteogenic function in vivo, contains a 38 amino acid nuclear matrix targeting signal (NMTS) that directs Runx2 to active subnuclear foci to form stable complexes. Furthermore, the subnuclear trafficking of Smads to Runx foci requires interaction with Runx2. However, the precise structural basis for this Runx2-Smad interaction has not been resolved. We have carried out studies to define the sequence required for Smad interaction by generating sequential C terminal deletion mutations of wild type Runx2 protein (aa 1-528) and constructing two internal deletions of the NMTS domain. Functional cooperation between the BMP2 responsive Smad1 and Runx2 wild type and mutant proteins was determined using a luciferase reporter under the control of a promoter with multiple copies of Smad and Runx responsive elements. Our data show 90% reduction of BMP-mediated transcriptional activity of mRunx2 1-432 that retains the NMTS domain, when co-expressed with Smad1 in HeLa cells which lack endogenous Runx expression. However, Smad1-mRunx2 1-432 complexes can still be formed, as determined by co-immunoprecipitation studies. In situ immunofluorescence also revealed that Smad1 was targeted to subnuclear foci in the presence of Runx2 1-432. Further deletion of the C-terminus to 361 amino acids, which lacks the NMTS, results in a complete loss of transcriptional activity when co-expressed with Smad1 and loss of complex formation at subnuclear sites. These data suggest that the Smad functional activity resides largely in the C-terminus and overlaps the NMTS. Internal deletions of the NMTS region of Runx2 abrogate association with subnuclear foci and transcriptional activity is significantly reduced by 2-3 fold. Notably, Smad1 interaction with these Runx2 mutants is retained in the nucleus by in situ and by co-immunoprecipitation studies. These findings indicate that the Smad interacting domain overlaps the NMTS and that the NMTS interaction site is a significant component for the transcription mediated by the Runx2 complex. Thus, in conclusion, these biochemical and in situ nuclear trafficking studies provide conclusive evidence for the temporal-spatial requirement of the NMTS of Runx2 for interaction with Smad for biological activity.

Disclosures: ***F. Afzal****, None.*

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The Transcription Factor STAT-1 Is a Novel Negative Regulator of Bone Mass and Bone Formation. L. Xiao[1], T. Naganawa*[1], E. Abogunde*[1], G. Gronowicz[2], J. D. Coffin[3], M. M. Hurley[1]. [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Orthopedic, University of Connecticut Health Center, Farmington, CT, USA, [3]Pharmaceutical Sciences, University of Montana, Missoula, MT, USA.

Signal transducers and activators of transcription (STATS) play a central role in the biological responses to a number of cytokines and growth factors. Growth hormone, FGF2, IL-6, MCSF and Interferon γ are important factors that regulate bone cell function that also activate STAT1-dependent DNA binding activity. However, there are no studies on the role of STAT1 in bone formation. To assess the role of STAT1 in bone, we examined wild type Stat1 (+/+) and homozygous Stat1 null (-/-) male mice that are maintained on an inbred 129S6/SvEv background. Body wt and femur length were similar in 13-14 week old Stat1+/+ and Stat1-/- mice. Western blot analysis confirmed the absence of the 92 kDa STAT1 protein in bones from Stat1-/- mice. Bone mineral density (BMD) and bone mineral content (BMC) were determined by DEXA analysis of femoral and vertebral bones from 13-14 week old mice of both genotypes. Femoral BMD was significantly increased by 17% and BMC was increased by 10% in Stat-/- mice when compared with Stat1+/+ mice. Verte-

bral BMC was also significantly increased by 19% in Stat1-/- mice compared with +/+ mice. Micro-computed tomography revealed a marked increase in cortical width of femurs of Stat1-/- mice. As determined by the Structure Model Index, there was significantly more plate like structure of trabecular bone in femoral metaphysis of Stat1-/- mice. There was a 15% increase in trabecular thickness (0.0672±0.00 vs 0.0573+0.00 p<0.05) in Stat1-/- mice but trabecular number was not increased when compared with Stat1+/+ mice. Bone volume/trabecular volume was increased in the Stat1-/- mice compared with bones from Stat1+/+ mice (18.4± 0.025 vs 12.2 ± 0.019). Preliminary histomorphometry of distal femurs showed that osteoblast surface/bone surface (34.5 ± 2.0 vs 19.2 ± 4.2 p< 0.05) and mineralized surface/ bone surface (23.4 ± 1.4 vs 17.5 ± 0.01 p< 0.05) was significantly increased in Stat1-/- compared with Stat1+/+ mice. There were no significant differences in osteoclast number or osteoclast surface/bone surface. In addition calvarial width was significantly increased by 28% in Stat1-/- mice compared with Stat1+/+ mice. These data suggest that STAT1 is a negative regulator of bone mass and bone formation in mice. It is likely that STAT1 is a novel important transcription factor in osteoblast function. Characterization of the role of STAT-1 in bone may have implications for the development of therapeutic targets for treatment of metabolic bone disorders.

Disclosures: ***L. Xiao****, None.*

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Sost **Tissue Expression and Regulation of Wnt Signaling.** M. Shen*[1], D. S. Miao[2], D. Goltzman[2], A. C. Karaplis[1]. [1]Medicine, SMBD-Jewish General Hospital, McGill University, Montreal, PQ, Canada, [2]Medicine, MUHC, McGill University, Montreal, PQ, Canada.

The *SOST* gene encodes a protein that is mutated in patients with sclerosteosis. Loss of function of SOST leads to the formation of massive amounts of normal bone throughout life, implicating suppression of bone formation as its most likely physiological role. Therefore, this protein might become an important tool in the development of therapeutic strategies for osteoporosis.

To further explore SOST function, murine *Sost* cDNA encompassing the entire coding region was cloned from mouse kidney total RNA by PCR. Digoxigenin labeled sense and antisense riboprobes were generated from a 151-bp *Sma*I *Sost* fragment and used for *in situ* hybridization of E-15.5 embryo and 2-month-old adult mouse sections. *Sost* mRNA expression was observed in keratinocytes, kidney tubular epithelial cells, skeletal muscle, growth plate chondrocytes and in bone, where it localized specifically in osteoblasts and osteocytes.

The tissues that express *Sost* are also known sites for *Wnt* gene expression. Wnts are secreted glycoproteins that exert their effects on neighboring cells by binding to the Frizzled (Fz) transmembrane receptor family. Single-pass transmembrane proteins of the LDL receptor-related protein family (LRP5 and -6,) have also been implicated in the reception of the Wnt signal, a key pathway involved in various developmental processes, including osteoblast proliferation and function. Therefore, we next examined whether interplay exists between Sost and Wnt signaling. For these experiments, cultured C3H10T1/2 murine mesenchymal cells were co-transfected with a luciferase reporter gene construct containing Tcf/Lef transcription factor binding site sequences and either Sost expression vector or empty vector alone. Following transfection, the cells were treated with Wnt-3A and luciferase activity was measured. Wnt-3A strongly potentiated Tcf/Lef-mediated luciferase gene transcription (>5-fold) while no activation was observed when the Tcf binding sites were mutated. In the presence of Sost expression, Wnt-mediated transcriptional activation was markedly impaired, thereby identifying Sost as a potent inhibitor of Wnt action.

Based on these observations, it is likely that in the absence of SOST, WNT activity in inducing osteoblast proliferation and function continues unabated, leading to the progressive sclerosing bone dysplasia of sclerosteosis.

Disclosures: ***A.C. Karaplis****, None.*

1024

High Bone Mineral Density in SOST Knock-out Mice Demonstrates Functional Conservation of Osteocyte Mediated Bone Homeostasis in Mouse and Human. N. Sun*, Y. Gao*, J. Pretorius*, S. Morony, P. J. Kostenuik, S. Simonet, D. L. Lacey, I. Sarosi*, C. Kurahara*, C. Paszty*. Metabolic Disorders, Amgen Inc., Thousand Oaks, CA, USA.

In humans, complete lack of the protein sclerostin due to homozygosity for null mutations in the SOST gene is responsible for causing sclerosteosis, a rare genetic disease characterized by increased bone mineral density (BMD) throughout the skeleton. Sclerostin is a secreted protein and has been previously shown to be expressed in bone by osteocytes, to bind bone morphogenetic proteins (BMPs) and to inhibit osteoblast differentiation/function in cell culture. Based on these genetic and biochemical data a novel bone homeostatic pathway in humans has been proposed in which osteocytes, by secreting sclerostin, negatively modulate osteoblast mediated bone anabolic activity.

To determine the effect of a complete lack of sclerostin in mice we have generated knock-out mice homozygous (-/-) for a deletion of the SOST gene. The -/- mice appear normal compared to controls and are fertile. Qualitative radiographic assessment of 4-month old male and female -/- mice showed normal bone morphology but with a generalized increased radiodensity in all portions of the skeleton (skull, axial skeleton, ribs, pelvis, long bones) as compared to wild-type (+/+) littermates. Peripheral quantitative computed tomography of tibia from -/- mice showed a statistically significant increase in total BMD (38%), trabecular BMD (66%), cortical BMD (22%) and cortical area (56%) as compared to +/+ littermates.

This high bone mineral density phenotype in mice recapitulates the major clinical feature found in humans with sclerosteosis, and demonstrates functional conservation of sclerostin secretion by osteocytes as an important component in the control of bone homeostasis in mouse and human. As such, these sclerostin knock-out mice should serve as a useful tool for gaining a deeper understanding of the basic biology surrounding this novel pathway, as well as it's therapeutic potential in the anabolic treatment of osteoporosis.

Disclosures: ***C. Paszty****, Amgen Inc. S, E.*

1025

Smurf2-Mediated Loss of TGF-β Signaling Stimulates Articular Chondrocyte Maturation. Q. Wu, M. J. Zuscik, J. F. Baden*, E. M. Schwarz, H. Drissi, R. J. O'Keefe, J. E. Puzas, R. N. Rosier. Orthopaedics, University of Rochester, Rochester, NY, USA.

Cartilage within diarthroidal joints is maintained by articular chondrocytes (ACs) which produce matrix and are prevented from undergoing terminal maturation. During osteoarthritis (OA), unknown maturational constraints are lost and ACs recapitulate the differentiation process that occurs during normal endochondral ossification. The aims of this study were to identify these unknown maturational constraints and to define the molecular processes at work during inappropriate stimulation of AC hypertrophy. To address these aims, we developed a cell culture model utilizing 5-azacytidine (Aza) to induce maturation in cultured chick ACs. Aza is known to un-mask expression of silenced genes by replacing cytidine in the DNA, thus blocking the suppressive effect of cytidine methylation on gene transcription. ACs, which do not spontaneously express markers of chondrocyte maturation such as indian hedgehog, type X collagen (colX) or alkaline phosphatase, were induced to express all three of these markers by a 48 hr exposure to 15 μg/ml Aza. Since it is a well established paradigm that TGF-β strongly suppresses chondrocyte maturation, we investigated whether Aza-induction of AC maturation may be due to a loss of TGF-β signaling. We tested this hypothesis i) by comparing Aza effects with those induced by blocking signaling through the TGF-β Smads (2 and 3) using a dominant negative (Δ) approach, ii) by examining the impact of Aza on the expression of Smad2 and 3 and the ubiquitin E3 ligase Smurf2, a protein that targets Smad2 and 3 for degradation, and iii) by measuring the rate of Smad2 and 3 degradation following Aza treatment by using metabolic labeling and ubiquitin immunoprecipitation methods. We found that over-expression of ΔSmad2 or ΔSmad3 mimics Aza by inducing expression of the hypertrophic marker colX. Furthermore, Aza-treated ACs display reduced Smad2 and 3 and increased Smurf2 protein levels. Finally, Aza mimics Smurf2 by ubiquitinating and degrading Smad2 and 3. These results indicate that AC maturation is caused by Smurf2-induced degradation of Smad2 and 3 and raise the possibilty that Smurf2 up-regulation during OA may be an initiator of the disease process. This is supported by immunohistochemical studies which demonstrate that Smurf2 protein expression is significantly increased in human OA cartilage relative to normal cartilage controls. We conclude that AC maturation is induced by Smurf2-dependent loss of TGF-β signaling, suggesting that inhibition of Smurf2 function may represent a novel therapeutic approach for treatment of OA.

Disclosures: ***Q. Wu****, None.*

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Overexpression of the Novel Collagen Triple Helix Repeat Containing Gene (CTHRC1) Indicates a Role in Cartilage and Bone Formation via Regulation of BMP Expression. V. Lindner*[1], P. Pyagay*[1], D. P. Moore*[1], M. L. Mancini*[1], T. N. Wight*[2], L. Liaw*[1]. [1]Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, ME, USA, [2]Vascular Biology, The Hope Heart Institute, Seattle, WA, USA.

The purpose of the present study was to understand the function of the novel gene Cthrc1 using both in vitro and in vivo approaches.

Transgenic mice overexpressing Cthrc1 were generated and analyzed using established methods. Routine cell transfection procedures were used to establish CTHRC1 overexpressing and CTHRC1 depleted cell lines.

CTHRC1 is a secreted 26.5kDa protein that is glycosylated and highly conserved from lower chordates to mammals. The sequence contains a short collagen domain which prompted the name 'collagen triple helix repeat containing 1'. Cthrc1 mRNA expression is regulated by TGF-ß family members. Localization of CTHRC1 protein showed remarkable overlap with known sites of bone morphogenetic protein (BMP) expression, such as in the bone matrix and at epithelial-mesenchymal interfaces in the skin and chorioid plexus. In the mouse embryo, CTHRC1 was abundant in the developing heart, proliferating chondrocytes and in the surrounding periosteum. Immunoreactive CTHRC1 was abundant in the bone matrix where it was expressed by osteocytes and osteoblasts. In transgenic mice overexpressing Cthrc1, a striking lack of Alcian blue staining of cartilagenous structures was observed, particularly noticeable in the spinal column indicating a lack of cartilage proteoglycan content and this was confirmed by ultrastructural analysis. The axial skeleton was particularly affected with defects in neural tube closure. Other symptoms included dwarfism, brittle bones, reduction in bone marrow cells and detachment of the epidermis. Downregulation of CTHRC1 in MC3T3-E1 osteoblasts was associated with loss of BMP4 expression in these cells whereas overexpression of CTHRC1 in C3H10T1/2 cells was accompanied with increased BMP4 levels. Furthermore, in co-transfection experiments BMP4 promoter activity was significantly increased in the presence of CTHRC1. Increased levels of BMP4 and the downstream target Msx1, known to be involved in axial development, were also increased in skeletal tissues of Cthrc1 transgenic mice.

In conclusion, we identified Cthrc1 as a novel gene with important functions in cartilage and bone formation, most likely by regulating BMP mediated signaling events.

Disclosures: ***V. Lindner****, None.*

1027

Role of Reg Growth Factors and Receptor in Osteoarthritis Pathogenesis. A. Moreau[1], D. Wang*[1], S. Jacques*[1], M. Rosec*[1], M. Lacroix*[1], C. Chassaing*[1], J. Martel-Pelletier*[2], J. Fernandes*[3]. [1]Stomatology and Biochemistry, Université de Montréal, Montréal, PQ, Canada, [2]Pharmacology, Université de Montréal, Montréal, PQ, Canada, [3]Surgery and Biomedical Sciences, Université de Montréal, Montréal, PQ, Canada.

The key features of osteoarthritis (OA) are the focal destruction of the articular cartilage and the abnormal growth of the subchondral bone producing outgrowths. Since in humans OA develops and changes very slowly, it is difficult to follow that disease over any length of time. Besides that, the heterogeneity of the disease results in controversy as its aetiology and progression. Thus, study of early events of the degenerative process cannot be made in humans and recourse must be made to animal models. We have recently inactivated the transcription factor Pitx1, which is highly expressed in articular and growth plate chondrocytes during mouse development. Pitx1-null mice displayed poorly developed joints, which are markedly apoptotic. Interestingly, Pitx1+/- mice that are phenotypically normal at birth, exhibit with aging clinical features of OA such as progressive joint stiffness associated with an abnormal fibrillation and calcification of their articular cartilage. Histological analysis of mineralized adult femurs and tibiae revealed also an increased thickening of subchondral, trabecular and cortical bone.
At the molecular level, expression analysis of Pitx1-null and Pitx1+/- mice allowed to identify and characterize a novel molecular cascade involved in OA pathogenesis. Indeed, expression analysis using RNA isolated from articular cartilage of patients with OA and age and gender-matched subjects showed the expression of Pitx1 only in matched controls. Moreover, the lack of Pitx1 in OA articular chondrocytes leads to a marked up regulation of Reg I growth factor and its receptor, which has been confirmed at the protein level by immunohistochemistry assays with human Reg I antibodies on OA articular cartilage. We have also demonstrated *in vitro* that the gain-of-function of Reg receptor enhances by several folds the activation of NF-κB induced by TNF-α or IL-1β suggesting that Reg signaling activity is a key mediator of pro-inflammatory cytokine action in OA pathogenesis. This was further supported by the fact that gain-of-function of Pitx1 abrogates Reg I, Reg II and Reg receptor expression completely, which may explain why pro-inflammatory cytokines cannot activate NF-κB in cells devoid of Reg receptor. Taken together these results revealed the role of new emerging transcription and growth factors involved in OA pathogenesis, which could lead to a more rational approach for the development of better therapeutic compounds to prevent and cure OA.

Disclosures: ***A. Moreau****, None.*

1028

Soluble VEGF Isoforms Are Essential to Establish Vascularization of Cartilage Elements and Regulate Development and Survival of Growth Plate Chondrocytes. C. Maes*[1], I. Stockmans*[1], K. Moermans*[1], R. Van Looveren*[1], N. Smets*[1], P. Carmeliet*[2], R. Bouillon[1], G. Carmeliet[1]. [1]Laboratory of Experimental Medicine and Endocrinology, Katholieke Universiteit Leuven, Leuven, Belgium, [2]The Center for Transgene Technology and Gene Therapy, V.I.B., Katholieke Universiteit Leuven, Leuven, Belgium.

Vascular endothelial growth factor (VEGF) exists as 3 major isoforms, VEGF120, VEGF164 and VEGF188, which differ in matrix- and receptor-binding. Inhibition of VEGF or expression of only the soluble VEGF120 isoform resulted in impaired bone vascularization associated with reduced trabecular bone volume and an enlargement of the hypertrophic chondrocyte zone of the growth plate.
Here we report that neonatal mice that express exclusively VEGF164 showed no bone abnormalities. However, mice expressing only the matrix-associated isoform VEGF188 (VEGF188/188 mice) showed dwarfism, completely disrupted growth plate development and severe knee joint dysplasia. Developmental analysis of VEGF188/188 mice demonstrated increased and ectopic hypoxia in the epiphyseal cartilage elements, preceding massive apoptosis of immature chondrocytes in the interior of the growth plates. Although metaphyseal angiogenesis was not altered, vascularization surrounding the epiphysis was impaired likely causing the increased hypoxia. Concomitantly, peripheral growth plate chondrocytes showed increased proliferation and decreased differentiation in vivo. In agreement, VEGF188/188 hindlimbs cultured in hypoxic conditions lacked growth inhibition and showed increased apoptosis in the resting chondrocyte zone as compared to WT limbs. Because expression of both VEGF and the isoform-specific VEGF164 receptor neuropilin-1 was detected in immature growth plate chondrocytes, VEGF164 may directly regulate cellular proliferation and survival in hypoxic cartilage. However, abnormal expression of genes of the parathyroid hormone-related peptide (PTHrP) - Indian hedgehog (Ihh) pathway was observed in VEGF188/188 mice suggesting an indirect effect of VEGF. These findings indicate that the insoluble VEGF188 isoform is insufficient to establish an epiphyseal vascular network that meets the oxygen needs of cartilage elements in growing bones, resulting in increased hypoxia. Additionally, our data provide in vivo and in vitro evidence for a previously unknown role for VEGF isoform-specific signalling, regulating chondrocyte development. We conclude that the integrated actions of the three VEGF isoforms are essential to couple metaphyseal and epiphyseal vascularization, chondrocyte development and ossification during endochondral bone formation.

Disclosures: ***C. Maes****, None.*

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Specific Role of Indian Hedgehog in Bone versus Cartilage *in vivo*. A. Eichbichler*[1], D. W. Soegiarto*[2], C. Carr*[1], T. Kobayashi[3], A. P. McMahon*[4], T. L. Clemens[5], B. Lanske[1]. [1]Oral and Developmental Biology, HSDM and Forsyth Institute, Boston, MA, USA, [2]Munich Biotech AG, Neuried, Germany, [3]Endocrine Unit, MGH, Boston, MA, USA, [4]The Biolabs, Harvard University, Cambridge, MA, USA, [5]Div. of Endocrinology, Univ. of Cincinnati, Cincinnati, OH, USA.

Mice lacking the Indian hedgehog gene (Ihh) survive only until birth and exhibit markedly reduced chondrocyte proliferation, abnormal chondrocyte maturation, and absence of mature osteoblasts. Since Ihh is expressed in chondrocytes as well as in osteoblasts, current animal models do not allow us to distinguish whether Ihh has a direct effect on osteoblasts or whether the effects on bone are indirectly mediated through chondrocytes during endochondral ossification. Furthermore, the postnatal function of Ihh is still unclear. We have generated mice using the *cre*-loxP/*flp*-frt gene technology which will allow us to specifically ablate, *in vivo*, the Ihh gene from several types of cells in the chondrocyte and osteoblast lineage, providing a way to uncover the exact role of Ihh in cartilage versus bone. We have established six independent floxed Ihh mouse lines (derived from J1 and R1 ES cell clones) and mated them to several *cre* transgenic mouse lines. Offspring from matings to the ubiquitously expressed *cre* transgenic animals driven by the phosphoglycerate-kinase I-(PGK-1) promoter resembled the phenotype of the conventional Ihh ko animals and established that we had efficient excision of the floxed Ihh gene by the *cre* recombinase. Breeding to collagen type I- and osteocalcin– *cre* transgenics have been performed as well. These mice selectively lack Ihh function in osteoblasts of varying degrees of maturity, and allow us to define the role of Ihh in osteoblasts independent of chondrocytes. Collagen type I-*cre* and osteocalcin-*cre* conditional Ihh ko animals survive postnatally, have shorter long bones and weigh slightly less. Serum ionized calcium levels do not show any major differences. Histological and μCT evaluation of bone density and microarchitecture are in progress. These novel mouse models should allow us to uncover the specific role of Ihh in cartilage versus bone, and this new information will provide a more secure framework for understanding Ihh regulation of chondrocyte and osteoblast development and activity.

Disclosures: ***A. Eichbichler****, None.*

1030

Combination of SOX5, SOX6 and SOX9 Is Sufficient for Chondrogenesis. T. Ikeda*[1], H. Kawaguchi[2], S. Kamekura*[2], I. Kou*[1], K. Hoshi[2], K. Nakamura*[2], S. Ikegawa*[1], U. Chung[2]. [1]Riken SNP Res. Ctr., Tokyo, Japan, [2]Tissue Engineering & Orthopaedic Surgery, Univ. of Tokyo, Tokyo, Japan.

To better understand skeletal development and devise treatment for irreversible cartilage damage requiring cartilage regeneration such as osteoarthritis, it is essential to know both the requisite and sufficient conditions for chondrogenesis at molecular levels. Although recent genetic studies have shown the necessity of SOX5, 6 and 9 for chondrogenesis *in vivo*, it is still unknown whether they are sufficient for chondrogenesis or other factors are also needed. To address this question, we expressed various combinations of SOX5, 6 and 9 in a variety of primary and immortalized cells by adenovirus vectors, and cultured them in serum-free medium without known chondrogenic supplements such as insulin, BMP and TGF-ß. Co-expression of SOX5, 6 and 9 (the SOX trio) started to induce differentiation of human mesenchymal stem cells (hMSC) into chondrocytes within 2 days; they expressed cartilage-specific mRNAs of type II, IX, XI collagens, aggrecan, chondromodulin-1, and matrilin-3 as well as cartilage-specific matrix by histology, to a level comparable to articular cartilage and chondrocytic cell lines within 2 weeks. Expression of SOX9 alone induced chondrogenesis to a much lesser extent, while that of SOX5 and/or 6 completely failed to do so. The SOX trio differentiated embryoid bodies derived from mouse ES cells into chondrocytes. Even cells committed to other lineages could be differentiated into chondrocytes: non-chondrogenic human immortalized cell lines HeLa, HEK293, HuH-7, and primary human dermal fibroblasts. Some non-chondrogenic cells responded to expression of SOX9 alone, but the response was correlated with endogenous expression levels of SOX5 and 6, further supporting the essential role of the SOX trio. Although the best efficiency of differentiation was achieved in 3-dimensional culture on atelocollagen gel, the SOX trio induced substantial chondrocytic differentiation even in monolayer culture, which is usually hostile to chondrogenesis. Contrary to the conventional methods, the SOX trio inhibited marker genes for hypertrophic chondrocytes and osteoblasts such as type I & X collagens, bone sialoprotein, Runx2, and osteopontin. A single application of the SOX trio adenovirus to a cortical bone defect in the mouse tibia produced a substantial cartilage within 1 week, while the LacZ virus failed to do so. These lines of evidence strongly suggest that the SOX trio is not only necessary but also sufficient for chondrogenesis both *in vitro* and *in vivo*. Rapid and potent induction of permanent cartilage from hMSC and dermal fibroblasts by transduction of the SOX trio may innovate cartilage tissue engineering.

Disclosures: ***T. Ikeda****, None.*

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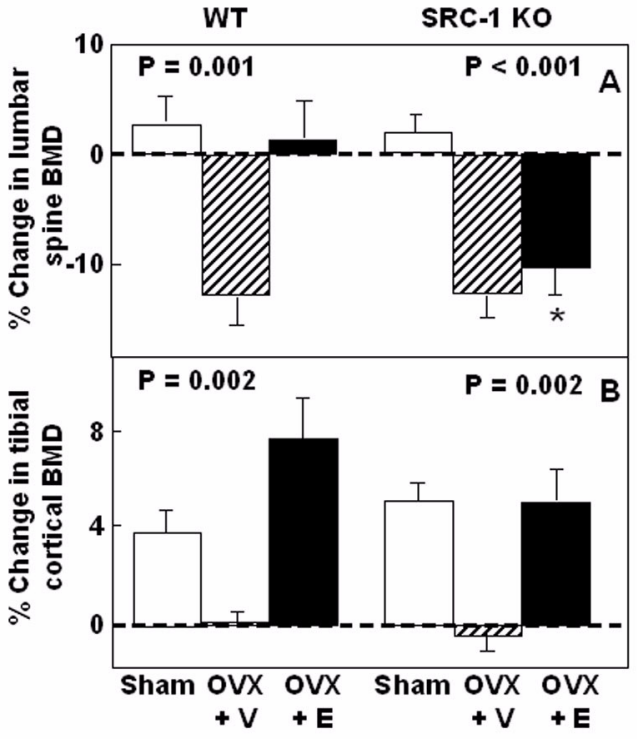
Steroid Receptor Coactivator (SRC)-1 Knockout Mice Have an Impaired Response to Estrogen in Cancellous but not in Cortical Bone. U. I. L. Moedder[1], B. L. Riggs[1], A. E. Kearns[1], A. Sanyal[1], J. D. Sibonga[2], J. Xu*[3], B. W. O'Malley*[4], T. C. Spelsberg[5], S. Khosla[1]. [1]Endocrinology, Mayo Clinic, Rochester, MN, USA, [2]Orthopedic Research, Mayo Clinic, Rochester, MN, USA, [3]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA, [4]Department of Molecular and Cellular Biology, Baylor College of medicine, Houston, TX, USA, [5]Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA.

SRC-1 is an important nuclear receptor coactivator that enhances estrogen (E) action in many tissues, but its role in mediating E effects on bone is unknown. We recently demonstrated that in osteoblastic cells, SRC-1 potentiates the activity of co-expressed ER-α/β or ER-β alone, with little or no potentiation of ER-α. Since cancellous bone contains both ER-α and -β, whereas cortical bone contains predominantly ER-α (JCEM 86:2309, 2001), we tested whether E action on bone would be impaired primarily in cancellous bone in SRC-1 knock out (KO) mice. Initial dose ranging studies in wild type (WT) C57BL/6 mice established an estradiol (E_2) dose of 10 μg/kg/d as the minimal physiologic E dose needed to prevent bone loss. Next, 3 month old WT and SRC-1 KO mice underwent either sham surgery, ovx with vehicle (V), and ovx with a 10 μg/kg/d E_2 pellet for 60 days (n = 9-11 per group). BMD was measured at baseline and the end of the study by DXA and pQCT. While this dose of E completely prevented loss of cancellous bone at the spine in WT mice, it was entirely ineffective in the SRC-1 KO mice (Panel A; P-values are for within strain ANOVAs; *P < 0.02 for comparison with WT, ovx + E group). However, E effects on cortical bone at the tibia were similar in the SRC-1 KO and WT mice (panel B). Bone histomorphometry confirmed the BMD measurements. Consistent with previous studies, we also found by RT-PCR that in mice, cancellous bone (at the vertebrae) contained both ER-α and -β, whereas only ER-α was detectable in cortical bone (at the femur shaft). We thus conclude that SRC-1 deficiency impairs the skeletal response to E in cancellous but not in cortical bone. We hypothesize that this may be due to the relative expression of ER-β versus -α in cancellous and cortical bone and the specific interactions of SRC-1 with these receptor isoforms in bone cells. Moreover, similar to mice, variations in SRC-1 levels or function may also determine the skeletal response to E in cancellous bone in humans.



Disclosures: ***U.I.L. Moedder****, None.*

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Recombinant hCG, but not DHT Stimulates Osteoblastic Collagen Synthesis in Older Men with Partial Age-Related Androgen Deficiency. C. Meier[1], P. Y. Liu*[2], L. P. Ly*[2], J. de Winter-Modzelewski*[1], M. Jimenez*[2], D. J. Handelsman*[2], M. J. Seibel[1]. [1]Bone Research Program, ANZAC Research Institute, University Sydney, Concord NSW, Australia, [2]Department of Andrology, ANZAC Research Institute, University Sydney, Concord NSW, Australia.

The relative contributions of androgens and estrogens on bone metabolism are still controversial. Within the setting of two double-blind, placebo-controlled studies, we evaluated the effect of DHT and recombinant human chorionic gonadotropin (rhCG) on bone turnover in healthy, community-dwelling older men with partial androgen deficiency (total testosterone [TT] ≤15 nmol/l twice).In the first study, 35 men (mean age 68.3±6.8 yrs, mean baseline TT 13.9±3.3 nmol/l) were randomized to receive either daily transdermal DHT gel (Andractim, Lab. Besins-Iscovesco; 70 mg; n=17) or placebo for 3 months. In the second study, 40 men (mean age 67.4±5.4 yrs, mean baseline TT 11.4±2.2 nmol/l) were randomized to receive either twice weekly rhCG sc (Ovidrel, Serono; 5000 IU; n=20) or placebo for 3 months. The following parameters were measured in serum before, at monthly intervals and 1 month after treatment: Aminoterminal propeptide of type I procollagen (PINP), osteocalcin (OC), carboxyterminal crosslinked telopeptide of type I collagen (ICTP), free (FT) and total testosterone, estradiol (E2), LH, and FSH. Compared to placebo, treatment with DHT resulted in a significant increase of serum DHT (15-20 nmol/l above baseline), paralleled by a suppression in LH, FSH, TT and FT, but not in E2 levels. Markers of both bone formation (serum PINP and OC) and bone resorption (serum ICTP) remained unchanged throughout the study. In contrast, administration of rhCG induced a significant rise in TT, FT and E2 levels. In these men, a significant rise of PINP levels with a max. increase after 1 month (p=0.02 compared to placebo) was observed, while serum levels of OC and ICTP did not change. The change in PINP (ΔPINP) was independent of baseline FT, TT and E2 levels, but correlated with the change in E2 levels (r=0.59, p=0.02). No significant association was found between ΔPINP and ΔTT (r=0.20, p=0.68) or ΔFT (r=0.40, p=0.14).
We conclude that in healthy older men with age-related androgen deficiency, pharmacological DHT doses suppress TT and FT without altering E2 levels or bone metabolism consistent with enhanced aromatization or direct DHT effects via androgen receptor (AR) to maintain stable bone turnover. In contrast, rhCG stimulates osteoblastic collagen formation proportional to increased blood estradiol without changing bone resorption. We conclude that while estradiol has prominent effects on bone in older men, direct AR-mediated androgen effects cannot be excluded.

Disclosures: ***C. Meier****, None.*

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Estrogen Prevents Bone Loss by Direct Targeting of TNF Producing $CD4^+$ Lymphocytes, and $CD4^+$ Dependent Regulation Of $CD8^+$ Activation. G. Toraldo[1], Y. Gao[1], W. Qien[2], K. Dark[2], M. Weitzmann[2], R. Pacifici[2]. [1]Division of Bone and Mineral Diseases, Washington University, St. Louis, St. Louis, MO, USA, [2]Division of Endocrinology, Emory University School of Medicine, Atlanta, GA, USA.

A key mechanism by which ovariectomy (ovx) induces bone loss is by expanding the population of TNF producing T cells via stimulation of T cell activation and proliferation. However, the involved T cell sub-populations remain unknown. FACS analysis of the spleens and bone marrow from sham operated mice demonstrated that ~ 40 % of both $CD4^+$ and $CD8^+$ T cells produce TNF. Ovx induced a ~ 2 fold expansion in both $CD4^+$ and $CD8^+$ T cells, although, no change in the % of TNF producing cells within each group was observed. Thus both $CD4^+$ and $CD8^+$ contribute to the enhanced TNF production of ovx mice. However, as there are ~ twice as many $CD4^+$ as $CD8^+$ T cells, TNF is mostly $CD4^+$ derived. To investigate how estrogen (E) regulates $CD4^+$ and $CD8^+$ populations, T cell deficient nude mice were reconstituted with either $CD4^+$ or $CD8^+$ by means of adoptive transfer of $CD4^+$ or $CD8^+$ cells harvested from intact WT mice, and then ovx or sham operated. Effective engraftment of was verified by FACS at 4 weeks after transplantation. Transplanted nude mice were found to contain 6-8 fold higher numbers of $CD4^+$ or $CD8^+$ T cells compared to non-transplanted nude controls, a number equal to ~ 50 % of that of normal WT mice. In vivo BrdU incorporation studies revealed that ovx induced a ~ 2.5 fold increase in the proliferation of both $CD4^+$ and $CD8^+$ cells in WT mice. Likewise, nude mice reconstituted with $CD4^+$ T cells showed enhanced T cell proliferation in response to ovx. In contrast, ovx failed to activate T cell proliferation in $CD8^+$ reconstituted nude mice, suggesting that activation of $CD8^+$ T cells requires signals generated by $CD4^+$ cells. This hypothesis was confirmed by in vivo bone density measurements by DEXA. We found nude mice to be completely protected against ovx induced bone loss, while WT controls lost ~10 % of their bone density in 4 weeks after ovx. In nude mice reconstituted with $CD4^+$ T cells ovx elicited a ~8 % bone loss ($p<0.05$), while sham operation was followed by a non significant ~2% increase in BMD. In contrast, ovx failed to induce bone loss in nude mice reconstituted with $CD8^+$ T cells as their BMD at 4 weeks after ovx was unchanged from baseline. We conclude that ovx regulates TNF production by $CD4^+$ T cell by directly inducing activation and proliferation of these lymphocytes. In contrast, CD8 activation is a secondary event induced by $CD4^+$ lymphocytes. Although, $CD4^+$ T cells are responsible for the majority of TNF production and ovx induced bone loss, $CD8^+$ cells contribute to this process by producing additional TNF as a result of signaling between $CD4^+$ and $CD8^+$ cells.

Disclosures: ***G. Toraldo****, None.*

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Osteopenia and Impaired Fracture Healing in the EP4 Receptor Knockout Mice. M. Li, D. R. Healy*, Y. Li*, H. A. Simmons, D. T. Crawford*, H. Z. Ke, L. C. Pan, T. A. Brown, D. D. Thompson. Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT, USA.

Prostaglandin E_2 (PGE_2) is a known bone anabolic agent and it binds to 4 receptor subtypes, EP1-EP4. In vitro and animal studies have suggested that the EP4 receptor subtype plays a critical role in PGE_2's bone anabolic effects. However, its role in the maintenance of bone mass and fracture healing is not known. Therefore, the studies reported here were designed to address this issue by characterizing the skeletal phenotype of EP4 receptor knockout (KO) mice and by comparing fracture healing in KO mice with wild type (WT) mice. Male EP4 KO and strain-matched WT mice at 15 to 16 months of age were used in these studies. There were no significant differences in body weights and femoral length between KO and WT mice. Lower bone mass was seen radiographically in both axial and

long bones of KO mice relative to WT mice. Micro-CT images of the distal femurs showed thinner cortices, fewer trabeculae, and deteriorated trabecular network in KO mice. The pQCT analysis showed significant decreases in total mineral content (-27%), trabecular content (-24%) and cortical content (-25%) of the distal femoral metaphyses in KO mice compared with WT controls. Histomorphometric measurements of the distal femoral metaphyses showed that trabecular bone volume, trabecular number, trabecular thickness, mineralizing surface, and bone formation rate were significantly decreased and osteoclast number was significantly increased in KO mice compared with WT mice. These data indicate that knocking out the EP4 receptor results in decreased bone formation and increased bone resorption, leading to loss of cancellous and cortical bone mass. To investigate whether EP4 receptor plays a role in fracture healing, KO and WT mice were subjected to transverse femoral fracture under general anesthesia. The fracture healing was examined by radiography and histomorphometry at 1, 2, 3, and 4 weeks after fracture. Callus formation was significant delayed as evidenced both radiographically and histologically in the fractured femurs of KO mice compared with those of WT mice. Histological measurements of fracture calluses showed significant decreases in total callus area and bony callus area by 31% and 43% at 2 weeks, and by 68% and 77% at 3 weeks, respectively, in KO mice compared with WT mice after fracture. By 4 weeks, complete bony bridging was seen in WT mice but not in KO mice. Our data demonstrate that the absence of EP4 receptor decreases bone mass and impairs fracture healing in mice. This finding suggests that the EP4 receptor is a positive regulator in the maintenance of bone mass and fracture healing.

Disclosures: ***M. Li****, None.*

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A Crucial Role for Thiol Antioxidants in Estrogen-Deficiency Bone Loss. J. M. Lean*, J. T. Davies*, K. Fuller, C. J. Jagger*, B. Kirstein*, G. A. Partington*, Z. L. Urry*, T. J. Chambers. Cellular Pathology, St George's Hospital Medical School, London, United Kingdom.

The mechanisms through which estrogen prevents bone loss are uncertain. In several other tissues, the beneficial effects of estrogen on lipids, endothelial cells and neurons are considered to occur through suppression of oxidant stress. Although high levels of reactive oxygen species (ROS) damage cells, lower levels serve as signaling molecules, and the ROS hydrogen peroxide stimulates osteoclastic bone resorption. Thus, estrogen might prevent bone loss by increasing antioxidant defenses in bone. We therefore tested the effects of ovariectomy and estrogen on the antioxidant defenses of rodent bone marrow. We found that glutathione and thioredoxin, the major thiol antioxidants, and glutathione and thioredoxin reductases, the enzymes responsible for maintaining these in a reduced state, fell substantially after ovariectomy. Levels were rapidly normalized by replacement doses of exogenous 17β-estradiol, but not by the inactive stereoisomer 17α-estradiol. To test whether these altered thiol antioxidant levels were causally related to bone loss, we administered N-acetyl cysteine (NAC) or ascorbate, antioxidants that increase tissue glutathione levels, or L-buthionine-(S,R)-sulphoximine (BSO), a specific inhibitor of glutathione synthesis, to mice. NAC and ascorbate abolished ovariectomy-induced bone loss. In contrast, BSO administration provided a phenocopy for estrogen-deficiency: it decreased bone marrow glutathione, increased osteoclast number and caused substantial bone loss.
The osteoclast is a prime candidate as target for antioxidant-mediated regulation, because osteoclastic function is dependent upon NFκB, which is regulated by ROS. We therefore tested the effects of estrogen on the thiol antioxidant system in osteoclasts. We found that 17b-estradiol strongly stimulated glutathione and thioredoxin reductases and glutathione in osteoclast-like cells in vitro.
Expression of cytokines such as IL-1, TNFα and IL-6, that have been shown to be crucial for the bone loss of estrogen-deficiency, is enhanced by ROS-mediated activation of NFκB. We found that NAC prevented activation of NFκB in osteoclasts. BSO did the opposite. Moreover, 17b-estradiol suppressed expression of TNFα and IL-1 in osteoclasts in culture. These observations strongly suggest that estrogen deficiency causes bone loss by lowering thiol antioxidants in osteoclasts, which leads to ROS-enhanced activation of NFκB and the expression of resorptive cytokines in osteoclasts, thereby promoting osteoclastic resorption while simultaneously impairing osteoblastic function.

Disclosures: ***J.M. Lean****, None.*

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Spatial Mediation of Intracortical Resorption. K. A. King*, N. A. Rabaia*, Z. Zhang*, S. Srinivasan, T. S. Gross. Orthopaedics, University of Washington, Seattle, WA, USA.

Intracortical resorption induced by disuse is highly focused within specific portions of the cortex, but the mechanism underlying this phenomenon is unknown. Recently, we have found that osteocytes upregulate the extracellular matrix protein osteopontin (OPN) in response to disuse and direct oxygen deprivation. Given the role of OPN in osteoclast chemotaxis, we hypothesized that intracortical resorption is temporally preceded by spatially co-localized elevations in osteocyte OPN expression. We assessed the validity of this hypothesis with a series of experiments ranging from the molecular to tissue levels. First, we used semi-quantitative competitive RT-PCR to assess the time course of OPN mRNA elevations in osteocyte-like MLO-Y4 cells exposed to direct oxygen deprivation for up to 24 hr. Normalized to β-actin expression, OPN mRNA was elevated 40% following 6 hr and 104% by 24 hr of oxygen deprivation compared to control normoxic cells. Next, we used image analysis to assess the spatial distribution of intracortical resorption sites induced by 6 wks of disuse in the midshaft of adult male turkey ulnae (n=3). Two sites of interest were identified, the first demonstrating a minimal number (mean + S.E.) of intracortical resorption events (central caudal cortex; 0.3 + 0.3), while the second site demonstrated profound intracortical resorption (cranial/ventral cortex; 20.3 + 1.8). We then turned to immunohistochemistry and confocal microscopy to assess osteocyte OPN expression in ulnae exposed to either 1 d (n=2) or 7 d (n=3) of disuse. Previously, we have found that approximately 16% of osteocytes stain positive for OPN in intact control bones. In the caudal cortex of bones exposed to disuse, 25.8 + 6.9% of osteocytes stained positive for OPN expression. In contrast, 48.2 + 12.9% of osteocytes demonstrated OPN expression in the cranial/ventral cortex. In summary, osteocytes rapidly upregulated OPN mRNA expression within a few hours of exposure to hypoxia. Within days, disuse induced osteocyte OPN upregulation in a spatially non-homogenous manner. Critically, specific locations of minimal and maximal osteocyte OPN expression observed at 1 and 7 d of disuse (prior to intracortical resorption) corresponded with the locations of minimal and maximal intracortical resorption observed following 6 wk of disuse. We conclude that these data support our initial hypothesis across a time scale ranging from hours to weeks and speculate that osteocyte OPN expression can be used to predict specific locations of intracortical resorption. As a result, it may be possible to identify novel strategies to minimize intracortical resorption, a disproportionate contributor to the degradation of bone strength associated with bone loss pathologies.

Disclosures: ***T.S. Gross****, None.*

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Mechanical Loading Induces the Expression of Wnt Pathway Genes and Activation of the Wnt Pathway in Osteoblasts Enhances Loading Responses. J. Robinson[1], M. Chatterjee-Kishore*[2], C. Li*[2], V. Gironda*[1], P. Green*[1], F. Bex[1]. [1]Women's Health Research Institute, Wyeth Research, Collegeville, PA, USA, [2]Genomics Division, Wyeth Research, Cambridge, MA, USA.

Increases in bone mass in response to skeletal loading is an important adaptive response which ensures the integrity of bone under vigorous physical stress. However, the molecular events that translate a mechanical stimulus into a biological response are not clear. Mechanical stress has been shown to induce genes including nitric oxide synthase (eNOS), cycloxygenase-2 (COX2) and prostacyclin synthase (PTGS). Low density lipoprotein receptor related protein (LRP) 5 and the Wnt/B-catenin pathway have recently been shown to play an important role in bone metabolism and have been implicated in the response of bone cells to stress. Therefore we designed experiments to determine whether mechanical loading could regulate Wnt/B-catenin related genes and whether activation of the Wnt/beta-catenin pathway could further modulate the expression of known stress responsive genes. Using a Flexercell strain (FX-3000) instrument, we applied mechanical strain (3,400 microstrain, 2Hz, 7200 cycles/hr) for 5 hrs to MC3T3 cells and analyzed by real time PCR the gene expression of known stress responsive genes as well as Wnt/B-catenin related genes immediately post-load or 24 hr post-load. The application of strain on MC3T3 cells elicited a significant induction of COX-2, eNOS, PTGS, connexin 43, jun, c-fos, cyclin D1, Wnt 10B and secreted frizzled related protein 1 (SFRP1) expression compared to non-loaded controls. We next tested whether activating the Wnt pathway in an osteoblast prior to applying a mechanical load would have a synergistic effect on gene expression following a loading stimulus. In these experiments, MC3T3 cells were treated with a glycogen synthase kinase-3β inhibitor (GSK3i) to increase B-catenin nuclear translocation and thereby activate the canonical Wnt/B-catenin pathway. Immediately following GSK3i administration, the cells were subjected to load (3,400 microstrain, 2Hz, 7200 cycles/hr) for 5 hrs. The GSK3β inhibitor treatment resulted in increases in COX-2 (3X), eNOS (3X), connexin 43 (2X), jun (3X), c-fos (3X), cyclin D1 (2X), Wnt 10B (2X) and SFRP1 (3X) above the load response alone.
In summary, these experiments further support the effects of mechanical load on the expression of genes known to influence osteoblast growth and differentiation and now show that transcriptional activation of Wnt/B-catenin pathway genes may also be involved in a loading response. Additionally, our data suggest that activation of the canonical Wnt/B-catenin pathway in osteoblasts makes cells more responsive to a loading stimulus.

Disclosures: ***J. Robinson****, None.*

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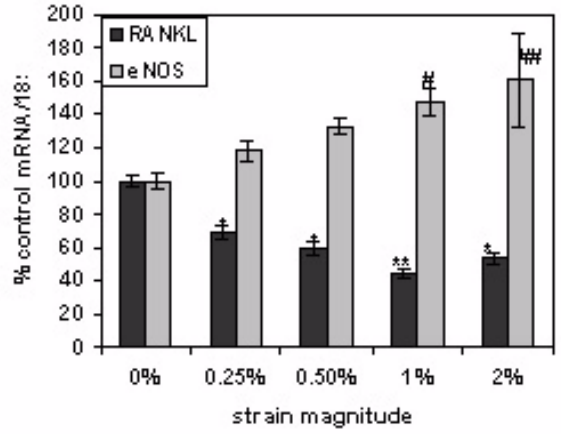
Mechanical Strain Differentially Regulates eNOS and RANKL Expression via ERK1/2 in Primary Bone Stromal Cells. J. Rubin, T. C. Murphy*, L. Zhu*, E. Roy*, M. S. Nanes, X. Fan. Medicine, VAMC & Emory University, Decatur, GA, USA.

Complex interactions between signals controlling both osteoblast and osteoclast functions are ultimately responsible for the adaptive response of the skeleton to its loading environment. We here evaluate the cellular response to mechanical input in terms of 2 signals which affect bone coupling, nitric oxide (NO) and RANKL. NO, released after mechanical stimulation from many cell types, has been implicated in both repression of osteoclast activity and stimulation of osteoblast function. In mature bone, NO is generated from endothelial nitric oxide synthase (eNOS). In contrast, RANKL expression promotes resorption. We have previously shown that straining bone stromal cells diminishes RANKL expression via ERK1/2 activation. Here we found that application of 2% strain (BioFlex, Flexcell) to primary murine stromal cultures increased NO secretion and eNOS expression as measured by real-time PCR. iNOS was unchanged. The effect of strain on eNOS was magnitude-dependent increasing to 161 ± 28% that of control eNOS (see figure). Concurrently, 0.25% strain significantly inhibited RANKL expression with increased repression at 1% strain (44 ± 3% cf with controls). Both responses to strain were blocked when an ERK1/2 inhibitor was present during strain application. To confirm ERK1/2's central role, cells were treated with an adenovirus encoding constitutively active MEK (Ad.caMEK). Ad.caMEK increased basal ERK1/2 activation resulting in increased eNOS expression and NO production by >4-fold and decreased RANKL expression by half. In contrast, inhibition of JNK failed to prevent strain effects. Thus we have shown that strain has a magnitude-specific ability to control two genes in entirely divergent directions. For the coordinate regulation of eNOS and RANKL, the mechanical effect relies on activation of ERK1/2. The divergent gene expression resulting from ERK1/2 activation suggests that the final regulatory result is determined by recruitment of specific co-regulators. In total, in

vitro strain represents a paradigm for understanding how loading the skeleton results in positive bone remodeling.



Disclosures: ***J. Rubin****, None.*

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VEGF-Mediated Angiogenesis Is a Key Event for Exercise-Induced Bone Gain. Z. Yao*[1], M. Lafage-Proust[1], J. Plouët*[2], C. Alexandre[1], L. Vico[1]. [1]Lbto, INSERM E366, Fac Med, St Etienne, France, [2]CNRS UMR 5089, Toulouse, France.

Appropriate physical training is able to increase bone mass and strength. But its mechanism remains unclear. Although increased capillary supply during training-induced muscle hypertrophy is well described, no parallel mechanism was studied in bone. Among vasoactive factors, VEGF has multi-functional activities on wall vessel cells and is an osteoblastic growth factor. We hypothesized that bone vascular and VEGF changes are involved in bone remodelling alteration leading to increased bone mass. Nine-week male Wistar rats underwent treadmill training experiment at 60% of their VO2max. (regimen showed to be osteogenic after 5 weeks, Bourrin et al., JBMR 1995). Exercise-induced BMD changes were assessed longitudinally by Dexa and bone cellular activities and vessel number and area - after India ink/barium sulphate infusion - were analyzed by histomorphometry (n=8/group) in tibial metaphyses. VEGF and Flt1/R1 and KDR/R2 receptor expressions in tibia were evaluated by on line RT-PCR (n=6/group). We tested several running period durations: 14 and 10 days, and 5 weeks. In the latter experience, a VEGF inhibitory antibody (RUN-anti-VEGF) or a placebo (RUN) was administrated IP twice a week. Results obtained in running rats were compared to those of sedentary rats (SED). After a 14d. running period metaphyseal vessel number increased by 20% in RUN vs. SED ($p<0.05$), this increase was associated with an increased bone formation rate ($p<0.001$) and a decreased active resorption surfaces ($p<0.03$), while trabecular bone mass had not changed yet. Mineralizing surfaces positively correlated with vessel number ($r= 0.85$, $p<0.001$) in running rats. A 10-day training period induced an up-regulation of VEGF (153%) and Flt1 (83%, $p<0.01$) in the tibial periosteum and metaphysis while KDR expression increased not significantly. After a 5-week training period, metaphyseal tibial BMD did increase by 9% in RUN rats only vs. SED. Cancellous bone mass increased by 21% in RUN compared to RUN-anti-VEGF and SED groups ($p<0.03$). Anti-VEGF antibody also prevented the increase in vessel number observed in RUN tibial metaphysis (25%, $p<0.02$ vs. SED). In RUN group, bone formation rate resumed at this time while osteoclastic surfaces continued to decline (22%, $p<0.05$) as compared to both SED and RUN-anti-VEGF. We showed that an osteogenic exercise regimen induced an early VEGF expression increase in bone tissue, followed by an increased cancellous vessel network which was associated to bone remodelling uncoupling. These bone and vascular events preceded the exercise-induced increase in bone mass and are fully prevented by a VEGF antagonist.

Disclosures: ***L. Vico****, None.*

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PTH Replaces Cancellous Bone Lost During 28 Days of Hindlimb Unloading in Skeletally Mature Rats. S. A. Bloomfield[1], M. R. Allen[1], H. Lemmon*[2], H. A. Hogan[2]. [1]Dept of Health & Kinesiology, Texas A&M University, College Station, TX, USA, [2]Dept of Mechanical Engineering, Texas A&M University, College Station, TX, USA.

Skeletally mature rats subjected to 28 days of hindlimb unloading (HU) experience a significant loss of proximal tibia bone mineral density (BMD) and plantar flexor muscle strength. Although muscle strength recovers within 14 days of reambulation, there is no sign of BMD recovery through 28 days. The purpose of this study was to evaluate the effectiveness of daily PTH (1-34; 80µg/kg/day) administration on the recovery of bone parameters following 28 days of HU. Six-month-old male Sprague Dawley rats were divided into 3 groups: 28 day HU (HU; n=4), 28 day HU + 28 day recovery with vehicle (VEH; n=8) and 28 day HU + 28 day recovery with PTH (PTH; n=8). Both recovery groups received daily subcutaneous injections for 28 days during recovery from HU. In vivo peripheral quantitative computed tomography (pQCT; Stratec, Research M) scans of the proximal tibia metaphysis, providing total and cancellous bone mineral density (BMD), were obtained at baseline, after 28 days of HU (R+0), and at recovery days 14 (R+14) and 28 (R+28). In vivo peak isometric torque (ISO) and mass of plantar flexor muscles were obtained at sacrifice. VEH- and PTH-treated groups both had non-significantly higher body mass at R+28 compared to HU (+11 and 14%, respectively). After HU (R+0), there was a significant loss of total and cancellous BMD (-7 and 9%, respectively). At R+14, total and cancellous BMD of VEH-treated animals (544 ± 14 and 223 ± 10 mg/cm^3, respectively) remained similar to R+0 values while PTH-treated animals significantly increased both total and cancellous BMD (635 ± 17 and 257 ± 6 mg/cm^3, respectively) to baseline levels. By R+28, BMD of VEH animals remained at R+0 levels while values had significantly increased over baseline values in PTH-treated animals (+11 and 21% for total and cancellous BMD). Muscle mass was significantly greater in both VEH and PTH groups compared to HU (+37 and 29%, respectively) while ISO was non-significantly higher (+3 and 4%, respectively). A subset of bones (n=2/gp) were analyzed ex vivo using microCT (EVS, Explore RS) at the same location as studied by pQCT. PTH-treated animals had significantly higher cancellous BV/TV (+ 141%), accounted for by higher trabecular thickness (+ 33%) and number (+ 83%), compared to VEH-treated animals; there was no difference between HU and VEH-treated values. These data demonstrate that daily PTH injections significantly enhance the recovery of unloading-induced decrements in bone density, apparently due to significant increases in cancellous bone volume.

Disclosures: ***S.A. Bloomfield****, None.*

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Genetic Variations Regulate Bone Loss and Gene Expression Related to Skeletal Unloading. M. Squire[1], L. Donahue[2], C. Rubin[1], S. Judex[1]. [1]Biomedical Engineering, SUNY Stony Brook, Stony Brook, NY, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA.

The influence of the genome on the skeleton is highlighted not only by variations in bone morphology but also by a differential sensitivity to altered levels of mechanical loading. Using genetically distinct strains of inbred mice, we previously demonstrated that *female* BALB/cByJ (BALB) mice are highly sensitive to their mechanical environment (losing up to 60% of femoral trabecular bone in 3wk) while trabecular and cortical bone of C3H/HeJ (C3H) mice is less responsive. Here, we subjected *male* adult BALB and C3H mice (16wk old, n=10 each) to hindlimb unloading to determine the influence of genetics and gender on the magnitude of bone loss and to investigate whether alterations in bone morphology are paralleled by changes in the expression of candidate genes. After 21 days of unloading, indices of bone morphology in the distal femur were assessed via high-resolution (11 µm) micro computed tomography scanning (µCT). Total RNA was extracted from the proximal tibia (including bone marrow and cartilage, n=4 each) and relative changes in gene expression (normalized to GAPDH) were determined using real-time reverse transcription polymerase chain reaction. In stark contrast to our previous data from female mice, male BALB mice subjected to hindlimb unloading did not lose trabecular bone from the distal femoral metaphysis, yet C3H mice lost 22% ($p<0.03$) of metaphyseal trabecular bone. Additionally, both male BALB and C3H mice lost significant amounts of epiphyseal trabecular bone (23% and 15%, respectively, $p<0.008$), but loss of functional weight bearing did not produce changes in metaphyseal cortical bone of either strain. At the molecular level, hindlimb unloading down-regulated the expression of collagen type I (α1) by 23% ± 13% (mean ± SE) in BALB mice. Expression levels of bone morphogenic protein 2 (BMP-2) and receptor activator of NF-κB ligand (RANKL) were also lower in disuse BALB than in controls (31% ± 18% and 18% ± 12%, respectively). In C3H males, however, collagen I expression levels were not different between disuse and control mice, while BMP-2 and RANKL expression levels were 25% ± 9% and 26% ± 19% higher, respectively, in disuse mice. These data indicate a strong influence of genetics and gender on both the susceptibility of bone to disuse osteoporosis and the specific sites within bone that are most heavily affected. The differential expression of genes involved in bone formation and resorption further demonstrate the strain specificity of bone's response to unloading. The future exploration of the underlying molecular and genetic basis may enable the early identification of individuals most likely to suffer from osteoporosis based on their genotype.

Disclosures: ***M. Squire****, None.*

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Rest-Insertion Augments Periosteal Bone Formation Rates in Response to Short-Term High-Frequency Loading. J. M. LaMothe*[1], G. M. Peters*[1], T. S. Gross[2], S. Srinivasan[2], R. F. Zernicke[3]. [1]Faculty of Kinesiology, University of Calgary, Calgary, AB, Canada, [2]Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, WA, USA, [3]Faculties of Kinesiology, Medicine, and Engineering, University of Calgary, Calgary, AB, Canada.

High-frequency vibrations can stimulate bone growth, and insertion of short rest periods between consecutive loading cycles may also augment bone formation rates. Thus, we investigated if rest-insertion increased bone growth in response to short-term high-frequency loading in the mature murine skeleton. Right tibiae of skeletally mature (16 wk) female C57BL/6 mice were loaded in cantilever bending at 800 µ (*in vivo* strain gauge calibration; CV = 14%), 30 Hz, 5 d/wk for 3 wk. Mice were randomized into one of two groups: continuous high-frequency (HF) stimulation for 100 s (n = 9), or 1-s pulses of high-frequency stimulation followed by 10 s of rest (rest inserted = RI) for 100 s (n = 9). Upon completion of the 3 wk loading protocol, mice were allowed 4 d of cage ambulation before euthanization. Calcein injections were administered on days 1 and 21; label incorporation was used histomorphometrically to assess (blinded) periosteal bone formation rate (BFR) with bone surface as a referent. Pilot data showed that anaesthesia and immobilization in the loader did not alter indices of bone formation, and that the contralateral limb suitably represented the normal control situation. Non parametric Mann Whitney tests identified differences between groups, and Wilcoxon tests identified differences between left and right limbs ($p < 0.05$). All values were represented as means ± standard deviation. HF stimulation alone significantly increased BFR (0.43 ± 0.20 µm/day) compared to the

contralateral control limb (0.13 ± 0.10 μm/day). Similarly, RI loading significantly ($p < 0.05$) increased BFR (0.76 ± 0.31 μm/day) compared to the contralateral control limb (0.35 ± 0.19 μm/day). BFR in response to RI loading was significantly increased compared to HF loading (77%; $p < 0.05$). Thus, short-term, moderate-magnitude high-frequency loading augmented periosteal bone formation rates in skeletally mature female C57BL/6 mice. Furthermore, high-frequency stimulation was more osteogenic if applied in short pulses with intermittent rest periods, despite a ten-fold decrease in the absolute number of loading cycles. Optimization of osteogenesis in response to exogenous loading may underpin the development of non-pharmacological regiments designed to increase bone strength in individuals with compromised bone structures.

Disclosures: ***J.M. LaMothe****, None.*

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The COMT Val158Met Polymorphism Is Highly Associated with Maturation and Musculoskeletal Growth in Early Pubertal Girls. A. L. Eriksson[1], M. Suuriniemi[2], A. Mahonen[3], S. Cheng[2], C. Ohlsson[1]. [1]Centre for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]LIKES-Foundation for Sport and Health Sciences and Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [3]Department of Medical Biochemistry, University of Kuopio, Kuopio, Finland.

Estrogen plays a vital role in the growth of adolescents. Catechol-O-Methyltransferase (COMT) is a key enzyme in the degradation of estrogens. A functional polymorphism in the COMT gene (val158met) decreases the activity of the enzyme by 60-75%. The aim of the present study was to investigate the influence of the COMT polymorphism on maturation and growth in early pubertal Finnish girls.

246 healthy 10-12 year old girls in Tanner stage 1-3 were genotyped for the COMT val158met polymorphism. They were classified as homozygous for the low activity genotype ($COMT^{LL}$), heterozygous ($COMT^{HL}$) or homozygous for the high activity genotype ($COMT^{HH}$). The relationship between COMT genotype and height, bone phenotype, hormone levels and pubertal development was investigated.

Girls with $COMT^{LL}$ were 5,4 cm taller than girls with $COMT^{HH}$ and 2,4 cm taller than $COMT^{HL}$ (p ANOVA = 0,001). DEXA measurements showed an increase in BMC and bone area in total body, femur neck, total femur, and L2-L4 spine in $COMT^{LL}$ compared to $COMT^{HH}$. $COMT^{HL}$ was an intermediate group. No major effect was seen on aBMD. pQCT measurements of the tibia showed an increased BMC in $COMT^{LL}$. This was due to an increased cross sectional cortical area which in turn was caused by an increased periosteal circumference. No influence was seen on cortical or trabecular vBMD suggesting that the effects were caused by an increased longitudinal and radial growth.

$COMT^{LL}$ had higher levels of free estradiol and serum IGF-1, and they were more likely to be in Tanner stage 2 or 3 than $COMT^{HH}$. Statistical regression models indicated that the effects of COMT genotype on bone parameters were exerted via a regulation of estrogen and/or IGF-1. The COMT genotype also influenced cross sectional muscle area. The effect on cortical bone vanished when the muscle area was included in a linear regression model suggesting that the COMT effect on cortical bone is mediated via increased muscle mass.

In conclusion the COMT polymorphism is highly associated with height, cortical bone mass and pubertal development in early pubertal girls. Most of these effects can be explained by altered estradiol and IGF-1 status. We propose that a low activity COMT phenotype results in increased estradiol levels which in turn increase the activity in the GH/IGF-1 axis, resulting in increased longitudinal and radial bone growth and earlier pubertal development.

Disclosures: ***A.L. Eriksson****, None.*

1044

Risk Factors for Upper Limb Fracture in Children: A Population Based Case-Control Study. D. Q. Ma, G. Jones. Menzies Research Institute, Hobart, Australia.

Fractures in younger life are very common especially those involving the upper limb. However, the causes of these fractures are poorly understood. The aim of this population based case-control study was to evaluate the association of both bone dependent and independent factors with upper limb fracture risk in children 9-16 years of age. Areal (aBMD) and apparent (BMAD) bone mineral density were measured by dual energy X-ray absorptiometry (DXA). Skeletal age (SA) and metacarpal index (MI) was determined by hand radiograph. Types and patterns of physical activity, risk taking and diet were assessed by interview-administered questionnaires. Coordination was measured using the 8-point movement ABC. A total of 321 fracture cases and 321 randomly selected age- and gender-matched controls were recruited. Fracture sites were as follows: hand: n=91, wrist and forearm: n=190, upper arm: n=40. Conditional logistic regression analysis showed wrist and forearm fracture risk was significantly associated with lumbar spine BMAD (OR: 1.52/SD reduction, 95% CI 1.18-1.96), MI (OR: 1.43/SD reduction, 95% CI 1.12-1.79), television, computer and video viewing (OR: 1.58/category, 95% CI 1.14-2.20), light physical activity (OR: 0.81/unit, 95% CI 0.65-1.00), cola consumption (OR: 1.43/category, 95% CI 1.03-1.97) and walking backwards score (OR: 1.17/unit, 95% CI 1.02-1.34). Hand fracture was significantly associated with the difference between skeletal age and chronological age (OR 1.45/year reduction, 95%CI 1.05-1.96), high-risk sport participation (1.43/sport, 95% CI 1.08-1.88) and total risk taking score (OR: 1.94/unit, 95% CI 1.01- 3.72). Upper arm fracture risk was only significantly associated with high-risk sport participation (OR: 2.73/sport, 95% CI 1.27-5.84) but conclusions may be limited by sample size considerations. Obesity or overweight was not associated with any fracture type. For total fractures, there was a gender discordant effect of sports participation with an increased fracture risk in boys and decreased fracture risk in girls which reached statistical significance for total, contact, non-contact and high-risk sports participation as well as four individual sports (soccer, cricket, surfing and swimming). In conclusion, both bone dependent and independent factors are important determinants of upper limb fracture risk in children. There is heterogeneity of cause for both gender and different fracture sites which will necessitate different approaches to prevention.

Disclosures: ***D.Q. Ma****, None.*

1045

A Co-Twin Calcium Intervention Trial in Young Females: Effects Measured by Hip Structural Analysis. J. D. Wark[1], L. M. Paton*[1], T. J. Beck[2], C. Nowson*[3], M. Cameron*[1], S. Kantor*[1], H. A. McKay[4], M. Forwood[5]. [1]Medicine, University of Melbourne, Parkville, Australia, [2]Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [3]School of Health Sciences, Deakin University, Burwood, Australia, [4]School of Human Kinetics, University of British Columbia, Vancouver, BC, Canada, [5]Anatomy and Developmental Biology, University of Queensland, Brisbane, Australia.

A high dietary calcium intake is widely recommended for children to enhance peak bone mass but the effect of calcium on the biomechanical properties of the proximal femur is not widely reported. We conducted a randomized, placebo-controlled trial of calcium supplementation (1000 - 1200mg daily) in 79 pairs of female twins aged (mean (SD)) 12.2 (2.0) years at baseline, using a powerful co-twin control design. Bone properties were able to be evaluated by Hip Structural Analysis (HSA) of proximal femur densitometry scans in 64 twin pairs (34 monozygotic, 30 dizygotic) up to 18 months intervention. HSA parameters were measured at the narrowest segment of the femoral neck (NN), intertrochanteric (IT) and upper femoral shaft (FS) sites. All data were adjusted for age, height and weight, none of which differed between groups at any time point. After 18 months at the IT and FS sites, there were significant within- pair differences in percent change from baseline comparing calcium – placebo treated twins, respectively: at the IT site, in areal bone mineral density (ABMD) [2.0 %, $p = 0.04$] and the average buckling ratio [-4.2 %, $p = 0.002$], respectively; at the FS site in ABMD [2.7 %, $p = 0.01$], endocortical diameter [-3.1%, $p = 0.02$], average cortical thickness [3.4%, $p = 0.01$] and average bucking ratio [4.7%, $p = 0.009$], respectively. At 6 months there was a difference in NN subperiosteal width and endocortical diameter but this was not maintained. Therefore, observed effects were limited to the IT and FS sites. At the femoral shaft there was inhibition of modelling at both endosteal and periosteal surfaces, resulting in narrowing of the femoral shaft and a thicker cortex. The implications for adult bone health are uncertain since the effects were more evident at the shaft site rather than the more proximal sites where osteoporotic fractures are common. Our findings complement the sparse body of literature that, importantly, investigates the geometric adaptation of growing bone to lifestyle factors. It is possible that calcium given during growth may reduce bone fragility in later life through an increase in cortical thickness.

Disclosures: ***J.D. Wark****, None.*

1046

Adult Bone Structure and Strength are Influenced by Lean Mass, Exercise History, and Estradiol Levels: The 10-year Longitudinal Penn State Young Women's Health Study. M. A. Petit[1], T. J. Beck[2], H. Lin*[1], C. Bentley*[1], T. Lloyd[1]. [1]Dept. of Health Evaluation Sciences, Penn State University, Hershey, PA, USA, [2]Dept. of Radiology, Johns Hopkins University, Baltimore, MD, USA.

We used longitudinal data from the 10-year Penn State Young Women's Health Study to establish determinants of adult bone mass, structure and strength. 112 participants were enrolled in the study at age 12 and measurements made bi-annually for 6 years and annually thereafter. Proximal femur DXA scans (Hologic QDR 2000) were taken from age 17-22y and analyzed using a hip structure analysis program to assess areal BMD (g/cm^2), subperiosteal width, cortical thickness, bone axial strength (cross-sectional area, CSA) and bone bending strength (section modulus) at the femoral neck and femoral shaft regions. Height and weight were measured by standard procedures and body composition taken from DXA total body scans. Other variables were measured at each time point and the average of all data from age 12-22y used including: calcium and total calorie intake from 3-day food records; estradiol and testosterone values converted to log transformed and then standardized scores; sports exercise score by questionnaire; and age at menarche. Backward regression models were used to assess determinants of change in bone variables and absolute bone values at age 21-22y, controlling for height and weight in each model. Models were run both with and without lean mass. Femoral neck section modulus (+3.5%) and width (+1.6%), but not BMD (-0.8%), increased significantly from age 17-22y. At the shaft, all variables increased (+1.0-4.0%, $p < 0.01$) from age 17-22y. After controlling for changes in body weight and height, none of the variables contributed significantly to predicting change. Absolute bone values at age 22y were predicted primarily by lean mass, sports exercise scores, and average estradiol levels (controlling for height and weight). At both the neck ($r^2 = 0.48$, $p < 0.01$) and the shaft ($r^2 = 0.67$, $p < 0.01$), only lean mass predicted section modulus. When lean mass was removed from the model, sports exercise score replaced lean mass as a predictor at both neck ($r^2 = 0.41$, $p < 0.01$) and shaft ($r^2 = 0.61$, $p < 0.01$) sites. For neck cortical thickness, BMD, and CSA, both estradiol and sports exercise history were significant predictors ($r^2 = 0.20$-0.40, $p < 0.01$), and for bone width testosterone (negative) and lean were significant ($r^2 = 0.48$). Results were similar for each geometric variable at the shaft site. These data suggest bone adapts its bending strength primarily to mechanical loading either through lean body mass or adolescent exercise.

Disclosures: ***M.A. Petit****, None.*

1047

Maternal Vitamin D Status During Late Pregnancy and Accrual of Childhood Bone Mineral. M. K. Javaid[1], S. R. Shore*[1], P. Taylor*[2], C. Gale*[1], B. J. Boucher*[3], K. Noonan*[3], K. M. Godfrey*[1], N. K. Arden*[1], C. Cooper[1], &. The Princess Anne Hospital Study Group*[1]. [1]MRC Environmental Epidemiology Unit, University of Southampton, Southampton, United Kingdom, [2]Medical Physics and Bioengineering, Southampton University Hospitals NHS Trust, Southampton, United Kingdom, [3]Metabolic Medicine, Royal London Hospital, London, United Kingdom.

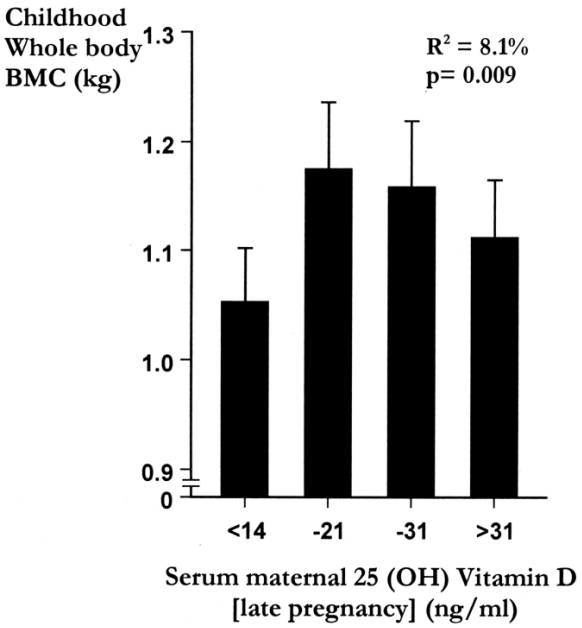
Enhancing peak bone mass is a key component of strategies for the primary prevention of osteoporotic fractures in later life. There is growing evidence that the childhood skeletal growth trajectory is established during early life, and epidemiological studies suggest that the mechanism may involve programming of the vitamin D axis.

We therefore investigated specific maternal and neonatal determinants of childhood bone mass in 211 children (113 boys), who were measured at birth and for whom maternal anthropometry, lifestyle characteristics and serum 25OH vitamin D concentrations (25OHD) during late pregnancy were determined. Now aged nine years, the children have had body composition measured using DXA.

There was a significant positive (r=0.21; p=0.008) association between maternal 25OHD in late pregnancy and the whole body bone mineral content (WBBMC) of the children nine years later. However, there was a threshold in the relationship such that mothers with serum 25OHD concentrations below 14 ng/ml (lowest quartile) had children with significantly (p=0.001) lower WBBMC than those born to mothers more vitamin D replete (Figure). Maternal use of vitamin D supplements and maternal sunshine exposure were significant (p<0.01) determinants of maternal vitamin D status, as well as of childhood WBBMC (p<0.05).

These observations support the hypothesis that a mothers vitamin D status during pregnancy has persisting effects on bone mass accrual rates of her child. The use of vitamin D supplements during pregnancy may increase bone mass accrual during childhood and reduce that child's risk of osteoporotic fracture in later years.

Maternal 25OHD concentration during late pregnancy predicts whole body bone mass of her child nine years later



Disclosures: ***M.K. Javaid****, None.*

1048

Effects of Use and Discontinuation of Depot Medroxyprogesterone Acetate Injectable Contraception on Bone Density in Adolescents: Results from a Longitudinal Study. D. Scholes[1], A. Z. LaCroix[1], L. E. Ichikawa*[1], W. E. Barlow*[1], S. M. 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 (Depo-Provera, DMPA) is associated with decreased bone mineral density (BMD), but bone effects in adolescents have received little study. We report on a prospective evaluation of the effects of DMPA on bone in a cohort of 174 adolescents. Participants, ages 14-18 at baseline, were enrollees of a Washington state HMO and were selected using computerized databases. We recruited 81 participants who were using DMPA (range 1-39 mo. at baseline) and 93 comparison women. Hip, spine and whole body BMD were measured by DEXA every 6 months for up to 36 months. We collected data on DMPA use, bone density, and other variables via questionnaire and exam. At baseline, DMPA users were significantly more likely to be African-American, current smokers, to have been pregnant, and to have lower calcium intake; BMD at baseline did not differ significantly between the exposure groups. Data were available for 157 (90%) participants at 6 mos, 146 (84%) at 12 mos, and 135 (78%) at 24 mos. Relative to comparison women, continuous users of DMPA showed significantly decreased BMD at the hip and spine after 12 months (see table). After 24 months, hip BMD for DMPA users declined by 4.5%. Women who discontinued DMPA had 12-month mean percent increases in BMD which were significantly greater than non-users at the hip and whole body.

Adjusted Mean Percent Change in BMD after 12 months, by DMPA Exposure Status

DMPA status	N	Total hip	Spine	Whole body
Continuous use	44	-2.31*	-1.49**	+0.28
Discontinued	29	+1.61*	+2.73	+3.02*
Non-use (comparison)	73	-0.46	+1.81	+1.02

Adjusting for: baseline BMD, age, ethnicity, gravidity, smoking, physical activity, calcium intak

P-values, relative to non-use: *p<0.005; **p<0.0001

These longitudinal data provide evidence that DMPA use among adolescents is strongly associated with bone density loss. These losses may be largely reversible, as indicated by the significant increases in BMD 12 months post-discontinuation.

Disclosures: ***D. Scholes****, None.*

1049

Regulation of Bone Formation by Wnt Signaling. D. Glass*[1], M. Patel[1], F. Long*[2], M. M. Taketo*[3], A. McMahon*[4], G. Karsenty[1]. [1]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA, [2]Washington University Medical School, St. Louis, MO, USA, [3]Biomedical Genetics, The University of Tokyo, Tokyo, Japan, [4]Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA.

Lrp5 loss-of-function mutations lead to low bone mass with fractures while Lrp5 gain-of-function mutations lead to high bone mass and fracture resistance thus identifying Lrp5 as one of the most important regulators of skeletal physiology discovered to date. Lrp5 is thought to signal in response to Wnt proteins but this has not been demonstrated in vivo. To better understand the nature of the Lrp5 signaling pathway we directly activated the canonical Wnt signaling pathway only in osteoblasts in vivo. These mice, harboring a constitutively active allele of β-catenin, have a high bone mass phenotype that, when crossed onto an Lrp5 null background, completely rescues the low bone mass of Lrp5 deficiency. In a converse experiment, we generated an osteoblast-specific deletion of β-catenin; our preliminary analyses indicate that these mice recapitulate the low bone mass of Lrp5 deficiency. To further demonstrate the role of β-catenin in Lrp5 signaling, we also generated transgenic mice expressing the high bone mass $LRP5^{G171V}$ allele in osteoblasts. If β-catenin is indeed downstream of Lrp5, then selective deletion of β-catenin in the osteoblasts of these high bone mass $LRP5^{G171V}$ mice should rescue their phenotype. To test this we are crossing $LRP5^{G171V}$ transgenics to mice with a conditionally null allele of β-catenin in the presence of an *α(I)1 collagen-cre* transgene. Lastly, we used a candidate gene approach to identify the transcriptional partner for β-catenin in osteoblasts. These gene deletion experiments identified a member of the Lef/Tcf family as the putative DNA binding partner of β-catenin. Our results so far strongly suggest that Lrp5 signals through the canonical Wnt signaling pathway in vivo, thus identifying Wnt proteins as critical regulators of differentiated osteoblast function.

Disclosures: ***D. Glass****, None.*

1050

The Osteogenic Activity of β-catenin Requires Interaction with BMP-2 Effectors. G. Mbalaviele, S. Sheikh, S. Cheng, J. Stains, R. Civitelli. Bone and Mineral Diseases, Washington University, St. Louis, MO, USA.

Mutations of critical elements of the *Wnt* pathway (Lef1, LRP-5) affect skeletal development and bone mass acquisition, and β-catenin, a component of *Wnt* signaling controls osteogenic differentiation. We have previously reported that a transcriptionally active N-terminal truncated β-catenin mutant (ΔN151) commits mesenchymal stem cells to the osteogenic pathway, while inhibiting adipogenesis. Retroviral transduction of ΔN151 in the uncommitted, multipotential C3H10T1/2 cells has no effect on osteogenesis, but it synergistically stimulates BMP-2 induction of osteoblast genes and matrix mineralization. We now find that in metatarsal bone rudiments from 17-day mouse fetuses, transduction of ΔN151 by itself enhances mineralization 1.5-fold relative to LacZ transduced metatarsals, an effect similar to that of BMP-2. Thus, β-catenin signaling alone is an insufficient activator of osteoblast differentiation *in vitro*, but it is active *in vivo* probably because it synergizes with endogenously produced activators. To test this hypothesis, we studied β-catenin-BMP-2 interactions in C3H10T1/2 cells using promoter-luciferase constructs. As expected, ΔN151 was able to stimulate Tcf-Lef dependent transactivation (2-fold relative to LacZ transduced cells), but BMP-2 was not. By contrast, SMAD-dependent transactivation was stimulated 7-fold by BMP-2, but not by ΔN151. Consistent with a Tcf/Lef independent interaction, transfection of a constitutively active Tcf3 construct in C3H10T1/2 cells did not reproduce the synergism with BMP-2, and a dominant-negative Tcf3 mutant did not prevent BMP-2 induction of alkaline phosphatase. Using the osteocalcin promoter (-637 to –32) as a reporter of tissue-specific transactivation, we found that ΔN151 was a marginal activator (1.2-fold), but it synergized with BMP-2 (1.8-fold increase over BMP-2 alone). However, this synergism was lost when a –199 to –32 osteocalcin promoter (which contains the Tcf/Lef consensus site) was used, while BMP-2 stimulation was retained. Finally using an anti-SMAD4 antibody, we were able to co-immunoprecipitate β-catenin. In sum-

mary, the osteoinductive action of β-catenin does not require Tcf/Lef transactivation. The synergism between β-catenin and BMP-2 is mediated by non-canonical interactions with components of the BMP-2 signaling system and determine the fate of mesenchymal stem cells, shifting differentiation towards the osteogenic lineage. This novel model of osteoinduction offers a mechanism by which β-catenin, a ubiquitous transcription factor, modulates tissue specific events.

Disclosures: ***R. Civitelli****, None.*

1051

NFAT: A Major C-Fos Target Gene During Osteoclast Formation. D. L. Galson[1], C. Zhao*[2], L. Peng*[3], C. Laplace*[3], M. A. Bachler*[4], H. Amano[5], H. Aburatani*[6], H. Ishikawa*[2], E. F. Wagner*[4], K. Matsuo[2]. [1]Center for Bone Biology, Dept. of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [2]Dept. of Microbiology and Immunology, Keio University School of Medicine, Tokyo, Japan, [3]Dept. of Medicine, Beth Israel Deaconess Medical Center & Harvard Medical School, Boston, MA, USA, [4]Research Institute of Molecular Pathology (IMP), Vienna, Austria, [5]Showa University School of Dentistry, Tokyo, Japan, [6]Research Center for Advance Science and Technology, The University of Tokyo, Tokyo, Japan.

Mice lacking c-Fos (*Fos*$^{-/-}$ mice) are osteopetrotic and lack osteoclasts. Microarray analysis using osteoclastogenic cultures revealed that along with the decreased expression of known osteoclast marker genes such as *Acp5* (tartrate-resistant acid phosphatase; TRAP), *Calcr* (calcitonin receptor; CTR), *Ctsk* (cathepsin K), *Car2* (carbonic anhydrase 2), and *Mmp9* (matrix metalloproteinase 9), the expression of *Nfatc1* is abolished in *Fos*$^{-/-}$ precursors. The lack of NFATc1 expression was confirmed at the protein level by Western blotting. NFAT binding sites were identified in the osteoclast-specific promoters of TRAP and CTR. These sites were shown by EMSA to bind NFATc1 and both the TRAP and CTR promoters were transactivated by cotransfection into RAW264.7 cells with a constitutively active form of NFAT (ΔNFAT). Site-specific mutation of the NFAT binding sites demonstrated a functional role for these sites in responding to NFAT. Furthermore, activation of endogenous NFAT by the Ca^{2+} ionophore A23187 induced transfected CTR promoter-reporters. This was dependent on the identified NFAT binding sites and was inhibited by CsA. Earlier work has shown that transient transfection of ΔNFAT into RAW264.7 cells induced the expression of the endogenous TRAP and CTR genes in the absence of RANKL. Notably, in this report, introduction of a nuclear form of NFAT into *Fos*$^{-/-}$ precursors by retroviral gene transfer restored transcription of most osteoclast marker genes (including *Acp5*, *Calcr*, *Ctsk*, and endogenous *Nfatc1)* and rescued bone resorptive function. These data indicate that the lack of NFATc1 is a major reason for the differentiation block of *Fos*$^{-/-}$ osteoclast precursors.

Disclosures: ***D.L. Galson****, None.*

1052

Decreased Bone Formation and Osteopenia in Mice Lacking aCGRP. S. Liese*[1], T. Schinke[1], P. Catala-Lehnen[1], M. Priemel[1], J. M. Rueger[1], R. B. Emeson*[2], M. Amling[1]. [1]Dept. of Trauma, Hand, and Reconstructive Surgery, Hamburg University School of Medicine, Hamburg, Germany, [2]Dept. of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA.

Cell-type-specific alternative splicing of the primary transcript of the Calca gene results in the expression of two polypeptides, calcitonin and aCGRP. Whereas the hormone calcitonin is secreted by thyroidal C-cells, the neuropeptide aCGRP is widely distributed in the central and peripheral nervous system. We have recently analysed the bone phenotype of Calca-deficient mice that fail to produce both calcitonin and aCGRP. Surprisingly, these mice display a high-bone-mass phenotype caused by an increased bone formation rate. As these results are not consistent with the classical role of calcitonin as an inhibitor of bone resorption, we speculated that the bone phenotype of the Calca-deficient mice is caused by the absence of aCGRP. To address this question we analysed a aCGRP-deficient mouse model where calcitonin expression is not affected. In contrast to the Calca-deficient mice we found that the bone volume (BV/TV) of the aCGRP-deficient mice is significantly decreased compared to wildtype littermates at the age of 6 months (8.7 ± 1.5 vs. 13.3 ± 1.3 %). This osteopenic phenotype is caused by a reduction in the bone formation rate (86.5 ± 4.5 vs. 111.5 ± 11 µm3/µm2/year) whereas bone cell numbers are not affected. Serum levels of calcitonin are not elevated in the aCGRP-deficient mice demonstrating that aCGRP is a physiologic activator of bone formation. Taken together these data suggest that aCGRP is one important player in the regulation of bone formation by the sympathetic nervous system. Most importantly however, they demonstrate that the high-bone-mass phenotype of the Calca-deficient mice is indeed caused by the absence of calcitonin thereby confirming its physiologic function as a negative regulator of bone formation.

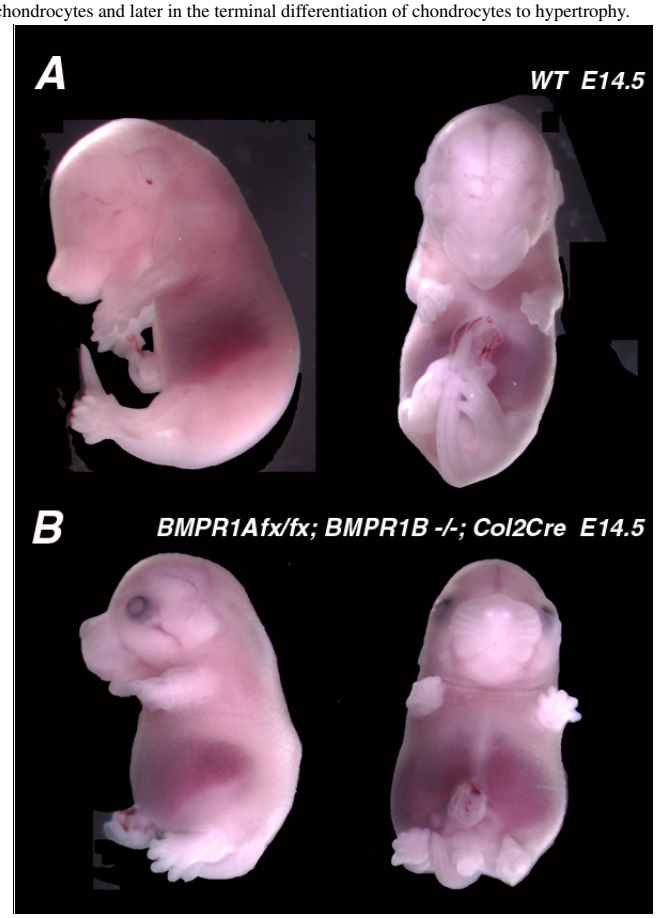
Disclosures: ***S. Liese****, None.*

1053

BMPR1A and BMPR1B Have Overlapping Functions and Are Required for Chondrogenesis in vivo. B. S. Yoon*[1], Y. Mishina*[2], R. R. Behringer*[2], K. M. Lyons[1]. [1]Molecular, Cell, and Development Biology, University of California Los Angeles, Los Angeles, CA, USA, [2]Department of Molecular Genetics, University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA.

Bone Morphogenetic Proteins (BMPs) are members of the Transforming Growth Factorβ (TGFβ) superfamily, that signal through complexes of type I and type II receptors. There are three type I receptors that transduce BMP signals: BMPR1A, BMPR1B, and ActRI. The sequence similarity of BMPR1A and BMPR1B, and their coexpression during skeletal development raise the possibility of functional redundancy. However, other studies suggest that they have distinct functions in chondrogenesis.

In order to examine the roles of BMPR1A and BMPR1B in skeletal development, we generated mice null for BMPR1B and conditionally null for BMPR1A in cartilage (*Bmpr1b*$^{-/-}$*; Bmpr1a*$^{fx/fx}$; *Col2Cre*). *Bmpr1b*$^{-/-}$ mice are viable and have a mild skeletal phenotype. *Bmpr1a*$^{fx/fx}$; *Col2Cre* mice die postnatally and have general chondrodysplasia. This phenotype is exacerbated by the loss of one allele of *Bmpr1b*. In addition, *Bmpr1b*$^{+/-}$*; Bmpr1a*$^{fx/fx}$*; Col2Cre* mice have expanded hypertrophic zones in apendicular skeletal elements, demonstrating a role of BMPs for the terminal differentiation of chondrocytes *in vivo*. In contrast to these relatively mild phenotypes, double mutants exhibit profound defects in chondrogenesis. In these mice, mesenchymal cells condense but do not differentiate into chondrocytes. This is demonstrated by severely diminished production of Collagen II, Link Protein and Aggrecan. These results show that BMPR1A and BMPR1B are functionally redundant in skeletal development, that BMP signaling is required for chondrocyte differentiation *in vivo*, and that these two receptors are primarily responsible for mediating BMP signaling throughout chondrogenesis. We also show that BMP signaling is required at various stages throughout chondrogenesis, first in the differentiation of condensing mesenchymal cells to chondrocytes and later in the terminal differentiation of chondrocytes to hypertrophy.



Disclosures: ***B.S. Yoon****, None.*

1054

Selective Deficiency of Runx2-II Isoform Causes Limited Skeletal Abnormalities Involving Terminal Events in Bone Development. Z. Xiao, L. G. Simpson*, L. D. Quarles. Medicine, Duke University, Durham, NC, USA.

Runx2 (also known as *Cbfa*1) is a complex gene in which two promoters transcribe two major isoforms, designated *Runx2*-II and *Runx*2-I, that differ in their respective 5' untranslated regions and N -termini. *Runx*2-I and -II are presumed to have distinct functions, based on the predominant expression of the Type II isoform in bone and the Type I isoform in non-osseous tissues. Previous *Runx*2 null mouse models produced by targeted disruption of the common *Runt* domain results in the deletion of both isoforms. Double homozygous *Runx*2-I and -II -deficient mice exhibit a global loss of mineralized bone due to the absence of osteoblasts, whereas the double heterozygous *Runx*2-I and -II-deficient mice display skeletal abnormalities that are limited to defects in the skull and distal clavicles. While these studies indicate the importance of *Runx*2 gene products in skeletal development, they do not establish the separate functions of the *Runx*2-I and -II isoforms. In current study, we created mice selectively deficient for the "bone-related" *Runx*2-II gene product by deleting a 2.6 kb region of the *Runx*2 gene containing the P1 promoter, the 5'UTR region, and exon 1 which encodes the N –terminus of *Runx*2-II. Male and female heterozygous *Runx*2-II$^{+/-}$-deficient mice were crossed to create F2 wild-type (*Runx*2-II$^{+/+}$), heterozygous (*Runx*2-II$^{+/-}$), and homozygous (*Runx*2-II $^{-/-}$) -deficient mice. We found that *Runx*2-II$^{+/-}$ mice were fertile, viable and without gross abnormalities of skeletal development. Indeed, heterozygous *Runx*2-II$^{+/-}$ mice were indistinguishable from wild-type mice in their gross appearance,

body weight, and length at birth and had no abnormalities of the skull or clavicles. In contrast, homozygous *Runx* 2-II $^{-/-}$ mice exhibited a high perinatal mortality and a partial arrest of mineralized bone formation that was limited to specific skeletal locations. *Runx*2-II$^{-/-}$ mice were smaller than their littermates, with shortened tails and extremities. Examination of alizarin red/alcian blue stained whole skeletal mounts revealed that the homozygous *Runx*2-II$^{-/-}$ deficient mice had absent formation of the phalangeal bones, distal ribs, caudal spine, hyoid bone, dysplastic distal clavicles, and occipital regions of the skull, but otherwise exhibited complete formation of cervical and lumbar spine, proximal ribs, long-bones, and other regions of the skull, suggesting a site specific arrest of bone formation. These findings support separate functions of *Runx*2-I and -II isoforms. *Runx*2-II appears to be more important in regulating terminal events in bone development, whereas the *Runx*2-I isoform is sufficient to form regions of the skeleton that develop earlier during embryogenesis.

Disclosures: ***Z. Xiao****, None.*

1055

Response of Markers of Bone Turnover and Bone Density to Teriparatide in Postmenopausal Women Previously Treated with an Antiresorptive Drug. B. Ettinger[1], J. San Martin[2], G. G. Crans*[2], I. Pavo*[2]. [1]Division of Research, Kaiser Permanente Medical Care Program, Oakland, CA, USA, [2]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.

To determine whether prior treatment with long-term antiresorptive therapy affects the response to teriparatide [rhPTH(1-34),TPTD], we are conducting an 18-month trial in postmenopausal women who started TPTD 20 µg s.c. once-daily immediately after stopping antiresorptive therapy. Six-month results were presented at the ASBMR 2002 meeting. Patients had used either alendronate (ALN, n=33) 10mg/d or raloxifene (RLX, n=26) 60mg/d for ≥18 months prior to entering the trial. Bone mineral density (BMD) T-scores were <-2.5 prior to start of antiresorptive therapy and ≤-2.0 at initiation of TPTD. At baseline, subjects were similar in age, BMD, and duration of antiresorptive therapy (28 months), but markers of bone turnover were significantly lower in the ALN than the RLX group.

Bone markers exhibited rapid significant increases from baseline in both groups during TPTD treatment. After 12 months of TPTD treatment, the RLX-pretreated group increased BMD in the spine and total hip, whereas the ALN-pretreated group increased BMD only at the spine. Despite evidence of significant TPTD-induced stimulation of bone formation in the ALN-pretreatment group, the increase in BMD was less than that observed in RLX-pretreated patients. The discrepancy between bone marker and BMD responses after long-term ALN-pretreatment may be the result of both marked suppression of bone turnover and increase of matrix mineralization at the time these women began TPTD. Stimulation of bone remodeling by TPTD may have removed highly mineralized bone from some surfaces while depositing new, not yet fully mineralized, bone at other surfaces. In addition, low bone turnover may have caused a delayed anabolic response. Preliminary 18-month data suggest that both groups continue to increase in bone density, but cumulative changes appear considerably less in the ALN-pretreated group.

		Duration of teriparatide treatment			
	Previous therapy	1 month	3 months	6 months	12 months
Bone markers		Median absolute change from baseline			
PINP	ALN	22.5*	44.3*	93.4*	118.0*
	RLX	48.5*†	81.1*	142.7*	139.9*
NTX	ALN	1.5*	4.2*	9.0*	7.2*
	RLX	2.9*	6.5*	14.3*	8.6*
BMD		Mean percentage change from baseline			
Lumbar spine	ALN		0.6	0.5†	2.5*†
	RLX		2.1*†	5.2*†	7.7*†
Total hip	ALN		-0.3	-1.8*†	-1.0†
	RLX		0.4	0.5†	1.5*†

Change from baseline within (*P<0.05) and between (†P<0.05) groups

Disclosures: ***B. Ettinger****, Eli Lilly and Company 2, 5, 8.*

1056

The Neutralization of FGF-23 Ameliorates Hypophosphatemia and Rickets in *Hyp* Mice. Y. Aono*[1], T. Shimada*[2], Y. Yamazaki*[2], R. Hino*[2], Y. Takeuchi[3], T. Fujita[3], S. Fukumoto[4], N. Nagano[1], M. Wada[1], T. Yamashita[2]. [1]Pharmaceutical Development Labs., KIRIN Brewery CO.,LTD., Takasaki, Japan, [2]Pharmaceutical Research Labs., KIRIN Brewery CO.,LTD., Takasaki, Japan, [3]Dept. of Medicine, Univ. of Tokyo school of Medicine, Tokyo, Japan, [4]Dept. of Laboratory Medicine, Univ. of Tokyo Hospital, Tokyo, Japan.

FGF-23 is the latest member of the FGF family and plays a pathogenic role in the development of tumor-induced osteomalacia and autosomal dominant hypophosphatemic rickets/osteomalacia. X-linked hypophosphatemia (XLH) is the most common form of vitamin D-resistant hypophosphatemia characterized by renal phosphate wasting, inappropriately low 1,25-dihydroxyvitamin D (1,25D) levels and rachitic bone lesions. Recent studies have shown that serum FGF-23 levels were elevated in most patients with XLH. Furthermore, based on an ELISA, we showed about 10-fold increase of serum FGF-23 level in *Hyp* mice, a murine homologue of XLH, as compared to normal littermates. These lines of evidence strongly suggest that increased FGF-23 also plays a pathogenic role in XLH. To investigate the therapeutic potential to neutralize excess FGF-23, we examined effects of anti-FGF-23 neutralizing antibodies on phosphate and skeletal abnormalities in *Hyp* mice. Two monoclonal antibodies with neutralizing activity against FGF-23, which recognize different sites of FGF-23, were mixed and used as a neutralizing antibody preparation (NAb). *Hyp* mice and normal littermates were subcutaneously injected with NAb or the isotype-matched control antibody (CAb). A single injection of NAb caused dose-dependent elevations of serum phosphate and 1,25D levels in *Hyp* mice. These findings were supported by evident accumulation of renal type IIa sodium phosphate co-transporter protein in renal brush border membrane fractions and significant elevation of renal 1α-hydroxylase mRNA level. Furthermore, 4-week treatment of *Hyp* mice with 5-time subcutaneous injections of NAb led to the amelioration of rachitic bone lesions and the stimulation of bone elongation (see table). These findings demonstrate that neutralization of elevated FGF-23 can ameliorate hypophosphatemia and rickets in *Hyp* mice and has potential to become a novel therapeutic approach for XLH and other FGF-23-related diseases.

Table: Effects of 4-week Treatment of NAb on Skeletal Parameters of Hyp Mice (Mean ± SEM)

	Normal-CAb	*Hyp*-CAb	*Hyp*- NAb (2 mg/kg)	*Hyp*- NAb (8 mg/kg)
Δ Tail Length (mm)	12.9 ± 1.3**	5.2 ± 0.5	11.8 ± 2.0**	16.5 ± 1.3**
Femoral Ash wt/Dry wt (%)	56.9 ± 0.4**	36.7 ± 0.9	48.4 ± 0.9**	53.2 ± 0.6**
Tibial GPl.Th (µm)	64.1 ± 4.3**	135.1 ± 8.8	91.3 ± 8.8*	70.7 ± 3.4**
Tibial Md.V/BV (%)	97.9 ± 0.5**	58.8 ± 5.2	90.0 ± 1.9**	95.7 ± 1.3**
Tibial O.Th (µm)	1.94 ± 0.14**	8.84 ± 0.81	3.65 ± 0.21**	2.59 ± 0.56**
Tibial MS/BS(%)	11.04 ± 1.25	-	4.88 ± 1.30	11.17 ± 1.03
Tibial BFR/BS (mm3/cm2/Y)	1.75 ± 0.34	-	0.17 ± 0.10	1.19 ± 0.33

*p<0.05, **p<0.01 compared to *Hyp*-CAb. -: cannot be determined

Disclosures: ***Y. Aono****, KIRIN Brewery CO.,LTD. 3.*

1057

Lifestyle, Clinical Factors & Long-Term Risk for Hip Fracture in Women & Men: 50 Years of Follow-Up from the Framingham Study. E. J. Samelson[1], L. A. Cupples*[2], M. T. Hannan[1], K. E. Broe[1], D. T. Felson*[3], D. P. Kiel[1]. [1]Hebrew Rehab Ctr for Aged, Boston, MA, USA, [2]BU Sch Pub H, Boston, MA, USA, [3]BU Sch Med, Boston, MA, USA.

Studies that have identified risk factors for hip fracture (HFx), including low weight, weight loss, tall height, smoking, and alcohol, have examined factors associated with relatively short-term risk of HFx in elderly persons, age >65 years. It is not known whether these factors, measured in young and middle age (30-65 years) can predict risk of HFx over the long-term. Our goal was to identify lifestyle and clinical factors in young to middle age women and men that predict long-term risk of HFx.

Participants included 2461 women and 1937 men who enrolled in the Framingham Study in 1948 and attended a 10-y follow-up visit in 1958 (mean age 48, range 32-65). Every 2 y, cohort members receive physical exams and complete questionnaires. Subjects without previous HFx were followed for 50 y for first occurrence of HFx, last contact, death, or closing date (6/98). Baseline characteristics (weight, height, BMI, smoking, alcohol, physical activity, grip strength, and heart rate) were averaged over measurements taken at 5 visits (10 y) from 1948-58. Weight change was calculated as 1958 - 1948 weight. Cox regression was used to calculate hazard ratios (HR) and 95% confidence intervals (CI) for risk of HFx associated with each factor, adjusted for age, weight, height, smoking, alcohol, and menopause (women).

During 50 y of follow-up, 371 HFx occurred (295 women, 76 men). In women, lower weight and greater height during young to middle age were associated with slightly increased long-term risk of HFx (Table). In men, only smoking was associated with long-term HFx risk. No other characteristics examined during young to middle age (BMI, 10-y weight change, alcohol, physical activity, grip strength, and heart rate) affected long-term risk of HFx.

Height and weight, easily measured in clinical settings, in young to middle age women predict subsequent hip fracture risk in old age. Men who smoke during young to middle age incur increased risk of hip fracture in the long-term. Factors identified previously as important short-term predictors of hip fracture, such as weight loss and alcohol consumption, were modestly or un-related to long-term incidence over 50 years, suggesting these factors play a more significant role in short- rather than long-term risk of hip fracture.

HR (95% CI) for Long-Term Risk of HFx (through 1998)

Baseline Characteristics (1948-58)	Women	Men
Height (2 inch increase)	1.13 (1.02-1.25)	1.04 (0.85-1.27)
Weight (10 lb decrease)	1.07 (1.01-1.14)	0.99 (0.88-1.11)
10-Y Weight Change (10 lb decrease)	1.04 (0.98-1.10)	1.13 (0.90-1.43)
Smoking (10 cigs/day increase)	1.07 (0.92-1.26)	1.29 (1.08-1.55)
Alcohol (2 oz/wk increase)	1.04 (0.98-1.10)	1.01 (0.95-1.06)

Disclosures: ***E.J. Samelson****, None.*

1058

IL-7 Induces Bone Loss in Estrogen Deficient Mice by a Mechanism involving Activation and Expansion of TNF Producing T Lymphocytes. R. Sheperd*[1], Y. Gao[1], W. Qien[1], K. Dark[2], M. Weitzmann[2], R. Pacifici[3]. [1]Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA, [2]Division of Endocrinology, Emory University School of Medicine, Atlanta, GA, USA, [3]Division of Endocrinology and Metabolism, Emory University School of Medicine, Atlanta, GA, USA.

Ovariectomy (ovx) leads to bone loss through an expansion of the pool of TNF producing T cells but the responsible mechanism remains poorly defined. It is now known that IL-7 is elevated in ovx mice, and that neutralization of IL-7 in vivo, prevents ovx induced bone loss (J. Clin. Invest. 2002). As IL-7 regulates T cell activation and proliferation, we have investigated if IL-7 plays a central role in the expansion of T cells critical for ovx induced bone loss. Twelve week old female C57BL6/J mice were ovx or sham operated and treated with the neutralizing IL-7 antibody M-25, or with isotype matched irrelevant antibody (1 mg IP 3 times/week) for 4 weeks. We then performed in vivo BrdU incorporation studies and FACS quantification of the T cell activation markers CD25+ and CD69+ to assess T cell proliferation and activation. Our data revealed that ovx increases T cell activation, proliferation by ~2.5 fold. As a result ovx also increased by ~3 fold the total number of T cells in the bone marrow and spleen and consequently by 3 fold the total number of TNF producing T cells. IL-7 neutralization in vivo completely prevented the ovx-induced increase in T cell number, activation, proliferation, and TNF production, while treatment with irrelevant antibody did not. Since it has been reported that enhanced macrophage antigen presenting cell activity (APC), drives the increased T cell proliferation and the resulting bone loss associated with ovx (ASBMR 2002), we investigated if IL-7 regulates macrophage APC activity. We found that ovx resulted in a 2 fold increase in macrophage APC activity, which was completely reversed by treatment with anti IL-7 antibody, but not with irrelevant antibody. As ovx upregulates macrophage APC activity by increasing production of IFNγ, we investigated if ovx upregulates IFNγ through a IL-7 dependent mechanism. Our data show that in vivo IL-7 neutralization completely prevented the ovx induced increase in IFNγ. In summary, IL-7 is central for the upregulation of IFNγ induced macrophage APC activity and the resulting increase in T cell activation, proliferation and TNF production which follow ovx. Thus, IL-7 is a newly recogmized key "upstream" target through which estrogen regulates immune functions critical for bone homeostasis. Together the data demonstrate that IL-7 plays an essential role in the mechanism by which estrogen deficiency induces bone loss.

Disclosures: ***R. Pacifici****, Eli Lilly & Company 8.*

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Reduction of Type II Collagen Degradation in Postmenopausal Women with Osteoporosis by Alendronate Once-Weekly and Risedronate. P. Garnero[1], M. Valimaki*[2], D. Hosking[3], A. Daifotis[4], C. Peverly*[4], L. Zaru*[4], A. Santora[4]. [1]Synarc SAS, Lyon, France, [2]Helsinki University Central Hospital, Helsinki, Finland, [3]Nottingham City Hospital, Nottingham, United Kingdom, [4]Merck Research Labs, Rahway, NJ, USA.

Urinary type II collagen C-telopeptide breakdown products (CTX-II), reflecting cartilage type II collagen degradation is a potential marker for osteoarthritis (OA) disease activity. Antiresorptive agents such as bisphosphonates may decrease the cartilage destruction associated with OA by reducing subchondral bone turnover and/or by a direct effect on cartilage metabolism. Reduction in urinary CTX-II levels has been previously demonstrated with alendronate use in postmenopausal women (Lehmann et al, Ann Rheum Dis 2002). We report urinary CTX-II results in a head-to-head trial designed to compare the efficacy of alendronate and risedronate for the treatment of osteoporosis. This randomized, double-blind, multicenter, multinational study enrolled 558 postmenopausal women, 60-90 years old (mean, 69), with osteoporosis defined by low BMD T-score (either lumbar spine or total hip/femoral neck ≤ -2.5, or ≤ -2.0 at both sites). Patients were randomized into three treatment groups: alendronate 70 mg† once weekly using standard am dosing (ALN); risedronate 5 mg daily dosed 2 hours after a meal and at least 2 hr before the next (RIS); or matching placebo (PBO) for each. Creatinine (Cr) corrected CTX-II (ng/mmol Cr) (Cartilaps) was measured at baseline, Month 3, and Month 6 in a randomly selected subset of study patients. Per-protocol analysis of urinary CTX-II/Cr was similar for both time points; Month 6 results are shown in the table.

Percent Change in Urinary CTX-II/Cr from Baseline at Month 6

Alendronate N=61		Risedronate N=59		Placebo N=61	
Mean	95% CI	Mean	95% CI	Mean	95% CI
-36.92***	(-43.37, -29.74)	-14.17***	(-23.10, -4.20)	-5.74**	(-15.38, 4.99)

Within-group test of mean percent change: ** not significant; ***p ≤ 0.001
Between treatment difference: ALN vs RIS and ALN vs PBO p ≤ 0.001; RIS vs PBO not significant

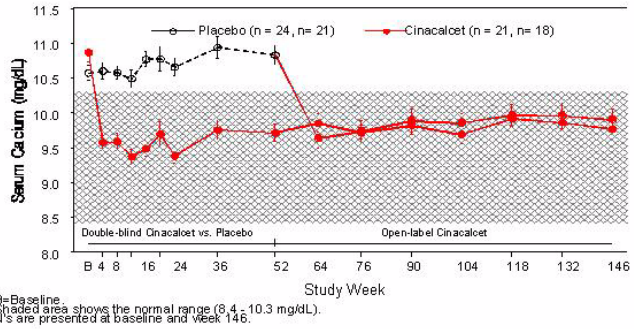
In this study, alendronate treatment resulted in twice the reduction of urinary CTX-II compared to risedronate (between-group difference p≤ 0.001). Decreases in both the alendronate and risedronate groups were significant from baseline but only the alendronate group was significantly different from placebo (p≤ 0.001). We conclude that treatment with alendronate, at the currently approved dose for osteoporosis, significantly reduces a biochemical marker of cartilage destruction, suggesting a possible role as a disease-modifier in the management of osteoarthritis.
†Manufactured by Merck & Co., Inc., Whitehouse Station, NJ

Disclosures: ***P. Garnero****, None.*

1060

Long-Term Control of Primary Hyperparathyroidism with Cinacalcet HCl (AMG 073). M. Peacock[1], S. Scumpia*[2], M. A. Bolognese*[3], M. A. Borofsky*[4], D. M. Guo*[5], L. C. McCary*[5], L. E. Schwanauer*[5], D. M. Shoback[6]. [1]Indiana Univ Sch of Med, Indianapolis, IN, USA, [2]Ctr for Clin Res, Austin, TX, USA, [3]Bethesda Health Res, Bethesda, MD, USA, [4]Clin Res Ctr of Reading, LLP, West Reading, PA, USA, [5]Amgen Inc., Thousand Oaks, CA, USA, [6]Univ of California, San Francisco, Dept of Veterans Affairs Med Ctr, San Francisco, CA, USA.

Cinacalcet HCl is a calcimimetic that specifically binds and modulates the calcium-sensing receptor on the parathyroid gland to increase its sensitivity to calcium, resulting in decreases in intact parathyroid hormone (iPTH) and serum calcium in patients with primary hyperparathyroidism (HPT). This prospective study evaluated the long-term effects of cinacalcet HCl in patients with primary HPT. Patients were initially randomized into a 1-yr double-blind, placebo-controlled dose-titration study (maximum dose 50 mg bid). At the end of 1 yr, patients were able to enroll in an extension study in which all patients received cinacalcet HCl (no placebo). The effects of cinacalcet HCl on serum calcium, iPTH levels, and bone mineral density (BMD) were examined over a 3 yr period.
Forty-five patients completed 1 yr of double-blind therapy and enrolled in the extension study. The mean (SE) baseline serum calcium was 10.8 (0.1) mg/dL and iPTH was 110 (7) pg/mL. Reductions in serum calcium were observed immediately following initiation of cinacalcet HCl treatment (mean [SE] serum calcium presented in the figure) in the initial double-blind study and in placebo patients who switched to cinacalcet HCl after 1 yr. Thirty-nine patients remained on study for 3 yrs, and 87% of those patients had a serum calcium within the normal range. Mean pre-dose iPTH was reduced by 7% from baseline after 3 yrs. BMD was unchanged from baseline. The majority of patients (~60%) remained on 30 mg bid. Therapy appeared safe and well tolerated in this study with no serious adverse events considered related to cinacalcet HCl.



B=Baseline
Shaded area shows the normal range (8.4 - 10.3 mg/dL).
N's are presented at baseline and week 146.

Primary HPT is frequently asymptomatic, but complications including bone loss, gastrointestinal distress, kidney stones, muscle weakness, and neurobehavioral disorders may exist. In this study in patients with primary HPT, cinacalcet HCl administration was safe and effective, normalizing serum calcium levels and maintaining this effect for 3 yrs. Cinacalcet HCl offers potential as a novel therapy for the treatment of hypercalcemia in patients with primary HPT.

Disclosures: ***M. Peacock****, Amgen Inc. 5.*

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Treatment of c-Fos Over-expressing Osteoclast Precursors with Cytokines Induces Osteoclast Formation and Abrogates Bisphosphonate-induced Osteoclast Apoptosis. T. Yamashita[1], L. Xing[1], K. Matsuo[2], K. Matsuo[2], E. F. Wagner[3], B. F. Boyce[1]. [1]Path, Univ Rochester, Rochester, NY, USA, [2]NILS, Aichi-ken, Japan, [3]Res. Inst. Molec. Path., Vienna, Austria.

Expression of NF-κB and c-Fos is required for osteoclast (ocl) formation during development and is upregulated by cytokines, such as TNF and IL-1, at sites of inflammation. NF-κB in turn can upregulate expression of IL-1, TNF and IL-6 in ocls. Thus, in inflammatory bone diseases, cytokines could initiate self-perpetuating up-regulatory cycles involving c-Fos and NF-κB in ocls to maintain bone resorption along with or independent of RANKL. To explore this possibility, we used retroviral gene transfer to infect M-CSF-dependent splenocytes from NF-κB p50 and p52 double knockout (dKO) or wild-type (wt) mice with c-Fos or a GFP control vector and treated the cells with IL-1 or TNF. Numerous multinucleated ocls (50-100/96 well) formed in c-Fos-expressing dKO cultures treated with TNF or IL-1, but not in GFP-infected controls, indicating that c-Fos can substitute for NF-κB to mediate cytokine-induced ocl formation. No ocls formed in wt or dKO cultures infected with c-Fos or GFP retroviral supernatants alone. Expression of Fos family members, c-jun, fra-1 and fra-2, but not c-Fos or Fosb was higher in dKO than wt types splenocytes, suggesting that NF-κB may regulate c-jun, fra-1 and fra-2 expression. To examine if c-Fos expression is upregulated in ocls in inflammatory arthritis, we stained bone sections from TNF transgenic mice with a c-Fos antibody. c-Fos was strongly expressed in ocls eroding bone in the joints of arthritic mice, but not in ocls in the adjacent normal metaphysis nor in the metaphyses of wt mice. We found a similar staining pattern for IL-6 in ocls in the transgenic mice, suggesting that TNF expression in the inflamed joints induces increased ocl expression of c-Fos and IL-6. Previous studies suggest that bisphosphonates have limited efficacy in preventing inflammation-mediated bone loss which we hypothesize is due to cytokine-mediated increased c-Fos expression in ocls rendering them more resistant to bisphosphonates. To test this, we treated c-Fos- or GFP-expressing wt ocls with Zoledronate and found that the c-Fos-expressing ocls survived at 10^{-7} M, while the GFP-

expressing ocls died at this concentration. Furthermore, alendronate-treated TNF-transgenic mice had significantly more apoptotic ocls in their metaphyses vs PBS controls but not in their inflamed knee joints. Our findings indicate that when c-Fos expression is upregulated in ocls cytokines can induce ocl formation directly. They also support our hypothesis that cytokines may induce c-Fos expression in ocls and that c-Fos protects them from bisphosphonate-induced death.

Disclosures: ***T. Yamashita****, None.*

1062

The NFAT Family of Transcription Factors May Be Key Regulators of the RANKL Gene in T Cells. A. R. Pettit*[1], C. Manning*[1], S. R. Goldring[1], T. Libermann*[1], S. L. Peng*[2], E. M. Gravallese*[1]. [1]Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA, USA, [2]School of Medicine, Washington University, St. Louis, MO, USA.

Receptor activator of NF-kB ligand (RANKL) is an essential factor for osteoclast differentiation, and plays a major role in physiologic bone remodeling and in pathologic bone loss in conditions such as inflammatory arthritis and cancer. There is limited understanding of the pathways regulating transcription of the human RANKL gene. Therefore, we have performed analyses to identify transcription factors responsible for RANKL gene regulation in T cells, an important source of RANKL in several diseases associated with bone loss. Jurkat T cells, a human T lymphoma cell line, constitutively express low RANKL mRNA levels and are a useful cell system for investigating RANKL gene regulation. In these cells, RANKL mRNA was induced by stimulation with phorbol 12-myristate 13-acetate and ionomycin (P/I) or by plate-bound anti-CD3/CD28. We cloned a 1902 base pair fragment of the RANKL putative promoter, which contains a nuclear factor of activated T cells (NFAT) and an NFAT/activator protein (AP)-1 consensus element, into a luciferase reporter vector (pXP2-R1902). pXP2-R1902 luciferase activity was assessed in transient transfections of resting and activated Jurkat T cells. Inhibition of the activiation of NFATs activation was achieved by blockade with cyclosporine A (CsA), which inhibits calcineurin activation and subsequently NFAT nuclear translocation. In Jurkat T cells transfected with pXP2-R1902 we observed up to a 40-fold increase in luciferase activity above basic vector. An additional 6- to 8-fold induction of luciferase activity was seen when transfected Jurkat cells were stimulated with P/I, correlating with our mRNA observations. P/I induction of RANKL mRNA and pXP2-R1902 luciferase activity were inhibited by CsA blockade, implicating NFAT transcription factors as regulators of RANKL mRNA induction. We also examined regulation of RANKL mRNA expression by GeneChip analysis in P/I stimulated murine CD4+ T cells isolated from wild type (WT) or NFATc1 and c2 double-deficient (-/-) mice. GeneChip analysis demonstrated that RANKL mRNA expression in stimulated NFATc1-/-/c2-/- CD4+ T cells was 3.5-fold less than expression in stimulated WT CD4+ T cells. Similarly, induction of RANKL protein expression, as assessed by flow cytometry, was impaired in NFATc2-/- CD4+ T cells activated with anti-CD3/CD28 compared with activated WT CD4+ T cells. These data support the hypothesis that the NFAT family of transcription factors may be important transcriptional regulators of the RANKL gene in T cells and may provide novel therapeutic targets for inhibiting RANKL expression.

Disclosures: ***A.R. Pettit****, None.*

1063

Unequivocal Demonstration of Estrogen Signaling Through the Androgen Receptor in Osteoblasts and Osteoclasts. J. Chen[1], S. Kousteni[1], L. Han[1], H. Peng*[1], L. K. McCauley[2], S. C. Manolagas[1]. [1]Div Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, CA Veterans Healthcare System, Univ of Arkansas for Med Sciences, Little Rock, AR, USA, [2]School of Dentistry, Univ of Michigan, Ann Arbor, MI, USA.

Estrogens or androgens control the survival of osteoblasts and osteoclasts in a non—sex-specific manner. And 4-estren-3α,17β-diol (estren), a synthetic ligand of the estrogen (ERα or ERβ) or androgen receptor (AR) which reproduces the nongenotropic effects of sex steroids without affecting classical transcription, reverses gonadectomy-induced bone loss in both females and males. To search for a molecular explanation of the non-sex-specific nature of these phenomena we employed osteoblasts and osteoclasts from double estrogen receptor knockout (DERKO) mice, kindly provided by P. Chambon, and investigated whether estrogen may signal kinase-mediated control of bone cell survival via the AR. We report that estradiol (E2), dihydrotestosterone (DHT), or the estren stimulated ERK phosphorylation and osteoclast apoptosis and attenuated osteoblast apoptosis in DERKO cells, with identical potencies, over a concentration range of 10-11 to 10-7 M. The effects of E2 or DHT, or estren could be blocked by either the ER antagonist ICI 182,780 or the AR antagonist flutamide. Silencing the AR in DERKO osteoblasts using the small interfering RNA technique (siRNA), but not the non-essential cytoskeletal protein lamin A/C- used as a negative control, abrogated the anti-apoptotic effect of all three compounds. Conversely, the anti-apoptotic effect of all three compounds was restored upon rescuing the AR by introducing a human AR cDNA into DERKO cells in which the endogenous (murine) AR was silenced. The effects of E2, DHT or estren in AR-silenced DERKO cells could be also restored by transfecting an expression construct encoding for the ligand binding domain (LBD) of the human ERα fused to membrane localization sequence. However, transfecting the human ERα LBD fused to a nuclear localization sequence did not rescue the effect of the compounds. Transfection of a Src kinase dead dominant negative mutant into the DERKO cells abrogated the anti-apoptotic effect of either E2 or DHT, as did the transfection of a Src mutant missing the SH3 domain which is required for the physical association of Src to the AR. In contrast, a Src mutant missing the SH2 domain which is required for the association of Src to the ER was ineffective. These results demonstrate unequivocally that the effects of estrogen in the DERKO cells are indeed mediated by the nongenotropic actions of the AR involving activation of cytoplasmic kinases.

Disclosures: ***J. Chen****, None.*

1064

Osteoclastogenic Activity of NFAT Family Requires a Partnership with c-Jun/c-Fos. F. Ikeda[1], T. Matsubara*[1], T. Watanabe*[2], T. Kukita[2], K. Yoshioka*[3], S. V. Reddy[4], S. Tanaka[5], J. Inoue*[6], R. Nishumura[1], T. Yoneda[1]. [1]Dept Biochem, Osaka Univ Grad Sch Dent, Osaka, Japan, [2]Sec Oral Mol Biol, Facult Dent Kyushu Univ, Fukuoka, Japan, [3]Cancer Res Inst, Kanazawa Univ, Kanazawa, Japan, [4]Div Hematol Oncol, Univ Pittsburgh Cancer Inst, Pittsburgh, PA, USA, [5]Dept Orthop Surg, Univ of Tokyo, Tokyo, Japan, [6]Inst Med Sci, Univ of Tokyo, Tokyo, Japan.

RANKL signaling plays a central role in the regulation of osteoclast differentiation and function. Recently, NFAT2, one of the family members of the transcription factor of nuclear factor of activated T cell (NFAT), is found to be a target of RANKL and regulate osteoclast differentiation. However, involvement of other NFAT family members is unknown. More importantly, molecular events by which RANKL activates NFAT and NFAT controls osteoclastogenesis are unclear. Determination of the expression of NFAT families in osteoclast lineages by western analysis showed that osteoclasts and RAW264 murine monocytic cells expressed not only NFAT2 but also NFAT1. Soluble RANKL (sRANKL) promoted the nuclear translocation of NFAT1 and stimulated the transcriptional activity of NFAT. Moreover, TRAF6 overexpression dramatically enhanced NFAT transcriptional activity. These results suggest that NFAT activation by RANKL is mediated through TRAF6. Overexpression of either NFAT1 or NFAT2 in RAW264 cells using adenovirus system induced TRAP-positive multinucleated osteoclast-like cell formation and stimulated TRAP gene promoter activity. On the other hand, a specific NFAT inhibitor VIVIT peptide markedly suppressed sRANKL induced-osteoclastogenesis. These results indicate that NFAT is a stimulator of osteoclastogenesis. However, the stimulatory effects of NFAT1 or NFAT2 on osteoclast formation were substantially less than sRANKL, suggesting that an involvement of additional molecules associated with RANKL signaling is required for NFAT1 or NFAT2 to optimally function. In support of this notion, co-overexpression of c-Jun and c-Fos, which were activated by sRANKL, with NFAT1 or NFAT2 enhanced osteoclastogenesis. In contrast, overexpression of dominant-negative c-Jun or c-Fos abolished the NFAT-promoted osteoclastogenesis and TRAP promoter activity. Finally, analyses using a reporter construct driven by sequential deletion mutants of the TRAP gene promoter identified a putative NFAT/AP-1 binding element critical to TRAP promoter activation. In conclusion, our results suggest that both NFAT1 and NFAT2 are important transcription factors that mediate RANKL-induced osteoclastogenesis and that an establishment of a partnership with c-Jun/c-Fos complex optimizes osteoclastogenic activity of NFAT.

Disclosures: ***F. Ikeda****, None.*

1065

Vav3 Regulates Osteoclast Function by Controlling Cytoskeleton Reorganization Downstream of M-CSF and αvβ3 Signaling. R. Faccio[1], K. Fujikawa*[1], A. Zallone[2], S. L. Teitelbaum[1], W. Swat*[1], F. P. Ross[1]. [1]Pathology, Washington University, St Louis, MO, USA, [2]Department of Human Anatomy, Bari, Italy.

Vavs are Guanosine Exchange Factors (GEFs), which mediate the conversion from the inactive GDP- to the active GTP-form of Rho, Rac and Cdc42, known regulators of the actin cytoskeleton. Osteoclasts (OCs) are highly motile cells, which attach to the bone matrix, undergo polarization and resorb bone. These events require several changes in the OC cytoskeleton, and previous studies using overexpression of Rho or the specific inhibitor, C3 exoenzyme, have shown that actin ring assembly is controlled by this GTPase. To overcome the potential problems of Rho, Rac or Cdc42 overexpression, we generated mice lacking Vav1-3, animals in which one or more of the small GTPases may not be activated. We found that Vav3 is the predominant form of Vavs in osteoclasts, more highly expressed than in other hematopoietic cells, and that, compared to WT mice, Vav3 -/- long bones show a marked increase in trabecular bone volume (>2.7 fold, $p<0.001$). While osteoclast differentiation is not affected by lack of Vav3, either in vitro or in vivo, confocal analysis of Vav3-/- OCs reveals that these cells are less spread, with numerous but incomplete actin rings. Podosomes are replaced by irregular actin clusters, and microtubules are completely disorganized. Thus, Vav3-/- OCs fail to polarize when plated onto bone and resorb poorly, explaining the increased trabecular volume in vivo. Both the resorptive and spreading phenotypes are rescued by retroviral transfection of Vav3, but not Vav1, indicating specificity within this family. Since Rho, Rac and Cdc42 have been reported to control cell polarization and spreading, we analyzed the activation of these small GTPases in OCs treated with M-CSF. While cells lacking Vav3 continue to generate GTP-Rho in response to the cytokine, they fail to activate either Cdc42 or Rac. Finally, the same null OCs also exhibit defective αvβ3 signaling in response to cell adhesion, namely failure to phosphorylate either c-Src or ERKs when plated on osteopontin. Thus Vav3, while not regulating OC differentiation, appears to play a key role in controlling cytoskeletal reorganization, cell adhesion-mediated signaling and bone resorption. The fact that Vav3 regulates cytokine-stimulated activation of Cdc42 and Rac, events that correlate with osteoclastic bone resorption, suggests a new role for these GTPases in osteoclast function and M-CSF signaling. Taken together our data suggest that Vav3 is a possible new therapeutic target for osteoporosis.

Disclosures: ***R. Faccio****, None.*

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Control of Multi-Nucleated Osteoclast Formation by RAIN, a Novel RANK-Associated Inhibitor that Regulates Actin Polymerization. A. T. Poblenz*, B. G. Darnay. Bioimmunotherapy, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA.

Formation of multi-nucleated osteoclasts is a central process in bone homeostasis, which is governed by receptor activator of NF-κB (RANK) and its ligand, RANKL. The process of osteoclastogenesis involves at least four basic events: (1) proliferation and migration of precursor cells, (2) early signaling events of differentiation including activation of transcription factors, (3) homotypic cell-cell recognition and membrane fusion in conjunction with the structural reorganization of the multi-nucleated cell, and (4) activation of the multi-nucleated osteoclast to resorb bone. The proteins affecting osteoclast differentiation and activation have been well characterized; however, considerably little is known how triggering RANK causes the fusion and formation of the multi-nucleated osteoclast. We have identified a novel RANK-interacting protein termed RAIN (RANK-Associated Inhibitor), which prevents multi-nucleated osteoclast formation without affecting early RANKL signaling. In contrast, cells lacking RAIN develop a rapid and increased number of osteoclasts after RANKL stimulation. Furthermore, RANKL induces the expression of RAIN at a time when cellular fusion is observed. Consistent with the biological properties of RAIN, *in vitro* studies with recombinant RAIN indicated its ability to co-sediment with G-actin and its ability to prevent the polymerization of actin. On close inspection of the amino acid sequence of RAIN, we observed an actin-binding motif in RAIN when aligned with cofilin and twinfilin, two proteins that cause actin depolymerization. Based upon the potential actin-binding domain in RAIN, a series of point mutations in RAIN (K90/91A, K174/R176A/A180T, and R194A) were constructed, and RAW264.7 cells stably expressing these mutants were generated. Unlike the overexpression of wild-type RAIN in RAW264.7 cells, all RAIN mutants differentiated into TRAP-positive, multi-nucleated osteoclasts and resorbed bone upon RANKL treatment. To our knowledge, this is the first report of a molecule linking RANK to the cellular machinery controlling the formation of the multi-nucleated osteoclast. RAIN's ability to regulate formation of polymerized actin filaments indicates a strong prerequisite for actin turnover in the formation of the multi-nucleated osteoclast.

Disclosures: ***B.G. Darnay****, None.*

1067

Transient versus Sustained Activation and Nuclear Accumulation of ERKs Underlie the Anti- Versus the Pro-Apoptotic Effects of Estrogens on Osteoblasts/Osteocytes and Osteoclasts, Respectively. J. Chen, L. I. Plotkin, J. I. Aguirre, L. Han, H. Peng*, S. Kousteni, T. Bellido, S. C. Manolagas. Div Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, CA Veterans Healthcare System, Univ of Arkansas for Med Sciences, Little Rock, AR, USA.

Sex steroids exert anti- apoptotic effects on osteoblasts/osteocytes and pro -apoptotic effects on osteoclasts. In spite of such divergent outcomes in both cases the effect of sex steroids requires activation of the extracellular signal regulated kinases (ERKs). To explain the mechanistic basis of this divergence, we searched for potential differences in the kinetics of activation and/or in the subcellular localization of the activated ERKs in response to 17β-estradiol (E2) in the two cell types. To this end, we used the osteocytic MLO-Y4 cell line and osteoclasts generated from murine bone marrow progenitors by treatment with MC-SF and soluble RANKL. In MLO-Y4 cells, E2 produced a rapid and transient phosphorylation of ERKs beginning at 2 min, reaching a peak at 5 minutes and returning to base line by 30 min. In osteoclasts on the other hand, E2 induced ERK phosphorylation within 2 min but the magnitude of this effect increased progressively thereafter until at least 24 h. We then shortened the duration of ERK activation in osteoclasts by pre-treatment for 30 min with 1 mg/ml cholera toxin (CT) which ADP-ribosylates and activates the αs subunit of G proteins leading to activation of the adenyl cyclase/cAMP/PKA pathway, which in turn suppresses ERK phosphorylation. ERK phosphorylation in response to E2 in CT-treated osteoclasts was indeed converted from sustained to transient, with a rapid zenith reached by 2 min, followed by a return to base line by 30 min and remaining low thereafter until at least 24 h. Strikingly, in CT treated-osteoclasts the pro-apoptotic effect of E2 was abrogated. Next we converted transient to prolonged ERK activation in osteocytes by a) inhibiting CRM/exportin1-mediated export of ERKs from the nucleus with leptomycin B, and b) overexpressing a GFP-ERK2 mutant that resides permanently in the nucleus. In either case, instead of preventing, E2 stimulated apoptosis; and, this effect was blocked by the ERK inhibitor PD98059. Overexpression of a wild type GFP-ERK2 or a GFP-ERK2 mutant with impaired translocation into the nucleus did not affect the anti-apoptotic properties of E2. We conclude that the kinetics of ERK phosphorylation and the length of time that phospho-ERKs are retained in the nucleus determines specific downstream patterns of phosphorylation of target transcription factors or other substrates, and thereby defines the pro- versus anti-apoptotic effects of sex steroids on bone and perhaps other cell types.

Disclosures: ***J. Chen****, None.*

1068

ER-α Negatively Regulates TGF-β Signaling by Blocking Smad4 Binding to DNA. Y. Wu[1], L. Wu[1], W. E. Gathings*[2], X. Cao[1]. [1]University of Alabama at Birmingham, Birmingham, AL, USA, [2]University of Alabama at Huntsville, Huntsville, AL, USA.

Estrogen is recognized to be essential for bone formation and maintenance since estrogen deficiency can lead to postmenopausal osteoporosis. TGF-β is one of the most abundant cytokines embedded in the bone matrix. It regulates both osteoblast and osteoclast cell proliferation and differentiation as either an autocrine or paracrine factor. Estrogen also enhances TGF-β secretion by osteoblasts and osteocytes. Here we show that ER-α interacts with Smad4 as a transcription cofactor that mediates a direct crosstalk between the estrogen and TGF-β pathways in osteoblasts. Immunoprecipitation assays demonstrated that ER-α co-precipitates with Smad4 in the 2T3 osteoblast cell line. To examine the potential function of this interaction, a TGF-β-responsive luciferase reporter plasmid (pLuc-3Tp) was transfected into 2T3 cells with overexpression of ER-α and treated with TGF-β. The results indicate that ER-α inhibits TGF-β-mediated transcriptional activity from 13 fold to 4 fold. Similar results were obtained when a reporter construct bearing Smad-binding elements (SBE) were used. We also examined endogenous TGF-β downstream gene transcription in 2T3 cells using semi-quantitative RT-PCR. We found that estrogen inhibits TGF-β-induced transcription of these genes (e.g., PAI-1 and Smad7). Mapping of the interaction domains indicates that the N-terminal Mad homologous domain 1 (MH1) and linker region of Smad4 are essential for its interaction with ER-α. Since MH1 is the Smad4 DNA binding domain, these results suggest that interaction of ER-α with Smad4 interferes with Smad4 DNA binding activity. We, therefore, performed Chromatin Immunoprecipitation to examine the effects of ER-α on binding of Samd4 to DNA *in vivo*. TGF-β induced Smad4 binding to the Smad binding elements of the PAI-1 promoter, and co-treatment with estrogen abolished the binding. These results were confirmed with DNA precipitation assays. Smad4 protein co-precipitated with a DNA fragment containing Smad binding elements, and TGF-β enhanced the precipitation. Co-treatment with both estrogen and TGF-β inhibited the formation of the Smad4/SBE complex. In summary, we found that ER-α directly interacts with Smad4. The interaction inhibits TGF-β-induced binding of Smad4 to DNA. Consistent with these observations, estrogen also inhibits TGF-β-induced endogenous gene transcription. Our data suggest that the interaction between ER-α and Smad4 acts as a cross-talk mechanism between estrogen and TGF-β signaling pathways. This cross-talk mechanism may help to further elucidate the roles of TGF-β and estrogen in bone development and remodeling as well as in the pathogenesis of osteoporosis.

Disclosures: ***Y. Wu****, None.*

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Steroid Receptor Coactivator-1 (SRC-1) Contributes to the Maintenance of Bone Volume by Sex Hormones in Both Males and Females. T. Yamada[1], H. Kawano[1], K. Sekine*[2], S. Kato[2], H. Kawaguchi[1]. [1]Orthopaedic Surgery, Univ. of Tokyo, Tokyo, Japan, [2]IMCB, Univ. of Tokyo, Tokyo, Japan.

Although coactivators are known to modulate nuclear receptor activities intracellularly, their physiological roles in skeletal tissues remain unknown. Steroid receptor coactivator-1 (SRC-1) is the first identified coactivator that interacts with steroid receptors and enhances their transcriptional activities. In this study we initially created floxed SRC-1 mice in which the SRC-1 gene locus was flanked by loxP sites, and succeeded in generating SRC-1 deficient mice (SRC-1KO) by mating them with CMV-Cre transgenic mice. SRC-1KO developed normally without abnormalities of major organs except for moderate atrophy of testis in males. In long bones and vertebrae, however, bone densitometry and 3D-μCT analyses revealed significant decreases of trabecular and cortex bone volumes in SRC-1KO as compared to wild-type (WT) littermates at 24 weeks of age in both males and females. Histomorphometric analysis at the proximal tibiae showed that bone volume (BV/TV) of SRC-1KO was about 20% lower than that of WT. Parameters for both bone formation (Ob.S/BS & MAR) and resorption (Oc.N/B.Pm & ES/BS) were higher in SRC-1KO males and females than in WT littermates, indicating a high turnover of osteopenia. Although SRC-1KO showed higher levels of serum and urinary bone metabolic markers (ALP, osteocalcin, deoxypyridinoline), the serum levels of testosterone in males (+30%) and estradiol (E_2) in females (+40%) were also elevated as compared to those of WT littermates. To examine the involvement of SRC-1 in the actions of sex hormones and glucocorticoid on bone, we performed hormone administration experiments. After gonadectomies at 16 weeks of age, bone volumes of WT and SRC-1KO were decreased during the next 8 weeks to the same levels in both sexes. When slow releasing pellets of dihydrotestosterone and E_2 were subcutaneously implanted to gonadectomized males and females, respectively, bone volumes of WT showed full restoration to the sham WT levels; however, in SRC-1KO the restorations were limited to approximately one half, suggesting that osteopenia by SRC-1 deficiency may be due to a partial insensitivity to sex hormones in both sexes. Contrarily, when a slow releasing pellet of prednisolone was implanted, bone volume decrease was similar in WT and SRC-1KO during the following 8 weeks. We conclude that SRC-1 deficiency cannot be compensated by other cofactors in the anabolic actions of sex hormones on bone, although it is not essential in the catabolic action of glucocorticoid. SRC-1 might be involved in the pathophysiology of sex hormone-deficient osteoporosis and therapeutic effects of hormone replacement therapies in humans.

Disclosures: ***T. Yamada****, None.*

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A Deficiency in 1,25-Dihydroxyvitamin D3 Production or Action Is Associated with Parathyroid Hormone Resistance at the Level of Osteoblast-induced Osteoclast Formation. N. K. Shevde, J. A. Reading*, J. M. Prahl*, J. W. Pike, H. F. DeLuca. Biochemistry, University of Wisconsin-Madison, Madison, WI, USA.

Vitamin D and PTH are the two major regulators of mineral homeostasis acting in concert on overlapping tissue targets to regulate serum calcium and phosphorus levels. Early studies support an important physiological interplay between vitamin D3 and PTH. This interplay is highlighted in vitamin D3 deficiency that results in PTH resistance. The mechanism of this resistance remains unknown. To explore this problem, we evaluated the effects of PTH on osteoblast-induced osteoclast (OC) formation in the absence of functional 1,25(OH)2D3 that occurs in 25-hydroxyvitamin D3-1alpha-hydroxylase (1-OHase) null or in vitamin D receptor (VDR) null mice. Bone marrow cells from wildtype (WT) and

VDR null mice were isolated and cultured (106 cells/ml) in the presence of 1,25(OH)2D3 (10-8 M) or PTH (10-7 M). OC formation was assessed after 8 to 10 days by quantitating the total number of multinucleated, TRAP+ cells. Marrow-derived monocytes (105 cells/ml) were also isolated and cultured in the presence of both RANKL and M-CSF to assess the effects of the VDR null phenotype on OC precursor number (OCP). OC were induced by 1,25(OH)2D3 in cultures derived from WT marrow but not from VDR null marrow, as expected. Interestingly, PTH had little effect on VDR null marrow cultures while strongly stimulating OC formation in WT marrow cultures. OCP number was comparable in monocytes derived from both animal sources. These results indicate that the inability of VDR null mice to process 1,25(OH)2D3 signaling impairs the ability of PTH to induce osteoblast-mediated OC formation. We next tested the ability of 1,25(OH)2D3, PTH or the combination to induce OC formation from marrow cells derived from WT and 1-OHase null mice. OC were formed in equal numbers in response to either 1,25(OH)2D3 or the combination of the two hormones from both cell sources. PTH alone, however, induced OC formation only in marrow cells derived from WT mice; no OC were formed from cells derived from 1-OHase null mice. These experiments indicate that a deficiency in either the production or action of 1,25(OH)2D3 reduces the ability of PTH to promote OC formation. This resistance does not appear to be the result of changes in PTH receptor mRNA expression, but may be related to elevated OPG mRNA levels observed in the 1,25(OH)2D3 deficient state. These findings suggest that vitamin D-deficiency may limit the ability of PTH to modulate osteoclast formation, bone resorption and remodeling.

Disclosures: ***N.K. Shevde****, None.*

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1a,25(OH)2D3 Mediated Rapid Responses in Osteoblasts and Ligand Binding to Caveolae Enriched Membrane Fractions Are Abrogated in VDR Knock-Out Mice but not Wild Type. L. P. Zanello, J. A. Huntakangas*, C. X. Olivera*, J. E. Bishop, A. W. Norman. Biochemistry, University of California-Riverside, Riverside, CA, USA.

The steroid hormone 1a,25(OH)2D3 (1,25D) is known to mediate both genomic responses (hrs-day) via interaction with the classical nuclear vitamin D receptor (VDR) and rapid (sec-min) responses (RR) via interaction with a membrane associated putative steroid receptor VDRmem. A challenging project has been to biochemically identify the VDRmem and to define the RR signal transduction pathway(s) to which it is linked. Here we report comparative studies performed in wild type (WT) and VDR knock-out (KO) mice to assess functional RR (opening of chloride channels and changes in whole cell capacitance) in calvarial osteoblasts and ligand binding [[3H]1,25D] studies to a lipid raft/caveolae enriched membrane fraction (CMF) of intestine (I), kidney (K) and lung (L). We conducted whole-cell patch-clamp measurements in a total of 27 KO and 55 WT primary cultured osteoblasts obtained from 15 and 9 genotyped animals (5 KO couples and 5 WT) respectively. WT osteoblasts expressed Cl- currents that were significantly potentiated by 1,25D, 5nM (n=12 of 12 cells) at depolarizing potentials within the first 1-5 min. However, equivalent Cl- currents in KO osteoblasts were not modified by 0.5-50nM of1,25D. We also found that 1,25D, 0.5-50nM, promoted a significant transient increase in whole cell capacitance value (DCap=2.33 + 1.1pF, n=8 of 12 cells), which is a measure of the cell secretory activity in WT osteoblasts, but not in KO cells (DCap 0.58+0.09, n=8 cells). Using sequential PercollTM and OptiPrepTM gradient ultracentrifugations we prepared a CMF as a source of the VDRmem. Saturable in vitro binding of [3H]-1,25D was detected in CMF of I, K and L (Kd 1.4±0.6 nM and Bmax ≈ 28±10 fmol/mg protein, n=2). VDR knockout (KO) mice displayed a remarkably reduced saturable binding of [3H]-1,25D in both the nuclear fraction (NF) and CMF in all 3 tissues compared with WT mice; for I, K, and L, respectively, WT and KO results were for NF (100% vs. 14±10%, p<0.05 and 100% vs. 28±4%, p<0.05 and 100% vs. 15±18%, p<0.05) and for CMF (100% vs. 50±11%, p=0.05 and 100% vs. 44±39% p=0.15 and 100% vs. 15±5%, p<0.05). Binding of [3H]-1,25D in NF of all WT tissue vs. KO tissue was reduced 100% vs. 19±11%, p<0.001 and in CMF 100% vs. 36±25%, p<0.001.). In western blot analysis expression of classical VDR in chick CMF from I and K was clearly detectable, but was lower in L CMF. These data suggest that a nuclear VDR or slightly altered form may be the VDRmem present in the CMF and that its presence is essential for RR in osteoblasts.

Disclosures: ***L.P. Zanello****, None.*

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Identification of Calcium Independent Actions of the Vitamin D Endocrine System in Mice with Targeted Disruption of the 1α-Hydroxylase and VDR Genes. D. Panda, D. Miao, I. Bolivar*, J. Li*, G. N. Hendy, D. Goltzman. Department of Medicine, Calcium Research Lab, McGill University, Montreal, PQ, Canada.

Normalization of serum calcium by a "rescue diet" has been reported to reverse abnormalities in mice homozygous for targeted disruption of the VDR gene ($VDR^{-/-}$). To assess whether similar reversal can also occur in mice deficient in the major ligand for the VDR, $1,25(OH)_2D$, we compared parameters of skeletal and calcium homeostasis in $VDR^{-/-}$ mice with the same parameters in mice homozygous for targeted disruption of the 1α- hydroxylase gene ($1\alpha OHase^{-/-}$). Mice were fed either a "high calcium intake diet" (1.5% calcium in the drinking water plus 1% in the diet) or a "rescue diet" (2% calcium plus 20% lactose in the diet) for 3 months after weaning. Both mouse models exhibited hypocalcemia, rickets or osteomalacia and secondary hyperparathyroidism on the high calcium intake. On the rescue diet, serum calcium levels were normalized in both models (11.6mg±0.9 /dl in $1\alpha(OH)ase^{-/-}$ and 12.6mg/dl in $VDR^{-/-}$) and mineralization of bone was normalized. PTH levels decreased 8.4 fold (1430±184 pg/ml to170±19 pg/ml) in the $1\alpha OHase^{-/-}$ mice and 14.4 fold (720±95 pg/ml to 50.0±0.5 pg/ml) in the $VDR^{-/-}$ mice. On the rescue diet, parathyroid gland size normalized in $VDR^{-/-}$ mice, but remained enlarged in $1\alpha(OH)ase^{-/-}$ animals. In addition the cartilaginous growth plate was normalized in $VDR^{-/-}$ mice but remained enlarged and distorted in $1\alpha(OH)ase^{-/-}$ animals. At 4 months of age the increased trabecular bone volume and increased osteoblast numbers observed in both $1\alpha(OH)ase^{-/-}$ and $VDR^{-/-}$ mice on the high calcium intake were decreased below wild type levels in animals fed a rescue diet. These studies confirm that $1,25(OH)_2D$ deficiency and VDR deficiency do not produce identical phenotypes and that parathyroid gland size and the cartilaginous growth plate cartilage abnormalities are normalized by correcting hypocalcemia in VDR deficient but not in $1,25(OH)_2D$ deficient animals. In addition, the reduction of trabecular bone volume below normal in both models, when serum calcium is normal and PTH is measurable, suggests a role for the $1,25(OH)_2D$/VDR system in bone anabolism.

Disclosures: ***D. Panda****, None.*

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Association of an IGF-I Gene Promoter Polymorphism and Hip Bone Geometry and Its Influence on the Risk of Hip Fracture: The Rotterdam Study. F. Rivadeneira[1], J. Janssen*[2], T. J. Beck[3], J. Houwing*[1], A. Hofman*[1], H. Pols[2], C. M. Van Duijn*[1], A. Uitterlinden[2]. [1]Epidemiology & Biostatistics, Erasmus MC, Rotterdam, Netherlands, [2]Internal Medicine, Erasmus MC, Rotterdam, Netherlands, [3]Radiology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Previously, we genotyped 7012 individuals for a CA-repeat polymorphism in the promoter region of the IGF-I gene and found it associated to bone mineral density (BMD) and frailty fracture with gender specific patterns. We now examined if the association between IGF-I genotypes and hip fracture is related to differences in hip bone geometry (HBG). Structural geometry of the femoral neck (FN) was estimated indirectly from standard DEXA outputs in 2372 men and 3134 women. The computational method assumes the FN as a uniform right circular cylinder and provides estimates of FN properties including *neck width* (*W*), *cross-sectional area* (*CSA*), *cortical thickness* (*T*), and indexes of bone stability and strength such as the *buckling ratio* (*BR*) and *section modulus* (*Z*), respectively. Mean HBG parameters adjusted for age, height and weight were compared among fracture cases vs. non-cases and IGF-I promoter genotypes using ANCOVA. Hip fracture cases in both genders show (highly significant) fracture-predisposing patterns of HBG in all outcomes except for *W* (Table 1). Overall, non-carriers of the *192-bp* allele have patterns of HBG that predispose them to fracture as compared to homozygotes for the allele. In males (Table 2) there is a significant allele dose effect over *W* and *Z*, while in females (Table 3) with *T* and *BR*. These findings suggest that this promoter polymorphism or another polymorphism in linkage disequilibrium influences the distribution patterns of bone mass in a gender specific manner: in males, with a greater influence on size and bone strength, and in females, more on the process of cortical thinning with bone instability. HBG contributes to explain partially the mechanism by which IGF-I genotype differences relate to hip fracture risk in the elderly.

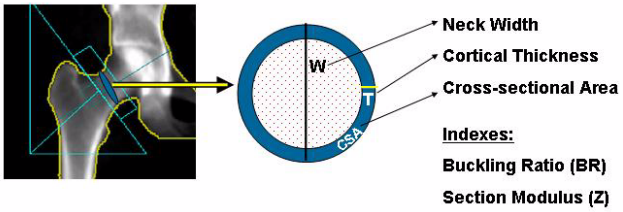

Table 1

	MALES			FEMALES		
	No fracture n=2338	Fracture n=34		No fracture n=3029	Fracture n=105	
BMD (g/cm²)	0.88	↓ 10.5%	p<0.01	0.81	↓ 8.2%	p<0.01
W (cm)	3.03	↑ 1.5%	NS	2.69	↓ 0.3%	NS
CSA (cm²)	2.54	↓ 9.4%	p<0.01	2.09	↓ 8.1%	p<0.01
T (mm)	1.70	↓ 11.5%	p<0.01	1.58	↓ 8.7%	p<0.01
BR	9.17	↑ 13.5%	p<0.01	8.77	↑ 10.5%	p<0.01
Z (cm²)	1.35	↓ 6.3%	p<0.05	0.98	↓ 7.0%	p<0.01

Table 2

MALES

	192-bp ALLELE						
	Homozygotes n=1041		Heterozygotes n=1049		Non-carriers n=282		Trend
BMD (g/cm²)	0.88	↓	0.2%	↓	0.3%		NS
W (cm)	3.04	↓	0.5%	↓	1.0%		p<0.05
CSA (cm²)	2.55	↓	0.7%	↓	1.2%		NS
T (mm)	1.69	↓	0.1%	↓	0.1%		NS
BR	9.21	↓	0.2%	↓	0.7%		NS
Z (cm²)	1.36	↓	1.2%	↓	2.2%		p<0.05

Table 3

FEMALES

	192-bp ALLELE						
	Homozygotes n=1355		Heterozygotes n=1392		Non-carriers n=387		Trend
BMD (g/cm²)	0.82	↓	0.8%	↓	1.7%		p<0.05
W (cm)	2.68	↑	0.6%	↓	0.6%		NS
CSA (cm²)	2.09	↓	0.3%	↓	2.0%		NS
T (mm)	1.58	↓	0.9%	↓	1.7%		p<0.05
BR	8.72	↑	1.5%	↑	1.6%		p<0.05
Z (cm²)	0.97	↓	0.3%	↓	2.1%		NS

Disclosures: ***F. Rivadeneira****, None.*

1074

Predicting Long Term Hip Fracture Risk with Bone Mineral Density and Hip Structure in Post-Menopausal Women: The Study of Osteoporotic Fractures (SOF). T. A. Hillier[1], T. J. Beck[2], T. Oreskovic*[2], J. H. Rizzo*[1], K. L. Pedula*[1], D. Black[3], K. L. Stone[3], J. A. Cauley[4], D. C. Bauer[3], B. C. Taylor*[5], S. R. Cummings[3]. [1]Center for Health Research, Portland, OR, USA, [2]Johns Hopkins, Baltimore, MD, USA, [3]UCSF, San Francisco, CA, USA, [4]U Pittsburgh, Pittsburgh, PA, USA, [5]U MN, Minneapolis, MN, USA.

Although BMD is a strong predictor of hip fracture, the majority of fractures occur in older women that do not have osteoporosis by BMD criteria (Wainwright SA ASBMR 2001). The current study seeks to determine if we can improve the prediction of hip fracture in older women with measures of bone geometry and structure.

We applied our hip structural analysis program (Beck TJ et al, JBMR 16(6), 2001) to hip scans obtained in 7646 women participating in SOF who had BMD measured by DXA (Hologic 1000) in 1989-90. Participants were contacted every four months to ascertain falls and fractures, and more than 98% of these follow-up contacts were completed. Hip fractures were adjudicated from radiology reports. We used Cox-proportional hazards model (PH), with stepwise selection, to identify the subset of non-collinear geometry variables that best predict fracture. These variables: cross-sectional area (CSA), subperiosteal width (W), buckling ratio (BR) and neck shaft angle (NSA), were used in subsequent PH models to estimate hazards ratios (HR), per unit SD, for each geometry variable individually, and as a combined group with and without BMD.

Over an average of 10.4 years of follow-up, there were 642 incident hip fractures. After multivariate (MV) adjustment for covariates previously demonstrated to be significant in predicting fracture, the hazard of hip fracture was significant ($p<0.001$) in each model for femoral neck BMD (HR 2.0 per 1 SD decrease), CSA (HR 1.8 per 1 SD decrease), W (HR 1.2), BR (1.6), and NSA (HR 1.2). A MV model of combined geometry variables (CSA, W, BR, NSA) either with or without BMD did not improve the % variance explained (<6% for all models); BMD was the strongest predictor. In a subset of 908 women who reported no falls during the SOF study, the explained variance increased to 8% with the combined geometry variables, and the HR markedly increased for BR (HR 3.7); BMD was not a significant predictor.

We conclude that hip structure variables derived from HSA are strong individual predictors of fracture, but did not significantly improve the overall prediction of hip fracture above BMD alone. However, there are certain subgroups where HSA may be particularly useful in predicting fracture. Future research should continue to focus not only on understanding important structural differences among persons that fracture, but also on finding ways to improve our current ability to predict only a minority of fractures with BMD.

Disclosures: ***T.A. Hillier****, None.*

1075

Cumulative Risks of Fracture in Patients Using Oral Glucocorticoids. T. P. van Staa[1], P. Geusens[2], H. Pols[3], C. de Laet[3], H. G. M. Leufkens*[4], C. Cooper[5]. [1]Procter&Gamble Pharmaceuticals, Egham, United Kingdom, [2]University Hospital, Maastricht, Netherlands, [3]Erasmus Medical Center, Rotterdam, Netherlands, [4]Utrecht University, Utrecht, Netherlands, [5]Medical Research Council, Southampton, United Kingdom.

The aim of this study was to estimate 5-year risks of fractures in patients using oral glucocorticoids (GC).

The study population consisted of 191,752 patients aged ≥40 years prescribed an oral GC (in the UK General Practice Research Database). The period of follow-up was divided into time-periods of current (i.e. duration of individual prescription plus 3 months) and no exposure, with patients moving between these exposures. Using Cox proportional hazards models, a risk score, indicating the association to risk, was initially estimated from daily and cumulative GC dose of each exposure period, body mass index (BMI), and disease and drug history. Then, the 5-year risk of fracture (Cox survival function) was estimated for each sum of scores.

The risk scores for osteoporotic (O) and hip (H) fracture are summarised in the table (GC daily dose in mg/day [DD], cumulative dose in grams [CD]; GC scores for a person aged 65 years).

DD	CD	O	H		O -6	H -6
< 2.5	≤ 1	0	0	Age (10 years)	4	8
	> 1	3	1	Fall history	8	8
2.5-4.9	≤ 1	5	4	Prior fracture	6	5
	> 1	3	4	BMI < 20	3	6
5-7.4	≤ 1	2	5	RA	2	4
	> 1	5	5	IBD	1	2
7.5-14.9	≤ 1	6	9			
	> 1	7	10			
15-29.9	≤ 1	4	9			
	> 1	9	11			
≥ 30	≤ 1	2	2			
	1-5	6	4			
	> 5	12	12			

The 5-year risk of osteoporotic fracture for patients with scores of 30, 40, 50, and 60 was 7.2, 18.5, 42.7, and 78.1%, respectively. Hip fracture scores of 60, 70, 80, and 90 corresponded to 5-year hip fracture risks of 1.7, 4.6, 11.8, and 28.6%, respectively. The 5-year risk of osteoporotic fracture for a 65-year female with RA, low BMI, and fracture and fall history using 7.5 mg GC (total score of 52) was 49.4% (male with similar history 31.2%). Hip fracture risk was 18.6% (male 10.8%). A 80-year old female with COPD and low BMI who used 2.5 mg for 2 years had a 5-year risk of osteoporotic fracture of 12.8% (male 7.2%). Hip fracture risk was 5.1% (male 2.8%). Pulse dosing (defined as short-term use with DD ≥ 30 mg) was associated with only a small increased risk of osteoporotic fracture in first-time users (relative rate 1.20; 95% CI 0.98-1.46) and in patients with pulse dosing at least 3 months after end of prior GC use (1.21; 1.04-1.42).

In conclusion, this risk score allows for assessing an individual's risk of fracture.

Disclosures: ***T.P. van Staa****, None.*

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A Meta-analysis of Body Mass Index (BMI) as a Predictor of Fracture Risk. C. E. D. De Laet, H. Johansson*, O. Johnell, J. Kanis, E. V. McCloskey, D. Mellstrom*, L. J. Melton, A. Oden*, P. Delmas, P. Garnero, A. Oglesby, J. Eisman, H. Pols, J. Reeve, A. Silman, A. Tenenhouse. Dept of Public Health, Erasmus MC, Rotterdam, Rotterdam, Netherlands.

Today, body mass index (BMI) is a well-documented risk factor for future fracture. The aim of this study was to quantify this effect and to explore the relationship of fracture risk (all osteoporotic fractures and hip fracture) with age, gender and bone mineral density (BMD) from an international perspective using world-wide data. We studied 45,000 men and women from nine prospective population-based cohorts comprising Rotterdam, EVOS/EPOS, CaMos, Rochester, Sheffield, Dubbo, EPIDOS, and two cohorts from Gothenburg with a total follow-up of almost 190,000 person years. The effect of BMI, BMD and age on osteoporotic fracture risk and on hip fracture risk was examined using a Poisson model in each cohort separately. The results of the different studies were then merged using weighted coefficients. Without information on BMD, low BMI was associated with a significantly increased risk of all osteoporotic fractures, controlled for age. But, this risk increase was not linearly distributed across BMI values as shown in the table. At the extremes the risk ratios increased at a steeper rate than around the median. In the common range of BMI (around 26 kg/m²) the risk only modestly increased with decreasing BMI. Also for hip fracture risk, BMI was a significant risk factor, especially at the extremes of the BMI spectrum. We observed no significant differences in risk ratio between men and women. These risk ratios by BMI were markedly reduced when adjusted for BMD suggesting that BMD is an important intermediary.

Risk ratio in men and women combined controlled for age (BMI=25 as reference)

	Osteoporotic fracture		Hip fracture	
BMI	Without BMD	With BMD	Without BMD	With BMD
15	1.95	1.18	4.18	1.64
20	1.30	1.04	1.96	1.32
25	**reference**	**reference**	**reference**	**reference**
30	0.90	0.98	0.83	1.08
35	0.75	0.93	0.76	1.32
40	0.59	0.92	0.52	1.19

We conclude that low BMI confers a risk of fracture of substantial importance that is independent of age and sex. The significance of BMI as a risk factor varies according to the level of BMI. Its validation on an international basis permits the use of this risk factor in case finding strategies. These data also show that there is no conflict between advice for weight control with reasonable target values (such as for the prevention of diabetes or CVD) and the prevention of osteoporotic fractures.

Disclosures: ***C.E.D. De Laet****, None.*

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Increased Fracture Risk among Breast Cancer Survivors -Results from the Women's Health Initiative. Z. Chen[1], M. Maricic[1], T. L. Bassford*[1], C. Ritenbaugh*[2], A. M. Lopez*[1], M. S. Leboff[3], M. Gass*[4], D. H. Barad*[5]. [1]University of Arizona, Tucson, AZ, USA, [2]Kaiser Center for Health Research, Portland, OR, USA, [3]Brigham and Women's Hospital, Boston, MA, USA, [4]University of Cincinnati, Cincinnati, OH, USA, [5]Albert Einstein College of Medicine, Bronx, NY, USA.

Breast cancer survivors have an increased risk of low bone density, however, except for vertebral fractures assessed by a radiographic method, the risk of other fractures among breast cancer survivors has not been previously reported. We hypothesized that postmenopausal women with a history of breast cancer have a higher risk for fractures compared to women of the same age who have not had cancer. The study population was a prospective multiethnic cohort of postmenopausal women from the Women's Health Initiative Observational Study (WHI-OS). Among them, participants who replied affirmatively to the question " Did you ever have breast cancer?" at baseline were considered to be breast cancer survivors (n = 5,298). The reference group included WHI-OS women who had not had any type of cancer at baseline (n = 80,848). The average follow-up was 5.1 years. Information on fractures was self-reported from a yearly updated medical questionnaire. The confirmation rate of all self-reported fractures was approximately 70% based on results from a subset of WHI participants. Self-reported hip fractures were further confirmed by reviewing medical records. At baseline, breast cancer survivors were older (65.0 vs. 63.4 years), more years past menopause (17.6 vs. 15.9 years), and heavier (72.4 vs. 71.5 kg) than the women in the reference group. Results from Cox regression models indicated that breast cancer survivors had more than 28% increased risk for all types of fractures except hip fractures after adjusted for age, years since menopause, weight, ethnicity and geographic region of enrollment into the study (Table 1). In conclusion, compared to women without any cancer history, breast cancer survivors are at an increased risk for fractures, other than hip fractures. Our results suggest that prevention is needed to reduce fracture risk in the growing population of breast cancer survivors.

Table 1. Adjusted Results from the Cox Proportional Hazard Models

Fracture Outcomes	Breast Cancer Survivors (N = 5298)	None Cancer Reference Group (N = 80848)	Hazard Ratio	95% CI		p value
	% Fractures	% Fractures		Lower	Upper	
Hip	0.6%	0.6%	0.912	0.621	1.339	0.766
Clinical spine	1.4%	1.0%	1.287	1.001	1.655	0.049
Lower arm / wrist	3.2%	2.3%	1.323	1.119	1.564	0.001
All other	9.7%	7.6%	1.317	1.119	1.447	0.000
Total	13.6%	10.5%	1.305	1.205	1.414	0.000

Disclosures: ***Z. Chen****, None.*

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A Novel Association Between a Frizzled Related Protein Amino Acid Variant and Vertebral Fracture Risk in Older Women. J. M. Zmuda[1], W. C. Hsueh*[2], L. Lui[2], K. L. Stone[2], J. A. Cauley[1], T. A. Hillier[3], S. Germer*[4], R. Higuchi*[4], D. R. Greene*[4], J. Li*[4], J. D. Allard*[5], T. Nikolcheva*[5], J. Mangaccat*[4], N. Umblas*[4], G. Peltz*[5], S. R. Cummings[2]. [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of California, San Francisco, CA, USA, [3]Kaiser Permanente Center for Health Research, Portland, OR, USA, [4]Roche Molecular Systems, Alameda, CA, USA, [5]Roche Palo Alto, Palo Alto, CA, USA.

Frizzled related protein (FRZB; aka secreted frizzled related protein 3) is a soluble antagonist of Wnt, a secreted growth factor involved in tissue differentiation including skeletal formation. Recently, FRZB has been implicated in the determination of bone mineral density (BMD) in inbred mice (G. Peltz et al. manuscript in preparation). In addition, transfection of FRZB into mesenchymal stem cells can obviate the Wnt-induced formation of osteoblasts instead of adipocytes in-vitro. In the present study, we tested polymorphisms within FRZB and found that a C→G polymorphism which creates an Arg324Gly amino acid substitution is associated with vertebral fracture among women aged 65 years and older in the Study of Osteoporotic Fractures.

Incident hip fractures over an average of 10.8 years of follow-up were validated by review of X-ray films. Incident vertebral fractures were defined by morphometry using lateral spine radiography at baseline and an average of 3.7 years later. Women with an incident hip fracture (n=275), vertebral fracture (n=262) or low (T score <-2.5) total hip BMD (n=276) were compared to 278 control women without a history of fracture and a total hip BMD above the top 20% percentile of the BMD distribution (Z score >1.28). The Arg324Gly polymorphism was genotyped using allele specific, kinetic polymerase chain reaction (Germer, et al., Genome Res 10:258-266, 2000). Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) associated with at least one 324Gly minor allele. The minor allele frequency was 0.10 among controls. There was no significant association between the Arg324Gly polymorphism and either low hip BMD or hip fracture risk. In contrast, women with at least one 324Gly allele had 51% lower age-adjusted risk of vertebral fracture (95% CI: 0.30, 0.82) compared to women with the Arg324Arg genotype. Additional adjustments for body mass index, use of hormone therapy, and lumbar spine BMD did not alter the relationship between the Arg324Gly polymorphism and vertebral fracture risk (OR: 0.39; 95% CI: 0.17, 0.88). We conclude that the Arg324Gly amino acid polymorphism in FRZB, or a closely linked allelic variant, may contribute to the genetic susceptibility to vertebral fracture among older women.

Disclosures: ***J.M. Zmuda****, None.*

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Gain-of-Function Mutation of FGFR2IIIc: A Mouse Model of Crouzon Syndrome. R. M. Locklin*[1], V. P. Eswarakumar*[2], A. Harmelin*[3], G. M. Morriss-Kay*[1], P. Lonai*[2]. [1]Human Anatomy and Genetics, University of Oxford, Oxford, United Kingdom, [2]Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel, [3]Veterinary Services, Weizmann Institute of Science, Rehovot, Israel.

Crouzon syndrome, the most common craniosynostosis syndrome, is associated with dominant mutations of fibroblast growth factor receptor 2 (FGFR2). Most are in exon IIIc, encoding part of the external ligand-binding domain, and cause receptor dimerization leading to ligand-independent activation.

FGFR2 is alternately spliced, having IIIb or IIIc forms with unique binding specificity and expression patterns. IIIc is expressed in pre-osteogenic cartilage rudiments and binds epithelial FGFs. To investigate the role of FGFR2IIIc in craniosynostosis, we generated a gain of function mouse mutant. The homozygous mutation was perinatal lethal, with pronounced brachycephaly, cleft palate, multiple skeletal synostoses and disorders of other organs. The heterozygote was viable and fertile, exhibiting craniosynostosis, a domed skull vault, ocular proptosis and a milder brachycephaly. No limb defects were apparent. The heterozygous mutant is therefore a good model for the human disorder.

For detailed examination, skeletal preparations of heterozygotes were stained with alizarin red. Coronal sutures were found to be completely fused and lambdoid sutures partially fused. Sagittal sutures, which do not close in the mouse, remained partially separable but contained wormian bones. Parietal bones were larger, and frontal bones, smaller, than wild types, while the skull base was shortened and thickened.

Effects on the proliferation-differentiation balance in developing craniofacial bones was investigated using bromodeoxyuridine (BrdU) immunohistochemistry to label proliferating cells. The proportion of BrdU-labelled nuclei at embryonic day 14 (E14), E16 and postnatal day 1 (P1) was quantitated using an image analysis system. At E14 proliferation at the coronal suture was increased in heterozygotes compared to wild types, while by P1 this appeared to be reversed. Thus the mutation could result in an early increase in proliferation followed by an early switch to differentiation, leading to premature fusion of the suture. In contrast, at the basioccipital-basisphenoid synchondrosis, the heterozygotes exhibited a highly significant decrease in proliferating chondrocytes at E14, as well as an increase in the thickness of the periosteum. This may explain the shortened and thickened skull base.

FGFR2IIIc is important in bone formation but exerts different effects on endochondral and intramembranous ossification. The FGFR2IIIc gain-of-function mutant provides a valuable model of Crouzon syndrome.

Disclosures: ***R.M. Locklin****, None.*

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Ubiquitous Overexpression of *Phex* Does Not Fully Rescue the *Hyp* Mouse Phenotype. R. G. Erben[1], D. Mayer*[1], K. Weber*[1], T. Johnson*[1], K. Jonsson[2], H. Jüppner[2], B. Lanske[3]. [1]Institute of Animal Physiology, University of Munich, Munich, Germany, [2]Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [3]Harvard School of Dental Medicine, The Forsyth Institute, Boston, MA, USA.

Mutations of Phex, a phosphate regulating gene with homologies to endopeptidases on the X chromosome, are responsible for X-linked hypophosphatemia (XLH) in humans, and its mouse homologue, Hyp. Recently, it has been shown that targeted overexpression of Phex in osteoblasts is not sufficient to rescue the Hyp phenotype. Therefore, the aim of this study was to examine whether ubiquitous overexpression of Phex in transgenic mice would fully rescue the Hyp phenotype. To test this hypothesis, we generated two mouse lines overexpressing the human Phex gene under the control of a human β-actin promoter. With the exception of brain, RT-PCR analyses showed expression of the transgene in Phex transgenic (Phex-tg) mice in all tissues examined. Subsequently, we crossed female heterozygous Hyp mice with male heterozygous Phex-tg mice to produce wildtype (WT), Phex-tg,

Hyp, and Hyp/Phex-tg offspring. Twelve-week-old male mice were used for all studies. Compared with their WT littermates, Phex-tg mice had normal bone mineral density (BMD) as measured by peripheral quantitative computed tomography, normal bone histology, and normal serum and urinary phosphate. Hyp mice demonstrated the known phenotype characterized by reduced body weight, hypophosphatemia, hyperphosphaturia, and rickets. Hyp/Phex-tg mice had normal body weight relative to WT controls, and showed a dramatic, albeit not complete, improvement in femoral BMD. Total BMD of the femoral shaft increased from 384 ± 28 in Hyp to 541 ± 27 in Hyp/Phex-tg mice, and total BMD of the femoral metaphysis from 315 ± 38 in Hyp to 407 ± 25 mg/cm³ in Hyp/Phex-tg mice (means ± SD). In WT littermates, the BMD of the femoral shaft and the femoral metaphysis was 596 ± 32 and 455 ± 56 mg/cm³, respectively. Compared with Hyp mice, bone histomorphometry in Hyp/Phex-tg mice revealed pronounced improvements in structural bone parameters, a normal growth plate, and a dramatic reduction in osteoid volume, although some disturbances in bone mineralization remained. Surprisingly, however, Hyp and Hyp/Phex-tg mice had comparable reductions in tubular reabsorption of phosphate, and were hypophosphatemic relative to WT controls. All results were similar for both Phex-tg mouse lines. Thus, our data further strengthen the notion that the pathophysiological mechanisms behind renal phosphate wasting and the bone mineralization defect in XLH may involve different factors.

Disclosures: ***R.G. Erben****, None.*

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High-Throughput Analysis of Bone Phenotypes in Gene Knockout Mice to Identify Novel Osteoporosis Drug Targets. R. Brommage[1], Z. Z. Shi*[2], G. Liu*[1], D. R. Powell*[1]. [1]Endocrinology, Lexicon Genetics, The Woodlands, TX, USA, [2]Target Validation, Lexicon Genetics, The Woodlands, TX, USA.

Screening gene function *in vivo* is a powerful approach to discover novel drug targets in the human genome (Zambrowicz and Sands, Nature Review Drug Discovery 2:38-51; 2003). Using both gene-trapping and homologous recombination technologies, 926 knockout (KO) mouse lines have been generated, involving genotyping ~255,000 mice. Mice are F2 crosses from C57BL/6J and 129 SvEv parental strains. Of these 926 KO lines, 229 (25%) are embryonic/neonatal lethal or have reduced viability. All 697 viable lines have been analyzed by PIXImus DEXA at ~14 weeks of age, when peak BMD has been attained. Using a Scanco μCT40, trabecular bone parameters in the fifth lumbar vertebrae (LV5) have been analyzed in 279 lines and midfemur cortical thickness (CT) in 243 lines. DEXA analyses are performed on both male and female mice, but only males are analyzed by μCT. For the vast majority of lines, 4 KO and 2 wild-type control mice are analyzed for each gender. For DEXA screening, volumetric BMD in KOs is compared to both littermate (LM, n = 668 lines) and historical (HC, n = 697 lines) wild-type controls. Volumetric BMD in KOs averages 99.7% of LM values with a standard deviation (SD) of 2.9% and 99.8% of HC values with a SD of 4.4%. LV5 trabecular bone volume (TBV) in KOs averages 20.3% with a SD of 4.2%. Femoral CT averages 253 μm with a SD of 15 μm. Lines with high bone mass resulting from geometrically large bones are of low interest for drug development. These high throughput screens successfully identified two genes known to be important in bone metabolism. LRP5 KOs have low bone mass (vBMD = 89% of LM and 91% of HC, CT = 226 μm) and *klotho* KOs have high vBMD (119% of LM and 133% of HC) and high TBV (43%) but low CT (165 μm). KO of the amino-bisphosphonate target farnesyl diphosphate synthase is embryonic lethal. Using these high throughput procedures, a recently identified gene (LG881) coding for an enzyme has been observed to influence bone mass. TBV in female KO mice is elevated by 52% in LV5 (22.2% ± 1.5%, n = 14 vs 14.6% ± 1.1%, n = 13; $P < 0.001$) and 144% in the distal femur metaphysis (7.8% ± 1.4%, n = 14 vs 3.2% ± 0.7%, n = 13; $P = 0.01$). At both sites, increased trabecular number is more dramatic than changes in trabecular thickness. No nonskeletal phenotypes have been identified. The high bone mass phenotype resulting from genetic ablation of this enzyme provides a novel target for the development of drugs to treat osteoporosis.

Disclosures: ***R. Brommage****, Lexicon Genetics 3.*

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The Bone Morphogenetic Protein 2 Gene Contributes to Bone Density and Osteoporotic Fractures. U. Styrkarsdottir[1], J. Cazier*[1], O. Rolfsson*[1], H. Larsen*[1], E. Bjarnadottir*[1], V. D. Johannsdottir*[1], M. S. Sigurdardottir*[1], K. Jonasson*[1], M. L. Frigge*[1], A. Kong*[1], J. R. Gulcher*[1], G. Sigurdsson[2], K. Stefansson*[1]. [1]deCode Genetics, Reykjavik, Iceland, [2]National University Hospital, Reykjavik, Iceland.

The purpose of this study was to map a locus for osteoporosis and isolate the corresponding gene.

A genome wide scan was performed in extended Icelandic osteoporotic families with a framework scan of 1000 microsatellite markers. We used multipoint, affected-only allele-sharing methods to assess the evidence for linkage, obtained using the program Allegro and the Decode Genetic map. An initial set of 207 pedigrees was assembled containing 1334 study individuals. Linkage analysis was performed by defining an osteoporotic phenotype and considering only those meeting criteria as affected. Phenotypes aimed at mapping genes specific to skeletal sites (hip and spine), and mapping genes causing generalized osteoporosis (moderate and severe). Age-sex-and-weight corrected BMD at the hip or spine, below a certain cut-off (lowest 16th for hip, spine, or moderate phenotype and lowest 10th for the severe phenotype) was used as the basic phenotype, adding osteoporotic fractures and bisphosphonate users as affected. Strongest linkage was identified to chromosome 20p12 in all four phenotypes, and after adding more markers to the region, thereby increasing the information content to over 95%, resulted in LOD scores of 2.87, 4.56, 3.75 and 4.96 (P value 8.8×10^{-7}), for spine, hip, moderate and severe phenotypes, respectively.

The 9 cM region defined within the drop of one in LOD score corresponds to a 2.2 MB segment containing 6 known genes (*BMP2*, *CHGB*, *LOC51605, C20orf154*, *C20orf155*, and *C20orf42*), the *BMP2* gene being a strong candidate. However, expression analysis showed that four of the genes in the region are expressed in bone marrow or in an osteoblast cell line (*BMP2, C20orf42, C20orf154, and CHGB*). A very dense set of polymorphic markers distributed across the 1-LOD drop, 30 microsatellites and 99 SNPs, were used for identifying the osteoporosis gene by association analysis and LD analysis. A case-control association analysis in 800 affected (severe phenotype) and 800 randomly collected controls identified haplotypes spanning the *BMP2* gene associated with osteoporosis. *BMP2* is associated with many definitions of an osteoporotic phenotype, including osteoporotic fractures as well as low bone mineral density (BMD), both before and after menopause, and in men. Thus, *BMP2* gene variation appears to be a major risk factor for osteoporosis and osteoporotic fractures in Iceland.

Disclosures: ***U. Styrkarsdottir****, None.*

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Analysis of *GCMB* Gene Allelic Variants in Isolated Hypoparathyroidism. C. Ding[1], Z. Deng*[1], M. A. Levine[2]. [1]Pediatrics, Johns Hopkins University, Baltimore, MD, USA, [2]Pediatrics, Cleveland Clinic Foundation, Cleveland, OH, USA.

Mutations of the genes encoding PTH and the calcium sensing receptor are associated with isolated hypoparathyroidism (IH), but many cases remain unexplained thus implicating other genes in the molecular pathogenesis. One candidate is the *GCMB* gene located at chromosome 6p23-24, which encodes a human ortholog of the *Drosophila glial cells missing (gcm)* gene, especially as this protein is expressed predominately, if not exclusively, in parathyroid cells and is critical for development of the parathyroid glands. We previously reported homozygous intragenic deletion of the *GCMB* gene as the basis for autosomal recessive IH in one kindred (Ding *et al*, J Clin Invest 2001). To investigate the prevalence of *GCMB* mutations we isolated genomic DNA from 25 patients with sporadic or familial IH from 16 families, and analyzed the 5 exons, intron-exon boundaries, and the promoter of the *GCMB* gene by direct sequencing of PCR products. We identified variant nucleotide changes in 10 patients, including heterozygous promoter mutations (-74 G to T and -149 G to A), neutral mutations (codons 154 AAG to AAA; 203 GGT to AGT) and missense mutations (G203S, I227V, Y282D, and N315D). Among the 8 patients with missense mutations, 6 are heterozygous and 2 are compound heterozygotes. Linkage analysis of *GCMB* mutations in one extended kindred excluded both the Y282D and N315D variants as causative of IH, however neither variant was present as a common polymorphism in a screen of genomic DNA from 50 unrelated subjects. To examine the transactivating potential of the *GCMB* variants, we used site-directed mutagenesis to insert the missense mutations into an epitope-tagged wild type human GCMB cDNA. Wild type or mutant GCMB cDNA's were transiently expressed in HEK293T cells with a luciferase reporter plasmid carrying six tandemly arranged GCM binding sites (6x gbs luc). RT-PCR and immunoblot showed that variant forms of GCMB with amino acid substitutions were expressed at levels similar to that of wild type GCMB. GCMB variants with I227V or Y282D were as effective as wild type GCMB in stimulating luciferase activity (50 to 150-fold stimulation). By contrast, the G203S and N315D variants stimulated luciferase activity at levels that were 40% and 50% lower than wild type GCMB, respectively. Co-transfection of HEK293T cells with equal amounts of wild type and variant GCMB did not show dominant inhibitor activity. Our results suggest that *GCMB* gene mutations are an unusual cause of IH. On the other hand, our study indicates that there are many allelic variants for GCMB, some of which appear to have reduced transactivating potential. The prevalence, and significance, of these allelic variants remains to be determined.

Disclosures: ***M.A. Levine****, None.*

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Deletion of *Mepe* in *Hyp* Mice Fails to Correct Hypophosphatemia but Partially Rescues Abnormal Mineralization *Ex Vivo*. S. Liu[1], T. A. Brown*[2], Z. Xiao[1], R. Guo*[1], L. D. Quarles[1]. [1]Medicine, Duke University, Durham, NC, USA, [2]Department of Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT, USA.

X-linked Hypophosphatemia (XLH) is characterized by hypophosphatemia, impaired mineralization and aberrant $1,25(OH)_2D_3$ regulation. Inactivating mutations of PHEX is the cause of XLH. PHEX deficiency leads to the accumulation of a phosphaturic factor, called phosphatonin. The matrix extracellular phosphoglycoprotein *Mepe* (also called *Of45*) is increased in the *Hyp* mouse homologue of XLH and is a candidate for phosphatonin. To gain further insights into the biological relevance of MEPE in the pathogenesis of the XLH, we transferred *Mepe*-deficient mice onto the *Hyp* mouse background. *Hyp* mice had hypophosphatemia (5.5±0.5 mg/dl), lower serum calcium (7.1±0.1 mg/dl), increased serum PTH (44 ± 6 pg/ml), and inappropriately normal $1,25(OH)_2D_3$ levels (213±40.5 pM) compared to wild-type controls (9.1±0.6 mg/dl, 7.7±0.1mg/dl, 33±9 pg/ml and 217 ±37 pM). *Mepe*$^{-/-}$mice had normal serum phosphorus (9.9±0.6 mg/dl), calcium (7.7±0.1 mg/dl) and $1,25(OH)_2D_3$ (262 ±43 pM), and unexpectedly reduced serum PTH levels (17±3 pg/ml). Transfer of *Mepe* deficiency onto the *Hyp* mouse background failed to correct the hypophosphatemia (5.3±0.3 mg/dl), and resulted in serum calcium (7.4±0.1mg/dl), PTH (34.0 ±9.2 pg/ml) and $1,25(OH)_2D_3$ (221±11 pM) levels in the *Mepe* $^{-/-}$*/Hyp* mice indistinguishable from values in *Hyp* mice. Femoral BMD (g/cm^2) was significantly lower in *Hyp* mice (0.048±0.002) compared to wild-type controls (0.065 ±0.009). BMD in *Mepe*$^{-/-}$mice (0.062±0.001) was not increased and *Mepe*$^{-/-}$*/Hyp* mice retained low BMD values (0.043±0.002). In contrast, serum osteocalcin (pg/ml) and urinary Dpd/creatinine were significantly greater in *Hyp* (249±30 and 92 ±25) compared to respective values in wild-type controls (175±13 pg/ml and 13.5 ±3.9) and *Mepe*$^{-/-}$mice (200±18 and 20±4). *Mepe*$^{-/-}$*/Hyp* mice had further increases in osteocalcin (348±29) but not Ddp/Cr (59±15) levels. Bone marrow stromal cultures (BMSCs) derived from *Hyp* mice displayed impaired mineraliza-

tion compared to wild-type and *Mepe*$^{-/-}$ mice, whereas BMSCs from *Mepe*$^{-/-}$*/Hyp* mice had increased mineralization indistinguishable from wild-type controls. These results demonstrate that M*epe* is not mediating the hypophosphatemia in *Hyp* mice, and that persistent hypophosphatemia in M*epe*$^{-/-}$*/Hyp* may mask the effects of *Mepe* deficiency to improve bone mineralization, since rescue of abnormal mineralization occurred in *Hyp* and *Mepe*-deficient BMSCs. These results demonstrate that *Mepe* is not phosphatonin, but may be involved in the pathogenesis of defective mineralization in *Hyp* mice.

Disclosures: ***S. Liu****, None.*

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Evidence for a Physiological Role of FGF-23 in the Regulation of Renal Phosphate Reabsorption and Plasma Calcitriol in Healthy Humans. S. L. Ferrari, J. P. Bonjour, R. Rizzoli. Department of Geriatrics and Internal Medicine, Division of Bone Diseases, Geneva, Switzerland.

FGF-23 appears to be responsible for tumor-induced osteomalacia (TIO) and is also implicated in autosomal-dominant hypophosphatemic rickets (ADHR), two disorders characterized by hypophosphatemia, renal phosphate (Pi) wasting and inappropriately low plasma calcitriol. Pi homeostasis is regulated by tubular Pi reabsorption and calcitriol production, two regulators which respond to dietary Pi intake independently of PTH. To investigate whether FGF-23 contributes to the physiological regulation of Pi homeostasis in humans, we measured the renal handling of Pi, calciotropic hormones and serum FGF-23 in 29 healthy male volunteers (mean age ±sem, 24.3 ±0.5 yrs) submitted to a dietary modification trial for two weeks. After 2 days on their regular diet, subjects were advised to a low Pi diet, including a Pi binder (Al and Mg hydroxide, 1g before each meal) for 5 d. During this period, dietary calcium (Ca) was also restricted in order to minimize concomitant changes in PTH. This was followed by 2 d. of regular diet and 5 d. of high Pi diet including a supplement of 1000 mg Pi/d. Measurements were performed on the last day of each diet period after an overnight fast. Serum FGF-23 was measured by ELISA recognizing the COOH-terminus (aa 180-251) of the molecule (Immunotopics Inc. (San Clemente, CA), with a lower limit of detection of 3 RU/ml and an inter-assay CV of 5 to 7.3%.
On regular, low and high Pi diet, 24 hr. urinary (U) Pi excretion was 1.99 ±0.07, 1.03 ± 0.08 and 3.68 ±0.16 (mmole/mmoleCreat/d, p<0.0001), respectively, and plasma Pi was 1.26 ±0.03, 1.14 ±0.03 and 1.23 ±0.03 (mmole/L, p=0.0033), whereas plasma iCa remained unchanged. Among subjects adherent to the dietary changes (27/29), FGF-23 levels decreased by 29 ±6% and increased by 31 ±9% on low and high Pi diets, respectively, compared to regular diet (p<0.0001), and were positively correlated with 24 hr. UPi excretion (p=0.0003) but not with 24 hr. UCa excretion. In contrast, PTH increased non-significantly on both low and high diets. FGF-23 was negatively correlated with tubular Pi reabsorption (TmPi/GFR, p=0.0028) and plasma calcitriol (p=0.05), whereas PTH was not. By multiple regression analysis, plasma Pi and FGF-23 were both negatively correlated to calcitriol (p=0.0021 and 0.04, respectively), whereas PTH was not.
In conclusion, FGF-23 plasma levels vary in response to changes in dietary Pi. FGF-23 negatively correlates with both renal Pi reabsorption and plasma calcitriol, suggesting that this phosphaturic factor plays a physiological role in Pi homeostasis.

Disclosures: ***S.L. Ferrari****, None.*

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Parathyroid Hormone (1-34; Teriparitide) Enhances Experimental Fracture Healing. Y. M. Alkhiary[1], L. C. Gerstenfeld[2], D. M. Cullinane[2], D. Nathanson*[1], E. Krall[2], M. Sato*[3], B. Mitlak*[3], T. A. Einhorn[2]. [1]Restorative Sciences & Biomaterials, Boston University School of Dental Medicine, Boston, MA, USA, [2]Boston University Medical Center, Boston, MA, USA, [3]Lilly Research Laboratories, Indianapolis, IN, USA.

Substantial progress has been made in the use of surgically implanted recombinant proteins to enhance fracture (fx) healing where there is nonunion or substantial loss of bone. However, a systemically administered therapy that could promote fracture healing would be desirable. We therefore tested the hypothesis that a once-daily subcutaneous injection of teriparatide could enhance fx healing. Two hundred fifty-two male Sprague Dawley rats underwent standard, closed mid-diaphyseal femoral fxs. Immediately post-fx, animals were divided into three groups and administered daily injections of 5 ug/kg teriparatide, 30 ug/kg teriparatide, or aqueous vehicle (control) for a maximum of 35 days treatment. Each group was further divided into three subgroups, which were euthanized on day 21, 35, or 84 post fx respectively. At necropsy, bones were harvested and the calluses subjected to QCT and mechanical torsion testing to failure. Additionally, calluses from three animals in each group were analyzed by histomorphometry. The 30 ug dose produced significant increases on day 21 post-fx for bone mineral content (BMC; p=0.006), bone mineral density (BMD; p=0.001), and percent cartilage in the callus (p=0.004) relative to controls. On day 35, both the 30 ug (p=0.001) and the 5 ug (p=0.01) groups showed increases in BMC and BMD. At this time, the 30 ug group also showed a significant increase in torque strength (p=0.001). While dosing was discontinued after day 35, analyses performed after 84 days in rats previously treated with 30 ug showed sustained increases over control for BMC (p=0.001) and BMD (p=0.002). Histological analysis and geometric measurements of the calluses further showed that there was no change in osteoclast number, while overall cortical diameters in the teriparatide treated animals were greatest at 84 days. These data show that daily subcutaneous administration of low-dose teriparatide, PTH (1-34), enhances fracture healing by increasing BMD, BMC, and strength, and produces a sustained anabolic effect throughout the remodeling phase of fracture repair.

Disclosures: ***Y.M. Alkhiary****, Eli Lilly Research Laboratories 2.*

1087

MEPE Regulates Bone Mineralization and Phosphate Transport: PHEX and the MEPE ASARM-Peptide. P. S. N. Rowe[1], Y. Kumagai[2], G. Gutierrez[3], I. R. Garrett[3], R. Blacher*[2], D. Rosen*[2], D. Chen[3], M. K. Drezner[4], L. D. Quarles[5], G. R. Mundy[6]. [1]Periodontics, UTHSCSA, San Antonio, TX, USA, [2]Acologix, Emeryville, CA, USA, [3]Osteoscreen, San Antonio, TX, USA, [4]Univ of Wisconsin, Madison, WI, USA, [5]Duke Univ, Durham, NC, USA, [6]UTHSCSA, San Antonio, TX, USA.

Matrix extracellular phosphoglycoprotein (MEPE) expression is markedly elevated in X-linked hypophosphatemic rickets (Hyp) osteoblasts and in oncogenic hypophosphatemic osteomalacia (OHO) tumors. It is also expressed exclusively in osteoblasts, osteocytes, and odontoblasts. Since the Hyp defect is associated with osteoblast-secreted factors that impair mineralization and renal phosphate excretion and OHO has many phenotypic similarities to Hyp, we examined the effects of recombinant full-length human-MEPE (Hu-MEPE) on serum and urinary phosphate in-vivo, 33PO4-uptake in renal proximal-tubule-cultures and mineralization of osteoblast cultures. Dose dependent hypophosphatemia and hyperphosphaturia occurred in mice following intraperitoneal (IP) administration of Hu-MEPE (up to 400 ug/kg/31h), similar to mice given the phosphaturic hormone PTH (80 ug/kg/31h). Also the fractional excretion of phosphate (FEP) was stimulated by MEPE (65.0% (P<0.001)) and PTH groups (53.3% (P<0.001) relative to the vehicle group (28.7% (SEM 3.97)). In addition, Hu-MEPE significantly inhibited 33PO4-uptake in primary human proximal-tubule renal-cells (RPTEC) and a human renal cell-line (Hu-CL8) in vitro (Vmax 53.4% inhibition; Km 27.4 ng/mL, and Vmax 9.1% inhibition; Km 23.8 ng/mL respectively). Moreover, Hu-MEPE dose dependently (50 to 800 ng/mL) inhibited BMP2 mediated mineralization of a murine osteoblast cell-line (2T3) in vitro. Inhibition of mineralization was localized to a cathepsin-B released carboxy-terminal acidic MEPE-peptide containing the acidic-serine-aspartate-rich motif (ASARM-peptide). We have demonstrated previously that PHEX and a carboxy-terminal fragment of PHEX protects MEPE from cathepsin B cleavage and subsequent release of the acidic ASARM-peptide. Also, PHEX is defective in HYP and there is a marked increase in expression of MEPE and specific proteases (cathepsin D, ECEL1/DINE and neprilysin), potentially resulting in elevated levels of ASARM-peptide and in consequence rickets/osteomalacia. We propose that a novel PHEX-MEPE cell-surface protective-interaction is responsible for modulating mineralization and renal phosphate-handling. The distinct activities of these MEPE-peptides may be of use for the treatment of phosphate-handling and mineralization disorders in teeth, bone, kidney and also in the prevention of ectopic-mineralization.

Disclosures: ***P.S.N. Rowe****, Acologix N; Osteoscreen 5.*

1088

Glitazones, the Anti-Diabetic PPAR-gamma Agonists, Pose a Significant Risk of Bone Loss; Differential Effects of Rosiglitazone and Netoglitazone. B. Lecka-Czernik, L. J. Suva, D. Gaddy, S. O. Rzonca*. Geriatrics, Orthopaedic Surgery, and Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Glitazones compose a new class of oral anti-diabetic agents that are used for treating type 2 diabetes. These compounds sensitize cells to insulin through specific activation of PPAR-γ nuclear receptor. One of its isoforms, PPAR-γ2, is expressed in marrow mesenchymal stem cells and controls osteoblast and adipocyte development. PPAR-γ2 activated with rosiglitazone, an FDA-approved anti-diabetic glitazone, inhibited osteoblast and promoted adipocyte differentiation *in vitro* and *in vivo*. Rosiglitazone administration to non-diabetic C57BL/6 adult mice (6 mo) at a dose of 20 μg/g body weight/day for 7 weeks led to a significant decrease in bone mineral density (BMD) and bone mineral content (BMC). Detailed analysis of the microarchitecture of the proximal tibia using microcomputed tomography (microCT) revealed a significant decrease in BV/TV (32%), trabeculae number (22%) and connectivity (21%), and an increase in the spacing between trabeculae (33%) (p<0.05). Changes in bone structure were accompanied by changes in the expression of adipocyte and osteoblast marker genes measured in the whole tibia using real time PCR. The expression of fatty acid binding protein aP2 was increased 3-fold, whereas expression of osteoblast-specific genes, such as Runx2/Cbfa1, Dlx5, osteocalcin and collagen I, was decreased by 50%. Interestingly, although rosiglitazone administration to animals did not change the *ex vivo* potential of isolated marrow mesenchymal stem cells to differentiate toward osteoblasts and adipocytes, it significantly decreased the fraction of osteoblastic precursors that are sensitive to rosiglitazone in culture. These cells may represent plastic precursors capable of interconversion between adipocyte and osteoblast phenotypes. A similar decrease in the same cell fraction was seen in non-treated 24 mo old, compared to 6 mo old animals. Another agonist, netoglitazone, which binds to PPAR-γ with lower affinity, yet its anti-diabetic efficacy is 50-times greater than rosiglitazone, was examined. Netoglitazone was over 100-times less effective than rosiglitazone in both the inhibition of osteoblastogenesis and induction of adipogenesis in the model of marrow mesenchymal cell differentiation, U-33/γ2 cells. Netoglitazone administration to mice led to much smaller decreases in BMD and BMC, compared to the similar rosiglitazone dose. These results indicate that glitazones induce changes in bone that resemble age-related bone loss, and suggest that there may be effective anti-diabetic PPAR-γ agonists with little or no adverse effects on bone.

Disclosures: ***B. Lecka-Czernik****, None.*

1089

Transgenic Mice Overexpressing Human FGF23(R176Q) Recapitulate a Severe Form of the ADHR Phenotype. X. Y. Bai*[1], D. S. Miao[2], J. R. Li*[2], D. Goltzman[2], A. C. Karaplis[1]. [1]Medicine, SMBD-Jewish General Hospital, McGill University, Montreal, PQ, Canada, [2]Medicine, MUHC, McGill University, Montreal, PQ, Canada.

Renal phosphate wasting is associated with the hereditary disorders X-linked (*XLH*) and autosomal dominant (ADHR) forms of hypophosphatemic rickets and the acquired condition of tumor-induced osteomalacia (TIO). In addition to hypophosphatemia, patients with these diseases exhibit decreased or inappropriately normal serum levels of 1,25-dihydroxy vitamin D3 [1,25$(OH)_2D_3$], as well as rickets/osteomalacia. The genes responsible for XLH and ADRH have been identified as *PHEX* and *FGF23*, respectively. In ADHR, mutations in FGF23 prevent proteolytic cleavage, increase stability, and enhance the biological potency of the circulating protein. Elevated circulating serum levels of FGF23 have now been reported in XLH patients and FGF23 is abundantly expressed in tumors associated with TIO. To explore the biological actions of FGF23 in the intact organism, the cDNA encoding the R176Q form of FGF23 described in patients with ADHR, was introduced in a transgenic mouse model by targeting its expression in the liver.

The transgene was constructed in the pLIV.7 plasmid, which contains 3-kb of 5' flanking sequence, exon 1, part of exon 2, a 254-bp fragment of the 3' end, including the poly(A) signal, and 1.7-kb hepatic control region (HCR) of the human apolipoprotein E3 gene. Human FGF23-myc tag was mutated to encode FGF23(R176Q)-myc, and ligated into pLIV.7. The transgene was then microinjected into C57B/6J x CBA single cell embryos.

Animals carrying the transgene developed features characteristic of rickets and osteomalacia, such as shortened long bones and tail, whose severity correlated with the copy number of the transgene. Biochemically, they exhibited marked elevation in serum FGF23 levels, serum alkaline phosphatase activity, and PTH, while demonstrating normocalcemia with hypophosphatemia and increased urinary phosphate excretion. Histologically, in the long bones there were widened metaphyses, smaller epiphyses, thicker growth plates with irregular chondrocyte columns, disorganized hypertrophic zone lacking mineralization, and thickened trabeculae with large amount of unmineralized osteoid, consistent with extensive rickets/osteomalacia. Analysis of other tissues identified diffuse parathyroid hyperplasia and renal cysts in the mice carrying the transgene.

These animals recapitulate the morphological and biochemical features characteristic of a severe form of AHDR and will serve as a useful tool for dissecting the molecular signals that alter renal phosphate handling and vitamin D metabolism in the renal phosphate wasting states of XLH, ADHR, and TIO.

Disclosures: ***A.C. Karaplis****, None.*

1090

Expression of the Measles Virus Nucleocapsid (MVNP) Gene in Osteoclasts Increases Osteoclast Formation and Activity In Vitro and In Vivo. G. D. Roodman[1], N. Kurihara[1], S. V. Reddy[1], F. Singer[2], T. Cundy[3], H. Zhou*[4], D. Dempster[4], J. Windle*[5]. [1]Medicine-Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Endocrine, John Wayne Cancer Ctr, Santa Monica, CA, USA, [3]Medicine, University of Auckland, Auckland, New Zealand, [4]Pathology, Helen Hayes Hospital, New York, NY, USA, [5]Human Genetics, Virginia Commonwealth University, Richmond, VA, USA.

We reported that OCL from Paget's patients (PD) express MVNP and that OCL formed by human OCL precursors transfected with the Edmonston (Ed) strain of MVNP gene express a pagetic phenotype (increases in OCL numbers, nuclei/OCL, and sensitivity to 1,25-$(OH)_2D_3$). We recently reported the full-length MVNP sequence from a PD, which contained mutations in the C-terminus that differed from the Ed-MVNP and were also present in three other PD. To determine the effects of expressing MVNP and the PD mutations on OCL in vivo, we targeted the Ed or the PD-MVNP to cells in the OCL lineage in transgenic mice using the tartrate resistant acid phosphatase promoter (TRAP). Bone marrow from transgenic mice or nontransgenic littermates (controls) were tested for their capacity to form OCL, their sensitivity to 1,25-$(OH)_2D_3$, and their bone resorbing capacity in vitro. Both TRAP-PD -MVNP and Ed-MVNP marrow formed increased numbers of highly multinucleated OCL (30-50 nuclei/OCL). Interestingly, TRAP-PD-MVNP marrow required only 10^{-11}M 1,25$(OH)_2D_3$ to form large numbers of OCL while TRAP-Ed-MVNP marrow cells required 10^{-10}M. Marrow from both types of transgenic mice formed more OCL (three to fourfold) and were hyper-responsive to 1,25$(OH)_2D_3$ than marrow from nontransgenic littermates. Furthermore, $TAF_{II}17$, a potential coactivator of the vitamin D receptor that confers hyperresponsivity to 1,25-$(OH)_2D_3$ in OCL precursors from PD, was increased more than 50% in TRAP- PD-MVNP marrow cells than TRAP-Ed-MVNP mice but was not increased in control mice. Histomorphometric analysis of vertebrae, femurs and tibiae from TRAP-PD-MVNP mice showed increased OCL/mm bone (1.99 ± 0.8 vs. 0.89 ± 0.26 mean $\pm$ SD $p < .01$) and OCL surface ($5.04 \pm 2.11\%$ vs. $2.56 \pm 0.26\%$ $p < .01$) compared to nontransgenic littermates. Similar results were seen with TRAP-Ed-MVNP mice. Bone resorption lacunae were deeper and more numerous in both types of transgenic mice than controls. These data demonstrate that expression of the MVNP gene in OCL increases OCL formation and bone resorption in vivo, and mutations in the MVNP sequence present in PD increase the capacity of OCL precursors to respond to 1,25-$(OH)_2D_3$. These data support an important role for the MVNP gene in the pathogenesis of Paget's disease, and suggest that the "pagetic" mutations in MVNP may have physiologic significance.

Disclosures: ***G.D. Roodman****, None.*

1091

Stimulation of Cyclooxygenase-2 Expression by TGF-Beta Enhances Bone Metastases in Breast Cancer. T. Hiraga, T. Yoneda. Dept Biochem, Osaka Univ Grad Sch Dent, Osaka, Japan.

Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme of prostaglandin (PG) synthesis. Recent studies have shown that COX-2 is involved in angiogenesis, growth, apoptosis and invasiveness in breast cancer, suggesting the importance of COX-2 in malignant behavior of breast cancer. However, the role of COX-2 in distant metastasis in breast cancer is still unclear. Bone is one of the commonest sites of metastasis in breast cancer patients. Clinical studies have shown that the PG production by breast cancer cells positively correlates with the occurrence of bone metastases. These data raise the possibility that COX-2 expression in breast cancer cells contributed to the pathophysiology of bone metastases. To approach this, we studied the MDA-MB-231 (MDA-231) human breast cancer cells that caused bone metastases following heart inoculation. Immunohistochemical examination showed that MDA-231 cells in the close vicinity of residual bone expressed intense COX-2 in bone metastases. In contrast, COX-2 expression was undetectable in MDA-231 tumors developed in orthotopic site. Histomorphometrical examination revealed that a selective COX-2 inhibitor NS-398 (20mg/kg/day for 3weeks, ip) significantly reduced bone metastases with decreased number of osteoclasts, suggesting a critical role of COX-2 in bone metastases. Of note, the bisphosphonate ibandronate reduced COX-2 expression in MDA-231 cells in bone metastases, suggesting that COX-2 expression is dependent on bone resorption. Since evidence is accumulating that bone resorption releases bone-stored growth factors which in turn modulate the metabolism of metastatic cancer cells in bone, we next determined the effects of TGF-beta (TGFb), one of the most abundant growth factors stored in bone, on COX-2 expression in MDA-231 cells in culture. TGFb increased COX-2 expression in MDA-231 cells, whereas IGF, FGF and PDGF that are also stored in bone showed no effects. ELISA demonstrated that TGFb significantly increased PGE2 production and NS-398 inhibited PGE2 production in a dose-dependent manner in MDA-231 cells. Conditioned medium harvested from TGFb-treated MDA-231 cells significantly stimulated osteoclast-like cell formation in mouse bone marrow cultures, while the conditioned medium of MDA-231 cells treated with TGFb in the presence of NS-398 exhibited no effects. In conclusion, our results suggest that bone-derived TGFb up-regulates COX-2 expression in breast cancer cells colonizing bone, thereby increasing PG production, which in turn stimulates osteoclastic bone destruction, leading to the progression of bone metastases. Our results also suggest that suppression of COX-2 is a potential therapeutic intervention for bone metastases in breast cancer.

Disclosures: ***T. Hiraga****, None.*

1092

Lymphoproliferative Disease Induces Spontaneous Osteolytic Bone Lesions in Tax Transgenic Mice. L. Gao*[1], H. Zhao[2], H. J. Deng*[1], S. Kaushik*[1], J. Harding*[1], L. Ratner*[1], K. Weilbaecher[1]. [1]Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA, [2]Department of Pathology, Washington University School of Medicine, St. Louis, MO, USA.

Adult T-cell leukemia/lymphoma (ATLL) is associated with expression of the HTLV-1 viral oncogene, tax, and with hypercalcemia and bone invading (osteolytic) lesions in 70% of patients. Tax transgenic mice have been previously generated utilizing the human granzyme B promoter that limits tax gene expression to the mature T-cell. Tax mice develop LGL leukemia and soft tissue tumors. We found that 70% of Tax mice developed numerous osteolytic bone lesions throughout the tail vertebre identified by X-ray analysis, between the ages of 4-12 months (Fig.1A). Bone mineral density measured by DEXA in the affected tail regions showed significant bone loss. TRAP staining on paraffin embedded tissue sections showed increased number of osteoclasts (indicated by arrows) at the interface of tumor and bone (Fig.2). Tax and wild type cultured osteoclasts were indistinguishable for the ability to form multinucleated TRAP positive cells capable of resorbing calcium phosphate matrix. Soft tissue tumor expression of osteoclast activating factors such as IL-6, PTHrP, M-CSF, and TNFa were similar in mice with and without osteolytic bone lesions. However, we detected higher RANKL expression by RT-PCR in soft tissue tumors associated with osteolytic bone lesions compared to soft tissue tumors without bone lesions. Furthermore, Tax+/Interferon gamma null mice developed widespread bone lesions and soft tissue tumors at earlier ages associated with more severe bone destruction compared to Tax+/Interferon gamma wild type mice (Fig.1B). We conclude that Tax mice develop spontaneous osteolytic bone metastases that are associated with enhanced tumor expression of RANKL. Interferon gamma gene deletion resulted in enhanced osteolytic tumor formation. Tax mice provide a new animal model for dissecting the pathogenesis of spontaneous bone osteolysis and for screening of new therapeutics targeted against tumor associated bone destruction.

Fig.1

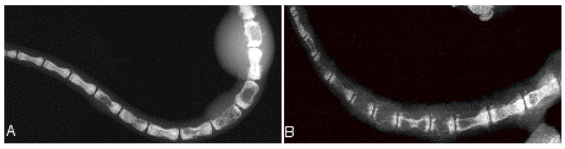
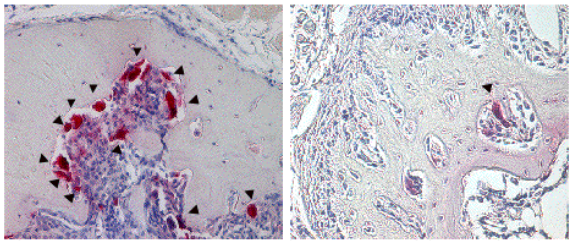


Fig.2



Disclosures: ***L. Gao****, None.*

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PTHrP Stimulates New Bone Formation by Molecular Mimicry of Endothelin-1. K. S. Mohammad, T. A. Guise, J. M. Chirgwin. Medicine/ Endocrinology, Univ of Virginia, Charlottesville, VA, USA.

Prostate cancer metastases to the skeleton are frequently osteoblastic, despite abundant expression of the osteolytic factor parathyroid hormone-related protein, PTHrP. We propose a new explanation for this paradox: N-terminal fragments of PTHrP stimulate new bone formation by activating the endothelin A (ETA) receptor. Endothelin-1 (ET-1) is a 21 aa peptide which potently stimulates new bone formation via the ETA receptor. ET-1 residues 6-9 [LMDK] and PTHrP residues 8-11 [LHDK] share strong sequence homology. We tested PTHrP 1-16 in an ex vivo assay. Hemi-calvariae [n=4] of 4 day old mice were cultured on grids in medium + 0.1% BSA and test factor for 4 or 7 days. They were then fixed, decalcified, and embedded in paraffin. Sections were cut parallel to the sagittal suture, stained with H&E;, and analyzed by computerized histomorphometry to determine new bone area and osteoblast number. Inclusion of 100nM PTHrP 1-16 caused a 3-fold stimulation of new bone area at 4 days (0.0095 vs 0.0035mm2 for untreated control, $p<0.05$) and 7 days (0.014 vs 0.0035mm2 for untreated control, $p<0.001$). Osteoblast numbers were correspondingly increased. The response was equivalent to that caused by 100nM ET-1 positive control. There was no additional response to 1000nM PTHrP 1-16 or to the combination of 100nM each PTHrP 1-16 and ET-1. The effects on new bone area and osteoblast number were blocked by the inclusion of 0.01mM ABT627, a selective ETA receptor antagonist, which did not block new bone formation stimulated by FGF-2. We found similarly strong anabolic responses to 25nM PTHrP 1-20 and 1-23, while PTHrP 1-34 instead caused extensive osteolysis. Our data demonstrate that N-terminal peptides of PTHrP exert potent anabolic effects on bone via the ETA receptor. The sequence of PTHrP 18-23, RRRFF, is cleaved by prostate-specific antigen. Proteolysis at this site frees residues 8-11 to bind to ETA, providing a molecular explanation for the osteoblastic phenotype of PTHrP-positive prostate cancer bone metastases. The mimicry of ET-1 by PTHrP N-terminal peptides suggests that ETA receptor antagonists could be effective in treating prostate cancer, both against the actions of endothelin itself and against the anabolic effects of PTHrP fragments.

Disclosures: ***K.S. Mohammad****, None.*

1094

A Novel 25-Hydroxyvitamin D_3 Analog for Prostate Cancer. R. Ray*[1], N. Swamy*[1], T. Chen[1], S. Peleg[2], S. Christakos[3], N. Weigel*[4]. [1]Endocrinology, Diabetes and Nutrition, Department of Medicine, Boston University School of Medicine, Boston, MA, USA, [2]M.D. Anderson Cancer Center, Houston, TX, USA, [3]New Jersey Medical School, Newark, NJ, USA, [4]Baylor College of Medicine, Houston, TX, USA.

25-hydroxyvitamin D_3 (25-OH-D_3) is not considered to be therapeutically important due to its low vitamin D receptor (VDR)-binding affinity. Recently we developed an analog of 25-OH-D_3, 25-hydroxyvitamin D_3-3-bromoacetate (25-OH-D_3-3-BE) which engages VDR by cross-linking to its hormone-binding pocket. Preliminary studies with 25-OH-D_3-3-BE demonstrated that it inhibited the growth of prostate cancer cells (LNCaP, PC-3. LAPC-4), immortalized normal prostate cells (PZ-HPV-7), breast cancer cells (MCF-7), and primary skin cells (keratinocytes) similar to 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$). Furthermore, we observed that 25-OH-D_3-3-BE was cytotoxic only towards PC-3, LNCaP and LAPC-4 cells. DNA-fragmentation and caspase assays showed that 25-OH-D_3-3-BE induced apoptosis. VDR-transfection assays in COS-7 cells demonstrated that 25-OH-D_3-3-BE modulated 24-hydroxylase promoter activity similar to 1,25$(OH)_2D_3$. Furthermore, in a pull-down assay 25-OH-D_3-3-BE stimulated interaction of VDR with RXR and GRIP-1 in PC-3 cells. These results strongly suggested that prostate cancer cell-specific effects of 25-OH-D_3-3-BE were mediated by VDR. Serum calcium measurement of CD-1 mice dosed with various amounts of 25-OH-D_3-3-BE showed that at least 3.3µg/20 gm of this compound did not raise serum calcium beyond control. Furthermore, 25-OH-D_3-3-BE drastically changed the cellular structure of tumor-cells in nude mice bearing PC-3-induced prostate tumor without eliciting significant systemic toxicity. Tissue-specific effects of 1,25$(OH)_2D_3$ and its analogs are not common due to the ubiquitous nature of VDR. We have demonstrated that 25-OH-D_3-3-BE, a non-calcemic analog of 25-OH-D_3 displayed strong prostate cancer cell-specific effects. It is also noteworthy that various human prostate cancer cells respond to 1,25$(OH)_2D_3$ differently, potentially limiting their use. In contrast, 25-OH-D_3-3-BE was equally effective in AR-positive LNCaP and AR-negative PC-3 and LAPC-4 cells. Therefore, 25-OH-D_3-3-BE could potentially be developed as a therapeutic agent for androgen sensitive and androgen-refractory prostate cancer

Disclosures: ***R. Ray****, None.*

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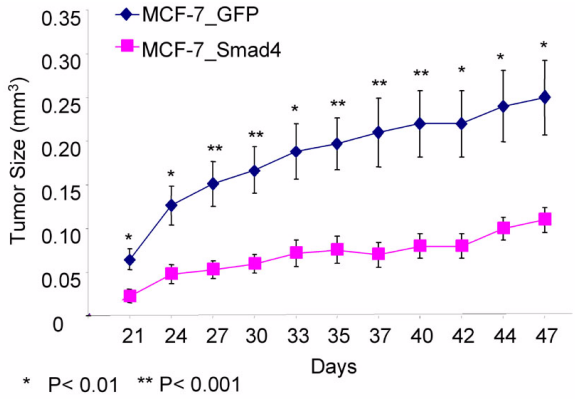
Tumor Suppressor Smad4/*DPC4* as a Corepressor for Estrogen Receptor α. L. Wu[1], Y. Wu[1], Q. Li*[1], W. Gathings*[2], X. Cao[1]. [1]Univ. of Alabama at Birmingham, Birmingham, AL, USA, [2]Univ. of Alabama in Huntsville, Huntsville, AL, USA.

Antiestrogen compounds are widely used for the treatment of osteoporosis, breast cancer and other diseases. Recent studies suggest that novel yet unidentified cofactors for the estrogen receptor may contribute to the tissue-specific activity of antiestrogens. We have demonstrated that Smad4, a common signal transducer in the TGF-β/BMP signaling pathway, functions as a transcription corepressor for estrogen receptor α (ERα). Endogenous ERα co-immunoprecipitates with Smad4 and the interaction can be induced by the antiestrogen ligands, tamoxifen, raloxifene and droloxifen. The observation was confirmed in chromatin immunoprecipitation assays. Smad4 and ERα form a complex when ERα binds to an estrogen responsive element in the estrogen target gene promoter. Importantly, overexpression of Smad4 enhances the antiestrogens inhibitory effect on luciferase reporter activity and down regulates the estrogen target gene transcription in breast cancer cells. Mapping of the interaction domains indicates that the N-terminal activation function domain 1 (AF1) of ERα and the N-terminal Mad homologous domain 1 (MH1) and Linker region of Smad4 are essential for their interaction. Estrogen receptor β (ERβ), however, does not interact with Smad4, and neither TGF-β nor Smad4 has any effect on ERβ-mediated transcription, suggesting that ERβ is not involved in the cross-talk between estrogen and TGF-β. We have also developed MCF-7 cell lines that overexpress Smad4 (MCF-7_Smad4) or GFP (MCF-7_GFP) mediated by retrovirus. By using a cell proliferation assay, we observed that overexpression of Smad4 enhances antiestrogen-induced cell growth arrest. Smad4 overexpression also enhances antiestrogen-mediated apoptosis as demonstrated in TUNEL assay. We also examined the effect of Smad4 on breast tumor growth in nude mice. MCF-7_GFP and MCF-7_Smad4 cells were inoculated into the flanks of 15 mice with 1×10^5 cells per site. The formula, length × width × height ×0.5236, was used to calculate tumor volume. As shown in the figure, Smad4 significantly inhibited tumor growth. In summary, we found that Smad4 functions as a transcription corepressor for ERα, and that Smad4 repression activity is differentially regulated by antiestrogens. Thus, TGF-β may regulate cell fate through Smad4-mediated cross-talk with the estrogen signaling pathway.



Disclosures: ***L. Wu****, None.*

1096

Ectopic Runx2 Regulates Breast Cancer Metastasis Associated Osteolytic Disease. G. L. Barnes[1], K. E. Hebert*[1], A. Javed[2], M. H. Kamal*[1], T. A. Einhorn[1], J. B. Lian[2], G. S. Stein[2], L. C. Gerstenfeld[1]. [1]Department of Orthopaedic Surgery, Boston University Medical Center, Boston, MA, USA, [2]Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA.

Metastatic breast cancer cells preferentially localize to skeletal sites and predominantly produce osteolytic disease. Metastasis associated lytic bone disease is the result of cancer cell mediated disruptions of the normal homeostatic interactions between osteoblasts and osteoclasts. We have recently reported that human breast cancer cells ectopically express the transcription factor Runx2, a factor associated with skeletal cell differentiation. We propose that the ectopic expression of Runx2 by human breast cancer cells is a key regulator of the interactions between cancer and bone cells that lead to osteolytic disease. In order to test this hypothesis we have disrupted the endogenous ectopic Runx2 activity in human

breast cancer cells through the use of several independent Runx dominant-negative constructs. In an in vivo intramedullary injection model system 90% of animals injected with control MDA-MB-231 breast cancer cells, which constitutively express Runx2, produce large lytic lesions associated with TRAP+ osteoclasts. In contrast, only 15% of mice injected with the dominant-negative Runx expressing MDA-MB-231 cells display detectable lesions. Furthermore, the lesions that do appear are smaller in size and are not associated with active osteoclasts. We have used an in vitro co-culture system with marrow stromal cells to assess the homeostatic interactions between osteoblasts and osteoclasts in the presence of human breast cancer cells. These studies demonstrate that breast cancer cells disrupt normal skeletal cell homeostasis and produce lytic disease by inhibiting new osteoblast differentiation and enhancing osteoclast differentiation and activity. In these co-cultures we observe a greater than 60 % reduction in osteoblast associated mineralized nodule formation and osteoblast gene expression (BSP, OC and Col I) together with a 2-3 fold increase in osteoclast associated TRAP expression and the expression of the pro-resorptive cytokines TNFa, RANKL and IL-6. Over-expression of the Runx dominant-negative construct in these cancer cells abolishes these cancer cell mediated effects in our in vitro system. Together both the in vivo and in vitro studies demonstrate that Runx2 is a central regulator of breast cancer cell mediated disruptions in normal skeletal homeostasis, specifically those that lead to osteolytic disease.

Disclosures: ***G.L. Barnes****, None.*

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Cystatin 10, a Novel Chondrocyte-Specific Protein, Contributes to Pathogenesis of Osteoarthritis and Ectopic Ossification through Chondrocyte Calcification. T. Yamada[1], H. Kawano[1], T. Fukuda*[2], K. Yoshimura*[2], T. Nakamura*[2], Y. Koshizuka[1], S. Kamekura*[1], U. Chung[1], S. Kato[2], H. Kawaguchi[1]. [1]Orthopaedic Surgery, Univ. of Tokyo, Tokyo, Japan, [2]IMCB, Univ. of Tokyo, Tokyo, Japan.

In efforts to elucidate the molecular mechanisms of endochondral ossification, we recently identified a novel gene, cystatin 10 (Cst10), which was upregulated with ectopic ossification of the mouse auricular cartilage. Expression of Cst10 was specific to cartilage, especially to hypertrophic chondrocytes of the growth plate. In the culture of mouse chondrogenic ATDC5 cells, Cst10 expression was seen simultaneously with that of type X collagen, and increased in synchrony with maturation thereafter. Overexpression of Cst10 cDNA in ATDC5 cells induced calcification as determined by Alizarin red and von Kossa stainings. To further investigate the physiological role of Cst10, we created mice lacking the Cst10 gene ($Cst10^{-/-}$ mice). $Cst10^{-/-}$ mice developed normally without abnormalities of major organs. Immunohistochemical and in situ hybridization analyses of the growth plate showed no abnormality in proliferation or hypertrophy of chondrocytes; however, calcification of hypertrophic chondrocytes was impaired in the $Cst10^{-/-}$ growth plate and the height of calcified layer was about 60% that of wild-type (WT) littermates at 8 weeks of age. Bone densitometry, histomorphometry and 3D-μCT analyses revealed a significant decrease in the volume of primary spongiosa adjacent to the growth plate by Cst10 deficiency. In the culture of primary chondrocytes derived from the growth plate, calcification of $Cst10^{-/-}$ cells was suppressed compared to that of WT cells although their proliferation and earlier differentiation determined by Alcian blue staining were normal. Despite these *in vivo* and *in vitro* abnormalities, bone growth and turnover of $Cst10^{-/-}$ mice remained similar to those of WT during the observation period up to 52 weeks of age. However, when osteoarthritis was induced by producing instability in the knee joint of 8-week-old mice through ligament transection and menisectomy, substantial osteophyte formation due to endochondral ossification was seen in the WT knee 10 weeks after the operation, while this was rarely seen in the $Cst10^{-/-}$ knee. In addition, although all WT mice exhibited ossification of the tendon insertion of patella and calcaneus at 52 weeks of age, none of the $Cst10^{-/-}$ mice showed this disorder (n=4, each). We thus conclude that Cst10, an inducer of chondrocyte calcification, contributes to the pathogenesis of osteoarthritis and ectopic ossification without affecting the physiological bone growth or turnover, suggesting that this molecule could be a therapeutic target for these disorders.

Disclosures: ***T. Yamada****, None.*

1098

In Vivo Characterization of Human SOST: SOST-Transgenic Mice Exhibit Skeletal Defects and are Osteopenic. M. S. K. Sutherland[1], C. Yu*[1], J. C. Geoghegan*[1], M. Jonas*[2], D. G. Winkler[1], B. R. Kovacevich*[2], A. Snell*[3], M. Appleby*[3], M. E. Brunkow*[4], J. A. Latham[1]. [1]Gene Function & Target Validation, Celltech R & D, Inc., Bothell, WA, USA, [2]Molecular Biology, Celltech R & D, Inc., Bothell, WA, USA, [3]Discovery Biology, Celltech R & D, Inc., Bothell, WA, USA, [4]Genomics, Celltech R & D, Inc., Bothell, WA, USA.

Sclerosteosis is a severe skeletal disorder characterized by progressive bone overgrowth and high bone mineral density due to a loss of function of the SOST gene. The gene product, sclerostin, which shares homology to the DAN family of BMP antagonists, is highly expressed in osteocytes. To explore the role of sclerostin in bone homeostasis in an *in vivo* animal model, SOST-transgenic mice were generated by selectively targeting the expression of human SOST to bone with the mouse osteocalcin gene 2 (OG2) promoter. We hypothesized that overexpression of human SOST in mice would result in decreased deposition of mineral and lower bone mineral density. A series of four independent transgenic founders were identified with gene copy numbers ranging from 4 to 30. Bones from the mice were shown to express the transgene by RT-PCR analysis and sclerostin protein was detectable by Western blot. SOST-transgenic mice exhibited kinky tails and were smaller at birth compared to age-matched wildtype littermates. All other visible phenotypes were normal. Alizarin red staining of the developing skeleton (E15.5 to 1 week of age) as well as X-ray analyses of weaned and adult mice revealed that the transgenic mice had extra floating ribs along with collapsed and fused vertebral bodies in the lumbar and sacral regions. Histological analyses of cross-sections of the lumbar vertebrae showed that the bones of SOST-transgenic mice had thinner cortices and reduced amounts of trabecular bone. The lumbar vertebrae (L3 to L5) and femurs of transgenic mice also displayed a significant reduction in bone mineral density as measured by PIXImus. Biomechanical testing of the vertebrae and femur by compression and 4-point bending, respectively, revealed that the bones of the transgenic mice were more fragile and less resistant to fracture compared to normal littermates. This is the first data bridging the role of sclerostin in rodents with man where the gene was originally characterized. These results highlight the important role that sclerostin plays in bone formation and maintaining the integrity of bone, and suggests that agents that neutralize this protein may be of therapeutic benefit in clinical indications associated with loss of bone.

Disclosures: ***M.S.K. Sutherland****, Celltech R& D, Inc. 1, 3.*

1099

Gender Dependent Neuroregulation of Bone Mass: Evidence from the Neuropeptide Y Y2/Y4 Double Knockout Mouse. P. A. Baldock[1], A. Sainsbury*[2], R. F. Enriquez*[1], M. Couzens*[2], H. Herzog*[3], E. M. Gardiner[1]. [1]Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney NSW, Australia, [2]Neurobiology Program, Garvan Institute of Medical Research, Sydney NSW, Australia, [3]Neurobiogy Program, Garvan Institute of Medical Research, Sydney NSW, Australia.

Neuropeptide Y (NPY) Y2 receptor has previously been shown to regulate cancellous bone formation in the mouse by a centrally mediated mechanism. However, the manner in which this elevation takes place is unknown. Elevated circulating levels of pancreatic polypeptide, the major ligand for NPY Y4 receptor, implicated the Y4 in the Y2 knockout (KO) bone phenotype.

This study compared the effect of NPY Y2 KO, Y4 KO and Y2/Y4 double KO on the regulation of bone mass in male and female mice. Distal femora from 4 month old knockout and wildtype mice were examined. Values [units] are given: mean (SEM).

Y4 KO in male mice did not affect cancellous or cortical bone mass. BV/TV [%] in Y4 KO mice 8.2 (1.2) did not differ from wildtype 6.6 (1.4). Both these values were lower than in Y2 KO mice 12.4 (1.7). In contrast, cortical bone was not different from wildtype in either Y4 or Y2 KO mice. Cortical area [mm2] in Y4 knockout 1.1 (0.1) did not differ from wildtype 1.2 (0.1) or Y2 KO mice 1.1 (0.1).

Y2/Y4 KO in male mice had a synergistic effect on cancellous bone. BV/TV in Y2/Y4 KO mice 17.4 (2.1) was elevated compared to Y4 KO 8.2 (1.2) and Y2 KO 12.4 (1.7), ($p< 0.06$). In contrast, cortical area was reduced in Y2/Y4 KO 0.92 (0.03) compared to Y4 KO 1.12 (0.04) and Y2 KO 1.09 (0.05). This effect was associated with reduced cortical thickness.

Interestingly, in female Y2/Y4 KO mice, there was no synergistic increase in cancellous BV/TV, with Y2/Y4 mice 10.6 (0.6) not different from Y2 KO females 10.8 (2.1), but with both greater than wildtype 5.9 (0.3). Moreover, cortical area 0.91 (0.2), femoral length [mm] 16.0 (0.1) and mid-femoral circumference [mm] 5.2 (0.1) in female mice were not different from male Y2Y4 KO mice 0.92 (0.03), 16.0 (0.1) and 5.2 (0.1), despite significant sex differences in cortical bone in both Y2KO and Y4KO mice.

Although the Y4 receptor pathway does not independently regulate bone mass, these data suggest that in males there is an interaction between the Y2 and Y4 pathways to reduce cancellous bone volume and increase cortical bone mass. Furthermore these data suggest an interaction between the control of bone mass by sex hormones and the NPY pathways.

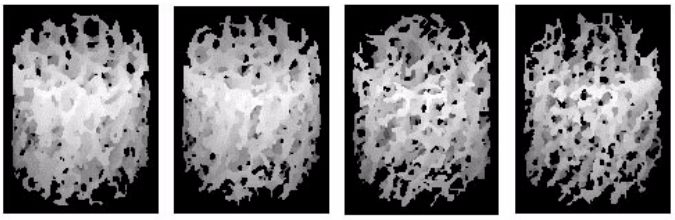
Disclosures: ***P.A. Baldock****, None.*

1100

Longitudinal Changes in Trabecular Bone Architecture Detected by Micro-MRI Based Virtual Bone Biopsy. F. W. Wehrli[1], A. M. Popescu*[1], B. Vasilic*[1], B. R. Gomberg*[1], P. K. Saha*[1], B. Zemel[2], B. Bunker*[1], A. C. Wright*[1], P. J. Snyder[3], H. Peachy*[3]. [1]Radiology, University of Pennsylvania, Philadelphia, PA, USA, [2]Nutrition and Growth, Children's Hospital of Philadelphia, Philadelphia, PA, USA, [3]Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Estrogen depletion following menopause is accompanied by bone loss and architectural deterioration of trabecular bone (TB). Conversely, hormone replacement therapy (HRT) protects against such bone loss. Here we report preliminary data on the first in vivo detection of such skeletal changes in an ongoing study involving early postmenopausal women, half of whom are getting HRT and half who do not (controls), using a protocol whereby BV/TV as well as parameters expressing TB topology are quantified at the distal radius and tibia by means of a MRI-based virtual bone biopsy. DXA BMD at the spine and hip were measured as well. From the MR images acquired at 137x137x410 μm3 voxel size, bone volume fraction maps were generated which were subvoxel processed to yield a final voxel size of 62x62x103 μm. These images were binarized, skeletonized and subjected to 3D topological analysis. Skeletonization converts rods to curves and plates to surfaces. Parameters extracted comprised the volume density of curves (CURV) and surface voxels (SURF) as well as composite parameters surface/curve ratio (S/C) and "erosion index" (EI). Apparent TB thickness (Tb.Th) was derived as well. Subjects were imaged at baseline and 11-13 months later. So far, 11 control and 5 HRT subjects afforded images of adequate quality. Relative differences from baseline were computed for each subject and the mean differences compared between groups. Measurements at the wrist indicated a relative decrease in CURV ($p=0.01$) and decrease in EI ($p=0.05$) in HRT relative to controls. Tb.Th was suggestive of being greater in the HRT group ($p=0.08$). Other structural parameters were not different. These preliminary findings are consistent with the known protective effects of HRT ensuring maintenance of a more plate-like TB architecture. The observations in the radius were not paralleled by similar findings in the tibia. BMD, though not statistically significant, indicated the same protective effect of HRT. This work suggests that MRI-based in vivo micromorphometry of trabecular

bone may be useful for monitoring osteoporosis treatment.



Virtual bone biopsy cores in a HRT (a, b) and control subject (c, d) taken at baseline (a, c) and one year later (b, d).

Disclosures: ***F.W. Wehrli****, None.*

1101

Identification of *Alox15* as a Gene that Regulates Peak Bone Mass in Mice. R. F. Klein[1], J. D. Allard*[2], A. S. Carlos*[1], Z. Avnur*[2], D. A. Olson*[1], R. Waters*[2], D. Dinulescu*[1], T. Nikolcheva*[2], M. Shea[1], B. Hansen*[1], J. Kansagor*[1], J. D. Belknap*[1], G. Peltz*[2], E. S. Orwoll[1]. [1]Oregon Health & Science University, Portland, OR, USA, [2]Roche Bioscience, Palo Alto, CA, USA.

The identification of genes underlying quantitative-trait loci (QTL) for complex diseases, such as osteoporosis, is a challenging and difficult task. We have previously identified a major QTL on chromosome 11 that is associated with variation in peak bone mineral density (BMD) in C57BL/6 (B6) and DBA/2 (D2) mice. In the present study, we used complementary genetic and genomic approaches to identify a novel molecular pathway affecting peak BMD. Selective breeding was used to transfer the chromosomal fragment containing the chromosome 11 QTL interval from B6 onto a D2 genetic background. Comparison of the resulting congenic mouse strain with the background D2 strain established that a 30 megabase B6 genomic interval on chromosome 11 in isolation confers increased BMD (68.3 ± 0.6 mg/cm^2 *vs*. 65.4 ± 0.4 mg/cm^2, $p < 0.0001$). Genome-wide microarray expression profiling identified a significant reduction (~20-fold) in the expression of *Alox15* in the high BMD congenic strain compared to the background D2 strain. *Alox15* encodes a lipid-peroxidizing enzyme of previously unknown biological function (leukocyte 12-lipoxygenase; L-12-LO or 12/15-LO). Sequencing of the *Alox15* cDNA revealed several differences between the D2 and B6 strains. Compared to B6 mice, calvarial cells isolated from D2 mice exhibited increased mRNA expression for *Alox15* and *CD36* (a gene induced by naturally-occurring lipid oxidation products of 12/15-LO activity). Cross-breeding experiments employing *Alox15* knockout mice with absent *Alox15* expression rescued mice from the low bone mass phenotype associated with the *Alox15* D2 allele. Moreover, pharmacological inhibition of 12/15-LO *in vivo* resulted in higher BMD and improved bone strength in a murine model of osteoporosis. These results provide strong presumptive evidence that 12/15-LO is an important determinant of peak bone mass in mice, raise the possibility that inherited variation in the 12/15-LO signaling pathway contributes to the pathogenesis of osteoporosis in humans, and suggest that pharmacological modulation of 12/15-LO activity may be useful in the prevention/therapy of osteoporosis.

Disclosures: ***R.F. Klein****, Merck & Co., Inc. 8; Procter & Gamble 8; Aventis 8; Eli Lilly and Co., Inc. 8.*

1102

TGFβ1 Prevents Ovariectomy Induced Bone Loss by Repressing T Cell Activation. Y. Gao[1], G. Toraldo[1], W. Qian*[2], K. Dark*[2], R. Shepherd*[1], M. N. Weitzmann[2], D. A. Towler[1], R. Pacifici[2]. [1]Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA, [2]Division of Endocrinology and Metabolism, Emory University School of Medicine, Atlanta, GA, USA.

Enhanced production of TNF and RANKL by activated T cells plays a pivotal role in ovariectomy (ovx) induced bone loss but the responsible mechanism is unknown. TGFβ1 may be a key regulator of ovx-induced T cell activation since estrogen (E) stimulates its production and TGFβ1 represses T cell function. To investigate the role of TGFβ in ovx induced bone loss we blocked the decrease in TGFβ1 induced by ovx by injecting mice IM with pCMV-TGFβ1 plasmid which elevates serum TGFβ levels. Injection of this plasmid led sham and ovx mice to attaine equally high levels of serum TGFβ1 for 4 weeks. Ovx induced significant bone loss and increased bone turnover in mice injected with mock plasmid, but not in mice treated with pCMV-TGFβ1, suggesting a role for TGFβ1 in preventing ovx-induced bone loss. Ovx increases T cell activation through enhancement of macrophage antigen presenting cell (APC) activity and expression of the APC regulating gene CIITA (ASBMR 2002). We thus investigated if E suppresses APC activity and CIITA through TGFβ. Our data shows that E effects on APC activity and CIITA were abolished by a neutralizing TGFβ1 antibody, thus suggesting that E regulates CIITA expression and APC activity through a TGFβ1 dependent mechanism. To further implicate TGFβ in the mechanism by which E represses T cell activation and bone loss we adoptively transferred T cells, insensitive to TGFβ due to a dominant negative TGFβ receptor type II (dnTGFβRII) transgene, into T cell-deficient nude mice. As expected, no bone loss was detected in ovx and sham nudes and in sham nudes reconstituted with WT T cells. In contrast, transplantation of T cells from either WT or dnTGFβRII mice into ovx nudes was followed by increased T cell activation and a bone loss equal to that of WT ovx mice. Importantly, transplantation of T cells from dnTGFβRII mice into sham nudes was also followed by T cell activation and bone loss equal to those observed in WT ovx mice. These findings demonstrate that silencing the repressive effects of TGFβ1 on T cells renders them insensitive to E and capable of inducing bone loss in vivo. Further attesting to the relevance of TGFβ signaling in T cells, the bone density of intact dnTGFβRII mice was significantly lower than that of WT littermates. In summary, the data confirm that T cells play an essential role in ovx induced bone loss and suggest that one mechanism by which E prevents bone loss is by stimulating the production of TGFβ. This cytokine mediates the repressive effect of E on T cell activation thus preventing T cell production of bone wasting cytokines.

Disclosures: ***Y. Gao****, None.*

1103

Induction of Osteoblast Lineage Commitment and Differentiation by 4-estren-3α,17β-diol (Estren), but Not 17β-estradiol:A Putative Explanation of the Bone Anabolic Properties of ANGELS. S. Kousteni, L. Han, M. Almeida, J. Chen, H. Peng*, R. Jilka, S. C. Manolagas. Div Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, CA Veterans Healthcare System, Univ of Arkansas for Med Sciences, Little Rock, AR, USA.

Estren, a compound that reproduces the nongenotropic effects of sex steroids without affecting classical transcription, increases BMD, mechanical strength and serum osteocalcin, in ovariectomized mice above the level of the estrogen-replete state, without affecting reproductive organs. As shown elsewhere in this meeting estren potentiates Wnt signaling in osteoblasts, by a) suppressing the activity of c-jun, and thereby decreasing the transcriptional activity of the Wnt antagonist Dickkopf-1 (Dkk-1) and b) increasing the expression of Wnt-1 and Wnt-2 and their receptor Frizzled-10. These actions in turn lead to the potentiation of β-catenin mediated transcription. Here, we report that estren, but not 17β-estradiol (E2), at 10-8M induced alkaline phosphatase (AP) activity and osteocalcin in the uncommitted mesenchymal progenitor cell line C2C12. These effects were blocked by either ICI 182,780 or flutamide as well as inhibitors of each one of 3 different cytoplasmic kinases known to be activated by estren: the Src kinase inhibitor PP1, the PI3 kinase inhibitor wortmannin, and the JNK kinase inhibitor SP600125. Further, estren, but not E2, induced Runx2 expression, increased AP activity and also induced mineralization (in the presence of ascorbic acid and β-glycerophosphate) in cultures of the committed pre-osteoblastic cell line MC3T3-E1. The potency of all these effects of estren was similar to that produced by a maximal dose of BMP-2 (300 ng/ml). Most strikingly, induction of AP and osteocalcin by estren in C2C12 cells was completely abrogated by the BMP-2 antagonist noggin (100 ng/ml) as well as the Wnt antagonist Dkk-1 (50 ng/ml), indicating that estren exerts its pro-differentiating effects on osteoblast precursors by stimulating both BMP-2 and Wnt signaling. Consistent with this contention and the possibility that Wnt signaling is a downstream effector of BMP-2 action, the BMP-2-induced stimulation of AP and osteocalcin in C2C12 cells was blocked by Dkk-1. Based on these findings, as well as the evidence that Wnt signaling is a critical determinant of bone mass in rodents and humans, we conclude that the anabolic effects of ANGELS, as distinguished from the anti-remodeling/anti-catabolic effects of estrogens and androgens, may result in part from the ability of ANGELS to induce lineage commitment and differentiation towards osteoblasts via kinase-mediated potentiation of a BMP-2/Wnt signaling cascade.

Disclosures: ***S. Kousteni****, Anabonix, Inc. 4.*

1104

The *Wnt* Signaling Pathway Modulates *TWIST* Expression to Maintain and Expand Osteoprogenitor Populations during Osteogenesis. T. Yan*[1], S. D. Flanagan*[2], T. Linkhart[3], D. D. Strong[3], C. A. Glackin[1]. [1]Molecular Medicine, City of Hope Beckman Research Institute, Duarte, CA, USA, [2]Neurosciences, City of Hope Beckman Research Institute, Duarte, CA, USA, [3]Musculoskeletal Disease Center, JL Pettis VA Medical Center, Loma Linda, CA, USA.

Osteoblasts originate from a common osteoprogenitor or from mesenchymal stem cells (MSCs). The formation of osteoblasts requires that MSCs differentiate into mature osteoblasts through the process of osteogenesis. Osteogenesis requires that a series of transcriptional events take place to induce bone specific signaling pathways and gene expression, resulting in the development of the mature osteoblast phenotype in remodeling bone. We have focused on how the basic helix loop helix (bHLH) transcription factor family (e.g. *TWIST*) regulates gene expression in the early stages of osteogenesis and how signaling mechanisms (e.g. *Wnt* signaling) affect bHLH factors to stimulate or inhibit osteoblast differentiation. Since *Wnt-11* is highly expressed in the developing human skeletal system, we have examined the role of *Wnt-11* in the regulation of *TWIST* expression during osteogenesis. We have obtained evidence that *TWIST* is a positive regulator of important osteoprogenitor genes such as periostin and collagen, proteins required for cell adhesion, spreading and matrix formation. Based on our recent findings, we hypothesize that *Wnt* signaling molecules (including *Wnt-11*) play a significant role in inhibiting *TWIST* expression, which allows Runx2/Cbfa1 to facilitate osteoblast differentiation. To examine our hypotheses, murine 2T3 cells were stably transfected with plasmid vectors that over or under-express *TWIST*, periostin, *Wnt-11*, and sFRP2 and their phenotypes characterized. Runx2/Cbfa1 and *TWIST* promoters has been analyzed using *in vivo* footprinting techniques by LMPCR. Our preliminary data indicate that over-expressing *Wnt-11* in murine 2T3 cells causes a dramatic reduction of *TWIST* and an increase in Runx2/Cbfa1, ALP, and osteocalcin expression. By contrast, reduced *Wnt-11* expression prevents osteoblast differentiation by up-regulating *TWIST* and maintaining Runx2/Cbfa1 expression. The cellular morphology of the anti-sense *Wnt-11* is similar to that observed for the *TWIST* over-expressing osteoblasts suggesting that both *TWIST* and Runx2/Cbfa1 together with *Wnt-11* signaling may be important for the recruitment and maintenance of osteoprogenitors in remodeling bone. Based on these findings, we propose that *Wnt* signaling affects *TWIST* and Runx2/Cbfa1 expression to either maintain osteoprogenitor populations or, in the absence of *TWIST*, to enable positive activation by Runx2/Cbfa1 of downstream genes leading to osteoblast differentiation.

Disclosures: ***T. Yan****, None.*

1105

Δ2ΔFosB, a Truncated Isoform of ΔFosB Lacking Transactivation Domains, is Sufficient to Induce Osteosclerosis and Decreased Adipogenesis in Transgenic Mice. M. Kveiborg, R. Chiusaroli, G. Sabatakos, M. Wu*, W. M. Philbrick, W. C. Horne, R. Baron. Yale University School of Medicine, New Haven, CT, USA.

The AP-1 family of transcription factors consists of Fos- and Jun-related proteins, which play important roles in bone cell differentiation. As reported previously, ΔFosB and Δ2ΔFosB are naturally occurring splice isoforms of FosB. Although both maintain the ability to heterodimerize with Jun proteins and bind consensus AP-1 sites, they lack the major C-terminal transactivation domain of FosB. In addition, a potential N-terminal transactivation domain is absent in Δ2ΔFosB. Transgenic mice overexpressing ΔFosB under the control of the neuron-specific enolase (NSE) promoter develop an osteosclerotic phenotype as well as decreased adipose tissue. Furthermore, transgenic mice overexpressing ΔFosB specifically targeted to osteoblasts demonstrated that these effects are mediated by cell autonomous and independent mechanisms.

To determine the relative importance of Δ2ΔFosB versus ΔFosB in mediating the fat and bone phenotypes, we have generated transgenic mice overexpressing only Δ2ΔFosB under the control of the NSE-promoter. Overexpression of Δ2ΔFosB in bone and adipose tissues was confirmed by western blotting and histomorphometric analysis showed that the mice develop a severe osteosclerosis, characterized by a more than 4-fold increase in trabecular bone volume, doubling of trabecular thickness and number, and increased dynamic bone formation parameters. NSE-Δ2ΔFosB transgenic mice also exhibited a decrease in adipose tissue and serum leptin levels. Bone marrow cultures established from NSE-Δ2ΔFosB transgenic mice revealed a reduced ability of the cells to differentiate into adipocytes. Specifically, the expression levels of PPARγ and C/EBPα, two important adipogenic transcription factors were decreased, whereas the osteoblastic transcription factor, Runx2 was increased. Thus, overexpression of the Δ2ΔFosB isoform, which conserves the DNA-binding and heterodimerization capacity but lacks any known transactivation domains, is sufficient to induce both the osteosclerotic phenotype and the decreased adipogenesis to the same degree as previously observed in NSE-ΔFosB transgenic mice. These results suggest that the mechanisms by which ΔFosB affects osteoblast and adipocyte differentiation do not require intrinsic transcriptional activity. Since we have shown ΔFosB and Δ2ΔFosB interact with other AP-1 family members and with Smads, Runx2 and C/EBPβ, we conclude that they affect cell differentiation by interfering with the activity of other transcription factors or co-factors.

Disclosures: ***M. Kveiborg****, None.*

1106

Osteopenia in Transgenic Mice with Osteoblast-Targeted ICER Expression. Y. Huang[1], G. Gronowicz[2], J. R. Harrison[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]Orthodontics, University of Connecticut Health Center, Farmington, CT, USA.

ICER is a cAMP inducible member of the CREM transcription factor superfamily. Alternative splicing of the ICER transcript gives rise to 4 isoforms: ICER I, Iγ, II, and IIγ. Since ICER contains predominantly the basic leucine zipper region for DNA binding and dimerization, it acts as a dominant negative regulator of cAMP-dependent gene transcription in most systems. Previously, we reported that ICER mRNA and protein are induced by PTH via the cAMP-PKA signaling pathway in cultured osteoblasts and in calvariae *in vivo*. The goal of the present study was to assess the function of ICER in bone *in vivo* by overexpressing ICER in osteoblasts of transgenic mice. ICER I and ICER II cDNAs with an N-terminal FLAG epitope tag were cloned downstream of a fragment containing 3.6 kb of the rat Col1a1 promoter and 1.6 kb of the rat Col1a1 first intron to produce the pOBCol3.6-F-ICER I and II transgenes, respectively. The 3.6 kb Col1a1 promoter fragment targets ICER expression broadly to cells of the osteoblast lineage. Multiple lines of pOBCol3.6-F-ICERI and II transgenic mice were generated and characterized. In general, the phenotypes of ICER I and ICER II transgenic mice were similar. Transgenic mice at 8 weeks of age had a smaller body weight than wild-type littermates. At 8 weeks, all mice had significantly decreased bone mineral density of femurs and vertebrae as measured by DEXA. Static histomorphometry showed that transgenic mice had almost no trabecular bone and reduced cortical bone compared to non-transgenic littermates ($p<0.01$). There were no significant changes in osteoblast or osteoclast number per unit bone surface in the secondary spongiosa. However, transgenic calvariae and femoral cortical bone and primary spongiosa demonstrated exuberant TRAP staining. Dynamic histomorphometry showed a decreased bone formation rate in transgenic femurs. *Ex vivo* analysis of osteoblastic differentiation using bone marrow stromal cells revealed impaired formation of mineralized nodules under osteogenic differentiation conditions. Moreover, the formation of TRAP-positive multinucleated cells was enhanced in transgenic bone marrow stromal cells cultured for 5 days in the presence of M-CSF and RANK ligand. In summary, transgenic mice with osteoblast-targeted overexpression of ICER demonstrated osteopenia characterized by a profound loss of trabecular architecture and a reduction in cortical bone width. We speculate that osteoblast-targeted ICER overexpression caused dysregulation of both bone formation and resorption.

Disclosures: ***Y. Huang****, None.*

1107

Regulation of G Protein Signaling in Osteoblasts In Vivo. R. A. Nissenson, M. Bencsik*, P. Nguyen*, J. Peng*, B. Halloran. Endocrine Research Unit, VA Medical Center/UCSF, San Francisco, CA, USA.

Intracellular signaling by G proteins is thought to provide critical regulation of osteoblast differentiation and function in response to extracellular signals. However, the presence of multiple G proteins and their cognate receptors has made it difficult to ascribe direct functional activities to specific G protein signals. We have initiated an effort to regulate G protein signaling by controlling the expression of an activated G protein coupled receptor (GPCR) in osteoblasts of transgenic mice. Several founder lines of mice expressing the tetracycline transactivator (tTA, tet off) gene driven by the 2.3 kb mouse α1Col I promoter were established. F1 mice from the line displaying the highest level of expression of tTA mRNA in bone (line 139) were mated with mice expressing a tTA driven gene encoding a GPCR (termed R01) known to activate Gi. Genotypic analysis of progeny demonstrated appropriate transmission of the tTA and R01 transgenes, but failed to identify any double transgenic pups (0/43), indicating that R01 expression during embryogenesis resulted in embryonic/perinatal lethality. However, if the mothers were maintained on doxycycline (doxy, 200 mg/kg chow) during the entire course of their pregnancy to suppress transgene expression, double transgenic pups were obtained at approximately Mendelian frequency (11/47). Only a very low level of expression of the R01 gene was detected in the bones of double transgenic pups when maintained on the +doxy diet. However, when the diet was switched to a –doxy diet at 4 weeks of age, R01 expression in bone was induced by 20 fold within 2 weeks. Phenotypic analysis of the skeleton of mice expressing the R01 transgene is currently in progress. These results suggest that excessive skeletal Gi signaling during embryogenesis results in lethality. Further, we have demonstrated the feasibility of controlling the expression of key osteoblast signaling pathways in vivo. This approach promises to allow direct investigation of the impact of specific G protein signals in osteoblasts on the structure and function of bone in vivo.

Disclosures: ***R.A. Nissenson****, None.*

1108

Inhibition of Glycogen Synthase Kinase-3β (GSK3β) Induces Bone Formation in a Mouse Calvarial Model. W. Zhao[1], B. Bhat[1], M. Wasko*[1], P. Yaworsky[2], Y. Kharode[1], P. Green*[1], J. Robinson[1], P. Zhang*[3], H. Fletcher*[3], V. Coleburn*[1], J. Wrobel*[3], F. Bex[1]. [1]Bone metabolism, Wyeth Research, Collegeville, PA, USA, [2]Genomics Division, Wyeth Research, Cambridge, MA, USA, [3]Chemical Sciences, Wyeth Research, Collegeville, PA, USA.

Glycogen Synthase Kinase-3 (GSK3β) is an important negative regulator of Wnt signaling through its action to destabilize β-catenin. Recent evidence from studies of the G171V LRP5 mutation suggests that activation of Wnt signaling may be involved in the resulting osteogenic skeletal phenotype. To determine in vivo whether Wnt pathway activation through GSK3β inhibition induces osteogenesis, the local administration of a GSK3β inhibitor (GSKi) on mouse calvariae were examined. We show here that inhibition of GSK3β induced expression of alkaline phosphatase(ALPase) activity in osteoblasts and increased calvarial thickness. GSKi at 1mg/kg or vehicle was injected sc daily for 18 days over the right side of the calvaria in 4 week-old male Swiss-Webster mice. The effect of GSKi on calvarial bone was assessed in histological sections by ALPase enzyme histochemical staining, quantitative histomorphometry and β-catenin expression by immunohistochemistry. Human PTH (1-34) at 20μg/kg/day, sc served as a positive control for the model and produced a significant increase in calvarial thickness. An increase in calvarial thickness was noted on the right hemicalvarium injected with GSKi when compared to either the left noninjected hemicalvarium of the same animal(11.8%, $p<0.005$) or calvaria from mice injected with vehicle alone (6%). ALPase was markedly enhanced in osteoblasts following either GSKi or PTH administration. Immunohistochemistry of calvaria injected with GSKi revealed strong β-catenin expression in pre-osteoblasts and osteoblastic cells lining the periosteum and the sagittal suture. In contrast, PTH had no effect on levels of β-catenin expression. Thus inhibition of GSK3β by local injection of GSKi has a bone anabolic effect that is associated with an increase in the level of β-catenin leading to the induction of osteoblast function. These results further support the significance of the Wnt signaling pathway in bone and the therapeutic potential of regulating this pathway in treating osteoporosis.

Disclosures: ***W. Zhao****, None.*

1109

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

Osteoclastogenesis is regulated by an integrated network of numerous bone metabolic factors, among which 1α,25 dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$) promotes osteoclastogenesis by reciprocally upregulating the expression of the receptor activator of NF-κB ligand (RANKL) gene and downregulating that of the osteoprotegerin (OPG) gene. To analyze the mechanism of OPG gene suppression by $1\alpha,25(OH)_2D_3$, we characterized cis-acting elements of the mouse OPG gene, and assessed the posttranscriptional modification process by actinomycin D studies. Both Northern blot and transient transfection analyses revealed that OPG gene expression was rapidly and transiently suppressed by $1\alpha,25(OH)_2D_3$ treatment. Additionally, actinomycin D studies revealed that $1\alpha,25(OH)_2D_3$ significantly shortened the half-life of OPG mRNA by 4 hours. Deletion mutant studies and electrophoretic gel motility shift assay revealed that mainly the c-Jun homodimer bound to the AP-1 binding site (TGACTGA, -293/-287) and maintained the steady-state

transcription of the OPG gene. Furthermore, the construct with the mutated AP-1 site (TGACTGA to CTCCCTC) was resistant to $1\alpha,25(OH)_2D_3$ -driven OPG gene suppression. In agreement with transient transfection studies, the amount of phosphorylated c-Jun protein (phospho-c-Jun) was decreased by vitamin D_3 treatment of ST2 stromal cell lines by 6 hours, while keeping the total amount of c-Jun protein constant. The amounts of the phosphorylated Jun N–terminal kinase (JNK) and the phosphorylated SAPK/Erk kinase (SEK1), on the other hand, were almost unchanged by vitamin D_3 treatment. Taken together with the fact that the OPG promoter has no consensus negative vitamin D-responsive elements, these data suggest that vitamin D_3 transrepresses the mouse OPG gene by reducing the proportion of the phospho-c-Jun protein in a JNK-independent manner. Our data indicated that short-term treatment with vitamin D_3 effectively downregulated OPG gene expression both by accelerating the degradation of OPG mRNA and by transrepressing the OPG gene through the AP-1 binding site in the catabolic phase. The OPG gene became insensitive to vitamin D_3 treatment, however, and reverted to its steady-state expression level in the long run, leading to the anabolic phase of the effect of $1\alpha,25(OH)_2D_3$ on bone.

Disclosures: ***T. Kondo****, None.*

1110

1,25-Dihydroxyvitamin D3 Stimulates Dynamic Vitamin D3 Receptor/ Retinoid Receptor DNA-Binding and Coactivator Recruitment in Intact Osteoblasts. S. Kim*, H. Yamamoto, N. K. Shevde, J. W. Pike. Biochemistry, University of Wisconsin-Madison, Madison, WI, USA.

Hormonal 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) induces gene expression through the vitamin D receptor (VDR), which binds as a retinoid X receptor (RXR) heterodimer to specific DNA sequences within target gene promoters and facilitates the recruitment of protein complexes essential for transcriptional modulation. In these studies, we explore the dynamics of this 1,25(OH)2D3-induced process on the 25-hydroxyvitamin D3-24-hydroxylase (Cyp24) and osteopontin (OPN) gene promoters in intact osteoblastic MC3T3-E1 target cells using chromatin immunoprecipitation techniques. 1,25(OH)2D3 rapidly stimulates the production of mRNA for both Cyp24 and OPN through direct actions on each gene's promoter. 1,25(OH)2D3 also induces rapid association of the VDR with both the Cyp24 and the OPN gene promoters. This DNA binding appears to cycle at early stages of transcriptional initiation with a periodicity of approximately 1 hr. Proteosome inhibitors such as MG132 reduce this cycling, suggesting that proteolysis is essential to the cycling process and transcriptional activation. VDR binding correlates directly with the accumulation of RXR on both promoters and double immunoprecipitations indicate the presence of both VDR and RXR on the same DNA fragment. 1,25(OH)2D3 treatment is also associated with rapid recruitment of coregulators such as the p160 coactivators SRC-1, 2 and 3 and the histone acetyltransferases p300 and CBP. The pattern of retention of these coactivators on the two promoters differs from that of VDR, however. DRIP205 is also rapidly recruited to these promoters, a process that correlates directly with the presence of RNA polymerase II. Interestingly, cotreatment of osteoblasts with 1,25(OH)2D3 and ZK159222, a vitamin D antagonist that blocks transcriptional activation by 1,25(OH)2D3, significantly reduces VDR DNA binding, limits the recruitment of coactivators such as SRC-1 and blocks the recruitment of RNA polymerase II. Lack of coactivator recruitment does not appear to be accompanied by increased association of corepressors such as SMRT or NCoR indicating that this antagonist may be a passive blocker of transactivation. These studies establish that 1,25(OH)2D3-induced transactivation in intact cells is a dynamic process that involves both RXR and a surprising number of coactivator complexes previously believed through molecular biological studies to associate with the VDR.

Disclosures: ***S. Kim****, None.*

1111

Expression of the Pagetic Phenotype in Osteoclasts (OCL) Requires VDR Dependent Transcription. N. Kurihara[1], S. V. Reddy[1], P. Friedman[2], A. W. Norman[2], G. D. Roodman[1]. [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of California, Riverside, CA, USA.

OCL in Paget's disease (PD) are abnormal morphologically and contain paramyxoviral transcripts. OCL precursors from Paget's patients are hyper-responsive to 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) and can form OCL at concentrations of 1,25-$(OH)_2D_3$ that are at least 1 to 2 logs less than those required for normal marrow cultures. We found that the hyper-responsivity to 1,25-$(OH)_2D_3$ was not due to increased vitamin D receptor (VDR) numbers or a VDR mutation. This hyper-responsivity to 1,25-$(OH)_2D_3$ allows increased OCL formation at physiologic levels of 1,25-$(OH)_2D_3$ that do not induce RANK ligand (RANKL), something that occurs at very low levels in normals. This hypothesis suggests that VDR dependent gene transcription is required for expression of the Paget's phenotype in OCL. In support of this, we have recently shown that transduction of normal CFU-GM with retroviral constructs containing the cDNA for the measles virus nucleocapsid (MVNP) gene, can induce these cells to form OCL that express a pagetic phenotype and are hyper-responsive to 1,25-$(OH)_2D_3$. Using a GST-VDR chimeric protein with lysates from MVNP transduced normal OCL precursors (CFU-GM derived cells) and marrow cells from involved bones from Paget's patients, we have isolated a potential coactivator of VDR, TAF_{II}-17, that was not expressed in empty vector (EV) transduced normal cells. The data suggested that VDR plays a key role in the abnormal OCL activity in PD. To test this hypothesis, OCL precursors from VDR -/- and VDR +/+ mice were transfected with the MVNP gene, and the characteristics of OCL formed examined. GST-VDR pull-down assays of MVNP transduced VDR-/- and VDR+/+ osteoclast precursors demonstrated that both cell types expressed high levels of TAF_{II}-17, a potential coactivator of VDR, which did not occur in EV transduced VDR-/- and VDR+/+ cells. OCL formation was significantly decreased in bone marrow cultures from VDR -/- mice treated with RANKL and M-CSF compared to the marrow cultures from VDR +/+ mice (32 ±7 vs. 61 ±6). The OCL that formed in marrow cultures of VDR -/- mouse transfected with the MVNP gene were small and did not express a pagetic phenotype (increased rate of formation, increased nuclear number, hyperresponsivity to RANKL). In contrast, MVNP transfected VDR +/+ mouse cells treated with RANKL/M-CSF formed large numbers of abnormal OCL that expressed a pagetic phenotype. As expected VDR -/- marrow cells did not form OCL in response to 1,25-$(OH)_2D_3$. These data suggest that VDR mediated gene transcription is required for optimal levels of OCL formation and that VDR mediated gene transcription is required for expression of a pagetic phenotype in OCL.

Disclosures: ***N. Kurihara****, None.*

1112

Vitamin D Receptor Isoforms Mediate the Cell-Selective Actions of the Vitamin D Analog Ro-26-9228. A. Ismail*[1], C. V. Nguyen*[1], M. R. Uskokovic*[2], S. Peleg[1]. [1]Endocrine Neoplasia & HD, M. D. Anderson Cancer Center, Houston, TX, USA, [2]Roche Inc., Nutley, NJ, USA.

The vitamin D analog Ro-26-9228 is tissue- and gene-selective in vivo. In culture, the vitamin D receptor (VDR)-mediated transcriptional activities of this analog were 30 to 100 times greater in human osteoblastic cell lines (MG-63 and hFOB) than in the human colon carcinoma cell line, Caco-2. We found that analog-bound VDR from hFOB cells had significant ability to interact with the p160 coactivator, GRIP, whereas analog-bound VDR from Caco-2 cells did not. We hypothesized that the cellular environment in the two cell types may differently regulate the events associated with acquisition of conformational changes that promote recruitment of coactivators of transcription to the VDR-ligand complexes. This activity was examined in the cytoplasmic and in the chromatin-bound receptor subpopulations of the two cell types. We found that in hFOB cells, unoccupied VDR was cytoplasmic and both 1,25-dihydroxyvitamin D3 (1,25D3) and the analog Ro-26-9228 induced a rapid accumulation of VDR in the chromatin. The ligand-bound (1,25D3 or Ro-26-9228) VDR from either cytoplasm or chromatin of hFOB cells had the ability to interact with GRIP. In contrast, the VDR in Caco-2 cells was present in both the cytoplasm and the chromatin in a ligand-independent manner. However, the treatment of Caco-2 cells with 1,25D3 selectively induced an accumulation of a smaller VDR form (form B) in the chromatin, whereas the analog's ability to induce accumulation of form B was poor. Examination of GRIP-interacting ability of VDR from Caco-2 cells revealed that the cytoplasmic and chromatin-associated large receptor form (form A) had ligand binding activity but did not have the ability to interact with GRIP, although the chromatin-bound form B did. To determine if form B was a proteolytic product of form A, we treated Caco-2 cells with the proteasome inhibitor MG132 and found, unexpectedly, that it caused a chromatin-exclusive accumulation of form B of VDR. Ligand treatment together with MG132 treatment appeared to promote a transformation of form A into form B. Furthermore, MG132 restored the coactivator-binding ability of the analog-treated VDR from Caco-2 cells to the same level as that induced by 1,25D3. Similar experiments with hFOB cells suggested that their VDR existed in a single, proteasome-sensitive form B. We hypothesize that the cell selectivity of Ro-26-9228 action is due to its limited ability to transform the inactive, proteasome-resistant form A of hVDR in Caco-2 cells into an active proteasome-sensitive form B, whereas in hFOB cells this step is not rate-limiting for VDR activation by the analog.

Disclosures: ***S. Peleg****, None.*

1113

Evidence for a Role of C/EBP Beta in the Cross Talk between Parathyroid Hormone and $1,25(OH)_2D_3$ that Involves Enhancement of Protein Kinase a Mediated Induction of Vitamin D Receptor Transcription. P. Dhawan*[1], X. Peng*[1], M. Huening*[1], M. Centrella*[2], T. L. McCarthy*[2], S. Christakos[1]. [1]Depart ment of Biochemistry, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA, [2]Yale University Medical School, New Haven, CT, USA.

C/EBP beta is a newly identified target of $1,25(OH)_2D_3$ action in osteoblastic cells. The induction of C/EBP beta in osteoblastic cells by $1,25(OH)_2D_3$ precedes the induction of 25-hydroxyvitamin D_3 24-hydroxylase [24(OH)ase] mRNA, consistent with a role of C/EBP beta as a mediator of the $1,25(OH)_2D_3$ response of the 24(OH)ase gene. In previous studies we showed that C/EBP beta cooperates with the vitamin D receptor (VDR) in the activation of 24(OH)ase transcription. A C/EBP beta site was identified in the rat 24(OH)ase promoter at –395/-388. Mutation of this site inhibited C/EBP beta binding and markedly attenuated the transcriptional response to C/EBP beta. In addition we have found cooperation of CBP/p300 with C/EBP beta by direct protein-protein interaction in regulating VDR mediated 24(OH)ase transcription. We now report that not only $1,25(OH)_2D_3$ but also parathyroid hormone (PTH) can induce C/EBP beta expression in osteoblastic cells. A peak induction of 9 fold is observed at 1h after PTH treatment (25 nM; first significant induction at 10 nM) in UMR106 osteoblastic cells with a return to basal levels by 9h. A similar induction of C/EBP beta by PTH was observed in primary osteoblasts. Treatment with $1,25(OH)_2D_3$ (10^{-8}M) for 16h followed by 4h with PTH (25nM) results in a 3-4 fold enhancement of C/EBP beta mRNA and 24(OH)ase mRNA as well as a 3-4 fold enhancement of VDR mediated 24(OH)ase transcription over the levels observed with $1,25(OH)_2D_3$ alone. Our data using the hVDR promoter (-1500/+60 that contains 2 putative C/EBP beta sites) indicate that C/EBP beta (5µg) can enhance PKA mediated transcription of hVDR 4 fold. Dominant negative C/EBP beta was found to inhibit the C/EBP beta mediated enhancement of VDR transcription. Thus, C/EBP beta plays an important role as an activator of $1,25(OH)_2D_3$ induced 24(OH)ase transcription as well as in PTH modulation of $1,25(OH)_2D_3$ action in osteoblastic cells. Our findings indicate, for the first time, a role for C/EBP beta in the cross talk between PTH and $1,25(OH)_2D_3$ that involves enhancement of PKA induced VDR transcription. Our findings also suggest that C/EBP beta may be a mediator of other PTH actions that affect skeletal integrity and osteoblast function.

Disclosures: ***P. Dhawan****, None.*

1114

Functional Cooperation Between Cbfa1 and the Vitamin D Receptor in the Regulation of Osteopontin Transcription. Q. Shen*, S. Christakos. Dept. of Biochemistry, UMDNJ-New Jersey Medical School & GSBS, Newark, NJ, USA.

Osteopontin (OPN) is a glycosylated phosphoprotein present in bone extracellular matrix that has been reported to modulate both mineralization and bone resorption. In osteoblastic cells the transcription of OPN is stimulated in response to 1,25dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) via the VDR/RXR complex. OPN expression is also regulated by the osteoblast specific differentiation regulator, Runx2/Cbfa1. Targeted disruption in mice of the vitamin D receptor (VDR) or Cbfa1 results in a marked inhibition of OPN expression in osteoblasts. In this study we addressed possible cross talk between VDR and Cbfa1 in regulating OPN transcription. Studies were done using COS-7 cells (that lack endogenous VDR and Cbfa1) transfected with the OPN promoter (-777/+79; VDRE –757/-743) and hVDR and/or Cbfa1 expression vectors. In $1,25(OH)_2D_3$ treated (10^{-8}M, 24h) VDR transfected COS-7 cells OPN transcription is induced 4.2±1.1 fold ($p<0.05$ compared to basal). OPN transcription was maximally induced 8.3±1.4 fold ($p<0.05$) by co-transfection of Cbfa1 expression vector in the absence of $1,25(OH)_2D_3$. Coexpression of Cbfa1 and VDR and treatment with $1,25(OH)_2D_3$ (10^{-8}M, 24h) resulted in a 20.1±1.6 fold induction of OPN transcription, suggesting functional cooperation between Cbfa1 and VDR. In COS-7 cells, the enhancement of the inductive action by $1,25(OH)_2D_3$ and Cbfa1 was dose dependently inhibited by AML1-ETO that acts as a repressor of Cbfa1. In ROS17/2.8 cells, that contain endogenous Cbfa1, 300ng pCMV AML1-ETO significantly diminished the $1,25(OH)_2D_3$ induction of OPN transcription ($p<0.05$). A Cbfa1 site was noted in the OPN promoter (AACCACA at –136/-130). Gel shift assays demonstrated that Cbfa1 binds to this site. A protein-DNA complex was not observed using a mutated Cbfa1 site (AAGAACA) as a probe. Using a mutant OPN promoter with the Cbfa1 site mutated as described for the gel shift assay and Cbfa1 and VDR co-transfected COS-7 cells, we found that cooperative activation between $1,25(OH)_2D_3$ and Cbfa1 was not observed. Our study demonstrates for the first time that the osteoblast specific transcription factor Cbfa1 has cooperative effects and cross talk with the VDR/RXR complex in $1,25(OH)_2D_3$ induced OPN transcription and suggests that Cbfa1 and VDR may cooperate in vivo to regulate the expression of the OPN gene.

Disclosures: ***Q. Shen****, None.*

1115

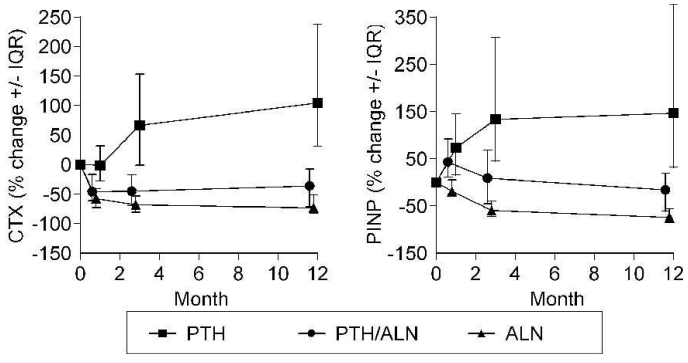
Effect of PTH, Alendronate, or PTH Plus Alendronate on Biochemical Markers of Bone Turnover: One Year Results of the PaTH Study. D. C. Bauer[1], P. Garnero[2], J. P. Bilezikian[3], S. L. Greenspan[4], J. A. McGowan[5], L. Palermo*[1], D. M. Black[1]. [1]UCSF, San Francisco, CA, USA, [2]Synarc, Lyon, France, [3]Columbia University, New York, NY, USA, [4]University of Pittsburgh, Pittsburgh, PA, USA, [5]NIAMS, Bethesda, DC, USA.

Parathyroid hormone (PTH) promotes and bisphosphonates inhibit bone remodeling, yet both agents increase bone mass and reduce fracture risk. The effects of combined PTH and bisphosphonate therapy on markers of bone turnover have not been reported.

We randomized 238 postmenopausal women (not currently using bisphosphonates) with low hip or spine BMD (T-score <-2.5 or <-2.0 with a risk factor) to a daily regimen of: 100 µg rhPTH(1-84) (NPS)(n=119); alendronate (ALN) 10 mg (Merck)(n=60); or PTH and ALN together (n=59). BMD at the spine and hip were assessed by DXA. Fasting serum levels of N-terminal propeptide of type 1 collagen (P1NP, Roche) and C-terminal telopeptide of type 1 collagen (sCTX, Roche) were used to monitor formation and resorption, respectively, at baseline and 1, 3, and 12 months after randomization. An uncoupling index (z-score for P1NP minus z-score for sCTX) was calculated at each visit. Follow-up was complete in 95% of those randomized; analyses are by ITT.

Bone formation and resorption increased with PTH treatment and decreased with ALN treatment (Figures). For combination therapy at 1 month, bone resorption decreased close to levels observed with ALN alone, and formation increased close to levels observed with PTH alone. By 3 months, marker response to combination therapy mirrored changes observed with ALN therapy alone, but to a lesser extent. In each treatment group, correlations with 12 month change in spine BMD were higher with 1 month uncoupling index (r=0.43-0.61, $p<0.05$) than with 1 month change in P1NP or sCTX, or any parameter measured at later time points.

We conclude that after 1 month of combined PTH/ALN therapy the predominant effect on bone turnover is an increase in bone formation (PTH-driven) and a reduction in bone resorption (ALN-driven), but thereafter ALN blunts the anabolic effects of PTH. One month uncoupling index may best predict the 1 year change in spine BMD.



Disclosures: ***D.C. Bauer****, Merck R; GSK R.*

1116

A Rapid and Profound 6-Month Suppression of Bone Turnover Is Seen with a Single SC Dose of AMG 162 in Postmenopausal Women. P. J. Bekker[1], D. L. Holloway[1], A. S. Rasmussen*[1], R. Murphy*[2], P. Leese*[3], G. Holmes*[4], C. R. Dunstan[5], A. M. DePaoli*[1]. [1]Clinical Research, Amgen Inc, Thousand Oaks, CA, USA, [2]Biostatistics, Amgen Inc, Thousand Oaks, CA, USA, [3]Quintiles, Lenexa, KS, USA, [4]SFBC International, Inc., Miami, FL, USA, [5]ANZAC Research Institute, Concord NSW, Australia.

RANKL (Receptor Activator of NF kappa B Ligand) is an essential osteoclastic differentiation and activation factor. The bone anti-resorptive activity, safety and tolerability of AMG 162, a fully human monoclonal antibody to RANKL, were evaluated in postmenopausal women in this randomized, double-blind, placebo-controlled, single-dose, dose escalation study.

Six cohorts of 8-9 women randomly received a single subcutaneous (SC) injection of either AMG 162 or placebo (3:1 ratio). AMG 162 doses were 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg. Adverse events, chemistry, hematology, urinary N-telopeptide/creatinine (uNTX; Osteomark®), serum N-telopeptide (sNTX), and serum bone-specific alkaline phosphatase (BSAP, Ostase®) were followed for 6 months in all cohorts and 9 months in the 3 highest dose cohorts.

Forty-nine women were enrolled. uNTX mean % changes (+/- SE) from baseline are displayed.

	AMG 162, mg/kg single SC dose						
Visit	Placebo (n=12)	0.01 (n=6)	0.03 (n=6)	0.1 (n=6)	0.3 (n=6)	1.0 (n=6)	3.0 (n=7)
Day 2	-10 ± 7	-9 ± 7	-32 ± 8	-32 ± 11	-25 ± 27	-38 ± 19	-73 ± 5***
Week 1	-9 ± 5	-32 ± 8**	-61 ± 5 ***	-66 ± 9***	-56 ± 24***	-80 ± 3***	-78 ± 6***
Week 2	-7 ± 7	-43 ± 8***	-66 ± 8***	-75 ± 6***	-69 ± 17***	-82 ± 2***	-83 ± 4***
Month 1	-7± 6	-35 ± 6 **	-61 ± 8 ***	-76 ± 5***	-57 ± 24***	-69 ± 7***	-82 ± 4***
Month 3	9 ± 9	3 ±10	-15 ± 17	-35 ± 7*	-65 ± 16***	-78 ± 2***	-84 ± 3***
Month 6	-10 ± 10	-22 ± 5	20 ± 18	-6 ± 14	-22 ± 27	-59 ± 8*	-81 ± 5***
Month 9	-36 ± 12	Not done	Not done	Not done	-42 ± 18	-38 ± 12	-69 ± 5

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. placebo

Similar results were observed for sNTX. At Month 6, the mean change was 2%, –43 % and –56% in the placebo, 1.0 and 3.0 mg/kg AMG 162 groups, respectively. BSAP levels did not decrease remarkably until after the third week. Intact PTH levels increased up to ~4-fold by Day 5 in the 3.0 mg/kg dose group, but returned towards baseline with follow-up. Albumin-adjusted serum calcium did not decrease more than 10% on average in any group and no subject had values below 2 mmol/L. AMG 162 was very well tolerated. No related serious adverse events occurred; there was no evidence of an increased infection risk. No clinically meaningful laboratory changes, other than those described above, occurred. No subject had evidence of anti-AMG 162 antibodies.

In summary, a single SC dose of AMG 162 resulted in a dose-dependent rapid and sustained decrease in bone turnover from baseline. The tolerability profile was excellent. AMG 162 has potent anti-resorptive activity and could be a convenient treatment for osteoporosis.

Disclosures: ***P.J. Bekker****, None.*

1117

The Risk vs. Benefit Profile of Estrogen Plus Progestin Does Not Differ by Underlying Risk of Osteoporosis. The Women's Health Initiative. J. Cauley[1], J. Robbins[2], Z. Chen[3], S. Cummings[4], R. Jackson[5], A. LaCroix*[6], M. LeBoff[7], C. Lewis[8], J. McGowan[9], J. Neuner*[10], M. Pettinger*[11], M. Stefanick*[12], J. Wactawski-Wende[13], N. Watts[14]. [1]U Pitt, Pittsburgh, PA, USA, [2]UC Davis, Sacramento, CA, USA, [3]U AZ, Tucson, AZ, USA, [4]UCSF, San Francisco, CA, USA, [5]OSU, Columbus, OH, USA, [6]FHCPC, Seattle, WA, USA, [7]Harvard, Boston, MA, USA, [8]UAB, Birmingham, AL, USA, [9]NIAMS, Bethesda, MD, USA, [10]U WI, Milwaukee, WI, USA, [11]FHCRC, Seattle, WA, USA, [12]Stanford, Palo Alto, CA, USA, [13]SUNY, Buffalo, NY, USA, [14]U Cincinatti, Cincinnati, OH, USA.

In the Women's Health Initiative Estrogen plus Progestin (E+P) trial, the overall benefit vs. risk profile, summarized in a global index, was a central focus. The global index was defined as the first occurrence of coronary heart disease, invasive breast cancer, stroke, pulmonary embolus, endometrial and colorectal cancer, hip fracture or death due to other causes. Overall, the global index showed a nominally significant 15% increase in the E+P group indicating more harm than benefit. The reduction in fractures and colorectal cancer were not sufficiently large to counterbalance the increased risk of heart disease and breast cancer. In the current analysis, we tested the hypothesis that the risk-benefit profile differs in women who are at "high" risk of fracture. We formed a summary risk score factor score using age (≥70), race (White/Asian), weight (<127 lbs), current smoking, positive family history of fractures, positive personal history of fractures; low calcium intake (<600 mg/day), self report of ≥2 falls in the past year; and never use of HRT prior to the trial. We assigned a score of one to each risk factor and summed the total number of risk factors (0-9). We divided the groups into tertiles (number of risk factors 0-2, 3, 4-9). The annual incidence of all fractures in placebo subjects was 1.4%, 1.8%, and 2.6% from tertile 1 (lower risk) through tertile 3 (higher risk), showing the validity of the scores. There was no evidence that the global index was more favorable in women who were considered at high risk of fractures. (Table) We conclude that the benefit of fracture reduction does not outweigh risks for heart disease and breast cancer even in women at higher risk of fracture.

Table: Relative Hazard of Global Index by Randomization Assignment, Stratified by Summary
Osteoporosis Risk Score
N = number with event
ann% = annualized incidence (%)

	E+P (N = 8506)		Placebo (N = 8102)		Hazard Ratio	95% CI	
						Lower	Upper
Summary risk score (9 items)	N	(ann. %)	N	(ann. %)			
Low (0-2 factors)	202	(1.27%)	182	(1.28%)	1.02	0.83	1.24
Mid (3 factors)	282	(1.87%)	233	(1.59%)	1.18	0.99	1.40
High (4-9 factors)	369	(2.16%)	304	(1.87%)	1.16	1.00	1.35

Disclosures: ***J. Cauley****, Merck 2, 8; Eli Lilly 2, 8; Novartis 2, 5; Pfizer 2.*

1118

Changes in BMD at Different Skeletal Sites After Discontinuation of Treatment With rhPTH (1-34) in Postmenopausal Osteoporotic Women. J. R. Zanchetta, L. Rubio*, A. Mango*, C. E. Bogado*. Clinical Research, IDIM, Buenos Aires, Argentina.

Treatment with rhPTH (1-34) induces dramatic bone density increases in postmenopausal osteoporotic women. Assessment of the effects of treatment discontinuation on BMD is not easy. Patients remain free of treatment for different periods of time and thereafter receive different medications, making difficult the follow-up of BMD changes.
Ninety three patients who participated in a large, international rhPTH (1-34) vertebral fracture prevention trial at our site were follow-up with DXA measurements during 18 months. They have been randomly assigned to receive daily, self-injected doses of 20 (n=37) or 40 (n=25) ug of rhPTH or placebo (n= 31), plus 1000 mg calcium and 400 to 1,200 IU of vitamin D. After a media of 21 months of treatment, rhPTH treatment was stopped and patients entered in an observational follow-up study, receiving calcium and vitamin D supplements only. BMD measurements at lumbar spine (LSBMD)and femoral neck (FNBMD) and whole body bone mineral content (TBMC)were performed 6 and 18 months after treatment discontinuation.
rhPTH administration significantly increased BMD at all skeletal sites evaluated. LSBMD increased by 12.9 % (p< 0.001) and 9.0% (p<0.001), while FNBMD increased by 5.1% (p<0.001) and 4.3% for the 40 and 20 ug doses respectively. TBMC increased 5.6% (p<0.01) in the 40 ug group and by 3.3% in the 20 ug group. There were no significantly changes in the placebo group.
During follow-up LSBMD decreased by –3.04% and –3.42% after 6 months and by –4.32% and –0.88% after 18 months, in the 40 and 20 ug groups respectively. Despite bone loss, LSBMD remained significantly higher by 4.6% (40 ug group) and 3.8% (20 ug group) as compared with baseline after 18 months without treatment. FNBMD decreased by –1.2% after 6 months and –1.1 after 18 months in the 20 ug group. In the 40 ug group it changed by +1.03 and –2.4 after 6 and 18 months respectively. After 18 months FNBMD values remained 3.4% and 1.8% higher as compared with baseline in the 40 and 20 ug groups respectively. TBMC decreased by –2.3% and –1.79 after 6 months in the 40 and 20 ug groups respectively, but did not change significantly thereafter. No significantly changes were evident in the placebo group.
Our results confirm that patients loss bone after rhPTH(1-34) treatment is discontinued. However, 18 months after discontinuation, BMD remains significantly increased as compared with baseline at lumbar spine and femoral neck. The differences in the pattern of bone loss between lumbar spine and femoral neck or whole body, might reflect a different response of trabecular and cortical bone tissue after treatment discontinuation.

Disclosures: ***J.R. Zanchetta****, Eli Lilly and Company 5.*

1119

Effects of Simvastatin Treatment in Postmenopausal Osteopenic Women: A Randomized Controlled Trial. L. Rejnmark[1], N. H. Buus*[2], P. Vestergaard[1], F. Andreasen*[2], L. Heickendorff*[3], M. L. Larsen*[4], L. Mosekilde[1]. [1]Dept. of Endocrinology and Metabolism, Aarhus Amtssygehus, Aarhus, Denmark, [2]Center for Clinical Pharmacology, Aarhus University, Aarhus, Denmark, [3]Dept. of Clinical Biochemistry, Aarhus Amtssygehus, Aarhus, Denmark, [4]Dept. of Medicine and Cardiology, Aarhus Amtssygehus, Aarhus, Denmark.

Statins have been suggested as potential agents in the management of osteoporosis as statins have been reported to cause bone anabolic as well as antiresorptive effects.
In a double-blinded design, 82 healthy postmenopausal women with osteopenia or osteoporosis (T-score < -1), aged 53 to 74 years, were randomized to one-year of treatment with simvastatin 40 mg/day or placebo. Participants were recruited from the general population. None of the participants had known hyperlipidemic diseases. Bone mineral density (BMD) and plasma levels of cholesterol, PTH and biochemical markers of bone turnover were measured at baseline, after one year of treatment (week 52), and 26 weeks after withdrawal of treatment (week 78). Calcium supplements (400 mg/day) were administrated to all participants during the entire 1.5-year study period. Seventy-eight women completed the one-year of treatment. After one year, plasma cholesterol was reduced with simvastatin but not placebo (-27% vs. +0.7%, p<0.001). After withdrawal of treatment (week 78), plasma cholesterol returned to baseline levels in the simvastatin group and did no longer differ from the placebo group (p=0.83). However, plasma PTH levels as well as plasma levels of biochemical markers of bone formation (osteocalcin and bone-specific alkaline phosphatase) and bone resorption (CTx) did not differ between groups at week 52 or at week 78. Compared with placebo, simvastatin did not cause significant changes in BMD at the lumbar spine, total hip, femoral neck or whole body at week 52 or at week 78. Only at the forearm, BMD increased in response to simvastatin. At the total forearm, BMD was increased by 1.1% in the simvastatin group at week 52 (vs. –0.3% in the placebo group, p=0.01) and by 1.8% at week 78 (vs. +0.4% in the placebo group, p=0.02). At the ultradistal forearm, BMD was increased by 2.9% in the simvastatin group at week 52 (vs. +0.1% in the placebo group, p=0.02) and by 3.4% at week 78 (vs. +1.1% in the placebo group, p=0.04). Within the statin group, changes in cholesterol levels did not correlate to BMD changes at any measurement site. Thus, our results do not support a general beneficial effect of simvastatin on bone metabolism. Therefore, statin treatment is most likely not a therapeutic option in the treatment of osteoporosis.

Disclosures: ***L. Rejnmark****, None.*

1120

Daily versus Cyclic PTH Combined with Alendronate versus Alendronate Alone for Treatment of Osteoporosis. F. Cosman[1], J. W. Nieves[2], M. M. Luckey[3], M. Zion*[2], L. Woelfert*[1], R. Lindsay[1]. [1]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Clinical Research Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [3]St. Barnabus Hospital, Livingston, NJ, USA.

During the early response to PTH administration, bone formation is stimulated more dramatically than bone resorption, which catches up by 6 months. Therefore, it is possible that repeated 3 month cycles of PTH therapy could produce an equal or greater skeletal response to that produced by daily PTH treatment. We embarked on a 15 month treatment trial involving 126 patients (mean age 67.7 years) with osteoporosis, already treated with alendronate for at least 1 year (mean = 3.2 years). While alendronate was continued (70mg/week), patients were randomly assigned to receive (1-34)hPTH 25 mcg by subcutaneous injection daily (DAILY PTH), 25 mcg PTH daily for cycles of 3 months on and 3 months off (CYCLIC PTH), or alendronate alone (A). Data are presented from the first 83 patients completing 9 months of the trial. Baseline demographic, biochemical and BMD variables were similar in the 3 groups. Serum levels of bone formation (osteocalcin, bone alkaline phosphatase, and aminoterminal propeptide of Type I procollagen); the urinary bone resorption marker, crosslinked n-telopeptide (NTX); and BMD were measured every 3 months. Bone turnover markers did not change significantly in the A group throughout the 9 months. In the DAILY PTH group, bone formation markers increased rapidly during the first 3 months and continuously throughout the 9 months (increases ranging from 80-300% for different markers, all p<0.001). In the CYCLIC PTH group, bone formation markers increased within the first 3 months of treatment similarly to the DAILY PTH group, declined during the off PTH cycle, and then rose again robustly during the second PTH cycle (60-270% from baseline, all p<0.001). NTX increased more modestly than bone formation markers in the PTH groups at 3 months and thereafter followed a similar pattern to bone formation markers in DAILY PTH vs CYCLIC PTH groups. Lumbar spine BMD rose progressively in the DAILY PTH group. In the CYCLIC PTH group, lumbar BMD rose to 3 months, declined slightly to 6 months and rose again from 6 to 9 months. At 9 months, there were no significant differences in BMD change between the 2 PTH groups; spine BMD increased 4.3% in the DAILY PTH group (p<0.0001), and 3.8% in the CYCLIC PTH group (p<0.0001). There was a smaller increase in BMD in patients in the A group (2%, p<0.04). These data indicate that PTH stimulates bone formation and increases BMD in the presence of long-term established alendronate. Furthermore, short cyclic challenges with PTH might be an efficient and economical way to use PTH for treatment of osteoporosis.

Disclosures: ***F. Cosman****, None.*

1121

Delayed Fracture and Osteotomy Healing in Pediatric Osteogenesis Imperfecta Patients Receiving Pamidronate. C. F. J. Munns, F. Rauch, L. Zeitlin, F. Fassier*, F. H. Glorieux. Shriners Hospital for Children and McGill University, Montreal, PQ, Canada.

Treatment with cyclical intravenous pamidronate infusions has led to marked improvements in the clinical management of children and adolescents with osteogenesis imperfecta. However, the effect of treatment on fracture healing is not well characterized. In the present study we evaluated bone healing following radiologically confirmed lower limb fractures and after osteotomies performed for the placement of intramedullary rods. Delayed healing was defined as the radiographically apparent persistence of a fracture or osteotomy line 12 months following the event. A total of 197 fractures (132 femur, 65 tibia) in 85 patients with moderate to severe forms of OI (median age at the time of fracture 4.8 years; range 0.0 to 19.9 years; 45 girls) were evaluated. Incomplete fracture healing was more than twice as frequent when pamidronate therapy had been started before the fracture occurred (42 of 155 (27%) vs 5 of 42 (11%); P=0.04 by Chi square test). During pamidronate treatment, fractures that subsequently demonstrated delayed healing occurred at an older age (8.1 vs 4.3 years), a taller height (107 vs 85 cm), a heavier weight (19.3 vs 12.2 kg) and after a longer duration of treatment (2.1 vs 1.1 years) than fractures that healed normally (P<0.001 for all comparisons by U-test). 48% of fractures in OI type IV patients and 19% of fractures in OI type III patients showed delayed healing (P<0.001 by Chi square test). Osteotomy healing was assessed after 200 intramedullary rodding procedures in 79 OI patients with moderate to severe forms of OI (median age at the time of surgery 5.4 years; range 1.2 to 19.8 years; 42 girls). Delayed osteotomy healing was almost four times more frequent when pamidronate had been started before surgery (97 of 162 (60%) vs 6 of 38 (16%); P<0.001 by Chi square test). During pamidronate treatment, factors associated with delayed osteotomy healing were older age (6.5 vs 3.9 years), taller height (94 vs 82 cm) and heavier weight (15.9 vs 12.2 kg) (P<0.001 for all comparisons by U-test). Duration of pamidronate treatment was not associated with delayed osteotomy healing (1.4 vs 1.7 years; P=0.4 by U-test). Delayed osteotomy healing occurred after 83% of rodding procedures in OI type IV patients compared to 45% in OI type III patients

(P<0.001 by Chi square test). This study demonstrates that cyclical intravenous pamidronate therapy is associated with a significant delay in fracture and osteotomy healing in children and adolescents with OI. The clinical sequelae of the delay in healing require investigation.

Disclosures: ***C.F.J. Munns****, None.*

1122

Treatment of OIM Mice with Osteoprotegerin. J. Quenzer*[1], K. Boachie-Adjei*[1], A. Hao*[1], C. Dunstan[2], C. L. Raggio*[1], N. P. Camacho[1]. [1]Research Division, Hospital for Special Surgery, New York, NY, USA, [2]Amgen Corp., Thousand Oaks, CA, USA.

Osteogenesis imperfecta (OI) is a heritable disease caused by molecular defects in type I collagen that can result in severe bone fragility. Several studies have established that bisphosphonates are effective in reducing fracture risk and improving bone properties in OI. The purpose of the current study was to explore the possibility that treatment of an animal model of OI with the anti-resorptive agent osteoprotegerin (OPG) would also improve bone properties and reduce fractures. Mice homozygous for the *oim* mutation *(oim/oim)* are deficient in proα2(I) collagen and model moderate-to-severe OI. *Oim/oim* (n = 7) and *oim/+* mice (n = 9) were treated with 1 mg/kg/day recombinant OPG fusion protein (Fc-OPG) (generously supplied by Amgen Corp., Thousand Oaks, CA) injected s.c. 3x/week from 6 weeks to 14 weeks of age. At sacrifice, whole body AP radiographs were taken and fractures counted. Dissected femora were radiographed with a density standard, digitized, and analyzed with SigmaScan software (Jandel Scientific). The mineralized phase was evaluated histologically in tibial metaphyses with alcian blue staining. Data from this study were compared to data from saline-treated (control) mice. All the mice tolerated the OPG treatment very well, with consistent weight gain throughout the study. There was variability in response of both the *oim/+* and *oim/oim* mice to OPG, as evidenced by the quantity of new metaphyseal bone formation. Histologically, some mice exhibited little new bone growth, while some mice had metaphyseal bone with persistent calcified cartilage and thickened trabeculae. This was also reflected in the radiographic densities, which ranged from 1.19 to 3.14 (mean = 2.26 $\pm$.73) for the *oim/+* mice, and from 0.84 to 2.09 (mean = 1.17 $\pm$.48) for the *oim/oim* mice. The OPG *oim/oim* mice sustained between 1 – 4 fractures/mouse, compared to 1 – 7 fractures/mouse in the saline-treated *oim/oim* mice. *Oim/+* mice do not typically fracture, and consistent with that phenotype, sustained no fractures with OPG treatment. Previous studies have shown that Fc-OPG, which contains a human OPG domain, can be antigenic in mice after some time. Thus, the variability of response in the current study could be attributed to that fact. Nevertheless, these preliminary results support the notion that optimization of the dosing regime of OPG could result in consistent new bone formation and fracture reduction in the *oim/oim* mice.

Disclosures: ***N.P. Camacho****, None.*

1123

The Effects of a Lifestyle Intervention for Teen Girls on Bone Mineral Density. C. Ritenbaugh*[1], L. DeBar*[1], M. Aickin*[1], P. J. Elmer*[1], D. Elliott*[2], E. Orwoll[2]. [1]Kaiser Permanente Center for Health Research, Portland, OR, USA, [2]Oregon Health and Science University, Portland, OR, USA.

YOUTH is a lifestyle intervention for increasing bone mineral density (BMD) among adolescent women (14-16 years of age), with a primary end point of change in BMD by DEXA at two years. Higher risk female adolescents (Body Mass Index between 16 and 23) and their parent(s)/guardian(s) were individually recruited among members of a large HMO in the Pacific Northwest. Eligible teens (N=228) were randomized into a lifestyle intervention or to general health education (control). The intervention targets are: (1) improving diet (increasing consumption of fruits, vegetables, and high calcium foods; and decreasing soft drink consumption), and (2) increasing high-impact physical activity and spinal motion. The control group receives general health information.

Interim results of the intervention on BMDare shown below for the first 140 girls (72 control, 68 intervention) who had BMD assessment at one year (Hologic 4500). At baseline (mean $\pm$ S.D.): overall age = 14.9 $\pm$ 0.6 yrs.; overall bmi = 20.0 $\pm$ 1.6; months since menarche 26.7 $\pm$ 14.5. In both groups, 76% of girls self-identified as white. There were significant 1-year differences in diet changes for the intervention group compared to control (intervention group: increased fruits and vegetables and calcium, decreased soda), but no significant differences in changes in high-impact or total activity levels or spinal motion. The table below shows baseline BMD values by group, the adjusted mean difference at one year, and the p-value for the 1-year difference. The adjusted mean difference is estimated from a conditional change regression (ANCOVA), adjusting for BMD at baseline and time since menarche.

Bone Mineral Density Site	Baseline Control X $\pm$ S.E.	Baseline Intervention X $\pm$ S.E.	Adjusted mean difference	p value
Total body	1.1 $\pm$ 0.08	1.11 $\pm$ 0.07	.0023 $\pm$. 0035	0.513
Femoral neck	0.89 $\pm$ 0.12	0.89 $\pm$ 0.12	-.0006 $\pm$ .0054	0.906
Trochanter	0.77 $\pm$ 0.10	0.75 $\pm$ 0.11	.0097 $\pm$ .0042	0.021
Ward's Triangle	0.88 $\pm$ 0.14	0.88 $\pm$ 0.14	-.0067 $\pm$ .0076	0.382
Total hip	0.96 $\pm$ 0.11	0.96 $\pm$ 0.12	.0060 $\pm$ .0039	0.130
Spine L1-L4 (mean)	0.97 $\pm$ 0.10	0.95 $\pm$ 0.09	.0177 $\pm$ .0043	0.000

Conclusion: Teenage girls can achieve lifestyle dietary changes for one year that result in enhanced bone mineral density at predominantly trabecular bone sites.

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Disclosures: ***C. Ritenbaugh****, None.*

1124

Interdependence between Calcium Intake and Menarcheal Age on Bone Mass Gain: A 8 Years Follow-up Study from Pre-puberty to Post-menarche. T. Chevalley[1], J. P. Bonjour[1], D. Hans*[2], D. Slosman*[2], R. Rizzoli[1]. [1]Div. of Bone Diseases, Geriatric and Internal Medicine, Univ. Geneva, Switzerland, [2]Div. of Nuclear Medicine, Radiology, University of Geneva, Switzerland.

Both early menarche and high Ca intake during growth are considered as factors reducing the risk of osteoporosis, probably by increasing peak bone mass. Recent observation suggests a possible interdependence between these two factors. We investigated whether Ca intake could influence menarcheal age (MENA) and, conversely, whether the response of bone mineral mass accrual to increased Ca intake could vary according to MENA. 144 girls aged 7.93±0.04 yrs (mean±sem) were randomized in a double blind controlled trial to receive either a Ca supplement (Calsup) of 850mg/day (n=77) or placebo (n=67) during one year. Areal bone mineral density (aBMD) was determined by DXA at 6 skeletal sites: radius metaphysis, radius diaphysis, femoral neck, trochanter, femoral diaphysis and L2-L4 vertebrae. The mean yearly aBMD gains at these 6 sites were 21±2 and 27±2 (p<0.005) in the Placebo and Calsup group, respectively (intention to treat cohort). The subjects were reexamined at 1.0, 3.5 and 7.5 yrs after the end of intervention and MENA was recorded. Spontaneous Ca intake was assessed by frequency questionnaires at baseline, 6 and 12 months allowing us to calculate the total Ca intake (spontaneous±Calsup, in g/yr) during the intervention phase, taking into account the individual degree of compliance in the Calsup group. Further frequency questionnaires made at each follow-up visit indicated a steady individual position within the spontaneous Ca intake range. We now report on results obtained when the subjects were 16.38±0.07 and 16.34±0.06 yrs in the placebo (n=58) and Calsup (n=67) groups, respectively. A highly significant negative correlation was found between Ca intake and MENA (R=-0.35, p<0.0001), and between MENA and gain in aBMD from age 8.0 to 16.4 yrs at all 6 skeletal sites (R range: from - 0.41 to - 0.22, p<0.0001- p<0.016). The positive effect of Calsup on mean axial and appendicular aBMD gain from baseline remained significantly greater in girls below (Early) but not in those above (Late) the median of MENA: Early, placebo (MENA=12.2±0.2 yrs) 286±8 mg/cm^2, Calsup (MENA=12.1±0.1 yrs) 317±8, p<0.01. Late, placebo (MENA=13.9±0.1 yrs) 284±10 mg/cm^2, Calsup (MENA=13.9±0.1yrs) 276±16, p>0.05. In conclusion, our study shows for the first time a relationship between Ca intake during pre-puberty and menarcheal age. It also indicates that early menarche is associated with lasting bone mass gain in response to Ca supplementation. Thus, both early menarcheal age determinants and high Ca intake appear to positively interact on bone mineral mass accrual.

Disclosures: ***T. Chevalley****, None.*

1125

Femoral Neck Bone Strength Changes in Prepubertal Boys: A Randomized Controlled Trial of a 2-Year, School-Based Exercise Intervention. K. J. MacKelvie[1], M. A. Petit*[2], K. M. Khan[1], H. A. McKay[1]. [1]University of British Columbia, Vancouver, BC, Canada, [2]Pennsylvania State University, Hershey, PA, USA.

Exercise during growth has a positive influence on bone mineral accrual, yet little is known about how bone structure and bone strength adapt to loading during growth. Our previous work has shown that, in early pubertal girls, the primary response to loading was bone accrual at the endosteal surface of the femoral neck, conferring a significant increase in bone bending strength over 8 months. Our objective was to compare changes in proximal femur bone structure and bone strength between 31 prepubertal (Tanner Stage 1) boys who participated in a school-based, high impact circuit intervention (12 minutes, 3x/week) for 20 months and 33 maturity-matched controls. We assessed structural variables and bone strength at the narrow neck (NN), intertrochanteric region and femoral shaft regions by applying the Hip Structural Analysis program to dual energy x-ray absorptiometry scans (DXA, Hologic QDR 4500) of the proximal femur. We derived total body lean mass and fat mass from DXA total body scans, and measured height and weight by standard methods, physical activity by questionnaire (PAQ-C), calcium intake by a food frequency questionnaire, and maturity by Tanner staging self-assessment of pubic hair. Intervention boys (10.2±0.5 years old) and control boys (10.1±0.5 years old) had similar baseline height (140.8 vs 141.3 cm) and weight (36.9 vs 35.4 kg) and similar average 20-month physical activity scores and calcium intakes (861 vs 850 mg/day). Twenty-month height and weight changes did not differ between groups; lean mass changed more (P<0.05) in intervention (22.8%) than control boys (18.6%). We compared 20-month bone changes with ANCOVA, adjusting for height change, final Tanner stage and baseline bone values. At NN region, intervention boys had greater expansion at both the periosteal (+2.6%, P=0.1) and endosteal (+2.7%, P=0.2) surfaces, resulting in significantly greater changes in section modulus (bone bending strength) (+7.5%, P=0.02). Changes at the intertrochanteric and femoral shaft regions did not differ significantly between groups. Twenty-month change in NN bone bending strength was correlated positively with change in lean mass (r=0.47, P<0.001), but negatively with change in fat mass (r=-0.33, P=0.01). A 2-year school-based, high impact exercise program implemented 3 x/week for 12 minutes is an effective strategy for site-specific gains in bone strength at the femoral neck region of the proximal femur. The bone structural adaptation may be mediated by changes in lean body mass. These data further highlight the surface-, site- and sex-specific bone adaptation to loading during growth.

Disclosures: ***K.J. MacKelvie****, None.*

1126

Influence of Sex Hormones on Bone Geometric Properties and Mineral Density in Early Pubertal Girls. Q. Wang*[1], P. Nicholson*[1], M. Suuriniemi[2], A. Lyytikäinen[1], E. Helkala*[1], M. Alen*[3], H. Suominen[1], S. Cheng[1]. [1]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [2]Department of Cell Biology, University of Jyväskylä, Jyväskylä, Finland, [3]PEURUNKA-Medical Rehabilitation Center, Jyväskylä, Finland.

The effects of sex hormones on bone geometric properties and density in early adolescent girls are not yet fully understood. The present study aimed to evaluate the correlations among serum 17β-estradiol (E2), testosterone, bone geometric properties and mineral density in pre- and early pubertal girls. E2, testosterone, and sex hormone binding globulin (SHBG) were measured using time-resolved fluoroimmunoassays (Delfia, Wallac Oy, Turku) in 245 10-12 year-old Finnish girls without history of disease or medication known to affect bone metabolism. The left tibial shaft was measured by peripheral quantitative computed tomography (Stratec XCT-2000). The cortical bone area and marrow cavity area were measured and expressed as proportions of the total tibial cross sectional area (CSA). Cortical thickness and total volumetric BMD (vBMD) were also determined. These tibial geometric and densitometric measures were correlated against the serum sex hormone concentrations controlling for age, body weight and height. The results showed that E2 was significantly associated negatively with marrow cavity proportion ($r = -0.19$, $p = 0.003$), positively with cortical proportion and thickness as well as total vBMD ($r = 0.24$, $p < 0.001$; $r = 0.18$, $p = 0.004$, and $r = 0.23$, $p < 0.001$, respectively). Free E2 showed similar correlations with these bone variables as total E2. However, testosterone and free testosterone were not associated with these bone variables. On the other hand, SHBG was associated negatively with vBMD, cortical thickness, and cortical proportion ($r = -0.18$, $p = 0.005$; $r = -0.22$, $p <0.001$; and $r = -0.16$, $p = 0.012$, respectively), positively with marrow cavity proportion ($r = 0.16$, $p = 0.010$). Total bone CSA did not correlate with E2, testosterone or SHBG. These results suggest a positive effect of E2 on bone cortical development by suppressing bone turnover at the endosteum during the early pubertal period, and that the effects of E2 on bone are more pronounced than that of testosterone.

Disclosures: ***Q. Wang****, None.*

1127

The Paternally Imprinted Autosomal Dominant Form of Pseudohypoparathyroidism Type-Ib Is Associated With a 2998-bp Deletion Centromeric of *GNAS1*. M. Bastepe, L. Fröhlich*, H. Jüppner. Endocrine Unit/Medicine, Mass. General Hosp., Harvard Med. Sch., Boston, MA, USA.

Most cases of pseudohypoparathyroidism type-Ib (PHP-Ib), unlike some other PHP forms, are not caused by coding Gs-alpha mutations. However, a familial form of PHP-Ib with autosomal dominant inheritance and paternal imprinting (AD-PHP-Ib) has been mapped to a ~2.5-Mb locus on 20q13.3 comprising a portion of *GNAS1*, the gene encoding Gs-alpha. Moreover, sporadic and familial PHP-Ib cases show a loss of methylation at *GNAS1* exon A/B, and recent studies have shown that the mutation leading to this epigenetic abnormality in AD-PHP-Ib kindreds resides at least 56 kb upstream of exon A/B. Taken together, these findings implicate a mutation affecting a long-range, *cis*-acting element that controls imprinting of *GNAS1*. We have now refined the AD-PHP-Ib locus to an ~280 kb interval based on a new recombinant individual in kindred W. Subsequent analysis of markers in this region revealed a heterozygous 2,998-bp deletion in kindred F, which was present only in affected members and unaffected carriers. The same deletion was also found in patients and healthy carriers of 12 other unrelated AD-PHP-Ib kindreds, but not in 114 healthy controls. For each patient affected by AD-PHP-Ib, the deletion was inherited maternally and associated with loss of methylation at exon A/B without defects at other DMRs of *GNAS1*. The deletion was also found in two apparently sporadic PHP-Ib cases with the exon A/B methylation defect. These cases, on further analysis of clinically unaffected parents, appeared to have inherited the mutation from their mothers. Many other sporadic PHP-Ib cases, despite having the exon A/B methylation defect, lack the deletion or any similar mutations. Interestingly, kindred W, which strongly links to the *GNAS1* region (lod score: 2.53), also does not show the deletion or any mutations within the deleted region, although the epigenetic abnormality in this kindred is identical to that in kindreds in whom the deletion is present. It is thus likely that, while the identified deletion disrupts a *cis*-acting regulatory element necessary for establishment and/or maintenance of the methylation imprint at exon A/B, there are additional imprinting control elements at *GNAS1*. *In vivo* studies will help elucidate the relationship between the identified mutation and the loss of exon A/B methylation.

Disclosures: ***M. Bastepe****, None.*

1128

Noncoding Deletion Present in van Buchem Patients Removes Essential Regulatory Elements Required for Bone-specific Expression of BMP-antagonist Sclerostin. G. G. Loots*[1], M. Kneissel*[2], H. Keller[2], M. Brunkow*[3], J. Chang*[4], D. Ovcharenko*[4], I. Plajzer-Frick*[4], V. Afzal*[4], E. M. Rubin*[5]. [1]Genomics Division, LLNL, Livermore, CA, USA, [2]Bone Metabolism Unit, Novartis Pharma, Basel, Switzerland, [3]Celltech Inc., Bothell, WA, USA, [4]Life Sciences Division, LBNL, Berkeley, CA, USA, [5]DOE, Joint Genome Institute, Walnut Creek, CA, USA.

Sclerosteosis is a generalized progressive bone overgrowth disorder due to the loss of function of the *SOST* gene product sclerostin. Van Buchem disease is a similar skeletal disorder characterized by milder sclerosteosis-like phenotypes and is associated with the presence of a 52 kb deletion (VBDel) located ~35kb downstream of the *SOST* transcript and ~10kb upstream of the *MEOX1* gene on human chromosome 17p21. Human-mouse comparative sequence analysis revealed several highly conserved noncoding elements present in the VBDel region suggesting that van Buchem disease is caused by the removal of essential *SOST*-specific regulatory elements. Using in vitro BAC-recombination techniques we have engineered a ~160kb human BAC containing the *SOST* and *MEOX1* transcripts by removing the ~52kb intergenic region absent in patients suffering from van Buchem Disease. We have generated several lines of transgenic animals carrying either the wildtype human *SOST* BAC or the VBDel modified BAC. Following the expression pattern of the endogenous sost mouse gene, we have investigated the expression pattern of the human transgenes in these two types of transgenic animals. Similar to the murine *SOST* expression, the human sost transcript from the wildtype BAC is predominantly expressed in the mineralized bones of fetal, neonatal and adult mice, as well as in the apical ectodermal ridge of the developing embryo. Transgenic animals carrying the modified VBDel BAC fail to express the human sost transcript in mineralized bone, while the embryonic expression of this transgene is unaffected. Using comparative sequence analysis, transient transgenic technology and transient transfection experiments in osteoblastic cells we are currently in the process of testing all the evolutionary conserved noncoding elements present in the VBDel for the potential to drive expression in the adult skeletal structures. Our findings suggest that van Buchem disease is caused by a regulatory mutation that diminishes osteoblast-specific expression of the BMP-antagonist sclerostin.

Disclosures: ***G.G. Loots****, None.*

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Engraftment of Wild-Type Bone Marrow Stromal Cells in Akp2-/- Mice: A Potential Therapy for Infantile Hypophosphatasia. D. Harmey, S. Narisawa*, J. L. Millan. The Burnham Institute, La Jolla, CA, USA.

Hypophosphatasia is a rare heritable disease for which there is currently no treatment. This disease results from loss-of-function mutations in the tissue non-specific alkaline phosphatase (TNAP) gene. TNAP hydrolyzes the mineralization inhibitor inorganic pyrophosphate (PP_i) and thereby promotes mineralization. There is wide variation in the severity of this disease, the most severe form of which is perinatal/infantile hypophosphatasia. We have developed a mouse model of infantile hypophosphatasia by ablating the TNAP (*Akp2*) gene. *Akp2*$^{-/-}$ mice have poorly mineralized bones, suffer spontaneous fractures and display elevated levels of PP_i. These mice appear normal at birth but are growth retarded from day 5, suffer epileptic seizures and do not survive weaning. The mineralization defects in these mice lies with the bone forming osteoblasts, which are unable to mineralize matrix. Given that bone marrow stromal cells (BMSCs) are a source of multipotential progenitors that under osteogenic conditions can differentiate into mature osteoblasts, we hypothesized that BMSCs from wild-type (WT) mice may be used as donor cells to treat the hypophosphatasia mice. BMSCs were isolated from the long bones of 6-week old WT mice. To ensure that these cells successfully differentiated into osteoblast-like cells, we cultured BMSCs in the presence of ascorbic acid (50 μg/ml) and β-glycerophosphate (10 mM). Under these conditions BMSCs differentiated into osteoblastic-like cells expressing characteristic osteoblast markers, *i.e.,* TNAP, osteocalcin (OCN) and core binding factor (CBFA-1). Based on these observations, we performed bone marrow transplants into *Akp2*$^{-/-}$ mice. To aid BMSC engraftment in the host mice, we sub-lethally irradiated 4-day old *Akp2*$^{-/-}$ mice. Following a 24-hour recovery period the mice were injected with a mixture of BMSCs that had been cultured under osteogenic conditions, along with freshly isolated BMSCs, these injections were performed daily for 3 weeks. Mice were sacrificed and long bones dissected and longitudinal frozen sections were examined for TNAP activity to determine whether engraftment of donor cells had occurred. Clusters of donor-derived TNAP positive cells were observed within the bone marrow cavity in some of the treated mice. While clearly we need to optimize the conditions for engraftment, these findings are encouraging in terms of the potential use of BMSCs as a means of replacing TNAP-deficient osteoblasts by wild-type osteoblasts with the goal of ameliorating the hypomineralization of infantile hypophosphatasia.

Disclosures: ***D. Harmey****, None.*

1130

Osteoporosis Pseudoglioma Syndrome: 3 Siblings with a Novel LPR5 Mutation. E. A. Streeten[1], H. Morton*[2], D. J. McBride*[1]. [1]Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD, USA, [2]Pediatric Genetics, Clinic for Special Children, Strasburg, PA, USA.

Osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder of severe juvenile osteoporosis and congenital blindness, due to mutations in the LRP5 gene. Approximately 30 cases of OPPG are known worldwide. We report 3 siblings with OPPG, members of a conservative Mennonite church in Pa. The proband (A) is an 11 9/12 yo boy who was noted to be blind at 7 weeks of age and bilateral retinal detachments were diagnosed. At age 6 yrs, he had his first fracture, of a clavicle after a mild fall. At 7 9/12 yo, he fractured his proximal R femur after a mild fall, and over the next 7 months, had atraumatic fractures of the left femur and tibia and spine at multiple levels. At age 8 9/12, DXA showed a Z score of –6.1 in the spine and metabolic evaluation was normal. He was started on oral risedronate and over the next year, his BMD improved 78% and he had no fractures. In the 2nd year on risedronate, BMD increase was smaller, total 103% in the first 2 years (Z score –7.25 to –4.27), and he had another 3 femur fractures. His treatment was then changed (8 months ago) to IV pamidronate 1 mg/kg x 3 every 4 months and he has had no fractures since then. Rods have been placed in both femurs but he has been non-ambulatory since age 7 ½ yo. The proband's brother (B), now 7 yo, had congential blindness due to retinal detachment and at age 4 9/12 yo, DXA showed a spine Z score of -5.0. He was started on oral risedronate, and after 2 years, his Z score increased to -3.7. Now aged 7 yo, he has had no fractures to date. The 3rd affected sibling (C), a girl now 12 mos old had congenital blindness due to bilateral retinal detachment and has not yet had a DXA.

A DXA study on the father was normal (spine T score -0.5, hip +0.3); the mother had mild osteopenia (spine T score -1.2, hip -0.6). There are 5 unaffected siblings to the proband.
To study the LRP5 gene in this family, primers were designed to include flanking intron sequence for each of the 23 LRP5 exons. PCR products were generated from a control sample, both parents, and an affected child using genomic DNA. Sequence analysis demonstrated a novel exon 6 allelic variant (Trp-425-X) in the affected child. The parents were heterozygous for the variant allele; the control sample was homozygous for the wild type allele.
In summary, we report 3 siblings with OPPG and a novel LRP5 mutation. The heterozygote mother has mild osteopenia and further studies on heterozygotes in this family may help to clarify whether heterozygotes in OPPG have low bone mass.

Disclosures: ***E.A. Streeten****, None.*

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Variation in Bone Brittleness Among Chromosome Substitution Mouse Strains. J. Ryan*[1], A. Hill*[2], J. Nadeau*[2], K. Jepsen[1]. [1]Orthopaedics, Mount Sinai School of Medicine, New York, NY, USA, [2]Center for Computational Genomics, Case Western Reserve University, Cleveland, OH, USA.

Genetic background is an important determinant of bone mechanical properties; these properties are complex both genetically (multigenic) and mechanically (dependent upon many traits). To understand how genes interact to create a mechanically functional bone, we examined a set of 21 B6.AJ-Chr Chromosome Substitution Strains, CSS, (19 autosomes, x, y) that were developed for complex trait analysis [1]. Each of the B6.AJ-Chr(i) strains is a homozygous inbred strain that is identical to the B6 strain except that chromosome 'i' has been replaced (intact) with the corresponding AJ chromosome. If B6.AJ-Chr(i) differs from B6 for a trait, there must be at least one QTL on chromosome 'i' related to the trait. We tested for variation in bone brittleness among the CSS panel given that femurs from AJ (brittle) and B6 (ductile or tough) differ significantly for this clinically relevant trait [2]. Femurs from 14-16 wk old male and female B6 and AJ mice (n=10-25/group) and from each of the chromosome substitution strains (n=7-16/group), except for chromosomes 5, 7, and 13 (female), were failed in 4-point bending. Post-yield deflection (PYD), the deformity before failure, was calculated as a measure of bone brittleness. The data revealed that for females, the B6.AJ-Chr12, -Chr16, -Chr18, and -ChrX strains have PYD values significantly smaller (more brittle) than B6 ($p<0.05$, ANOVA). For males, B6.AJ-Chr18 was the only strain to show a significantly reduced PYD relative to B6 ($p<0.01$, ANOVA). In contrast to females, male B6.AJ-Chr16 showed reduced PYD compared to B6, but the difference was not significant, and male B6.AJ-Chr12 and B6.AJ-ChrX showed similar PYD values compared to B6. Analysis of the CSS panel revealed that genes on certain chromosomes of the AJ background interacted with the remaining genes of the B6 background and this lead to a brittle phenotype. Importantly, variation in brittleness among the CSS panel was gender specific; our data suggest there are important genes on chromosomes 12, 16, 18, and X for females and 18 for males that contribute to bone brittleness. Variation in femoral PYD among inbred strains correlates with mineral content [3]; thus, variation in PYD among the CSS panel may indicate that certain genes within the AJ background contributed to an alteration in matrix composition and consequently material quality. Future work will quantify these matrix changes that accompany the chromosome substitutions and determine how these changes are responsible for the increased bone fragility. [1] Nadeau et al, Nat Genet 24(3), 2000. [2] Jepsen et al, JBMR 16(10), 2001. [3] Jepsen et al, Mamm Genome 14(2), 2003.

Disclosures: ***K. Jepsen****, None.*

1132

Fine Mapping *Igfsl1*, a QTL with Low BMD and Low Serum IGF-1, Using Two Different Breeding Strategies. C. L. Ackert-Bicknell[1], K. L. Shultz[1], R. Klein[2], E. Orwoll[2], E. C. Akeson*[3], W. G. Beamer[1], C. J. Rosen[4]. [1]The Jackson Laboratory, Bar Harbor, ME, USA, [2]Oregon Health Sciences University, Portland, OR, USA, [3]The Jackson Laboratory, Bangor, ME, USA, [4]Maine Center for Osteoporosis, St. Joseph Hospital, Bangor, ME, USA.

Serum IGF-1 levels are correlated with BMD, femoral cross-sectional area and fracture risk. We have previously reported that serum IGF-1 level is a polygenic trait, with Quantitative Trait Loci (QTLs) on Chr 1, 6, 10 and 15. The B6.C3H-6T (6T) congenic strain is a C57BL/6J (B6) mouse carrying a portion of the C3H/HeJ (C3H) Chr 6 (*D6Mit93* to *D6Mit150*), and has a 20% lower serum IGF-1, reduced periosteal circumference, shorter femurs and reduced trabecular bone as compared to B6. There was no difference in IGF-1 transcript levels in either the liver or long bone, as measured by RT-PCR and RNase protection assay, in 6T as compared to B6. Two congenic sublines were created in an attempt to fine map this QTL. The B6.C3H-6-1 subline, (*D6Mit93* to *D6Mit124*) does not contain this QTL, whereas the B6.C3H-6-2 (6-2) subline (*D6Mit124* to *D6Mit150*) does. To further fine map, 584 offspring from a 6T by B6 cross were generated but no recombination in the 6-2 congenic region was found, a phenomenon also seen in the original B6xC3H F2 genome wide scan. Fluorescent in situ hybridization was performed on chromosome spreads from the 6T and control strains to find an explanation for this lack of recombination. Probes were generated from BACs selected from the Roswell Park Cancer Institute B6 Female mouse BAC library. BACs were chosen based on chromosomal location in relation to the above three D6Mit markers as determined from the Ensembl published mouse genome. An inversion on Chr 6 in the B6.C3H-6T strain of mouse was discovered, explaining the lack of genetic recombination. As an alternate strategy, the serum IGF-1 levels in 23 of the B6 by DBA/2J (D2), recombinant inbred (BXD RI) strains were measured and a strong QTL for serum IGF-1 was mapped to the region of *D6Mit10* (LOD 6.0), 2.4 cM proximal to *D6Mit150*. As in the C3H cross, it was the B6 allele that conferred higher serum IGF-1 in the B6xD2 cross. Coincidentally, this same region contained a previously published QTL for femoral cross-sectional area. We are now exploring this D2 Chr 6 region for low serum IGF-1 in order to fine map *Igfsl1* and to complement previous gene expression data from B6.C3H-6T. As work towards the goal of producing a B6.D2-6 congenic progresses, it has been noted that this region will undergo genetic recombination, indicating that D2 does not carry the chromosomal inversion. The similarity in phenotype between mice D2-like or C3H-like for this region on Chr 6 suggests that *Igfsl1* is not a function of the inversion, but rather is a gene or genes with multiple alleles.

Disclosures: ***C.L. Ackert-Bicknell****, None.*

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Anti-IL-8 Therapy Inhibits Breast Cancer Growth and Osteolysis *In Vivo*. M. S. Bendre[1], R. A. Skinner*[1], R. Singh*[2], D. Gaddy[1], L. J. Suva[1]. [1]Orthopaedic Surgery, UAMS, Little Rock, AR, USA, [2]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA.

Bone is one of the most common sites for breast cancer metastasis. Skeletal metastases connote a dramatic change in the prognosis for the patient and significantly increase the morbidity associated with breast cancer. Understanding the factors contributing to the metastatic behavior of breast cancer cells to bone is essential for the development of interventions that may prevent or impede the progression of these lesions. Recently we reported that elevated levels of the chemokine interleukin 8 (IL-8) but not parathyroid hormone related protein (PTHrP) correlate with increased bone metastasis in a population of human breast cancer cells (MDA-MET; Bendre et al, 2002). When added to cultures of human peripheral blood mononuclear cells, IL-8 stimulates osteoclastogenesis and bone resorption by both RANKL dependent and independent mechanisms. This process is presumably associated with the expression of the IL-8 specific receptor CXCR1 by human osteoclasts and their precursors (Bendre et al., 2003). Thus, we hypothesized that IL-8 may be responsible for the osteolysis induced by breast cancer cells colonizing bone. To test this hypothesis in vivo, MDA-MET cells were injected in the tibia of nude mice. The mice were treated with either a monoclonal antibody directed against IL-8 (35ug), or a control IgG (35ug), or no treatment every alternate day for 28 days following tumor cell inoculation. Mice were sacrificed and tibiae evaluated radiologically and histologically. All injected mice receiving no treatment (7/7) or treated with control IgG (6/6) developed large osteolytic bone tumors. In the IL-8 antibody treated group 44% did not show any tumor (3/7). Small tumor foci with no demonstrable osteolysis were observed in 2 mice (28 %) and 2 /7 (28%) of mice developed osteolytic tumor. The total area of osteolytic lesions and tumor burden was significantly lower in mice treated with IL-8 antibody compared with mice receiving control IgG or no treatment. These data suggest that IL-8 may have an important pathogenic role in the development and growth of osteolytic bone lesions. The efficacy of anti IL-8 antibody provides a strong rationale for the utility of anti-IL-8 therapy in the inhibition of tumor growth in bone, and suggests a novel therapeutic opportunity for the treatment of bone metastasis.

Disclosures: ***M.S. Bendre****, None.*

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Increased Survival and Decreased Metastasis Formation after Ten Years in Patients with PTHrP-positive Breast Cancer; a Prospective Study in 526 Patients. M. A. Henderson*[1], J. A. Danks[2], J. Slavin*[3], T. Harris*[2], J. L. Hopper*[4], T. J. Martin[2]. [1]Surgical Oncology, Peter MacCallum Cancer Institute, Melbourne, Australia, [2]St Vincent's Institute of Medical Research, Melbourne, Australia, [3]Pathology, St Vincent's Hospital, Melbourne, Australia, [4]Public Health and Community Medicine, University of Melbourne, Melbourne, Australia.

The aim was to determine in a prospective clinical study whether PTHrP production by primary breast cancers is predictive of skeletal metastases. In a prospective study of 526 consecutive patients with operable breast cancer, the significance of positive PTHrP staining by immunohistology has been evaluated with respect to other prognostic factors, and to the pattern of metastatic disease and overall survival. 79% of tumors stained positively for PTHrP. Ten year survival of patients with PTHrP positive tumors (median follow-up 7.5 years) was 74% (95% CI 68-80%), and for those with PTHrP negative tumors was 47% (95% CI 35-56%) ($p<0.0001$). Patients with PTHrP negative primary tumors were more likely to develop metastases at all sites, including bone ($p=0.008$), lung ($p=0.002$), liver ($p=0.016$) and soft tissue ($p=0.002$). Multivariate analysis using a Cox Proportional Hazards Model identified the following as independently significant prognostic variables: tumor size (RR 1.01, $p<0.0001$), node status (RR 1.1, $p<0.0001$), PTHrP status (RR 0.43, $p<0.0001$), PR (RR 0.6, $p = 0.02$) and vascular invasion (RR 1.74, $p = 0.006$). PTHrP status was associated with ER ($p = 0.001$), PR ($p = 0.03$) and menopausal status ($p = 0.01$); not significantly associated were tumor size ($p = 0.06$), vascular invasion, tumor grade and patient age. Other clinical studies that relate PTHrP expression by the primary tumor to development of bone metastases are characterized by any or all of small numbers, case selection, limited follow-up and retrospective accrual. The present study is the only long-term, prospective study of consecutive patients which investigated primary tumor PTHrP status and skeletal complications and survival. The conclusion is that increased production of the multifunctional protein, PTHrP, by breast cancers confers upon them a less invasive phenotype. Possible mechanisms for this are being actively explored. The likely contribution of PTHrP to establishment of bone metastases represents a specific, separate mechanism of PTHrP action, by which cancer cells in bone marrow are able to promote osteoclast formation and activity, thereby providing the specific property necessary to complement the general invasive properties of the cancer cells.

Disclosures: ***T.J. Martin****, None.*

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Wnt/beta-catenin Signaling Antagonist DKK1 Is Secreted by Plasma Cells and Contributes to Osteoblast Dysfunction and Osteolytic Lesions in Multiple Myeloma. J. D. Shaughnessy*[1], F. Zhan*[1], E. Tian*[1], R. Walker*[1], E. Rasmussen*[2], B. Barlogie[1]. [1]Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Cancer Research and Biostatistics, Seattle, WA, USA.

Background: Multiple myeloma (MM) is a plasma cell (PC) malignancy that grows in the bone where it causes osteolytic lesions, which lead to intractable bone pain, life-threatening hypercalcemia, and a risk for pathological fractures. Lytic lesions are only found adjacent to medullary plasmacytoma, suggesting that PC secrete factors that directly affect osteoclast and/or osteoblast biology and function.
Methods: Oligonucleotide microarray profiling and biochemical analyses were used to identify molecular determinants of osteolytic lesions in 174 cases of newly diagnosed MM.
Results: Logistic regression and permutation analysis of global gene expression patterns in patients with no MRI-defined focal lesions (n = 36) and those exhibiting >= 1 focal lesions (n = 137) identified 58 genes distinguishing the two groups. The soluble Wnt/b-catenin signaling antagonist, dickkopf-1 (DKK1), represented the only gene coding for a secreted factor in 28 genes significantly (P < .0001) over-expressed in PC from patients with focal lesions. Immunohistochemistry and immunofluoresence microscopy showed DKK1 expression to be unique to PC in both MM and normal bone marrow. Elevated DKK1 protein in MM bone marrow plasma was strongly correlated with both gene expression patterns and MRI-focal lesions. Incubation of osteoblast precursor cells with bone marrow plasma from patients with elevated serum DKK1 could inhibit BMP-2 induced alkaline phosphatase production in a DKK1-dependent fashion.
Conclusions: Given the critical role of Wnt/b-catenin signaling in osteoblast development and function, data presented here suggests that malignant PC contribute to the lytic bone destruction process in MM, in part, through the secretion of the Wnt signaling antagonist DKK1. These data also provide support for the notion that DKK1 represents a potential target for the prevention or treatment of malignancy-related osteolysis, as well as osteoporosis.

Disclosures: ***J.D. Shaughnessy****, None.*

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Antibody to CXCR4 Blocks Prostate Cancer Metastasis to Osseous Sites *In Vivo*. N. Osman*, Y. X. Sun*, A. J. Koh-Paige*, A. Schneider*, K. Cook*, J. Wang*, L. K. McCauley, R. S. Taichman. Periodontics/Prevention/Geriatrics, University of Michigan Dental School, Ann Arbor, MI, USA.

Previously we determined that the SDF-1/CXCR4 chemokine axis is activated in prostate cancer (CaP) metastasis to bone. Moreover, SDF-1 triggers the adhesion of CaPs to marrow endothelial cells and is related to increasing tumor grade.
To delineate the role of SDF-1/ CXCR4 receptor in CaP disease, two experiments were performed. First, to establish a positive correlation between SDF-1 expression and tumor metastasis, SDF-1 tissue levels were characterized for a variety of murine tissues by ELISA. Expression of SDF-1 was highest in the pelvis, tibia, femur, liver, adrenal/kidneys and lungs of those tissues examined with concentrations of SDF-1 ranging from 5-25 µg/mg total protein.
Second, an *in vivo* metastasis model was examined with two experimental groups. PC-3^{luc+} cells ($2x10^5$) were either pre-incubated with neutralizing antibody to CXCR4 (R&D Systems (MBA173)) at 10 µg/animal, or an isotype matched control. Bone metastases were established by administering the cells into the left cardiac ventricle in HSD:athymic nu/nu mice. Equal doses of the antibodies were administered I.P. at 2 and 24 h post inoculation. At 4 weeks, the mice were injected with luciferin. Bioluminescence generated by the luciferin/luciferase reaction served as a locator for cancer growth and was used for quantification using a Xenogen IVIS system and LivingImage™ software. Signal intensity was quantified as the sum of all detected photons within the region of interest during a 1 min. luminescent integration time. The animals were subsequently radiograph and sacrificed. Bones were evaluated by histomorphometric analysis to confirm cancer involvement.
Antibody to CXCR4 significantly reduced the total metastatic load compared to IgG control. Examination of individual sites of bone metastasis also demonstrated that antibody to CXCR4 significantly reduced the luminescent signal further demonstrating that the SDF-1/CXCR4 in part regulates osseous metastasis including femur/tibia regions, spine and maxilla/mandible areas (not presented). Preliminary histology and radiographic analyses confirm these findings. These *in vivo* metastasis data directly provide critical proof of principle to support a role for SDF-1/CXCR4 in osseous metastasis.

	Photons Per Area		
Tx	Whole Animal	Femur/Tibia	Spine
IgG	$1.8 \pm 0.2 \times 10^8$	$6.0 \pm 5.7 \times 10^7$	$1.0 \pm 0.2 \times 10^7$
Anti-CXCR4	$5.0 \pm 5.0 \times 10^{6}$*	$5.9 \pm 4.0 \times 10^{6}$*	$1.2 \pm 0.4 \times 10^{5}$*

*Sig.diff.from IgG Tx ($p < 0.05$)

Disclosures: ***R.S. Taichman****, None.*

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Hypogonadism Causes Bone Loss and Increased Bone Metastasesin a Model of Mixed Osteolytic/Osteoblastic Metastases: Prevention by Zoledronic Acid. S. S. Padalecki, M. R. Carreon*, B. G. Grubbs*, T. A. Guise. University of Texas Health Science Center, San Antonio, TX, USA.

There is accumulating evidence that bone resorption is an important component of osteoblastic metastases. The precise role of this bone resorption, whether it is essential in the pathophysiology of the osteoblastic response and whether inhibiting it would block the development of osteoblastic metastases, is of practical clinical importance. Bone is a repository for growth factors, which are released as a consequence of osteoclastic bone resorption. In men with advanced prostate cancer, gonadal ablation increases systemic bone resorption, thereby releasing these growth factors. Therefore, gonadal ablation may also make bones of a more fertile environment for prostate cancer metastasis.
To test the hypothesis that increased osteoclastic bone resorption due to hypogonadism causes a more fertile environment for mixed osteolytic/osteoblastic bone metastases, we developed a mouse model which mimics the clinical situation of men rendered hypogonadal as a result of treatment for prostate cancer. Surgical castration of male nude mice resulted in reduced trabecular bone volume and increased osteoclast numbers over a 4-week period compared with eugonadal controls. To investigate the role of hypogonadism in the development of mixed osteolytic/osteoblastic bone metastases, male nude mice underwent orchiectomy or sham surgery 4 weeks prior to intracardiac inoculation with TSU-PR1 bladder cancer cells that cause mixed metastases. Mice were treated daily with zoledronic acid or vehicle from the time of surgery. Radiographs, bone mineral density and bone histomorphometry were used to assess bone mass and tumor osteolysis. Hypogonadism resulted in lower bone mineral density compared with sham-controls. This was prevented by zoledronic acid. TSU-PR1 bone metastases were increased and developed faster in hypogonadal mice treated with vehicle compared to sham-controls. Zoledronic acid decreased bone metastases in hypogonadal TSU-PR1-inoculated mice but had no effect on soft tissue metastases or survival.
This work provides in vivo evidence that hypogonadism causes bone loss and may increase metastases to the skeleton that are mixed in nature. Treatment with zoledronic acid not only prevented bone loss due to androgen deprivation but also reduced bone metastases in this model. These data support the hypothesis that increased bone resorption due to androgen deprivation may result in a more fertile environment for the development of bone metastases. Bone resorption inhibitors, such as bisphosphonates, may benefit hypogonadal men with advanced prostate cancer to prevent bone loss as well as skeletal metastases.

Disclosures: ***S.S. Padalecki****, None.*

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Osteoclasts Enhance Angiogenesis in Concert with Myeloma Cells through Osteopontin and VEGF. M. Abe, T. Hashimoto*, T. Oshima*, H. Shibata*, E. Sekimoto*, Y. Tanaka*, T. Hara*, K. Kitazoe*, S. Ozaki*, S. Kido*, D. Inoue, T. Matsumoto. Department of Medicine and Bioregulatory Sciences, University of Tokushima Graduate School of Medicine, Tokushima, Japan.

Multiple myeloma (MM) develops in the bone marrow, and causes devastating bone destruction by enhancing osteoclastic bone resorption. We have reported that osteoclasts (OCs) induced by MM cells in turn enhance survival and growth of MM cells. Similarly, angiogenesis has been demonstrated to be enhanced in the bone marrow in patients with MM, which has drawn considerable attention as a potential therapeutic target. Both angiogenesis and osteolysis develop in the MM bone marrow in parallel with the tumor expansion, suggesting a link between OC and vascular cell activities. In addition, we previously demonstrated that OCs constitutively secrete high levels of an angiogenic noncollagenous matrix protein, osteopontin (OPN), and that MM cells secrete VEGF, a potent angiogenic factor. We therefore investigated a role of OCs in enhancement of angiogenesis in MM with a particular focus on OPN and VEGF.
Vascular tubule formation was assayed using Angiogenesis Kit (KZ-1000) obtained from KURABO (Osaka, Japan). Conditioned media (CM) from either peripheral blood mononuclear cell-derived OCs or MM cell lines (ARH77, U266 and RPMI8226) at 10 % enhanced vascular tubule formation equipotently 1.5-fold. Interestingly, CM from co-cultures of both cells further enhanced it 2.5-fold, suggesting a role of the interaction between OCs and MM cells in angiogenesis. Although no MM cell lines secreted detectable OPN, MM cell contact enhanced OPN production by OCs beyond the already high basal levels (about 10 vs. 7 microg / 10E5 cells for 3 days). On the other hand, all MM cell lines secreted VEGF whose levels were not changed when co-cultured with OCs. Since VEGF has been demonstrated to induce an OPN receptor, alphaVbeta3 integrin, on endothelial cell surface and to enhance OPN-induced endothelial cell migration, we next examined the effects of OPN and VEGF on angiogenesis. OPN (1 microg/ml) or VEGF (10 ng/ml) alone enhanced vascular tubule formation 1.3- and 1.5-fold, respectively, and both in combination further enhanced it 2-fold, suggesting cooperative action of both factors on angiogenesis. Consistently, antibodies against alphaVbeta3 integrin and VEGF each alone partially and both in combination completely abrogated tubular formation enhanced by CM from the co-cultures of OCs and MM cells.
In conclusion, OCs enhance angiogenesis in concert with MM cells, which is largely mediated by OPN and VEGF derived from OCs and MM cells, respectively. Therefore, impairment of OCs may inhibit MM progression not only directly but also through suppression of angiogenesis.

Disclosures: ***M. Abe****, None.*

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Calcitonin/Calcitonin Gene-related Peptide Protect the Maternal Skeleton from Excessive Resorption During Lactation. J. P. Woodrow*[1], C. S. Noseworthy*[1], N. J. Fudge*[1], A. O. Hoff*[2], R. F. Gagel[2], C. S. Kovacs[1]. [1]Memorial University of Newfoundland, St. John's, NF, Canada, [2]MD Anderson Cancer Center, Houston, TX, USA.

Surgical models of calcitonin deficiency had suggested that lack of calcitonin lowered bone mass after a cycle of pregnancy and lactation, leading to the hypothesis that calcitonin protects the maternal skeleton from excessive resorption during pregnancy and lactation. That postulated role has never been fully accepted because of inconsistent results, confounding effects of thyroid ablation, and recognition that extrathyroidal sources of calcito-

nin were not obliterated.
We utilized the CT/CGRP gene ablation model (*Ct*-null mice) to re-explore the possible role of calcitonin (and CGRP) in regulating maternal bone metabolism during pregnancy and lactation.
Ct-het mice were back-crossed into outbred *Black Swiss* strain. *Ct*-het males and females were mated to generate wt, *Ct*-het and *Ct*-null females. These mice were studied after 10 weeks of age and were mated to *Ct*-het males. Serial daily measurements of bone mineral content (BMC) and density were made during pre-pregnancy, pregnancy, lactation, and after weaning (approx 60 day cycles). A PIXIMUS densitometer (DXA scaled for mice) assessed total and regional BMC and density. A baseline BMC was determined for each mouse from her pre-pregnancy measurements, to which all subsequent readings were normalized (expressed as % of baseline). Ionized calcium was measured during pre-pregnancy, pregnancy, and lactation.
Baseline BMC trended higher in *Ct*-null (0.360 ± 0.01 g vs 0.333 ± 0.02 g in *Ct*-het and 0.301 ± 0.03 g in wt, p=NS). BMC increased in pregnancy to a significantly lower peak in *Ct*-null (112.1 ± 1.6 % vs. 120.4 ± 2.1 % in *Ct*-het and 132.7 ± 3.7 % in wt, p< 0.01). During lactation, BMC plummeted to 45.7 ± 3.3 % of baseline in *Ct*-null versus 94.4 ± 0.4 % of baseline in *Ct*-het and wt (p< 0.02). Within 2 weeks of weaning, BMC returned to baseline in *Ct*-null, *Ct*-het and wt. Maternal ionized calcium did not change between pre-pregnancy to late pregnancy. Ionized calcium rose significantly post-delivery in *Ct*-null to 1.42 ± 0.02 mmol/l but not in *Ct*-het or wt, and returned to normal (1.29 ± 0.02 mmol/l) by one week post-delivery.
In summary, *Ct*-null mice gained less BMC during pregnancy and plummeted to 45% of baseline BMC during lactation, a net excursion (from peak to trough) of 67% of BMC. This excess bone resorption was accompanied by maternal hypercalcemia. BMC returned to baseline after weaning in *Ct*-null as promptly as in wt and *Ct*-het.
We conclude that calcitonin/CGRP prevent severe drops in maternal BMC and increased skeletal fragility during lactation, but are not required in the long-term because the maternal skeleton recovers to baseline.

Disclosures: ***J.P. Woodrow****, None.*

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Inhibition of Vascular Calcification In LDLR -/- Mice by Treatment With Human PTH(1-34). J. S. Shao, N. Charlton-Kachigian*, Y. Gao, S. L. Cheng, D. A. Towler. Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA.

Vascular calcification is a common response to dysmetabolic stimuli such as diabetes and hypercholesterolemia. We identified that high fat diets induce insulin - resistant diabetes, hyperlipidemia, and aortic calcification in LDL receptor knockout (LDLR -/-) mice. Diabetes and aortic calcification are more pronounced in male animals, with vascular calcification dependent upon BMP2 - Msx2 signaling that promotes osteogenic differentiation of aortic myofibroblasts. We examined the regulation of vascular calcification and aortic osteogenesis by human PTH(1-34), a prototypic osteoanabolic hormone, in LDLR -/- mice fed diabetogenic diets. Methods: Six week old male LDLR -/- mice (n= 5 / arm) were fed mouse chow or high fat diet (42% calories from fat; 0.15% cholesterol by weight) for 4 weeks. Mice were treated s.c. with vehicle, or 0.4 mcg / kg of PTH(1-34) dosed 5 days / week. At sacrifice, bone mineral content (BMC) was determined by DXA. Osteogenic gene expression was quantified in RNA from single aortas and hind limbs by fluorescence RT-PCR. Vascular calcification was determined by Alizarin red (AR) staining of cardiac valve sections, using image analysis to quantify leaflet mineralization. Circulating regulators of inflammation and mineral deposition (osteopontin/OPN; osteoprotegerin/OPG; leptin) were measured by ELISA. Results: Serum glucose and lipid levels increased significantly in response fatty diets. These diets upregulated aortic expression of BMP2, Msx2, and OPN. As in humans, diabetes upregulated circulating OPG and leptin levels ca. 4-fold; no change in circulating OPN was noted. PTH(1-34) increased BMC in diabetic LDLR-/- mice, with concomitant induction of osseous Msx2, osseous OPN, and serum OPN; no change in lipids, glucose, OPG, or leptin was noted. By contrast, PTH(1-34) treatment suppressed aortic OPN and Msx2 mRNA accumulation > 50%, indicating reciprocal regulation of orthotopic vs. heterotopic osteogenic programs. Valve calcification mirrored vascular osteogenic gene expression responses. As compared to vehicle, PTH(1-34) decreased cardiac valve calcification ca. 80% (8.3 +/- 1.5 % vs. 1.5 +/- 0.5 % valve area calcified; p < 0.001). Analyses of spleen cells showed that PTH(1-34) decreased CD68+ OPN+ monocytes by 50% (p = 0.08), suggesting that anti-inflammatory actions may contribute to vascular PTH responses. Summary: PTH(1-34) inhibits valve calcification and aortic osteogenic differentiation during the initiation of diabetic vascular calcification. Intermittant PTH(1-34) may exert beneficial actions on vascular calcium homeostasis at the early stages of macrovascular responses to diabetes.

Disclosures: ***D.A. Towler****, None.*

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PTH Receptor Internalization by Inactive PTH Fragments. Regulation by NHERF1 (EBP50). W. Sneddon[1], C. Syme*[1], A. Bisello[1], C. E. Magyar*[1], E. J. Weinman*[2], P. A. Friedman[1]. [1]Universities of Pittsburgh, Pittsburgh, PA, USA, [2]and Maryland, Baltimore, MD, USA.

PTH effects on kidney and bone are mediated by the Type 1 PTH receptor (PTH1R). PTH1R is internalized in a cell- and ligand-specific manner. In kidney distal tubule (DCT) cells and some bone cell lines (e.g. ROS17/2.8), both the agonist PTH(1-34) and the antagonist PTH(7-34) induce PTH1R internalization. In contrast, in renal proximal tubule (PCT) and SaOS2 cells, only PTH(1-34) internalizes the PTH1R. Mahon and Segre reported (Nature '02) that Na/H exchanger regulatory factors (NHERFs) bind to the PTH1R through a C-terminal PDZ recognition domain and have a role in signaling. NHERF1 is a cytoplasmic adapter protein that has been implicated in both protein targeting and the assembly of protein complexes. NHERF1 possesses tandem PDZ domains and an ERM domain that binds to the actin-associated proteins, ezrin, radixin, and moesin. NHERF1 is expressed by proximal tubules and by SaOS2 cells, but not by distal tubules or ROS17/2.8 cells. We mutated the PTH1R PDZ domain from ETVM to ETVA to determine the role of NHERF1 on PTH1R internalization. In cells lacking NHERF1, the ETVA/PTH1R internalized after challenge with PTH(1-34) or PTH(7-34). However, in cells expressing NHERF, PDZ mutation abolished the ability of NHERF1 to interact with the PTH1R. PTH(7-34) now efficiently internalized the PTH1R/ETVA receptor. We hypothesized that NHERF1 mediates the cell- and ligand-specific pattern of PTH1R internalization. Heterologous expression of full-length NHERF1 in DCT or ROS17/2.8 cells inhibited PTH(7-34)-stimulated PTH1R internalization without affecting PTH(1-34)-induced endocytosis. To determine if the ERM domain is involved in ligand-induced PTH1R internalization, we expressed ERM-deficient NHERF1 (NHERFdERM) in DCT cells. NHERFdERM did not inhibit PTH(7-34)-induced receptor endocytosis suggesting that the ERM domain is required for tethering the PTH1R. Because the ERM domain binds actin-associated proteins, we theorized that disrupting the actin cytoskeleton would release the PTH1R from NHERF and permit PTH(7–34) to internalize the receptor. As predicted, cytochalasin D, PTH(7–34) promoted receptor internalization in cells expressing NHERF1. Cytochalasin did not affect PTH(1-34)-induced endocytosis or EVTA/PTH1R sequestration. We conclude that NHERF1 acts as a molecular switch for the conditional efficacy of PTH fragments. Cells expressing NHERF do not internalize the PTH1R in response to PTH(7-34), whereas the PTH1R is internalized in cells lacking NHERF. The magnitude and pattern of NHERF expression may have important implications for understanding PTH1R function in kidney and bone in states of calcium wasting and aging.

Disclosures: ***W. Sneddon****, None.*

1142

Parathyroid Hormone-Induced Recruitment of Osteoblasts from Bone Marrow Fibroblasts. R. T. Turner, S. Lotinun. Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

Pulsatile administration of parathyroid hormone (PTH) to rats results in a rapid increase in cancellous bone formation, largely due to modulation of existing bone lining cells to express the osteoblast phenotype. In contrast, continuous infusion of PTH results in hypercalcemia, focal bone resorption and severe peritrabecular marrow fibrosis (parathyroid bone disease). The purpose of the present study was to determine the origin of the PTH-induced fibroblasts as well as their fate following termination of PTH treatment. Studies were performed in which PTH was continuously infused into adult female rats for 1 w (40 µg/kg/d) using sc implanted osmotic pumps. The animals were co-infused with 3H-thymidine (2 mCi/rat) to label cells progressing through the cell cycle. Groups of PTH-treated and control rats were sacrificed 0 d and 7 d following termination of PTH treatment. In spite of a dramatic increase in osteoblast (OB) number and cancellous bone formation in PTH-treated rats, negligible numbers of OB and osteocytes (OCy) were labeled at d 0 in either treated or control animals. This result indicates that continuous as well as pulsatile PTH induces modulation of bone lining cells to osteoblasts. Peritrabecular fibroblasts were not present in the controls. In the PTH-treated rats, 85% of the fibroblasts, which formed multiple cell layers and lined 70% of the cancellous bone surface, were labeled with thymidine, indicating proliferation. Gene array analysis revealed significant PTH-induced increases in mRNA levels for genes associated with fibroblast differentiation and migration (e.g., PDGF-A, lysyloxidase, collagen XII, frizzled and phosphodiesterase 1). Fibroblasts were absent on bone surfaces in the PTH-treated rats on d 7, and 85% of the OB on and 73% of the OCy within active remodeling sites were labeled, numbers dramatically greater than in the controls (15% and 4%, respectively, p<.01). These data provide evidence that fibroblasts recruited to bone surfaces in response to continuous PTH are capable of differentiating to osteoblasts. To further test this possibility we performed an experiment in which PTH was infused for 1 w and bone histomorphometry and mechanical properties evaluated after 4 w. At the end of the study, the PTH-treated animals had normal serum Ca and PTH, no evidence of parathyroid bone disease, increased bone volume and increased strength. These findings demonstrate that continuous PTH can recruit osteoblasts by at least two mechanisms; 1) modulation of lining cells; and 2) recruitment of marrow fibroblasts to bone surfaces where they undergo proliferation and subsequent differentiation to osteoblasts. The latter mechanism represents a novel target for development of new therapies to treat osteoporosis.

Disclosures: ***R.T. Turner****, None.*

1143

Targeted Overexpression of G protein-coupled Receptor Kinase 2 in Osteoblasts Promotes Bone Loss. L. Wang*, L. D. Quarles, R. F. Spurney. Department of Medicine, Duke University Medical Center, Durham, NC, USA.

G-protein coupled receptors (GPCR's) play a key role in bone remodeling. The osseous effects of GPCR systems may be regulated by the rate of receptor desensitization in osteoblasts. GPCR desensitization is largely mediated by direct phosphorylation of GPCR proteins by a family of enzymes termed GRK's. To investigate the role of GRK's in regulating bone formation in vivo, we overexpressed the potent GPCR regulator GRK2 in osteoblasts using the osteocalcin gene 2 (OG2) promoter to target expression to mature osteoblasts. Four founder lines were established that expressed enhanced levels of GRK2 mRNA in calvaria. Overexpression of GRK2 in osteoblasts resulted in a significant reduction in bone mineral density (BMD) in transgenic (TG) mice (40.1 ± 0.6 mg/cm2) compared to non-TG littermate controls (42.7 ± 0.4 mg/cm2; P<0.005). The decrease in BMD affected predominantly trabecular bone and was most pronounced in female mice. To investigate the mechanism of GRK-dependent bone loss, we assessed parathyroid hormone (PTH)-induced cAMP generation by the PTH/PTH-related peptide (PTHrP) receptor in mouse calvaria ex-vivo. Calvaria from GRK2 TG mice had an attenuated response to PTH-induced cAMP generation. This reduction in GPCR responsiveness was associated with an increased osteoprotegrin (OPG) to OPG ligand (OPGL) mRNA ratio in calvaria consistent with

reduced osteoblast coupling to osteoclasts. In support of the lack an osteoclastic effect, urinary deoxypyridinoline (DPD) excretion was similar in TG mice (20 ± 1.4 nM DPD/mM creatinine) and control animals (22 ± 3.6 nM DPD/mM creatinine; P=NS). Taken together, these data suggest that enhancing GRK activity in mature osteoblasts diminishes the anabolic effects of GPCR systems leading to bone loss.

Disclosures: ***L. Wang****, None.*

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PTH/PTHrP Receptor Delays Chondrocyte Hypertrophy via both Runx2-dependent and Independent Pathways. J. Guo, U. Chung, A. Szczepanik*, D. Yang*, F. R. Bringhurst, H. M. Kronenberg. Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

Targeted disruption of either the PTHrP or PTH/PTHrP receptor (PTHR) genes in vivo leads to acceleration of chondrocyte differentiation, while chondrocyte hypertrophy is delayed or severely disturbed in Runx2 deficient mice. Because the effect of the Runx2 knockout on chondrocyte differentiation is opposite to that of the PTHR or PTHrP knockout, we considered the possibility that the delayed differentiation induced by PTHR activation might be mediated in part by downregulation of Runx2 expression. To examine this possibility, cultured embryonic tibia and femur were acutely treated with PTH, and Runx2 expression then was analyzed by in situ hybridization. Treatment of cultured rudiments with rat PTH(1-34) at a dose of 100 nM dramatically suppresses Runx2 mRNA levels in hypertrophic chondrocytes; Runx2 mRNA in adjacent bone collar was unchanged. The maximal effect occurred at 4-8 hr of treatment. Similar repression of (mutated) Runx2 mRNA by PTH was observed in Runx2-deficient tibia (in which inactive but detectable mRNA is found), indicating that Runx2 action is not needed for the regulation of Runx2 mRNA by PTH. Runx2 protein levels in wild type tibia, measured by Western blot, were not affected by 6-hr treatment with PTH. PTH treatment exhibited no effect on Runx2 expression in the presence of Actinomycin D, an inhibitor of RNA synthesis; addition of Actinomycin D alone led to a rapid fall in Runx2 mRNA levels. These data suggest that the effect of PTH on Runx2 expression was mainly due to altered transcription. Moreover, the same effect of PTH on Runx2 mRNA levels was also found in PLC-defective PTHR mutant rudiments.

To explore Runx2-independent effects of PTHrP on chondrocyte hypertrophy we examined E17.5 femurs from littermates with or without PTHrP and/or Runx2 gene deficiency. Vascular invasion and many chondrocytes that express collagen type X and osteopontin mRNA were observed in the double knockout femurs, whereas no vascular invasion and no cells expressing either collagen type X or osteopontin were found in Runx2-deficient femurs.

Our data indicate that Runx2 promotes but is not required for chondrocyte hypertrophy and that the delayed hypertrophy induced by PLC-independent PTHR activation may be mediated by both Runx2-dependent and independent mechanisms.

Disclosures: ***J. Guo****, None.*

1145

Biglycan Mediates the WNT Signaling Pathway in Osteoblastic Cells. S. Wadhwa, X. D. Chen, M. F. Young. Craniofacial and Skeletal Diseases Branch, NIDCR, Bethesda, MD, USA.

Biglycan (bgn) is a member of the small leucine-rich proteoglycan family and is abundantly expressed in the extracellular matrix of bone. Previous work has indicated that mice who are deficient in bgn have low bone mass. However, the mechanism for this effect is relatively unknown. We suspect that changes in bone's extracellular matrix leads to altered cell signaling. It was recently found that bgn directly binds to a wnt induced soluble protein, wisp-1, suggesting that there exists a relationship between bgn and the wnt signaling pathway. In addition, studies have shown that defects in the wnt signaling pathway leads to decrease bone mass. In this study, we wanted to test the hypothesis that bgn is directly involved in the regulation of the wnt signaling pathway. In order to determine which components of the wnt signaling pathway might be affected we examined changes in mRNA profiles in osteoblastic cultures obtained from the calvaria of newborn wild type and bgn deficient mice. Using a microarray database we had previously designed, comparing bgn deficient and wildtype osteoblastic cells, we discovered that wnt-1, wnt-3a and wisp-1 mRNA levels were lower in bgn deficient osteoblasts compared to osteoblasts from wild type mice. These results were confirmed in three independent experiments using semi quantitative RT-PCR. In contrast, also by three independent experiments, we found by western blot analysis that beta catenin, a central player in the wnt signaling pathway, was upregulated in osteoblasts from bgn deficient mice. To test whether bgn effects were direct, we constructed an adenovirus encoding the full-length murine bgn gene. The adenovirus was then used to isolate native recombinant protein, which was added back to bgn deficient osteoblastic cultures. The addition of bgn (2ug/ml) for 24 h caused wnt-1, wisp-1 and beta catenin levels to approach the levels of wild type osteoblastic cultures. Interestingly, in a gain of function approach, the addition of recombinant bgn (2ug/ml) for 24 h to MC3T3-E1 cells caused a significant reduction in beta catenin levels. Taken together these results suggest that the wnt signaling pathway maybe modulated by proteoglycans in the extracellular matrix and that biglycan and wnt may act cooperatively to regulate bone mass.

Disclosures: ***S. Wadhwa****, None.*

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The Heritage of Cells within Postnatal Tendon and Bone Formation. D. L. Glaser*[1], R. Ramachandran*[2], X. Jiao*[1], T. W. Lin*[1], L. J. Soslowsky*[1], E. M. Shore[1], F. S. Kaplan[1], D. J. Goldhamer*[3]. [1]Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, USA, [2]University of Pennsylvania, Philadelphia, PA, USA, [3]Molecular & Cell Biology, University of Connecticut, Storrs, CT, USA.

A fundamental question in the cellular pathogenesis of postnatal tissue repair and cell differention is the identity of progenitor cells responsible for new tissue. In fibrodysplasia ossificans progressiva (FOP), heterotopic ossification affects both tendon and muscle. While several stem cell populations could contribute to this process, the lineages of these cells remain undefined. This study examined the identity of progenitor cells responsible for post-natal tendon repair and ectopic bone formation.

To generate animal models with tagged cells of specific lineages, transgenic mice that express Cre recombinase under control of a tissue-specific promoter/enhancer were mated to Rosa26-LacZ reporter mice, resulting in cell-specific LacZ expression. Once activated, LacZ expression remains constitutive for the life of the cell and its descendants, regardless of phenotypic changes. Transgenic mouse lines with the following promoters (and cell specific expression) were generated: MyoD (satellite cells); Smooth Muscle Myosin Heavy Chain (smooth muscle cells, pericytes); and Tie2 (endothelial cells, hematopoietic stem cells, side population cells). To determine the role of bone marrow-derived cells, chimeric mice were produced by transplanting bone marrow (BMT) from constitutive Rosa26-LacZ mice into irradiated C57BL/6 hosts. The fates of the LacZ tagged cells were followed during BMP induced ectopic ossification and in a patellar tendon injury model. Animals were examined histologically at various intervals from 4 days to 12 weeks. Specimens were stained for beta-galactosidase activity to identify participation of specific cell lineages.

In MyoD-cre/R26R mice, labeled cells were consistently found in cartilage and bone but not in tendon. In SMMHC-cre/R26R mice, labeled cells were found in healing tendon but not in heterotopic ossification. In Tie2-cre/R26RA, labeled cells were abundant in fibroproliferative tissue that proceeds both bone and tendon formation. In BMT animals, labeled inflammatory cells were observed early in both models, however, fibroproliferative, cartilage, bone and tendon cells were not labeled; the bone marrow elements within ectopic bone ossicles were labeled.

Taken together, these lineage tracing studies provide the first definitive evidence of the heritage of cells in postnatal tendon repair and ectopic ossification and provide direct cellular targets for therapeutic approaches that regulate the regenerative response in these tissues.

Disclosures: ***D.L. Glaser****, None.*

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Expression of mCSF-1 in op/op Mice Leads to Amelogenesis Imperfecta Phenotype. S. L. Abboud[1], K. Woodruff*[1], J. Dickson*[2], N. Ghosh-Choudhury[1], J. Schulze*[2], L. Cardenas*[2], M. MacDougall*[2]. [1]Pathology, University of Texas, San Antonio, TX, USA, [2]Pediatric Dentistry, University of Texas, San Antonio, TX, USA.

CSF-1-mediated osteoclastogenesis is critical for tooth eruption. In op/op mice, absence of soluble (s) and membrane-bound (m) forms of CSF-1 decreases osteoclasts and leads to osteopetrosis and failure of tooth eruption. Using a transgenic approach, we showed that human (h) mCSF-1, expressed in bone of op/op mice (op/opT) using the osteocalcin promoter, caused significant regression of osteopetrosis and restored tooth eruption. However, the biologic effect of each isoform on tooth formation is unknown. To determine the role of CSF-1 in tooth development, teeth from 3 wk op/op, op/opT and wt mice were compared for enamel/dentin integrity and matrix protein expression. The osteocalcin promoter targets odontoblasts and high hCSF-1 protein was detected in op/opT teeth. At 3 wks, op/opT showed an amelogenesis imperfecta (AI) phenotype characterized by malaligned, chalky white incisors, with twisted tips prone to breakage. By scanning EM (SEM), morphology of op/opT molars was similar to wt. However, SEM of fractured molars in op/op mice showed abnormal enamel and dentin. The enamel was thin, lacked normal parallel crystals and fractured on a different plane than dentin, indicating a faulty dentin/enamel junction. In contrast, op/opT molars showed normal dentin and thicker more organized enamel that fractured in the same plane as dentin similar to wt. Although op/opT enamel was improved, it was thinner and less organized than wt enamel. To determine if the AI phenotype was associated with altered dental matrix proteins, their expression profile was assessed using real time PCR. Compared to wt, op/op mice showed a decrease in dentin-enriched proteins, DSPP and Dmp-1 and an increase in enamel-enriched proteins, enamelin, ameloblastin and EMSP-1. Expression of mCSF-1 in op/opT increased DSPP and Dmp-1 close to wt levels. Expression of enamelin was almost normalized, whereas expression of ameloblastin and EMSP-1 remained similar to that in op/op mice. Regulation of enamelin in op/opT likely occurred via interaction of mCSF-1 on the surface of odontoblasts with its cognant receptor on ameloblasts. By RT-PCR, ameloblast, but not odontoblast, cell lines expressed the c-fms receptor. These findings indicate that CSF-1 is essential for normal tooth matrix development. Expression of mCSF-1 in op/opT partially corrected the enamel defect seen in op/op mice and likely contributed to the AI phenotype; sCSF-1 may be required for complete resolution. Targeting CSF-1 to teeth may provide a useful therapeutic strategy for regulating enamel/dentin matrix proteins and eruption in dental disorders.

Disclosures: ***S.L. Abboud****, None.*

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Dentin Matrix Protein-1 (Dmp1) Mice Display Multiple Abnormalities during Early and Late Skeletal Development. L. Ye*[1], H. Huang*[1], Y. Xie*[1], Y. Lu*[1], D. Chen[2], S. L. Dallas[1], L. F. Bonewald[1], Y. Mishina*[3], J. Q. Feng[1]. [1]School of Dentistry, UMKC, Kansas City, MO, USA, [2]Molecular Medicine, UTHSCSA, San Antonio, TX, USA, [3]NIEHS/NIH, Research Triangle Park, NC, USA.

Previously we reported that adult mice lacking Dmp1 gene exhibit the characteristics of chondrodysplasia, osteoarthritis, and show severe defects in mineralization. Although newborns appear grossly normal, upon further detailed examination it was found that these animals also exhibit skeletal abnormalities, including increased trabecular bone in metaphysis as well as a delay in secondary ossification.
To gain a complete understanding of temporal roles of Dmp1 in skeletal development and the potential mechanisms for the observed skeletal defects, Dmp1 null mice from embryonic day 3 to 1 year old were examined. Radiographs showed osteopetrosis-like changes in metaphyses of mutants starting from day 10, which were confirmed by von kossa and alizarin red/alcian blue staining as well as alkaline phosphatase histochemistry. Dmp1 mutants also showed abnormalities in the length and diameter of their long bones as well as a failure in remodeling to form the normal hour glass shape as seen in WT littermates. In addition, Dmp1 nulls showed a dramatic delay in secondary ossification in long bones, vertebrae and tails. To determine the mechanisms underlying these skeletal abnormalities, we first examined expression of markers of bone formation as well as bone ECM proteins by both in situ hybridization and/or immunohistochemistry at 3-wk after birth. Cbfa1 expression as well as proteoglycans, such as decorin, biglycan and fibromodulin was increased in metaphyses. This suggests that one of the major abnormalities in Dmp1 mice may be overexpression of ECM proteoglycans. We also examined expression of caspase 3 and found a dramatic reduction in the number of hypertrophic chondrocytes expressing this marker of apoptosis. To examine the role of Dmp1 in mineralization, double calcein labeling was performed at 3-wk and 3-mo after birth. In WT animals a clear double label was observed on the endosteal surface. In contrast in Dmp1 null mice, calcein label was incorporated diffusely on both the endosteal and the periosteal surfaces. This aberrant periosteal mineralization may contribute to the bony protrusions observed in mutants. To examine if delayed secondary ossification was due to impaired vascular invasion, PECAM was used as a marker. The mutants showed reduced numbers of blood vessels in epiphyses.
These data show that in addition to its role in the adult skeleton, Dmp1 plays critical roles in early bone development through its effects on vascular invasion, chondrocyte apoptosis, mineralization and expression of bone ECM molecules.

Disclosures: ***L. Ye****, None.*

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Genome-wide Generation of Knock-out Mice and in vivo Expression Profiling using VelocigeneTM, a New High-throughput Method, Identifies Novel Genes Expressed in Bone and Cartilage. R. Raz*, N. C. Adams*, P. Kraus*, N. W. Gale*, D. Frendewey*, W. Auerbach*, W. T. Poueymirou*, H. Su*, Y. Xue*, T. M. DeChiara*, D. M. Valenzuela*, G. D. Yancopoulos*, A. N. Economides*. Regeneron Pharmaceuticals, Tarrytown, NY, USA.

A novel method, termed VelocigeneTM, has been developed which allows for the rapid generation of genetically modified mice. This method utilizes bacterial homologous recombination for precise engineering of modified genes in bacterial artificial chromosomes (BACs), to generate BAC-based targeting vectors (BACvecs). ESC clones targeted by homologous recombination with BACvecs are identified using quantitative PCR. Use of BACvecs in place of traditional targeting vectors affords many advantages, such as increased targeting frequency, ability to make very large deletions in a single step, and bypass the need for negative selection and isogenicity. In addition, the whole process is highly scalable, automated, and industrialized, allowing the generation of 500 *knock-out* lines per year at our current throughput. Since the targeted gene is typically replaced by a reporter such as *lacZ*, its expression pattern can be visualized. We have used this technology to target more than 425 genes to date. Among them, 225 have been surveyed for expression. Of those, 24 are expressed in cartilage but not in bone, 5 are expressed in bone and not cartilage, whereas 8 are expressed in both. Detailed analysis has revealed intricate expression patterns. For example, the Monocyte Chemotactic Protein-3 (MCP3) is expressed only in the vertebral disks but not in other cartilagenous structures, whereas the Placental Growth Factor (PLGF) is only present in the growth plates of the long bones. This expression profiling has also shown that genes widely characterized in other systems are also expressed in cartilage or bone, where their function had been largely ignored; examples are EphA2, Angiopoietin Y1, Pigment Epithelium Derived Factor (PEDF) and Cocaine/Amphetamine-Regulated Transcript (CART). Detailed expression profiling for these genes will be presented. Additionally, *knock-out* mice have been generated for a number of these genes and their phenotypes are being analyzed in bone and cartilage. In conclusion, our high-throughput approach for generating and analyzing *knock-out* mice where genes of interest are replaced by a reporter provides precise and comprehensive information about their expression patterns, to an extent superior to other methods.
*Raz, Adams, and Kraus contributed equally to this work

Disclosures: ***R. Raz****, Regeneron Pharmaceuticals 1, 3.*

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Dynamic Imaging of Extracellular Matrix Molecules in Osteoblasts and Role of Fibronectin as an Orchestrator of Extracellular Matrix Assembly. P. Sivakumar*[1], A. Czirok*[2], B. J. Rongish*[2], D. M. Peters*[3], S. L. Dallas[1]. [1]Univ. Missouri, Kansas City, MO, USA, [2]Kansas Univ. Med. Ctr., Kansas City, KS, USA, [3]Univ. Wisconsin, Madison, WI, USA.

The extracellular matrix (ECM) has classically been viewed as a static three dimensional scaffold whose role is to hold tissues together and act as a barrier to cell movement. However, recent dynamic imaging studies in early embryos and fibroblast cultures have demonstrated that the ECM is not static and that ECM molecules form structures that continually undergo movement and deformation. Here we have used time lapse fluorescence imaging to study the dynamics of ECM proteins in living osteoblast cultures.
Latent TGFβ binding protein-1 (LTBP1) is an ECM protein that binds TGFβ and regulates its bioavailability in bone. LTBP1 time dependently co-localizes with fibronectin, which is one of the earliest ECM proteins to be assembled and is critical for osteoblast differentiation. Dynamic imaging, using fluorescently labeled fibronectin or LTBP1 antibodies as probes, revealed a large amount of cell movement in postconfluent FRC cultures. This resulted in large displacements of the LTBP1 and fibronectin-positive fibrils, which continually stretched and contracted to accommodate cell movements. Occasionally, stretched fibrils were seen to break, followed by contraction of the fibrils to 25% of their extended length. These studies suggest that fibronectin fibrils are highly stretched in living osteoblast cultures and have elastic properties. Stretching of fibronectin molecules has been proposed to expose cryptic self assembly sites as well as binding sites for other ECM proteins. We hypothesized that a major pathway by which fibronectin regulates bone cell function is by orchestrating the assembly of other ECM proteins. To examine this a fibronectin-null cell model was used. Fibronectin-null embryonic fibroblasts failed to incorporate LTBP1 and fibrillin-1 into their ECM as shown by immunofluorescent staining, immunogold EM, and Western blotting. Incorporation of type I collagen was also dramatically impaired. Addition of 10μg/ml plasma fibronectin rescued incorporation of all three ECM molecules. Rescue experiments using a panel of fibronectin fragments showed that only fragments which assembled into ECM fibrils were able to rescue LTBP1 incorporation.
These data show that fibronectin may act as a template for assembly of a number of ECM proteins, which may explain in part the importance of fibronectin in osteoblast differentiation. Time lapse imaging studies have highlighted the dynamic nature of fibrillar bone ECM proteins, which undergo surprisingly large movements and distortions associated with osteoblast movement.

Disclosures: ***P. Sivakumar****, None.*

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Differential Effects of Teriparatide and Alendronate on Markers of Bone Remodeling and Areal and Volumetric Bone Density in Women with Osteoporosis. M. McClung[1], P. Miller*[2], R. Civitelli[3], M. Warren*[4], S. Greenspan*[5], L. Haddock*[6], J. Tamayo*[7], D. Donley*[8], J. San Martin[8]. [1]Oregon Osteoporosis Center, Portland, OR, USA, [2]Colorado Center for Bone Research, Lakewood, CO, USA, [3]Washington University School of Medicine, St. Louis, MO, USA, [4]Physicians East, Greenville, NC, USA, [5]University of Pittsburg Medical Center, Pittsburg, PA, USA, [6]University of Puerto Rico, San Juan, Puerto Rico, [7]COMOP Osteoporosis Research Center, Mexico D.F., Mexico, [8]Eli Lilly and Company, Indianapolis, IN, USA.

Teriparatide (rDNA origin) injection [TPTD; rhPTH (1-34)], a stimulator of bone formation, and alendronate (ALN), a bisphosphonate, both increase bone mineral density (BMD) and decrease fracture risk. We conducted an 18-month randomized, double-blind, prospective clinical trial comparing the effects of TPTD or ALN on markers of bone remodeling, lumbar spine, hip areal and volumetric BMD in postmenopausal women with osteoporosis. Final patient visits will occur in May 2003 and full 18-month data will be presented. During a 6-month interim analysis, double-labeled iliac crest bone biopsies were obtained from a subset of patients: TPTD (n=8) or ALN (n=9). Histomorphometric analysis revealed pronounced stimulation of formation in patients on TPTD, while patients on ALN revealed pronounced suppression. This was reflected in significant differences in mineralizing surface (8.1±4.4% (mean ± SD) vs. 0.2 ± 0.3%, P<.001), bone formation rate (0.062 ± 0.036 vs. 0.002 ± 0.002 μm/d, P<.05) and activation frequency (0.99±0.57/yr vs. 0.02±0.03/yr, P<.001)(TPTD vs. ALN). Markers of bone formation revealed a 217% increase in serum N-terminal procollagen type I extension peptide (PINP) in the TPTD group (n=76) versus a 67% decrease (P<.001) in ALN treated patients (n=85). Markers of bone resorption revealed a similar trend, urinary N-telopeptide (NTx) increased by 59% with TPTD treatment (n=73) compared to a 72% decrease with ALN treatment (n=84)(P<.001). Contrasting effects on bone remodeling between TPTD and ALN translated into increases in BMD. A greater increase in areal lumbar spine BMD, measured by DXA, was present in the TPTD group (n=84) compared with the ALN group (n=87), 4.7% vs. 3.2%, respectively (P<.01). Volumetric lumbar spine trabecular BMD was measured by QCT in a subset of patients and results showed even more drastic differences between therapies. Volumetric BMD was 5-fold higher with TPTD (n=16) than with ALN (n=19), 14.6% vs. 2.9%, respectively (P<.01). In conclusion, our results underscore the different mechanisms of action between TPTD and ALN, and confirm that stimulation of bone formation with TPTD increases areal and volumetric BMD more than suppression of bone turnover with ALN.

Disclosures: ***M. McClung****, Aventis 2, 5; Eli Lilly and Company 2, 5; Merck & Co., Inc. 2, 5; Procter & Gamble Pharmaceuticals 2, 5.*

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Teriparatide (rhPTH [1-34]) Treatment Improves the Structure of the Proximal Femur in Women with Osteoporosis. L. M. Semanick*[1], K. Uusi-Rasi*[1], J. R. Zanchetta[2], C. E. Bogado*[2], E. F. Eriksen[3], M. Sato[3], T. J. Beck[1]. [1]Department of Radiology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]IDIM and USAL University, Buenos Aires, Argentina, [3]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.

Postmenopausal women with osteoporosis enrolled in the Fracture Prevention Trial who received daily injections of teriparatide (rhPTH [1-34]) showed significantly lower rates of vertebral and non-vertebral fragility fractures compared to placebo controls. These observations suggest that teriparatide improves bone mechanical strength. A subset of postmenopausal women (n=558) in the randomized double-blind multicenter study (mean age 70 ± 7 years) were randomized to a once-daily self-administered subcutaneous injection of placebo (n=189), teriparatide 20 μg (TPTD20; n= 86) or teriparatide 40 μg (TPTD40; n=183) for a median of 20 months. Dual energy x-ray absorptiometry (DXA) hip scans were analyzed with the Hip Structure Analysis (HSA) program to derive structural geometry. This HSA program measures BMD and the geometric properties of cortical bone within narrow regions across the femoral neck, intertrochanter and femoral shaft from images acquired by DXA. Linear models were used to assess the effects of teriparatide treatment on bone structure using baseline values as covariates. There were no significant differences in body size or age between treatment groups at any time point. At the femoral neck, teriparatide increased bone mass and improved bone strength in a dose-related manner, compared to placebo. The mean increase (95% CI) in bone mineral density was 4.2% (2.4% to 6.0%) in the TPTD20 group and 7.2% (5.4% to 9.1%) in the TPTD40 group, compared to placebo. The mean differences in axial strength (bone cross-sectional area) were 3.5% (1.8% to 5.3%) and 6.3% (4.5% to 8.2%), and in bending strength (section modulus) 3.6% (1.4% to 5.8%) and 6.8% (4.6% to 9.1%) in the TPTD20 and TPTD40 groups respectively. The mean increase in cortical thickness was 4.5% (2.6% to 6.4%) and 7.7% (5.7% to 9.7%), while buckling ratio (local cortical instability) decreased by 5.5% (3.5% to 7.5%) and 8.6% (6.6% to 10.5%) in the TPTD20 and TPTD40 groups respectively. Changes at the intertrochanteric region were comparable to those at the narrow neck. Teriparatide treatment effects did not reach significance at the cortical shaft. In conclusion, compared to placebo, teriparatide treatment increased bone mass, improved bone strength, increased cortical thickness and reduced the buckling ratio at the intertrochanteric region and the femoral neck.

Disclosures: ***L.M. Semanick****, Eli Lilly and Company 2.*

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A Novel Vitamin D Analogue Restores Bone Mass and Adds Extra Bone by Markedly Stimulating Bone Formation in Ovariectomized Rats with Established Osteopenia. H. Z. Ke[1], H. Qi*[1], D. T. Crawford*[1], G. Xu*[1], M. Li[1], L. plum[2], M. Clagett-Dame[2], H. F. DeLuca[2], D. D. Thompson[1], T. A. Brown[1]. [1]Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT, USA, [2]University of Wisconsin-Madison/Deltanoid Pharmaceutical, Inc., Madison, WI, USA.

It has been reported that a novel vitamin D analogue, 2-methylene-19-nor-(20S)-1-a,25(OH)2D3 (2MD), stimulates osteoblastic bone formation in vitro and increases bone mass in ovariectomized (OVX) rats with minimal effect on serum calcium. The purpose of this study was to test whether 2MD can restore bone mass in OVX rats with established osteopenia. Sprague-Dawley female rats were sham-operated (sham) or OVX at 4 months of age. Beginning at 8 weeks post-surgery, OVX rats were orally dosed with 2MD at 0.5, 1, 2.5, 5 or 10 ng/kg/d for 16 weeks. Compared with sham and OVX controls, serum calcium did not differ in OVX rats treated with 2MD at 0.5 or 1 ng/kg/d, while slightly but significantly increased at 2.5, 5 or 10 ng/kg/d. Treatment with 2MD in OVX rats significantly increased total body bone mineral density by 4%, 7%, 14%, 23% and 31% at 0.5, 1, 2.5, 5 or 10 ng/kg/d, respectively. These observations were confirmed by pQCT analysis of distal femoral metaphysis (DFM) and femoral shafts (FS). Total bone density in DFM dose-dependently increased in 2MD-treated OVX rats (up to +73%) at doses equals to or greater than 1 ng/kg/d compared with OVX controls. Similarly, total bone content in FS increased significantly at all doses of 2MD treated OVX rats (up to +33%). Compared with OVX controls, there were significant increases in trabecular bone volume and trabecular connectivity determined by micro-CT analysis of DFM in 2MD treated OVX rats at doses as low as 1 ng/kg/d. Furthermore, bone formation on periosteal, endocortical and trabecular surfaces determined by histomorphometric methods dose-dependently increased in OVX rats treated with 2MD compared with OVX and sham controls. Interestingly, 2MD induced new trabecular bone formation in the marrow cavity of tibial shafts where there was no trabecular bone in sham or OVX rats, indicating that the pre-existing bone surface was not required for 2MD induced bone formation. This differs from parathyroid hormone, another bone anabolic agent, which requires pre-existing bone surface for its bone anabolic effect to be manifested. In summary, we found that 2MD not only restored both trabecular and cortical bone mass but also added extra bone to the osteopenic, OVX rats by stimulating bone formation on all bone envelopes. These data indicate that 2MD may have therapeutic potential in human skeletal disorders such as osteoporosis.

Disclosures: ***H.Z. Ke****, Pfizer 3.*

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Proteasomal Degradation of Runx2 Shortens the Anti-apoptotic Signal of PTH in Osteoblasts: Why Intermittent Administration is Needed for Bone Anabolism. T. Bellido, L. I. Plotkin, A. A. Ali, C. A. O'Brien, 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 Medical Sciences, Little Rock, AR, USA.

Intermittent PTH causes bone anabolism, whereas sustained elevation of the hormone has a catabolic effect. However, an explanation for this dichotomy has remained elusive. Based on earlier evidence that the anabolic effect of intermittent PTH in mice is due at least in part to attenuation of osteoblast apoptosis, we examined whether continuous elevation of the hormone also reduced osteoblast death. We report that administration of PTH to Swiss Webster mice with an osmotic pump, or feeding them a calcium-deficient diet to increase the level of endogenous hormone, did not affect the prevalence of osteoblast apoptosis as determined 6 days after initiation of treatment by in situ end labeling. Consistent with this *in vivo* evidence, PTH (or dibutyryl-cAMP) provided only temporary (6-12 h) protection against etoposide- or anoikis-induced death of osteoblastic calvaria cells or OB-6 cells. The anti-apoptotic effect of PTH on OB-6 cells required Runx2-mediated transcription of genes like Bcl-2. Remarkably, PTH also caused an increase in ubiquitinated Runx2 marked for proteasomal proteolysis, and a decline in total Runx2 that reached 30% of controls at 3-6 h as determined by Western blot analysis. Acceleration of Runx2 degradation by overexpression of Smurf1 (the ubiquitin E3 ligase that targets Runx2 for degradation) blocked the anti-apoptotic effect of PTH. Conversely, abrogation of the PTH-induced decline in Runx2 using the proteasome inhibitor lactacystin, or by transfection of a dominant negative mutant of Smurf1, extended the anti-apoptotic effect of PTH to at least 24 h. Overexpression of either wild type Runx2, or a Runx2 mutant lacking the Smurf1 recognition site, also extended the anti-apoptotic effect of PTH. Moreover, Smurf1 reversed the prolonging effect of wild type Runx2 but not that of the mutant Runx2. We conclude that the duration of the anti-apoptotic effect of PTH is short-lived because it depends on the level of Runx2, which in turn is decreased by PTH via Smurf1-mediated proteasomal proteolysis. The self-limiting nature of PTH-induced survival signaling in osteoblasts explains why intermittent administration of the hormone is required for bone anabolism.

Disclosures: ***R.L. Jilka****, Anabonix, Inc. 4.*

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Isosorbide Mononitrate Increases Bone Formation and Decreases Bone Resorption in Postmenopausal Women: A Randomized Trial. S. A. Jamal[1], S. R. Cummings[2], G. A. Hawker[3]. [1]Endocrinology, St. Michael's Hospital, University of Toronto, Toronto, ON, Canada, [2]San Francisco Coordinating Center and Research Institute at the California Pacific Medical Center, San Francisco, CA, USA, [3]Rheumatology, Women's College Ambulatory Care Centre, University of Toronto, Toronto, ON, Canada.

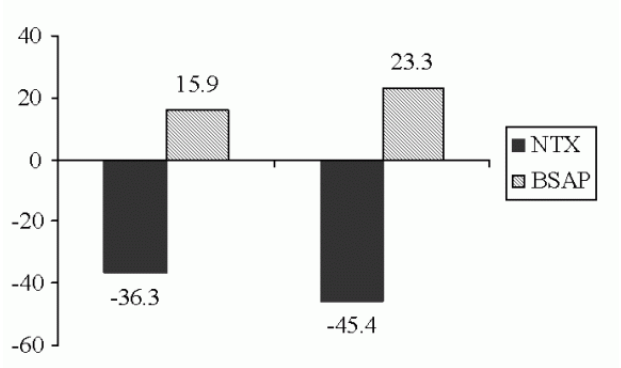
Nitric oxide (NO) both stimulates bone formation and inhibits bone resorption in vitro. NO donors (nitrates) are inexpensive, safe, and widely available but their value for postmenopausal osteoporosis has never been evaluated in a randomized trial.

We enrolled women who were at least 3 years postmenopausal with hip bone mineral density (BMD) T-score between 0 and -2.5. We randomly assigned them to 5 mg or 20 mg per day of oral isosorbide mononitrate (ISMO), or placebo for 12 weeks. We measured changes in urine N-Telopeptide, (NTx), a marker of bone resorption and serum Bone Specific Alkaline Phosphatase (BSAP), a marker of bone formation.

Compared with women randomized to placebo, women randomized to 20mg of ISMO had a 45.4 % decrease in NTx (95% confidence interval [CI]: 25.8 to 64.9) and a 23.3% increase (95% CI: 8.9 to 37.8) in BSAP. Women randomized to 5mg of ISMO had a 36.3% decrease in NTx (95% CI: 14.8 to 57.8) and a 15.9% increase in BSAP (95% CI: 1.1 to 30.7) (Figure 1). 21% of women taking ISMO stopped treatment because of quickly reversible headache.

We conclude that ISMO decreases bone resorption to a degree similar to estrogen but also increases bone formation. This suggests that nitrates may substantially reduce fracture risk in postmenopausal women.

Figure 1. Changes in urine NTX and serum BSAP in women randomly assigned to 5 or 20 mg of ISMO daily compared with change in women assigned to placebo.



Disclosures: ***S.A. Jamal****, None.*

1156

Estren Is a SERM with the Capacity to Excert Genomic Effects. S. Movérare[1], J. Dahllund*[2], N. Andersson[1], S. Nilsson*[2], J. Gustafsson[3], C. Ohlsson[1]. [1]Centre for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]KaroBio AB, Huddinge, Sweden, [3]Department of Biosciences at Novum & Department of Medical Nutrition, Karolinska Institute, Huddinge, Sweden.

It was recently reported that 4-estrene-3α,17β-diol (estren) increases bone mass without affecting classical transcription (1). The aim of the present study was to compare the physiological effects of estradiol and estren on a variety of physiological responses in ovariectomized (ovx) wild type (WT) and estrogen receptor (ER) inactivated mice ($ER\alpha^{-/-}$, $ER\beta^{-/-}$ and $ER\alpha^{-/-}\beta^{-/-}$). Ovx mice of the different genotypes were treated for four weeks with estradiol (0.7µg/mouse/day) or estren (75 µg/mouse/day). As expected, estradiol increased the uterine weight (816 ± 97 %), the trabecular BMD (88 ± 21 %) and the cortical BMC (17 ± 4 %) while it decreased the gonadal fat weight (-62 ± 5 %) compared with vehicle in WT mice. Estren exerted a clear effect on the uterine weight (132 ± 28 %) and the trabecular BMD (19 ± 4 %) but no effect on the gonadal fat weight or the cortical BMC in WT mice. Comparison of the effect of estren in WT mice and ER inactivated mice demonstrated that the effects on uterus and trabecular BMD were exerted via ERα.

The effect of estren on the activation of an estrogen-responsive element (ERE)-driven reporter gene (ALP) was tested in ERα and ERβ reporter cell lines (2). Estren activated the reporter gene both via ERα (EC_{50} 70 nM) and via ERβ (EC_{50} 77 nM). Both the effect via ERα and the effect via ERβ were completely blocked by the estrogen receptor antagonist ICI 182780. Thus, estren has *in vitro* the capacity to exert genomic effects via both ERα and ERβ.

In conclusion, estren is a SERM with effects on trabecular BMD and uterus and these effects *in vivo* are mediated via ERα. Furthermore, *in vitro* data demonstrate that estren has the capacity to exert genomic effects.

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Disclosures: ***S. Movérare****, None.*

1157

Severely Decreased Bone Formation and Cortical Bone Content in Mice Lacking the Protein Kinase Akt2. H. Z. Ke, G. Xu*, D. T. Crawford*, H. Qi, H. A. Simmons, T. A. Brown, D. D. Thompson. Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT, USA.

The phosphoinositide-dependent serine-threonine protein kinase Akt (also known as PKB) has been proposed to be a key mediator of cell proliferation and survival. However, the regulatory role of Akt on trabecular and cortical bone development is not known. The purpose of this study was to elucidate the effects of knockout of Akt2 (PKB-beta), one of the three isoforms of Akt, on cortical and trabecular bone in female and male mice. Skeletal phenotypes of knockout (KO) female mice at age of 4, 8 and 12 months and KO male mice at 8 months of age were characterized and compared with their wild-type (WT) littermates. Compared with WT controls, body weight was significantly lower by 10%, 20% and 17% at 4, 8 and 12 months of age, respectively, in KO females and by 28% at 8 months of age in KO males. At 8 months of age, fat body mass decreased significantly by 50% and 55% in KO females and males, respectively, compared with WT. Femoral length did not differ between KO and WT in females and males, indicating that Akt2 does not regulate longitudinal bone growth. PQCT analysis of the distal femoral metaphysis (DFM) showed that KO females had significantly lower total bone content (-19%), total bone density, cortical bone content and cortical bone area compared with WT at 4 months of age. These parameters increased with age between 4 and 8 months of age, while they decreased with age between 8 and 12 months of age in both KO and WT females. However, these parameters remained significantly lower in KO compared with WT in all age groups. Similarly, PQCT analysis of the femoral shaft (FS) showed that KO females had significantly lower total bone content, total bone area, cortical bone content, cortical bone area, periosteal and endocortical circumferences compared with WT at 8 and 12 months of age. In 8-month-old KO male mice, total bone content and cortical bone content decreased significantly by 36% and 38% in DFM, and 23% and 22% in FS, respectively, compared with WT. Cortical bone histomorphometric analysis indicated that there was a 90% decrease in periosteal bone formation rate and a 57% decrease in endocortical bone formation rate in KO males compared with WT. There was a 59% decrease in trabecular bone formation rate determined by histomorphometric analysis of DFM and a 44% decrease in trabecular bone volume determined by micro-CT analysis of DFM in 8-month-old KO males compared with WT. In summary, Akt2 knockout severely decreased bone formation and bone mass in mice. Thus, activation of Akt2 may provide therapeutic potentials for skeletal disorders such as osteoporosis.

Disclosures: ***H.Z. Ke****, Pfizer 3.*

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Capsaicin Sensitive Sensory Neurons Maintain Normal Bone Integrity. W. S. Kingery[1], S. Offley*[1], T. Guo*[1], A. Joseph*[1], D. P. Lindsey*[2], C. R. Jacobs[2]. [1]PM&R Service (117), VAPAHCS, Palo Alto, CA, USA, [2]Rr&d, VAPAHCS, Palo Alto, CA, USA.

Familial dysautomia is an autosomal recessive disease in which patients suffer from extensive unmyelinated sensory neuron loss, reduced bone density, and frequent fractures. It has been proposed that the loss of neuropeptides synthesized by unmyelinated neurons adversely affects bone integrity in this hereditary syndrome. To determine whether unmyelinated neurons can contribute to bone density and strength we treated skeletally mature male rats with either capsaicin (250mg/kg s.c.) or vehicle. Capsaicin activates the vanilloid receptors (VR1) that are expressed by many of the small diameter sensory afferents, including most of the neuropeptide containing neurons. Activation of the VR1 causes an influx of cations into the neuron resulting in the excitotoxic destruction of unmyelinated and small myelinated afferents. Four weeks after capsaicin treatment bone mineral density (measured by DXA scanning) in the metaphyses of the tibia and femur was reduced. There was also a reduction in distal femur density and a loss of bone strength (41% decrease in ultimate stress on biomechanical testing). No changes occurred in body weight, 24 h grid-crossing activity, hindpaw weight bearing, or gastrocnemius and soleus muscle mass after capsaicin treatment, data indicating that skeletal unloading did not contribute to the loss of bone density and strength. Peroneal nerve histomorphology was evaluated by light and electron microscopy 4 weeks after capsaicin treatment. Capsaicin treatment caused a 57% loss of unmyelinated sensory axons and a 8% loss of myelinated axons. Capsaicin also reduced the substance P content (measured by EIA) of the sciatic nerve by 78% and diminished sciatic nerve stimulation evoked extravasation responses by 64%. There was also a 68% reduction of substance P content in the proximal tibia at 4 weeks after capsaicin treatment. These results support the hypothesis that capsaicin sensitive afferent neurons contribute to the maintenance of metaphyseal bone density and strength. Capsaicin sensitive neurons have efferent functions in the tissues they innervate, effects mediated by the release of neuropeptides from the peripheral nerve terminals. We postulate that the deleterious effects of capsaicin treatment on cancellous bone density and strength are mediated by reductions in local neuropeptide content and release.

Disclosures: ***W.S. Kingery****, None.*

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Aromatase Inhibition Does not Alter Bone Turnover, Serum OPG or Bone Density in Elderly Men with Mild Hypogonadism. B. Z. Leder[1], J. L. Rohrer*[1], S. D. Rubin*[2], J. Gallo*[3], J. S. Finkelstein[1]. [1]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [2]AstraZeneca Pharmaceuticals, Wilmington, DE, USA, [3]Eximias Pharmaceuticals, Berwyn, PA, USA.

The reduction in serum gonadal steroid levels that occurs in aging men may contribute to various aspects of age-related physiological decline, including bone density. Recent studies suggest that estradiol (E) may be a crucial regulator of bone turnover in elderly men though testosterone (T) likely also plays a role. Thus, the effects of aromatase inhibition in men are difficult to predict. To assess the skeletal effects of aromatase inhibition in elderly men with mild hypogonadism (screening T < 350 ng/dL), we randomized 37 subjects (ages 62-74) to receive either anastrozole (a potent aromatase inhibitor) 1 mg QD, anastrozole 1 mg twice weekly, or placebo for 12 wk. Gonadal steroid levels, biochemical markers of bone turnover, osteoprotegerin (OPG), and total body bone density (TBBD) were measured at baseline and after 12 wk of treatment.

Both doses of anastrozole increased serum bioavailable T (BioT) levels (and total T- data not shown) in these men and decreased serum estradiol (E) levels. (*p<0.01 vs Group 3. **p <0.01 vs Group 3 and P<0.05 vs Group 2)

Changes in hormone levels (mean ± SD)

	Baseline BioT (ng/dL)	Week 12 BioT (ng/dL)	Baseline Estradiol (pg/mL)	Week 12 Estradiol (pg/mL)
Group 1 (anastrozole 1 mg QD) (n=12)	99 ± 31	207 ± 65**	26 ± 8	17 ± 6*
Group 2 (anastrozole 1 mg 2/wk) (n=11)	115 ± 37	178 ± 55*	27 ± 8	17 ± 5*
Group 3 (placebo QD) (n=14)	100 ± 35	97 ± 30	23 ± 4	25 ± 6

Despite these hormonal changes, no changes in osteocalcin (OC), bone specific alkaline phosphatase, or amino-terminal propeptide of type I procollagen were observed in any group. Similarly, no changes were observed in serum N-telopetide (NTX) or urinary deoxypyridonoline. (Data shown below for NTX and OC only.)

Changes in bone turnover markers (mean ± SD)

	Baseline NTX (nmol/L)	Week-12 NTX (nmol/L)	Baseline OC (ng/mL)	Week-12 OC (ng/mL)
Group 1	10.8 ± 2.3	11.0 ± 2.1	17.5 ± 3.8	17.8 ± 3.0
Group 2	11.8 ± 2.9	11.2 ± 3.0	18.9 ± 5.4	18.0 ± 5.4
Group 3	11.2 ± 3.0	10.9 ± 2.3	20.5 ± 6.8	21.5 ± 6.5

Serum OPG and TBBD also did not change in any group. These data suggest that increasing T and decreasing E for 12 weeks in mildly hypogonadal elderly men does not adversely affect bone metabolism. Furthermore, despite published reports that E increases and T decreases OPG expression *in vitro*, serum OPG levels are also unaffected by aromatase inhibition. Additional study is needed to assess the long-term effects of this method of T replacement on the skeleton and other gonadal steroid responsive tissues.

Disclosures: ***B.Z. Leder****, AstraZeneca Pharm. 2; Merck 8.*

1160

Leptin Modulates both Resorption and Formation while Preventing Disuse-Induced Bone Loss in Tail-Suspended Female Rats. T. Thomas, A. Martin*, R. de Vittoris*, M. Lafage-Proust, C. Alexandre, L. Vico. INSERM E0366, Saint-Etienne, France.

We previously demonstrated that peripheral administration of leptin was able to prevent disuse induced bone loss over 2 weeks of tail-suspension in female rats. In accordance with in vitro data, we tested the hypothesis that leptin preventive action on bone loss could be related to effects on both resorption and formation activities taking advantage of the uncoupling pattern of bone remodeling in this model. One hundred female Wistar rats were randomly assigned to 1 of the following groups: tail-suspended or non-suspended rats treated either with leptin or vehicle for 3, 7 and 14 days, and 2 baseline groups. After 1 week of adaptation, rats were suspended and human recombinant leptin was continuously administered using intraperitoneal Alzet pumps. Animals were measured at baseline, day 7 and day 14 using PIXImus (Lunar Corp.), and tibia samples were collected for histomorphometry after sacrifice. After 7 and 14 days, suspension induced a decrease in bone mineral density measured at the tibial metaphysis, a trabecular bone site, as compared to baseline (0.162±0.003 vs 0.173±0.004 and 0.147±0.003 vs 0.181±0.004; P<0.01, respectively) whereas leptin administration for 7 and 14 days prevented this decrease (0.181±0.005 and 0.177±0.014, respectively; NS). These data were confirmed by histomorphometry. This preventive effect of leptin on disuse-induced bone loss was partly explained by a significant prevention of the transient increase in bone resorption observed in the suspended group at day 7 (N.Oc/B.Ar: 43.0±5.3 vs 124.3±6.7; P<0.01). These leptin effects could be mediated by the osteoprotegerin (OPG) pathway as suggested by the 3-fold increase in OPG gene expression under leptin administration, as measured by direct RT-PCR in tibial osteoblastic cells harvested at day 3 from the 2 suspended groups. Only at day 14, leptin prevented the decrease in bone formation rate induced by suspension (BFR/BS: O.21±0.02 vs 0.11±0.01 um3/um2/d; P<0.05). The latter effects could be related to leptin role, in mediating the reciprocal differentiation between adipocytes (Ad) and osteoblasts demonstrated in vitro. Indeed, we observed no significant difference in mineral apposition rate whereas leptin prevented the concurrent increase in adipogenesis observed in the suspended group treated with vehicle (Ad.S/B.Ar: 3.1±2.0 vs 4.9±2.1 %; P<0.05). In summary, these data suggest that peripheral administration of leptin could prevent disuse-induced bone loss with combined effects on both bone resorption and bone formation. Further studies are now needed to clarify the putative dual leptin effects on bone depending on its central brain-mediated vs peripheral effects.

Disclosures: ***T. Thomas****, None.*

1161

PPAR-Alpha Agonists Increase Bone Mineral Density in Female Rats. U. Syversen, I. Bakke*, G. Aune*, L. Thommesen*. Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Faculty of Medicine, 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 14,643 and fenofibrate, the gamma agonist pioglitazone and the combined PPAR alpha, gamma and delta agonist tetradecylthioacetic acid (TTA) on bone mineral density (BMD) in female rats.

Fifty female Fischer rats were divided into 5 groups and were given methocel (control group), Wyeth 14,643, fenofibrate, TTA and pioglitazone (50 mg/kg body weight) for 4 months. BMD was measured by double x-ray absorptiometry. Body weight was registered throughout the study. There was no difference in body weight between control rats and the treatment groups. After 4 months femoral BMD was significantly higher in rats treated with Wyeth 14,643 (7%, p=0.0002), fenofibrate (5.7%, p=0.0035) and TTA (5%, p=0.0116) than in control rats. In rats treated with pioglitazone, femoral BMD tended to be lower than in control rats (-3%, p=0.1591). Total body BMD was significantly lower in the pioglitazone treated group compared to controls (p<0.0001), while there was no difference between the control group and the other groups. Wyeth 14,643 was found to inhibit the differentiation of monocytes into osteoclasts in a dose-dependent manner, and also inhibited the proliferation of the preosteoclast cell line.

In conclusion, treatment with the PPAR-alpha agonists Wyeth 14,643 and fenofibrate, and the combined PPAR alpha, gamma and delta agonist TTA, significantly increased femoral BMD in female rats, while treatment with the PPAR gamma agonist pioglitazone resulted in a significantly decrease in total body BMD.

Disclosures: ***U. Syversen****, None.*

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β2-Adrenergic Agonists Have Negative Effects on Bone Architecture and Density in Rat. N. Bonnet*, B. Brunet-Imbault*, C. J. Parnaud*, G. Lemineur*, A. Arlettaz*, C. Chappard, D. Courteix, C. L. Benhamou. Inserm ERIT-M 0101, Orleans, France.

β2-receptors have recently been demonstrated to influence bone metabolism and bone mass via the central nervous system. β2-agonists have shown a negative effect on bone mass (1), but the effect on bone architecture has not been studied. The aim of this study is to quantify the effects of two β2-agonists (clenbuterol and salbutamol) on bone mass, bone length, morphological and topological trabecular bone parameters and bone strength.

34 ten weeks old Wistar female rats were studied. During 6 weeks SC daily injections were performed : 10 control rats receiving normal saline (2mg/kg/day) were compared to 13 rats receiving salbutamol (4mg/kg/day) and 11 rats receiving clenbuterol (2mg/kg/day). High-resolution BMD was measured by dual-energy X-ray absorptiometry on the *ex-vivo* rat femurs. The femur lengths were measured with a digital calliper. Trabecular bone microarchitecture was characterized by X-ray micro-computed tomography(μCT) (Skyscan 1072) : morphological (BV/TV, Tb.N, Tb.Th) and topological (SMI, TBPf, connectivity, degree of anisotropy (DA)) parameters were measured on the femur distal metaphysis. The radiological projections obtained by μCT were used to measure the cortical thickness at the mid diaphysis. The mechanical strength was measured using three-point bending tests.

Compared to controls, salbutamol and clenbuterol respectively induced significant reductions of 6.6% and 6.9% in BMD (p<0.001), 3.7% and 3.7% in the length (p<0.001) and 6% and 4.7 % in the cortical thickness (p<0.01). Salbutamol had no significant effect on morphological and topological parameters nor fracture load whereas clenbuterol induced a significant reduction of 45.8% of BV/TV (p<0.001), 46.6% of Tb.N (p<0.001), 18.5% of connectivity (p<0.02), 9.9% of fracture load (p<0.04) and a significant increase of TBPf (p<0.04). DA and SMI are not significantly influenced.

These results indicate that clenbuterol had an additive effect on microarchitecture. BV/TV, TbN were reduced whereas TbTh remained constant suggesting a predominant mechanism of trabeculae removal. The TBPf evolution in the clenbuterol group also indicates changes in the trabecular network shape. Salbutamol doesn't modify significantly the trabecular microarchitecture whereas the salbutamol concentration has been doubled compared to clenbuterol.

These data confirm the negative influence of β2-agonists on bone mass, and show the deleterious effect of the clenbuterol on trabecular bone microarchitecture, with potential pharmacological consequences in medicine and doping.

(1) Takeda S. *et al*., Cell, 2002, 305-17.

Disclosures: ***C.L. Benhamou****, None.*

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Role of TGF-β and Menin in the Proliferation and Secretion of Human Parathyroid Cells. H. Sowa[1], H. Kaji[1], R. Kitazawa[2], S. Kitazawa[2], T. Tsukamoto*[1], S. Yano*[1], G. N. Hendy[3], T. Sugimoto[1], K. Chihara*[1]. [1]Endocrinology, Kobe University, Kobe, Japan, [2]Molecular Pathology, Kobe University, Kobe, Japan, [3]Medicine, McGill University, Montreal, PQ, Canada.

Primary hyperparathyroidism is a common endocrine disorder caused by parathyroid tumor and an excess of parathyroid hormone (PTH) secretion. However, the mechanism of tumorigenesis in parathyroids is still unknown. Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder with multiple endocrine tumors, and somatic mutations of the MEN1 gene have also been found in 10-20 % of sporadic primary parathyroid tumors. We previously demonstrated that menin, a product of the MEN1 gene, interacts with Smad3, and that inactivation of menin blocks TGF-β-signaling (PNAS, 2001). The present study was therefore performed to clarify the role of TGF-β and menin in the proliferation and PTH secretion of parathyroid cells by employing human parathyroid cells from the patients with secondary hyperparathyroidism. Western blot analysis of parathyroid tissues demonstrated that parathyroid cells express TGF-β. TGF-β significantly inhibited the proliferation of parathyroid cells in MTT assay and ^{3}[H]-thymidine incorporation. Menin inactivation by menin antisense (AS) oligonucleotides (oligo) abrogated TGF-β-inhibited proliferation and PCNA expression in these cells. Moreover, the staining of PCNA and PTH by immunocytochemistry of parathyroid cells showed that menin inactivation with AS oligo antagonized TGF-β-inhibited proliferation in PTH-positive cells. As for secretion of PTH, TGF-β inhibited the expression of PTH (Western blot) and its level in the medium (RIA) of parathyroid cells. Inactivation of menin with AS oligo increased basal PTH secretion and TGF-β-inhibited PTH secretion On the other hand, control sense oligo did not affect the proliferation and PTH secretion of parathyroid cells. The present study demonstrates that menin inactivation antagonizes TGF-β-inhibited proliferation and PTH secretion in human parathyroid cell cultures. These findings suggest that TGF-β functions as an autocrine- or paracrine- inhibitory factor of growth and PTH secretion in parathyroid tissues, and that blockage of TGF-β signaling by menin inactivation results in tumor formation.

Disclosures: ***H. Sowa****, None.*

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COX-2 Inhibitors and Fracture Healing: Reversibility of Effects After Short Term Treatment. L. C. Gerstenfeld[1], D. M. Cullinane*[1], E. A. Krall*[1], J. Fitch*[1], M. Thiede[2], T. A. Einhorn[1]. [1]Boston University Medical Center, Boston, MA, USA, [2]Pharmacia Corp, Chesterfield, MO, USA.

Reports have suggested inhibition of experimental fracture (fx) healing with both COX-2-specific inhibitors (coxibs) and nonspecific non-steroidal anti-inflammatory drugs (NSAIDs). Because drug specificity, dose, and duration may influence fx healing, and because patients with fxs require analgesia for only short periods of time after initial stabilization, we tested the hypothesis that short-term dosing is reversible. Two experiments were conducted: in the first, 150 male SD rats underwent production of standard closed mid-diaphyseal femoral fx. Animals were then divided into three treatment gps and dosed by daily gavage with either the NSAID, ketorolac (4 mg/kg/d); the coxib, valdecoxib (5 mg/kg/d); or vehicle. In each gp, rats were administered drugs or vehicle for the first 7 days post-fx and euthanasia was performed on half of the animals on day 21 and the other half on day 35. In a second experiment, rats (n=150) were administered drug or vehicle for the first 21 days post-fx and euthanized on days 21 and 35. Methods of data analysis included manual testing of the integrity of the fx calluses, mechanical torsion testing to failure, and histomorphometry. Fisher's exact test was used to measure the association of % of nonunions with treatment. In rats treated for 7 days, there were no significant differences in % of nonunions after 21 days (16% of ketorolac, 22% of valdecoxib, and 8% of vehicle-treated animals, p=0.47), nor after 35 days (5% of ketorolac, 0% of valdecoxib, and 9% of

vehicle-treated, p=1.0). In rats treated for 21 days, there were differences in % of non-unions after 21 days (13% of ketorolac, 36% of valdecoxib, and 4% of vehicle-treated, p=0.01), but the differences disappeared by 35 days (0% in ketorolac and valdecoxib gps, 5% in vehicle-treated, p=1.0). Torsional strength in the bones which had united showed no significant differences among any of the treatment gps at either 21 or 35 days. Histomorphometry showed a strong correlation between non-union rate and percent cartilage in the calluses. These data suggest that both coxibs and NSAIDs delay fx healing and the magnitude of the effect as measured by non-union in this study is related to the duration of treatment. However, upon discontinuation of treatment, healing normalizes and if treatment is administered for up to 3 weeks post-fx, healing will occur within the expected time-frame (35 days in rats). Extrapolation of these findings to a clinical setting further suggests that management of fracture-associated pain with inhibitors of COX-2 should neither impair nor delay healing as long as the duration of treatment is consistent with current standards of care.

Disclosures: ***T.A. Einhorn****, Pharmacia 2, 5.*

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Clinical Characteristics of 94 Subjects with Autosomal Dominant Osteopetrosis Type II (ADO2) and Chloride Channel 7 Gene Mutations. S. G. Waguespack[1], K. A. Buckwalter*[2], M. J. Econs[3]. [1]Endocrine Neoplasia and Hormonal Disorders, U.T. M.D. Anderson Cancer Center, Houston, TX, USA, [2]Radiology, Indiana University, Indianapolis, IN, USA, [3]Medicine, Indiana University, Indianapolis, IN, USA.

ADO2 is caused by mutations in the chloride channel 7 (ClCN7) gene. The clinical characteristics of this disease remain poorly understood. We studied 94 individuals with ClCN7 gene mutations from 11 ADO2 families. We collected clinical data through patient interviews, medical records, and/or self-reported responses on a questionnaire. We assessed for a history of fractures (FX), osteomyelitis (OM), visual loss (VL), and bone marrow (BM) failure in adults and children. A score of "severe" for the fracture category was given if there was a history of > 10 fractures or > one hip/femur fracture. We compared the data between clinically affected, unaffected gene carriers and normal control subjects. Individuals were defined as affected or unaffected based on the presence or absence of classic radiographic findings and/or abnormal serum biochemistries, respectively.

Subjects <18 yrs. of age

	N	Age ± SD	FX (Any)	FX (Severe)	OM	VL	BM failure
Affected	19	8.9 ± 4.5	11 (58%)	3 (16%)	1 (5%)	8 (42%)	1 (5%)
Carrier	3	7.9 ± 7.3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Control	67	7.7 ± 4.2	11 (16%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Subjects >18 yrs. of age

	N	Age ± SD	FX (Any)	FX (Severe)	OM	VL	BM failure
Affected	43	47.1 ± 16.4	42 (98%)	14 (33%)	10 (23%)	4 (9%)	1 (2%)
Carrier	29	43.3 ± 15.6	17 (59%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Control	150	42.7 ± 15	68 (45%)	0 (0%)	2 (1%)	0 (0%)	0 (0%)

In this cross sectional analysis, almost all patients with ADO2 have a fracture at some point of their lives. Although fractures occur in all groups, the presence of a severe fracture history was identified only in affected patients. As expected, fractures in unaffected gene carriers did not differ from controls. Osteomyelitis was most commonly identified in the mandible and/or maxilla (8/10 affected adults); the other cases involved the lower extremity, two of which occurred after surgical repair of a fracture. Visual loss appears to occur at a relatively high rate, particularly in the pediatric age group. Finally, although rare, BM failure can occur in ADO2 patients. In conclusion, ADO2 is a disease of significant morbidity that can be quite severe in its clinical course; given the high rates of visual loss in subjects < 18 yrs., it is paramount to completely assess vision in any child with ADO2.

Disclosures: ***S.G. Waguespack****, None.*

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Long-term Infusion of Secreted Frizzled Related Protein 4 (sFRP-4) Reduces Serum Phosphate Concentrations without a Compensatory Change in Vitamin D Metabolism. T. Berndt*[1], T. A. Craig*[1], A. E. Bowe*[2], J. Vassiliadis*[2], D. Reczek*[2], R. Finnegan*[2], S. M. Jan De Beur[3], S. C. Schiavi[2], R. Kumar[1]. [1]Medicine, Mayo Clinic, Rochester, MN, USA, [2]Receptor Ligand Therapeutics, Genzyme Corporation, Framingham, MA, USA, [3]Division of Endocrinology, Johns Hopkins University, Baltimore, MA, USA.

Tumor induced osteomalacia (TIO) is a syndrome associated with hypophosphatemia, excessive phosphate excretion, osteomalacia, and abnormal vitamin D metabolism. Recent studies have identified sFRP-4 as a protein that is over expressed in tumors from patients with TIO. Therefore, the present studies were performed to determine the effects of long-term intravenous infusion of sFRP-4 on renal phosphate handling and vitamin D metabolism in rats. Normal rats were anesthetized and prepared for solute clearance studies. After a 90-minute stabilization period, a control clearance was taken and then either vehicle (n=5) or sFRP-4 (0.3 microgram/kg/hr, n=7) was infused. After a 1-hour equilibration period, one-hour clearances were taken at 4 and 7 hours after the initiation of the infusion. At the end of the experiment, blood was collected for the measurement of 1 alpha, 25-dihydroxyvitamin D, and the kidneys were removed for the isolation and assessment of 25-hydroxyvitamin D 1 alpha hydroxylase cytochrome P450 and 25 hydroxyvitamin D 24-hydroxylase cytochrome P450 mRNA concentrations by quantitative real-time RT-PCR. In the vehicle infused group, basal fractional excretion of phosphate (FEPi) was 7 ± 2% and plasma Pi was (2.0 ± 0.2 mM) and were stable throughout the experiment. With long-term infusion of sFRP-4, FEPi significantly increased from 9 ± 3% to 20 ± 4%, p<0.05 four hours after initiation of the sFRP-4 infusion and was 18 ± 4% at 8 hours. Infusion of sFRP-4 decreased serum Pi from 2.0 ± 0.1 mM to 1.5 ± 0.1 mM, p<0.05, at 4 hours and to 1.5 ± 0.1 mM, p<0.05, at 8 hours. Serum 1 alpha, 25-dihydroxyvitamin D concentrations and renal 25-hydroxyvitamin D 1 alpha-hydroxylase cytochrome P450 mRNA were similar in both groups. We conclude that long-term infusion of sFRP-4 is associated with increased phosphate excretion, hypophosphatemia, and that the vitamin D endocrine system fails to respond to the hypophosphatemic stimuli following sFRP-4 infusion. Sodium and calcium excretions were not changed. Thus, sFRP-4 has the biological properties of "phosphatonin".

Disclosures: ***T. Berndt****, Genzyme Corporation 2.*

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The Role of Local Corticosteroid Generation in Inflammation-Associated Bone Loss. M. S. Cooper, P. Emery*, M. Hewison, P. M. Stewart*. Division of Medical Sciences, University of Birmingham, Birmingham, United Kingdom.

Generalized and periarticular osteoporosis are common features of inflammatory arthritis. These effects appear to be mediated by high levels of pro-inflammatory cytokines. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an intracellular enzyme that generates active cortisol from inactive cortisone. By generating active glucocorticoids locally, osteoblastic 11β-HSD1 is an important determinant of the effects of glucocorticoids on osteoblasts. In vitro, osteoblastic 11β-HSD1 expression is induced by pro-inflammatory cytokines. We thus hypothesized that inflammation-associated bone loss may reflect a localized form of corticosteroid-induced osteoporosis. Corticosteroid metabolism was examined in patients presenting with early rheumatoid arthritis (age 59±13 (mean±SD), n=31) or mechanical, non-inflammatory, joint conditions (n=9). None of the patients had been treated with glucocorticoids or bone active medications. Serum markers of inflammation and clinical measures of disease severity were measured. Corticosteroid metabolite profiles were determined by gas chromatography/ mass spectrometry on urine samples giving measures of total corticosteroid metabolite production, 11β-HSD1 activity ((THF+alloTHF)/THE ratio), 11β-HSD2 activity (urinary free cortisol/urinary free cortisone (UFF/UFE)) and 5α-reductase activity (THF/THE ratio).

The (THF+alloTHF)/THE ratio (indicating 11β-HSD1 activity) was significantly increased in patients with inflammatory arthritis compared with controls (1.54±0.08 (mean±SE) vs 1.20±0.11; p<0.05). There was no difference in total corticosteroid metabolite production, 11β-HSD2 activity or 5α-reductase activity. Amongst patients with inflammatory arthritis significant correlations between inflammatory markers and 11β-HSD1 activity were found with high inflammatory markers predicting increased 11β-HSD1 activity (r=0.40 for ESR, r=0.39 for CRP, both p<0.05). In contrast, there was no correlation of inflammatory markers with corticosteroid metabolite production or 5α-reductase activity.

11β-HSD1 activity is elevated in patients with early untreated inflammatory arthritis and increases with the level of disease activity. This activity appears to reflect local generation of active corticosteroids from circulating inactive precursors. Increased 11β-HSD1 activity and thus chronic local corticosteroid excess may account for the periarticular and generalized osteoporosis seen in inflammatory arthritis.

Disclosures: ***M.S. Cooper****, None.*

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Increased Circulating Level of FGF-23 in Patients with McCune –Albright Syndrome. T. Yamamoto[1], Y. Imanishi[2], H. Koshiyama*[3], Y. Miyoshi*[4], N. Shimizu[5], E. Kinoshita*[6], Y. Nakagomi[7], A. Miyauchi[8], K. Satomura[5], M. Inaba[2], T. Sugimoto[9], Y. Nishizawa[2], H. Juppner[10], K. Ozono[4]. [1]Pediatrics, Minoh City Hospital, Minoh, Japan, [2]Metabolism, Endocrinology & Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan, [3]Medical Research Institute, Kitano Hospital, Osaka, Japan, [4]Pediatrics, Osaka University Graduate School of Medicine, Suita, Japan, [5]Second Division of Pediatrics, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan, [6]Pediatrics, Nagasaki University School of Medicine, Nagasaki, Japan, [7]Pediatrics, Yamanashi University School of Medicine, Kofu, Japan, [8]Medicine, National Hyogo-Chuo Hospital, Sanda, Japan, [9]Division of Endocrinology/Metabolism, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan, [10]PediatricEndocrine Unit, Department of Medicine, Mass. General Hospital and Harvard Medical School, Boston, Boston, MD, USA.

McCune-Albright syndrome (MAS) is sometimes complicated with hypophosphatemia. We previously reported the presence of a humoral factor which inhibited intestinal phosphate transport (Yamamoto T et al : J Bone Miner Metab 19:287,2001). Recently, fibroblast growth factor 23(FGF23) was reported as a phosphaturic factor and we recently reported increased circulating serum FGF23 levels in patients with oncogenic and X-linked hypophosphatemia. Thus, we measured serum FGF23 levels in patients with MAS accompanying hypophosphatemia. As a control for hypophosphatemia, we also investigated the serum FGF23 levels in 2 patients with hereditary hypophosphatemic rickets with hypercalciuria(HHRH).

Serum phosphate levels were clearly decreased in 7 MAS patients with hypophosphatemia compared with 14 normal controls. Mean serum phosphate values for MAS and normal controls were 3.0 and 4.5 mg/ml respectively (P=0.0002). Mean serum ALP and plasma FGF23 levels were clearly increased in MAS patients respectively (ALP:1357 vs 732 IU/l ; P=0.0251, FGF23: 307 vs 81 RU/ml ; P=0.0104). In HHRH, plasma mean FGF23 levels were within normal range(67 RU/ml). In MAS patients, plasma FGF23 levels were negatively correlated to serum phosphate levels (r= -0.553, P=0.0127) but positively correlated

to serum ALP levels(r=0.668, P=0.0052). Serum ALP levels were not correlated to serum phosphate levels. Oral phosphate loading test in two MAS patients revealed impaired maximal increase of serum phosphate levels compared to normal controls. These data suggest that FGF23 is a possible causal factor for hypophosphatemia in MAS patients. However, the mechanism of FGF23 on the regulation of serum phosphate levels in MAS patients remains further investigation.

Disclosures: ***T. Yamamoto****, None.*

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Mechanical Stimulation Promotes Osteocyte Survival: Requirement of Nuclear Targets of the Src/ERK Pathway. L. I. Plotkin, J. I. Aguirre, B. Strotman*, S. C. Manolagas, T. Bellido. Endocrinology, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. Arkansas for Med. Sci., Little Rock, AR, USA.

Mechanical signals activate the extracellular signal regulated kinases (ERKs) in osteocytes. Since mechanical loading might regulate osteocyte apoptosis *in vivo* and ERKs are required for osteocyte survival induced by systemic factors, we investigated whether mechanical stimulation influences the response of osteocytic cells to pro-apoptotic stimuli and whether ERKs are involved. MLO-Y4 osteocytic cells stably expressing nuclear green fluorescent protein (GFP) were first stretched for 10 min at 5% elongation, and subsequently treated with the proapoptotic stimuli etoposide or dexamethasone. Whereas either pro-apoptotic agent increased the percentage of cells exhibiting apoptotic features after 6 h in unstretched cultures, cells subjected to mechanical stimulation were protected from the effect of the pro-apoptotic agents. Inhibition of ERKs with PD98059 or with a dominant negative (dn) form of MEK (the kinase responsible for ERK activation) abolished the protection from apoptosis induced by stretching. On the other hand, inhibition of PI3K or p38 MAPK, two other kinases activated by mechanical signals, had no effect. Consistent with the previously demonstrated involvement of Src in ERK activation induced by stretching, cells treated with the inhibitor of Src kinases PP1 or transfected with a Src mutant lacking kinase activity did not exhibit the protective effect of stretching. Strikingly, cells transfected with a dn Src mutant lacking the SH2 domain (which mediates Src interaction with the estrogen receptor) also lost the responsiveness to stretching, whereas cells transfected with a dn Src mutant lacking the SH3 domain (which mediates Src interaction with the androgen receptor) remained responsive. These results are consistent with evidence (shown elsewhere in this meeting) that the transduction of mechanical signals into ERK activation involves the estrogen receptor, but not the androgen receptor. In addition, mechanical stimulation induced rapid translocation and nuclear accumulation of ERK2 fused to GFP, which increased between 1-20 min of stretching, and lasted for about 1 h after 10 min of stimulation, declining to control levels by 3 h. Furthermore, the anti-apoptotic effect of mechanical signals was abolished by actinomycin D or cycloheximide, inhibitors of RNA or protein synthesis, respectively. Taken together, these results indicate that mechanical signals promote osteocyte survival via transcription-dependent mechanisms downstream of the Src/ERK signaling pathway.

Disclosures: ***L.I. Plotkin****, None.*

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Blockade of Anabolic Mechanotransduction Pathway in COX-2 Deficient Mice. Y. Mikuni-Takagaki[1], H. Sekiya[2], K. Naruse*[3], M. Itoman*[3], K. Seto*[2]. [1]Oral Biochemistry, Kanagawa Dental College, Yokosuka, Japan, [2]1st Dept. of Oral and Maxillofacial Surgery, Tsurumi University School of Dental Medicine, Yokohama, Japan, [3]Orthopedic Surgery, Kitasato University School of Medicine, Sagamihara, Japan.

Cyclooxygenase-2 (COX-2) has been shown to play an essential role in bone formation during fracture repair.
We have reported that low-intensity pulsed ultrasound (US), which mechanically accelerate fracture healing 40% by time, targets osteoblasts but not osteocytes. The anabolic response of osteoblasts was obliterated either by blocking COX-2 with NS398 or by inhibiting p38 with SB203580 (JBMR, 2003). In order to substantiate a fundamental role of COX-2 in the mechanotransduction pathways, acceleration of fracture repair by US was studied in ***COX-2***$^{-/-}$ mice (B6; 129S7-*Ptgs2*^{tm1Jed}, Jackson Laboratories), which were subjected to the mid-diaphyseal femoral fracture with intramedullary fixation at relatively old age of 52±2 weeks. Half of the animals in each group were exposed to the ultrasound for 20 min a day, from the fourth day of surgery. Both exposure and control groups were anesthetized prior to the treatment using a SAFHS (Smith & Nephew Inc.) ultrasound generator equipped with probes 13-mm across (Teijin Limited). Conditions were identical to those used for patients and the bone cell cultures. Homozygous mutant and control mice were maintained on a mixed genetic background of 129S/C57BL/6J, and neither COX-2 mRNA nor protein was detected in tissues from homozygous mutant mice. Bone formation during the skeletal repair was monitored by contact X-ray, microCT, histochemical analyses, and real-time PCR. Radiographs and histologic sections of ***COX-2***$^{-/-}$ and wild-type mice after 10-day postfracture point showed detectable delay in healing of ***COX-2***$^{-/-}$ femora with and without the exposure to US. By day 21, the difference became apparent: 1) All wild-type femora exposed to US attained bony union. 2) If not treated with pulsed ultrasound, ***COX-2***$^{-/-}$ femora did not produce union. Only some of them resulted in mineralized callus formation. 3) Untreated wild-type and ***COX-2***$^{-/-}$ femora exposed to US were in between 1) and 2) in that order. Furthermore, we found that the amplified late upregulation of mRNAs such as IGF-I, which we reported in mechanically loaded cells, was lost in ***COX-2***$^{-/-}$ osteoblasts. The defect in upregulation, however, was at least in part rescued by the added PGE_2 at 1 hr, when the immediate early induction of COX-2 results in PGE_2 secretion. Taken together, these results suggest that COX-2 not only plays an important role in fracture repair, but also functions as an essential mediator of mechanotransduction leading to bone formation.

Disclosures: ***Y. Mikuni-Takagaki****, None.*

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Unloading-induced Bone Loss Occurs though the Central Control via Sympathetic System. H. Kondo[1], K. Tsuji[1], K. Kitahara[1], S. Rittling[2], A. Nifuji[1], D. Denhardt[3], G. Karsenty*[4], M. Noda[1]. [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Rutgers University, Piscataway, NJ, USA, [3]Dept of Nutritional Sciences, Rutgers University, New Brunswick, NJ, USA, [4]Dept of Molecular and Human Genetics, One Baylor Plaza, Huston, TX, USA.

Unloading in bed-ridden patients results in disuse osteoporosis, the number of which is soaring in our modern aging society. Although, it is well known that unloading causes rapid bone loss in vivo, the mechanisms underlying this phenomenon are not fully understood. Tail suspension enhances excretion of deoxypyridinoline into urine suggesting that not only bones in the hind limb but also systemic skeletal elements would be subjected to central control which would be regulated by tail suspension. One of the candidates for such systemic control may be central nervous system that stimulates sympathetic nerve to suppress bone formation. This paper examined whether sympathetic nervous system is involved in unloading-induced bone loss in tail suspension. Adult 129 mice were treated either with vehicle or with guanethidine which depletes noradrenaline and chemically impairs sympathetic system. Guanethidine alone did not affect bone volume levels. Tail suspension reduced cancellous bone volume (BV/TV) in vehcle-treated mice. In contrast, in guanethidine treatmented mice, such reduction in BV/TV was no longer observed even after tail suspension. Furthermore, propranolol, an antagonist against beta adrenergic receptor, prevented tail suspension-induced cancellous bone loss. Propranolol by itself did not alter basal levels of cancellous bone volume. Propranorol treatment prevented tail suspension-induced reduction in bone mineral density in the proximal end of tibia. In order to examine the cellular mechanisms underlying these observations, we conducted bone marrow cell cultures in the presence of ascorbic acid and beta glycerophosphete. Tail suspension suppressed mineralized nodule formation in the cultures of bone marrow cells compared to the cells oblained from control loaded mice. In contrast, bone marrow cells obtained from mice treated with propranolol no longer exhibited suppression of nodule formation by tail suspension. Similarly, bone marrow cells obtained from guanethidine treated mice did not show tail suspension-induced suppression in the levels of mineralized nodule formation in culture. These data indicated that central sympathetic control mediates the unloading–induced bone loss.

Disclosures: ***H. Kondo****, None.*

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Rescuing the Phenotype of Biglycan-Deficient Mice via Exercise. D. H. Kohn*[1], J. M. Wallace*[2], R. M. Rajacher*[2], X. Chen[3], S. Shi*[3], M. R. Allen*[4], S. A. Bloomfield*[4], P. G. Robey[3], M. F. Young[3]. [1]Biologic & Materials Sciences, University of Michigan, Ann Arbor, MI, USA, [2]Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA, [3]Nidcr, NIH, Bethesda, MD, USA, [4]Health and Kinesiology, Texas A & M University, College Station, TX, USA.

Biglycan-deficient mice exhibit lower bone volume than wild types, due to a deficiency in bone forming cells. We hypothesized that mechanical stimulation could rescue the phenotype of these knock-outs. At 8 wk of age, bgn-deficient and wild type male mice (C57BL6/129 background; 10 mice/group; NIDCR animal approval 001-151) were randomly assigned to control or exercise (treadmill running 10 m/min, 30 min/day, 21 days) groups. Bones were harvested for QCT and mechanical testing (4 point bending). Phenotypic changes in bgn-deficient mice were primarily exhibited in the tibia (Table). KO mice also have a trend towards a larger cortical area that is more isotropically distributed. However, this material is of lower quality, causing the bone to have lower strength and energy dissipation. This phenotype may be due to an upregulation of other matrix proteins responsible for promoting mineralization, causing over-mineralization and weaker, more brittle bone. This hypothesis is supported by the QCT data showing a significant increase in BMC and BMD in the KOs. Exercise lead to an increase in cross sectional area and post-yield deformation in the wild types, at the expense of strength. Exercise had a less significant effect on the geometry of the knockouts, but was accompanied by increases in yield force (p=0.005), post-yield displacement (p=0.044), total displacement (p=0.045), and ultimate energy (p=0.045). These properties were greater or equal to wild type control values, and the increase in properties due to exercise was at least as large in KOs as wild types. Exercise was therefore able to rescue the phenotype of bgn knockouts.

Tibiae Geometric and Mechanical Properties (mean ± SEM)

Property	WT Control	WT Run	KO Control	KO Run
Cortical Area (mm^2)	0.52 ± 0.04	0.63 ± 0.03[b]	0.62 ± 0.03	0.65 ± 0.02
Moment of I (mm^4)	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01[a]	0.06 ± 0.01
Yield Force (N)	23.1 ± 2.8[b]	15.9 ± 1.0	17.3 ± 2.2	23.3 ± 1.9[b]
Ultimate Force (N)	24.3 ± 2.6	22.5 ± 2.8	23.2 ± 2.1	28.5 ± 2.1
Elastic Disp. (mm)	0.08 ± 0.14	0.05 ± 0.01	0.05 ± 0.00[a]	0.058 ± 0.005
Plastic Disp. (mm)	0.02 ± 0.01	0.09 ± 0.03[b]	0.02 ± 0.00	0.036 ± 0.007[b]
Yield Energy (mJ)	1.18 ± 0.35	0.50 ± 0.07	0.58 ± 0.09[a]	0.88 ± 0.12
Ultimate Energy (mJ)	1.58 ± 0.44	1.88 ± 0.46	1.15 ± 0.15	1.81 ± 0.26[b]
Yield Stress (N/mm^2)	150 ± 33	85 ± 9	90 ± 12[a]	118 ± 12

a = significant effect of genotype; b = significant effect of exercise via 2-way ANOVA

Disclosures: ***D.H. Kohn****, None.*

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Knockout of Cyclooxygenase-2 (COX-2) in Mice Results in a Skeletal Phenotype, but Does not Influence Mechanical Responsiveness. I. Alam[1], S. J. Warden*[1], A. G. Robling[2], C. H. Turner[1]. [1]Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Anatomy & Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA.

Cyclooxygenase-2 (COX-2) is inductively expressed to control a rate-limiting step in the production of prostaglandins (PGs). PGs have effects on both bone formation and resorption, which are mediated through the proliferation and differentiation of osteoblasts and the regulation of osteoclast differentiation. Previous studies have shown that COX-2 mediated production of PGs is essential for the healing of fractures, and that inhibition of COX-2 interferes with skeletal mechanical responsiveness. This study compared adult COX-2 knockout (COX-2-/-) mice to wild-type (COX-2+/+) littermate controls to investigate the effect of COX-2 on bone mass and size, and explored the role of COX-2 in mechanotransduction. We identified that COX-2-/- mice had 11% lower body weight compared to COX-2+/+ mice ($p<0.01$). They also had smaller bones with long bone length being 4-5% shorter ($p<0.01$). On bone mass assessment, whole femoral and tibial BMC (normalized by body weight) was 22-29% lower in COX-2-/- mice by DXA ($p<0.01$), and femoral, tibial and humeral midshaft vBMD was 8-11% lower by pQCT ($p<0.05$). Similarly, vBMD in the proximal femur, tibia and humerus was 6-10% lower in COX-2-/- mice ($p<0.05$). Exposing ulnas of COX-2-/- and COX-2+/+ mice to an identical mechanical stimulus, no influence of genotype was observed on histomorphometric measures of bone formation (relative BFR/BS— COX-2+/+ 134.0±28.1 mm3/mm2/yr; COX-2-/- 180.8±41.1 mm3/mm2/yr). The results of this study are interesting from a couple of perspectives. Firstly, COX-2 is not believed to have a role in homeostasis as it is inductively expressed. However, we found COX-2-/- mice to be lighter and to have greater mortality than COX-2+/+ mice suggesting a role in normal physiological functioning. Secondly, we found the deletion of the gene that encodes for COX-2 to have no effect on skeletal mechanical responsiveness. This contradicts previous findings using COX-2 specific inhibitors. One explanation for this opposing finding is that COX-2-/- mice may have developed compensatory pathways for the absence of COX-2, pathways not developed when COX-2 is blocked temporarily using inhibitors. These findings highlight the need for further exploration of the role of COX-2 in the skeleton.

Disclosures: ***I. Alam****, None.*

1174

OPN Deficiency Increases Bone Formation and also Protects Tooth from Severe Root Resorption during Mechanical Stress-Induced Tooth Movement. C. Chung*[1], K. Kitahara[1], S. R. Rittling*[2], K. Tsuji[1], A. Nifuji[1], K. Soma*[3], D. T. Denhardt[2], M. Noda[1]. [1]Molecular pharmachology, Tokyo Medical and Dental Univ., Tokyo, Japan, [2]Rutgers Univ., Piscataway, NJ, USA, [3]Orthodontic Science, Tokyo Medical and Dental Univ., Tokyo, Japan.

Previously we have developed a new mechanical stress-induced tooth movement model in mice, which induces a reproducible mesial directed movement of the maxillary first molar accompanied by bone resorption on the pressure (mesial) side, and new bone apposition on the tension (distal) side resulting in a reverse pattern of bone remodeling within the alveolar bone. Since OPN, a major non-collagenous bone matrix protein, is induced by mechanical stress, affecting osteoclastic bone resorption and also bone formation, we have applied this model to an OPN-deficient mice to elucidated the direct role of OPN during mechanical stress induced tooth movement along with the underlying alveolar bone remodeling. Even though OPN is a well-known chemotactant of osteoclast precursors *in vitro*, OPN-deficiency did not affect the number of osteoclasts nor delay the tooth movement *in vivo*. On the contrary, tooth movement within the alveolar socket was significantly increased ($p<0.05$) and the tooth was protected from severe root resorption, with significantly less odontoclasts ($p<0.05$) in the OPN-deficient mice. Because OPN was also shown to regulate osteoblastic bone formation in calvaria to mechanical stress, we have also investigated bone formation during mechanical stress-induced tooth movement in the alveolar bone by using calcein/xylenol orange triple labeling. As the tooth moved within the alveolar socket, new bone apposition was detectable in the distal tension side in both WT and OPN-deficient mice, while the unloaded internal control did not. Mineral apposition rate (MAR) and bone formation rate (BFR) was significantly increased ($p<0.05$) in the OPN-deficient mice, suggesting increased movement to mechanical stress was in part due to increased bone apposition. We further investigated the mineral contents using energy dispersive spectrometer (EDS) attached to scanning electron microscope (SEM), however the basal level of Ca/P in tooth, alveolar bone, and periodontal ligament was similar in both group.

During clinical orthodontic treatment and in case of traumatic occlusion, root resorption is one of the most common and serious complications, because unlike bone, tooth cannot undergo remodeling and recover once it is severely destroyed. Since our data shows OPN deficiency to protect tooth from root resorption without disturbing the movement itself and to even increases bone formation to mechanical stress, regulation of OPN may be a way to secure smoother tooth movement *in vivo*.

Disclosures: ***C. Chung****, None.*

1175

Functional Modulation of LRP5-Wnt-Dkk1 Activity by Various Mutations in LRP5 Beta-Propeller1. B. M. Bhat[1], K. M. Allen*[2], J. Graham*[3], W. Liu*[3], A. Morales*[2], A. Anisowicz*[2], H. Lam*[1], C. McCauley*[1], V. Coleburn*[1], M. Cain*[3], E. Fortier*[3], H. Wang*[2], F. J. Bex[1], P. Yaworsky*[3]. [1]Women's Health and Bone, Wyeth Research, Collegeville, PA, USA, [2]Human Genetics, Genome Therapeutics Corp., Waltham, MA, USA, [3]Genomics, Wyeth Research, Cambridge, MA, USA.

Allelic forms of low-density lipoprotein receptor-related protein 5 (LRP5) have been identified in a spectrum of human bone density traits. A single point mutation (G to T) in the LRP5 gene results in a glycine to valine amino acid change (G171V) that is responsible for an autosomal dominant High Bone Mass (HBM) trait in two independent kindreds. Recently, 6 additional amino acid changes in LRP5 have been associated with increased bone mineral density in several sclerosing bone disorders. Loss of function mutations in LRP5 result in an autosomal recessive disorder of reduced bone mass, osteoporosis pseudoglioma syndrome (OPPG). LRP5 acts as a co-receptor with Frizzled family members to propagate cellular signals upon Wnt protein binding. This signaling can be antagonized by Dickkopf 1 (DKK1), a known LRP5 ligand. In the presence of Wnt, expression of LRP5 or the HBM variant, (LRP5-G171V mutant), induces nuclear translocation of beta-catenin and T cell factor (TCF)-luciferase reporter activity. When DKK1 is introduced, the HBM variant-mediated TCF activity is inhibited to a lesser degree than that with LRP5. Structural analysis of LRP5 revealed that the HBM mutation lies in the first beta-propeller domain. Additional substitutions of G171 to 171K, F, I or Q also resulted in HBM-like activity in the presence of Wnt and DKK1. This indicates the importance of this position to LRP5 receptor function. In order to determine if other amino acid changes might similarly affect function, we modeled the effect of the HBM mutation within beta-propeller1. Interestingly, mutations A65V, A214V and M282V but not E128V and G199V resulted in an HBM–like TCF-activity. We also simulated the G171V mutation in the other beta-propellers of LRP5, as well as LRP6. These substitutions all function as the wild type receptor. In conclusion, by mutation analysis and in vitro Wnt-Dkk1-TCF-functional studies, we have identified other mutations in beta-propeller1 that result in HBM-like properties. Interestingly, all the mutations associated with increased bone density lie in the first beta-propeller. Structural analysis of the LRP5 family member LDLR suggests that this first beta-propeller domain interacts with the ligand binding domains. Therefore, further analysis of additional mutations in the molecule may lead to a better understanding of the function of LRP5, and its interaction with Wnts and Dkks.

Disclosures: ***B.M. Bhat****, None.*

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The Osteoblast Homeoprotein Msx2 Promotes Osteogenic Differentiation of Aortic Myofibroblasts. S. Cheng, J. Shao, N. Charlton-Kachigian*, D. A. Towler. Div. of Bone and Mineral Diseases, Dept. of Int. Med, Washington University School of Medicine, St. Louis, MO, USA.

Calcific vasculopathy is prevalent in diabetes, contributing to cardiovascular mortality. In a murine model of type II diabetes, diabetogenic diets heterotopically activate an aortic osteogenic gene regulatory program that includes expression of the bone morphogen BMP2 and its genomic target Msx2 in aortic adventitial myofibroblasts. To evaluate the roles of BMP2 and Msx2 signaling in aortic calcification, we studied effects of BMP2 and Msx2 on primary aortic adventitial myofibroblasts. These myofibroblasts express vascular smooth muscle cell (VSMC) markers, and respond to BMP2 by upregulating Msx2 mRNA accumulation and enhancing osteogenic differentiation. Transduction of primary aortic myofibroblasts with a retrovirus expressing Msx2 also promotes osteogenic differentiation. Expression of osteoblast-specific differentiation factor osterix is upregulated 10-fold by Msx2, and mRNA level for the early osteoblast phenotypic marker alkaline phosphatase (ALP) increases >50-fold. ALP enzyme activity is also stimulated, and mineralized matrix nodule formation is enhanced 30-fold by Msx2. Whereas osteopontin and Runx2 gene expression is unchanged by Msx2, SM22α and VSMC α-actin are stimulated 3-4 fold. In contrast, PPARγ expression is drastically down regulated by Msx2 to 5% of the control level. Msx2 actions were further studied in the C3H10T1/2 model of mural mesenchymal lineage allocation. As in primary aortic myofibroblasts, Msx2 enhances osteogenic differentiation of C3H10T1/2 cells. Adipogenesis – revealed by lipid accumulation and expression of PPARγ and adipsin – is completely inhibited by Msx2 (confirmed in 3T3-L1 preadipocytes). While Msx2 activation of early osteogenesis is cell-autonomous, inhibition of adipogenesis occurs in part via a secreted adipostatic activity. In conclusion, Msx2 regulates lineage allocation of aortic myofibroblasts. Migratory adventitial myofibroblasts capable of adipogenic and osteogenic differentiation can be diverted to the mineralizing osteogenic lineage by Msx2, and thus contribute to the medial and neointimal calcification of diabetes.

Disclosures: ***S. Cheng****, None.*

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Osteoblast Gene Transcription from Connexin-Response Elements (CxREs) is Regulated by the PKC/Raf/MEK/ERK Pathway. J. P. Stains, R. Civitelli. Bone and Mineral Diseases, Washington University, St. Louis, MO, USA.

We have previously demonstrated that recessive (gene deletion) or dominant (connexin45 overexpression) disruption of connexin43 (Cx43) function results in osteoblast dysfunction and abnormal expression of osteoblast genes, including downregulation of osteocalcin and type I collagen transcription. Using ROS17/2.8 cells, which are highly coupled by Cx43, and ROS/Cx45 cells, where gap junctional communication is altered by

Cx45 overexpression, we have identified transcription elements in the osteocalcin and collagen (I)α1 proximal promoters which sense changes in gap junctional communication. These connexin-response elements (CxREs) provide gap junction sensitivity to heterologous promoters, and in non-osteoblastic cell contexts as well. Transcription via CxREs is regulated by recruitment of the ubiquitous transactivator Sp1 and the repressor Sp3. By chromatin immunoprecipitation, we demonstrate that perturbation of gap junctional communication by overexpression of Cx45 in an endogenous Cx43 background results in transcriptional repression consequent to increased Sp3 bound to the CxRE, while in a highly coupled environment (where Cx43 prevails) Sp1 is preferentially bound to the CxRE. We find that gap junctional communication alters Sp1/Sp3 recruitment via post-translational effects. Immunoprecipitations reveal decreased serine/threonine phosphorylation of Sp1 in Cx45 overexpressing cells, while acetylation of Sp3 is increased; both modifications affect Sp1/Sp3 binding affinity and transcriptional activity in a fashion compatible with transcriptional downregulation via CxRE. Since such post-translational modifications are modulated by MAPK activities, we used anti-phospho-PKC, -Raf and -ERK antibodies to show that PKC/Raf/ERK activation is reduced in a communication-deficient background. Furthermore, using luciferase reporter assays we show that inhibition of the Raf/MEK/ERK signal pathway with the MEK inhibitor U0126 results in markedly reduced transcription from the CxRE, whereas overexpression of a constitutively active MEK1, or treatment with PMA, an activator of the PKC pathway, increase CxRE-driven transcription. In contrast, p38 MAP Kinase and JNK/SAPK pathways do not regulate transcription from CxREs. Thus, gap junctional communication controls gene transcription by specifically modulating the PKC/Raf/MEK/ERK signal transduction cascade, which controls the activity of CxRE-binding transcription complexes. These findings offer a molecular mechanism by which intercellular communication via gap junctions modulate the response of bone forming cells to hormonal or local signals.

Disclosures: ***R. Civitelli****, None.*

1178

Regulation of the Anabolic Action of Calcineurin. L. Sun[1], H. C. Blair[2], B. S. Moonga[1], P. J. R. Bevis*[1], E. Abe[1], S. Epstein[1], M. Zaidi[1]. [1]Medicine, Mount Sinai School of Medicine and the Bronx VA GRECC, New York, NY, USA, [2]Pathology, University of Pittsburgh and Pittsburgh VA Medical Center, Pittsburgh, PA, USA.

Calcineurin, a ubiquitous calcium/calmodulin activated phosphatase, is inhibited by the commonly used immunosuppressants, cyclosporine A and FK506, both of which cause dramatic bone loss. Deletion of the calcineurin Aα gene thus resulted in severe osteoporosis arising mainly from a ~40% reduction in bone formation evident in tetracycline double labeling studies. Over-expression of calcineuirn Aα, in contrast, stimulated osteoblast differentiation. Profound, up to ~300-fold increases in osteoblast differentiation markers, namely, Runx-2, osteocalcin, bone sialoprotein, and alkaline phosphatase, were noted upon transduction of MC3T3.E1 osteoblasts with a calcineurin Aα-TAT fusion protein. All three isoforms of calcineurin A, α, β and γ, as well as those of calcineurin B, B1 and B2, were expressed in both primary osteoblasts and MC3T3.E1 cells. Traditionally, calcineurin dephosphorylates and *permits* the nuclear translocation of the transcription factor, NFATc. Here, we establish a new pathway for calcineurin action – its dephosphorylation of IκBβ, the inhibitory subunit of NFκB family of transcription factors. Dephosphorylated IκBβ binds to and *prevents* the nuclear translocation of p65, p50, and c-Rel. Through the use of complementary techniques – co-immunoprecipitation, chemical cross-linking, and phosphatase assays – we have demonstrated that calcineurin Aα binds to and dephosphorylates IκBβ, and that both effects are inhibited by NFATc. To understand the mechanism of calcineurin Aα-IκBβ binding, we used (a) peptide RII that selectively inhibits calcineurin's phosphatase activity by acting on its 18 amino acid-long, protein kinase domain, and (b) several IκBβ mutants either lacking the PEST domain (ΔPEST) or containing single amino acid substitutions within it (S313A and S315A). Peptide RII potently inhibited the calcineurin-induced dephosphorylation of IκBβ. Likewise, the ΔPEST and S313A mutants, but not the S315A mutant, blocked calcineurin-IκBβ binding and enhanced the nuclear translocation of the NFκB heterodimers, p65, p50 and c-Rel. Finally, whereas transfection with calcineurin Aα up-regulated the expression of ryanodine receptors, a family of Ca^{2+} release channels, the IκBβ mutant, S313A, when co-transfected with calcineurin Aα, blocked this effect. Together, the evidence appears compelling that calcineurin binds to, and dephosphorylates IκBβ, as an alternate substrate, *via* its PEST domain (Ser313). This selective dephosphorylation of IκBβ might, in fact, regulate the extent of calcineurin's novel anabolic action.

Disclosures: ***L. Sun****, None.*

1179

MSG-Sensitive Hypothalamic Neurons Contribute to the Control of Bone Mass. F. Elefteriou*[1], S. Takeda[1], X. Liu*[1], D. Armstrong*[2], G. Karsenty[1]. [1]Molecular and Human Genetics and Bone Disease Program of Texas, Baylor College of Medicine, Houston, TX, USA, [2]Pathology, Baylor College of Medicine, Houston, TX, USA.

Using chemical lesioning we previously identified hypothalamic neurons that are required for leptin antiosteogenic function. In the course of these studies we observed that destruction of neurons sensitive to monosodium glutamate (MSG) in arcuate nuclei did not affect bone mass. However MSG treatment leads to hypogonadism, a condition inducing bone loss. Therefore the normal bone mass of MSG-treated mice suggested that MSG-sensitive neurons may be implicated in the control of bone mass. To test this hypothesis we assessed bone resorption and bone formation parameters in MSG-treated mice. We show here that MSG-treated mice display the expected increase in bone resorption and that their normal bone mass is due to a concomitant increase in bone formation. Correction of MSG-induced hypogonadism by physiological doses of estradiol corrected the abnormal bone resorptive activity in MSG-treated mice and uncovered their high bone mass phenotype. Since Neuropeptide-Y (NPY) is highly expressed in MSG-sensitive neurons and since Y2-receptor deficient mice display a high bone mass phenotype, we tested whether NPY regulates bone formation. Surprisingly, NPY-deficient mice had a normal bone mass. This study reveals that distinct populations of hypothalamic neurons are required for the control of bone mass and demonstrates that MSG-sensitive neurons control bone formation in a leptin-independent manner. It also indicates that NPY deficiency does not affect bone mass.

Disclosures: ***F. Elefteriou****, None.*

1180

Evidence that the Mechanism Whereby Insulin-Like Growth Factor Binding Protein (IGFBP)-6 Markedly Inhibits Osteoblast Differentiation Is Through Sequestration of an Activator of Osteoblast Differentiation, the LIM Mineralization Protein-1 (LMP-1). D. D. Strong, T. A. Linkhart, Y. Gunn*, R. Kim*, J. Rung-Aroon*, S. Mohan, D. J. Baylink, Y. Amaar. Musculoskeletal Disease Center, Jerry L. Pettis VA Medical Center, Loma Linda, CA, USA.

Recent *in vitro* studies to overexpress IGFBP-6 (BP-6) in human osteoblasts, or to suppress BP-6 expression with antisense RNA have shown that this IGFBP strongly inhibits osteoblast differentiation (ie. it can completely halt osteoblast differentiation and it does so through an IGF-independent action). BP-6 is located primarily in the nucleus by virtue of its novel nuclear localization sequence. Thus we sought to test the hypothesis that BP-6 mediates its actions through inhibitory interactions with transcription factors or co-activator proteins that regulate osteoblast marker gene expression. To test this hypothesis, we utilized a yeast two-hybrid screen to identify intracellular proteins that bound to BP-6. BP-6 fused to the Gal-4 DNA binding domain was expressed in a yeast 2-hybrid bait vector and used to screen a U2 human osteosarcoma cDNA yeast library. BP-6 strongly interacted with a protein encoded by a cDNA fragment containing the LIM domain regions of LMP-1. Most importantly, LMP-1 was shown previously to induce osteoblast differentiation *in vitro* and induce bone formation *in vivo* (Boden et al., 1998). Northern blot analysis indicated that LMP-1 was expressed in human osteoblasts suggesting the hypothesis that BP-6 inhibits osteoblast differentiation by blocking the differentiation actions of LMP-1. Co-immunoprecipitation studies with lysates from SaOS-2 cells overexpressing HA-tagged-LMP-1 with recombinant BP-6 protein confirmed that BP-6 bound to the LMP-1 in mammalian cells. Co-immunoprecipitation studies also revealed that LMP-1 interacted strongly with BP-6 but not with IGFBP-5, demonstrating BP-6 specificity for this inhibitory interaction. When BP-6-red-fluorescent and LMP-1-green fluorescent fusion protein expression vectors were transiently co-transfected into osteoblasts, LMP-1 protein and BP-6 co-localized in the nucleus. In conclusion, the present findings that LMP-1 strongly binds to BP-6, is a strong inhibitor of osteoblast differentiation and past findings that LMP-1 is a positive regulator of osteoblast differentiation is consistent with the hypothesis that BP-6 sequesters LMP-1 to inhibit osteoblast differentiation.

Disclosures: ***D.D. Strong****, None.*

1181

Different Roles of Integrin Subunits to Stimulate Proliferation and Differentiation by Mechanical Stress in Osteoblasts. P. Müller*[1], C. Schmidt*[1], B. Nebe*[1], J. Rychly[2]. [1]Department of Internal Medicine, University of Rostock, Rostock, Germany, [2]Experimental Research Center, University of Rostock, Rostock, Germany.

To evaluate the role of integrin receptors in the mechanisms that are involved in the regulation of osteoblast physiology by mechanical stress we used a magnetic drag force device to apply physical forces on defined integrin receptors without stretching the whole cell. Magnetic beads were coated with different antibodies to integrin receptors and attached to the receptors on the dorsal surface of adherent cells. Forces on the receptors were directed in parallel to the cell surface. Application of physical forces to the β1 integrin subunit in cells of the osteoblastic cell line MG-63 induced an enhanced expression of collagen I as well as of proteins involved in the cell cycle regulation. The effects were increased compared with only clustering the receptor by incubation with β1 integrin antibody coated beads. This indicates that β1 integrin mediates mechanical stress to induce both differentiation and proliferation and the effects depend on the force strength. To see, whether different α integrin subunits play a specific role to mediate mechanical stress we applied physical forces to α2, α3 and α5 integrin subunits which were expressed in similar densities on the cell surface. Forces applied to all three α subunits induced an increased expression of cyclin D1, cyclin E and induced the phosphorylation of Rb protein. This indicated that the three integrins which we tested transduced physical forces to stimulate cell proliferation. However, application of forces to these integrin subunits revealed that only α5 but not α2 and α3 induced an enhanced expression of collagen I. Further analyses of signalling events demonstrated that only stress to integrin α5 provoked an activation of MAP-kinases and this was required for collagen expression due to mechanical stress. In addition, we found that only stress on integrin α5 induced the activation of the adaptor protein Shc. In contrast, forces to all three α integrin subunits activated the focal adhesion kinase (FAK) and induced the cytoskeletal immobilization of this protein. In conclusion, we found differences in the function of integrins to mediate mechanical stress to stimulate cell proliferation and collagen I expression. We suggest that collagen expression due to mechanical forces is mediated by α5 and requires the Shc pathway, whereas for proliferation various α integrin subunits may function as mechanotransducers which involves the activation of FAK.

Disclosures: ***J. Rychly****, None.*

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Caspase-3 Deficiency Causes Osteopenia by Attenuating Differentiation of Osteoblast Progenitors. M. Miura*[1], X. Chen[1], S. Gronthos[2], Y. Bi*[1], M. R. Allen*[3], S. Lakhani*[4], R. Flavell*[4], X. Feng*[5], P. G. Robey[1], M. Young[1], S. Shi*[1]. [1]Csdb, NIDCR, Bethesda, MD, USA, [2]Institute of Medical and Veterinary Science, South Australia, Australia, [3]3Texas A&M University, Galveston, TX, USA, [4]Yale University School of Medicine, New Haven, CT, USA, [5]Baylor College of Medicine, Houston, TX, USA.

Caspase-3 is a critical enzyme for apoptosis and cell survival. Apoptosis has important biological roles in the development, homeostasis of cell population, and in pathogenesis. Caspase-3 deficient (CPP32-/-) mice survive for 3-5 weeks and have various phenotypes including decreased apoptosis of neural cells and peripheral T cells and severely stunted growth. However, whether caspase-3 deficiency results in any bone defect is unclear. In this study, we tested the hypothesis that caspase-3 plays an important role in maintaining integrity of bone and found that both homozygous (CPP32-/-) and heterozygous (CPP32+/-) mice have reduced bone density. To determine the cellular basis for the reduced bone mass we examined pre-osteoblasts isolated from the calvarias of newborn normal and mutant mice and tested the relative levels of proliferation, differentiation and cell survival. Our results showed that caspase-3 deficient cells had increased proliferation rates, but decreased capacity to form a mineralized matrix. Examination of the intracellular signals elicited in response to TGF-beta revealed that caspase-3 deficient cells exhibited an increased expression of phospho-Smad2 and P21 as well as decreased expression of CDC2 and CDK4. Furthermore, caspase-3 deficient cells showed increased cell senescence and attenuated TGF-beta-regulated Cbfa1 expression. Inhibition of Smad2 expression by siRNA transfection was able to partially rescue TGF-beta-mediated down-regulation of CDK4 and CDC2, indicating that the Smad2 pathway is responsible for the TGF-beta-mediated cascade in caspase-3 deficient cells. In contrast, BMP2-induced phospho-Smad1 signaling was not impaired in pre-osteoblasts of CPP32-/- or CPP32+/- mice. Bone marrow stromal stem cells (BMSSCs), another population of osteogenic progenitors, showed similar results in terms of proliferation, differentiation and response to the TGF-beta induction. Moreover, the reduced bone density in caspase-3 deficient mice was not associated with an elevated osteoclast activity. Taken together, these findings demonstrate for the first time that caspase-3 is crucial for the proliferation and differentiation of osteogenic progenitors. Deficiency of caspase-3 may lead to osteopenia by attenuating differentiation of osteogenic progenitors through activation of the TGF-beta/Smad pathway.

Disclosures: ***M. Miura****, None.*

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c-Cbl-PI3-kinase Interaction Is Required for M-CSF-induced Osteoclast Migration and for Bone Resorption. A. Sanjay[1], T. Miyazaki[1], G. DeBlasi[1], K. Henriksen*[2], M. Ensig*[2], W. C. Horne[1], R. Baron[1]. [1]Orhtopaedics, Yale University School of Medicine, New Haven, CT, USA, [2]Nordic Biosciences, Copenhagen, Denmark.

The non-receptor tyrosine kinase Src and the proto-oncogene product c-Cbl, Src's major substrate in osteoclasts (OCs), are required for osteoclast motility and bone resorption. Phosphatidylinositol 3' kinase (PI3K), which is also necessary for normal OC migration and function, associates with c-Cbl. Both the SH2 and SH3 domains of the PI3K p85 regulatory subunit have been implicated in mediating the c-Cbl-PI3K interaction. c-Cbl's tyrosine 731 occurs in a sequence (YEAM) that is consensus motif for phosphorylation by Src and, when phosphorylated, for binding the SH2 domain of p85. In the present study, we show that mutating tyrosine 731 to phenylalanine, inhibiting Src kinase activity, by expressing a dominant-negative Src mutant or mutating the Src binding site on c-Cbl blocked the binding of PI3K to c-Cbl. Thus, the Cbl-PI3K association appears to require both the binding of Src to c-Cbl and the subsequent phosphorylation of tyrosine 731 of c-Cbl by Src. We then investigated the functional role of the c-Cbl-PI3K interaction in M-CSF-induced OC migration and in bone resorption, using c-Cbl-/- osteoclast-like cells (OCLs) reconstituted with various c-Cbl mutants. M-CSF (50 ng/ml), induced a 60% increase in migration of WT OCLs in a Boyden chamber migration assay. In contrast the migration of c-Cbl-/- OCLs, intrisically low, was not increased by the presence of M-CSF. As expected, expression of WT c-Cbl in c-Cbl-/- OCLs with the adenovirus system rescued the phenotype and M-CSF induced a 65% increase in migration, comparable to the response of WT OCLs to M-CSF. In contrast, over-expression of a c-Cbl molecule in which the tyrosine 731 is mutated, CblY731F, failed to rescue the M-CSF-induced motility of the c-Cbl-/- OCLs, demonstrating that the Src-dependent phosphorylation of Y731 and the recruitment of PI3-kinase by c-Cbl are required for osteoclast migration in response to M-CSF. Aws a consequence of impaired migration, the pit forming capability of Cbl Y731F-expressing-OCLs was also reduced to 30% of control. Taken together these results indicate that the Src kinase-dependent interaction of c-Cbl with PI3K is important for OC migration and bone resorption.

Disclosures: ***A. Sanjay****, None.*

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Regulation of Osteoclast Apoptosis and Bone-Resorbing Activity by Ubiqutination of Proapoptotic BH3-only Bcl-2 Family Member Bim. T. Akiyama[1], P. Bouillet*[2], U. Chung[1], A. Fukuda[1], H. Oda*[1], K. Nakamura*[1], A. Strasser*[2], S. Tanaka[1]. [1]Dept. of Orthopaedic Surgery, University of Tokyo, Tokyo, Japan, [2]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

Osteoclasts (OCs) are terminally differentiated cells with a short life span, and undergo rapid apoptosis without trophic factors such as macrophage colony-stimulating factor (M-CSF). There is accumulating evidence that pro-apoptotic and anti-apoptotic Bcl-2 family proteins play essential roles in regulating apoptosis, and we found that OC apoptosis was associated with a rapid and sustained increase in the pro-apoptotic BH3-only Bcl-2 family member Bim. This increase in Bim level was due to the reduced level of ubiquitination of Bim, and M-CSF treatment dramatically enhanced its ubiquitination. Overexpression of a degradation-resistant lysine-free Bim mutant in OCs completely abrogated the anti-apoptotic effect of M-CSF, while wild type Bim did not show such effects, suggesting that ubiquitination-dependent regulation of Bim levels is critical for regulating OC apoptosis.
In an attempt to clarify the role of Bim in the skeletal homeostasis, we analyzed the skeletal tissues of *bim-/-* mice. Histological examination and X-ray analysis revealed that *bim-/-* mice had mild osteopetrosis although the number of OCs was increased. Histomorphometry of *bim-/-* mice demonstrated that they showed low levels of both bone formation and bone resorption markers, leading to abnormally low bone turnover. In situ hybridization showed clear expression of *bim* gene in OCs but not in osteoblasts or chondrocytes in normal mice bone, indicating that the osteopetrosis of *bim-/-* mice is primarily caused by the abnormal function of OCs. Consistent with this in vivo observation, OCs differentiated from bone marrow cells of *bim-/-* animals showed significantly reduced bone-resorbing activity compared to *bim+/+* OCs in spite of their marked prolongation of survival, and adenovirus-mediated Bim introduction not only induced apoptosis of *bim-/-* OCs, but also normalized their bone-resorbing activity. These results show that Bim is regulated in a ubiquitination-dependent manner in OCs, and works as a key regulator of activation of OCs as well as their apoptosis.

Disclosures: ***T. Akiyama****, None.*

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Role of IKK Alpha in RANK-Mediated Osteoclastogenesis. M. L. Chaisson*, M. E. Tometsko*, A. P. Armstrong*, D. G. Branstetter*, W. C. Dougall. Cancer Biology, Amgen, Seattle, WA, USA.

Signaling through RANK is required for osteoclast, mammary gland, and lymph node development. However, the extent to which RANK utilizes similar signaling pathways in these different tissues remains to be determined. For instance, it has recently been shown that mice expressing a kinase inactive form of IκB kinase α (IKKα) have similar defects in mammary gland and lymph node development as RANK-null mice, but no apparent osteopetrotic phenotype. To further analyze the role of IKKα in RANK signaling in osteoclasts, we obtained fetal liver cells containing hematopoetic precursors from IKKα-null and wild-type E18.5 embryos. The fetal liver cells were cultured with CSF-1 and RANK ligand (RANKL) for 6 days and stained for TRAP activity. In wild-type fetal liver cells, this treatment led to formation of large, multinucleated $TRAP^+$ osteoclasts. However, in IKKα-null fetal liver cells, only small, primarily single-nucleated $TRAP^+$ cells were observed. TRAP expression, as assessed by quantitative RT-PCR, was 10-fold less in IKKα-null fetal liver cells treated with CSF+RANKL compared to wild-type cells. Similar decreases were observed for other osteoclast markers, including β3-integrin, MMP-9, and calcitonin receptor. Additionally, we observed a requirement for IKKα in RANK-mediated regulation of several chemokine genes. RANK upregulated expression of SDF-1 and MIP-1γ in wild type cells, and down-regulated expression of MIP-1β, MCP-1, and MIP-1α, effects that were dramatically decreased in IKKα-null cells. These effects were not due to a lack of osteoclast precursor potential in IKKα-null cells, as similar numbers of cells expressing macrophage markers F4/80 and CD11b were observed in wild-type and knockout fetal liver cultures after 1 week growth in CSF-1. As IKKα is a known regulator of NF-κB, we next analyzed the contribution of IKKα to RANK-mediated NF-κB activation in fetal liver cells grown in CSF-1. In both wild-type and IKKα-null cells, RANKL treatment caused complete degradation of IκBα by 15 minutes, followed by re-synthesis at 30 minutes, suggesting that osteoclastogenic defects observed in IKKα-null cells were not due to lack of classical NF-κB activation. Additionally, RANKL-treatment of IKKα-null cells resulted in normal phosphorylation of p38, JNK, and ERK, although a slight decrease in phospho-AKT was seen compared to wild-type. Although classical NF-κB activity was not impaired in IKKα-null cells, we did observe a 4-fold decrease in RANKL-mediated upregulation of p100 in IKKα-null cells compared to wild type. These data indicate that IKKα is required for RANK-mediated osteoclastogenesis *in vitro*, likely through activation of a non-canonical NF-κB pathway.

Disclosures: ***M.L. Chaisson****, Amgen 1, 3.*

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A New Mechanism for Osteoclast and Myeloma Cell Elimination. H. Das*, J. F. Bukowski*. Medicine, Harvard Medical School, Boston, MA, USA.

Mechanisms regulating osteoclast homoestasis in health and disease are not fully understood. Balance between bone formation and bone resorption is essential to maintaining healthy bone. Nitrogen-containing bisphosphonates (N-bisphosphonates) are directly toxic to osteoclasts, and act by blocking farnesyl diphosphate synthase, a key enzyme in the mevalonate pathway, thereby inhibiting prenylation of proteins important in intracellular trafficking. However, complete inhibition, and death of osteoclasts can take up to 72 hours. Gamma delta T lymphocytes comprise 2 to 5% of T lymphocytes in human blood and bone marrow. The predominant subset of these lymphocytes co-express V gamma 2 and V delta 2 T cell receptor chains on their cell surface, which uniformly recognize N-bisphosphonates. Engagement of this receptor by N-bisphosphonates triggers the secretion of large amounts of interferon gamma, a cytokine that inhibits osteoclast activity and inhibits tumor cell growth. These T cells can kill myeloma cells that have bound the N-bisphosphonate risedronate to their cell surface. Gamma delta T cells kill osteoclasts within 3 hours after contact, and pretreatment of osteoclasts with risedronate enhances this efficiency of killing by 3-fold.
Incubation of osteoclasts or monocytes on plastic plates coated with hydroxyapatite, then pretreated with risedronate and washed extensively, also renders these cells 3-fold more sensitive to killing by gamma delta T cells. Since monocytes do not resorb bone and do not

form pits in these hydroxyapatite plates as do osteoclasts, the data strongly suggest that bone resorption is not necessary for risedronate to release from hydroxyapatite and bind to cell surfaces in sufficient quantity to be detected by gamma delta T cells. In addition, direct exposure of osteoclasts, monocytes, or myeloma cells to soluble risedronate followed by extensive washing renders these cells susceptible to killing by gamma delta T cells.
Thus, a function of gamma delta T cells is to kill osteoclasts and myeloma cells. Exposure to N-bisphosphonates enhances the susceptibility of osteoclasts and myeloma cells to killing. These data suggest that N-bisphosphonates bound to bone can release from bone, and bind to osteoclasts or myeloma cells, rendering them susceptible to specific recognition and elimination by gamma delta T cells. This may be a mechanism whereby N-bisphosphonates inhibit bone resorption and decrease metastases to bone.

Disclosures: ***J.F. Bukowski****, Procter & Gamble Pharmaceuticals 3.*

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RGS12 Is Essential for Signaling by RANKL for Terminal Differentiation of Osteoclasts via Ca^{2+} Oscillation Pathway. S. Yang*, W. Chen*, Y. Abe*, Y. P. Li. The Forsyth Institute, Harvard School of Dental Medicine, Boston, MA, USA.

RANKL is essential for terminal differentiation of monocytes/macrophages into osteoclasts. Despite recent progresses in studies of the RANKL signaling mechanism, little is known about how RANKL specifically induces terminal differentiation of osteoclasts. To gain insight into RANKL signaling in differentiation of osteoclasts, we employed a genome-wide screening approach to identify genes that are specifically or predominately expressed in osteoclasts, using GeneChip. In particular, we focused on signaling factors in this screening. The most notable gene from the screening was RGS12. The expression of RGS12 in osteoclasts was found to be18 fold higher than that in stromal cell. Northern Blotting analysis confirmed the result obtained from Microarray assay. Immunocytochemistry using RGS12-specific antibodies showed that RANKL induced RGS12 expression in RAW264.7, monocyte progenitor cells. To demonstrate the importance of the RGS12 function in osteoclast differentiation, we used RNA interference (RNAi) technology to silence (knockdown) RGS12 expression in RAW264.7cells. Using a mammalian expression vector for small interfering RNA (siRNA), pAVU6+27 siRNA expression vector, we constructed mouse RGS12 siRNA vectors and introduced these into RAW264.7cells. The stable clones were tested for RGS12 expression by Immunostaining. In contrast to wild-type cells, those transfected with RGS12 siRNA contained undetectable RGS12 protein under conditions of RANKL induction. These RGS12 knockdown cells failed to differentiate into tartrate-resistant acid phosphatase positive multinucleated osteoclast like-cells. It was previously reported that RGS12 is a multifunctional protein capable of direct interactions through its PTB domain with the tyrosine-phosphorylated calcium channel. A recent study showed that RANKL induces terminal differentiation of monocyte/macrophage into osteoclasts by evoking Ca^{2+} oscillations via TRAF6 and c-Fos signaling pathways. These pervious works in other Laboratories led us to investigate RGS12 role in Ca^{2+} oscillations induced RANKL in osteoclast differentiation. Our data showed that strong Ca^{2+} oscillations occurred in RAW264.7 cells induced by RANKL for 24 hours. In contrast to RAW264.7 control cells, Ca^{2+} oscillations in RAW264.7 cells transfected with RGS12 siRNA-c6, which displayed a low level of RGS12 expression under RANKL induction, were markedly reduced. Furthermore, RAW264.7 cells transfected with RGS12 siRNA-c6 failed to form extracellualr acidification. Our results thus reveal that RGS12 is essential for terminal differentiation of osteoclasts induced by RANKL via Ca^{2+} oscillation pathway.

Disclosures: ***S. Yang****, The Forsyth Institute 3.*

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Mechanism of Action of TSH (Thyrotropin) on Bone. E. Abe[1], W. Yu[1], Y. Li[2], R. Marians*[1], T. Ando*[1], H. C. Blair[2], T. F. Davies*[1], M. Zaidi[1]. [1]Medicine, Mount Sinai School of Medicine, and the Bronx VA GRECC, New York, NY, USA, [2]Pathology, University of Pittsburgh and Pittsburgh VA Medical Center, Pittsburgh, PA, USA.

Deletion of the TSH receptor in mice resulted in a dramatic skeletal phenotype characterized by severe osteoporosis and focal sclerosis. Ex vivo bone marrow cultures showed enhancements in both osteoclast and osteoblast formation. Real time RT-PCR and FACS analysis revealed presence of TSH receptors on both osteoclast and osteoblast precursors and their concentration was increased during cell differentiation. Activation of these receptors by recombinant TSH resulted in a marked inhibition of the formation of mature cells. Together, the data point towards a primary role for TSH in the control of bone remodeling. Here, we have explored the molecular mechanism underlying the inhibition of osteoclast and osteoblast formation by TSH. For studies on osteoclast formation, we used TSH receptor-positive RAW-C3 cells, a sub-clone of RAW264.7 cells, which undergoes RANK-L-induced osteoclast differentiation. TSH inhibited both osteoclastogenesis and osteoclast survival in a concentration-dependent manner. TSH also attenuated the expression of several markers of osteoclast differentiation, namely cathepsin K, β_3 integrin, TRAP, and calcitonin receptor. Finally, TSH inhibited RANK-L-induced phosphorylation of Janus N-terminal kinase (JNK) within 15 minutes. This was accompanied by an equally impressive inhibition of the nuclear translocation of the transcription factor, c-jun. The other RANK-L-induced pathways involved in osteoclast formation and survival, namely, Akt, p38, and Erk1/2 remained unaffected, as did the nuclear translocation of c-fos. The anti-osteoclastogenic and pro-apoptotic actions of TSH thus appear to be exerted through reduced JNK phosphorylation, diminished c-jun nuclear translocation, and likely, the attenuated transcription of genes whose promoters have AP-1-binding sites. We next explored the mechanism of TSH-induced reduction in osteoblast differentiation using mouse calvarial osteoblasts. TSHR expression in the cells was confirmed by real time RT-PCR. TSH reduced the expression of the osteoblast differentiation markers alkaline phosphatase, bone sialoprotein and osteocalcin. It also potently inhibited the expression of osterix as well as Runx-2, a critical pro-differentiation transcription factors, whilst the expression of LRP-5 remained unchanged. Collectively, therefore, we have identified TSH as a powerful negative regulator of bone remodeling that inhibits osteoclast and osteoblast formation using distinct molecular pathways.

Disclosures: ***E. Abe****, None.*

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Identification of Three RANK Cytoplasmic Motifs Mediating Osteoclast Differentiation. W. Liu*, D. Xu, P. Patel*, H. Tsay*, X. Feng. Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

RANKL and its receptor RANK play a pivotal role in osteoclast (OC) differentiation and function. As a member of the TNF receptor superfamily, RANK may mediate intracellular signaling by recruiting signaling molecules such as TRAFs. Several groups previously attempted to characterize the RANK domains that interact with various TRAFs. Collectively, these groups showed that distinct RANK cytoplasmic regions interact with TRAFs 1, 2, 3, 5 and 6 in transformed cells in the context of over-expression and/or in *in vitro* binding assays. However, the data from these studies are conflicting, undermining the biological significance of these data. Moreover, specific RANK cytoplasmic motifs mediating OC differentiation and function have not been functionally identified. We have previously developed a chimeric receptor approach that permits us to carry out a detailed structure/function study of the RANK cytoplasmic domain in OC differentiation and function using authentic OCs. RANK cytoplasmic domain contains six putative TRAF-binding motifs (PTM): PTM1, ILLMTREE at a.a.286-293; PTM2, PSQPS at a.a.349-353; PTM3, PFQEP at a.a.369-373; PTM4, VYVSQTSQE at a.a.537-545; PTM5, PVQEET at a.a.559-564; PTM6, PVQEQG at a.a.604-609. Using this chimeric receptor approach, we showed that mutation of only one PTMs in RANK cytoplasmic domain had no effect on the RANK's ability to mediate OC differentiation, suggesting that some of the PTMs may be functionally redundant in regulating OC differentiation. To test this possibility, we constructed a mutant in which all six PTMs are mutated. Osteoclastogenesis assays with this mutant showed that this mutant failed to mediate OC formation, confirming that some of the PTMs are indeed functionally redundant. To elucidate which PTM is functional, we further engineered six more mutants: W1, W2, W3, W4, W5 and W6. In each of these 6 mutants only one PTM is NOT mutated. Specifically, PTM1 is not mutated in W1 while PTM2 is not mutated in W2, and so forth. These mutants would allow us to determine which PTM alone is able to induce OC formation in osteolastogenesis assays. Our data showed that W3, W5 and W6 are capable of mediating OC formation while W1, W2 and W4 failed to do so, revealing that PTM3, PTM5 and PTM6 are functional RANK cytoplasmic motifs that are capable of inducing OC formation. Significantly, our work presented here represents the first study establishing a functional link between specific RANK cytoplasmic motifs and a physiologically relevant readout: OC formation. More importantly, the identification of these functional RANK motifs has laid the foundation for delineating the downstream signaling pathways leading to OC formation in a more physiologically relevant manner.

Disclosures: ***W. Liu****, None.*

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Rescue of the Phenotype of RANKL-Deficient Mice by Hepatic Expression of Soluble RANKL Transgene. H. Yasuda[1], A. Minamida*[1], J. M. Penninger*[2], Y. Iwakura*[1]. [1]Institute of Medical Science, University of Tokyo, Tokyo, Japan, [2]Princess Margaret Hospital, University Health Network, Toronto, ON, Canada.

RANKL, a member of the TNF family, is a key regulator of osteoclastogenesis, lymphocyte development and lymph node (LN) organogenesis. RANKL is a membrane-bound ligand but its soluble form is released from different type of cells by shedding. RANKL knockout (KO) mice showed severe osteopetrosis, with no osteoclasts, marrow spaces, or tooth eruption, and exhibited profound growth retardation. These mice also showed defects in early differentiation of T and B cells, and lacked all LNs. We previously reported that transgenic (TG) mice overexpressing soluble RANKL (sRANKL) only in the liver after birth showed severe osteoporosis with an increase of osteoclasts. In human increases of serum sRANKL level were reported in patients with rheumatoid arthritis and juvenile Paget's disease, but the physiological and pathological roles of sRANKL are unknown. To understand the bona fide roles of sRANKL, we analyzed the phenotype of KO mice aged 4 to 6 weeks with hepatic expression of sRANKL transgene (hereafter called TG/KO mice). Expression of sRANKL in KO mice rescued osteoclast development entirely in long bones with marrow cavities but failed to restore tooth eruption and growth retardation. Dual-energy X-ray absorptiometry revealed that bone mineral densities of femur and tibia in TG/KO mice were markedly decreased compared with those of TG, KO and wild-type mice. In TG/KO mice the defects in differentiation of T and B cells were partially restored. The expression of sRANKL restored LN organogenesis in KO mice and TG/KO mice developed all LNs examined; e.g. inguinal, axillary, mandibular, and mesentery LNs. It is of interest that the serum sRANKL concentrations in TG/KO mice were variable (0.3 to 50 ng/ml) in individuals. Rescue of osteoclastogenesis and LN organogenesis was observed in all TG/KO mice examined. Surprisingly there were sRANKL concentration thresholds required in restoration of the defects in differentiation of T and B cells in TG/KO mice. The sRANKL concentration in wild-type mice was about 0.1 ng/ml, ranging 0 to 0.2 ng/ml. Rescue of the phenotype of KO mice with the minimal concentration (0.3 ng/ml) of hepatic-expressed sRANKL suggests that sRANKL may play an important role in osteoclastogenesis and LN organogenesis in physiological conditions. Hepatic expression of sRANKL rescued most of the phenotype of KO mice, except for tooth eruption and growth retardation, indicating that the membrane-bound RANKL is not essential for the restorations. In addition, the strict regulation of time- and tissue-specific expression of the RANKL gene may not be important for the functions of RANKL or sRANKL.

Disclosures: ***H. Yasuda****, None.*

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Overexpression of a Human *SOST* BMP-antagonist Results in Severe Limb Deformities in Mice. G. G. Loots*[1], M. Kneissel*[2], M. M. Glatt*[2], M. Khokha*[3], R. M. Harland*[3], E. M. Rubin*[4]. [1]Genomics Division, LLNL, Livermore, CA, USA, [2]Bone Metabolism Unit, Novartis Pharma, Basel, Switzerland, [3]Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA, USA, [4]DOE, Joint Genome Institute, Walnut Creek, CA, USA.

Sclerosteosis is a generalized progressive bone overgrowth disorder due to the loss of function of the *SOST* gene product sclerostin. Null mutations in the BMP-antagonist *SOST* result in occasional polydactyly and in a substantial progressive increase in bone mass. Accumulation of abnormal bone mass begins in adulthood and affected patients display increased bone formation, while bone resorption is undisturbed. To investigate the physiological function of this BMP antagonist, we generated several lines of transgenic mice carrying a ~160kb human *SOST* BAC. Following the expression pattern of the endogenous murine *SOST* gene, we investigated the expression pattern of the human transgene in several independent lines of transgenic animals. Similar to the murine *SOST* expression, the human *SOST* transcript was robustly expressed in the mineralized bones of fetal, neonatal and adult mice. Transgenic lines overexpressing human *SOST* grew to maturity with normal body size and weight and bone mass. Preliminary results from ongoing ageing studies revealed a small age-related decrease in bone mineral density in the appendicular and axial skeleton compared to non-transgenics from four to six months of age as evaluated by DEXA and pQCT. Further evaluations will show whether this trend will become more pronounced with ageing. When bred to homozygosity, transgenics showed congenital limb defects. The fore and hind limbs of these animals were severely deformed displaying a wide range of fused and missing digits as visualized by autoradiography and microCT. Since limb development is initiated prior to ossification of the skeleton, we examined the expression of the endogenous mouse *SOST* gene and the human transgene in the developing embryo. We found *SOST* to be expressed as early as E9.5dpc, predominantly in the mesenchyme tissue of the developing limb bud. In addition first results from a limited number of animals demonstrated decreased bone mineral density in the tibia of young adult homozygous mice. These initial characterizations suggest that excessive production of *SOST* can cause limb defects and possibly osteoporotic phenotypes and that transgenic mice overexpressing this BMP-antagonist could serve as a useful animal model for studying limb development and potentially bone formation.

Disclosures: ***G.G. Loots****, None.*

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Hox Proteins as BMP Downstream Transcription Factors. X. Li*, X. Cao*. Department of Pharmacology, University of Alabama at Birmingham, Birmingham, AL, USA.

BMPs are potent growth factors that induce osteoblast differentiation and bone formation. BMPs transduce their signals into the cell through a family of mediator proteins, Smads. Upon phosphorylation by BMP receptor, R-Smads such as Smad1 interact with Smad4 and translocate into the nucleus where it activates gene transcription and induces osteoblast differentiation. We have reported that Hoxc-8 acts as a transcription repressor. Smad1 interaction with Hoxc-8 dislodges Hoxc-8 from its element, resulting in the induction of gene expression leading to bone cell differentiation. Smad6 interacts with Hoxc-8 as a heterodimer when binding to DNA, and Smad6/Hoxc-8 complex inhibits interaction of Smad1 with Hoxc-8 and Smad1-induced transcription activity. There are 39 members within the Hox gene family arranged into four clusters. Based on the sequence similarity and position on the chromosomes, genes in the four clusters are divided into 13 paralogs. In this study, we systematically examined the potential interactions of Smad1, Smad4 and Smad6 with 13 Hox proteins that were chosen from each of the 13 paralogs including HoxA1, HoxA2, HoxB3, HoxB4, HoxA5, HoxB6, HoxB7, HoxA9, HoxD10, HoxA11, HoxD12 and HoxD13. We generated the 13 Hox expression vectors in both bacteria and mammalian cells. Each of the 13 purified GST-Hox proteins was examined for its interaction with Smad1, Smad4, and Smad6. Just like Hoxc-8, all of the Hox formed heterodimers with Smad6 when binding to osteopontin Hox binding element. Both Smad1 and Smad4 interact with all of the 13 Hox proteins and block binding of these Hox proteins to DNA element. These data suggest that all of the 39 Hox proteins interact with Smad1, Smad4 and Smad6, not TGF-β specific R-Smads, as major BMP downstream transcription factors. Furthermore, we examined the potential function of the interactions between Smads and Hox proteins in gene transcription and BMPs stimulated Osteoprotegrin (OPG) transcription. It has been shown that Hox sites mediate both OPG promoter construct activity and endogenous OPG gene expression in response to BMP stimulation. OPG promoter reporter construct was co-transfected with HoxA1, HoxB4 and HoxB7 expression plasmids in C3H10T1/2 cells treated with BMP-2. As expected, BMP-2 stimulated OPG promoter transcriptional activity, and Hox proteins inhibited BMP-2-mediated transactivation. Thus, our data indicate a fundamental Smad-mediated transcription mechanism, which likely is involved in all of the 39 Hox transcription factors. Our findings may help to understand the mechanism of BMP-regulated tissue patterning and bone development.

Disclosures: ***X. Li****, None.*

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Menin Is Required for BMP2-Induced Osteoblastic Differentiation Through the Interaction with Smad1/5 and Runx2. H. Sowa[1], H. Kaji[1], L. Canaff[2], G. N. Hendy[2], T. Tsukamoto*[1], T. Komori[3], K. Miyazono[4], T. Sugimoto[1], K. Chihara*[1]. [1]Endocrinology, Kobe University, Kobe, Japan, [2]Medicine, Mc Gill University, Montreal, PQ, Canada, [3]Molecular Medicine, Osaka University, Osaka, Japan, [4]Molecular Pathology, University of Tokyo, Tokyo, Japan.

Menin is the product of the MEN1 gene, which is responsible for multiple endocrine neoplasia type1. Menin is ubiquitously expressed, and homozygous menin knockout mice exhibit cranial and facial hypoplasia, suggesting the importance of menin in the regulation of bone formation. We previously demonstrated that menin interacts with the TGF-β signaling molecule, Smad3 (PNAS, 2001). Moreover, our recent study indicated that menin is required for the commitment of multipotential mesenchymal stem cells into the osteoblast lineage and inhibits the later differentiation of osteoblasts (JBC, 2003). In that study, inactivation of menin antagonized BMP-2-induced osteoblast differentiation in C3H10T1/2 cells, mouse mesenchymal cells, but not in mouse osteoblastic MC3T3-E1 cells. Smad1/5 and Runx2 are critical components of the BMP signaling pathway in osteoblast differentiation. The present study was therefore performed to clarify the mechanism by which menin inactivation antagonizes BMP-2-induced osteoblast differentiation. In mouse bone marrow stromal cell-lines, ST-2 and PA-6 cells, inactivation of menin with menin antisense oligonucleotides antagonized BMP-2-induced expression of osteocalcin and Runx2, and ALP activity, although it did not affect the chondrogenic and adipogenic differentiation induced by BMP-2. Menin was co-immunoprecipitated with Smad1 and Smad5 in ST-2 and MC3T3-E1 cells. Inactivation of menin with transfection of menin antisense DNA (AS) abrogated the Smad1/5-driven luciferase expression of 3GC2-lux in ST-2 cells but not in MC3T3-E1 cells. These results suggest that menin physically and functionally interacts with Smad1/5 in uncommitted mesenchymal stem cells, but not in well-differentiated osteoblasts. Secondly, menin was also co-immunoprecipitated with Runx2 in ST-2 cells but not in MC3T3-E1 cells. Moreover, AS antagonized the Runx2-driven luciferase expression of OSC-luci in ST-2 cells but not in MC3T3-E1 cells. These results indicate that menin interacts with Runx2 physically and functionally in uncommitted mesenchymal cells but not in well-differentiated cells. In conclusion, the present study suggests that menin is involved in BMP-2-induced commitment of multipotential mesenchymal stem cells into the osteoblast lineage, and this may be mediated by its interaction with Smad1/5 and Runx2.

Disclosures: ***H. Sowa****, None.*

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Regulation of FGF23 Expression but Not Metabolism by Phex. S. Liu, R. Guo*, Z. Xiao, L. D. Quarles. Medicine, Duke University, Durham, NC, USA.

Inactivating mutations of the type II cell-surface protease Phex is the cause of X-Linked Hypophosphatemia (XLH) and the disease phenotype in the Hyp-mouse homologue. FGF23, a phosphaturic factor, is increased in subjects with XLH and correlates with the degree of hypophosphatemia. Increments in FGF23 could result either from inactivating mutations of Phex that prevent the normal degradation of FGF23 or from increased biosynthesis of FGF23 caused by intermediate steps downstream of Phex. To distinguish between these two possibilities, we assessed Phex-dependent metabolism of FGF23 and measured FGF23 message levels in various tissues derived from normal and Hyp mice. We generated recombinant FGF23 and Phex proteins in COS-7 cells and translated FGF23 and Phex in rabbit reticulocyte lysates in vitro. In COS-7 cells full-length FGF23 was present in total cell lysates, but N-terminal and C-terminal fragments generated from cleavage at the RXXR site predominated in conditioned media (CM). Decanoyl-Arg-Val-Lys-Arg-chloromethyl-ketone (CMK) added to the culture media resulted in a dose-dependent inhibition of FGF23 processing in COS-7 cells, indicating that an endogenous furin-type convertase is responsible for FGF23 metabolism. Co-transfection of Phex with FGF23 in COS-7 cells did not result in additional metabolism of FGF23, either in the presence or absence of CMK. In addition, CMK did not inhibit Phex-dependent cleavage of the synthetic substrate ZAAL-pNa. Using in vitro translated proteins, a reduction in band intensity of FGF23 occurred after incubation with reticulocyte lysates expressing wild-type Phex, the inactive Phex-3'M mutant or vector alone, indicating that the apparent cleavage was non-specific. Since we could not demonstrate Phex-dependent cleavage of FGF23 either in vivo or in vitro, we examined the alternative possibility that FGF23 expression is increased in Phex-deficient Hyp mice. Using real-time PCR we found that FGF23 expression was highest in bone compared to other tissues. In addition, Hyp mice displayed significantly higher levels of FGF23 transcripts than normal controls in bone but not in bone marrow. Differential expression of FGF23 was also observed in Hyp compared to normal osteoblasts, but the overall abundance of FGF23 was very low in cultured osteoblasts compared to bone tissue. Although further studies are needed to determine the cell-type in bone that expresses FGF23, our findings indicate that FGF23 is not a direct Phex substrate, but its expression may be indirectly regulated in bone by the actions of Phex on unidentified substrates.

Disclosures: ***L.D. Quarles****, None.*

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Gremlin, a Bone Morphogenetic Protein Antagonist, Causes Severe Osteopenia I*n Vivo* by Distinct Mechanisms. E. Gazzerro[1], R. C. Pereira[1], V. Jorgetti*[2], A. N. Economides[3], E. Canalis[1]. [1]Department of Research, Saint Francis Hospital and Medical Center, Hartford, CT, USA, [2]Laboratoria de Fisibatologia Renal, Universidade de Sao Paulo, Sao Paulo, Brazil, [3]Functional Genomics, Regeneron Pharmaceuticals, Inc., Tarrytown, NY, USA.

Bone morphogenetic proteins (BMP) induce differentiation of stromal cells toward osteoblasts and enhance osteoblastic function. As such, they play a key role in bone development and in the maintenance of skeletal homeostasis. However, BMPs in excess are detrimental and their actions need to be tempered by intracellular and extracellular proteins. Extracellular antagonists bind BMPs with high affinity and prevent their binding to specific cell receptors. Although BMP antagonists share this common mechanism of action, specific activities are likely for each antagonist. Gremlin, a member of the DAN family of BMP antagonists, plays a role in the control of limb outgrowth, but its activity in postnatal skeletal formation is not known. *In vitro* gremlin prevents the effects of BMPs on osteoblastic function. To define the function of gremlin *in vivo*, we created transgenic mice overexpressing gremlin under the control of the rat osteocalcin promoter. Gremlin overexpression was confirmed by Northern blot analysis of RNA from calvaria of transgenic mice. The growth of gremlin transgenics was severely impaired and their weight was reduced by 30% compared to wild type littermates. The femoral length was half the length of control mice. Transgenic mice displayed tooth fragility. Histomorphometric analysis, performed at 3.5 weeks of age, revealed up to 50% reduction in trabecular bone volume and number in the femurs of gremlin overexpressing mice compared to age matched controls. Trabecular thickness was normal. The decrease in trabecular bone volume was accompanied by a marked decrease in the number of osteoblasts, whereas osteoclast number was normal. Since the osteocalcin promoter becomes active in mature osteoblastic cells, the effect of gremlin is probably due to increased osteoblastic apoptosis and not due to a decrease in osteoblastic cell formation. Gremlin overexpression induces a skeletal phenotype different from that displayed by transgenic mice overexpressing the BMP antagonist noggin under the control of the same osteocalcin promoter. Noggin overexpressing mice have a marked reduction in trabecular bone volume with no changes in osteoblast number, suggesting that the effect is secondary to a decrease in osteoblastic function and not number. These differences underline that different functions of BMPs are regulated by different antagonists. In conclusion, gremlin overexpression *in vivo* causes severe osteopenia secondary to a decrease in osteoblast number.

Disclosures: ***E. Gazzerro****, None.*

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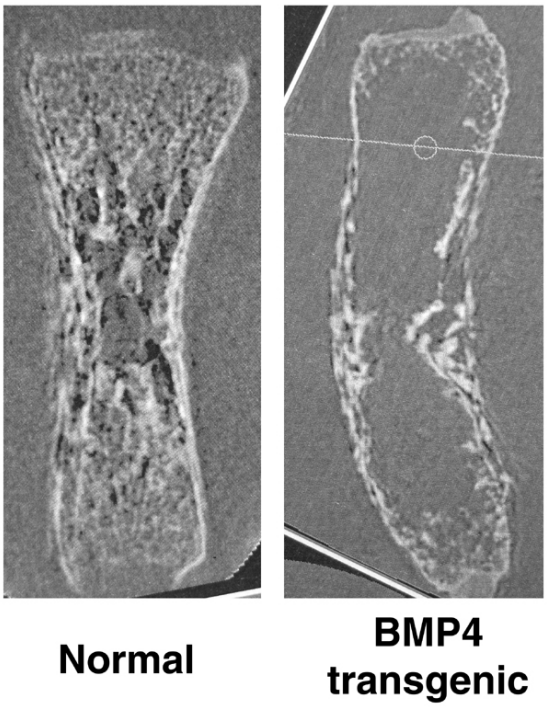
Overexpression of BMP4 in Osteoblasts Causes Severe Osteopenia in Transgenic Mice. N. Tsumaki*, M. Horiki*, Y. Hashimoto*, K. Kuriyama*, H. Yoshikawa. Orthopaedic Surgery, Osaka University Medical School, Suita, Japan.

Bone morphogenetic protein (BMP) family members such as BMP2, BMP4, and BMP7 are known to induce ectopic bone formation when implanted subucutaneusly. Extensive studies have revealed that BMPs promote non-committed cells to enter into osteoblastic lineage. These proteins thus have been candidates for medicine to treat bone diseases such as nonunion of fractures and osteoporosis. However, it remains to be clarified how BMPs control osteoblast differentiation, bone formation, and bone remodeling. To understand *in vivo* effect of BMPs on bone formation, we generated transgenic mice expressing BMP4 and Noggin to osteoblasts using the α1(I) collagen chain gene promoter sequence. Noggin is an antagonist of BMPs and believed to bind to various BMPs, preventing them to interact with their receptors.

[Materials and Methods] Transgene constructs were prepared by ligating BMP4 cDNA or Noggin cDNA to the 2.3 kb promoter sequence form the α1(I) collagen chain gene. By microinjecting these transgene inserts, transgenic mice were generated and analyzed.[Results and Discussion] Both the BMP4 and Noggin transgenic mice showed severe skeletal abnormalities and died at birth. Therefore we analyzed pleural generation zero (G_0) embryos at 18.5 d. p. c.. Histological analysis showed that normal mice limbs underwent endochondral ossification with solid bone formation in the center of each skeletal component at this stage. Limb skeleton of Noggin transgenic mice consisted of proliferative and hypertrophic chondrocytes, but lacked bone. In Noggin transgenic bone rudiments, BMP signals seemed to be blocked by excess amount of Noggin. These results may suggest that BMP signals are essential for bone development. BMP4 transgenic mice, on the other hand, showed bone formation. Analysis of longitudinal sections of humerus by using micro-CT showed that trabecular bone was significantly reduced in BMP4 transgenic mice than in Normal mice (Figure). Histological analysis revealed that BMP4 transgenic bone contained much less amount of blood vessels and larger number of TRAP-positive cells per bone than that of normal mice. Our results indicate that persistent expression of BMP4 in osteoblasts may cause osteopenia.



Disclosures: ***N. Tsumaki****, None.*

1197

Interferon γ Receptor Knock-Out Induces Bone Loss Through a Decrease in Bone Formation. G. Duque*[1], M. Macoritto*[1], N. Dion*[2], L. Ste Marie*[2], R. Kremer[1]. [1]Department of Medicine, Calcium Research Lab, McGill University, Montreal, PQ, Canada, [2]Centre Hospitalier de l'Universite de Montreal, Montreal, PQ, Canada.

Mesenchymal stem cells (MSCs) have the ability to differentiate into osteoblasts, adipocytes, chondrocytes or myocytes. In this study we first determine the genes involved in human MSC differentiation into osteoblasts by cDNA microarray analysis. We examined 12,000 human genes on the array Human Genome U95A (Affymetrix). In addition to the previously described pathways of osteoblast differentiation we found that a new set of genes known as "interferon-inducible genes" were rapidly and transiently up-regulated between 2.9 to 16.9 fold during this process. Furthermore we found that addition of interferon γ to human MSCs stimulates the expression of RunX2/Cbfa1, a key regulator of osteoblast differentiation. We next assessed the role of interferon γ on bone turnover in mice expressing the interferon gamma receptor knock-out phenotype ($IFGR^{-/-}$). $IFGR^{-/-}$ mice had over 40% reduction in bone mass at both hip and spine as compared to $IFGR^{+/+}$ mice. We also noted a significant decrease in structural parameters of trabecular mass by 3D micro-CT surface/volume (BS/BV) and trabecular thickness (Tb.Th) and a significant increase in trabecular separation (Tb.Sp), $p<0.01$. Histomorphometric analysis confirmed the decrease in BS/BV and Tb.Th and also demonstrated a significant decrease in cortical thickness (Ct.Wi) in $IFGR^{-/-}$ mice, $p<0.01$. Histological analysis of bone sections indicated a marked reduction of osteoblast staining (alkaline phosphatase and osteopontin) as well as a reduction in Cbfa1 and TRAPP staining ($p<0.01$) in $IFGR^{-/-}$ mice. Circulating levels of N-telopeptide RANKL and osteocalcin were also reduced by over 40% ($p<0.01$) in $IFGR^{-/-}$ mice. Finally bone marrow cells obtained from $IFGR^{-/-}$ mice and induced to differentiate into osteoblasts showed over 70% reduction in the number of mineralized nodules as compared to bone marrow cells obtained from $IFGR^{+/+}$ mice ($p<0.01$). Our data therefore demonstrate that interferon γ signaling regulates bone formation and that its deficiency results in low bone turnover osteoporosis.

Disclosures: ***G. Duque****, None.*

1198

Marrow Stromal Cells and Osteoclast Precursors Differentially Contribute to TNF-α Induced Osteoclastogenesis In Vivo. H. Kitaura, K. Aya, P. Zhou, S. Takeshita, F. P. Ross, S. L. Teitelbaum. Department of Pathology, Washington University, School of Medicine, St. Louis, MO, USA.

As documented primarily by in vitro experiments, marrow stromal cells, macrophages and T-cells are believed to participate in TNF-induced osteoclast recruitment. The relative contributions of each of the three cell types to inflammatory osteoclastogenesis and their roles as direct TNF targets, in vivo, are however unknown. To assess the role of stromal cells in TNF-induced osteoclast recruitment we generated two species of chimeric mice. Thus, wild type (WT) bone marrow, immunodepleted both T-cells and stromal cells was transplanted into irradiated mice lacking both TNF receptor type1 and type2 (WT to KO). In this condition osteoclast precursors but not stromal cells are capable of responding to TNF. As control, WT marrow was transplanted into WT mice (WT to WT). In each cir-

cumstance we prevented T-cell development by injection of anti-CD4 and anti-CD8 antibodies. Both groups of chimeric mice were administered increasing doses of TNF. At all doses of the cytokine the number of OCs measured histomorphometrically in vivo, is markedly reduced in WT to KO as compared to WT to WT chimeras (p<.001). Hence, TNF-responsive marrow stromal cells are essential for optimal TNF-induced osteoclastogenesis. Confirming this in vivo observation, a similar decrease in osteoclastogenesis occurs when marrow of TNF-injected WT to KO mice is cultured ex vivo in the presence of TNF (p<.001). TNF induces stromal cells to express RANKL (this meeting) suggesting the defect in OC formation in TNF-treated WT to KO mice reflects a relative paucity of RANKL. Consistent with this hypothesis, addition of RANKL to marrow cultures derived from TNF-treated WT to KO mice completely rescues their osteoclastogenic capacity. We have previously shown that RANKL sensitizes OC precursors to TNF-induced osteoclastogenesis. In the present exercise we find the converse, namely TNF sensitizes OC precursors to RANKL-induced osteoclastogenesis is also true. Thus, marrow derived from TNF treated both WT to WT and WT to KO mice generates substantially more osteoclasts in response to RANKL than does marrow from untreated mice (p<.001). The fact that the event is indistinguishable in marrow derived from WT to WT and WT to KO mice establishes that in contrast to TNF-induced RANKL production, TNF-induced RANKL sensitization is a stromal cell independent event. Thus, optimal TNF-induced osteoclastogenesis requires stromal cells which produce RANKL in response to TNF. On the other hand, TNF-induced RANKL sensitivity reflects direct targeting of TNF to OC precursors.

Disclosures: ***H. Kitaura****, None.*

1199

Hip Fracture Risk is Increased in Men at Both High and Low Levels of Serum Vitamin A. A. R. Opotowsky*, J. P. Bilezikian. Medicine, Columbia University, College of Physicians and Surgeons, New York, NY, USA.

One recent Swedish study suggests that high serum vitamin A levels are associated with increased risk of hip fracture among men. However, the population studied was notable for its generally high vitamin A intake and serum vitamin A levels. Therefore, it is unclear if these results apply to the US population in which the range of vitamin A is likely to be greater. We investigated the hypothesis that both high and low serum vitamin A predispose men to hip fracture. The NHANES I Epidemiologic Follow-up Study included 3,219 men 40 to 74 years old, of whom 51 had an incident hip fracture during the 22-year (μ=13.7±6.3) follow-up. As expected, vitamin A levels in this national sample were lower than those in the Swedish sample. The mean vitamin A level of the lowest quintile in this sample was 1.45 μmol/l compared with 1.72 μmol/l in the Swedish study. The relative risk (RR) of hip fracture for each quintile of baseline serum vitamin A as compared with the middle quintile was determined using Cox regression. There were 17 (2.6%), 12 (2.0%), 5 (0.8%), 4 (0.6%), and 13 (2.0%) incident hip fractures in the lowest to highest quintiles respectively. Controlling for age at exam, weight, height, and history of previous fractures, the RR (95% CI) of hip fracture for each quintile, compared with the middle quintile, respectively were 3.8 (1.4-10.6, p<0.01), 2.8 (1.0-8.1, p=0.06), 1.0 (reference), 0.7 (0.2-2.8, p=0.65), and 3.3 (1.2-9.5, p=0.03). We repeated the analysis using the parameters cited in the Swedish study linking high serum vitamin A to increased hip fracture risk. Those in the NHANES I sample who would have been classified in the highest quintile in the Swedish study (>2.64 μmol/l) were much more likely to sustain a hip fracture than those who would have been in the middle quintile in that study (2.17-2.36 μmol/l), RR=7.5 (1.7-33.8, p<0.01). However, subjects who would have been classified in the highest quintile in the Swedish study were no more likely to sustain a hip fracture than those in our lowest quintile, RR=0.8 (0.4-1.7, p=0.56). These results suggest that men with either high or low serum vitamin A are at increased risk of hip fracture. Prior studies concluding that only high serum vitamin A (or high vitamin A intake) confers increased risk may not be applicable to the general United States population. Rather, it appears that levels of vitamin A at both ends of the concentration range place men at significant risk for hip fracture.

Disclosures: ***J.P. Bilezikian****, None.*

1200

Smoking Influences the Association Between Estrogen Receptor Alpha Gene Polymorphisms and Bone Mineral Density: A Gene-Environment Interaction in the Framingham Offspring Study. A. M. Shearman[1], D. Karasik[2], L. A. Cupples*[3], S. Demissie*[3], S. Coven*[1], K. Gruenthal*[1], D. E. Housman*[1], D. P. Kiel[2]. [1]Ctr for Cancer Research, MIT, Cambridge, MA, USA, [2]Hebrew Rehab Ctr for Aged & Harvard Med Sch, Boston, MA, USA, [3]Biostatistics, BU Sch of Public Health, Boston, MA, USA.

There are conflicting results regarding the role of estrogen receptor alpha (ESR1) polymorphisms in the regulation of bone mineral density (BMD). Also, smoking affects estrogen metabolism, is associated with lower BMD and greater risk of osteoporosis, and may affect ESR1 transcription. We hypothesized that smoking might influence the association between ESR1 and BMD.

We studied 732 men and 792 women (mean age 60 ys, range 29-86) from the Framingham Study Offspring Cohort. BMD (g/cm^2) was measured by DXA at the hip (femoral neck, FN, trochanter, TR, Ward's area, WA) and L2-L4 lumbar spine (LS). DNA samples were genotyped for six polymorphisms: the TA repeat upstream of exon 1, +30T/C in exon 1, PvuII and XbaI in intron 1, +975C/G in exon 4, and *D6S440.* The two microsatellite repeat polymorphisms were recoded as presence/absence of the most frequent allele.

Information on age, height, body mass index (BMI), current cigarette smoking (y/n), and current estrogen use in women (y/n) was obtained at the time of BMD examination. We used ANOVA/ANCOVA to estimate the main effects of each polymorphism, as well as haplotype probabilities derived using the expectation-maximization algorithm, on crude and covariate-adjusted BMD in each sex separately.

High linkage disequilibrium was observed between the first four polymorphisms (D' = 0.82 - 0.99). 12.4% of men and 15.0% of women were current smokers.

No significant main associations between ESR1 polymorphisms and BMD were observed for any skeletal site, in either sex. In contrast, significant interactions between PvuII and XbaI genotypes, current smoking, and BMD (p = 0.004 to 0.014) were found in men. Interactions were also found between smoking, haplotypes px (frequency 55%) and PX (36%), and BMD at the hip in men (p = 0.002 and 0.031, respectively).

A substantial additive effect of combined PvuII and XbaI genotypes was found among male smokers (n=78): BMD was higher in pp-xx compared to PP-XX, difference of 12.3% (FN), 13.5% (TR), 19.8% (WA), and 8.5% (LS), pANOVA=0.06, 0.07, 0.03, and 0.05, respectively. No association was found in non-smoking men. In women, no interaction was found between ESR1 polymorphisms, current smoking, and BMD. In conclusion, the polymorphisms in intron 1 of the ESR1 gene are significantly associated with BMD in male smokers. These findings highlight the importance of considering smoking status when examining associations between ESR1 polymorphisms and BMD.

Disclosures: ***A.M. Shearman****, None.*

1201

Risk Factors of Hip Fracture in Women with High BMD: EPIDOS Study. J. A. Robbins[1], A. Schott*[2], P. Garnero[3], P. D. Delmas[4], P. J. Meunier[4]. [1]Internal Medicine, UC Davis, Sacramento, CA, USA, [2]Dept d'Info Med, Lyon, France, [3]Unit 403, INSERM/Synarc, Lyon, France, [4]Unit 403, INSERM, Lyon, France.

Older women with lower bone mineral density (BMD) are at greatest risk of hip fracture. Still, many fractures occur in those with higher BMDs. We used the EPIDOS data set to identify factors significantly predictive of hip fractures in women with high BMD but not those with low BMD. The cohort of 7562 non-institutionalized older French women, average age 80.5 (std, 3.8), was divided into 3 groups with equal numbers of incident hip fractures based on femoral neck BMD (97 ± 1 fractures/group). We did not make use of T-scores because of the many choices of available "normals". For this analysis follow-up averaged 3.6 years (std, 0.9) until study end, dropout, or hip fracture. All continuous variables were transformed to z-scores and were age adjusted to insure that the differences were not proxies for age. Cox proportional hazards examinations were carried out for each variable of possible interest in those in upper and lower ranges of BMD. A time dependent method was used to avoid confounding by mortality and/or drop out. Measures of strength, coordination, structures (ultrasound attenuation) and bone turnover, urinary deoxy pyridinoline (DPD) adjusted for creatinine in a sub-sample of the population, were investigated. Most factors traditionally associated with hip fracture were predictive in all groups; age, gait speed, right sided strength and coordination, and BMD measurements. The results for the extreme groups are presented in the table for those variables where they diverged. Hazards ratios (HR), and 95% confidence intervals (95% CI) for these HRs are shown.

	Low BMD (≤0.601gm/cm^2)				**High BMD (≥ 0.683 gm/cm^2)**			
Factor (per std or yes/no)	Sig	HR	95%CI		Sig	HR	95% CI	
Age	0.010	1.250	1.055	1.482	0.000	1.830	1.555	2.153
Urinary DPD	0.637	0.989	0.943	1.142	0.017	1.246	1.041	1.492
Ultrasound attenuation	0.160	0.818	0.618	1.083	0.000	0.577	0.453	0.734
Left foot coordination	0.226	1.119	0.933	1.342	0.017	1.246	1.040	1.492
Left grip strength	0.461	0.921	0.740	1.146	0.026	0.790	0.642	0.973
Couldn't heel/toe walk 4 steps	0.160	1.508	0.850	2.674	0.017	1.927	1.122	3.311
History of prior Fractures	0.140	0.738	0.493	1.105	0.031	1.550	1.042	2.309
Took sleeping pills regularly	0.006	1.786	1.183	2.697	0.083	0.702	0.407	1.048

In older women with what is usually accepted as non-osteoporotic range BMD, factors of weakness, in-coordination, and ultrasound, and bone turnover marker measurements, are associated with an increase in hip fractures. These factors are less important in those with lower BMDs. Sleeping pill use appears to be more associated with fractures in those with osteoporotic range BMDs. Low BMD remains the strongest associate of hip fracture but in those with higher BMD other factures related to fall risk and bone structure and turnover, which put women at risk for hip fractures, appear to play a role in hip fracture risk and may need to be addressed.

Disclosures: ***J.A. Robbins****, None.*

1202

Altered Sensitivity to Mechanical Load: A Potential Osteoporosis Phenotype. K. Uusi-Rasi[1], T. J. Beck[1], J. M. Zmuda[2], T. A. Hillier[3], L. Lui*[4], K. L. Stone*[4], L. M. Semanick*[1], S. R. Cummings[4]. [1]Johns Hopkins University, Baltimore, MD, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA, [3]Kaiser Permanente Center, Portland, OR, USA, [4]University of California, San Francisco, CA, USA.

Family history of fracture is an important risk factor for osteoporotic fractures, but the mechanism is unclear. We hypothesized that reduced sensitivity to skeletal load may produce weaker bones with thinner cortices that reach instability faster as bones expand and cortices thin with age. Differences in mechanosensitivity should alter the relationship between bone strength and skeletal load; estimated as the ratio of bending strength (section modulus - Z) to fat free mass (FFM by bioelectric impedance) as a crude index of muscle load. We evaluated hip geometries at the proximal femur of 2836 women from the Study of Osteoporotic Fractures who had repeated hip DXA scans (mean follow-up 3.7 y) analyzed with the Hip Structure Analysis (HSA) program and information on family history of hip

fracture. We studied whether women with incident hip fractures (Hip-Fx, n = 281) or other osteoporotic fractures (Other-Fx, n = 1324) have impaired mechanosensitivity (Z/FFM) than fracture free women (Fx-Free, n = 1231). To evaluate familial predisposition, we compared women who had a parent or sibling with hip fracture (+Hx, n = 650) to women without such history (-Hx, n = 2186) adjusting for age, knee-height and weight by ANCOVA. Hip-Fx had 8.8% (95% CI 6.7 to 11.0%) and Other-Fx 5.4% (3.9 to 6.8%) lower Z/FFM at the neck than Fx-Free. The respective values at the shaft were 5.1% (3.1 to 7.1%) and 3.6% (2.3 to 4.9%). Buckling ratio was 19% (15 to 24%) greater in Hip-Fx than in Fx-Free at both femoral neck and shaft with Other Fx intermediate. In family Hx groups, mean difference in Z/FFM was 2.5% (0.8 to 4.1%) at the neck and 1.8% (0.3 to 4.1%) at shaft in favor of -Hx group independent of Fx category. Buckling ratio was 5.6% (3.3 to 8.0%) and 3.3% (0.6 to 6.1%) greater among +Hx at neck and shaft, respectively. Bending strength declined and buckling ratio increased faster in Hip-Fx than in Fx-Free, mean differences were -0.8% (-1.2 to -0.4%) for bending strength and 0.9% (0.4 to 1.3%) for buckling ratio. No differences in rate of change in geometry were apparent between Hx groups. Our results suggest that mechanosensitivity is impaired in women with osteoporotic fractures compared to women who remain fracture-free. The association of mechanosensitivity with family Hx of hip fracture suggests an inherited trait while lack of an effect on rates of change suggests that mechanosensitivity levels may be fixed. The geometric conditions underlying differences in mechanosensitivity appear to lead to cortical dimensions that are more prone to buckling.

Disclosures: ***K. Uusi-Rasi****, None.*

1203

Higher 25-Hydroxyvitamin D Levels are Associated with Better Lower Extremity Function in Active and Inactive Ambulatory Elderly in the US. H. A. Bischoff[1], T. Dietrich*[2], E. J. Orav*[3], Y. Zhang*[4], E. W. Karlson*[1], B. Dawson-Hughes[5]. [1]Robert B. Brigham Arthritis and Musculoskeletal Clinical Research Center, Brigham and Women's Hospital, Boston, MA, USA, [2]Periodontology, Charité, Berlin, Germany, [3]Biostatistics, Div. of General Internal Medicine, Brigham and Women's Hospital, Boston, MA, USA, [4]Clinical Epidemiology Research and Training Unit, Boston University School of Medicine, Boston, MA, USA, [5]Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA.

Purpose: The aims of this study were to determine whether there is an association between 25-hydroxyvitamin D (25-OHD) levels and lower-extremity function in ambulatory elderly, and, if so, whether the association differs by activity level and whether there is an identifiable threshold in this association.
Subjects and Methods: Cross-sectional, representative sample of the US population (NHANES III; 4100 adults age 60-90+ years). Linear regression analyses and regression plots were used to assess functional performance by 25-OHD serum levels controlling for sex, age, race/ethnicity, BMI, calcium intake, poverty income ratio, number of medical comorbidities, self-reported arthritis, use of a walking device, month of measurement and activity level (inactive vs. active and METs in active elderly). Inactive subjects did not walk one mile without stop, swim, jog, bicycle, dance, exercise or garden in the last month (25% of subjects). Separate analyses were performed for the 8-feet walking speed (mean of 2 trials; in seconds [s]) and the sit-to-stand speed (5 repetitions; in s).
Results: Walking speed: compared to the lowest quintile of 25-OHD the highest quintile showed an average increase by 0.25s (test for trend; $p < .0001$). Sit-to-stand speed: compared to the lowest quintile of 25-OHD the highest quintile showed an average increase by 0.66s (test for trend; $p = .049$). In the regression plots, performance speed in both tests show the most pronounced improvement in the lowest 25-OHD levels. In the 25-OHD reference range of 22.5 to 94 nmol/l most of the improvement occurred in 25-OHD levels going from 22.5 to approximately 40 nmol/l. Further improvement was seen in the range of 40-94 nmol, but the magnitude was much less dramatic. In the adjusted analyses active subjects were 0.34s quicker in the walking test ($p < .0001$) and 0.80s quicker in the sit-to-stand test ($p < .0001$) than inactive subjects. However, there was no effect modification by activity level.
Conclusion: In both active and inactive ambulatory elderly subjects, 25-OHD levels above 40nmol/l and as high as 90 nmol/l, the upper end of the reference range, are associated with better musculoskeletal function in the lower extremities.

Disclosures: ***H.A. Bischoff****, None.*

1204

Secular Trends in Hip Fracture Incidence in California 1983-2000 for Different Ethnic Groups. S. L. Silverman[1], D. Zingmond*[2]. [1]OMC Clin Res Ctr, Beverly hills, CA, USA, [2]UCLA, Los Angeles, CA, USA.

Background: Rates of hip fracture have decreased among Nonhispanic White women in Rochester since 1970 (Melton, 2000). However, trends in hip fracture rates for ethnic minorities are unknown. The state of California has an ethnically diverse population and has maintained detailed data documenting hip fractures over the past two decades.
Purpose: To study secular trends in the incidence of hip fracture in California 1983-2000 among women and men from four different racial/ethnic groups: Nonhispanic White (NHW); African-American (AfrA); Hispanic American (HispA) and Asian American (AsA).
Methods: We used the California Office of Statewide Health Planning and Development annual hospital Patient Discharge Database, to identify all hospitalizations for acute hip fracture among individuals at non-Federal California general acute care hospitals from 1983 to 2000. Individual patient race/ethnicity, gender, and age are reported for each hospital discharge. Population estimates for race/ethnicity, gender, and age by year were estimated using U.S. Census and state Department of Commerce annual population estimates. For each race and gender, age adjusted rates were calculated restricted to those 55 years and older and standardized to the overall 2000 U.S. population age distribution by gender. Secular trends were fitted using weighted linear regression with robust variance estimators.
Results: Adjusted baseline rates for hip fractures among women were 733 fractures / 100,000 for NHW, 274 for AfrA, 245 for HispA, and 381 for AsA and among men were 251 for NHW, 156 for AfrA, 111 for HispA, and 111 for AsA. Estimated changes in hip fracture rates differ by both gender and by ethnicity. Among women over the past 18 years, hip fracture rates decreased by 4.5 fractures / 100,000 individuals / year in NHW ($p < 0.001$) and 4.3 in AsA ($p < 0.001$), were unchanged in AfrA (0.46 , $p = 0.560$), but increased by 5.4 in HispA ($p < 0.001$). Among men, rates increased among NHW by 1.57 ($p = 0.001$) and among HispA by 1.79 ($p < 0.001$). Changes in fracture rates for AfrA and AsA men were not significantly different from 0.
Conclusion: While hip fracture rates have declined significantly among NHW and AsA women over the past decade, rates have been unchanged among AfrA women and have increased significantly among HispA women. Further study is indicated to understand why risk of hip fracture is increasing among HispA women.

Disclosures: ***S.L. Silverman****, None.*

1205

Long-Term Effects of Poor Environmental Conditions During Early Life: Increased Prevalence of Hip Fractures among Holocaust Survivors. A. J. Foldes[1], J. Brodsky*[2], N. Bentur*[2]. [1]Osteoporosis Center, Hadassah University Hospital, Jerusalem, Israel, [2]JDC-Brookdale Institute of Gerontology, Jerusalem, Israel.

It has been suggested that senile osteoporosis is largely determined by peak bone mass which, in turn, is affected by a host of environmental factors. Comparing holocaust survivors to individuals of the same origin, who had not experienced the holocaust, provided us with a unique opportunity to test the relationship between detrimental conditions during early life and the prevalence of hip fractures (Fx) later in life.
Data were derived from a population survey that was conducted during 1997-8 on a sample of 2,669 Ashkenazi Jews (1,350 females, 1,319 males), 60 years of age or more (median age = 74), born in Europe before World War II. The prevalence of self-reported hip Fx (based on any previous diagnosis by a physician of a Fx at the pelvic bone or the femora) was recorded. Based on their personal history during World War II, the study participants were stratified into three groups: (1) Survivors of Nazi concentration or labor camps; (2) "Other" survivors, who lived under Nazi occupation, but did not experience camps; (3) Control group, consisting of those who did not live under Nazi occupation during WW II.

	Prevalence of hip Fx (%)				Odds ratio (camp survivors vs. controls)
	Total sample (n=2,669)	Camp survivors (n=452)	Other survivors (n=929)	Controls (n=1,288)	
Total sample	4.8	9.0	4.6	3.3	2.9
Females	5.7	11.4	5.5	3.9	3.0
Males	3.6	6.8	3.1	2.4	2.3
Age<75	2.8	9.4	2.2	0.8	13.1
Age≥75	8.1	7.9	9.5	7.3	NS

The following conclusions can be drawn: (1) An almost threefold higher prevalence of hip Fx was observed among survivors of Nazi camps (9.0%), as compared to controls (3.3%), with intermediate values for "other" survivors. The extent of this finding may even be underestimated, given the "survival of the fits" phenomenon. (2) The camp effect was limited to the younger cohort, which consisted of individuals who were 16-17 year-old or younger at the onset of WWII. This observation is also manifested by the unexpected finding, that among the camp survivors, hip Fx prevalence in the younger cohort exceeded that observed in the older cohort! (3) In the older cohort, that included individuals who were past the age of 16-17 at the onset of WW II, no camp effect was observed. (4) In the younger cohort, the prevalence of hip Fx in males (3.0%) approached that of females (2.7%), while in the older cohort, the expected higher prevalence of hip Fx in females (10.5%) compared to males (4.8%) was maintained.
The findings of this study support the notion that living under detrimental conditions during early life is associated with a higher risk of hip Fx later in life.

Disclosures: ***A.J. Foldes****, None.*

1206

Homecysteine, Bone Density and Fractures in Healthy Elderly People: The LASA Study. R. A. M. Dhonukshe-Rutten*[1], P. Lips[2], S. M. F. Pluijm*[3], L. C. P. de Groot*[1], W. A. van Staveren*[1]. [1]Human Nutrition and Epidemiology, Wageningen University, Wageningen, Netherlands, [2]Endocrinology, VU University Medical Center, Amsterdam, Netherlands, [3]EMGO Institute VU University Medical Center, Amsterdam, Netherlands.

Hyperhomocysteinemia may contribute to the development of osteoporosis. Some in vivo and in vitro studies support disturbance of cross-linking of collagen type I in bone by homocysteine (Hcy) as a possible mechanism. The relationship between homocysteine, bone mass and fracture incidence was studied in the Longitudinal Aging Study Amsterdam (LASA), a prospective cohort study which started in 1992. Subjects were 615 men and 652 women (mean age ± SD 76 ± 6.6 yr). Plasma samples were obtained in 1996 and
stored frozen until analysis. Plasma Hcy was measured by fluorescent polarisation immunoassay, osteocalcin was measured by IRMA and urine deoxypyrodinoline was measured

by competitive immunoassay.
Broadband ultrasound attenuation (BUA) was assessed in the heel bone (CUBA Clinical). A prospective follow-up of falls and fractures was done until 1999. Age- and sex-specific quartiles for (log-transformed) Hcy were used to calculate risk ratios for fractures. These were adjusted for age, BMI, smoking status, creatinine levels, falling and physical performance. Mean plasma Hcy ± SD was 13.5 ± 5.2 µmol/l. 42% of the men and 26% of the women had a high Hcy concentration (≥ 15.5 µmol/l). Serum osteocalcin and urine deoxypyrodinoline were positively associated with Hcy ($p < 0.001$). BUA was negatively associated with plasma Hcy (ß –1.8, $p< 0.02$ for men and ß –2.4, $p < 0.001$ for women). 21 Fractures occurred in men and 36 fractures occurred in women during the 3-years fracture follow-up. The adjusted risk ratio (95% confidence interval) for the highest homocysteine quartile for fractures was 3.3 (1.1;1.9) for men and 1.8 (0.8;3.9) for women and 2.2 (1.2;4.1) for the sexes combined, all compared with the lower three age- and sex-specific Hcy quartiles. There was no association between plasma Hcy and falls.
In conclusion, a high plasma Hcy was significantly associated with low BUA, high markers of bone turnover and increased fracture risk.

Disclosures: ***R.A.M. Dhonukshe-Rutten****, None.*

1207

Ultralow Estradiol Increases BMD and Decreases Bone Turnover in Older Women, Particularly those with Undetectable Estradiol: The ULTRA Trial. S. R. Cummings[1], V. Yankov*[2], K. Ensrud*[3], B. E. Ettinger[4], R. Wallace*[5], K. Johnson*[6], J. Macer*[1], E. Vittinghoff*[1], D. Grady*[1]. [1]Univ of Calif, San Francisco, CA, USA, [2]Berlex, Montville, NJ, USA, [3]Univ of Minn, Minneapolis, MN, USA, [4]Div of Research, Kaiser Permanente, Oakland, CA, USA, [5]Univ of Iowa, Iowa City, IA, USA, [6]Univ of Tennessee, Memphis, TN, USA.

Women with undetectable levels of estradiol (E2) have increased bone loss and fracture risk. Standard dose estrogen therapy reduces fracture risk but has serious adverse effects and, in women with a uterus, requires concomitant progestin to prevent endometrial cancer. We hypothesized that ultra low doses of E2 could be safely taken without a progestin and would improve bone resorption and BMD, particularly in those with undetectable E2 levels.
417 women age ≥ 60 years with hip BMD z-score >-2.0 and intact uteri were randomly assigned to ultra low TE2 (0.0125mg E2 daily via 3.25cm2 transdermal patch changed weekly) or placebo for 2 years. Follow-up measurements, including DXA and endometrial evaluation, were completed in 90% of participants and, of those, 99% used ≥75% of study patches. We measured pretreatment E2 by a double antibody assay that detects E2 ≥ 1.4 pg/ml, and measured SHBG by IRMA. We also analyzed outcomes stratified by undetectable vs. other quintiles of baseline E2/SHBG ratio, an index of bioavailable E2. We present results for undetectables vs. highest quintile of E2 at baseline.
In women assigned to ultra low TE2, E2 increased from a median of 4.8 pg/ml to only 7.5 pg/ml at 1 year (P<.001); E2 was undetectable in 13% at baseline and 4% at 1 year (P<.001). Overall, ultra low TE2 significantly decreased bone turnover and increased BMD (table). These effects were greater in women with undetectable baseline E2 than in those with higher E2/SHBG ratios (table). Safety and tolerability of TE2 was similar to that of placebo; there was no significant difference in incidence of endometrial proliferation or hyperplasia between groups.

		E2/SHBG Quintiles		*P-value for*
2-yr change*	All Women	*Undetectable*	*Highest*	*interaction†*
Osteocalcin	-15.6%	-33%	-9%	.005
Spine BMD	+2.4%	+3.5%	+1.2%	.10
Hip BMD	+1.5%	+2.4%	+0.9%	.11

*vs. placebo; P<.01 for all main effects. † Treatment-by-SHBG / E2 interaction

Ultralow TE2 decreased bone turnover and improved BMD in older women without causing endometrial proliferation. TE2 may produce greatest skeletal benefits in women with undetectable endogenous E2. Measurement of E2/SHBG may identify women with true 'estrogen-deficiency' who would substantially benefit from ultralow TE2.

Disclosures: ***S.R. Cummings****, None.*

1208

Short Term Response to Parathyroid Hormone (1-34hPTH) in Human Iliac Crest Bone using a Unique Quadruple (Double Double) Tetracycline Labeling Regimen and Single Biopsy. R. Lindsay[1], H. Zhou[1], F. Cosman[1], M. Bostrom[2], J. D. Cruz*[1], J. W. Nieves[1], D. W. Dempster[1]. [1]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Medicine, Hospital for Special Surgery, New York, NY, USA.

We describe here the early dynamic response of iliac crest bone to treatment with 1-34hPTH using a technique of quadruple labeling with two sets of tetracycline labels that allows longitudinal information from a single biopsy. Biopsies were obtained in 16 postmenopausal women (10 treated with 1-34hPTH [25mcg sc./day] for 7 weeks and 6 controls). Prior to treatment with PTH each individual received tetracycline labeling, using 2 different tetracyclines, following a standard protocol. Four weeks after initiation of treatment with 1-34hPTH, labeling was repeated with reverse order of tetracyclines, and biopsy performed 7 days after last label dose. Controls received identical labeling, but without PTH. Double labels were identified as first or second set based upon color sequence. Demographic data, and structural parameters in cancellous (Cn) and cortical (Ct) bone, were similar in both groups. In PTH treated individuals mineral apposition rate (MAR) and bone formation rate (BFR) increased from pretreatment (Cn 0.42 ± 0.09 to 0.64 ± 0.15 µm/day; 0.007 ± 0.002 to 0.025 ± 0.006 µm/µm2/day; $p<0.05$ and endosteal (En) 0.44 ± 0.09 to 0.66 ± 0.09; 0.025 ± 0.007 to 0.069+/-0.014: $p<0.05$) with PTH, while MAR and BFR remained constant or declined slightly in controls. The percent bone surface covered by labels increased in all subjects after PTH but not in controls. The average length of individual labels in the second set was greater than the first in Cn (0.66 ± 0.14 to 1.11 ± 0.27mm $p<0.02$) and in En (0.35 ± 0.07 to 0.54 ± 0.14, $p=0.01$) in PTH treated subjects but not in controls. In Ct the second set of labels was shorter after PTH, consistent with more rapid completion of Haversian systems. In PTH treated subjects, quadruple labels always occurred on eroded surface, but second labels also occurred 25% of the time on smooth surfaces, without evidence of prior resorption ($p<0.05$). Estimates of eroded surface were similar in both groups, suggesting little PTH induced increase in remodeling activation at 7 weeks. These data demonstrate the utility of quadruple tetracycline labeling to evaluate longitudinal short term changes in bone formation in a single iliac crest biopsy. The data support early stimulation of bone formation following administration of therapeutic doses of 1-34hPTH, without significant stimulation of resorption, and suggest strongly that the early PTH effects represent either surface annexation by osteoblasts spilling from remodeling sites and/or formation of bone on previously quiescent surfaces.

Disclosures: ***R. Lindsay****, Wyeth, P&G, Aventis, Lilly, Ilex, 2, 8.*

1209

Effects of Discontinuation of Teriparatide Treatment on Cortical Bone in Postmenopausal Women with Osteoporosis. C. E. Bogado*[1], A. Mango*[1], M. Sato[2], J. R. Zanchetta[1]. [1]Clinical Research, IDIM, Buenos Aires, Argentina, [2]Lilly Research Laboratories, Indianapolis, IN, USA.

We have previously reported the effects of teriparatide [rhPTH (1-34)] administration on cortical bone assessed by peripheral quantitative computed tomography (pQCT) in the distal radius of postmenopausal women with osteoporosis. Compared with placebo, patients showed significantly higher total bone mineral content, total and cortical bone areas, periosteal and endosteal circumferences and axial and polar moments of inertia.
Of the original study population, 93 women completed an 18-months observational study to determine the effects of discontinuation of teriparatide on the changes previously induced by the treatment on cortical bone. During the treatment phase patients received 1000 mg calcium and 400 IU vitamin D orally and either placebo (n= 26) or teriparatide at 20 (n= 37) and 40 ug (n= 28) as self-injected daily doses. Along the follow-up period patients did not receive any treatment for osteoporosis, or any drug that could affect bone metabolism other than the calcium and vitamin D supplements. Measurements were performed at the nondominant forearm, 6 and 18 months after discontinuation of therapy, at a site corresponding to 15% of the length of the ulna proximal to the radius distal end using a Stratec XCT 960 pQCT machine (Stratec Medizintechnik GmbH, Pforzheim, Germany).
There were no significant changes in the parameters of bone distribution and structural geometry during the study. Compared with placebo, total bone mineral content, total and cortical bone areas, periosteal and endosteal circumferences and axial and polar moments of inertia remained significantly higher 18 months after treatment discontinuation. There was a small, nonstatistically significant decrease in cortical density in the placebo group. In contrast, cortical bone density increased in the 40 ug group, although this change was not statistically significant. Cortical bone content did not change in any group.
In summary, there was no evidence of a detrimental effect on cortical bone density after treatment discontinuation. Moreover, the positive effects on bone distribution and structural geometry induced by teriparatide were still evident 18 months after teriparatide discontinuation. Since these favorable changes are associated with increased bone strength, our results are consistent with the reported evidence of durable effects of teriparatide for fracture risk reduction even after treatment is stopped.

Disclosures: ***C.E. Bogado****, None.*

1210

Osteogenic Activity of a Novel Synthetic Peptide Fragment of Human MEPE. T. Hayashibara*[1], T. Hiraga[1], B. Yi[2], M. Nomizu[3], Y. Kumagai[4], R. Nishimura[1], T. Yoneda[1]. [1]Dept Biochem, Osaka Univ Grad Sch Dent, Osaka, Japan, [2]Div Endocrinol, Univ Texas Hlth Sci Ctr, San Antonio, TX, USA, [3]Div Biosci, Hokkaido Univ Grad Sch Environ Earth Sci, Hokkaido, Japan, [4]Acologix, Inc., Emeryville, CA, USA.

Co-occurrence of disturbed phosphate homeostasis and skeletal disorders including rickets and osteomalacia in X-linked hypophosphatemic rickets and oncogenic hypophosphatemic osteomalacia suggests that the phosphate metabolism is associated with ossification. Although the mechanism of these diseases is largely unknown, it has been proposed that the yet-unidentified phosphaturic hormone named phosphatonin plays a role in the pathophysiology of these disorders. Matrix extracellular phosphoglycoprotein (MEPE) is one of the recently-identified candidates for phosphatonin. MEPE expression was found in osteoblasts/osteocytes and mice deficient in a MEPE homologue gene OF45 showed increased bone density, suggesting that MEPE produced in osteoblasts inhibits bone formation. In the present study, we examined the effects of a synthetic 23mer peptide (AC-100) containing RGD motif that corresponds to the region of 242-264 of human MEPE on bone formation in vitro and in vivo. Histological and histomorphometrical examination demonstrated that AC-100 (1, 10mg/ml) significantly promoted new bone formation with increased numbers of osteoblasts in organ cultures of neonatal mouse calvariae. These effects of AC-100 were comparable to those of rhBMP-2 (40ng/ml). Of interest, AC-100 enhanced PTH-rP-stimulated osteoclast-like cell formation in mouse bone marrow cultures. Repeated daily subcutaneous injections of AC-100 (20, 200mg) onto the mouse calvariae increased bone thickness and stimulated new bone formation as determined by calcein double-labeling to a similar extent to FGF-1 (12.5mg). A synthetic peptide in which the RGD sequence was scrambled into DRG failed to stimulate new bone formation, suggesting the interactions of the RGD sequence to integrins in osteoblasts are critical to

the osteogenic activity of AC-100. In support of this notion, AC-100 activated the integrin signaling molecules FAK and ERK in the MG-63 human osteoblastic osteosarcoma cells. Our results show that AC-100, a synthetic 23mer peptide fragment of human MEPE, increases new bone formation through activating osteoblasts, raising the possibility that MEPE, when cleaved, exhibits osteogenic activity in contrast to the full length MEPE. The results also suggest that the activation of integrin signaling in osteoblasts via RGD sequence is crucial to the osteogenic activity of AC-100. The anabolic effects of AC-100 may be beneficial for bone defects, fractures and bone diseases associated with decreased local bone mass.

Disclosures: ***T. Hayashibara****, None.*

1211

GLP-2 Is a Key Player in the Regulation of Bone Turnover: New Prophylaxis? D. B. Henriksen[1], P. Alexandersen*[2], T. L. Andersen*[1], I. Byrjalsen*[1], E. Gamwell Henriksen*[1], B. Hartmann*[3], C. Christiansen[2], J. Juul Holst*[3]. [1]Nordic Bioscience, Herlev, Denmark, [2]Center for Clinical & Basic Research, Ballerup, Denmark, [3]University of Copenhagen, Copenhagen, Denmark.

The circadian variation of bone resorption as derived from the collagen type I fragments measured in the CrossLaps (s-CTX) assay is significantly decreased in fasting individuals. This observation supports the idea that release of nutrients and minerals from the skeleton are tightly regulated to respond to food intake.

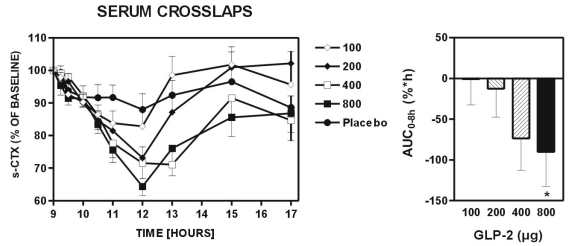
Studies of several gut hormones make it clear that these peptides are key regulatory hormones that transmit information generated during ingestion of food. Thus, the harvest from the skeletal reserve of both calcium and protein could be regulated by gut hormones to respond to ingestion of nutrition.

We have identified the gastrointestinal peptide, glucagon like peptide-2 (GLP-2) as a key factor in the regulation of bone resorption in response to food intake. This discovery lead to a placebo-controlled randomised study of the effect of single subcutaneous injection of GLP-2 in four different dosages or placebo (saline) in 60 postmenopausal women. GLP-2 caused a dose-dependent reduction in the s-CTX level from baseline. An area under the curve (AUC0-8h) analysis for s-CTX after GLP-2 injection confirm the dose-dependent decrease. Bone formation, as assessed by osteocalcin, was un-affected by the exogenous GLP-2 treatment.

The surprising acute effect of GLP-2 on bone resorption could indicate a direct action of the hormone on bone cells (osteoclasts and/or osteoblasts). We have located a GLP-2 receptor to be expressed by the osteoclast in both rat and human. Thus the mechanism of action for GLP-2 on bone turnover is most likely mediated through this bone tissue receptor.

These studies suggest that administration of GLP-2 results in a reduction of the bone resorption with no effect on the bone formation process and we interpret this action as a positive uncoupling of the bone turnover processes. A positive uncoupling of the bone turnover processes can potentially lead to a "passive" anabolic effect on the bone turnover, i.e. suppressing the bone resorption process and leave bone formation un-changed.

Thus, GLP-2 could provide a potential new drug for prevention and/or treatment of osteoporosis.



Disclosures: ***D.B. Henriksen****, Nordic Bioscience A/S 3.*

1212

An Orally Available, Non-Steroidal, Selective Androgen Receptor Modulator (SARM) Has Anabolic Activity in Osteopenic Female Rats. E. G. Vajda[1], W. C. Chang[1], E. C. Talaoc*[1], K. Burnett*[1], K. Griffiths*[1], E. Martinborough*[2]. [1]Pharmacology, Ligand Pharmaceuticals, San Diego, CA, USA, [2]Medicinal Chemistry, Ligand Pharmaceuticals, San Diego, CA, USA.

Both androgens and estrogens play a role in skeletal biology. Estrogen decreases bone remodeling in humans and animal models, but the role of androgens is not as clearly defined, particularly in women. Androgens have both anabolic and anti-resorptive activity in different models and may be useful in the treatment of osteoporosis. The potential for undesired prostate stimulation in men and virilizing activity in women however, has been a major limitation for the clinical use of androgens. In these studies, we report a novel, non-steroidal selective androgen receptor modulator (SARM) with tissue selectivity in castrated male rats and bone anabolic activity in osteopenic female rats. Nine week old male SD rats were castrated and immediately began treatment with the SARM or vehicle for 14 days. At doses that were fully efficacious at maintaining the levator ani muscle weight at intact levels (1 mg/kg p.o.), efficacy in the ventral prostate and seminal vesicle were 10% and 6% relative to intact controls, respectively. Efficacy in the preputial gland, a modified sebaceous gland, was 5%. Serum osteocalcin was elevated by castration and was restored to intact levels with SARM treatment, indicating changes in bone metabolism. Two month old female SD rats were ovariectomized (OVX) in order to induce osteopenia. Treatment with the SARM (0.1, 1, or 10 mg/kg p.o.), estradiol, or testosterone propionate was initiated 8-weeks post-OVX and continued for 12 weeks. Serum osteocalcin was significantly increased by OVX and significantly decreased by treatment. Serum total alkaline phosphatase was significantly increased with respect to OVX controls. DEXA scans at the mid-femur showed increased bone mineral density (8.3%) with respect to OVX control animals. Dynamic histomorphometry at the mid-femur revealed significantly increased periosteal bone formation (73.9% increase) in a dose-dependent manner. Femur bending strength was also significantly increased in biomechanical testing. In contrast to the effects of SARM treatment, periosteal bone formation rates were decreased and bending strength was unaffected in estradiol treated rats, demonstrating a distinctly different mechanism of action. Collectively, these results indicate that a SARM with tissue selective and potent bone anabolic activity may be a promising compound for the treatment of osteoporosis in men and/or women.

Disclosures: ***E.G. Vajda****, Ligand Pharmaceuticals 1, 3.*

1213

10.000 IU of Oral Vitamin D Per Day Are Required to Rapidly (3 months) Reach Adequate 25OHD in Osteoporotic Women. S. Mastaglia*, B. Oliveri, M. S. Parisi*, A. Cristófari*, C. Mautalen. Sección Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina.

Recently Heaney et. al. (J Am Coll Nutr 2003 Apr ,22(2):142-6) reported that 25OHD serum levels should be above 34 ng/ml (86 nmol/l) for adequate calcium absorption and beneficial for the treatment of osteoporosis. The dose of vitamin D required to rapidly reach adequate serum 25OHD levels (above 34ng/ml) has not been established to date.

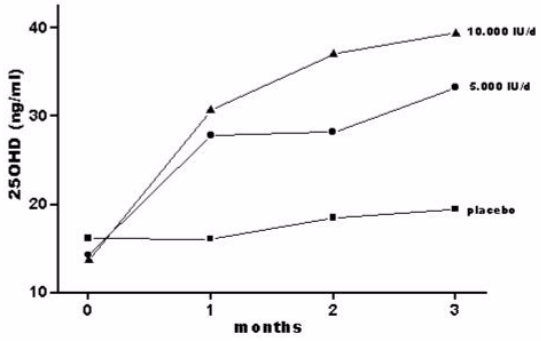
Thirty four women living in Buenos Aires (34° S) aged 61.7± 5.5 years, with 15.7± 6.1 years since menopause, average BMI: 27.7±4.7 and average T score of lumbar spine/ femoral neck: -2.5. The patients were randomized to receive placebo (G1) or vitamin D in a daily oral doses of 5.000 IU(G2) or 10.000 IU (G3). All the subjects received calcium supplementation (500mg/day/tablets).

Serum calcium, bone alkaline phosphatase (BALP), 25 hydroxivitamin D (25OHD), mm PTH, serum crosslaps (sCTX), and 24 hours urine calcium (uCa) and urine creatinine were measured at baseline and after 1, 2 and 3 months of treatment.

Average baseline 25OHD levels for the whole population was 14.2 IU ng/ml. All individual values were below 34 ng/ml. No differences in baseline measurements were found among the three groups. Serum and urinary calcium were within normal range. The average increments of 25OHD are shown in the figure.

At the end of 3 months 73% of group 3, 42% of group 2 and 0% of group 1 reached 25 OHD values above 34 ng/ml. Serum and urinary calcium values remained within normal range in all patients. No significant changes were observed in serum PTH, CTX or BAP.

In the light of the present results, the oral dose of vitamin D required to rapidly achieve adequate levels of 25OHD in osteoporotic postmenopausal females appears to be much higher than the usual recommended dose (800IU/day). Ten thousand units per day during 90 days was effective since 25OHD levels reached 39 ng/ml (range 28 to 55 ng/ml) and caused no side effects. Future studies should determine the maintenance dose, once the effective level has been achieved.



Disclosures: ***S. Mastaglia****, None.*

1214

Pre-treatment Bone Turnover and Fracture Efficacy of Alendronate: The Fracture Intervention Trial. D. C. Bauer[1], P. Garnero[2], M. C. Hochberg[3], A. Santora[4], P. Delmas[5], S. K. Ewing*[1], D. M. Black[1]. [1]UCSF, San Francisco, CA, USA, [2]Synarc, Lyon, France, [3]Univerisity of Maryland, Baltimore, MD, USA, [4]Merck Research Laboratories, Rahway, NJ, USA, [5]INSERM, Lyon, France.

Previous trials have demonstrated that bisphosphonate treatment reduces bone turnover and fracture risk among osteoporotic women. The effect of bone turnover rate prior to therapy on treatment efficacy has not been studied in large trials.

In the Fracture Intervention Trial, women age 55-80 with femoral neck BMD<0.68 g/cm2 were randomized to alendronate (ALN), 5-10 mg/d (n=3105) or placebo (PBO)(n=3081). At baseline 56% were osteoporotic, defined as those with prevalent vertebral fracture and/or BMDfn t-score <-2.5. Among these osteoporotic women, during a mean follow-up of 3.2 years, 492 non-spine fractures were documented and 285 incident vertebral fractures were identified on paired lateral spine x-rays. We used archived serum to measure pre-treatment levels of bone specific alkaline phosphatase (Bone ALP, Hybritech), and N-terminal propeptide of type 1 collagen (P1NP, Orion). C-terminal telopeptide of type 1 collage (sCTX, Crosslaps) was also measured, but only 20% of the cohort was fasting.

To determine the effect of pre-treatment bone turnover on fracture risk reduction, the risk

of spine and non-spine fracture was compared among ALN and PBO-treated subjects stratified into tertiles of baseline bone marker levels. Interactions between continuous baseline levels of markers and ALN efficacy were tested in age-adjusted proportional hazard and logistic models.
At baseline, mean (±SD) P1NP and bone ALP levels were 52.1±20.3 mg/dl and 14.0±4.5 mg/dl, respectively. The reduction in non-spine fractures with ALN treatment differed significantly among those with low and high levels of P1NP levels at baseline (Table), and similar trends were observed with bone ALP. Treatment efficacy for hip and morphometric spine fractures did not differ significantly according to baseline marker levels.
We conclude that in the Fracture Intervention Trial, reductions in non-spine fracture risk with ALN therapy are greater among osteoporotic women with high pre-treatment levels of bone turnover as assessed by P1NP.

Relative Risk of Non-spine Fracture (95% CI) for ALN vs. PBO by Tertile of Baseline Marker

Marker	Lowest Tertile	Middle Tertile	Highest Tertile	Tx*marker interaction p value
P1NP	0.89 (0.65, 1.21)	0.74 (0.54, 1.01)	0.54 (0.39, 0.74)	0.02
Bone ALP	0.83 (0.61, 1.13)	0.68 (0.49, 0.94)	0.61 (0.45, 0.83)	0.70

Disclosures: ***D.C. Bauer****, Merck R; GSK 2.*

1215

Hip Structure Analysis (HSA) Predicts Risk for Incident Hip Fracture in the Nursing Home. L. B. Yates[1], T. J. Beck[2], K. E. Broe[3], M. L. Bouxsein[4], D. P. Kiel[3]. [1]Harvard Medical School, Boston, MA, USA, [2]Johns Hopkins University, Baltimore, MD, USA, [3]Hebrew Rehabilitation Center for Aged, Boston, MA, USA, [4]Beth Israel Deaconess Medical Center, Boston, MA, USA.

Hip Structure Analysis (HSA) has been studied in community-dwelling populations to understand age-related differences in femoral geometry over the lifespan and to predict risk for hip fracture. To our knowledge, no study has applied HSA in an elderly nursing home population. We hypothesized that in this population older individuals would have less favorable HSA indices, and that these indices would predict incident hip fracture. We therefore performed DXA scans (Hologic QDR 1000) on 232 long-term care residents (168 women, 64 men), aged 67-102 y (mean 88 ± 6 y), and followed them for hip fracture, death or end of study (median follow-up 3.1 y). We divided subjects into three age groups: 67-84 y (n=61); 85-90 y (n=95); 91-102 y (n=76). We applied HSA to the DXA images to derive measurements at the narrowest point in the femoral neck, including BMD, cross-sectional area (CSA), subperiosteal width (W), endocortical diameter (ED), section modulus (Z, bending strength), buckling ratio (BR, cortical instability), and cortical thickness (CT). We used linear regression to analyze the association between age groups and HSA variables, and Cox proportional hazards to model risk for hip fracture, adjusting all models for baseline age, sex, height, weight, and need for assistance in ambulation (yes/no). Adjusted mean BR, ED, and W significantly differed ($p \leq 0.007$) by age group (oldest vs youngest, 4 to 7% difference), with the oldest group having the least favorable indices. There were no age group differences in CSA (p=0.56), BMD (p=0.34), or CT (p=0.33), although a trend was seen for Z (p=0.06). During follow-up 39 residents (17%) had hip fracture. For each standard deviation (SD) decrease in HSA variable at the femoral narrow neck, the hazard ratio (HR) and 95% CI for hip fracture are shown below.

Hip Fracture HR per -1 SD in HSA Variable (95% CI)

CSA	Z	BMD	CT	BR	W	ED
2.01	1.82	1.74	1.74	0.71	0.96	0.88
(1.14,3.53)	(1.03,3.21)	(1.09,2.77)	(1.09,2.77)	(0.53,0.95)	(0.58,1.57)	(0.56,1.40)

Our results indicate that age-related changes in hip structure occur even in very old persons and that measurements of femoral geometry predict risk for hip fracture in nursing home residents.

Disclosures: ***L.B. Yates****, None.*

1216

Hip Section Modulus, but not Bone Density, Shows the Effects a Decade Later of a Two-year Calcium Supplement Taken at the Time of Attainment of Peak Bone Mass. R. I. Price[1], N. K. Briffa[2], T. J. Beck[3], A. L. Mein*[2], B. C. C. Khoo*[1], R. L. Prince[1], S. S. Dhaliwal*[1]. [1]Sir Charles Gairdner Hospital, Nedlands, Australia, [2]Curtin University, Perth, Australia, [3]Johns Hopkins University, Baltimore, MD, USA.

Maximising premenopausal BMD is important for preventing fractures in old age. Calcium (Ca) intake is a significant agent for increasing BMD, but there is uncertainty as to the long term beneficial effect of a limited period of Ca supplement around the time of attainment of peak bone mass (PBM). Unlike DXA-derived areal BMD (aBMD), Hip Structural Analysis (HSA; [1]) describes bone structural geometry, in particular section modulus (Z; an index of bone strength), and may augment BMD as an assessor of bone fragility. This study examined changes in HSA and BMD in young women, over the decade after attainment of PBM.
Sixty-two women of mean (SD) age 27.8 (1.0) years were re-measured for aBMD and HSA, 9.4 (0.9) years following baseline measurements, on recruitment at age 18 into a 2-year randomised, controlled trial of Ca supplement (2). aBMD was measured at femoral neck (FN), trochanter (Tr) and intertroch. (IT). HSA variables were measured in duplicate at the "narrow neck" (NN) of FN, IT and shaft (Sh), using identical DXA (QDR1000W) images. Serial HSA data were unobtainable for 2 subjects. Thus 29 and 31 subjects were in the "placebo" (Pl) and "prior Ca supplement" followup groups. Dietary Ca was assessed at followup by questionnaire. Mean (SD) Ca intake was 895 (228) mg/d.
Focusing on section modulus, changes in Z over the decade were treatment and site dependent. NN Z increased from Mean (95% CI) of 1.33 (1.25, 1.41) cm3 to 1.43 (1.33, 1.53) (+0.8%/yr, p<0.003) for the Ca group, with a nonsignificant (NS) trend of +0.4%/yr in Pl. IT Z changes were NS for the Ca group (-0.7%, p<0.13), but IT Z increased from 3.71 (3.49, 3.93) to 4.09 (3.85, 4.33) (+1.1%/yr, p<0.04) for Pl. Sh Z increased from 2.01 (1.90, 2.12) to 2.23 (2.10, 2.36) (+1.2%/yr, p<0.03) for Pl, with NS for Ca. Prior Ca supplement had no effect on aBMD changes at any site (p<0.35) so treatment groups were combined in the BMD analysis. Over the decade, aBMD at hip sites declined [FN; -0.40%/yr (SD=0.62) (p<0.001): Tr; –0.34 (0.60) (p<0.001)], or was unchanged [IT; +0.12 (0.58) (p=0.10); Sh (at HSA shaft site); +0.32 (0.32) (NS)].
We conclude that, unlike hip aBMD which generally declined in the decade following attainment of peak bone mass, hip section modulus (and thus likely bone strength) generally increased. In sites of high cancellous content (eg FN) this increase was linked to prior Ca supplement, but at more cortical sites (eg Sh) it was conversely linked to the absence of Ca supplement. 1. Beck TJ et al, J Bone Miner Res 2001; 16: 1108. 2. Henderson NK et al, J Bone Miner Res 1995 10:1068

Disclosures: ***R.I. Price****, None.*

1217

Measurements of Plate and Rod Thickness in Human Trabecular Femoral Bone in osteoporosis and Osteoarthritis. A. Bonnassie*[1], F. Peyrin*[1], B. Brunet-Imbault*[2], C. Chappard[2], C. Benhamou[2]. [1]CREATIS UMR CNRS 5515, Villeurbanne, France, [2]Inserm ERIT-M 0101, Orléans, France.

3D X-ray microtomography which is increasingly used to analyze trabecular bone micro-architecture, enables computing architecture parameters without any model assumption. We propose a new geometrical analysis to identify the plate and rod structures in the 3D image and compute additional morphometric characteristics on each type of structure.
We analyzed two groups of subchondral bone in femoral head from osteoporostic patients (OP, n=8), and osteoarthritic patients (OAsc, n=8). Cylindrical cores (diameter 8mm) were imaged using 3D synchrotron radiation microtomography at the ESRF. In each 3D image (voxel size : 10.13 µm) a region of interest avoiding boundary effects related to bone cutting was selected (diameter : 6mm). 3D morphometric parameters of trabecular bone micro-architecture were computed using the Mean Intercept Length method and model independent techniques. We applied our new technique to label each voxel in the bone structure as a rod, a plate or a branching element. The result is illustrated in figure 1 where colors are associated to the different structures. After labeling, we computed the volume of each structure on the total volume, denoted PV/TV, NV/TV, RV/TV respectively for the plates, the rods and the branches. In addition the thickness of each type was also estimated as P.Th*, R.Th*, N.Th*.

Figure 1 : 3D display of the labeling of plates (light gray), rods (black) and branching (dark gray) structures in a femoral head bone in an osteoarthritic patient.
The two groups were found significantly different for all morphologic parameters. In addition, the geometrical analysis showed that the OAsc group presented a significantly higher volume of rods and branching component than the OP group. The trabecular thickness was also found significantly larger both for rods and plates in the Oasc group. In each structure (OP and Oasc), the percentage of plate and rod volume to the total volume were not significantly different, but the rod thickness was found significantly larger than the plate thickness.
The proposed method gives new insight in the morphologic analysis of trabecular bone micro-architecture.

Disclosures: ***F. Peyrin****, None.*

1218

Correlation of 2D Trabecular Structure Parameters with 3D μCT and Measurements of Bone Strength in Femoral Bone Cores. C. D. Arnaud[1], S. Liew*[1], D. Steines*[1], A. Nazarian*[2], R. Mueller*[3], B. J. Linder*[1], P. Lang*[1]. [1]Imaging Therapeutics, Inc., San Mateo, CA, USA, [2]Orthopedic Biomechanics Laboratory, Harvard Medical School, Boston, MA, USA, [3]Swiss Federal Institute of Technology, Zurich, Switzerland.

Bone mineral density measurements (BMD) identify subjects at risk for fracture and can help select those individuals who will benefit most from therapy. However, overlap exists in the distribution of BMD of patients with and without osteoporotic fracture, and BMD does not accurately predict the *presence* of fractures. Thus, factors other than BMD influence fracture risk. Key among those factors are alterations of trabecular structure. Most procedures for assessing bone structure are invasive (i.e. histomorphometry, 3D μCT) or expensive (i.e. MRI). We developed automated hip radiographic imaging technology that measures a host of parameters of the geometry and connectivity of trabecular structure, using routine proximal hip radiographs. It involves x-ray digitization, identification of regions of interest, background subtraction, extraction of trabecular structures, and binarization and skeletonization of those structures. To verify our ability to use this 2D x-ray technology to quantitatively assess trabecular architecture, we compared it with 3D μCT, a gold standard for such measurements. Furthermore, results from our techniques were compared to biomechanical bone properties.

Bone cores (n=49) were harvested from cadaveric proximal femora. Specimen radiographs were obtained and 2D structural parameters were measured on the radiographs. Cores were then subjected to 3D μCT and biomechanical testing. The 2D structural parameters measured included trabecular area ratio (AR), length (L), intensity (I) and thickness (T). Pearson's correlation coefficients (r) between 2D structure parameters and biomechanical failure load, biomechanical stiffness, and corresponding 3D μCT parameters, respectively, are listed in the table below.

We conclude that measurements of bone structure on femoral bone core radiographs correlate highly with bone failure loads and stiffness as well as 3D μCT measurements. These results suggest that inexpensive proximal femoral trabecular structural analysis from hip radiographs may yield vastly improved diagnostic assessment of osteoporosis and estimation of fracture risk.

*p<0.001	AR	L	I	T
Failure load	0.74*	0.81*	0.83*	0.81*
Stiffness	0.69*	0.74*	0.77*	0.75*
μCT	0.90 (with BV/TV)	0.84* (with BV/TV)	0.96* (with TbTh)	0.87* (with TbTh)

Disclosures: ***C.D. Arnaud****, None.*

1219

Chemical Composition Distinguishes Fracture from Non-Fracture Subjects Matched by Bone Mass. B. R. McCreadie[1], M. D. Morris*[2], T. Chen*[2], W. F. Finney*[2], E. Widjaja*[2], S. A. Goldstein[1]. [1]Orthopaedic Surgery, University of Michigan, Ann Arbor, MI, USA, [2]Chemistry, University of Michigan, Ann Arbor, MI, USA.

Although bone mineral density is used clinically to evaluate fracture risk, a large overlap exists between those who go on to fracture and those who do not. This study sought to determine whether the chemical composition of the bone extracellular matrix influences fracture risk.

Trabecular bone specimens were obtained from two groups of females, fractured (undergoing arthroplasty) and unfractured at death, matched for age and bone volume fraction. All specimens were taken from a location adjacent to the fracture site in most of the fracture cases. An epi-illumination Raman microspectroscopic imaging system was used to obtain Raman scatter spectra from each specimen. Ratios of the band areas of phosphate ν_1, carbonate ν_1 and collagen amide I were used as measures of carbonate/phosphate and mineral/matrix (phosphate/amide I).

A clear relationship was found between the carbonate/phosphate and phosphate/amide I ratios. The carbonate/phosphate ratio is essentially constant over much of the range, but increases considerably at the extreme low end of the phosphate/amide I ratio. The fracture specimens are concentrated at the low end of the carbonate/amide I range. A statistical comparison of carbonate/phosphate ratio yielded a p-value of 0.08, while for carbonate/amide I was 0.28.

In groups matched for bone mass, we were able to make a distinct separation between many females who did not fracture compared to those who did. The modest overlap in specimens may be a result of difficulties in defining a true control population. Some of the individuals that died without fracture would likely have sustained a fracture had they lived longer.

It is unclear whether the differences seen at the fracture site are local or systemic. If the differences in chemical composition are limited to the fracture site, these results suggest a large increase in remodeling prior to overt fracture. If the differences are systemic, it suggests that some individuals may be more susceptible to fracture based on the chemical constituency of bone matrix.

This study is the first to demonstrate a relationship between carbonate/phosphate and phosphate /amide I ratios. In addition, the results suggest a novel and potentially robust method for predicting risk of fracture, either alone or in combination with bone mineral density. It should be possible to develop spectroscopic instrumentation to conduct convenient non-invasive or minimally-invasive measurement of the relevant chemical constituents.

Disclosures: ***B.R. McCreadie****, Potential Commercialization of Technology 7.*

1220

Comparison of Areal and Volumetric Vertebral Bone Densities Measured by DXA and CT in Healthy Girls. T. A. L. Wren[1], A. Kovanlikaya*[2], X. D. Liu*[2], P. D. Pitukcheewanont*[3], V. Gilsanz[2]. [1]Orthopaedic Surgery and Radiology, Childrens Hospital Los Angeles, Los Angeles, CA, USA, [2]Radiology, Childrens Hospital Los Angeles, Los Angeles, CA, USA, [3]Endocrinology and Metabolism, Childrens Hospital Los Angeles, Los Angeles, CA, USA.

Dual-energy x-ray absorptiometry (DXA) is the most frequently used technique for measuring bone density in both children, although in growing subjects, areal bone mineral density (BMD) measurements are influenced by changes in bone size and soft tissue composition. The purpose of this study was to determine the agreement between DXA areal BMD values and CT volumetric bone density (BD) values. DXA areal BMD and Z-score (Z_{DXA}) were compared with CT volumetric BD and Z-score (Z_{CT}), and to CT measures of cross-sectional area (CSA) in 100 healthy girls, ages 5 - 20 years. Z-score represents the number of standard deviations (SD) the BMD or BD is above or below the mean for sex- and age-matched controls. Moderate correlations were observed between DXA and CT densities (r=.688, p<.0001) and between DXA and CT Z-scores (r=.624, p<.0001). A moderate correlation was also present between BMD and CSA (r=.662, p<.0001). The density correlations were weaker in subjects under 12 yr (r=.203, p=.54 for density; r=.449, p = .15 for Z-score) than subjects 12 yr and older (r=.668, p<.0001 for density; r=.652, p<.0001 for Z-score), while the correlations of BMD with CSA were stronger in subjects under 12 yr (r=.757, p=.0030) than older subjects (r=.394, p<.0001). Paired t-tests indicated that the DXA Z-scores were systematically lower than the CT Z-scores (p<.0001). We conclude that increases in DXA areal BMD of the lumbar vertebrae in girls under 12 yrs old are likely a reflection of changes in vertebral size rather than changes in density and that caution should be exercised before girls of any age are diagnosed with osteoporosis based on DXA measurements.

Disclosures: ***T.A.L. Wren****, None.*

1221

New Osteoporosis Screening Recommendations for Older Women: Targeted Screening May Be More Appropriate for Non-White Women. D. L. Broussard*, J. H. Magnus. Tulane University Health Sciences Center, New Orleans, LA, USA.

Universal osteoporosis screening in the U. S. is now recommended for all women ≥65 years of age, regardless of race/ethnicity. While some non-White women are at a greater risk of post-fracture disability and mortality than White women, non-White women have a lower overall fracture rate than White women. Furthermore, there is no consensus about what levels of bone mineral density (BMD) should be considered osteoporotic in different race/ethnic groups. Therefore, screening all older non-White women may not be cost effective, a circumstance that may be remedied by targeting screening only to those non-White women at high risk for osteoporosis. The purpose of this study was to determine if a set of low BMD risk factors for White women accurately identifies African American (AA) and Mexican American (MA) women with osteoporosis. Low body mass index, low calcium intake, current cigarette smoking, and no physical activity were found to be consistent low BMD risk factors for White women in published population-based studies. This set of four risk factors was evaluated among 252 AA, 232 MA, and 745 White women, aged 65 to 79 years, who participated in the Third National Health and Nutrition Examination Survey (NHANES III). Osteoporosis T-scores were calculated for each group as 2.5 SD below the race/ethnic specific young adult female (20-29 years) mean BMD for the total femur using published NHANES III data. The diagnostic accuracy of one or more of the four risk factors for identifying total femur osteoporosis in each of the race/ethnic groups of women was assessed by calculating sensitivity, specificity and positive and negative predictive values. Based on the race/ethnic group-specific T-scores, the prevalences of osteoporosis in this NHANES III population were 9.8%, 13.5%, and 13.1%, respectively, for AA, MA and White women. The sensitivities associated with the presence of one or more of the four risk factors were 87%, 92%, and 70%, respectively for AA, MA, and White women. Specificity was highest in White women (52%) while the specificities for AA and MA women fell below 50%. PPV ranged from 20% to 28%, and NPV ranged from 87% to 94%. About 23% of AA and 28% of MA women had no risk factors, and in a screening setting would not be referred to BMD testing. Of those with no risk factors, only 11% of AA and 6% of MA women had osteoporosis. Furthermore, the false negative rates for AA and MA women were low. These results suggest that applying the set of four risk factors studied as a prescreening risk assessment tool may be useful for identifying AA and MA women who are not likely to have osteoporosis and do not require a BMD measurement, which has the potential for reducing the economic burden of universal BMD screening.

Disclosures: ***D.L. Broussard****, None.*

1222

The Use of BMD for Therapeutic Decision Making. J. A. Kanis, A. Oden*, H. Johansson*, C. De Laet*, E. V. McCloskey, K. Kayan*, T. Jalava*, A. Oglesby*, O. Johnell. WHO Collaborating Centre for Metabolic Bone Diseas, University of Sheffield Medical School, Sheffield, United Kingdom.

Clinical risk factors are poor predictors of fracture risk, but not useless. They may have more utility in categorising individuals above or below risk thresholds. If so, BMD may not be required in all patients to make recommendations about treatment. The aim of this study was to develop a methodology to optimise the role of bone mineral density (BMD) measurements in a case finding strategy. We studied 2113 women aged 75 years or more randomly selected from Sheffield UK. Baseline assessment included hip bone mineral den-

sity (BMD) and clinical risk factors. Outcomes included death and fracture in women followed for 6723 person years. Poisson models were used to compute fracture probabilities. Women were categorised by fracture probability with and without a BMD assessment. An arbitrary 10 year fracture probability threshold of 35% was taken as an intervention threshold. Age, prior fracture, use of corticosteroids and low body mass index (BMI) were identified as significant clinical risk factors. 16.8% of women were classified as high risk on the basis of these clinical risk factors. The average BMD in these patients was approximately 1 SD lower than in low risk women. 21.5% of women were designated to be at high risk with the addition of BMD. 15% of all women were re-classified after adding BMD to clinical risk factors, most of whom lay near the intervention threshold. When a high probability of re-classification was accepted (without a BMD test) for high risk to low risk ($P_1 \leq 0.8$) and a low probability accepted for low to high risk ($P_2 \leq 0.2$), BMD tests would be required in only 21% of the population and the proportion of re-classified women would be reduced from 15 to 8%. The proportion of high risk individuals not detected would decrease from 46 to 13% which is 2.8% of all women. We conclude that the use of clinical risk factors can identify elderly women at high fracture risk and such patients have a low average BMD. BMD testing is required, however, in a minority of women – a fraction that depends upon the probabilities accepted for misclassification and the thresholds of risk chosen.

Disclosures: ***J.A. Kanis****, None.*

F002

Hypovitaminosis D in Healthy Adolescents. C. M. Gordon[1], K. DePeter*[2], E. Grace*[2], S. J. Emans*[2]. [1]Endocrinology & Adolescent Med., Children's Hospital, Boston, MA, USA, [2]Adolescent Medicine, Children's Hospital, Boston, MA, USA.

Vitamin D is essential for optimal bone accretion during adolescence. In a clinic sample of healthy adolescent girls and boys, we sought to determine the prevalence of hypovitaminosis D. We also examined whether the prevalence differed by gender, season or ethnicity, and examined anthropometric and lifestyle predictors of serum levels of 25-hydroxyvitamin D (25D). 282 adolescents (185 girls and 97 boys) who presented to an outpatient adolescent medicine practice in Boston, MA were evaluated at the time of a routine physical examination. Serum levels of 25D, parathyroid hormone (PTH), calcium, phosphorus and magnesium were obtained, and activity and nutritional questionnaires completed. The prevalence of hypovitaminosis D (<15 ng/ml) was 20% for the cohort. The problem was more prevalent in girls (22%) than boys (15%), and more prevalent in African- American (30%) than Asian (17%), Hispanic (18%), or White (2%) teenagers. There was a seasonal variation noted, with a prevalence of 9% during summer (July - Sept.) and 36% during winter (Jan.- March). The mean winter 25D level was significantly lower than that during summer (10.7 +/- 2.4 vs. 27.7 +/- 10.1 ng/ml, p<0.001), and PTH levels were higher (54.6 +/- 26.7 vs. 43.7 +/-19.9 pg/ml, p=0.006). There was a significant negative correlation between serum 25D and PTH (r= -0.27, p<0.001). There was a modest, but significant positive correlation between 25D levels and reported consumption of vitamins(r= 0.15, p=0.012) and calcium supplements (r= 0.15, p=0.013), and a negative correlation between 25D and both weight (r= -0.18, p=0.003) and body mass index (r= -0.17, p=0.004). There were no significant differences among boys and girls with respect to use of multivitamins or calcium supplements, but daily milk consumption was higher in the boys than girls (2.8 +/- 2.2 vs. 1.6 +/- 1.1 cups, p<0.001). Both the boys and girls with hypovitaminosis D reported significantly lower milk consumption than those who were replete (1.4 +/- 1.1 vs. 2.2 +/- 2.0 cups, p=0.039). Hypovitaminosis D occurred in 20% of otherwise healthy teenagers in a clinic sample from Boston, a city of northern latitude. The problem was more prevalent in girls than boys, and most prevalent among adolescents of African-American descent. These findings suggest that hypovitaminosis D is a significant problem among teenagers. Adolescent providers should take note of these findings, both of the high prevalence of this health problem and suboptimal nutritional practices, especially among girls and adolescents of certain ethnicities.

Disclosures: ***C.M. Gordon****, None.*

F004

Umbilical Vein Calcium Concentration Predicts the Bone Mass of Children at Age Nine Years. M. K. Javaid[1], S. R. Shore*[1], P. Taylor*[2], C. Gale*[1], F. O'Callaghan*[1], N. K. Arden*[1], K. M. Godfrey*[1], C. Cooper[1], &. The Princess Anne Hospital Study Group*[1]. [1]MRC Environmental Epidemiology Unit, University of Southampton, Southampton, United Kingdom, [2]Medical Physics and Bioengineering, Southampton Univeristy Hospitals NHS Trust, Southampton, United Kingdom.

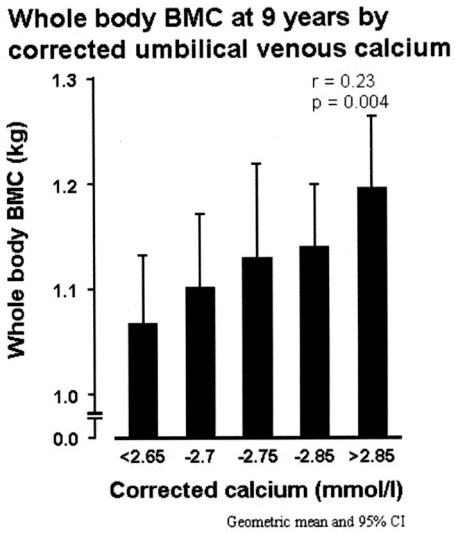
Osteoporotic fractures are a significant burden to society in the UK. Peak bone mass is a major determinant of bone strength in later life and epidemiological studies suggest that intrauterine and early postnatal environments influence skeletal trajectory and peak bone mass accrual. The aim of this study was to identify the specific maternal and neonatal determinants of bone mass in later childhood.

211 children (113 boys) were studied at age nine years. Their mothers anthropometry, diet and lifestyle had been characterized prospectively and the children were measured at birth. Now aged nine years, the children had their bone mass measured using DXA.

Boys were significantly taller (p=0.004) and had greater bone mass (p=0.002) than the girls. At nine years, birthweight remained a strong predictor of whole body bone mineral content (WBBMC) (r=0.40, p<0.001); lean mass (r=0.35, p<0.001) and fat mass (r=0.21, p=0.02) in both boys and girls. Lower maternal social class and educational attainment, smoking during late pregnancy, reduced prepregnant weight and height, and lower maternal fat stores during late pregnancy were significantly (p<0.05) associated with lower childhood WBBMC.

There were also strong positive associations between childhood WBBMC and umbilical venous calcium (p=0.002) and albumin (p=0.008) concentration. In contrast, umbilical venous phosphate, alkaline phosphate and creatinine did not predicted childhood WBBMC. In multivariate analyses, maternal smoking, socioeconomic status and fat stores were no longer associated with childhood WBBMC when umbilical calcium concentration was taken into account; male sex and tall maternal stature remained independent predictors of WBBMC.

These results suggest that umbilical venous calcium concentration is an important correlate of the trajectory of skeletal growth during the first nine years of postnatal life. The capacity to maintain a materno-fetal calcium gradient might be the physiological mechanism through which maternal smoking and nutrition adversely influence skeletal development in the offspring.



Disclosures: ***M.K. Javaid****, None.*

F008

Physical Activity Intensities and Femoral Neck Strength in Young Children: The Iowa Bone Development Study. K. F. Janz*[1], T. L. Burns*[2], S. M. Levy*[3], J. C. Torner*[4], M. C. Willing[5], T. J. Beck*[6], J. M. Gilmore*[3], T. A. Marshall*[3]. [1]Health and Sport Studies, University of Iowa, Iowa City, IA, USA, [2]Biostatistics, University of Iowa, Iowa City, IA, USA, [3]Preventive and Community Dentistry, University of Iowa, Iowa City, IA, USA, [4]Epidemiology, University of Iowa, Iowa City, IA, USA, [5]Pediatrics, University of Iowa, Iowa City, IA, USA, [6]Radiology, Johns Hopkins, Baltimore, MD, USA.

Intervention studies suggest that a threshold of vigorous-intensity physical activity is needed to impact bone strength in children. However, quantifying this relationship in population-based studies is difficult since children are not cognitively capable of accurately reporting activity intensities. In this observational study, accelerometry was used to investigate associations between activity intensities and femoral neck (FN) bone strength (426 children, mean age 5.2 yr, range 4 to 6 yr). We tested the hypothesis that within the scope of children's usual activity patterns, vigorous activity is more strongly associated with bone strength than other activity intensities.

Physical activity was measured using 4-d accelerometry readings. This method produced minute-by-minute movement count data. Movement counts were calibrated to oxygen uptake as a multiple of resting metabolic rate (METs). The predictor variables were minutes per day in sedentary and light (< 2.9 METs), moderate (3 to 5.9 METs), and vigorous activity (≥ 6 METs). FN aBMD, cross-sectional area (CSA), and section modulus (Z) were calculated from DXA outputs (Hologic 2000). Associations were examined using partial correlation coefficients and stepwise linear regression after adjustment for weight and height.

Partial Correlations Coefficients		Femoral Neck		
*P < 0.05, **P < 0.01, NS = not significant		aBMD (g/cm^2)	CSA (cm^2)	Z (cm^3)
Sedentary and Light (min)	Boys	-0.20**	NS	NS
	Girls	-0.30**	-0.19**	-0.19**
Moderate (min)	Boys	0.NS	NS	NS
	Girls	0.26**	0.20**	0.16*
Vigorous (min)	Boys	0.28**	0.27**	0.22**
	Girls	0.30**	0.26**	0.19**

In boys, only vigorous activity entered regression models that included weight and height, the proportion of variance explained after adjustments ranged from r^2 = 3.9 to 5.8%. In girls, vigorous activity also entered all models explaining 1.2 to 4.4% of the variance and sedentary and light activity inversely entered the FN aBMD model (r^2 = 5.7%). During young children's "everyday" activity, vigorous activity is more highly associated with FN bone strength than other activity intensities. In girls, sedentary and light activity negatively impacts bone strength. Public health guidelines emphasizing moderate activity may not be appropriate for children's bone health.

Disclosures: ***K.F. Janz****, None.*

F010

BMP-3 Is a Negative Regulator of Cell Maturation during Skeletogenesis. L. W. Gamer*, J. Nove*, V. Rosen. Oral and Developmental Biology, HSDM and Forsyth Institute, Boston, MA, USA.

Although BMP-3 is the most abundant BMP in bone matrix, little is known about its specific role in the skeleton. In vivo, BMP-3 knockout animals possess twice the trabecular bone mass of their wild type littermates. In vitro, BMP-3 inhibits osteoblast differentiation in bone marrow stromal cell cultures and blocks BMP-2 and 7 mediated osteogenic activity, suggesting that unlike osteogenic BMPs, BMP-3 has an inhibitory effect on its target cells. In order to study where, when and how BMP-3 might act, we performed detailed in situ hybridization analysis of BMP-3 mRNA during skeletogenesis in mouse and chick. Initially, BMP-3 transcripts outline the prechondrogenic mesenchymal condensations, marking the future perichondrial surface. As individual skeletal elements form, BMP-3 transcripts become restricted to the perichondrium adjacent to the central region of the developing skeletal element, and then localize to osteoblasts. At all stages of endochondral bone formation, BMP-3 expression is excluded from chondrocytes. Since BMP-3 appears to be a negative regulator of osteoblast differentiation, we hypothesized that BMP-3 secreted by perichondrial cells may also exert an inhibitory effect on adjacent chondrocytes. Using the chick limb as a model, we implanted beads loaded with recombinant BMP-3 protein into stage 20-22 chick limb and found a consistent shortening and fusion of skeletal elements at d7 and 11. We also constructed a BMP-3 retrovirus and used it to infect stage 22 wing buds and micromass cultures from stage 23-24 limbs. Infection of wing buds with the BMP-3 virus resulted in an expansion of the skeletal elements. In BMP-3 infected prechondrogenic cell cultures, we observed an increase in PNA positive cells at d2 and an increase in Alcian blue staining at d4 post infection, demonstrating that BMP-3 induces an early increase in cell adhesion followed by an increase in the accumulation of sulfated proteoglycans. Northern analysis of RNAs from BMP-3 infected cultures indicates BMP-3 may be delaying chondrocyte maturation as the levels of collagen type II and Ihh are elevated, while the level of collagen type X is decreased compared to untreated cells. These data support a model in which BMP-3, secreted by perichondrial cells, acts on prechondrogenic cells in the developing limb to control their maturation into hypertrophic chondrocytes, and modulates the responsiveness of these cells to osteogenic signals that direct bone formation.

Disclosures: ***V. Rosen****, None.*

F012

Bone Formation Induced by a Novel Strategy of Plasmid DNA-Controlled Release from Biodegradable Scaffolds. G. Pelled*[1], J. Jang*[2], Y. Zilberman[1], L. D. Shea*[2], D. Gazit[1]. [1]Skeletal Biotechnology Laboratory, Hebrew University-Hadassah Medical Center, Jerusalem, Israel, [2]Department of Chemical Engineering, Northwestern University, Evanston, IL, USA.

Novel strategies for bone regeneration are being investigated for the treatment of massive bone defects. Most gene-based approaches for bone repair involve the use of viral vectors encoding for osteogenic genes. A huge effort is being conducted in order to develop a non-viral method for gene delivery. Current non-viral techniques are limited in efficiency of transduction, and temporal or spatial control of DNA release using these vectors is impossible. We hypothesized that in vivo bone tissue formation could be achieved by a temporal and spatial controlled release of DNA from biodegradable scaffolds. A gas foaming process has been utilized to fabricate poly (lactide-co-glycolide) (PLG) porous scaffolds from polymeric microspheres. The DNA release kinetics from the scaffolds was analyzed using the Hoechst dye-binding assay. The ability of DNA released from the polymer scaffolds to transfect cells in vitro was examined using NIH/3T3 cells grown on scaffolds containing the reporter genes luciferase, beta-galactosidase, and GFP. For the in vivo experiments, scaffolds containing 50 ugr of hBMP2 plasmid were prepared. In order to non-invasively and quantitatively detect osteogenic activity in vivo, we implanted the scaffolds in transgenic mice, which express Luciferase under human Osteocalcin promoter. Since Osteocalcin is a late osteogenic marker, the detection of Luciferase bioluminescence by a cooled charge coupled device (CCCD) system can be quantitatively correlated to an osteogenic process. The implants were harvested after 30 days and analyzed using uCT and immunohistochemistry. We were able to control the kinetics of plasmid DNA release from the scaffold by varying the molecular weight of the polymer or the size of microspheres. Intense bioluminescence was quantified in the site of implantation in the transgenic mice indicating osteogenic activity. The uCT 3-D analysis demonstrated the presence of active osteogenesis within the implantation site correlated with the collagen I positive matrix, as revealed by immunohistochemistry. We conclude that the controlled release of plasmid DNA from PLG scaffolds could serve as an effective therapeutic tool for bone regeneration.

Disclosures: ***G. Pelled****, None.*

F014

BMP Antagonist—Noggin Enhances Regulated Bone Formation and Bone Healing Conferred by Self-activating Tet-on Retroviral Vector Expressing BMP4. H. Peng[1], A. Usas*[1], B. Gearhart*[2], B. Young*[1], J. Huard[1]. [1]Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA, USA, [2]Growth and Development Laboratory, Orthopaedic Surgery, Children's Hospital of Pittsburgh, Pittsburgh, PA, USA.

Regulated therapeutic gene expression is needed for many gene therapy applications. To develop an optimal regulated gene expression system we tried to optimize a self-inactivating, tet-on retroviral vector system capable of doxycycline (Dox)-regulated therapeutic gene expression in stem cells. We have systematically studied the effects of different regulatory elements in the 3' LTR on gene expression. This analysis has revealed that an intact U3 region is essential for high level gene expression in transfected cells. The introduction of an SV40 poly-adenylation signal cannot compensate for the deteriorative effects upon gene expression that result from a spectrum of U3 deletions. Further analysis of gene expression in the transduced cells showed that optimal, inducible gene expression by a self-inactivating retroviral vector requires the preservation of the DR1 and the TATA box. These findings enabled us to identify the best way to design self-inactivating vectors through which high level expression of bone morphogenetic protein 4 (BMP4) can be turned on and off via the addition or withdrawal of Dox. Muscle-derived stem cells (MDSCs) transduced with this vector system elicited bone formation in normal animals only following administration of Dox. More importantly, these cells healed critical-sized skull defects following administration of Dox. However, histological analysis revealed that there was isolated bone nodular formation in the absence of Dox, and bone overgrowth following Dox induction. To overcome these problems, we added MDSCs transduced with retroviral vector expressing noggin into the implants, along with MDSCs transduced with retro-Tet-on-BMP4. This novel strategy completely blocked the background bone formation and prevented bone overgrowth, but still preserved the capability of Dox-regulated bone formation. Thus, the amount of bone formation is dependent on the duration of Dox induction. In conclusion, we have identified the optimal design for a self-inactivating, tet-on retroviral vector that can efficiently transduce MDSCs and promote bone formation and bone healing through controllable BMP4 gene expression. More importantly, we have develop a novel strategy to improve the outcome of regulated BMP4 expression through the use of its specific antagonist—noggin. We believe this vector system and the improved regulated gene expression based on co-delivery of specific antagonist can also be applied to other systems where regulated therapeutic gene expression is needed.

Disclosures: ***H. Peng****, None.*

F016

Identification of the Gli family of Zinc Finger Proteins as Powerful Regulators of BMP-2 Expression in Osteoblasts. M. Zhao[1], D. Chen[1], I. R. Garrett*[2], G. Rossini*[2], B. O. Oyajobi[1], H. Sasaki*[3], G. R. Mundy[1]. [1]Cellular and Structural Biology, University of Texas Health Science center at San Antonio, San Antonio, TX, USA, [2]Osteoscreen Inc., San Antonio, TX, USA, [3]Institute for Molecular and Cellular Biology, University of Osaka, Osaka, Japan.

BMP-2 is important in skeletal morphogenesis and postnatal bone formation, but the regulation of its transcription in osteoblasts is not well understood. Since hedgehog (Hh) signaling modulates BMP-2 transcription, and Hh proteins mediate their effects on bone development through the Gli family of transcriptional regulators, we investigated the effects of Gli proteins on BMP-2 transcription. In this study, we examined the functions of Gli2 and Gli3 on BMP-2 expression in osteoblasts. The constructs of full length Gli2 or Gli3 ($Gli2^{act}$, $Gli3^{act}$) and their C'-terminal truncated forms ($Gli2^{rep}$, $Gli3^{rep}$), were co-transfected with BMP-2 promoter reporter constructs (-2712/+165-Luc) in C2C12 cells, and reporter activity was assessed. We found that $Gli2^{act}$ markedly and dose-dependently stimulated BMP-2 transcription, while $Gli3^{act}$ had a weak stimulatory effect. In contrast, $Gli3^{rep}$ decreased promoter activity by 80%, and $Gli2^{rep}$ by 55%. Analysis of BMP-2 promoter deletion cassettes from –2712/+165 to –150/+165, suggested that the functional sequence which responds to both activator and repressor forms of Gli2 and Gli3 is located in the region from –150 to –310. Mutational analysis indicated that three putative Gli binding sites in this region are responsible for the regulation of BMP-2 transcription. Gli2-mediated regulation of transcriptional activity of BMP-2 promoter was further confirmed by measurement of BMP-2 mRNA. We found that these Gli proteins undergo proteolytic processing in osteoblasts. IBMX induced $Gli3^{190}$ degradation and production of a short form of Gli3 ($Gli3^{85}$) in C3H10T1/2 cells, which we found required the E3 ubiquitin ligase Slimb, and was blocked by treatment with proteasome inhibitors PSI and epoxomicin. PKA, a negative regulator of Hh signaling, significantly attenuated $Gli2^{act}$-mediated activation of BMP-2 promoter activity. These results suggest that the regulation by Gli2 and Gli3 of BMP-2 expression is cAMP-, ubiquitin- and proteasome-dependent. $Gli2^{act}$ increased osteoblast ALP level in C2C12 cells whereas $Gli2^{rep}$ decreased it. In mouse calvarial cultures, we found that $Gli3^{rep}$ transfection significantly inhibited the effects of proteasome inhibition to stimulate new bone formation. Taken together, these data suggest that Hh signaling transductors Gli2 and Gli3 play important roles as regulators of BMP-2 expression, which may account in part for their multiple effects on skeletogenesis.

Disclosures: ***M. Zhao****, Osteoscreen Inc. 3.*

F020

Effect of Lepitn on Growth Plate and Chondrocyte Differentiation. Y. Kishida*, A. Myoui, N. Tamai*, A. Nampei*, M. Nishikawa*, M. Hirao*, T. Nakase, N. Shimizu*, H. Yoshikawa. Department of Orthopaedic Surgery, Osaka University Medical School, Suita, Japan.

Leptin is a 16-kDa protein encoded by the obese (ob) gene that hormonally regulates food intake and energy expenditure by negative feedback at the hypothalamic nuclei. It has been shown that leptin and its functional receptor (ObRb) are distributed widely and leptin mediates a variety of activities, including bone development. It was reported that femoral length in ob/ob mice, that had impaired leptin production, was shorter than in normal mice, which was rescued by locally administered leptin, suggesting that leptin has a direct effect on growth plate. Based on these observations, we first investigated the effect of leptin depletion on growth plate using ob/ob mice. Immunohistochemical analysis revealed that leptin was localized in prehypertrophic chondrocytes in normal mouse and Ob-Rb in hypertrophic chondrocytes both in normal and ob/ob mice. Although there was no difference in the microscopic appearance in growth plate and mRNA expression of type II, IX and X collagen by in situ hybridization, we found that the growth plates of ob/ob mice were more fragile than those of wild-type mice by mechanical test and easy to break at hypertrophic zone, suggesting that the matrix maturation is impaired in ob/ob mouse. To

obtain a better understanding of the role of leptin in enchondral ossification, we determined the effect of leptin on in vitro chondrocytic differentiation of murine ATDC5 cells that express Ob-Rb. We found that leptin, at a concentration of 1-10 ng/ml, which was an equivalent dose to normal serum concentration of leptin, abolished the matrix calcification in late stage of ATDC5 cell differentiation culture and profoundly inhibited and delayed cell apoptosis. Since parathyroid hormone-related protein (PTHrP) was reported to inhibit the terminal differentiation and apoptosis of chondrocytes as well as matrix mineralization, we next examined PTHrP mRNA expression by quantitative assay using a real-time polymerase chain reaction. In the presence of physiological concentration of leptin, PTH-rP expression by ATDC5 cells was gradually increased with time. On the other hand, without exogenous leptin, PTH-rP expression was decreased with time. Furthermore, the leptin effect on matrix mineralization was abolished by anti-PTH-rP neutralizing antibody. These findings suggest that leptin regulates matrix mineralization and apoptosis in ATDC5 cell differentiation culture via modulating the chronological expression pattern of PTH-rP. Taken together, our data suggests direct effect of leptin is essential in the normal chondrocyte differentiation and endochondral ossification.

Disclosures: ***Y. Kishida****, None.*

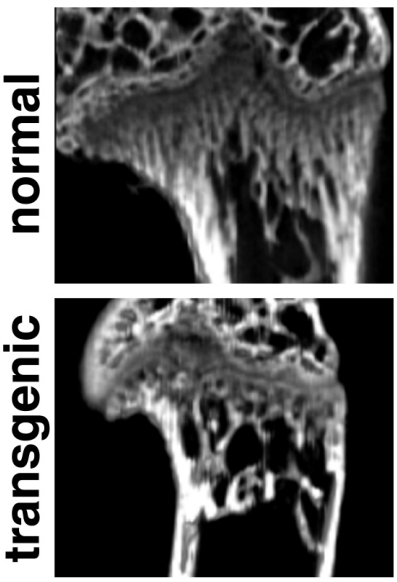
F027

Overexpression of Smad6 in Chondrocytes Distinctively Disturbs Terminal Differentiation of Chondrocytes and Causes Osteopenia in Transgenic Mice. M. Horiki*[1], N. Tsumaki*[1], A. Myoui[1], T. Imamura*[2], H. Yoshikawa[1]. [1]Orthopaedic Surgery, Osaka University Medical School, Suita, Japan, [2]Department of Biochemistry, The Cancer Institute, Tokyo, Japan.

Bone Morphogenetic Proteins (BMPs) are known to play an important role in promotion of cartilage proliferation and differentiation. There have been several reports showing that Smads mediate these BMP signals in chondrocytic cells *in vitro*. However, *in vivo* role of Smad proteins during endochondral bone formation has not been analyzed. In the present study, we tried to block Smad signaling in developing cartilage by overexpression of Smad6, an inhibitory Smad, in chondrocytes of transgenic mice.

Materials and Methods To prepare the Smad6 transgene construct, the complete Smad6 cDNA was linked to the promoter and enhancer sequences of theα2(XI) collagen chain gene. By microinjecting the transgene insert, transgenic mice were generated and analyzed.

Results and discussion Pleural lines of transgenic mice were established. Staining of skeleton showed that primordial cartilage of transgenic mice developed almost normally until birth. Calcification was slightly retarded in some skeletal components in transgenic mice. After birth, transgenic mice began to show dwarfism. At 3 weeks after birth, the average lengths of bony components were 30 % shorter in transgenic mice than in those of normal mice. Northern analysis revealed that the expression levels of Sox9 and type II collagen genes in transgenic mice were almost equal to those of normal mice, whereas expression of Type X collagen gene was reduced. Histological analysis showed that there was no marked difference in the zone of proliferating chondrocytes between normal and transgenic mice. Columnar alignment of hypertrophic chondrocytes in transgenic mice was slightly irregular when compared with that of normal mice. The primary spongiosa was significantly decreased in Smad6 transgenic mice than in normal mice, which was also confirmed by micro-CT analysis (Figure). Although transgene expression was detected in prolifertative and weakly hypertrophic chondrocytes, excess amount of Smad6 might interfere with signal transduction only at final stage of maturation process of cartilage. We speculate that impaired matrix of zone of hypertrophic chondrocytes might disturb proper replacement of cartilage by bone, leading to osteopenia.



Disclosures: ***M. Horiki****, None.*

F029

BIG-3, a Novel WD-40 Protein, Accelerates Chondrocyte Differentiation in Vitro. F. Gori, M. B. Demay. Endocrine Unit, Massachusetts General Hospital/ Harvard Medical School, Boston, MA, USA.

Among the local signaling pathways that regulate the onset of chondrogenesis and the sequential steps of chondrocyte differentiation is the bone morphogenetic protein (BMP) signaling pathway. We recently identified a BMP-2 induced gene, named BIG-3 (BMP-2 Induced Gene 3kb) that is expressed in a prechondroblastic cell line, MLB13MYC clone 17. BIG-3 is also expressed in proliferating and hypertrophic chondrocytes *in vivo* in the developing growth plate. We, therefore, undertook studies to address the role of BIG-3 during chondrocyte differentiation *in vitro* using mouse clonal chondrogenic ATDC5 cells. When treated with insulin, transferrin and sodium selenite (ITS) these cells undergo differentiation, becoming hypertrophic chondrocytes that make mineralized matrix. Upon BMP-2 treatment (200 ng/ml) BIG-3 protein was dramatically induced in ATDC5 cells. To determine whether stable expression of BIG-3 could alter the program of chondrocytic differentiation, ATDC5 cells were stably transfected with the full-length coding region of BIG-3 (ATDC5-BIG-3) cloned downstream of the CMV promoter in pcDNA3.1 or with the empty vector (ATDC5-EV). BIG-3 overexpression accelerated matrix proteoglycan synthesis at 14 d, as assessed by Alcian Blue staining, compared to pooled ATDV5-EV clones, where faint Alcian Blue staining was seen at 21 d. While ATDC5-EV clones cultured without ITS failed to show specific Alcian Blue staining after 30 d in culture, untreated pooled ATDC5-BIG-3 clones synthesized considerable amounts of Alcian Blue staining proteoglycans. Alkaline phosphatase (AP) mRNA expression was higher by 21 d in ITS-treated ATDC5-EV and ATDC5-BIG-3 clones compared to untreated clones. By 30 d AP mRNA expression was dramatically higher in ITS-treated and untreated ATDC5-BIG-3 clones compared to ITS-treated and untreated ATDC5-EV clones. Osteopontin (OP) mRNA expression, a marker of late hypertrophic chondrocytes was higher by 30 d in ITS-treated ATDC5-BIG-3 clones compared to ITS-treated ATDC5-EV clones. Untreated ATDC5-BIG-3 clones expressed higher OP mRNA levels compared to the untreated ATDC5-EV clones. Overexpression of BIG-3 also accelerates mineralized matrix formation as assessed by Alizarin Red staining in both the presence and the absence of ITS. These findings, which demonstrate that BIG-3 accelerates chondrocyte differentiation *in vitro,* combined with the observation that BIG-3 is expressed in a cell maturation dependent fashion in the growth plate during embryonic development, suggest that this novel protein plays a regulatory role in the maturation of growth plate chondrocytes *in vivo.*

Disclosures: ***F. Gori****, None.*

F032

Alteration in Bone Density of Mice due to Heterozygous Inactivation of LRP6. Y. P. Kharode[1], P. D. Green*[1], J. T. Marzolf*[1], W. Zhao[1], R. Askew*[2], P. J. Yaworsky*[2], F. J. Bex[1]. [1]Women's Health Research Institute, Wyeth Reserach, Collegeville, PA, USA, [2]Genomics, Wyeth Reserach, Cambridge, MA, USA.

Mutations in LRP5 have been associated with a continuum of bone phenotypes. LRP6 is a closely related homologue of LRP5, and like LRP5 has been identified as a co-receptor in the Wnt signaling pathway. Embryos homozygous for an inactivating mutation in LRP6 die at birth and exhibit a variety of severe developmental abnormalities including truncation of the axial skeleton, limb defects, microophthalmia and malformation of the urogenital system. Unlike the LRP6 mutants, mice homozygous for LRP5 disruption are viable but develop a low bone mass phenotype and retain embryonic eye vascularization. Clinically, inactivating mutations of LRP5 result in a severe reduction in bone mass and ocular pathology but, interestingly, heterozygous carriers of these mutations can have an intermediate low bone mass phenotype. To investigate potential similar dosage effects of LRP6 on the mouse skeleton, we evaluated bone density of the femur of LRP6 +/- males and females at 9, 17 and 26 weeks of age. The distal femurs of female LRP6 +/- mice at all ages had 9.5% to 12% lower total bone mineral density (BMD) and 20 to 31% lower trabecular BMD than age matched wild-type (WT) mice. At 9, 17 and 26 weeks of age, total BMD values of the femur as measured by pQCT for female LRP6 +/- mice (475±12, 488±13, and 552±17 mg/cm^3, respectively) were significantly lower ($p<0.05$) than total BMD of femur for WT (539±16, 611±9, and 619±11 mg/cm^3, respectively). Trabecular BMD of the femur in female LRP6 +/- mice (113±7, 128±8, and 152±8 mg/cm^3) was significantly lower ($p<0.01$) than the trabecular BMD for the age matched WT groups (163±13, 175±6 and 191±7 mg/cm^3, respectively). MicroCT analysis of the distal femurs revealed that at all time points BV/TV, Connectivity density and trabecular number were significantly lower and trabecular separation was significantly higher in LRP6 +/- compared to the corresponding WT group. In male LRP 6 +/- mice, the values for total BMD and for trabecular density were 3.5 - 7% and 8 - 27% lower, respectively, than those for the corresponding age-matched WT groups. Total and trabecular BMD in male LRP6 +/- mice were lower than corresponding WT groups; however, statistically significant differences between LRP6 +/- and WT were observed only in trabecular BMD at 17 weeks (128±10 mg/cm3 vs.175±9, $p<0.05$) and at 26 weeks (162±8 vs. 220±18 mg/cm^3, $p<0.05$). We conclude that (1) mice heterozygous for LRP 6 gene have lower BMD than WT and (2) the magnitude of this difference is more pronounced in females than in males.

Disclosures: ***Y.P. Kharode****, Wyeth Reasearch 3.*

F034

The Human LRP5 G171V Mutation in Mice Alters the Skeletal Response to Limb Unloading but Not to Ovariectomy. F. Bex[1], P. Green*[1], J. Marzolf*[1], P. Babij[2], P. Yaworsky*[2], Y. Kharode[1]. [1]Women's Health Research Institute, Wyeth Research, Collegeville, PA, USA, [2]Genomics Division, Wyeth Research, Cambridge, MA, USA.

We have demonstrated that transgenic mice expressing a G171V mutation in the low-density lipoprotein receptor related protein 5 (LRP 5) have a high bone mass phenotype strikingly similar to affected individuals in a kindred with this mutation. Previous studies have shown that these mice have an increased response to skeletal loading. To further characterize potential differences in mechanosensory regulation produced by the mutation, we have evaluated the skeletal response of these mice to unloading and have contrasted it with loading-independent effects of ovariectomy (ovx). Nine week old male transgenic (het) mice have significantly ($p<0.001$) higher total and trabecular bone mineral density (BMD) (570±7, 357±4 mg/cm^3, respectively) than their non-transgenic littermates (ntg) (360±13,147±4 mg/cm^3, respectively). To examine the effects of unloading, unilateral sciatic neurectomy was performed on the right limb of het and ntg male mice and femoral BMD measurements were taken after one and two weeks. At both time points, in both het and ntg mice, total and trabecular BMD were significantly decreased in the distal femur of the denervated limb compared to that for the contralateral intact limb. However, at both time points, the rate of bone loss due to unloading was greater for ntg than for het. After 2 weeks the denervation-induced decrease in total and trabecular density in ntg mice (16.3±1.6% and 19.7±2.2%, respectively) was significantly ($p<0.05$) greater than for the het mice (10.0±1.4% and 11.5±2.5%, respectively). To determine whether the mutation had any effect on ovx-induced osteopenia, het and ntg mice were ovx at 9 weeks of age and treated for 35 days with vehicle or 10 ug/kg/day 17β- estradiol (E2). Ovx resulted in significant bone loss in both het and ntg ovx groups compared to their respective sham- ovx controls and the rate of this bone loss was comparable for both groups. Ovx induced a 14.3% decrease in total BMD (696±6 to 597±7 mg/cm^3) and a 26.3% decrease in trabecular BMD (445±9 to 328±7 mg/cm^3) in the het group and a 14.9% decrease in total BMD (470±10 to 400±7 mg/cm^3) and 37.6% decrease in trabecular BMD (132±4 to 82±2 mg/cm^3) in the ntg group. Treatment with E2 completely prevented the ovx-induced bone loss in both het and ntg. In conclusion, the LRP5 G171V mutation does not substantially alter the rate of ovx-induced bone loss and responsiveness to E2 but it can attenuate bone loss due to unloading which would be in agreement with its suggested role in the response of bone to mechanical stress.

Disclosures: ***F. Bex****, None.*

F036

Histone Deacetylase (HDAC) 3 Interacts with the Amino Terminus of Runx2 and Represses Runx2-Dependent Transcription. T. M. Schroeder*[1], J. J. Westendorf[2]. [1]Graduate Program in Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA, [2]Orthopaedic Surgery and University of Minnesota Cancer Center, University of Minnesota, Minneapolis, MN, USA.

Runx2 (Cbfa1, AML-3), a member of the Runt Homology Domain protein family, is required for bone formation and osteoblast differentiation. As a transcription factor, Runx2 is capable of both activating and repressing osteoblast specific genes. Our lab is interested in defining the molecular mechanisms by which Runx2 represses transcription. As previously described, our studies indicate that Runx2 contains multiple autonomous repression domains, some of which are sensitive to histone deacetylase inhibitors. Histone deacetylases (HDACs) repress transcription by removing acetyl groups from lysine residues on histones and transcription factors, thereby facilitating chromatin condensation and altering protein function or stability. Co-immunoprecipitation and GST pulldown experiments revealed that HDAC3 interacts with the extreme amino terminus of Runx2, a region previously not defined as a repression domain. To study the effect of HDAC3 knockdown on osteoblast differentiation, RNA interference (RNAi) duplexes were designed against HDAC3. In a transient assay, one duplex significantly suppressed HDAC3 expression. This construct was used to in functional assays in which the effects of Runx2 and HDACs on the osteocalcin promoter were examined. As previously reported, Runx2 activates the bone-specific osteocalcin promoter three to five fold. HDAC3 did not affect the basal activity of the osteocalcin promoter but blocked Runx2-dependent activation of this promoter. Addition of the HDAC3 RNAi construct to the assay reversed HDAC3 repression of Runx2-mediated activation whereas a control RNAi construct had little effect. MC-3T3 cells stably expressing the HDAC3 RNAi duplexes are viable. The requirement of HDAC3 in osteoblast differentiation is being determined. Together, these data suggest that HDAC3 physically and functionally interacts with Runx2. Our model system will allow us to determine the role of HDAC3 in osteoblast differentiation and bone development.

Disclosures: ***T.M. Schroeder****, None.*

F038

Mechanisms Regulating Runx 1 and 2 Gene Expression During Mesenchymal Chondrogenesis. C. J. Lengner, C. Lepper*, A. J. van Wijnen, J. L. Stein*, J. B. Lian, G. S. Stein. Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, MA, USA.

The Runx family of transcription factors are key regulators of cell fate determination. Runx1 and Runx2 are both highly expressed in condensing mesenchyme (CM). Runx1 is transcriptionally active in perichondrium, whereas Runx2 is expressed in osteogenic lineage cells. In vivo, CM receives BMP2 signals for formation of skeletal rudiments prior to overt chondrogenesis. The role of Runx factors in pre-chondrocytic mesenchyme is unclear. We therefore addressed the responses of the Runx genes to chondrogenic regulatory signals using in vitro models of undifferentiated mesenchyme [primary mouse embryonic fibroblasts (MEFs) and C3H10T$^{1/2}$ cells] and their induction towards chondrogenesis by BMP2. All three Runx genes were found to be expressed in these cells. Contrary to conventional expectation, we observe that Runx2 is suppressed by BMP2 during chondrogenesis induced by micromass conditions, while Runx1 is up-regulated under the same conditions. In further support of this regulation, we demonstrate transcriptional inhibition of Runx2 is mediated by the pro-chondrogenic transcriptional repressor NKX3.2 (Bagpipe). Suppression of Runx2 by NKX3.2 is limited to undifferentiated mesenchymal cells (NIH3T3, C3H10T1/2) and is abrogated in committed osteoblasts (MC3T3E-1, ROS 17/2.8). Furthermore, we find an inverse correlation between endogenous expression of Runx2 and NKX3.2 at the onset of BMP-2 induced chondrogenesis. In conclusion, these data indicate Runx1 may be involved in promoting chondrogenesis, and that inhibition of Runx2 by NKX3.2 may be required for the development of the chondrogenic phenotype in condensing mesenchyme. Our studies suggest that the presence of Runx2 in mesenchyme prior to chondrogenesis may reflect a function to support a progenitor population which will differentiate later in development.

Disclosures: ***C.J. Lengner****, None.*

F041

Fibronectin Hep III Domain on Stromal Cells Is Required for Osteoclastogenesis at the Early Stage through an RGD-independent Manner. S. Arai*[1], Y. Azuma[2], A. Kudo[1]. [1]Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan, [2]Teijin institute for Biomedical Reserch, Tokyo, Japan.

RANKL is expressed on various tissues and hematopoetic cells. However, osteoclastogenesis is regulated mainly on bone marrow stromal cells. We tried to isolate surface molecules regulating osteoclastogenesis on stromal cells. 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 inhibited osteoclastogenesis dose-dependently at the early stage of osteoclastogensisis from osteoclast progenitors to mononuclear osteoclasts. However, inhibition was not observed in the stroma-free, sRANKL-induced osteoclastogenesis. We identified that the A15-1 antigen is fibronectin, a heterodimeric ECM glycoprotein and A15-1 binds its HepIII, which did not included domain. RGD sequence, an integrin-binding site. Antisense oligonucleotides against the mouse fibronectin mRNA were transfected into TSB13, resulting remarkable decrease of the ability of osteoclastogenesis. On the Hep III domain coated dish, osteoclastogenesis was stimulated. Moreover, the pro-B and pre-B cell lines that highly express RANKL on the surface were not able to support osteoclast differentiation, because the expression of the fibronectin is limited to mesenchymal lineage cells. Thus, the lack of fibronectin in other lineage cells implies the defect in osteoclastogenesis. In the in-vivo analyses, treatment with A15-1 significantly decreased both the number of mature osteoclasts and the area of erosion surface in PTH-induced hypercalcemia model mice. In this experiment, we firstly demonstrated the novel RGD-independent function of fibronectin on stromal cells in osteoclast differentiation. In adding to RANKL and M-CSF, fibronectin is necessary as the differentiation factor in osteoclastogenesis.

Disclosures: ***S. Arai****, None.*

F043

Promoter Region of Osteopontin that Directs Cell-specific and Developmental Regulation: An Analysis of Transgenic Mice. S. Nomura[1], Y. Higashibata*[1], T. Sakuma*[1], H. Kawahata*[1], S. Fujihara*[2], K. Moriyama[2]. [1]Pathology, Osaka university Medical School, Suita, Japan, [2]Orthodontics, School of Dentistry, University of Tokushima, Tokushima, Japan.

Osteopontin (OPN) is considered to plays important roles for controlling physiological and pathological calcification. OPN-expressing cell types in bone tissues are identified as osteoblasts, hypertrophic chondrocytes and osteoclasts. OPN expression is also detectable in a variety of cells such as epithelial cells in the developing and renal stone-forming kidney, granulated metrial gland cells (GMG cells) of maternal origin in placenta, glandular epithelial cells in mammary gland, macrophages in atherosclerotic plaques, and calcifying foci in breast cancer. Identification of the osteopontin promoter region controlling cell-specific expression in vivo provide us valuable information for the elucidation of the molecular mechanism in the calcification of the tissues. The transgenic mice carrying green fluorescent protein (GFP) gene under the control of 5.5-kb (Op5.5GFP), 3.1-kb (Op3.1GFP), 1.5kb Op1.5GFP) and 0.9-kb (Op0.9GFP) upstream region of mouse osteopontin gene were generated. Expression of GFP was investigated by means of Northern blotting, Western blotting, immunohistochemistry, in situ hybridization and by fluorescence. Localization of GFP-expressing cells was compared with those of OPN-expressing cells. GFP-expressing cell types in Op5.5GFP were identical to that of OPN-expressing ones. However, GFP expression was detected not only in hypertrophic chondrocytes but in proliferating and resting chondrocytes in Op3.1GFP, and fibroblastic cells expressed GFP in Op1.5GFP. No significant expression of GFP in bone tissue was detected in Op0.9GFP mice. Furthermore, the promoter region between 3.1kb and 1.5kb upstream was essential for the GFP expression in the kidney and placenta. The expression patterns obtained by the analysis of transgenic lines indicated the different promoter regions are involved in cell type specific expression of OPN gene. OPN-deficient mice carrying dominant negative OPN gene under the control of 5.5kb promoter showed significant abnormality in bone.

Disclosures: ***S. Nomura****, None.*

F046

Role of Collagenase-3 (MMP-13) in Cartilage Resorption as Observed During the Development of the Secondary Ossification Center in the Tibia of Young Rats. I. Synthesis and Secretion of Procollagenase-3 at Sites of Cartilage Erosion. E. R. Lee[1], L. Lamplugh*[1], B. Kluczyk*[1], J. S. Mort*[2], G. Murphy*[3]. [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.

The formation of a secondary ossification center in the cartilaginous epiphysis of long bones requires the excavation of canals and marrow space and, therefore, the resorption of cartilage. Earlier work from this laboratory has demonstrated with the assistance of anti-neoepitope antibodies, that the major cartilage component type II collagen is destroyed in this resorption by activated collagenase-3 cutting the fibrils (JBMR, vol17, S25, SA066, 2002). In the current study we have sought to identify the cell source of this collagenase-3 and to uncover details of its processing. Collagenase-3 is synthesized as a proenzyme bearing an inhibitory propeptide domain that is removed upon enzyme activation. We have reasoned that anti-peptide antibodies can be prepared against the propeptide and used to detect the proenzyme in the epiphysis by immunostaining, presumably at its cell source. Thus affinity purified antibodies have been prepared against the peptide HPVT-LAGILKKSTVTS corresponding to the amino acid residues 43 - 58 on the rat propeptide domain. With the aid of these antibodies we report here that the proenzyme is localized to newly developed sites of cartilage erosion precisely where the collagen is cleaved. Thus at 6 days of age, canals allowing the entry of capillaries are dug from the surface of the epiphysis (stage 1), while immunostaining indicative of the proenzyme appears at the blind end of each canal. By 8 days of age, the canal blind ends fuse to create a marrow space in the epiphysis (stage II), where the immunoreactivity occurs along the walls of the expanding marrow space (stage II). Highly reactive cells and matrix are found at the newly developed sites of cartilage erosion. The cells resemble septoclasts. They have a single nucleus, an abundance of rER and Golgi elements, contain dense bodies rich in cathepsin B and exhibit a deficiency of markers indicative of the macrophage-osteoclast lineage. The matrix in contrast, exhibits various stages of degradation and reveals reactivity indicative of the proenzyme along the surface of collagen fibrils. We conclude that a septoclast-like cell is involved in the synthesis and secretion of the collagenase-3 proenzyme at specified sites of cartilage resorption and that upon release, the proenzyme first associates with the fibril before becoming active and cleaving the collagen.

Disclosures: ***E.R. Lee****, None.*

F048

In Vivo Inhibition of Osteoblastic Metalloproteinases Leads to a Decreased Bone Turn-Over and an Increased Trabecular Bone Mass. V. Geoffroy*[1], C. Marty-Morieux*[1], N. Le Goupil*[1], P. Clement-Lacroix[2], C. Teraz*[3], M. Frain*[4], S. Roux[1], J. Rossert*[3], M. de Vernejoul[1]. [1]INSERM U349, Paris Cedex, France, [2]PROSKELIA, Romainville, France, [3]INSERM U489, Paris, France, [4]INSERM U368, Paris, France.

Although it has been suggested that matrix matalloproteinases (MMPs) may play a role in initiating the bone resorption process in vitro, there is to date no evidence that they play any role in in vivo bone maintenance. Mice specifically over-expressing TIMP-1 in osteoblasts have been generated to investigate the role of MMPs in bone in vivo.
We used an artificial promoter specifically driving cells of the osteoblastic lineage to overexpress the TIMP-1 (Tissue Inhibitor of MMPs) cDNA in mice. The level of expression of the transgene was about double that of the endogenous gene. Densitometric analysis, using DEXA and pQCT, and static and dynamic histomorphometry were used to evaluate the bone phenotype of mice from two independant transgenic lines. We showed that at 1 and 2.5 months of age only the female mice exhibited a bone phenotype. These mice displayed specific increases in the bone mineral density and volume of trabecular bone. This increase was accompanied by decreased trabecular separation, suggesting a decrease in bone resorption. Using an ex vivo resorption assay, we demonstrated that PTH-stimulated bone resorption was reduced in these mice. Evaluation of the bone histomorphometric dynamic parameters showed that the mineralizing surfaces and bone formation rate were both reduced. There was no change in the mineralization lag time or number of osteocyte lacunae. Using primary osteoblast culture and molecular analysis, we showed that the differentiation and function of osteoblasts from transgenic mice were normal, but that the ex vivo formation of mineralized nodules was delayed.
This model is the first to demonstrate that in vivo MMPs play a role in bone remodeling and bone balance. Moreover our data suggest that MMP activity could be involved in the hormonal regulation of the resorption by osteoblasts.

Disclosures: ***V. Geoffroy****, None.*

F052

Axial but not Peripheral Fractures Are Increased in Monoclonal Gammopathy of Undetermined Significance (MGUS). L. J. Melton[1], S. V. Rajkumar*[2], S. Khosla[2], S. J. Achenbach*[1], A. L. Oberg*[1], R. A. Kyle*[2]. [1]Health Sciences Research, Mayo Clinic, Rochester, MN, USA, [2]Internal Medicine, Mayo Clinic, Rochester, MN, USA.

Fractures in multiple myeloma patients result from lytic bone lesions, generalized bone loss and elevated bone turnover due to excessive osteoclast recruitment/activation by autocrine and paracrine cytokine production. Myeloma is typically preceded by the premalignant condition, MGUS, which is over 20 times more common. Elevated bone turnover, but not excessive bone loss, has been seen in patients with MGUS, but the implications for fracture have never been assessed. To test the hypothesis (H_o) that the skeleton is unaffected in MGUS, we estimated fracture risk among the 488 Olmsted County, MN residents with MGUS first diagnosed in 1960-94 (52% men; mean age, 71.4 $\pm$ 12.8 yr), who were subsequently followed in a population-based retrospective cohort study for 3901 person-years. During this period of observation, 200 patients experienced 385 fractures that were ascertained by review of complete (inpatient and outpatient) community medical records; follow-up was censored at progression to myeloma. Compared to expected fracture rates in the community, statistically significant increases were seen for fractures at most axial skeletal sites, e.g. vertebrae (standardized incidence ratio [SIR], 6.3; 95% CI, 5.2-7.5). There was a slight increase in hip (SIR, 1.6; 95% CI, 1.2-2.2) but not distal forearm fractures (SIR, 0.8; 95% CI, 0.4-1.5) so the overall increase in osteoporotic fracture risk (moderate trauma fractures of hip, spine or wrist $\geq$ age 35 yr: SIR, 2.5; 95% CI, 2.1-2.9) was accounted for by the increase in vertebral fractures. The relative risk (SIR) of any axial fracture was 2.7 (95% CI, 2.3-3.1) compared to only 1.1 (95% CI, 0.9-1.4) for all limb fractures combined. In a multivariate analysis, the independent predictors of any subsequent fracture were age (hazard ratio [HR] per 10-year increase, 1.4; 95% CI, 1.3-1.6) and corticosteroid use (HR, 1.8; 95% CI, 1.2-2.6); greater weight at diagnosis of MGUS was protective (HR per 10 kg, 0.8; 95% CI, 0.8-0.94). Baseline M-protein level, an important determinant of myeloma progression, did not predict fracture risk even in the univariate analysis (HR per 1 g/dL, 1.1; 95% CI, 0.9-1.5). Age independently predicted vertebral fractures (HR, 1.4; 95% CI, 1.2-1.7), while greater body mass index was protective (HR, 0.9; 95% CI, 0.9-0.96). Thus, the risk of axial, but not peripheral, fractures is increased among MGUS patients even before progression to myeloma. Further assessment of the pathophysiologic basis for this observation is needed since elevated bone turnover, for example, might be treatable with antiresorptive agents.

Disclosures: ***L.J. Melton****, None.*

F055

IL-3 Enhances Bone Destruction and Tumor Cell Growth in Myeloma Bone Disease. J. W. Lee*[1], S. J. Choi[1], H. Y. Chung[1], G. D. Roodman[2]. [1]Medicine-Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Medicine-Hematology/Oncology, University of Pittsburgh and Department of Veterans Affairs Medical Center, Pittsburgh, PA, USA.

We previously reported that Macrophage Inflammatory Protein (MIP)-1α is produced by myeloma cell lines and by patients with Multiple Myeloma (MM). MIP-1α induces human OCL formation independently of Receptor Activator of NF-Kappa B Ligand (RANKL), MIP-1α levels correlate with bone destruction in myeloma patients, and patients with elevated levels of MIP-1α have an extremely poor prognosis. Blocking MIP-1α expression in an in vivo model of human MM profoundly decreases disease progression and bone destruction by inhibiting both myeloma cell homing to the marrow as well as tumor cell growth. We recently reported that the regulation of MIP-1α gene expression is abnormal in patients with MM due to increased expression of the transcription factor Acute Myeloid Leukemia-1A (AML-1A) compared to AML-1B. We hypothesized that the increased expression ratio of AML-1A to AML-1B in myeloma also induced abnormal expression of other hematopoietic and bone specific genes that contribute to the poor prognosis of MM. We identified Interleukin-3 (IL-3) as a gene dysregulated by imbalanced AML-1A and AML-1B expression in myeloma. IL-3 mRNA expressions levels were increased in 8 out of 11 unfractionated bone marrow samples and 5 out of 7 highly purified samples of CD138+ myeloma cells from patients with MM who had decreased AML-1B expression. IL-3 was not consistently increased in patients with a normal AML-1A/B ratio. IL-3 protein expression levels were significantly increased in freshly isolated bone marrow plasma from patients with MM (66.4 $\pm$ 12 versus 22.1 $\pm$ 8.2, p=0.038). Consistent with these observations, IL-3 mRNA levels decreased where AML-1B was overexpressed in transfected myeloma cells compared to cells transfected with AML-1A or empty vector. Further more IL-3 (100 pg/ml) in combination with 200 pg/ml of MIP-1α and/or 10ng/ml of RANKL enhanced human OCL formation compared to MIP-1α or RANKL alone. Interestingly, huge OCLs that contained more than 10 nuclei formed in human OCL marrow cultures treated with IL-3 and MIP-1α or RANKL. These OCL demonstrated enhanced bone resorption on dentine slices. Importantly, IL-3 stimulated the growth of IL-6 dependent myeloma cells in the absence of IL-6, even though IL-3 did not induce IL-6 expression by myeloma cells. These data suggest that increased IL-3 expression in the bone marrow microenvironment of patients with MM by imbalanced AML-1A and AML-1B expression increases bone destruction and tumor cell growth in myeloma.

Disclosures: ***S.J. Choi****, None.*

F058

Menin, the MEN1 Gene Product, Interacts Directly with the TGF-beta Signaling Molecule, Smad3. L. Canaff, G. N. Hendy. Calcium Research Laboratory, McGill University, Montreal, PQ, Canada.

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by endocrine tumors of parathyroids, pancreas and pituitary. The tumor suppressor gene *MEN1* encodes a 610 amino acid nuclear protein called menin. Menin inactivation by antisense RNA antagonizes transforming growth factor type β (TGF-β) – mediated cell growth inhibition and menin co-immunoprecipitates with Smad3. Whether the interaction is direct and the sequence requirements for this are not known. To locate the Smad3 binding domain of menin, we generated truncated and deleted menin mutants and a panel of 12 naturally occurring menin missense mutants identified in MEN1 patients. Co-immunoprecipitation of transfected rat pituitary GH4C1 cells showed that menin Δ(1-40), menin Δ(1-100), menin Δ(277-477), menin Δ(195-610) and menin Δ(478-610) retained the ability to interact with Smad3 but the deletion of amino acids 1-195 from the N-terminus of menin abolished the interaction. Therefore, amino acids 101-195 are critical for menin-Smad3 interaction. All missense menin mutants retained the ability to co-immunoprecipitate with Smad3. To demonstrate direct interaction between menin and Smad3, we performed GST-

Smad3 pull-down reactions with *in vitro* translated ^{35}S-labeled full-length, deletion and missense mutants of menin. GST-Smad3 interacted with full-length menin, menin Δ(1-40), menin Δ(277-477), menin Δ(478-610) but not with menin Δ(40-277). All missense menin mutants interacted with Smad3. We previously showed that antisense menin suppressed, and co-expression of sense menin restored, TGF-β-induced transcriptional activity. Here, we investigated the ability of menin missense mutants to restore this activity. GH4C1 cells were transfected with a TGF-β responsive promoter reporter construct, antisense menin and wild-type or missense mutant menin constructs. Wild-type menin and missense mutants L22R, I86F, W126G, H139D, A242V, E255K, T344R, W423S, K502M and S555N were able to restore transcriptional activity. However, missense mutants A160P and A176P did not fully restore the activity. ^{35}S-labeled full-length Smad3, a Smad3 (EEVE) mutant that mimics the active phosphorylated molecule, and a Smad3ΔC mutant lacking aa 278-425 all interacted with GST-menin in pull-down assays. Therefore, the Smad3 MH2 domain and phosphorylation of the carboxyterminus are not essential for Smad3-menin interaction. These studies show that the interaction between menin and Smad3 is direct, the menin sequence 101-195 is critical for this, and identify amino acids within this region important for TGF-β stimulated transcriptional activity.

Disclosures: ***L. Canaff****, None.*

F061

Withdrawn

F063

A Low-molecular-weight Heparin Markedly Reduced Osteolytic Bone Destruction in a Mouse Model of Breast Cancer Bone Metastases. S. M. Käkönen[1], K. S. Mohammad[2], R. S. Käkönen*[3], A. Marjamäki*[4], A. Wärri*[4], M. Salmivirta*[4], T. A. Guise[2]. [1]University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [2]University of Virginia, Charlottesville, VA, USA, [3]Pharmatest Services, Turku, Finland, [4]BioTie Therapies, Turku, Finland.

Heparin treatment effectively prevents venous thromboembolism, but may also prolong survival in patients with malignant disease. Experimental studies support the hypothesis that cancer progression can be influenced by heparin and heparin-related compounds. The possible mechanisms include control of tumor cell growth by heparin-binding growth factors, tumor cell invasion by heparin-inhibitable enzyme systems, tumor cell metastasis by heparin-binding cell surface selectins and tumor angiogenesis. The effects of heparin compounds on cancer-induced osteolysis in bone metastases have not been examined. An unfractionated heparin has been shown to promote bone resorption, suppress bone formation and induce osteoporosis, whereas low-molecular-weight (LMW) heparin compounds have less adverse effects on bone. We have evaluated the therapeutic potential of dalteparin, a LMW synthetic heparin to inhibit osteolytic bone destruction in an experimental model of tumor metastases to bone.

MDA-MB-231 breast cancer cells were inoculated into left cardiac ventricle of athymic nude mice, which were then administered with LMW heparin (5mg/kg/d) or vehicle subcutaneously once daily. Animals were weighed weekly and the development of osteolytic lesions was monitored by radiography. Hind and fore limbs and soft tissue were collected for histomorphometry to evaluate tumor burden, bone area and osteoclast number.

The body weight of LMW heparin-treated mice was significantly higher compared to the vehicle group at sacrifice. In addition, only 18% of LMW heparin-treated mice were cachectic compared to 86% of the vehicle mice. Osteolytic lesion area as measured by radiographs was significantly lower in the LMW heparin-treated group when compared to the control. Histomorphometric examination revealed a significant reduction in the tumor burden in bone in the LMW heparin-treated mice compared to the control group. There were no changes in the total bone area between the groups. In addition, there were no differences in soft tissue metastases between the groups.

Our data demonstrate that LMW heparin treatment can efficiently reduce osteolytic lesion area and tumor burden in bone. Furthermore, it had clear beneficial effects on body weight and tumor-related cachexia in tumor bearing mice. LMW heparin could be a potential therapy to reduce the occurrence of bone metastases in addition for being used as thromboprophylaxis in cancer patients.

Disclosures: ***S.M. Käkönen****, None.*

F066

Fc-OPG Inhibits Osteoclast Activity, while Tumor-derived OPG Enhances Tumor Growth. J. L. Fisher*[1], R. J. Thomas-Mudge*[1], J. Elliott*[1], D. K. Hards*[1], N. A. Sims[1], C. R. Dunstan[2], J. Slavin*[3], T. J. Martin[1], M. T. Gillespie[1]. [1]St. Vincent's Institute of Medical Research, Fitzroy, Australia, [2]Amgen Corporation, Thousand Oaks, CA, USA, [3]Pathology, St. Vincent's Hospital, Fitzroy, Australia.

Osteoprotegerin (OPG) as a recombinant protein (Fc-OPG) has been demonstrated to inhibit osteoclast formation and activation both in vitro and in vivo. Since the local production of OPG may also be a means to limit tumor expansion in bone by preventing osteoclastogenesis, we used MCF-7 and MCF-7 cells overexpressing OPG, PTHrP or both OPG+PTHrP (MCF-7+OPG, MCF-7+PTHrP and MCF-7+PTHrP+OPG) to assess the contribution of tumor-cell derived OPG in tumor behavior in bone. Each of these cell lines were injected into the proximal tibiae of athymic mice, and animals monitored for 2.5 weeks, after which they were sacrificed and their limbs were assessed by radiology and histology. The cell lines were also inoculated into the mammary fat pad (MFP) to examine the effect of OPG overexpression at the primary site.

No osteolysis was observed radiologically following inoculation of the MCF-7 or MCF-7+OPG cells, although small intramedullary tumors were evident histologically in the latter group. MCF-7+PTHrP+OPG developed larger tibial tumors than the MCF-7 cells overexpressing PTHrP alone (18.6 ± 3.1 % osteolysis as determined by radiology, compared with 9.0 ± 2.2 %, $p<0.05$). The enhanced tumor growth afforded by OPG expression by MCF-7 cells was also observed in MFP tumor growth (50 days) whereby OPG overexpressing tumors were 2- to 3-fold larger in size.

The increased tumor growth in both bone and MFP as a consequence of OPG overexpression was abrogated by treatment with Fc-OPG (2.5 mg/kg/day, subcutaneous). Notably for tumor growth in bone, Fc-OPG treatment dramatically reduced osteoclast numbers at the tumor-bone interface in mice with MCF-7+PTHrP tumors, but osteoclast numbers were unaltered by Fc-OPG treatment in mice with tumors produced by MCF-7+PTHrP+OPG cells. Fc-OPG treatment of mice injected with either cell type resulted in a change of osteoclast morphology, with a flattened appearance adjacent to bone and reminiscent of a quiescent osteoclast. The effects of Fc-OPG were restricted to in vivo growth, as it had no effect upon cell growth in vitro.

These results indicate that full-length native OPG exerts dramatically different actions on tumor behavior than Fc-OPG, and that Fc-OPG acts upon stromal cells in the breast and may principally affect osteoclast activity (rather than formation) in established tumors in bone.

Disclosures: ***M.T. Gillespie****, None.*

F069

Soluble RANK Diminishes Progression of Prostate Cancer in SCID-Hu Animal Model. J. Zhang[1], J. Dai[1], Y. Lu*[2], W. Dougall*[3], Z. Yao*[2], E. T. Keller[1]. [1]University of Michigan, Ann Arbor, MI, USA, [2]Tianjin Medical University, Tianjin, China, [3]Department of Molecular Biology and Immunology, Amgen Inc, Seattle, WA, USA.

Prostate cancer (CaP) develops metastatic bone lesions with a mixture of osteosclerosis and osteolysis. We have previously demonstrated that targeting RANKL with OPG inhibits CaP-induced osteoclast activity and prevents tumor establishment in bone. However, OPG blocks TRAIL-mediated apoptosis. Thus methods to block RANKL activity, other than OPG, may be important. Accordingly, we evaluated ability of soluble murine RANK-Fc (sRANK) to prevent progression of established CaP in bone. Forty SCID mice had human fetal bone implanted subcutaneously. Four weeks later, LuCaP 35 prostate cancer cells (3 x 10^5) were injected into the implanted bone and allowed to develop into tumors. Six weeks after tumor implant, mice were palpated for presence of tumors and serum PSA was measured (28/40 mice had palpable evidence of tumors and were positive for PSA). The mice with tumors were randomly divided into three groups (n=9/group) to be either immediately sacrificed (BASE) or receive either sRANK-Fc (200 μg/mouse) or saline vehicle (V) by intraperitoneal injection three times a week. Mice were sacrificed after 6 weeks of sRANK administration. Serum PSA levels were decreased by 40% in sRANK-treated mice compared to V-treated mice. Osteocalcin and bone-specific alkaline phosphatase (bone formation marker) were decreased by approximately 30% and 25%, respectively, in the sRANK-treated mice compared to the V-treated mice. Urinary Ntx (bone osteolysis maker) were reduced by 30% in the sRANK-treated animals. Radiographs revealed primarily osteosclerosis; however, it was not possible to accurately quantify differences between groups based on radiographs. Bone mineral density was increased by 12% and 4% in the V and sRANK groups, respectively, compared to the BASE group. The trabecular volume/total bone volume (represents total mineralized bone in the trabeculae) was increased by 30% and 5% in the V and sRANK groups, respectively, compared to the BASE group. Tumor area compared to bone area tested by histomorphometric measurement was increased by 60% and 25% in the V and sRANK groups, respectively, compared to the BASE group (this represents continued tumor growth). The osteoclast perimeter (# osteoclasts/mm trabecular bone) was decreased by 0% and 90% in the V and sRANK groups, respectively, compared to the BASE group. Taken together, these data demonstrate that sRANK-Fc can diminish progression of an established osteoblastic CaP cell line in human bone.

Disclosures: ***J. Zhang****, None.*

F074

An Evaluation of the National Osteoporosis Society Position Statement on the use of Peripheral X-ray Absorptiometry. G. M. Blake, R. Patel, I. Fogelman. Osteoporosis Research Unit, Guy's, King's and St Thomas' School of Medicine, London, United Kingdom.

The recent United Kingdom National Osteoporosis Society (NOS) position statement on the use of pDXA devices proposes an algorithm that divides patients into 3 categories: (1) patients with a forearm T-score less than -2.5 who are recommended for treatment; (2) patients with a forearm T-score between -1 and -2.5 who are referred for spine and hip BMD measurements; (3) patients with a forearm T-score greater than -1.0 who are not treated. We have evaluated the clinical effectiveness of the NOS algorithm by comparing it with the alternative strategy of using forearm T-scores alone. The comparison was carried out using a mathematical model in which the population distribution of spine, hip and forearm BMD was described using a triaxial gaussian model. Correlation coefficients $r = 0.7$ were assumed between the different BMD measurements. Relative risks of fracture at each site were taken from the Marshall meta-analysis. The area under the curve (AUC) for the ROC curve showing the percentage of fracture cases plotted against the percentage of the whole population identified was calculated for the NOS algorithm and for forearm BMD alone. For identifying patients at risk of any type of fracture the AUC values were 0.62 and 0.59 respectively. For patients at risk of hip fracture they were 0.71 and 0.66, and for spine fracture 0.69 and 0.65. The small difference between the AUC values suggests that use of forearm BMD alone with an adjusted T-score threshold can reproduce the clinical outcome of the NOS algorithm. Detailed results of the model for discrimination of patients at risk of hip fracture are shown in the Table. For example, at age 65 20% of patients are treated on the basis of their forearm BMD, 55% are referred for spine and hip BMD, after which a

total of 33% of patients are treated. The forearm BMD measurements identify 30% of future hip fracture cases compared with 63% using the NOS algorithm. However, use of forearm BMD with a modified threshold of T = -2.1 would identify 56% of hip fracture cases while still treating 33% of the population. We conclude that use of forearm BMD alone with a modified T-score threshold of –2.1 would save the need for spine and hip scans and identify only slightly fewer fracture cases for treatment.

	% General Population Treated				% Hip Fracture Population Treated		
Age	Forearm T < -2.5	Referred for spine and hip	NOS algorithm	Adjusted T-Score	Forearm T < -2.5	NOS algorithm	Forearm $T < T_{adjust}$
55	7	45	14	-2.1	13	38	31
60	13	51	22	-2.1	21	50	43
65	20	55	33	-2.1	30	63	56
70	33	53	50	-2.1	46	78	72

Disclosures: G.M. Blake, None.

F076

Parameters of Hip Geometry and Bone Mineral Density Measured by Dual Energy X Ray Absorptiometry as Predictors of Hip Fracture Risk in Elderly Women from EPIDOS Cohort. F. Duboeuf*, P. Szulc, A. M. Schott*, P. Meunier, P. D. Delmas. U 403, INSERM, Lyon, France.

Bone architecture is an important determinant of bone strength . Cross sectional studies suggest that structural parameters of hip geometry derived from hip dual Energy X ray absorptiometry (DXA) may provide a simple method to assess the risk of hip fracture. The aim of our study was to test this hypothesis prospectively. We measured femoral neck bone mineral density (BMD) on a Lunar DPX at baseline and parameters of neck geometry in 65 elderly women who subsequently had a hip fracture and in 167 age matched controls, as part of the EPIDOS prospective cohort. We calculated the Estimated Cross Sectional Area (ECSA), the Estimated Endosteal Diameter (EED), the Estimated Cortical Thickness (EACT), and the Estimated Trabecular Volume Fraction (ETVF) according to the equations given by T Beck et al (1). Femoral neck diameter (ND) was measured with the Lunar DPX Software. Buckling ratio was derived from the following equation : (ND/2)/EACT. Results (see Table) shows that several parameters of hip structure (ECSA, EACT, and ETVF) predict the risk of hip fracture as well as hip BMD. Hip fracture risk seems related to a lower bone mineral content (ECSA) but not to a smaller bone size (neck width). On a morphological basis, low ECSA in hip fracture patients is associated with a decreased cortical thickness (EACT) which results in a decreased bending strength (lower section modulus) and an increased bone instability (higher buckling ratio). However, when these parameters were adjusted for hip BMD, none of the Odds ratio remained significant
Age, Weight and Height adjusted Odds Ratios (per 1 SD) for prediction of hip fracture

	All fractures (65)	Cervical fractures (42)	Trochanteric fractures (23)
Neck Diameter	NS	NS	NS
ECSA	2.0 (1.4-2.9)	1.6 (1.1-2.5)	2.9 (1.6-5.5)
EED	NS	NS	NS
EACT*	2.5 (1.7-3.7)	1.9 (1.2-3.0)	4.4 (2.1-9.2)
ETVF*	2.4 (1.6-3.6)	1.8 (1.2-2.9)	4.3 (1.9-9.6)
Sec Modulus	1.7 (1.2-2.4)	NS	2.3 (1.3-4.0)
Buckling Ratio	0.5 (0.3-0.7)	0.6 (0.4-0.8)	0.3 (0.2-0.6)
BMD*	2.5 (1.6-3.7)	1.9 (1.2-2.9)	4.3 (2.0-8.8)

*NS, Non significant, * Significantly different between cervical and trochanteric fracture*
We conclude that parameters of hip geometry derived from BMD measurements by DXA predict the risk of hip fracture in elderly women, but that this prediction is highly related to the BMD value. More reliable techniques to assess hip architecture, such as high resolution quantitative computerized tomography, might be necessary to explore the contribution of bone architecture to hip fragility, independently of bone mass.
References 1-Beck TJ, Ruff CB, Warden KE, Scott WW, Rao RU 1990, Invest Rad 25: 6-18.

Disclosures: ***F. Duboeuf****, None.*

F079

The Effect of 3D-XA Derived Geometric Parameters on Proximal Femur Failure Load Prediction. A. Le Bras*[1], S. Kolta[2], C. Roux[2], J. Fechtenbaum[2], W. Skalli*[1], D. Mitton*[1]. [1]LBM-ENSAM-CNRS, Paris, France, [2]Rheumatology, Cochin Hospital, Paris, France.

Bone mineral density (BMD g/cm²) measured by Dual energy X-ray Absorptiometry (DXA) has proven to be an important risk factor for hip fracture. Other structural factors such as bone micro and macro-architecture independently affect bone resistance. Bone macro-architecture has already been evaluated using 2 dimensional images, either on X-ray films or on DXA scans. Several geometric parameters have been evaluated as hip axis length (HAL), femoral neck axis length (FNAL), femoral neck diameter, neck-shaft angle, cortical wall thickness,… Many of these parameters predict fracture and some independently of BMD results. However, these 2D parameters greatly depend on patient positioning and inter individual anatomical differences. Therefore, their 3D evaluation would be more precise. A method of 3 Dimensional X-ray Absorptiometry (3D-XA) allowing 3D reconstruction of human proximal femurs using 2 orthogonal DXA scans was described (1). The aim of this study was to calculate whether adding the geometric parameters calculated on the 3D reconstruction of proximal femurs to areal BMD improve failure load prediction . 24 human proximal femurs were included 5 Males and 19 Females, aged 84±13 y. Two perpendicular DXA scans were acquired on a Delphi-W (Hologic) device. Areal density was measured as usual (g/cm²) and 3D reconstruction was done using both scans as previously described (1). On the 3D reconstruction we defined some geometrical parameters as the femoral neck axis length, the neck shaft angle, the femoral neck minimal cross sectional area and the femoral head diameter. Stance phase was selected for mechanical testing. Femoral shaft was maintained at an angle of 25° to the vertical. A vertical load was applied on femoral head at 12.7 mm/min. The vertical displacement and the compression strength were measured during the test. All the fractures were subcapital. Failure load was best correlated to femoral neck areal BMD ($r^2 = 0.62$, $p<0.0001$). Adding the 3D geometrical parameters in a linear multivariate regression model significantly improves failure load estimation ($r^2 = 0.73$, $p =0.0001$). This is the first study combining areal BMD and real 3D geometric parameters calculated from DXA images. It demonstrates that the measurement of geometric parameters using the 3D-XA method is possible and that adding these parameters to areal BMD allows better estimation of failure load for sucapital fracture in human femurs.
This work was supported by Biospace Instruments, France.
(1) 3 Dimensional X-Ray Absorptiometry (3D-XA) : Validation of 3D reconstruction of human proximal femurs using a DXA device. S Kolta, A Le Bras, V Bousson et al (ASBMR 2003)

Disclosures: ***J. Fechtenbaum****, None.*

F082

Interpretation of Peripheral Densitometry Devices in Clinical Practice: A Triage Approach to Diagnose Osteoporosis. J. A. Clowes, R. Eastell, N. F. A. Peel. Clinical Sciences (North), University of Sheffield, Sheffield, United Kingdom.

It is unclear how we might use peripheral densitometry in clinical practice. If we were to consider the T-score of the total hip as the 'gold standard' for the diagnosis of osteoporosis, then we could establish thresholds for each peripheral device above which the probability of osteoporosis is very low (<5%) or below which the probability of normal or osteopenic BMD is very low (<5%). We propose to use such a triage approach to identify individuals who require 1) additional central densitometry measurements (those between thresholds) or based on a peripheral measurement alone can be 2) recommended treatment (below lower threshold) or 3) appropriately reassured (above upper threshold).
We recruited 500 postmenopausal women (age 55-80 years) from the general population (population study) and 279 women with osteoporotic hip, vertebral, forearm and humeral fractures (fracture study). All subjects had measurements of spine, total hip (QDR4500A) and forearm DXA (DTX 200), forearm pQCT (XCT 2000) and QUS of the heel (Achilles+, DTU One, QUS 2, UBIS 5000), forearm (Omnisense) or phalanges (DBM Sonic).
In a population-based cohort (prevalence osteoporosis 9.8%) this triage approach correctly identified between 31% (DTU One SOS) and 73% (DTX 200, XCT 2000) of subjects in whom a treatment decision could be made without additional central DXA. The best performing QUS devices (QUS 2 BUA, UBIS 5000 BUA) identified 57% of subjects. In a fracture cohort (prevalence osteoporosis 36%) between 20% (DBM Sonic) and 41% (DTX 200, XCT 2000) of subjects did not require central DXA. The best performing QUS devices (UBIS 5000 BUA) identified 38% of subjects. The table shows thresholds for 9 measurements in the population study. Thresholds in the fracture population were slightly lower.
We conclude that a triage approach to interpreting peripheral densitometry results enables a treatment decision to be made in a substantial percentage of the population, but only with some devices.

Device	Threshold Upper (U) Lower (L)	Absolute value	Sensitivity (%)	Specificity (%)	% Not Requiring DXA
Spine DXA	U	0.983	96	38	43
(g/cm²)	L	0.707	41	95	
DTX 200 DXA	U	0.388	98	72	73
(g/cm)	L	0.286	32	95	
XCT 2000 QCT	U	279	88	75	73
(g/cm³)	L	195	22	98	
Achilles+ BUA	U	108	96	55	56
(dB/MHz)	L	94	22	96	
DTU One BUA	U	51	96	40	44
(dB/MHz)	L	35	28	95	
QUS 2 BUA	U	78	96	54	57
(dB/MHz)	L	55	35	95	
UBIS 5000 BUA	U	63	96	54	57
(dB/MHz)	L	56	39	95	
Omnisense SOS Radius	U	4067	96	32	35
(m/s)	L	3721	17	95	
DBM Sonic AdSOS	U	1876	96	33	35
(m/s)	L	1621	11	95	

Disclosures: ***J.A. Clowes****, None.*

F084

Metacarpal BMD and Cortical Index are Significant Predictors of Hip Fracture. S. Vasireddy, S. Bal, L. Reaney*, C. McGurk*, J. Kanis, E. McCloskey. University of Sheffield, Sheffield, United Kingdom.

The combination of assessments of skeletal strength and extra-skeletal risk factors optimises the identification of patients at high risk of future fracture. While hip BMD remains the reference standard, other more widely applicable skeletal measurements may be of value. In this study we have compared the ability of metacarpal BMD (mBMD) and cortical index (MCI) to that of hip BMD to predict incident hip fractures using a prospective case-control design.

The study cohort comprised 5212 women aged 75 years or older (mean 80 years, range 75-100) enrolled to a double-blind placebo-controlled study of the bisphosphonate, clodronate (Bonefos®). Baseline BMD at the total hip and distal forearm were measured by DXA (Hologic QDR4500 and Osteometer DTX200 respectively). Bilateral hand radiographs obtained at baseline were analysed using digital x-ray radiogrammetry (Sectra, Denmark) to produce automated measures of metacarpal BMD and MCI in 153 women who sustained incident hip fractures and in 532 randomly selected controls who remained free of hip fracture during a median follow-up of 4 years.

Cases with hip fracture had significantly lower hip BMD, forearm BMD, mBMD and MCI than controls. In univariate logistic regression analysis, the odds ratios (OR) for hip fracture per 1SD decrease in mBMD and MCI of 1.8 (95%CI 1.5-2.2, P<0.0001) and 1.7 (95%CI, 1.4-2.1, P<0.0001) respectively were similar to that of forearm BMD (1.9, 95%1.6-2.4, P<0.0001) while similar decreases in hip BMD demonstrated a larger gradient of risk (OR 2.4, 95%CI 1.9-3.0, P<0.0001). Adjusting for treatment with clodronate did not influence results. Metacarpal BMD and MCI remained significant predictors of hip fracture following adjustment for clinical predictors including age, height and body weight (OR 1.5, 95%CI 1.2-1.9, P=0.001 and 1.4, 1.1-1.7, P=0.002 respectively) but were not independent of forearm BMD or hip BMD. Following adjustment for the clinical variables, the ORs for the metacarpal indices remained comparable to that of forearm BMD (1.5, 95%CI 1.2-1.9) but was lower than that for hip BMD (2.0, 95%CI 1.6-2.6).

We conclude that metacarpal BMD and MCI are significant predictors of hip fracture independently of other extra-skeletal clinical risk factors. In the absence of access to hip BMD, mBMD or MCI may prove useful skeletal measures of fracture risk to include in the risk assessment of individuals.

Disclosures: ***S. Vasireddy****, None.*

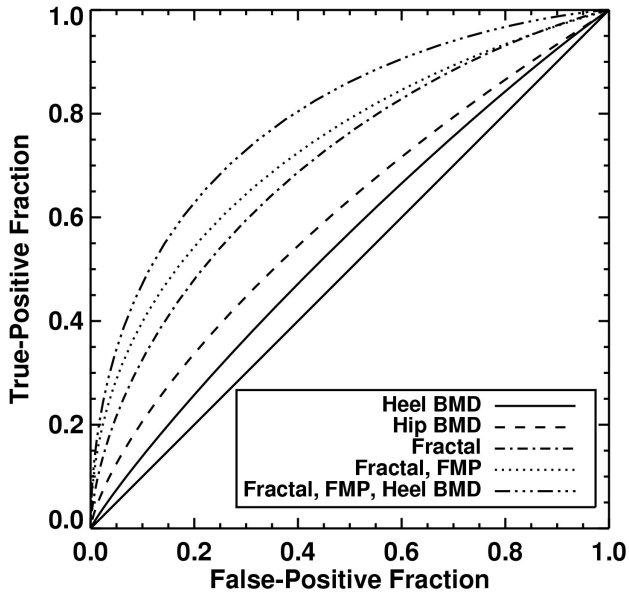
F087

Bone Mineral Density (BMD) and Radiographic Texture Analysis (RTA) of Densitometer Generated Calcaneus Images in Patients with and without Osteoporotic Fractures. T. J. Vokes[1], M. L. Giger*[2], M. R. Chinander*[2], M. J. Favus[1], V. Jaros*[1], D. Singh*[1]. [1]Medicine/Endocrinology, University of Chicago, Chicago, IL, USA, [2]Radiology, University of Chicago, Chicago, IL, USA.

Bone fragility is determined by bone mass, measured as BMD, and by bone structure, which cannot be easily assessed using currently available methods. RTA is an experimental method for evaluating bone structure. We have previously shown that radiographic texture analysis of densitometer-obtained heel images can differentiate subjects with and without vertebral fractures.

The purpose of this study was to determine whether RTA of densitometer-generated calcaneal images also differentiates subjects with and without any osteoporotic fractures (vertebral or peripheral). BMD of the lumbar spine, femoral neck and calcaneus was measured and RTA performed in 195 subjects referred for routine BMD testing (79 with and 116 without fractures). RTA yielded RMS (root mean square - a measure of the magnitude of variation in the trabecular pattern), FMP (first moment of the power spectrum – a measure of the coarseness of the trabecular pattern), and a fractal dimension, estimated using Minkowski dimension analysis. Fracture group had lower femoral neck (but not lumbar spine or calcaneus) BMD (p=0.001), and higher texture features fractal (p=0.001) and FMP (p=0.04), all by t-test. The same variables were significant predictors of presence of fractures in univariate analysis. In multivariate analysis, the presence of fractures was best predicted by a combination of femoral neck BMD (p<0.001) and fractal (p<0.001), and by the combination of fractal (p<0.001) and calcaneus BMD (p=0.001). In a subgroup of 93 women with untreated postmenopausal osteoporosis, texture features but not BMD measurements differed between 36 subjects with and 57 without fractures. In this subgroup, the highest area under the ROC curve Az=0.814 was achieved by a combination of calcaneus BMD, fractal, and FMP (figure).

We conclude that RTA differentiates subjects with and without fractures as well as BMD measurements. In postmenopausal women, a combination of calcaneal BMD and RTA, both obtained using a peripheral densitometer, provides a better assessment of fragility than the standard central BMD measurement.



Disclosures: ***T.J. Vokes****, Merck 2, S; Aventis 8.*

F089

Determinants of Proximal Femoral Strength in Elderly Women. T. F. Lang[1], J. H. Keyak*[2], A. Yu*[1], Y. Lu[1], L. Do*[1], J. Li*[1]. [1]Radiology, University of California, San Francisco, San Francisco, CA, USA, [2]Orthopaedic Surgery, University of California, Irvine, San Francisco, CA, USA.

DXA BMD estimates hip fracture risk by acting as a surrogate for bone strength. But this hybrid measure of bone density and size provides little information about how these factors individually determine hip strength. To address this, we used quantitative computed tomography (QCT) based finite element (FE) models, which encompass the full spatial distribution of material properties in the hip, to estimate failure load (FL) in 51 women (mean age 74.3±4.5 years). We related FL to integral and compartmental bone density and geometry quantified from the QCT scans.

Subjects underwent QCT imaging of the hip (GE 9800Q, 3mm slices). FE models of the left hip were built from the QCT scans, and were loaded in conditions simulating single-limb stance (producing subcapital fractures) and a fall backwards and to the side (trochanteric fractures). The FE procedure produced stance (FL_s) and fall (Fl_{fall}) failure load estimates in kN. To compare abilities of volumetric and areal BMD to predict hip strength we linearly regressed FL_s and Fl_{fall} against integral volumetric (viBMD) and integral areal BMD (aBMD). We built multivariate models relating FL_s and FL_{fall} to age, height and weight and to cortical BMD (cBMD), trabecular BMD (tBMD) and minimum femoral neck cross-sectional area (mincsa). The contribution of each parameter was given as standardized beta. Results are tabulated below.

aBMD and viBMD were roughly equivalent predictors of FL_s and FL_{fall} (r=0.7-0.8, $RMS_{error}FL_s$=1.7-2.0 kN, $RMS_{error}FL_{fall}$=0.43-0.44 kN). After height and weight correction, FL_{fall} declined with age (3%/yr, p=0.01) but not Fl_{stance}. Multivariate models including compartmental BMD and size parameters tended to predict FL better than aBMD or viBMD. In these models, tBMD, cBMD and mincsa independently predicted FL_s, but only tBMD and mincsa predicted FL_{fall}.

We observed different risk factors for hip fractures during single-limb stance and falls. These results agree with epidemiologic findings of different risk factors for cervical and trochanteric fractures.

FL type (kN) (mean± SD)	R (RMS_{error} kN)	tBMD β_{std} (p)	cBMD β_{std} (p)	mincsa β_{std} (p)
FL_s (11.5±3.03)	0.9 (1.53)	0.45 (<0.0001)	0.40 (0.02)	0.27 (0.007)
FL_{fall} (1.8±0.73)	0.9 (0.39)	0.60 (<0.0001)	0.27 (0.12)	0.30 (0.004)

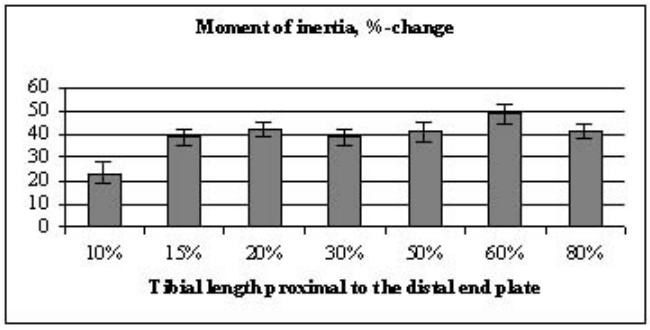
Disclosures: ***T.F. Lang****, None.*

F091

Geometric Characteristics of the Developing Tibia in Early Pubertal Girls: A Quantitative MRI Study. H. M. Macdonald*[1], A. Heinonen*[2], K. M. Khan*[3], K. J. MacKelvie*[4], H. Sievanen*[5], K. P. Whittall*[6], B. B. Forster*[6], H. A. McKay[7]. [1]Human Kinetics, University of British Columbia, Vancouver, BC, Canada, [2]UKK Institute, Tampere, Finland, [3]Family Practice, University of British Columbia, Vancouver, BC, Canada, [4]Endocrinology, University of British Columbia, Vancouver, BC, Canada, [5]Department of Health Sciences, University of Jyvaskyla, Tampere, Finland, [6]Radiology, University of British Columbia, Vancouver, BC, Canada, [7]Orthopaedics, University of British Columbia, Vancouver, BC, Canada.

INTRODUCTION: During puberty girls reach a peak bone mineral accrual velocity of approximately 325 g/yr. As bone mass, geometry and structure are all determinants of bone

strength it is essential that all parameters be characterized in the growing skeleton. Currently there is very little prospective data on bone geometry in growing girls.
AIM: Therefore, we studied change in tibial cross-sectional cortical area (CoA), total area (ToA), cortical to total area ratio (CoA/ToA) and cross-sectional moment of inertia (CSMI) in sixteen 10-11 yr old girls across two years of growth.
METHODS: We measured height and weight by standard methods and maturity by Tanner breast staging. We used a 1.5T MR system (GE Medical Systems) with a quadrature head coil to measure CoA, ToA and CSMI of slices located proximal to the distal tibia end plate at 10% (distal site), 15%, 20%, 30%, 50%, 60% (shaft sites) and 80% (proximal site) of the total tibial length. The sequence was T1-weighted, spin echo in the aforementioned seven transverse (tibial) planes, 3.0 mm sections with no gap.
RESULTS: After 2 years, the increase in ToA along the tibial length ranged from 17% to 23%. The CoA increased significantly more (19% to 24%) at the shaft sites compared with the distal site (9%). The CoA/ToA ratio increased significantly more at the shaft compared with both proximal and distal sites where it decreased by 4% and 8% respectively. Changes in CSMI are presented (Figure).
CONCLUSION: These MRI outcomes quantify the region specific change in bone parameters along the tibial length during growth in girls. Distally, changes in periosteal dimensions accounted for greater increases in bone strength whereas strength increases at the tibial shaft are explained by cortical enlargement. This reflects the axial compressive and bending moments, respectively, experienced at these two sites.



Disclosures: ***H.M. Macdonald****, None.*

F097

The Value of Bone Turnover Markers in the Prediction of Vertebral Fracture in Patients Using Oral Glucocorticoids. T. P. van Staa[1], R. Eastell[2], I. P. Barton*[1], R. Rizzoli[3], M. K. Javaid[4], C. Cooper[4]. [1]Procter&Gamble Pharmaceuticals, Egham, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom, [3]Hopitaux Universitaires, Geneva, Switzerland, [4]Medical Research Council, Southampton, United Kingdom.

Several bone turnover markers (BTM) predict fracture risk in postmenopausal osteoporosis, but their value in patients using oral glucocorticoids (GC) is unknown. The objective of the study was to evaluate whether BTMs, vitamin D and intact parathyroid hormone (PTH) predict fracture in GC users.
Data were obtained from two randomized clinical trials (risedronate prevention and treatment studies) with similar methods but different inclusion criteria with respect to prior CS exposure. The study population comprised of 306 patients with one-year vertebral fracture assessment (111 placebo and 195 risedronate patients) and included 30 patients with an incident fracture. The following bone turnover markers were measured at baseline and after 6 months: bone specific alkaline phosphatase (Bone ALP), osteocalcin (OC), deoxypyridinoline/creatinine (DPD/Cr), pyridinoline/creatinine (PYD/Cr)), and N-telopeptide of type I collagen (NTX). PTH and serum 25-hydroxy-vitamin D_3 [$25(OH)D_3$] were also measured at baseline. Cox regression analysis was used to determine whether these variables predicted incident vertebral fracture after 12 months.
In the placebo group, the age- and sex-adjusted relative rate (RR) of fracture in relation to baseline BTMs for 1 unit (standard deviation) increase were as follows: DPD/Cr 1.72 (95% confidence interval 0.99-2.99), NTX 1.76 (1.01-3.06), PYD/Cr 1.65 (1.03-2.64), Bone ALP 1.18 (0.71-1.96), OC 0.89 (0.49-1.63). PTH predicted fracture [age-sex-adjusted RR 1.80 (1.03-3.15), which became 4.62 (1.33-16.05) after BMD adjustment]. Vitamin D was not predictive [age-sex-adjusted RR 0.72 (0.38-1.37)]. In the risedronate group, both baseline BTMs and changes after 6 months of treatment did not predict fracture. The reduction in fracture risk in the risedronate group (relative to placebo) was similar across levels of baseline bone markers and vitamin D [e.g., the RR in patients with NTX ≤51 nmol BCE/mmol (median) was 0.31 (0.09-1.06), and > 51 was 0.37 (0.11-1.25)]. For baseline PTH, the RR in the patients with PTH ≤ 25 ng/mL was 0.98 (0.26-3.65), and 0.09 (0.02-0.34) for PTH > 25 (test for interaction P > 0.05).
In conclusion, markers of bone resorption predicted fracture better than markers of bone formation in the placebo group. BTM did not predict fracture in the risedronate group. Higher PTH levels were also predictive of fracture and appeared to be associated with a larger treatment effect of risedronate.

Disclosures: ***T.P. van Staa****, None.*

F100

Markers of Bone Turnover and History of Prior Fractures as Predictors of Long Term Fracture Risk in Postmenopausal Women Without Osteoporosis : The OFELY Study. E. Sornay-Rendu*, F. Munoz*, P. Garnero, F. Duboeuf*, P. D. Delmas. Unit 403, INSERM, Lyon, France.

Although a low bone mineral density (BMD) is the most important risk factor for fracture in postmenopausal women, about half of patients with incident fractures have baseline BMD above the WHO diagnostic threshold of osteoporosis (T score ≤ -2.5). Several studies have shown that bone turnover markers (BTM) and history of prior fracture are BMD independent risk factors for fractures. The aim of this study was to investigate whether the assessment of BTM and history of fracture could be used to identify, within women with a non osteoporotic BMD level, those at risk of fracture that may need a therapeutic intervention. We measured at baseline BMD by DXA at the spine and total hip and BTM in 668 postmenopausal women (mean age : 62 yr). Women were categorized in 3 groups according to their baseline BMD values : normal (T score at the spine and hip>-1), osteopenic (T score at the spine or hip between -2.5 and -1) and osteoporotic (T score at the spine and/or hip ≤-2.5). During a median 9.1 yr (IQ :2.9) of follow-up, 158 incident fractures including 50 vertebral and 108 non-vertebral fractures were recorded in 115 women : 10% in normal, 47% in osteopenic and 43% in osteoporotic women based on BMD.
In the whole population, baseline values of bone ALP in the highest quartile and history of prior fracture were associated with increased fracture risk with age adjusted hazard ratios [HR(95%CI)] of 1.5(0.98-2.2) and 2.4(1.5-3.7) respectively. The table shows the age adjusted HR, sensitivity and specificity of bone alkaline phosphatase, history of prior fracture and their combination for predicting fracture risk in women with BMD values in the osteopenic range. Similar results were obtained with serum osteocalcin and CTX. Thus, in women with osteopenia 59% of them with incident fractures could be identified with history of fractures and a single BTM measurement.

Predictors	HR (95% CI), p value	Sensitivity	Specificity
Bone ALP	2.2 (1.3-3.7), p<0.006	43	75
History of prior fracture	2.7 (1.4-5.0), p<0.002	29	89
Bone ALP and / or prior fracture	2.7 (1.5-4.6), p<0.0006	59	66

We conclude that in postmenopausal women with BMD values in the osteopenic range, a marker of bone turnover and history of prior fracture predict the risk of fracture up to 10 years later. Their assessment may play an important role in identifying women at high risk of fracture who could not be adequately detected by BMD measurement alone, and who may benefit from a therapeutic intervention.

Disclosures: ***E. Sornay-Rendu****, None.*

F109

Role of the Promoter –1997 G/T and -1663indelT Polymorphic Sites on the Transcription Regulation of the COL1A1 Gene. S. Balcells*[1], N. Garcia-Giralt*[1], A. Enjuanes*[2], X. Nogues[3], L. Mellibovsky*[3], A. Diez[3], D. Grinberg*[1]. [1]Genetics, University of Barcelona, Barcelona, Spain, [2]Urfoa, Institut Municipal d'Investigació Mèdica, Barcelona, Spain, [3]Hospital del Mar Universitat Autònoma de Barcelona, Barcelona, Spain.

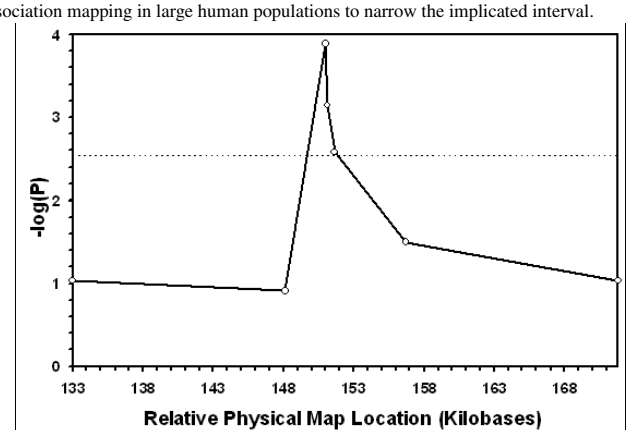
We identified two new polymorphisms in the upstream regulatory region of COL1A1 which were associated to BMD in postmenopausal women: one is a DIP (deletion/insertion polymorphism) at –1663 with two alleles containing 7 or 8 Ts, and the second, a SNP at –1997, with G or T alleles. We also showed that the sites containing these polymorphisms (PCOL1 and PCOL2) were binding sites for osteoblastic nuclear proteins, and that there were affinity differences between the two alleles of each polymorphism (JBMR 17:384-93, 2002). Here we present functional analyses of these regulatory regions using a luciferase reporter gene assay in MG63 cells. In order to compare the four different haplotypic combinations for the two polymorphisms (G/8T, G/7T, T/8T, T/7T), we prepared constructions of the full promoter (-2483 +40) or shorter ones in which a region from –1284 to –254, known to harbour a strong negative regulatory element (IR), was deleted. Preliminary results indicate that the two haplotypes bearing T at –1997(T/8T and T/7T) were the ones associated to a lower level of transcription, particularly haplotype T/8T in the absence of the IR. We have also tested constructions in which only one of the polymorphic sites was present, to compare the two alleles at each site. Transcription associated with the -1997T seemed to be lower than that associated with -1997G. Differences for the -1663 alleles were only observed in the absence of the IR: the 7T allele showed higher luciferase activity than the 8T allele. Finally, we have analysed different constructions bearing combinations of three different parts of the COL1A1 promoter in basal conditions or under different treatments (vitamin D, dexamethasone, TNFalpha, IL-1beta, estrogen, testosterone and PTH) to identify regions containing responsive elements. The main results were that vitamin D stimulated transcription only in the absence of the IR region, dexamethasone stimulated in all cases but has a greater effect if the polymorphisms were present, while TNFalpha and IL-1beta always decreased luciferase levels. In conclusion, variants –1997 T/G and -1663 indelT play a role in the transcription regulation of the COL1A1 gene, compatible with the BMD differences found among women bearing different genotypes at these sites.

Disclosures: ***S. Balcells****, None.*

F115

An X-Chromosome Locus Modulates Lumbar BMD in Postmenopausal Women, and Age-Dependent Decline of Spinal BMD in Mice. H. Mroczkowski*[1], C. A. Parsons*[2], O. M. E. Albagha*[2], D. M. Reid*[2], S. C. Manolagas[3], S. H. Ralston[2], R. J. Shmookler Reis[4]. [1]Biochem./Molec. Biol., Univ. of Arkansas for Medical Sciences and CAVHS, Little Rock, AR, USA, [2]Medicine & Therapeutics, Univ. of Aberdeen, Aberdeen, United Kingdom, [3]Medicine, Univ. of Arkansas for Medical Sciences and CAVHS, Little Rock, AR, USA, [4]Geriatrics, and Biochem./Molec. Biol., Univ. of Arkansas for Medical Sciences and CAVHS, Little Rock, AR, USA.

We recently identified several quantitative trait loci (QTL) for post-maturity change in spinal BMD of mice, by interval mapping in an interstrain-cross F2 population. From a significant mouse QTL on the X chromosome, we located the corresponding (conserved-synteny) region of the human X chromosome by reference to the working-draft human genome sequence. DNA sequencing and single base extension techniques, using protocols modified from those of the ABI SNaPshot and Molecular Dynamics SNuPe systems, were developed to genotype at single nucleotide polymorphisms (SNPs) across this human genomic interval. We then investigated the allele proportions for each SNP in a population of postmenopausal women from northeastern Scotland (n=3653). Subjects were ordered by lumbar-spine BMD values adjusted for age, height and weight, to identify individuals in the highest and lowest bone density deciles of the normalized population distribution. We performed initial genotyping in quadruplicate on replicate pools of DNA samples, created by combining equal amounts of DNA from each individual in a decile group. Analysis of the SNP allele peak height ratios implied a significant difference between the high and low DNA pooled samples at one SNP ($p<0.0002$), and this apparent difference was confirmed by testing the individual DNA samples comprising each pool ($p<0.004$). Reanalysis of pooled data, by a procedure that includes sampling and assay variance to calculate significance, gave 3 adjacent SNPs with p values <0.003 (Figure). Fine-scale mapping has thus narrowed the candidate region to <75 kilobase pairs and two possible genes. This study demonstrates the feasibility and benefit of transferring QTL information between the mouse and human genomes, at a stage well in advance of gene identification, relying on association mapping in large human populations to narrow the implicated interval.



Disclosures: ***R.J. Shmookler Reis****, None.*

F118

Completion of a Whole-Genome Scan for Linkage to Bone Mineral Density in Forty-two Caucasian Families. K. Sol-Church*[1], L. D. Spotila[2], H. Li*[1], G. N. Picerno*[1], D. L. Stabley*[1], K. Wang*[1], J. Korkko*[3], R. Kosich*[4], D. Prockop*[3], A. Tenenhouse*[5], C. McKay[6], M. Devoto*[1]. [1]Biomedical Research, Alfred I duPont Hospital for Children, Wilmington, DE, USA, [2]Drexel University, Philadelphia, PA, USA, [3]Tulane University, New Orleans, LA, USA, [4]Mcp, Hahnemann University, Philadelphia, PA, USA, [5]McGill Bone Centre, McGill University, Montreal, PQ, Canada, [6]Nephrology, Alfred I duPont Hospital for Children, Wilmington, DE, USA.

Osteoporosis of the femoral neck and lumbar spine is caused, in part, by low bone mineral density (BMD). In an attempt to identify the specific genes that might influence BMD, and thus osteoporosis, we and others have performed whole genome scans for linkage. The results of these studies are notable for the diversity of chromosomal loci with positive support for linkage. Included in this list are loci on chromosomes 1p, 1q, 2p, 4, 5q, 6p, 10, 11p, 11q, 12. Our initial study (EJHG (1998) 6:151) identified three loci in seven families recruited through a proband with low BMD. One of the loci, 1p36, was investigated further in a total of 42 families (HMG (2001) 10:2447) demonstrating further support for linkage of the hip BMD trait to 1p36.2-36.3. Here we report results of completion of a whole genome scan on the expanded sample of 42 families.

The families were recruited from the Metabolic Bone Centre of Montreal General Hospital, and were composed of from one to 12 nuclear families. Linkage mapping marker set v2.5 (Applied Biosystems) was used to genotype the available individuals, and data were compiled using Genotyper 2.1. Mendelian errors were detected by means of the Pedmanager software. Linkage was analyzed using the SOLAR package (AJHG (1998) 62:1198) that implemented a variance components approach. Data for chromosomes with lod-scores greater than 1 were also analyzed by means of the transmission disequilibrium test using the QTDT software (AJHG (2000) 66:279).

Heritability of BMD was 0.51 +/- 0.13 (femoral neck) and 0.79 +/- 0.1 (lumbar spine). Significant covariates included in the linkage and the QTDT analyses were age, sex, and BMI. In addition to lod = 2.85 at 1p36, two sites had lod scores greater than 1.5 for the femoral neck trait. These loci were 2p (34 cM from pter, lod = 1.76) and 22p (0 cM, lod = 1.94). The 2p locus also gave a lod score greater than 1 for the lumbar spine (35 cM, 1.30). Of these loci, none was significant in the QTDT analysis.

On the basis of our current data analysis, we conclude that there is some statistical support for loci on chromosomes 2p and 22p. The completion of this genome scan on this expanded set of families has served to place the observation of linkage at 1p36 in the context of a whole-genome scan.

Disclosures: ***K. Sol-Church****, None.*

F122

A QTL with Pleiotropic Effects on Serum Levels of Osteocalcin and Bone Alkaline Phosphatase in Baboons Maps to a Region Corresponding to Human Chromosome 6p21.1-21.3. L. M. Havill, M. C. Mahaney, J. Rogers. Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA.

We conducted a bivariate statistical genetic analysis of serum concentrations of bone specific alkaline phosphatase (Bone ALP), a highly specific marker of osteoblast function, and osteocalcin (OC), another osteoblast product and a marker of bone turnover, to search for genes with pleiotropic effects on these phenotypes. Using commercially available enzyme immunoassay kits, Bone ALP and OC were assayed in frozen serum samples obtained from 611 pedigreed baboons (*Papio hamadryas*) (437 females, 174 males) aged 5.5-30.0 years. We used a maximum likelihood variance decomposition approach to estimate the following parameters simultaneously for both traits: population (male) mean; mean effects of age, age^2, age-by-sex, age^2-by-sex, height, weight, and osteophytosis score; the proportion of the residual phenotypic variance due to the additive effects of genes (heritability (h^2)); unmeasured environmental factors (e^2), and the correlations between both traits due to shared genetic effects (ρ_G) and shared unmeasured environmental factors (ρ_E). Variation in serum Bone ALP and OC levels showed residual heritabilities of 0.17 (p=0.0005) and 0.25 (p=0.0006), respectively. A significant positive genetic correlation between the two phenotypes (ρ_G=0.42, p=0.003) provides strong evidence that 18% of the additive genetic variance in the two traits is attributable to the same gene(s). Initial univariate linkage screens localized possible QTLs for genes influencing variation in Bone ALP (LOD=2.80) and OC (LOD=2.71), to opposite ends of a 30 cM region of baboon chromosome 4 (homologous to human chromosome 6). Maximization of a multivariate multipoint linkage model, parameterized according to the bivariate model (above) and allowing for an additive genetic correlation due to the QTL, improved evidence for linkage (LOD=3.28) in this same region, localizing a single QTL intermediate to human microsatellite markers D6S422 and D6S259. This region of baboon chromosome 4 corresponds to human chromosome 6p21.1-21.3. Likelihood ratio tests reject the hypothesis of coincident linkage (p=<0.00001) supporting the pleiotropic effect of a single QTL on two markers of bone formation and turnover in the baboon. Given the close genetic and physiological similarity of this animal to humans, these results should be highly informative in our search for genes affecting skeletal maintenance and repair in humans.

Disclosures: ***L.M. Havill****, None.*

F126

Testing Association and Linkage of 8 Candidate Genes with BMD in 405 Caucasian Families Using Multiple SNP Markers and Haplotypes. L. J. Zhao*[1], J. R. Long*[2], H. Shen*[1], P. Y. Liu*[2], Y. Y. Zhang*[2], P. Xiao[1], D. H. Xiong[1], L. Elze*[2], R. R. Recker[2], H. W. Deng[1]. [1]Biomedical Sciences, Osteoporosis Research Center, Omaha, NE, USA, [2]Osteoporosis Research Center, Omaha, NE, USA.

With robust design and analyses, a large family sample, multiple SNPs (single nucleotide polymorphism) and haplotypes, this study is to test thoroughly 8 prominent candidate genes for BMD: vitamin D receptor (VDR), estrogen receptor α (ER-α), apolipoprotein E (APOE), type I collagen α 1 (COL1A1), transforming growth factor β 1 (TGF-β1), parathyroid hormone receptor type 1 (PTHR1), interleukin-6 (IL6) and tumor necrosis factor receptor 2 (TNFR2). Previous studies using a limited few markers, relatively small samples, and/or unreliable designs have yielded largely inconsistent results. 405 families with 1,874 Caucasians were recruited, phenotyped and genotyped. The program QTDT (quantitative transmission disequilibrium test) was used to test 28 SNPs in these genes (4 SNPs for the VDR gene, 6 for ER-α , 4 for APOE, 4 for PTHR1, 3 for COL1A1, 3 for TGF-β1, 2 for IL6 and 2 for TNFR2). Strong linkage disequilibrium was generally detected for SNPs within these genes. For the VDR gene, significant linkage with the ultra-distal BMD (UD BMD) was detected for all the four individual SNPs (p<0.005). Analyses of the haplotypes of the VDR confer further evidence for linkage of the VDR with the UD BMD (p<0.0001). Linkage was also detected for all the 4 SNPs of VDR with total body BMD (p<0.01), and for SNP rs2228570 with radius BMD (P<0.01). The haplotype ATAC of the VDR was associated with low BMD at intertrochanter (p<0.003), hip (p=0.005) and for the whole body (p<0.01). For the ER-α gene, SNP rs932477 was associated with wrist BMD variation (p=0.005). Association was also found for the ER-α haplotype CGCGAA with high body BMD (p<0.005), and haplotype CACGGG with low hip BMD (p<0.008) respectively. For the APOE gene, both individual SNPs and haplotype analyses suggested its linkage to whole body BMD (p<0.008). For the PTHR1 gene, the haplotype GATG was associated with high BMD at hip (p<0.002), femoral neck (p<0.0001), trochanter (p<0.01), intertrochanter (p<0.005), and whole body (p<0.003). Subjects carrying the haplotype GATG had femoral neck BMD 6.04% higher than none-carriers. Haplotype AACA of the PTHR1 gene was associated with high BMD at hip (p<0.006) and intertrochanter (p<0.005). In summary, our work supports that the VDR and APOE genes are linked to BMD variation and the ER-α and PTHR1 genes are associated with BMD variation in our Caucasian sample.

Disclosures: ***L.J. Zhao****, None.*

F131

Association of Apolipoprotein E Genotypes with Bone Mineral Density and Bone Loss in Early Postmenopausal Scottish Women. H. M. Macdonald[1], F. M. McGuigan*[1], P. N. Tasker*[1], A. Bassiti*[1], S. C. Main*[1], S. A. New[2], D. M. Reid[1], S. H. Ralston[1]. [1]Medicine and Therapeutics, University of Aberdeen, Aberdeen, United Kingdom, [2]School of Biological Sciences, University of Surrey, Guildford, United Kingdom.

The Apolipoprotein E4 (APOE 4) allele has been associated with reduced bone mineral density (BMD) in postmenopausal women from Japan and the Netherlands, and with rates of bone loss in peri- and postmenopausal American women. In this study we investigated whether APOE genotype influences BMD or bone loss using subjects from the Aberdeen Prospective Osteoporosis Screening Study (APOSS). A total of 3883 women underwent bone densitometry in 1990-3 (mean ± SD age: 48.5 ± 2.4 y) and again in 1997-2000 (mean ± SD duration of follow up: 6.3 ± 0.9 y). So far, 1755 of these women have been genotyped for APOE by DNA sequencing using the fluorescence-based MegaBACE™ system. Allele frequencies were: E3 78.2%; E4 14.0%; E2 7.8%. Genotypes in Hardy Weinberg equilibrium were as follows: E3/E3 1075 (61.3%), E3/E4 386 (22.0%), E2/E3 207 (11.8%), E2/E4 46 (2.6%), E4/E4 30 (1.7%), E2/E2 11 (0.6%). There was no association between APOE genotype and baseline BMD at either lumbar spine or femoral neck (FN), excluding an effect on peak bone mass. However FN bone loss was greatest in carriers of the E4 allele and least in carriers of E2 (Table). Reflecting this fact, FN BMD at follow-up was lowest in carriers of E4 and highest in carriers of E2. The differences remained significant after adjustment for age, weight, height, smoking, socio-economic status, physical activity and menopausal status/HRT use. There was no significant relationship between APOE genotype and bone loss at the lumbar spine (data not shown).

Table. Effect of APOE genotype on FN BMD

Mean BMD ± SD	APOE 2 (2/2 and 2/3)	APOE (3/3)	APOE 4 (4/4 and 3/4)	P
N	218	1075	416	
Baseline FN BMD (g/cm^2)	0.892 ± 0.14	0.886 ± 0.12	0.877 ± 0.12	0.250
Follow up FN BMD (g/cm^2)	0.855 ± 0.13	0.838 ± 0.12	0.827 ± 0.12	0.025
FN BMD change (%/y)	-0.60 ± 1.11	-0.83 ± 1.06	0.84 ± 0.91	0.007

The association between APOE 4 genotype, reduced BMD and increased early postmenopausal bone loss is confirmed in this large population based cohort of early postmenopausal women from Scotland. Further work is required to establish whether this effect is mediated through vitamin K transport, interaction with dietary fat/ cholesterol or an independent effect. Dietary information collected at follow-up for 3239 women from APOSS will allow us to investigate any interaction between dietary vitamin K, dietary fat and APOE genotype on BMD.

Disclosures: ***H.M. Macdonald****, None.*

F132

Relationship of ColIA1 Sp1 Polymorphism, BMD and Fracture Risk in Older Women: The Study of Osteoporotic Fractures. D. R. Greene*[1], L. Lui[2], K. L. Stone[3], P. Morin*[4], T. A. Hiller*[5], J. Li*[1], S. R. Cummings[6]. [1]Human Genetics, Roche Molecular Systems, Alameda, CA, USA, [2]University Of California, San Francisco, San Francisco, CA, USA, [3]University of California, San Francisco, San Francisco, CA, USA, [4]National Marine Fisheries Service, San Diego, CA, USA, [5]Kaiser Permanente Center for Health Research, Portland, OR, USA, [6]California Pacific Medical Center, San Francisco, CA, USA.

Osteoporosis accounts for significant mortality and morbidity and will continue to increase with projected increases in the elderly population. There are several treatment regimens for osteoporosis, some approved for prevention. However, the use of these agents has been limited to the prevention of initial or additional fractures after osteoporosis has developed. Better diagnostics for identifying osteoporosis would help to target individuals at high risk. There are several genetic polymorphisms associated with osteoporosis. One of the most closely studied is a polymorphism in the Sp1 binding region of the Collagen IA1 gene. We assessed the relationship of the ColIA1 polymorphism with bone density and fracture risk in 4428 older women participating in the multi-center Study of Osteoporotic Fractures. Blood samples collected in 1988-9 were genotyped for the polymorphism in the Sp1 binding site of Col1A1 using either a Taqman assay or real-time allele specific PCR. Bone mineral density of the hip and spine were assessed by DXA (Hologic, Inc.) Incident hip fractures were ascertained by tri-annual postcard follow-up, and subsequent confirmation based on review of radiology reports. Incident spine fractures were determined based on vertebral morphometry, using paired spine radiographs collected at the baseline exam (1986-88) and an average of 3.7 years later. Of the 4428 women included in this study, 266 hip fractures occurred during a mean follow-up of 11.3 years. After controlling for age, weight, use of estrogen and total hip BMD, we found that women who were homozygous for the minor allele of the ColIA1 polymorphism had an approximate 70% increase is risk of hip fracture compared to those homozygous for the major allele (RR=1.70; 95% CI 0.91 - 3.18 p=.09). Although a similar trend was observed for vertebral fracture, it was not close to statistical significance. Similarly, there was no observed association between the ColIA1 polymorphism and BMD. In conclusion, we found a borderline significant increased risk of hip fracture among women homozygous for the minor allele of the polymorphism in the Sp1 binding region of ColIA1. This result is comparable to the results from a meta-analysis of the literature on ColIA1, and illustrates the necessity of multiple studies and large cohorts to assess modest genetic effects.

Disclosures: ***D.R. Greene****, Roche Molecular Systems 3.*

F135

Genetic Variation in LDL Receptor-Related Protein 5 (LRP5) is a Major Risk Factor for Male Osteoporosis: Results from Cross-Sectional, Longitudinal and Case-Control Studies. U. Choudhury*[1], M. C. de Vernejoul[2], S. Deutsch*[1], T. Chevalley[3], J. P. Bonjour[3], S. E. Antonarakis*[1], R. Rizzoli[3], S. L. Ferrari[3]. [1]Division of Medical Genetics, Geneva University Medical School, Geneva, Switzerland, [2]INSERM U349, Hôpital Lariboisière, Paris, France, [3]Division of Bone Diseases, Department of Geriatrics and Internal Medicine, Geneva University Hospital, Geneva, Switzerland.

Mutations in the LDL receptor-related protein 5 gene (LRP5) cause osteoporosis-pseudoglioma (OPPG) and bone dysplasias with "high bone mass". The contribution of LRP5 polymorphisms to the population-based variation in bone mass and osteoporosis risk is unknown.

We investigated 5 LRP5 SNPs that were not in complete linkage disequilibrium and had a frequency above 2% in relation to areal bone mineral density (aBMD), bone mineral content (BMC) and bone area at the lumbar spine in 877 healthy Caucasian males and females aged 6 to 69 years. This sample included 332 growing children with longitudinal bone measurements for two years. An independent case-control study was performed in 86 men with early idiopathic osteoporosis, including 60 fractures, and 84 age-matched controls (mean age ±SD, 51 ± 13 yrs).

In the population-based study, two LRP5 missense substitutions (exon9 c.1999G>A and exon 18 c.3989 C>T) were associated with aBMD (P=0.01 and p=0.041, respectively), BMC (P=0.001 and P=0.028) and bone area (P=0.003 and P=0.043), as well as with stature (P=0.002 and P=0.072) (all analyses adjusted and standardized for age, gender and weight, Z-scores). LRP5 haplotypes were also significantly associated with all 3 skeletal measurements (P=0.012-0.002), with Z-scores differences between haplotypes greater than 2 Z-scores in men. In children, exon 18 genotypes were associated with two-years gain in BMC (P=0.042), vertebral area and height (P=0.005 and P=0.0013, respectively), and in stature (P=0.025). In adult males, LRP5 polymorphisms accounted for up to 15% of the population variance in these traits. In turn, exon18 T allele was associated with an increased multivariate relative risk (odds ratio 2.53, 95% CI [1.23-5.19], p=0.011, adjusted for age, weight and height) whereas LRP5 haplotype 1 was associated with a decreased risk (odds ratio 0.40, 95% CI [0.19-0.86], p=0.018) of osteoporosis in men.

In summary, LRP5 genetic variation contributes substantially to the determination of vertebral bone mass and stature, likely by influencing bone size during growth. We conclude that LRP5 genetic variation plays a major role in the determination of peak bone mass in Caucasians and represents an important susceptibility gene for osteoporosis in men.

Disclosures: ***S.L. Ferrari****, None.*

F137

Transcriptional Induction of the Gene Encoding α3 Chain of Type IX Collagen (*COL9A3*) by SOX9 Contributes to the Susceptibility of Knee Osteoarthritis. T. Ikeda*[1], A. Mabuchi*[1], A. Fukuda[2], S. Kamekura*[2], I. Kou*[1], H. Hiraoka*[2], A. Kawakami*[2], S. Yamamoto*[2], U. Chung[2], Y. Takatori*[2], M. Takigawa[3], H. Sakai*[4], A. Sudo*[5], A. Uchida*[5], K. Nakamura[2], H. Kawaguchi[2], S. Ikegawa*[1]. [1]Riken SNP Research Ctr., Tokyo, Japan, [2]Univ. of Tokyo, Tokyo, Japan, [3]Univ. of Okayama, Okayama, Japan, [4]Dokkyo Univ., Mibu, Japan, [5]Univ. of Mie, Tsu, Japan.

Although osteoarthritis (OA) is a common and serious skeletal disorder, its genetic and molecular backgrounds are little understood. Because mutations in type IX collagen genes (*COL9A1, COL9A2, COL9A3*) cause chondrodysplasia with early-onset OA phenotypes in humans and mice, we performed an association analysis on knee OA patients using single nucleotide polymorphisms (SNPs), and found a significant association of a marker SNP in *COL9A3* (1740C>T). Immunohistochemical analyses revealed focal induction of type IX collagen early in the repair stage of the OA articular cartilage of humans and mouse models, suggesting that *COL9A3* impedes OA progression. We further performed dense mapping of polymorphisms in the entire *COL9A3* gene and its 3-kb upstream sequences, and identified 62 polymorphisms. Association studies of these polymorphisms using 499 knee OA patients and 385 controls determined that a VNTR (variable number of tandem repeat)-like polymorphism in intron 25, located close to the marker SNP, showed the strongest association (p=0.001). Of this polymorphism, the one-repeat allele (1R) conferred high risk (p=0.004, OR=1.35) and the two-repeat allele (2R) low risk (p=0.001, OR=0.78) for OA. Since the repeat sequence had SOX9-binding motifs, we examined the transcriptional regulation of *COL9A3* by SOX9. SOX9 overexpression in human carcinoma cell lines induced *COL9A3* mRNA as well as *COL9A1*, *COL9A2* and *COL2A1* mRNA. Electrophoretic mobility shift assays with *in vitro*-translated SOX9 and luciferase-reporter assays in the SOX9-overexpressing cells transfected with the polymorphic repeat of *COL9A3* showed that this repeat strongly bound and responded to SOX9, both of which were abrogated by mutations in the SOX9-binding motifs. The reporter activity was dependent on the repeat number: it was stronger in cells transfected with 2R than those with 1R. In addition, overexpression of SOX9 in cultured dermal fibroblasts derived from individuals with different genotypes for the allele showed more rapid induction of *COL9A3* mRNA in cells with 2R than those with 1R. These findings strongly suggest that the SOX9-binding polymorphic enhancer in *COL9A3* affects type IX collagen transcription and thus contributes to the susceptibility of knee OA. SOX9 and its related molecules could therefore be targets of novel treatments of OA and cartilage regenerative medicine.

Disclosures: ***T. Ikeda****, None.*

F140

Contribution of the Vitamin D Receptor and Collagen I Alpha 1 Genes to Hip Fracture Risk. T. V. Nguyen, L. M. Esteban*, C. P. White, S. F. Grant, E. M. Gardiner, J. R. Center, J. A. Eisman. Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, Australia.

The risk of hip fracture risk is partly determined by genetic factors. Polymorphisms of the vitamin D receptor (VDR) and collagen 1α1 (COLIA1) genes have been associated with bone mineral density (BMD), and with fracture risk. It is not clear whether the two genes have additive effects on hip fracture risk. This study examines the contribution of VDR and COLIA1 genotypes to the liability to hip fracture in postmenopausal women.
Genotypes of the VDR (*TT, Tt, tt*) and COLIA1 (*SS, Ss, ss*) genes were determined in 677 (69 hip fracture and 608 non-fracture) women of Caucasian background, aged 70 ± 7 (mean ± SD), who were participants in the Dubbo Osteoporosis Epidemiology Study, Australia. Women with the *tt* genotype was significantly higher in the hip fracture group (26%) compared to those in the non-hip fracture group (14.4%; p = 0.03). Moreover, women with the *ss* genotype were over-represented in the hip fracture (10%) compared to those in the non-fracture group (4.4%; p = 0.04). After adjusted for age and femoral neck BMD in a multiple logistic regression model, women with *tt* genotype had a 2.6-fold (95% CI: 1.2 to 5.3), and women with *ss* genotype were associated with a 3.8-fold (95% CI: 1.3 to 10.8) increase risk of hip fracture. Approximately 33% of the liability to hip fracture was attributable to the presence of the *tt* genotype of the VDR gene and *ss* genotype of the COLIA1 gene.
Caucasian women with VDR *tt* genotype and COLIA1 *ss* genotype were associated with increased hip fracture risk, and the association was independent of BMD and age.

Disclosures: ***T.V. Nguyen****, None.*

F146

Endocannabinoid Regulation of Bone Remodeling. O. Ofek*[1], M. Fogel*[1], M. Attar-Namdar*[1], Y. Tam*[1], R. Müller[2], R. Mechoulam*[3], E. Shohami*[3], I. Bab[1]. [1]Bone Laboratory, Hebrew University of Jerusalem, Jerusalem, Israel, [2]Institute for Biomedical Engineering, Swiss Federal Institute of Technology (ETH) & University of Zürich, Zürich, Switzerland, [3]School of Pharmacy, Hebrew University of Jerusalem, Jerusalem, Israel.

Endocannabinoids, the endogenous ligands of cannabinoid receptors, are involved in complex central and peripheral physiologic systems. The role of endocannabinoids in the control of body weight and reproduction and the leptin negative regulation of both endocannabinoid levels and osteoblastic activity lead us to hypothesize that endocannabinoid signaling is involved in the regulation of bone remodeling. Indeed, micro-computed tomography of the distal femoral metaphysis of male and female knockout mice lacking the predominantly central CB1 cannabinoid receptor (*Cnr*) demonstrated a skeletal phenotype with a markedly reduced trabecular bone volume, number and connectivity. Normal mice given nine daily injections of the endocannabinoid 2-arachidonoyl glycerol (2AG) showed a dose (1-20 mg/Kg/day) dependent increase in trabecular bone formation rate consequent to increased mineralization perimeter, but not mineral appositional rate, suggesting 2AG stimulation of osteoblast number with no effect on osteoblast activity. To assess the occurrence of direct endocannabonoid signaling in bone, we measured the *Cnr* mRNA levels in the murine ST2 and MC3T3 E1 stromal cell lines and primary bone marrow derived stromal cell cultures. No expression could be detected in undifferentiated cells. However, progressive expression, specifically of the peripheral CB2 *Cnr* was clearly evident in cells grown in an osteogenic medium for 10-30 days. The cannabinoid radioligand [^{3}H]HU-243 bound competitively to the differentiated cells. An exceptionally high level of CB2 mRNA was found in bone marrow derived murine osteoclasts developing in the presence of M-CSF and RANKL and in their monocyte progenitors. Both the osteoblastic and osteoclastic cells also express the endocannabinoid degrading enzyme fatty acid amide hydrolase. Collectively, these data suggest potent endocannabinoid regulation of bone remodeling by direct signaling in bone cells as well as via central, yet unidentified pathway(s). The endocannabinoids appear to discriminate osteoblasts from other stromal cells, including osteoblast precursors, as portrayed by the restriction of cannabinoid receptor expression to differentiated osteogenic cells. The discovery of the bone endocannabinoid regulatory system will hopefully facilitate the development of a new generation of bone anabolic/antiresorptive strategies to gain a higher peak bone mass, prevent and rescue bone loss and accelerate bone wound healing.

Disclosures: ***O. Ofek****, None.*

F148

Cytokines Suppress Adipogenesis and Induce Osteoblastgenesis through the TAK1/TAB1/NIK Cascade. I. Takada*[1], M. Suzawa*[2], S. Kato[1]. [1]Institute of Molecular and Cellular Bioscience, University of Tokyo, Tokyo, Japan, [2]Department of Physiology, University of California San Francisco, Usa, CA, USA.

Pluripotent mesenchymal stem cells in bone marrow differentiate into adipocytes, osteoblasts and other cells. Balanced cytodifferentiation of stem cells is essential for the formation and maintenance of bone marrow but the mechanism of these control is largely unknown. We found that ST2 cells, a bone marrow derived mesenchymal stem cells differentiate into adipocyte with PPAR-gamma ligand (Troglitazone) but differentiate into osteoblasts expressing alkaline phosphatase when treated with both troglitazone and cytokines such as interleukin-1 (IL-1) and tumour-necrosis factor-alpha (TNF-alpha). The inhibitory effect of cytokines on adipogenesis in ST2 cells suggested that the ligand-induced transcriptional activity of PPAR-gamma might be suppressed by these cytokines.
To investigate this, we examined the transcriptional activity of PPAR-gamma in ST2 cells using a transiently expressed reporter assay and found that the ligand-induced transcriptional activity of PPAR-gamma was supressed by IL-1 and TNF-alpha, and that this suppression is mediated through NF-kappaB activated by the TAK1/TAB1/NF-kappaB-inducing kinase (NIK) cascade, a downstream cascade associated with IL-1 and TNF-alpha signalling.
In a co-immunoprecipitation assay treated with cytokines and troglitazone in ST2 cells, we detected an association between endogenous NFkappaB complex and endogenous PPAR-gamma. However, other nuclear receptors such as GR did not show this cytokine-induced association with NFkappaB in ST2 cells. Then we examined this association further with GST pull down assay and found that the DNA-binding C domain of PPAR-gamma was identified as a p65-interacting region. And unlike suppression of the PPAR-gamma transcriptional activity by MAP kinase-induced growth factor signalling through phosphorylation of the A/B domain, NF-kappaB blocked PPAR-gamma binding to DNA. Finally, we studied the molecular mechanism of the recruitment of NFkappaB to PPAR-gamma by examining potential PPAR-gamma coactivators and corepressors and found that PGC-2, a PPARgamma AF-1 specific coactivator associated with PPAR-gamma and NFkappaB complex.
These results indicate that the expression of IL-1 and TNF-alpha in bone marrow may alter the fate of pluripotent mesenchymal stem cells, directing cellular differentiation towards osteoblasts rather than adipocytes by suppressing PPAR-gamma function through TAK1/TAB1/NIK-NFkappaB cascade.

Disclosures: ***I. Takada****, None.*

F150

Specific Cytoplasmic Tyrosine Residues in c-Fms Regulate ERK Activation during Osteoclastogenesis. S. Takeshita, R. Faccio, S. L. Teitelbaum, F. P. Ross. Pathology, Washington University School of Medicine, St. Louis, MO, USA.

ERK signaling is pivotal for the proliferation and differentiation of many cell types. Recent reports reveal that ERK inhibition suppresses osteoclast (OC) formation from M-CSF-dependent bone marrow macrophages (MDBMs). Expanding on this information, we found that ERK activation is required only during the early stages of MDBM proliferation/differentiation. Given these observations and the fact that M-CSF is the strongest inducer of ERK activation in MDBMs, we analyzed the signaling pathways by which c-Fms activates this MAPK. To this end, we retrovirally transduced MDBMs with c-Fms chimeric receptors containing seven tyrosine (Y) residues (559, 697, 706, 721, 807, 921 and 974) mutated to phenylalanine (F), singly or in combination. Initial studies revealed that Y559F and Y807F decreased MDBM proliferation while Y559F, but not Y807F, reduced ERK phosphorylation. Interestingly, the Y559F/Y807F double mutation completely abolished proliferation, while continuing to stimulate ERK. These data led to experiments aimed at identifying the Y residue(s) responsible for initiating ERK activation. Using additional mutants we identified Y559 and Y697, which are c-Src and Grb2 binding sites, respectively, as critical residues for activation of the ERK pathway. The Y559F/Y697F double mutation blocked both cell division and ERK activation. While Y559F reduced ERK activity as described above, Y697F exhibited only minor effects. Surprisingly, chimeric receptors containing only Y559 or Y697, with all remaining Y mutated to F, stimulated ERK, but not MDBM division, establishing that the ERK pathway alone is insufficient to induce OC precursor proliferation. We next examined other signaling cascades downstream of M-CSF that may mediate these events. Since the kinases c-Src and PI3K regulate macrophage growth, we performed studies using the specific inhibitors PP2 (c-Src) and LY294002 (PI3K). Both compounds separately blocked ERK activation and MDBM proliferation. When combined, the two inhibitors synergistically suppress MDBM proliferation. Consistent with the known stimulatory effect of ERKs on OC differentiation, we found that PP2 also decreased this process. Lastly, the same Y to F mutants that suppress proliferation inhibit OC differentiation independently. In conclusion, we propose that OC precursor proliferation/differentiation requires (1) c-Fms signaling from the residues Y559 and Y807 or the combinations Y559/Y807 and Y559/Y697 and (2) activation of the c-Src and PI3K pathways. Finally, the ERK pathway, while necessary, is not sufficient for proliferation and differentiation of OC precursors.

Disclosures: ***S. Takeshita****, None.*

F154

RAS Association Domain Family Protein, RASSF1C, Is a Binding Partner and a Potential Intracellular Mediator of the Actions of IGF Binding Protein-5 (IGFBP-5) in Human Osteoblasts. Y. Amaar, S. Bala*, D. J. Baylink, S. Mohan. Musculoskeletal Disease Center, J.L. Pettis VAMC, Loma Linda, CA, USA.

Previous studies have shown that IGFBP-5 is a multifunctional protein that acts not only as a traditional binding protein but also functions as a growth factor independent of IGFs to stimulate bone formation. The intrinsic growth factor action of IGFBP-5 has been predicted to involve binding of IGFBP-5 to a putative receptor to induce downstream signaling pathways and/or nuclear translocation of IGFBP-5 to influence transcription of genes involved in osteoblast cell proliferation/differentiation. To identify the molecular pathway by which IGFBP-5 exerts its intrinsic biological activity in osteoblasts, we undertook studies to identify proteins that bound to IGFBP-5 using IGFBP-5 as bait in yeast two hybrid screen of U2 human osteosarcoma cell cDNA library. One of the clones that interacted strongly with the bait under high stringency conditions corresponded to the isoform C of the RAS association family 1 gene (RASSF1C) which encodes 270 amino acids. The interaction between RASSF1C and IGFBP-5 is confirmed by *in vitro* co-immunoprecipitation studies, using rIGFBP-5, IGFBP-5 polyclonal antibody, and cell lysates overexpressing RASSF1C. Northern blot and RT-PCR analysis showed that the RASSF1C is expressed in both untransformed normal human osteoblasts (derived from calvaria and rib) and in

osteosarcoma cell lines (TE85, SaOS-2 and U2), which are known to produce IGFBP-5. In order to determine if RASSF1C is an intracellular mediator of IGFBP-5 actions, we evaluated the effect of blockage of RASSF1C production on osteoblast proliferation. Addition of synthetic RASSF1C specific small interfering (si) RNA duplex caused a dose-dependent decrease in cell number (38% at 200 nM of siRNA, P<0.01) in human osteoblasts which produce abundant IGFBP-5.This effect was specific since a control siRNA duplex did not produce a similar effect. Conclusions: 1) Our study provides the first evidence that osteoblasts produce RASSF1C, a splice variant of RASSF1 that interacts with IGFBP-5 and regulates osteoblast proliferation. 2) Based on our findings and the recent findings that IGFBP-5 stimulates growth in human interstinal smooth muscle by RAS-dependent activation of p38 MAP kinase and Erk1/2 pathways, we predict that RASSF1C is an important intracellular mediator of the actions of IGFBP-5 and perhaps other growth factors in osteoblasts.

Disclosures: ***Y. Amaar****, None.*

F156

Insulin-Like Growth Factor-I Is Essential for Normal Bone Development. Y. Wang*, T. Sakata, B. P. Halloran, H. Z. ElAlieh*, S. J. Munson*, D. D. Bikle. Endocrine Unit, VA Medical Center , University of California, San Francisco, San Francisco, CA, USA.

Although insulin-like growth factor-I (IGF-I) has been identified clearly as an important growth factor for the skeleton, the role of IGF-I on embryonic bone development has not been evaluated in detail. To assess the role of IGF-I in modulating fetal skeletal development, we analyzed fetal bone tissue of mice homozygous for targeted ablation of the gene encoding IGF-I (IGF-I-/-) with respect to skeletal phenotype, and markers of chondrocyte proliferation and differentiation. In IGF-I -/- mice, skeletal malformations including short-limbed dwarfism were evident from E 14.5 to E18.5. Abnormal structures of spinal ossification centers, characterized by smaller hypertrophic chondrocyte size and number, were identified by H&E; staining on E18.5. A delay of mineralization in the spinal column, sternum and fore paws was identified by alizarin red-alcian blue staining in whole mount of skeletons on E14.5 to 18.5 and by von Kossa staining in bone sections of the spine on E18.5. The mRNA level of bone differentiation marker osteocalcin in the spine of IGF-I-/- mice was decreased to 16% of that of their wild type littermates (IGF-I +/+), however, no reduction was observed in mineralization of the long bones of IGF-I -/- mice. Although the extent of the proliferative zone in the growth plates of long bones and spinal ossification centers was normal in the IGF-I -/- mice, chondrocyte proliferation was reduced as assessed by PCNA staining. In the spinal ossification center, expression of collagen II was lower in IGF-I -/- mice, but not in the growth plates of long bones. No difference was observed in the expression of collagen X between IGF-I -/- and wildtype mice. Expression of Indian hedgehog (Ihh), which plays an important role in regulation chondrocyte differentiation, was reduced in IGF-I -/- mice in growth plates of long bones and the spinal ossification center, whereas expression of parathyroid hormone-related peptide (PTHrP), in a negative feedback loop with Ihh in regulating chondrocyte differentiation, was increased. Our results indicate that IGF-I is essential for normal embryonic bone development. IGF-I promotes chondrocyte proliferation, differentiation, and endochondral bone formation. Ihh and PTHrP are involved in regulating these effects of IGF-I on bone development. The effects of IGF-I on bone development are site specific in that lack of IGF-I leads to a greater delay in differentiation and mineralization of the spine than that of the long bones.

Disclosures: ***Y. Wang****, None.*

F159

Jab1 Enhances SCF E3 Ligase-Mediated Smad4 Degradation. M. Wan, Y. Tang*, C. Lu*, X. Cao. Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

TGF-β/BMPs exert a wide variety of biological activities, in particular, the regulation of skeletal development and bone remodeling. Smad4 is the critical common mediator shared in both TGF-β and BMP signaling pathways. Thus, the regulation of Smad4 protein steady state level is an important means of modulating TGF-β/BMP function. Protein degradation by the ubiquitin-proteasome pathway plays a vital role in regulating functional activity of signaling molecules, and substrate ubiquitination by a specific E3 ligase is a critical step in this pathway. Here we identified a specific SCF E3 ligase for Smad4 protein degradation. The SCF E3 ligase complex we identified contains four subunits: Skp1, Cul1, Roc1 and a substrate recognition subunit, β-TrCP. First, we examined the interaction between Smad4 and β-TrCP in yeast two-hybrid assays. Results of β-gal activity indicated that Smad4 specifically interacts with β-TrCP. We then confirmed the interaction between Smad4 and β-TrCP1 by immunoprecipitation assays in mammalian cells. The effects of SCF complex or its individual component on Smad4 steady-state level in cells were assessed by transient transfection and Western blotting. Our results indicate that Smad4 expression was almost abolished in the presence of three E3 ligase components (Cul1, Roc1 and β-TrCP). Roc1 with Cul1 or Cul1 with β-TrCP1 significantly down regulates Smad4, whereas, the individual components of SCF complex did not affect Smad4 expression level. To examine whether enhanced Smad4 turnover by SCF is due to its ability to promote ubiquitination of Smad4, *in vivo* ubiquitination assays were performed. Ubiquitin-conjugated Smad4 products were observed when both SCF components and ubiquitin were ectopically expressed. Thus, $SCF^{\beta\text{-}TrCP}$ is the specific E3 ligase that mediates Smad4 degradation. Jab1 (Jun activation domain binding protein 1) was reported to interact with Smad4 and induce its ubiquitination for degradation. To determine the molecular mechanism through which Jab1 exerts its Smad4 degradation activity, we first examined whether Jab1 has any effect on the Smad4-β-TrCP interaction by immunoprecipitation assay. As we expected, Jab1 enhances the interaction of endogenous Smad4 with β-TrCP in cells. In addition, overexpression of Jab1 in cells significantly enhances the degradation of Smad4 induced by SCF E3 ligase. Importantly, overexpression of the E3 ligase or Jab1 inhibits TGF-β- or BMP-induced gene transcription. Taken together, our data suggest that SCF is the E3 ligase mediating Smad4 degradation and Jab1 enhances its activity. Therefore, Jab1 and SCF may play critical roles in regulating cell proliferation and differentiation in response to TGF-β and BMP through a degradation pathway.

Disclosures: ***M. Wan****, None.*

F162

Inhibin A Prevents Orchidectomy-Induced Bone Loss in Mice. D. Gaddy[1], D. S. Perrien[1], N. S. Akel*[1], D. C. Montague[1], R. A. Skinner*[2], F. L. Swain*[2], L. J. Suva[2]. [1]Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Orthopaedic Surgery, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

We have previously demonstrated that Inhibin-A suppresses and Activin-A stimulates osteoblast and osteoclast differentiation in primary Swiss-Webster murine bone marrow cultures, as well as osteoblastogenesis in cultures of primary human bone marrow cells. These data led to our hypothesis that Inhibins act to suppress bone turnover and maintain bone mass through direct inhibitory effects on osteoblast and osteoclast development. To test this hypothesis in vivo in male mice, we utilized the GeneSwitch system which uses transgenic transactivator mice with liver-specific expression of a mifepristone-activated chimeric nuclear receptor (GLVP), crossed with transgenic target mice containing a GVLP-responsive promoter upstream of polio-virus IRES (internal ribosome entry site)-linked sequences coding for the alpha- and beta-subunits of inhibin A. This intercross produced "bigenic" mice capable of regulable expression of inhibin A from the liver, which when induced was associated with suppressed levels of FSH (Mol Endo, 2000 Jul;14(7):1075-85). We determined that both the GLVP only (monogenic) and the bigenic crossed mouse strains obtained peak bone mass at 5-6 months of age, as determined by bone densitometry using the Piximus (Lunar). At peak bone mass, baseline BMD measurements were performed prior to sham or orchidectomy (ORCH) of male mice, and the subcutaneous placement of mefipristone or vehicle-containing pellets (Innovative Research). Animals were followed for 4 weeks prior to obtaining femoral bone marrow for osteogenic culture, and tibial analyses of bone volume by microCT. Marrow cultures were initiated from both monogenic and bigenic mice, and cultured in the presence of ascorbic acid and betaglycerolphosphate, and in the presence or absence of Inhibin A, noggin or BMP2. As we previously reported (Endocrinology 2002 Jan;143(1):74-83), both Inhibin A and noggin suppressed recruitment of cells into the osteoblastic lineage (number of AP+ CFU-F), as well as mineralization (number of CFU-OB, and alizarin red normalized to protein). Induction of monogenic mice with mefipristone had no effect on either intact or ORCH bone volume or architecture in the proximal tibia. However, inhibin expression induced by mefipristone in bigenic mice protected against ORCH-induced volumetric bone loss (BV/TV) ($p<0.05$), which appeared to be due to a decrease in trabecular number. Together, these data provide direct in vivo evidence that Inhibin A regulates bone turnover and bone mass through bone marrow cell differentiation.

Disclosures: ***D. Gaddy****, Diagnostics Systems Laboratories 5.*

F165

The Anti-apoptotic Effects of Mechanical Stimulation in Osteoblasts/Osteocytes Are Transduced by the Estrogen Receptor (ER): A Novel Ligand-Independent Function of the ER. J. I. Aguirre[1], L. I. Plotkin[1], B. Strotman*[1], L. K. McCauley[2], I. Gubrig[1], S. Kousteni[1], S. C. Manolagas[1], T. Bellido[1]. [1]Endocrinology, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. Arkansas for Med. Sci., Little Rock, AR, USA, [2]School of Dentistry, University of Michigan, Ann Arbor, MI, USA.

Like estrogens, mechanical stimulation of osteocytic cells promotes their survival; and this effect is mediated via activation of the extracellular signal regulated kinases (ERKs), shown elsewhere in this meeting. Based on this and evidence that mice lacking the estrogen receptor (ER)α exhibit a poor response to mechanical loading, we examined here whether ER participates in mechanotransduction. MLO-Y4 osteocytic cells, which express ERα and ERβ, but not the androgen receptor (AR), were subjected to biaxial stretching (5% elongation for 10 min at 3 cycles/min) using a BioFlex loading station. Stretching increased ERK phosphorylation, as assessed by Western blot analysis, and also caused translocation and nuclear accumulation of ERK2, demonstrated by using a green fluorescent protein (GFP)-ERK2 fusion protein. Consistent with an ER-mediated action, pretreatment of the cells with the ER antagonist ICI182,780 for 20 min abolished stretching-induced ERK phosphorylation. Moreover, nuclear accumulation of GFP-ERK2 in response to stretching was abolished by silencing both the endogenous ERα and ERβ in MLO-Y4 cells by introducing small interference (si) RNAs. Introduction of siRNA for the non-essential protein lamin A/C, used here as a negative control, had no effect. Consistent with these findings, stretching increased ERK phosphorylation in osteoblastic cells derived from wild type mice, and ICI182,780 abolished this response. On the other hand, osteoblastic cells derived from mice lacking both ERα and ERβ (DERKO), but expressing normal levels of AR, did not exhibit stretching-induced ERK phosphorylation or nuclear accumulation of GFP-ERK2. Lastly, the anti-apoptotic effects of E_2 and stretching on MLO-Y4 cell apoptosis were additive, as a suboptimal concentration of E_2 (10^{-9}M) in combination with suboptimal stretching (only 1 min) increased nuclear GFP-ERK2 accumulation to a greater extent than the individual treatments. More importantly, whereas the suboptimal individual treatments did not prevent etoposide-induced apoptosis, their combination did. Taken together, these results indicate that the ligand-naïve ER, but not the AR, transduces mechanical signals; and that ligand-dependent as well as ligand-independent actions of the ER are involved in the regulation of the survival of osteocytes and osteoblasts.

Disclosures: ***J.I. Aguirre****, None.*

F167

Correlation of Osteocyte-like Cell Deformation due to *in-vitro* Fluid Flow Shear Stress with Attachment, Dendrite Extension, and PGE_2 Production. D. P. Nicolella[1], A. J. Siller-Jackson*[2], P. P. Cherian[2], D. Shin*[1], J. X. Jiang[2], E. Sprague*[2], J. Lankford*[1], L. F. Bonewald[3]. [1]Southwest Research Institute, San Antonio, TX, USA, [2]University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [3]University of Missouri at Kansas City, Kansas City, MO, USA.

In-vitro, osteocytes are highly responsive to fluid flow shear stresses suggesting that fluid flow within the bone matrix controls bone mechanotransduction. However, neither fluid flow rates nor shear stresses acting on cells and the resulting cell deformations have been quantified *in-vivo*. Thus, the relationship between *in-vivo* mechanical signals and *in-vitro* mechanical stimulation is not well known. We hypothesize that it is neither fluid flow nor matrix deformation per se, but rather the resulting cell deformation that causes the biological response. The purpose of this study was to quantify cell deformation due to *in-vitro* fluid flow mechanical stimulation. MLO-Y4 osteocyte-like cells were subjected to steady fluid flow generated shear stress using a parallel plate flow chamber at 0.2 Pa, 0.8 Pa, and 1.6 Pa. The cells were imaged with a digital camera attached to an optical microscope prior to the onset of flow and then again within 30 sec after the onset of flow (initial cell deformation). The cells were subjected to a total of 10 minutes of flow after which the flow was stopped, the cells were imaged, the flow re-started, and the cells were imaged again (cell deformation after 10 minutes). Using a digital image correlation technique, cell deformation was measured by comparing images captured prior to flow to images taken directly after the onset of flow at the beginning and at the end of the 10 min. of flow. Cell deformation increased as a function of fluid shear stress from 0.27% at 0.2 Pa to 4.9% at 1.6 Pa. This increase in cell deformation also correlates to an increase in biological responses, such as PGE_2 production. After ten minutes of flow, however, cell deformation was significantly reduced from 4.9% to 2.6% at 1.6 Pa. In addition, at the end of 10 min. of flow, cell branching was increased and dendritic processes were extended, in most cases making contact with neighboring cells. *In-vivo* fluid flow shear stresses in bone have been analytically estimated to be on the order of 0.2 to 3.0 Pa. When applied *in-vitro*, this level of fluid shear stress results in significant cell deformation, on the order of measured bone matrix deformation due to microdamage. In summary, the cells adapted to their *in-vitro* mechanical environment by reducing their compliance resulting in significantly lower cell deformation and by increasing the length of their dendritic processes to establish cell contacts in order to facilitate cell to cell communication.

Disclosures: ***D.P. Nicolella****, None.*

F169

Transcriptional Induction of FosB/DeltafosB Gene by Mechanical Stress in Osteoblasts. D. Inoue, S. Kido, S. Kato*, T. Matsumoto. Department of Medicine and Bioregulatory Sciences, University of Tokushima, Tokushima, Japan.

DeltafosB, a short splicing isoform of fosB, has been shown to increase bone mass by stimulating bone formation when over-expressed in transgenic mice. We have shown that deltafosB mRNA and protein expression is induced in osteoblast lineage cells both by fluid shear stress (FSS) in vitro and by mechanical loading in vivo. Therefore, deltafosB may play a role in mechanical stress-induced bone formation. In the present study, we further explored the mechanism of deltafosB induction. We found that fosB/deltafosB mRNA induction by FSS was inhibited by gadlinium (Gd), EGTA and BAPTA, but not by nifedipine, suggesting an involvement of calicium influx through Gd-sensitive cation channel. Moreover, we found that FSS activated ERK, which was mimicked by Ca ionophore and inhibited by EGTA and BAPTA, and that fosB induction was blocked by ERK inhibitors such as U0126. Thus, induction of fosB gene expression was dependent on Ca and ERK. To analyze transcriptional effects, we cloned the mouse fosB gene promoter region, subcloned into a luciferase reporter vector, and transfected into primary osteoblasts and MC3T3-E1 cells. We found that the (-1000-+307) and (-603-+307) but not the (-327-+307) fragments responded to FSS with more than two-fold stimulation, indicating that the sequences from –603 to –327 conferred the FSS effects. This region corresponds to the c-fos promoter region which has recently been suggested to confer shear stress response and contains similar DNA elements to the counterpart in the c-fos promoter: upstream CRE-like (CRE1: -469 to -462), SRE-like (-419 to -410), and downstream CRE-like sequences (CRE2: -404 to –397). Further transcriptional analyses with luciferase reporter gene assays using tandem oligonucleotides corresponding to each element suggested a major contribution of the CRE2 sequences. DNA precipitation assay revealed that FSS induced CREB1/ATF1 phosphorylation and subsequent binding of phosphorylated CREB to the CRE2 element in an ERK-dependent manner. Interestingly, although c-fos-derived consensus SRE responded to FSS, fosB SRE did not, suggesting that fosB gene transcription is subject to a different mode of regulation from that of c-fos gene. In conclusion, we have demonstrated for the first time that FSS induces fosB/deltafosB gene expression at the transcriptional level in osteoblasts through a Ca/ERK/CREB pathway in a manner distinct from c-fos gene. Thus induced deltafosB expression may contribute to mechanical stress-induced bone formation.

Disclosures: ***D. Inoue****, None.*

F173

Deficiency of CIZ, a Nucleocytoplasmic Shuttling Protein, Prevents Unloading-induced Bone Loss through the Enhancement of Osteoblastic Bone Formation in vivo. K. Hino*[1], T. Nakamoto*[2], M. Morinobu[3], K. Tsuji[1], A. Nifuji[1], H. Yamamoto*[3], H. Hirai*[2], M. Noda[1]. [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]University of Tokyo, Tokyo, Japan, [3]Ehime University, Matsuyama, Japan.

Unloading results in bone loss which leads to disuse osteoporosis, one of the major medical issues in our ageing society. However, the mechanisms underlying this phenomenon has not been fully elucidated. Mechanical stress could affect cells through their adhesion machinery and subsequently regulate gene expression. CIZ (Cas interacting zinc finger protein) is a nucleocytoplasmic protein, which localizes at cell adhesion plaques, associates with p130 Cas and recognizes specific DNA elements to regulate transcription of the genes encoding collagen and the MMP. CIZ also regulates BMP signaling. Thus, CIZ could be one of the possible candidate molecules to modulate bone metabolism under unloading and/or loading condition. To test the hypothesis that CIZ may be involved in the mechanical stress regulation of bone metabolism *in vivo*, we examined the effects of CIZ-deficiency on unloading-induced bone loss using tail suspension model. Two dimensional μCT analysis indicated that tail suspension reduced the levels of cancellous bone volume (BV/TV) in the distal end of the femur within the area of 800 ×350μm in wild type mice. In contrast, reduction in cancellous bone volume due to tail suspension was suppressed in CIZ KO mice. To address whether CIZ-deficiency suppression of the unloading-induced bone loss could be due to alterations in osteoblastic activities, bone marrow cells were cultured in the presence of ascorbate and beta-glycerophosphate after tail suspension. In wild type mice, tail suspension reduced the formation of alizarin red positive nodules. In contrast, CIZ-deficiency suppressed such reduction in the levels of nodule formation after tail suspension. Bone marrow cell cultures were also conducted in the presence of vitD and dexamethasone and TRAP positive cells were counted .TRAP positive cell numbers were not altered by the tail suspension regardless of the genotype. We also examined the effect of the CIZ deficiency on the cortical bone. μCT analysis of the cross section of the femur at the levels one forth from its distal end indicated that tail suspension suppressed cortical bone area in wild type mice. In contrast, CIZ deficiency suppressed such reduction in the cortical bone area in the femur after tail suspension. These data indicated that CIZ deficiency suppressed tail suspension-induced bone loss in cortical and cancellous bone possibly through CIZ actions on the cells in osteoblastic lineage to modulate bone response to unloading.

Disclosures: ***K. Hino****, None.*

F175

Pubertal Effects on the Muscle-Bone Mechanostat on Differentially Loaded Bones. N. J. Crabtree[1], N. Loveridge[2], M. S. Kibirige*[3], J. N. Fordham[3], N. J. Shaw[4]. [1]Queen Elizabeth Hospital, Birmingham, United Kingdom, [2]Cambridge University, Bone Research Group(MRC), Cambridge, United Kingdom, [3]South Cleveland Hospital, Middlesbrough, United Kingdom, [4]Birmingham Children's Hospital, Birmingham, United Kingdom.

The skeleton is a mechanically optimised organ such that its strength is regulated by loads routinely applied to it. According to the muscle-bone mechanostat theory, these loads mainly arise from muscle forces rather than gravitational weight. However, it is possible that other factors play an important role in this basic relationship. The aim of this study was to examine the possible effects of puberty and gender on the muscle-bone relationship on differentially loaded regions of the body.

637 healthy white children aged 5-18 years, had whole body & lumbar spine DXA. The ratio of bone mineral content to lean tissue mass was calculated for arms (ARMratio), legs (LEGratio) and the lumbar spine (LSratio). The data was analysed using MANOVA, including gender, puberty, lean body mass (LBM), fat body mass (FBM), age & height in the model.

There were no significant differences in the muscle-bone ratio in the prepubertal children. However, after adjustment for body size, significant gender and pubertal differences could be detected for both LSratio and ARMratio, with girls having more bone mineral per unit LBM in both regions. A similar trend was observed in the legs, although this did not reach significance even by late puberty. For the LEGratio the greatest variation explained by the model, was FBM closely followed by height. For the ARMratio the largest variation was explained by height and the least by FBM, however both were significant ($p<0.05$).

	Girls			Boys		
	Pre	Early	Late	Pre	Early	Late
LSratio	*0.90 (0.01)	‡*0.96 (0.02)	‡*1.07 (0.02)	0.85 (0.01)	‡0.79 (0.02)	‡0.83 (0.03)
LEGratio	5.4 (0.05)	5.5 (0.06)	5.5 (0.7)	5.3 (0.05)	5.4 (0.07)	5.3 (0.11)
ARMratio	5.9 (0.07)	‡6.0 (0.08)	‡6.0 (0.09)	5.7 (0.07)	‡5.1 (0.1)	‡5.2 (0.15)

$P<0.05$ diff. between *pre-, early- & post- puberty, ‡genders

Increases seen in the ratios with increasing height would suggest that the long bones are mechanically optimised for both the physical load and the local bending forces to which they are subjected. The differential effects of puberty on the muscle-bone relationship could in part be explained by the dramatic increase in circulatory estrogen observed in girls as they progress through puberty. However, this estrogenic effect appears to be modulated by the influence of gravitational load.

Disclosures: ***N.J. Crabtree****, None.*

F177

Rest-Inserted Loading and IGF-1 Synergistically Initiate Bone Formation in the Senescent Skeleton. S. Srinivasan, S. C. Agans*, K. A. King*, T. S. Gross. Orthopaedics, University of Washington, Seattle, WA, USA.

While counteracting age-related bone loss via general physical activities of daily living has attractive benefits, mild types of activity are ineffective in influencing bone cell dynamics. Interestingly, we recently observed that inserting 10-s of rest between low-magnitude loading cycles was sufficient to initiate significant bone formation in the aged skeleton. However, we were unable to further augment the response of aged bone (despite doubling of load magnitudes *and* 4-fold increases in loading cycles), which suggests an inherent age-related blunting of the osteogenic response. This result indicates that age-related deficits in osteoblastic cells may need to be explicitly counteracted to overcome this blunting, and importantly, to augment bone mass in the aged skeleton. We hypothesized that supplementing rest-inserted loading with an agent such as insulin-like growth factor-1 (IGF-1) capable of modulating osteoblast function would synergistically enhance bone formation induced in the aged skeleton. We examined this proposal using two groups of senescent female C57BL/6 mice (n=3/grp, 26 mo) that received 0.5 or 1 mg/Kg of rhIGF-1 (s.c.) 5 days/wk for 2-wk. Utilizing the non-invasive murine tibia loading device, the right tibiae of all animals were subject to a 50 cycle/d, low-magnitude loading protocol with a 10-s rest inserted between each cycle. The left tibiae served as internal controls for the effects of systemically administered IGF-1 while the right tibiae provided data regarding the interaction of IGF-1 with low-magnitude rest-inserted loading. Animals received calcein labels on days 3 and 12 for determination of dynamic histomorphometry parameters at the periosteal surface of the tibia mid-shaft. The minimal bone formation rates induced in control (left) bones in animals receiving 0.5 mg/Kg and 1.0 mg/Kg of IGF-1 were similar (0.038 ± 0.01and 0.042 ± 0.04 $\mu m^3/\mu m^2/d$, respectively). Rest-inserted loading supplemented with 0.5 mg/Kg IGF-1 only minimally increased bone formation (0.074 ± 0.07 $\mu m^3/\mu m^2/d$, only one of three animals responded). In contrast, rest-inserted loading supplemented with 1.0 mg/Kg IGF-1 resulted in a substantial increase in bone formation (0.135 ± 0.03 $\mu m^3/\mu m^2/d$, all three mice responded, range: 0.09 to 0.19 $\mu m^3/\mu m^2/d$). These results support our hypothesis and suggest that low-magnitude rest-inserted loading when supplemented with low-dose IGF-1, synergistically enhances bone formation in senescent animals. While preliminary, our observation of synergy holds promise as a means to initiate, enhance and sustain bone formation responses sufficiently to significantly influence bone mass and strength in the senescent skeleton.

Disclosures: ***S. Srinivasan****, None.*

F179

A School Curriculum Based Exercise Program Is Associated with Increased Bone Mineral Accrual and Bone Size in Girls During Early Growth – a 2 Years Prospective Controlled Intervention Study in 126 Girls. C. Lindén*, P. Gardsell, O. Johnell, M. K. Karlsson. Dept of Orthopaedics, Inst of Orthopaedics, Malmo, Sweden.

There are three previous published prospective exercise intervention studies in prepubertal girls, spanning 7 - 12 months where all but one evaluate girls with interest in exercised as they volunteered to participate in the exercise study. The purpose of this study was to evaluate an exercise intervention program within the school curriculum during the first school years, as to catch also girls not specific interested in exercise, and to extend previous observation to a 2 years prospective study, previously never published.

A population based cohort including 62 healthy Caucasian girls (92% participation rate) aged 7.6 (0.6) from four classes within the two first grades in one school were included in a school curriculum with 40 minutes physical activity every school day. The curriculum included general physical activity, ball games, running and jumps. A controls served 64 healthy age and gender matched girls within three schools in the same area subjected to the general Swedish curriculum of physical activity in these grades, 60 minutes per week. Bone mineral density (BMD; g/cm2) and bone size were measured with dual X ray absorbtiometry (DXA) at total body (TB), lumbar spine (LS), third lumbar vertebra (L3), femoral neck (FN) and leg before initiation of the intervention and after 2 years. Data is presented as mean (SD).

There were no differences in height, weight, total lean body mass, total fat content, BMD or bone size at baseline when comparing the groups. The annual gain in BMD was greater in the intervention group in comparison with the controls during the 2 year follow-up (annual gain in g/cm2 in TB BMD 0.03 (0.011) versus 0.02 (0.009), LS BMD 0.04 (0.031) versus 0.03 (0.016) and leg BMD 0.06 (0.019) versus 0.05 (0.015), all $p < 0.05$ respectively). Also bone size increased more within the intervention group (annual gain in cm2 in FN area 0.29 (0.243) versus 0.17 (0.267) and L3 area 0.75 (0.383) versus 0.46 (0.330), both $p < 0.05$ respectively). The discrepancies remained also after adjusting for differences in weight gain during the study period.

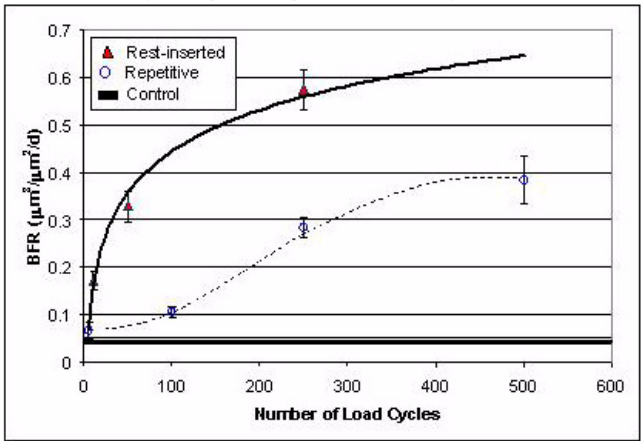
A school based exercise program within the general curriculum during the first school years seems to in girls be associaed with a greater increase in BMD and bone size.

Disclosures: ***C. Lindén****, None.*

F181

Rest Alleviates Tissue Saturation Due to Repetitive Mechanical Loading. S. L. Poliachik, S. C. Agans*, K. A. King*, T. S. Gross, S. Srinivasan. Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, WA, USA.

The response of bone to repetitive mechanical loading demonstrates both threshold and saturation behavior as the number of applied cycles increases. We hypothesized that rest-inserted loading can overcome the saturation normally observed in response to a high number of repetitive load cycles. To investigate the bone formation response to an increasing number of load cycles, we contrasted repetitive and rest-inserted loading using from 5 to 500 cycles at a single load magnitude. The right tibiae of 53 C57Bl/6J mice (female, 16wk) were externally loaded every other day (3 d/wk) for 3 weeks using a non-invasive murine tibia loading device. Calcein was administered on days 10 and 19, and animals were sacrificed on day 22. The animals were randomly assigned to one of 7 protocols, all generating a mean peak normal strain of 1200μ at the mid-diaphysis: repetitive loading of 5, 100, 250 or 500 cycles, or 10, 50 or 250 cycles with a 10s rest inserted between each cycle. Peak strains were determined using strain gage measurements and finite element analysis. Standard dynamic histomorphometry measures were used to determine bone responses at the mid-shaft of the right (loaded) and left (intact contralateral) tibiae. Periosteal bone formation in animals loaded with rest-inserted protocols exceeded the response of repetitive loading as the number of cycles increased (Figure). In repetitive loading protocols, 100 cycles were required to increase BFR above control levels, with the response saturating at 250 cycles (1.5-fold increase in number of cycles). In contrast, BFR induced by rest-inserted loading increased above control levels with only 10 load cycles, and the augmented response appeared to continue even after a 25-fold increase in applied cycles (10 to 250 cycles). We conclude that rest-inserted loading promotes a greater osteogenic response with an increase in the number of applied load cycles as compared to repetitive loading. In contrast, repetitive loading requires a greater number of cycles to exceed control values and the response saturates quickly, as others have noted. Although the mechanism is unclear at present, rest-inserted loading appears to alleviate the tissue saturation that normally limits the osteogenic response to repetitive cyclic loading.



Disclosures: ***S.L. Poliachik****, None.*

F185

Bcl-2 is a Pivotal Mediator of the Anti-apoptotic Effect of PTH on Osteoblasts: Evidence from RNA Silencing and Bcl-2-deficient Mice. A. A. Ali, L. I. Plotkin, I. P. Foote*, R. A. Wynne*, T. Bellido, C. A. O.Brien, 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 Medical Sciences, Little Rock, AR, USA.

Published evidence, as well as work presented elsewhere in this meeting, indicate that the anabolic effect of intermittent PTH administration is due at least in part to attenuation of osteoblast apoptosis; and that PTH exerts this effect via a Runx2-mediated increase in the transcription of anti-apoptosis genes like Bcl-2. To establish the pivotal role of Bcl-2 in the anti-apoptotic effect of PTH, we have decreased Bcl-2 expression in OB-6 osteoblastic cells using RNA silencing. We report that introduction of Bcl-2 silencing RNA (siRNA) caused a 70-80% reduction in the level of Bcl-2 protein as measured by Western blot analysis, compared to controls comprising mock-transfected cells or cells transfected with siRNA to lamin A/C. The transfection efficiency was >95% as indicated by fluorescence microscopy of cells co-transfected with FITC-labeled luciferase siRNA. Unlike the control cells, PTH failed to inhibit etoposide-induced apoptosis of cells transfected with Bcl-2 siRNA, as determined by enumeration of cells staining for active caspase-3. In full agreement with these findings, PTH failed to inhibit etoposide-, dexamethasone-, or anoikis-induced death of osteoblastic cells cultured from neonatal calvaria of Bcl-$2^{-/-}$mice (progeny of breeders from Jackson Labs), as determined by trypan blue staining or caspase-3 activity; whereas PTH did inhibit apoptosis of cells from wild type littermates. Consistent with a gene dose-response, calvaria cells from Bcl-2 heterozygotes (Bcl-$2^{+/-}$) exhibited a 40-50% reduction in the level of Bcl-2 protein, and attenuation of the anti-apoptotic effect of PTH by 50%. These results indicate that the absolute level of Bcl-2 is a critical determinant of the pro-survival effect of PTH on osteoblastic cells. Based on this and evidence that the anti-apoptotic effect of PTH also requires phosphorylation and thereby inactivation of the pro-apoptotic protein Bad, which normally binds to and neutralizes Bcl-2 during programmed cell death, we hypothesize that the pro-survival property of PTH is due at least in part to increased synthesis of Bcl-2 and reduced availability of Bad. The combined effect increases the total amount of free Bcl-2 available to sequester proteins like Bak and Bax that participate in the initial stages of the death program.

Disclosures: ***R.L. Jilka****, Anabonix, Inc. 4.*

F189

Transgenic Expression of Human Bcl-2 in Osteoblasts in Mice Prevents Glucocorticoid-Induced Apoptosis. G. Gronowicz, W. Zhang*, M. Y. Nahounou*, M. B. McCarthy*, A. C. Lichtler, A. G. Pantschenko*. Orthopaedics, University of Connecticut Health Center, Farmington, CT, USA.

High dose glucocorticoids leads to bone loss primarily by decreasing bone formation and increasing osteoblast apoptosis. Glucocorticoids have been shown to produce apoptosis by down-regulating bcl-2. To elucidate the role of apoptosis in bone, a transgenic mouse (Col2.3bcl-2) was developed with a 2.3 kb Col1a1 promoter fragment driving the the 1.7 kb region of the human bcl-2 in mature osteoblasts. Three founder lines were established. They appeared healthy and bred normally, however body weight, body length and femur length of Col2.3bcl-2 mice were smaller than their non-transgenic littermates (+/+) at 2 months, which had been similar at 1 month. IHC of human bcl-2 expression in femurs of 2-month heterozygote Col2.3bcl-2 mice (Tg/+) revealed transgene protein expression in osteoblasts at the growth plate, the endosteal surface and areas of active bone formation on the periosteal surface of the cortical bone. Human bcl-2 expression was also found in the calvarial osteoblasts. Static histomorphometric analysis of the calvaria demonstrated that calvaria width was reduced in Col2.3bcl-2 (103.4 ± 9.2 um vs. 130.7 ± 5.6 um, $p < 0.02$) compared to their +/+ littermates. Osteoclast number per bone surface and % osteoclast surface was reduced in the Col2.3bcl-2 mice compared to +/+ littermates (1.6 ± 0.5 % vs. 5.5 ± 1.2 %, $p<0.01$, and 4.7 ± 1.7 % vs. 15.5 ± 4.3%, $p < 0.04$, respectively). The decreased calvaria width in the transgenic mice suggest decreased bone resorption and formation. Administration of varying concentrations of dexamethasone (0.1, 0.3 and 1 mg/kg body weight) produce a dose-dependent increase in apoptotic osteoblasts in calvaria of +/+ mice visualized by TdT-mediated dUTP nick end labeling (TUNEL). 2-month-old Tg/+ and +/+ mice were treated with 1 mg/kg BW of dexamethasone or saline vehicle each day for 7 days and then sacrificed. Paraffin-embedded calvaria were evaluated for apoptotic osteoblasts (Ob) and osteocytes (Os). Few apoptotic cells were seen in the periosteum or sutures. No significant differences were found in the baseline apoptosis in either group treated with saline. Glucocorticoids were shown to increase osteoblast and osteocyte apoptosis in nontransgenic mice but this effect was significantly blunted in the transgenic mice, which express human bcl-2.

% TUNEL Positive Cells *p<0.05

	Dex +/+	Dex Tg/+	Veh +/+	Veh Tg/+
%Ob	39.8 ± 2.3	11.0 ± 3.1*	3.3 ± 1.6	3.7 ± 2.3
%Os	36.6 ± 5.3	14.7 ± 3.1*	2.5 ± 0.8	3.7 ± 1.0

Disclosures: ***G. Gronowicz****, None.*

F192

Phenotypic Characterization of Col2.3bcl-2 Transgenic Mice with Bone-Directed Expression of Bcl-2. A. G. Pantschenko*, W. Zhang*, M. Y. Nahounou*, F. Ledgard*, M. B. McCarthy*, A. C. Lichtler, G. Gronowicz. Orthopaedics, University of Connecticut Health Center, Farmington, CT, USA.

Bcl-2 plays an important role in preventing apoptosis in osteoblasts and is regulated by numerous growth factors and hormones that affect bone mass. To explore the role of bcl-2 in bone remodeling and development, we have established and characterized a CD-1 transgenic mouse that expresses human bcl-2 in mature osteoblasts. The Col2.3bcl-2 mouse was created with a 2.3 kb Col1a1 promoter fragment driving the 1.7 kb region of human bcl-2 cDNA. Three Col2.3bcl-2 founder lines were established which appeared healthy and bred normally. Body weight, body length and femur length were similar at 1 month of age. In general, the Col2.3bcl-2 heterozygote mice (tg/+) were significantly smaller than the corresponding nontransgenic littermates (+/+) at 2 and 6 months of age with the greatest difference observed at 2 months of age. To confirm transgene protein expression, human specific anti-bcl-2 antibody was used for immunohistochemistry. Human bcl-2 was expressed in osteoblasts at the growth plate, regions of the endosteal surface and areas of active bone formation on the periosteal surface of cortical bone. Non-transgenic littermates were negative for human bcl-2 expression. Static histomorphometric analysis of calvaria from 2 month-old mice showed a significant decrease in calvaria width in the tg/+ mice (103.4 ± 9.2 µm vs. 130.7 ± 5.6 µm) ($p < 0.02$), and a significant decrease in % osteoclast bone surface (4.7 ± 1.7 % vs. 15.5 ± 4.3 %) ($p < 0.04$) and osteoclast number per mm2 bone surface, (1.6 ± 1.2 vs. 5.4 ± 0.5) ($p < 0.01$). Therefore, Col2.3bcl-2 mice had thinner calvaria with fewer osteoclasts than +/+. In femurs, no significant differences were found between tg/+ and +/+ littermates. However, a comparison of sex differences in femurs of wild-type mice revealed that males had significantly more % trabecular bone (BV/TV, 13.1 ± 1.3 % vs. 8.5 ± 0.6 %) ($p < 0.002$), and decreased % osteoclast surface (15.3 ± 2.2 % vs. 21.0 ± 1.3 %) ($p < 0.03$) and % osteoblast surface (15.1 ± 0.8 % vs. 29.4 ± 1.7%) ($p < 0.001$) than +/+ females. However, transgenic Col2.3bcl-2 mice did not have any significant differences between males and females, as was observed for the +/+. Preliminary observations of the Col2.3bcl-2 mouse suggests that; 1) bone resorption is affected by bcl-2 expression in osteoblasts, 2) bcl-2 expression affects bone remodeling later in development (i.e. 2 months) rather than earlier, and 3) bcl-2 appears to abrogate the sex differences observed in the wild-type mice. Thus, the Col2.3bcl-2 mouse is a useful model for studying the role of apoptosis in bone development and remodeling.

Disclosures: ***A.G. Pantschenko****, None.*

F198

Transgenic Overexpression of ICER Affects cAMP-Inducible Gene Expression in Osteoblasts. F. Liu*, Y. Huang, B. E. Kream. Medicine, University of Connecticut Health Center, Farmington, CT, USA.

ICER, inducible cAMP early repressor, is a member of the CREM family of transcription factors that modulate cAMP-inducible gene expression. Four isoforms of ICER (I, Iγ, II, IIγ) arise by alternative splicing of a primary transcript that is initiated from the intronic P2 promoter of CREM. ICER acts as a dominant negative transcription factor since it contains almost exclusively the basic leucine zipper domain that specifies dimerization and DNA binding. We previously demonstrated that parathyroid hormone (PTH) induces the expression of all ICER isoforms in osteoblasts mainly through the cAMP-protein kinase A pathway. To study the role of ICER in cAMP-inducible gene expression in osteoblasts, we generated transgenic mice with ICER I and ICER II expression under control of 3.6 kb of the rat Col1a1 promoter and 1.6 kb of the rat Col1a1 first intron. The ICER I and II transgenes were engineered with the FLAG epitope tag to facilitate protein detection. Primary calvarial osteoblatic cells were prepared from 6-8 days old wild type (WT) and ICER transgenic (TG) mice. Immunostaining with an anti-FLAG antibody showed that transgenic ICER was expressed strongly in the nuclei of most TG cells. After 7 days of culture, cells were treated with 10 µM forskolin (FSK) for 0, 0.5, 1, 2 and 4 h. The expression of 3 cAMP-inducible genes, c-Fos, IL-6 and Cox-2, were examined by Northern blot analysis. All signals were normalized to actin expression. In TG cells, FSK induction of c-Fos and IL-6 was decreased by 20-30% at 1 h and by 30-40% at 2 h compared to WT cells. By contrast, FSK induction of Cox-2 was increased at all time points in TG cells: by 40% at 0.5 h, 70% at 1 h, 92% at 2 h and 110% at 4h. Similar results were seen in cells treated with 10 nM PTH. In summary, our results show that primary osteoblasts prepared from ICER TG mice had a lower magnitude of induction of c-Fos and IL-6 in response to FSK than WT cells. However, the response of Cox-2 to FSK and PTH was enhanced in TG cells. We hypothesize that ICER may interfere with a transcriptional inhibitor(s) in the Cox-2 promoter while antagonizing positive transactivating factors in the c-Fos and IL-6 promoters.

Disclosures: ***F. Liu****, None.*

F199

Epigenetic Regulation of Mouse RANKL Gene Expression. S. Kitazawa, A. Darwanto*, R. Kitazawa. Division of Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan.

Receptor activator of NF-kappaB ligand (RANKL) is a membrane-bound signal transducer requisite for differentiation and maintenance of osteoclasts. Except for some subpopulations of lymphocytic cells, RANKL expression is limited among stromal/osteoblasic cells which actively support osteoclast formation. To address the issue of what epigenetic mechanism gives the specificity of RANKL gene expression to stromal/osteoblastic cells in response to bone resorptional stimuli, we characterized the mouse RANKL gene promoter that contains three Runx2-binding sites, one CRE shared by the vitamin D response element (VDRE) and two CpG clustering regions (one around the transcription start site, and the other downstream of the VDRE). Using purified DNA from various normal mouse non-RANKL expressing tissues, we analyzed the CpG methylation status by Southern blotting after methylation sensitive (Hpa II) and resistant (Msp I) restriction enzymatic digestion, and by sodium bisulfite mapping. We found that DNA from most of the normal non-RANKL expressing tissues showed a methylation pattern in the 5'-flanking region of the RANKL gene by Southern blotting. Mirroring Southern blot analysis, sodium bisulfite mapping showed methylated cytosine heterogeneously scattered in both CpG loci in these tissues. Furthermore, in later passaged-ST2 stromal/osteoblastic cell lines that ceased to express RANKL gene in response to vitamin D3, a higher methylation rate was observed around Runx2-binding sites and the transcription start site than in early-passaged-ST2 cells. Since transcriptional induction of bone specific gene expression by Runx2 is achieved by recruiting histone acetylase, and since transcription repression by methylated-cytosine is achieved by recruiting histone deacetylase by metyl-CpG binding proteins, these results suggest that the histone acetylation status defined by the balance between bone-specific transcription factors like Runx2 and metyl-CpG binding proteins is an important epigenetic event defining bone tissue-specificity and vitamin D-dependency as well as the heterogeneity and diversity of the stromal/osteoblastic cells in RANKL gene expression.

Disclosures: ***S. Kitazawa****, None.*

F201

A Homeobox Gene Msx2 Stimulates the Osteoblastic Differentiation of Mesenchymal Cells in the Absence of Cbfa1/Runx2. F. Ichida*[1], K. Hata*[1], F. Ikeda[1], K. Yamashita*[1], T. Matsubara*[1], K. Hisada*[1], H. Yatani*[1], A. Yamaguchi[2], R. Nishimura[1], T. Yoneda[1]. [1]Osaka Univ, Suita, Japan, [2]Nagasaki Univ, Nagasaki, Japan.

Recent studies have revealed the importance of a homoeobox gene Msx2 in skeletogenesis. Mice deficient in Msx2 manifested persistent calvarial foramen with a defect in cortical and cancellous ossification in skull and a decrease in osteoblast number. Moreover, it is found that heterozygous mutations of Msx2 cause enlarged parietal foramina (PFM) characterized by oval defects in parietal bones in human. However, the molecular mechanism by which Msx2 regulates osteogenesis is still poorly understood. To understand the role of Msx2 in the regulation of osteogenesis, we studied the actions of Msx2 in an in vitro model in which the multipotent mesenchymal cell lines including C3H10T1/2 and C2C12 showed osteoblastic differentiation with increased alkaline phosphatase activity (ALP) upon treatment with BMP2. Msx2 expression was induced in C3H10T1/2 cells by the treatment with BMP2 in parallel with osteoblastic differentiation. Of note, introduction of Msx2 using adenovirus system promoted the osteoblastic differentiation of C3H10T1/2 and C2C12

cells. The effects of Msx2 were enhanced in the presence of BMP2 or co-introduction of Cbfa1. To determine whether Cbfa1 is essential for Msx2 to promote osteoblastic differentiation, we examined the effects of Msx2 on osteoblastic differentiation in the Cbfa1-deficient mesenchymal cell line named C2 that was established from Cbfa1 knock out mouse calvariae. BMP2 induced Msx2 expression and increased ALP activity in C2 cells. Furthermore, introduction of Msx2 alone also elevated ALP activity in C2 cells. These results suggest that Cbfa1 is not essential for the induction of Msx2 expression by BMP2 and that Msx2 leads the differentiation of mesenchymal cells toward osteoblastic direction in a Cbfa1-independent fashion. We next examined the effects of Msx2 overexpression on the mineralization of the primary neonatal mouse calvarial osteoblasts. Alizarin red staining demonstrated that overexpression of Msx2 increased the mineralization of primary calvarial osteoblasts in the presence of BMP2. On the other hand, overexpression of mutant Msx2, which is associated with PFM in human, inhibited BMP2-induced mineralization. In conclusion, our data suggest that Msx2 stimulates the osteoblastic differentiation of mesenchymal cells in the absence of Cbfa1 and also regulates the mineralization of osteoblasts. Msx2 may be an alternative molecule to Cbfa1 that is involved in the regulation of the differentiation of mesenchymal cells and osteoblast mineralization.

Disclosures: ***F. Ichida****, None.*

F202

The Effects of BIG-3 on Osteoblast Differentiation Are not Dependent upon Endogenously Produced BMPs. F. Gori, M. B. Demay. Endocrine Unit, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA.

Skeletal development *in vivo* occurs via two major processes, intramembranous and endochondral ossification. Among the local signaling pathways that play a role in these processes is the bone morphogenetic protein (BMP) signaling pathway. We recently identified a BMP-2 induced gene, named BIG-3 (BMP-2 Induced Gene 3kb) that encodes a novel protein belonging to the WD-40 repeat family of proteins. We have demonstrated that BIG-3 dramatically accelerates the program of osteoblastic differentiation when stably expressed in MC3T3-E1 cells. In addition, BIG-3 is developmentally expressed in cartilage and osteoblasts, suggesting that this protein plays a developmental role in both endochondral and intramembranous bone formation. Since BIG-3 is upregulated by BMP-2, we examined the potential interactions between BIG-3 and the BMP-2 signaling pathway during osteoblastic differentiation. To evaluate whether endogenous BMPs were required for the effects of BIG-3 on osteoblast differentiation, we treated MC3T3-E1 cells, stably transfected with the full-length coding region of BIG-3 (MC3T3E1-BIG-3) cloned downstream of a CMV promoter in pcDNA3.1, or MC3T3E1-cells transfected with the empty vector (MC3T3E1-EV), with noggin, a protein that blocks the actions of BMP-2, BMP-4 and BMP-7. Noggin treatment of pooled MC3T3E1-EV clones inhibited alkaline phosphatase (AP) activity by 57% fold compared to untreated pooled MC3T3E1-EV clones at 21 days in culture. Conversely, noggin treatment of pooled MC3T3E1-BIG-3 clones did not affect AP activity even at doses as high as 1 mg/ml. Noggin treatment also decreased the expression of Cbfa1 and type I collagen mRNAs by 1.9 and 2,3 fold, respectively, in pooled MC3T3E1-EV clones by 14 days but not in pooled MC3T3E1-BIG-3 clones. Addition of noggin to pooled MC3T3E1-EV clones significantly decreased mineralized matrix formation, reflected by a 5.5 fold decrease in Alizarin Red staining at 21 days compared to untreated clones. The effects of noggin on mineralized matrix formation persisted in pooled MC3T3E1-EV clones at 30 and 35 days (4.5 and 4.9 fold, respectively), whereas no effect was observed in pooled MC3T3E1-BIG-3 clones at any time point.
The observation that noggin treatment does not block the acceleration in differentiation seen in MC3T3-E1 cells stably expressing BIG-3, suggests that the actions of this novel WD-40 protein in accelerating osteoblastic differentiation are not dependent upon endogenously produced BMPs.

Disclosures: ***F. Gori****, None.*

F204

Dlx5 Function As a Key Switch Between BMP-2-Induced Osteoblast Differentiation and TGF-b1- Derived Antagonism. M. Lee, H. Park*, H. Kim*, H. Ryoo. Dept. of Biochemistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea.

BMP-2 not only blocks myogenic differentiation but also induces osteoblast differentiation in myogenic C2C12 cells. In contrast, TGF-b1 cannot support osteoblast differentiation and it even dramatically inhibits BMP-mediated osteoblast differentiation although Runx2 expression is induced by both BMP-2 and TGF-b1. Therefore the induction of Runx2 is not sufficient to explain the BMP-induced osteoblast differentiation and TGF-b1-derived antagonism. Our previous reports suggested that Dlx5 is a direct and specific target of BMP-signaling and the proximal target in turn, stimulated the downstream transcription factor Runx2, and consequently resulted in the bone marker gene expression in BMP-induced osteogenic transdifferentiation. In this study, we investigated whether the inhibitory role of osteoblast differentiation by TGF-b1 was also mediated by BMP-specific target, Dlx5. The induction of Dlx5 gene expression by constitutively active BMPR-IA or IB was completely or partially blocked by TGF-b1 treatment. Cycloheximide experiment showed that the inhibition of Dlx5 expression by TGF-b1 is dependent on new protein synthesis. Since Dlx5 induction by BMP-2 is observed as early as 1hr after stimulation and the new protein synthesized by TGF-b1 suppresses Dlx5 expression before being induced by BMP-2, we assumed that an immediate early gene product might be involved in the Dlx5 suppression by TGF-b1 and then we checked the involvement of AP-1 on Dlx5 suppression by blocking AP-1. A dominant negative c-fos (A-fos) that inhibits Jun-dependent transactivation was used for this AP-1 blocking. Interestingly, the exogenous A-fos significantly antagonized the Dlx5 suppression by TGF-b1. Moreover, one of AP-1 components, c-Jun protein level increased 1 hr after TGF-b1 treatment but not by BMP-2 in C2C12 and MC3T3-E1 cells. Finally we confirmed that c-Jun overexpression alone could suppress BMP-induced Dlx5 even in the absence of TGF-b1. Taken together, these results indicate that Dlx5 is the primary target where the opposing action of TGF-b1 on BMP-2-induced osteoblastic differentiation occurs and the c-Jun protein newly synthesized by TGF-b1 involves in the suppression of Dlx5.

Disclosures: ***M. Lee****, None.*

F205

PKIγ Knock Down Inhibits Termination of Immediate-Early Gene Expression Induced by PTH. X. Chen, J. C. Dai, S. A. Orellana*, E. M. Greenfield. Case Western Reserve University, Cleveland, OH, USA.

Immediate-early genes, such as IL-6 and c-fos, mediate both the catabolic and anabolic effects of PTH. We have shown that the primary mechanism responsible for termination of PKA activity, CREB phosphorylation, and immediate-early gene expression following stimulation by PTH acts downstream of receptor desensitization, adenylyl cyclase activation, and cAMP degradation (Am J Physiol Cell Physiol 283:1432-40, 2002). We therefore hypothesized that inhibition of PKA activity by the protein kinase inhibitor (PKI) family is responsible for termination of transcription factor phosphorylation and gene expression. We found that PKIγ mRNA and protein are strongly expressed in ROS17/2.8 and MC3T3-E1 osteoblastic cells, while little or no PKIα or PKIβ mRNAs are expressed. We also cloned and sequenced rat PKIγ from ROS17/2.8 cells (GenBank #AY150308). To test the role of PKIγ in termination of immediate-early gene expression, in vitro transcribed siRNA duplexes directed against the PKIγ coding region were transfected into ROS17/2.8 cells. Virtually complete knock down of PKIγ mRNA and protein was observed after 24 hours. This knock down increased the level of IL-6 and c-fos mRNAs induced at early time periods (≤1 hour) by PTH and extended the length of time that the mRNAs are induced (≥4 hours vs. 2 hours in mock-transfected cells). The effects of PKIγ knock down were confirmed using ROS17/2.8 clones stably transfected with PKIγ antisense constructs in the Tet-Off expression system. After 24 hours of tetracycline withdrawal, two independent antisense clones exhibited a similar temporal pattern of effects to that observed with siRNA except of somewhat reduced magnitude. Controls included antisense clones with tetracycline as well as sense and irrelevant clones ± tetracycline. The smaller effect on gene expression in these antisense transfection experiments is most likely due to partial knock down of PKIγ (~50% at both the mRNA and protein levels). To study the mechanism of action of PKIγ, PKA translocation was assessed by immunofluorescence and by western blotting of nuclear extracts. All 3 PKA catalytic domain isoforms were found to translocate into and out of the nucleus with maximal levels 15-30 minutes after exposure to PTH in ROS17/2.8 cells. These kinetics are consistent with PKIγ binding to nuclear PKA and transporting it back to the cytoplasm. Taken together, our results demonstrate that inhibition of PKA activity by endogenous PKIγ is a major mechanism responsible for termination of immediate-early gene regulation induced by PTH. These findings are the first in any cell type showing that endogenous levels of PKI are sufficient to regulate PKA signaling.

Disclosures: ***X. Chen****, None.*

F207

Negative Regulation of Runx2/Cbfa1 Activity by Src Signaling in Osteoblasts. S. K. Zaidi, A. J. Sullivan*, A. J. van Wijnen, J. L. Stein*, G. S. Stein, J. B. Lian. Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA.

Although Src tyrosine kinase signaling has an established role in osteoclast maturation, bone-forming osteoblasts isolated from Src deficient mice exhibit accelerated differentiation, with upregulation of several bone differentiation markers including osteocalcin. Here we have explored the mechanisms involving regulation of osteoblast differentiation by Src tyrosine kinase. Consistent with the upregulation of osteocalcin in Src null osteoblasts, we show that inhibition of Src tyrosine kinases in ROS 17/2.8 osseous cells by a chemical inhibitor or by a dominant negative inhibitor of Src tyrosine kinase (DN) results in the induction of endogenous osteocalcin transcript. We find that this inhibition of Src tyrosine kinase abrogates interaction of the bone related Runx2/Cbfa1 transcription factor with Yes-associated protein (YAP), a co-regulatory protein downstream of Src/Yes/Crk tyrosine kinases. Furthermore, the chromatin immunoprecipitation (ChIP) assay shows that Runx2 is required for the recruitment of YAP to the endogenous osteocalcin gene promoter. To further explore the potential role of YAP-Runx2 interaction in upregulation of the osteocalcin upon Src inhibition, we used a rat osteocalcin promoter (rOC) that contains three Runx binding elements and is upregulated in response to Runx2. We show that over-expression of YAP suppresses Runx2 mediated increase in rOC promoter activity in a dose dependent manner. To further examine the role of Src-YAP signaling cascade in regulation of Runx2 activity, a point mutation was introduced in YAP interacting domain of Runx2 (Runx2 Y433A). We find that the Runx2 Y433A mutant does not interact with YAP and does not respond to co-expression of YAP, but exhibits a higher transcriptional activation potential on the rOC promoter. This indicates that the endogenous YAP contributes to repression which is relieved in the Runx2 Y433A mutant. We then examined the possible role of endogenous YAP in maintaining Runx2 activity using a dominant negative inhibitor of YAP (YAP 1-301). This mutant, which resides in the cytoplasm, also relieves suppression of the rOC promoter activity thus demonstrating that the endogenous YAP plays a key regulatory role the maintenance of Runx2 activity. Taken together, our results provide molecular insights into the mechanism(s) underlying the induction of osteoblast differentiation markers in the absence of Src signaling.

Disclosures: ***S.K. Zaidi****, None.*

F209

A Glucocorticoid-Induced Transcription Factor Inhibits Adipocyte Differentiation and Stimulates Alkaline Phosphatase Activity in Mesenchymal Cells. X. Shi, W. Shi, Q. Li, B. Song*, M. Wan, S. Bai, L. Yang*, X. Cao. Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Gluocorticoids (GCs) are effective anti-inflammatory and immunosuppressive agents. However, long term GC usage causes fat accumulation in the bone marrow and accelerates trabecular bone loss, resulting in osteoporosis. Here we report that a GC-induced leucine zipper protein (GILZ) feedback inhibits GC effects on adipocyte differentiation and stimulates alkaline phosphatase (ALP) activity in mesenchymal cells. To investigate the mechanisms of GC-induced osteoporosis, we identified GILZ as a GC-induced antagonist. Gel shift assay showed that GILZ binds to a 40-bp DNA fragment containing a tandem repeats of the C/EBP binding site in the promoter region of PPARγ2 gene. Northern and Western analyses showed that the expression of GILZ mRNA and protein were rapidly and transiently induced by synthetic glucocorticoid dexamethasone. Upon its induction, GILZ binds to PPARγ2 promoter and represses PPARγ2 gene transcription. Importantly, the adipocyte differentiation was blocked when GILZ was overexpressed in C3H10T1/2 mesenchymal and 3T3-L1 preadipocytes. In accordance with the inhibition of adipogenesis, the expression of PPARγ2, C/EBPα, as well as adipocyte differentiation marker genes including adipsin and lipoprotein lipase were all inhibited in GILZ-expressing stable cells as measured by quantitative real-time RT-PCR. Interestingly, however, the ALP activity was significantly increased in GILZ-expressing C3H10T1/2 stable cell. The increase of ALP activity was even dramatic when these stable cells were treated with osteogenic supplements lacking dexamethasone. These data demonstrated that GILZ is a novel GC-induced antagonist that upon its induction, it blocks GC-induced adipogenesis of bone marrow stem cells and stimulates osteoblast differentiation, and therefore, GILZ is a prominent candidate for the development of new therapeutic agents to eliminate the side-effects of GCs on bone metabolism.

Disclosures: ***X. Shi****, None.*

F211

Functional Antagonism of Msx2 Repression by C/EBP for Regulation of OC Gene Transcription During Osteoblast Differentiation. M. Q. Hassan*, J. Liu*, G. S. Stein, J. L. Stein*, A. J. van Wijnen, J. B. Lian. Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, MA, USA.

The CAAT enhancer binding proteins C/EBPβ and C/EBPα are upregulated during osteoblast differentiation and are transcriptional activators of bone-related genes including osteocalcin. Formation of a Runx2-C/EBP complex in mature osteoblasts results in a 30 fold synergistic enhancement of OC transcription. The proximal (-208) OC promoter contains a Runx2/Cbfa1 site (-165/-159), and the C/EBP site (-105/-99) which is contiguous to the OC Box homeodomain (HD) element (-99/-76), a suppressor domain. The HD protein Msx2 represses OC transcription through sequence specific DNA binding, but can interact with other transcription factors including Runx2/Cbfa1. Therefore, we have addressed the interrelationships among these regulatory proteins during osteoblast differentiation by functional assays with WT and element specific mutations in -208 OC-CAT, and by chromatin immunoprecipitation (ChIP) assays at stages of primary calvarial cell differentiation. We confirmed by co-immunoprecipitation studies that Msx2 and Runx2 form a complex and that Msx2 is most associated with the OC promoter in proliferating osteoblasts by ChIP assays. The WT, mRunx2 and mRunx2-mC/EBP transgenes all exhibit a 2-fold repression in response to Msx2, indicative of the repressor activity at the HD site. However, a 2-fold repression is also observed when only the HD site is mutated, suggesting Msx2 is mediating repression through another element. Indeed, when both the Runx2 and the HD elements are mutated, repression by Msx2 is lost, indicating that both sites contribute to down regulation of OC. Thus, HD repression occurs through two different mechanisms: through homeodomain protein-DNA and protein-protein interactions. This finding is further supported by 4-fold repression observed in response to Msx2 when only the C/EBP element is mutated, suggesting that C/EBP can counteract HD mediated repression directly and through Runx2. During osteoblast differentiation, we find increasing C/EBPβ and δ factors associated with the OC promoter, and decreased Msx2 protein levels. Thus, our studies indicate that OC gene transcription is tightly regulated in early stage osteoblasts by HD protein repression through direct and indirect interactions. At later stages of osteoblast differentiation, C/EBP-Runx2 interactions predominate to upregulate OC transcription.

Disclosures: ***M.Q. Hassan****, None.*

F212

Genomic Regulatory Network for Early BMP2 Response Genes in Osteoblasts and Role of Dlx 5 and Related Family Members Using Retroviral Vectors. D. Guo*[1], M. A. Harris[1], A. Kishnaswamy[1], A. C. Lichtler[2], S. E. Harris[1]. [1]Oral Biology, U. of Missouri at Kansas City, Kansas city, MO, USA, [2]U. of Connecticut Health Center, Farmington, CT, USA.

A single short treatment of BMP2 to osteoblasts is capable of initiating a cascade of growth, cell migration, matrix rearrangement, and differentiation to a mineralizing matrix. Some of the earliest transcription factors induced in preosteoblasts by BMP signaling belong to the distalless related or Dlx family of transcription factors. Overexpression and knock-out experiments in mice have demonstrated a critical role for Dlx 5 and Dlx 6 in osteoblast growth and differentiation. We want to identify some of the genes directly regulated by Dlx5 and related family members. This will allow us to begin building a genomic regulatory network of early BMP action in osteoblasts. Previous studies have showed that Dlx5 and Dlx2 are two of the earliest genes induced by BMP2 in primary rat osteoblast and the mouse 2T3 osteoblast-like cells. To explore the gene regulatory network involved in BMP2 response, the homeodomain of Dlx5 was fused with Engrailed, a strong transcription repressor. This gene construct will in principle, inhibit genes regulated by Dlx5 and related family members. The ENG-Dlx5HD (DNA binding homeodomain only) construct, as well as a control empty vector were first packaged into a retrovirus, and infected into 2T3 cells. 90% of the cells were infected as determined by GFP fluorescence from a 3' IRES-GFP cassette in the virus. The infected control and ENG-Dlx5HD 2T3 cells were then exposed to 100ng/ml of BMP2 from 1 to 4 hours, followed by mRNA extraction, and micro-array assay of 5000 probes. A subset of 136 genes that were induced more than 1.6 to 20 folds by BMP2 in the control group at 1hr time point were first chosen for further analysis. Of this set of 136 genes, over 60 genes showed no or little response (lower than 50%) to BMP2 in the ENG-Dlx5HD group, while the remaining genes showed a BMP2 response in the presence of the ENG-Dlx5HD virus. This indicates that the 60 gene set is most likely dependent on Dlx transcription factors for some part of their regulation, while the remaining set are regulated by BMP2 by other sets of transcription factors and signals. Northern blottings of several genes was used to confirm several aspects of our genomic network model. By using mouse and human genomic comparisons of the these 136 genes (10kb of 5' flanking, the transcription unit, and 10kb of 3' flanking DNA) to search the Dlx/Msx, Smad 1, and Cbfa1 binding sites, we have demonstrated a significant increase in the number of clusters of Dlx/Msx sites in the gene set that is directly regulated by Dlx family members.

Disclosures: ***D. Guo****, None.*

F215

Abnormalities of Osteoblast Lineage Differentiation in Presenilin Heterozygous Knockout Mice. P. Liu, X. Jiang*, L. Wang*, D. Rowe. University of Connecticut, Farmington, CT, USA.

During embryogenesis, the osteoprogenitor lineage is required to produce a critical number of mature osteoblasts for normal limb development. Knock out mice with a phenotype of abnormal limb formation have revealed a number of genes and associated molecular pathways likely to be important to lineage differentiation. The presenilin KO (PSKO) has severe axioskeletal and brain abnormalities. This gene is a crucial member of the Notch signaling pathway which is known to influence lineage specification and differentiation, proliferation and programmed cell death. Because the osteoprogenitor lineage utilizes the same molecular pathways to maintain bone remodeling in adult tissues, we questioned whether mice with a hypomorphed PS gene could have a bone phenotype. Mice heterozygous for the PSKO mutation (PSKO/+) have normal size and no obvious loss of vertebral trabecular bone by μCT. To assess the ability of the osteoprogenitor lineage to generate mature osteoblasts, pOBCol3.6GFP and pOBCol2.3GFP transgenes were introduced producing PSKO/+ and +/+ littermates carrying these visual trangenes. Previously we have shown that the 3.6 transgene activates with a low intensity in preosteoblasts and brightens during early osteoblast differentiation. The 2.3 transgene activates in mature mineralizing osteoblasts.
CryoJane frozen sections of decalcified femur showed decreased numbers of 2.3GFP+ cells in the PSKO/+ mice relative to +/+ mice. The number of 3.6GFP+ cells was increased in PSKO/+ mice along the endosteal and trabecular bone surface and there was enhanced activity in the fibroblast-like cells of the periosteum. The number of BrdU positive cells lining the bone surfaces was also increased in the PSKO/+. Marrow stromal fibroblasts cultures were established from the PSKO/+ and +/+ mice. The number of cells expressing the 2.3 transgene in the PSKO/+ was significantly less than +/+, while the number of cells expressing the 3.6 transgene was remarkably increased in the PSKO/+ cultures. Mature cultures from the PSKO/+ carrying either transgene showed reduced mineralization and OC expression, while Col1a1 and BSP were enhanced. We interpret these results to indicate that the PSKO/+ mouse maintains a relatively normal bone mass in part by increasing the production of precursor cells sufficient to overcome an impaired conversion of early to mature osteoblasts. This inherent inefficiency in mature osteoblast production may act as an osteoporosis risk gene which, when combined with other factors affecting lineage progression (age or estrogen deficiency), will result in an osteoporosis phenotype. The use of the GFP marker gene may be a useful tool for identifying other genes important to lineage progression.

Disclosures: ***P. Liu****, None.*

F217

Murine Frizzled-1 is Upregulated by BMP-2 and Antagonises Wnt/β-Catenin Signalling in Murine Pluripotent Mesenchymal Cells. S. Roman Roman[1], D. Shi*[2], V. Stiot*[1], B. Vayssière*[1], T. Garcia[1], R. Baron[1], G. Rawadi*[1]. [1]Proskelia Pharmaceutical, Romainville, France, [2]CNRS, Paris, France.

Wnts are a family of secreted glycoproteins that play important roles in development by regulating cell growth and polarity. Wnts bind to receptor complex composed of Frizzled (Fz) and lipoprotein receptor-related protein (LRP) family members. Wnt signaling has been recently demonstrated to play a crucial role in osteoblast differentiation and bone formation. Mice and humans lacking LRP5 display a decreased bone mass and bone formation, whereas other mutations in the same gene lead to a high bone mass phenotype. We have previously shown that BMP2 induces the secretion of Wnt proteins in osteoblasts, suggesting that some of the bone anabolic properties of BMPs are exerted via a Wnt autocrine pathway. Genome-wide expression analysis of genes involved in the Wnt signaling cascade in response to BMP2 in mesenchymal pluripotent cell lines show that BMP-2, but not TGF-β1, induces the expression of murine frizzled-1 (mFz1) in C3H10T1/2 and C2C12 cell lines. Overexpression of mFz1 in the same cells represses BMP-2-mediated and Wnt-dependent alkaline phosphatase (ALP) induction. Accordingly, mFz1 overexpression significantly reduces Wnt3a-mediated ALP induction. To investigate the mechanism by which mFz1 inhibits ALP induction we examined the effect of mFz1 on the canonical β-catenin signaling cascade. Overexpression of mFz1 in mesenchymal cells significantly represses Wnt3a-mediated TCF-1 activation. Furthermore, microinjection of mFz1 tran-

scripts in *Xenopus* embryo inhibits the ability of Wnt1 to induce the expression of the Wnt/ß-catenin target gene *Siamois* in animal cap assay, and secondary axis formation in whole embryo. Using chimeric constructs in which N- and C-terminal segments of mFz1 were replaced by the corresponding parts of XFz3 (which is linked to the β-catenin pathway) we demonstrate that: i) the C-terminal cytoplasmic tail of mFz1 is sufficient to activate signaling through β-catenin and ii) the antagonistic activity of this receptor resides in the N-terminal part, within the cysteine-rich domain. Thus, a member of the frizzled receptor family, mFz1 is capable of antagonizing Wnt/β-catenin signaling. Our findings demonstrate that some members of the Frizzled family of receptors can negatively modulate Wnt signaling, while others are necessary for the activation of this signaling cascade. The fact that mFz1 is induced during BMP2-stimulated osteoblast commitment and differentiation therefore suggests the existence of a negative feed-back mechanism to down-regulate BMP-2-induced Wnt signaling, possibly playing an important role in the control of osteoblast differentiation.

Disclosures: ***G. Rawadi****, None.*

F219

Pleiotropic Functions of N-Cadherin in Osteoblasts. C. Lai-Huang, S. Cheng, R. Civitelli. Bone and Mineral Diseases, Washington University, St. Louis, MO, USA.

We have previously shown that expression of a truncated mutant of N-cadherin (Ncad) with dominant negative action globally inhibits osteoblast cadherins resulting in delayed acquisition of peak bone mass in mice and retarded osteoblast differentiation. To gain insights on the specific role of Ncad in bone, we used mice harboring a null mutation of the Ncad gene. Calvarial cells of heterozygous $Ncad^{+/-}$ mice (homozygous loss of Ncad is embryonically lethal) express lower abundance of Ncad protein relative to $Ncad^{+/+}$ cells. Whole body bone mineral density (BMD) by DEXA was only marginally (<4%; n.s.) lower in $Ncad^{+/-}$ mice relative to wild type littermate up to 6 months of age, implying that a single allele of Ncad is sufficient to maintain bone mass. The abundance of cadherin-11 and R-cadherin was not increased by single allele Ncad deletion, thus excluding compensatory changes of the other major osteoblast cadherins. Surprisingly, development of calcified nodules was faster and more intense in calvarial cells isolated from $Ncad^{+/-}$ mice relative to $Ncad^{+/+}$ cells, in the presence of ascorbic acid and β-glycerophosphate. Likewise, bone marrow stromal cells from heterozygous mice developed ~40% more alizarin red positive colony forming units than wild type cells, although the difference disappeared after 4 weeks of culture. In 2-week calvarial cultures, alkaline phosphatase (ALP) activity was 5-fold higher in $Ncad^{+/-}$ relative to $Ncad^{+/+}$ cells, whereas Cbfa1 mRNA abundance was not different. These in vitro data seem to suggest that Ncad may inhibit full osteoblast differentiation, keeping cells in a less differentiated state. Consistent with this view, Ncad mRNA expression, determined by real time RT-PCR, was progressively down regulated with time in differentiating $Ncad^{+/+}$ calvarial cells, while cadherin-11 mRNA remained unchanged. Interestingly, the mineralization time lag between heterozygous and wild type calvarial cells was not observed in the presence of BMP-2 (150ng/ml). Finally, phosphorylation of p38 MAP kinase by BMP-2 (a critical step in the action of BMP-2 in osteoblasts) was stronger in wild type than in $Ncad^{+/-}$ cells, as was up-regulation of alkaline phosphatase activity, while the effect on p-ERK was similar, suggesting that osteogenic stimulators may offset an inhibitory action of Ncad. In conclusion, Ncad has pleiotropic and complex functions in bone forming cells. While it is critically important in the early stages of osteoblast differentiation, Ncad may function as an inhibitor of late differentiation, perhaps contributing to modulate the timing of bone mineralization. In vivo, the positive effects of Ncad on osteogenic differentiation seem to prevail.

Disclosures: ***R. Civitelli****, None.*

F222

PTH Promotes Osteoblastic Differentiation/Commitment but not Proliferation in GFP-Marked Primary Osteoblast Culture. Y. Wang, Y. Liu*, S. Lee*, D. W. Rowe. University of Connecticut Health Center, Farmington, CT, USA.

Primary calvarial cell cultures derived from mice transgenic for pOBCol2.3GFP and pOBCol3.6GFP respond to PTH administered for the first 7 days of a 21-day culture period with an increase in both fluorescent markers of osteoblast differentiation. Either an expansion in the number of precursor cells capable of subsequent osteoblast differentiation or an enhancement in the proportion of precursors that become osteoblasts are the two contrasting possibilities that are addressed in the present study. The pOBCol3.6GFP transgene activates in early preosteoblastic cells and intensifies its activity as the early osteoblasts differentiate within the bone nodule. Using a computer controlled inverted microscope, serial digital images covering approximately 63% of the culture well recorded the progress of the developing bone nodules under identical photographic conditions. Individual images from multiple time points of culture were thresholded for intensity of GFP expression. The analysis revealed two distinct peaks of GFP expression that clearly separated differentiated bone nodules from surrounding preosteoblasts. Cultures exposed to PTH for 7 days showed a decrease in the area covered with low intensity GFP signal and a corresponding increase in the area with strong GFP expression. This result suggests that the preosteoblast population is not expanded by PTH but rather a greater proportion of precursor cells differentiate into osteoblasts. To validate this impression, parallel cultures were dispersed with trypsin/collagenase and aliquots were assessed for their DNA content or subjected to FACS analysis. Data showed that PTH treatment did not increase the DNA content of the culture and FACS analysis did not show a significant change in the proportion of GFP positive or negative cells. This result confirms that the 7-day PTH exposure does not increase the number of cell within the culture or change the proportion of GFP positive or negative cell. When cultures that had been exposed to PTH for 7 days were subjected to FACS sorting and replated for a 21-day culture period, both the GFP positive and negative population showed increased osteoblast differentiation relative to control FACS sorted cells. Similarly, untreated FACS sorted and replated GFP positive and negative cell population responded to a 7-day PTH exposure with a higher proportion of osteoblastic nodules relative to untreated control by day 21 of culture. The present study strongly suggests that the 7-day PTH treatment enhances the proportion of cells committed to enter osteoblastic differentiation whether they are at the preosteoblast (GFP positive) or pluripotential (GFP negative) level of differentiation.

Disclosures: ***Y. Wang****, None.*

F224

Calcyclin, a Ca^{2+}ion Binding Protein, Plays a Role for Anabolic Effects of Statin on Bone. R. Hwang*, E. Lee*, S. Li*, Y. Jin*, Y. Rhee*, S. Lee*, Y. Kim*, S. Chung*, S. Lim*. Division of Endocrinology, Department of Internal Medicine, College of Medicine, Brain Korea 21 Project for Medical Sciences, Yonsei University, Seoul, Republic of Korea.

The simvastatin is a pro-drug of a potent 3-hydroxy- 3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and inhibits cholesterol synthesis in humans and animals. In the previous studies, in vitro treatment of pharmacological dose of statins stimulated bone formation. To identify the mediators of the anabolic effect of simvastatin on osteoblasts, we tried to identify and characterize simvastatin-induced proteins by using proteomic analysis. Calvarial cells dissected from mice aged P1(postnatal 1) were treated with simvastatin in various doses(10^{-9}~10^{-6}M). For the proliferation assay, simvastatin was treated intermittently, 6 hour per 2 days, for 8 days totally because statins exert a number of effects 1) stabilizing cells 2) inhibition of cell proliferation, particularly lipophilic statins by multifaceted mechanisms. The proliferation of statin-induced OB cell was increased dose-dependently compared to the control without statin. The activation of osteoblast genetic markers such as alkaline phosphatase (ALP), type I collagen and osteoclacin in the presence of simsvastatin was remarkable in dose-dependant manner. The mouse calvarial cells with or without statin treatment were subjected to protein purification and subsequent two-dimensional(2D) electrophoresis. Two-dimensional protein patterns were analyzed by PDQuest (Bio-Rad) software and determined by mass-assisted laser desorption/ionization time-of-flight (MALDI TOF) analysis. Calcyclin was up-regulated more than 10 times. Up-regulated calcyclin mRNA after simvastatin treatment was validated by reverse transcription in mouse calvarial cell. In confocal microscope experiment, GFP-Calcyclin fusion protein was visualized in cytoplasm of MC3T3-E1 and was quickly shifted to nucleus 20 min. after the simvastatin treatment. Calcyclin cDNA were subcloned into pcDNA 3.0 to see whether this protein plays a role in osteoblastogenesis apart from statin. The expression of ALP mRNA was increased without treatment of simvastatin in MC3T3-E1 cells transfected with sense calcyclin DNA. It was also noticed that the proliferation was enhanced significantly in the presence of sense calcyclin gene. In conclusion, simvastatin stimulates the proliferation and early differentiation of osteoblast. Calcyclin is one of the candidate proteins playing a role in osteoblstogenesis in response to simvastatin, although the precise functions of calcyclin in osteoblast remain unknown.

Disclosures: ***R. Hwang****, None.*

F227

Potentiation of Wnt Signaling by Activation of the Nongenotropic Function of the Estrogen Receptor in Osteoblastic Cells. M. Almeida, J. Chen, L. Han, A. M. Vertino, H. Peng*, S. Kousteni, S. C. Manolagas. Div Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, CA Veterans Healthcare System, Univ of Arkansas for Med Sciences, Little Rock, AR, USA.

Mutations in LRP-5 and secreted Frizzled-related protein-1 revealed an important role of the Wnt signaling pathway in the regulation of bone mass. Estrogens regulate components of the Wnt signaling pathway, but it is unknown whether such regulation results from genotropic or nongenotropic mode of estrogen receptor (ER) action. We employed 4-estren-3α,17β-diol (estren), a compound that reproduces the nongenotropic effects of sex steroids without affecting classical transcription and exhibits bone anabolic properties and investigated its effects on Wnt signaling. In HeLa cells permanently transfected with either the wild type ERα or the ligand binding domain of the ERα fused to a membrane-localization sequence, estren, at 10-8M, upregulated the expression of Wnt-2 by 8-fold and Frizzled 10 (Frz10), a soluble Wnt receptor, by 3.5-fold , as determined by real time PCR. At the same concentration 17β-estradiol (E2) had a considerably weaker effect on Wnt-2 and Frz10 (1.5- and 2- fold stimulation respectively). In line with its effects on HeLa cells, estren upregulated Wnt-1, by 9-fold and in addition downregulated the Wnt antagonist Dickkopf (Dkk-1), by 2-fold, in MC3T3-E1 osteoblastic cells. Further, estren suppressed c-jun expression in MC3T3-E1 cells (2-fold) and c-jun phosphorylation in OB-6 osteoblastic cells. Moreover, it suppressed c-jun activity in murine vertebrae following short term administration to OVX mice (48 hours). The suppressive effect of estren on both c-jun and Dkk-1 expression in MC3T3-E1 cells was detectable within 1 hr, preceding an exponential upregulation of Wnt-1 at 14 and 24 hrs. E2 also had an early suppressive effect on c-jun and Dkk-1 at 1hr, but unlike estren, it did not upregulate Wnt-1 at 14 or 24 hrs. In agreement with the upregulating effect of estren on the expression of Wnt-1 and its suppressive effect on Dkk-1, it stimulated Wnt/β-catenin-mediated transcription by the T-cell factor (TCF) in MC3T3-E1 cells carrying a TCF reporter construct. This latter effect was at least 4-fold greater than the effect of E2 and it could be blocked by overexpression of Dkk-1. It is reported elsewhere in this meeting that estren induces osteoblast lineage commitment and differentiation and that this effect is prevented by Dkk-1. Based on this and evidence that c-jun downregulates Dkk-1, the results reported herein indicate that a c-jun-mediated decrease of Dkk-1 along with an increase of Wnt-1 and Wnt-2 and/or their receptor Frz10 by a nongenotropic action of the ER may account in part for the bone anabolic properties of ANGELS.

Disclosures: ***M. Almeida****, None.*

F229

Hyaluronan Increases RANKL Expression in Mouse Primary Osteoblasts Through CD44: A Potential Role in Age-related Bone Loss. J. Cao*[1], P. Singleton*[1], S. Majumdar*[2], A. Burghardt*[2], L. Y. W. Bourguignon*[1], B. P. Halloran[1]. [1]Endocrine Unit, Department of Medicine, University of California, San Francisco, San Francisco, CA, USA, [2]Magnetic Resonance Science Center, Department of Radiology, University of California, San Francisco, San Francisco, CA, USA.

Hyaluronan (HA), the major non-protein glycosaminoglycan component of the extracellular matrix in mammalian bone marrow, functions in part through its receptor, CD44, to stimulate a series of intracellular signaling events that lead to cell migration, adhesion, and activation. With aging, HA concentration and RANKL expression increase and OPG expression decreases. To determine whether HA activation of CD44 influences the receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG) expression, we measured the expression of RANKL and OPG in osteoblast-like cells from animals of different ages and from young CD44-/- mice treated with HA and anti-CD44 antibody. We also studied bone structure of wildtype and CD44-/- mice using micro CT.

Addition of HA dose-dependently increased RANKL mRNA (3.6-fold) and protein (3-fold) levels in osteoblast-like cells from young donors. Stimulation of RANKL could be blocked with anti-CD44 antibody. Treatment of cells with HA or anti-CD44 antibody had no significant effect on OPG mRNA levels. Compared to control mice, tibial mass, proximal tibial cancellous bone volume and structure (μCT) of CD44-/- mice are normal but the tibia was shorter, cortical bone was thicker, and medullary area was smaller. CD44-/- animals also expressed less RANKL in whole bone (-30%) and in osteoblast-like cells (-50%). Cells from CD44 knockout animals failed to respond to either HA or CD44 antibody treatment. With aging, the responsiveness of osteoblast-like cells to HA treatment were decreased. Cells from old mice showed no response to HA and CD44 antibody treatment.

In conclusion, HA can increase RANKL expression in osteoblasts through CD44, and the absence of CD44 in the CD44-/- mouse results in a bone phenotype consistent with decreased endocortical resorption. The interaction between HA and CD44 may play a role in age-related bone loss.

Disclosures: ***J. Cao****, None.*

F231

The bHLH Transcription Factor P8 Enhances Bone Formation by Promoting Osteoblast Cell Proliferation and Inhibiting Osteocalcin Expression. Y. Abe*[1], W. Chen[1], R. Isoda*[1], S. Yang*[1], M. Nishino*[2], Y. P. Li[1]. [1]The Forsyth Institute, Harvard School of Dental Medicine, Boston, MA, USA, [2]Department of Pediatric Dentistry, The University of Tokushima School of Dentistry, Tokushima, Japan.

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 a bHLH transcription factor, P8 that is predominantly expressed in mature osteoblasts. We confirmed that P8 exhibits similar up-regulated expression as osteocalcin during osteoblast differentiation by Northern Blot and *In Situ* Hybridization. To study the function of P8 in bone formation *in vitro* model, we generated a rat pre-osteoblast cell line ROBC10 derived from day 19 fetal rat calvaria. ROBC10 cells are able to differentiate to mature osteoblast that produce matrix proteins and form mineralized nodules in differentiation medium *in vitro.* Forced expression of P8 in ROBC10 cells resulted in 2.5 fold increases of mineralized nodules. In contrast, cells transfected with vectors containing P8 in antisense orientations resulted in 80% decreased mineralized nodules relative to cells transfected with vector alone. To evaluate the molecular basis of P8 bone formation function, we studied the effects of P8 on osteoblast cell proliferation, apoptosis and gene regulation. Our results showed that forced expression of P8 in ROBC10 cells resulted in the cells growing significantly more rapidly. The number of cells was about 2.5 fold when compared with that in the control group. ROBC10 cells transfected with vectors containing P8 in antisense orientations resulted in 40% decreased cell proliferation relative to cells transfected with vector alone. No apoptosis change was observed in the ROBC10 cells transfected either with vectors containing P8 in sense or antisense orientations. Cotranfection studies demonstrated that osteocalcin promoter activity was reduced to 20% of that of the control group without P8 expression. Previous studies in other laboratories indicated that osteocalcin is an inhibitor of bone formation. Our results suggested that P8 is a key regulator of bone formation. P8 enhances bone formation *in vitro* by promoting osteoblast cell proliferation and regulating osteoblast gene expression.

Disclosures: ***Y. Abe****, None.*

F235

Recruitment of Bone-Forming Cells from Remote Bone Marrow Sites During Fracture Healing. D. S. L. Shirley*, D. Marsh*, G. Jordan, G. Li. Department of Trauma and Orthopaedic Surgery, Queen's University of Belfast, Belfast, United Kingdom.

Osteoblast precursors reside in the marrow and periosteum and small numbers circulate in the blood. We have previously found an increase in circulating fibroblastic cells after fracture in humans, suggesting a systemic response following skeletal injury. This study tests the hypothesis that some of the osteoblasts integral to fracture healing, are recruited from remote bone marrow sites, via the peripheral circulation.

34 New Zealand White rabbits had tibial bone marrow harvested, 3 weeks prior to the creation of an ulnar fracture. The marrow stromal cells were isolated and cultured for 3 weeks. These autologous cells were fluorescently labelled, (PHK 26, Sigma, UK,) 1 day prior to re-implantation. The labelled cells were either re-introduced into the fracture gap (Group I, n = 9), into a vein (Group II, n = 12), or into a remote bone marrow cavity (Group III, n = 6). The control, (group IV, n = 7) did not receive any labelled cells. The fracture was established 48 hours prior to cell return. Animals were culled at either 3 or 12 weeks post-fracture. In group II, 3 animals were also sacrificed at 1 day and 1 week. Representative callus, lung, liver, spleen, blood, marrow and kidney tissues were harvested, from all animals. Solid tissues were cryo-sectioned and examined with a confocal microscope. The labelled cells were expressed as the average in 5 high power fields. Cytospins were made from the blood and marrow samples and the labelled cells expressed as a percentage of the total cells.

No labelled cells were identified in the group IV callus. Labelled cells were found in all group I callus, (mean 28.4 per high power field), all group II callus, (mean 10) and in all group III animals, (mean 23.5). There was no statistical difference in the number of cells found in the callus of groups I, or III. The labelled cells appeared on trabecular surfaces in all sections, and they were often surrounded by osteoid. A small number of labelled cells were found in the blood, marrow, lung, liver and spleen of all animals in groups I-III. No labelled cells were identified in the kidney tissue.

This study is a "proof of concept", demonstrating that bone-forming cells can be recruited from remote bone marrow sites and are integral in fracture healing. The presence of labelled cells in callus following venous delivery, supports their recruitment via the blood. The mechanisms of how these bone marrow cells are recruited to the site of injury needs further investigation. Understanding this phenomenon could provide new treatment alternatives for non-union and other bone pathology.

Disclosures: ***D.S.L. Shirley****, None.*

F237

Autoregulation of BMP Expression Mediates Osteogenic Differentiation of Murine Marrow Stromal Cells in Culture. C. M. Edgar, V. Chakravarthy*, G. L. Barnes, S. Kakar*, L. C. Gerstenfeld, T. A. Einhorn. Boston University School of Medicine, Boston, MA, USA.

The role of bone morphogenetic protein expression in the autoregulation of osteogenic differentiation of adherent murine marrow stromal cells (MSCs) in vitro was assessed. When grown in full osteogenic media (beta-glycerolphosphate, ascorbic acid, and 10^{-8} dexamethosome (Dex)), over 21 days these cells showed a temporal progression of osteogenic differentiation characterized by the development of mineralizing nodules, an increase in APase activity, increased expression of Dlx5 and Runx2, and a commensurate decreases in the expression of measured AP1 transcription factor family members. These changes were accompanied by sequential temporal increases in Col1α1, OPN, BSP and OC expression. As differentiation progressed, these cultures uniquely upregulated the expression of BMPs 2, 3B, 4, 6, 7, and 8A. Noggin treatment inhibited the nodule counts by 50%-70%; this was accompanied by reduced expression of osteoinductive transcription factors and phenotypic markers during MSC differentiation. In contrast, the addition of BMP-7 (OP-1) or BMP-2 increased nodule counts by 30%-50% and the expression of osteogenic markers and transcription factors from two to greater than ten fold depending on the specific mRNA(s) assayed. Interestingly, the addition of BMP -7 inhibited the expression of all of the endogenously expressed BMPs with the exception of BMP-2 while BMP-2 inhibited the expression of all of the endogenously expressed BMPs with the exception of BMP-7. A comparison of the effects of two common osteogenic culture additives (beta-glycerolphosphate and ascorbic acid (Asc+GP) or both Asc+GP and 10^{-8} Dexamethasome) demonstrated that each additive separately enhanced differentiation by ~20% and 50% respectively, as compared to 10% FBS only, based on both nodule counts and the expression of osteogenic mRNA. Interestingly, Dex led to a proportionate increase in the expression of multiple endogenously produced BMPs. A time course study of BMP expression over 1, 3, 6, 12, and 24 hours after a single treatment with Dex demonstrated that BMPs 8A and 3B were selectively upregulated two to four fold, while BMPs 2, 4, and 6 remained unchanged suggesting that BMPs 3B and 8A are selectively responsive to corticosteroid regulation. In summary, these data show that the osteogenic differentiation of MSCs is predominantly driven by the endogenous production of their own BMPs. Furthermore, the data demonstrate that the addition of exogenous BMPs downregulate endogenous BMP expression. Finally, these data suggest that corticosteroid induction of osteogenic differentiation may be mediated through the selective induction of BMPs 3B and 8A.

Disclosures: ***C.M. Edgar****, None.*

F239

Thrombospondin-2 Inhibits Marrow Stromal Cell Adipogenesis. W. Luow*[1], C. N. Bennett*[2], O. A. MacDougald*[2], J. D. Miller*[1], K. D. Hankenson[1]. [1]Orthopaedic Surgery, University of Michigan, Ann Arbor, MI, USA, [2]Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA.

Marrow stromal cells (MSC) are mesenchymal progenitors that differentiate to become endosteal osteoblasts and marrow adipocytes. A shift in the differentiation of MSC to adipocytes rather than osteoblasts is thought to contribute to the development of osteoporosis. Previous work has shown that recombinant thrombospondin-2 (TSP2), a secreted matricellular protein produced by MSC, promotes osteogenic differentiation, but inhibits adipogenic differentiation.

The purpose of this study was to further explore the significance of TSP2 in regulating MSC adipogenic differentiation. We utilized three different progenitor cell types, immortalized TSP2-null MSC (obtained from TSP2 knockout mice crossed with transgenic mice carrying a temperature sensitive SV40 large T antigen), immortalized WT MSC, and the preadipocyte line 3T3L1. Two different adipogenic induction protocols were utilized, MDI (isobutylmethylxanthine, dexamethasone, insulin), or MDIT (MDI + the PPARgamma agonist, troglitazone) a more potent adipocyte inducer than MDI alone. WT MSC showed

a 50% reduction in adipocyte formation relative to TSP2-null in the presence of MDI, but when induced with MDIT there was no difference between WT and TSP2-null. As well, the addition of 50 nM recombinant TSP2 inhibited TSP2-null MSC adipogenic differentiation 50% secondary to MDI, but not MDIT. Interestingly, recombinant TSP2 was not effective at reducing adipogenesis of potently preadipocytic 3T3L1 cells in MDI. The production of TSP2 by WT MSC secondary to adipogenic induction was studied using both real-time, reverse-transcriptase PCR and western blotting. WT MSC showed a reduction in TSP2 protein (75% less) and RNA (50% less) levels 24 hours following MDI treatment. This decrease continued until the cessation of the experiment at day 12. Rather surprisingly we found that TSP2 is a late-stage adipogenic inhibitor. TSP2 could be removed from the media as late as day 9 of a 12 day assay and the TSP2-null MSC retained normal adipogenic potential. Likewise, addition of TSP2 at day 9, during the maturation phase of lipid containing vesicles, inhibited differentiation. The treatment of TSP2-null MSC with truncated recombinant fragments of TSP2 shows that the type I repeats of the molecule are required for the inhibition of adipogenic differentiation. We hypothesize that the type I repeats of TSP2 interfere with late stages in adipogenic differentiation, either decreasing fatty acid synthesis or fatty acid uptake from the media, and that its effectiveness as an inhibitor is abrogated secondary to more potent adipogenic conditions.

Disclosures: ***K.D. Hankenson****, None.*

F243

PKCalpha is Downstream of Alphavbeta3 Integrin and Contributes to Adhesion-Induced ERK1/2 Activation in Osteoclasts. N. Rucci[1], L. Orrù*[1], C. DiGiacinto*[1], S. Migliaccio*[1], M. Longo*[1], A. Taranta*[1], R. Baron[2], A. Teti[1]. [1]Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy, [2]Department of Cell Biology and Orthopaedics, Yale University School of Medicine, New Haven, CT, USA.

Osteoclast alphaVbeta3 integrin engagement leads to cytoskeleton reorganization and activates a signalling cascade critical for bone resorption and cell survival. Several downstream pathways are presently known to transduce alphaVbeta3 signalling, but the potential role of serine-threonine protein kinases C (PKCs) has not yet been elucidated. Mouse primary bone marrow osteoclast-like cells (OCs) attached to immobilized LM609, a monoclonal antibody recognizing native alphaVbeta3, showed selective translocation of PKCalpha to the membrane and cytoskeletal compartments. Other PKC isoforms, representative of all classes of this family, did not appeared to be associated with alphaVbeta3 signalling. PKCalpha re-distribution was dependent on alphaVbeta3 since it occurred in alphaVbeta3 – stably transfected CHO cells, but not in parental cells which failed to attach to LM609. Immunoprecipitation revealed the presence of PKCalpha in a complex with the alphaVbeta3 integrin upon adhesion to LM609. This complex associated with the tyrosine kinases FAK in CHO cells and PYK2 in OCs. Adhesion to LM609 triggered ERK1/2 phosphorylation in the cytosolic as well as in the membrane and the cytoskeletal compartments. ERK1/2 activation did not appear to involve the Shc pathway as this adapter protein was not recruited by the alphaVbeta3 integrin, nor was it phosphorylated upon adhesion to LM609. The alphaVbeta3/PKCalpha immunocomplex was disrupted by chelation of intracellular calcium by BAPTA, which also inhibited PKCalpha translocation to the membrane/cytoskeletal compartments and subsequent ERK1/2 phosphorylation. In contrast, chelation of extracellular calcium had no effect. Neither PLCgamma nor PI3-K were implicated in PKCalpha translocation/recruitment by alphaVbeta3 and ERK1/2 phosphorylation. In contrast, inhibition of PKCalpha by the selective inhibitor Go6976 or by long-term treatment with phorbol 12-myristil 13-acetate significantly blunted ERK1/2 phosphorylation. Interestingly, these treatments failed to affect cell adhesion to LM609, suggesting a role of this PKC in events downstream of attachment to substrate. In conclusion, this study demonstrates the selective involvement of a PKCalpha cascade in the ERK1/2 phosphorylation pathway triggered by the alphaVbeta3 integrin. This cascade does not affect the ability of OCs to interact with the substrate but appears mandatory for ERK1/2-mediated pathways, which affect the differentiation and survival of OCs.

Disclosures: ***N. Rucci****, None.*

F248

OPG Tightly Regulates Levels of a Soluble form of RANKL In Vivo and In Vitro. Y. Nakamichi*[1], N. Udagawa[2], M. Mogi*[3], A. Togari*[3], M. Nakamura*[4], N. Sato*[3], Y. Kobayashi[1], N. Takahashi[1]. [1]Institute for Oral Science, Matsumoto Dental Univ., Shiojiri, Japan, [2]Biochemistry, Matsumoto Dental Univ., Shiojiri, Japan, [3]School of Dentistry, Aichi gakuin Univ., Nagoya, Japan, [4]Pediatric Dentistry, Matsumoto Dental Univ., Shiojiri, Japan.

Osteoprotegerin (OPG) knockout (-/-) mice exhibit aberrant bone metabolism characterized by accelerated bone resorption and formation. Recently, it was reported that the serum level of RANKL was markedly elevated in a patient of OPG-deficient juvenile Paget's disease (N Engl J Med 347:210, 2002). We examined how OPG regulates serum levels of RANKL using OPG (-/-), OPG (-/+) and wild type (WT) mice. The serum concentration of OPG in OPG (-/+) mice was significantly lower than that in WT mice [OPG (-/+) mice, 0.64±0.06 ng/ml; WT mice, 1.68 ±0.30 ng/ml], suggesting that the serum levels of OPG reflects the gene dosage. The serum concentrations of RANKL in OPG (-/-), OPG (-/+) and WT mice were 2.38±0.08 ng/ml, 0.37±0.01 ng/ml and 0.08±0.02 ng/ml, respectively. Thus, the serum level of RANKL was strikingly elevated in OPG (-/-) mice. When $1,25(OH)_2D_3$ ($1,25D_3$) was administered into OPG (-/-) and WT mice, serum RANKL concentrations were markedly increased in OPG (-/-) mice [11.6±5.09 ng/ml], but slightly in WT mice [0.13±0.02 ng/ml]. There was a possibility that OPG interfered with the interaction between anti-RAKL antibody and RANKL in the ELISA, when OPG formed a complex with RANKL in serum. Then, we established an ELISA for the complex of RANKL and OPG using anti-RANKL antibody-coated plates and peroxidase-linked anti-OPG antibody. The complex was detected only in serum of OPG (-/+) mice [0.32±0.04 ng/ml] but not of WT mice. Primary osteoblasts prepared from OPG (-/-), OPG (-/+) and WT mice were cultured in the presence or absence of 10^{-8} M $1,25D_3$. Concentrations of OPG, RANKL and the complex of OPG-RANKL in the conditioned medium showed the tendency similar to those in serum observed in vivo experiments. RANKL mRNA expression was similarly induced in response to $1,25D_3$ in osteoblasts from mice with three different genotypes. These results suggest that serum concentrations of RANKL is tightly regulated by circulating OPG at the post- translational level. OPG is proposed to be a potent inhibitor for the release of RANKL from RANKL expressing cells.

Disclosures: ***Y. Nakamichi****, None.*

F250

Decreased Bone Resorption and Increased Trabecular Bone Mass in Female Mice Lacking Placental Growth Factor. E. Daci*[1], P. Carmeliet*[2], S. Torrekens*[1], K. Moermans*[1], R. Van Looveren*[1], R. Bouillon[1], G. Carmeliet[1]. [1]Laboratory of Experimental Medicine and Endocrinology, Katholieke Universiteit Leuven, Leuven, Belgium, [2]Center for Transgene Technology and Gene Therapy, Katholieke Universiteit Leuven, Leuven, Belgium.

VEGF plays an important role in bone metabolism, whereas the role of its homologue, placental growth factor (PlGF) in this process is unknown. We investigated bone growth and remodeling in female mice lacking PlGF compared with wild type (WT) mice. PlGF deficiency resulted in decreased vascularization in the distal femoral metaphysis of newborn mice. Surprisingly, this was associated with an increase in trabecular bone volume (+ 18%) measured in the proximal tibia, an increase which became more pronounced in 12 weeks and 16 weeks-old PlGF-deficient mice (+ 43% and + 38%, respectively). These histomorphometry data were confirmed by pQCT analysis, showing that the trabecular bone mineral density was elevated in the knockout mice at 12 weeks (+ 30%) and 16 weeks (+ 40%), whereas cortical bone parameters were only minimally affected. However, serum osteocalcin levels measured in PlGF-deficient mice of different ages were 20% to 45% lower compared with WT mice. In agreement, mineral apposition rate and bone formation rate were 47% and 61% lower in the knockout bones, suggesting a low bone turnover in PlGF-deficient mice. Supporting a decrease in bone resorption, the urinary excretion of collagen cross-links was reduced by 29% and 43% in 12 and 16 weeks-old knockout mice, respectively. Bone demineralization, assessed by measuring calcium release in embryonic metatarsal cultures, was significantly decreased in PlGF-deficient explants, indicating that PlGF is required for osteoclastic bone resorption. Osteoclast formation in vitro was significantly decreased in PlGF-deficient cultures, and neutralizing antibodies against PlGF or its receptor Flt-1 partially inhibited osteoclast formation in WT cultures. Moreover, the size of osteoclasts and the number of nuclei/cell were considerably lower in PlGF-deficient cultures. On the other hand, PlGF deficiency did not affect the migration of mature osteoclasts, their survival or dentine-resorbing activity. Finally, in vivo administration of antiFlt-1, protected trabecular bone mass in ApoE-deficient mice from the high bone turnover and bone loss.
In conclusion, deficiency of PlGF in female mice results in decreased osteoclast formation, low bone turnover, and increased trabecular bone mass, indicating that PlGF is critical for the maintenance of normal bone mass through regulation of osteoclastic bone resorption.

Disclosures: ***E. Daci****, None.*

F253

Small GTP-binding Protein Rac1 Is Involved in the Cytoskeletal Organization and Activation of Osteoclasts. A. Fukuda[1], T. Akiyama[1], A. Hikita*[1], Y. Kadono[1], T. Miyazaki[1], M. Matsuda*[2], H. Oda*[1], K. Nakamura*[1], S. Tanaka[1]. [1]Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan, [2]Department of Tumor Virology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

Osteoclasts are terminally differentiated cells with a short life span, and in the absence of trophic factors, such as M-CSF or RANKL, they undergo rapid apoptosis. Various types of bisphosphonates, nonhydrolyzable analogs of pyrophosphate currently widely used in treating osteoporosis, have been reported to stimulate osteoclast apoptosis. Although the exact mechanism of action of bisphosphonates still remains elusive, recent studies have revealed that nitrogen-containing bisphosphonates inhibit posttranslational prenylation of small GTP-binding proteins (G-proteins), which may cause osteoclast apoptosis. We previously reported that Rac1, one of the Rho family members of small G-protein, is implicated in the M-CSF-induced pro-survival signaling in osteoclasts, and required for the activation of Akt in response to M-CSF. In the present study, we examined the role of Rac1 in the cytoskeletal organization and the bone-resorbing activity of osteoclasts using adenovirus vector expression system. We constructed adenovirus vector carrying cDNA of fusion protein of EGFP and dominant negative Rac1 ($Rac1^{DN}$) gene, and osteoclast-like cells (OCLs) generated in mouse co-culture system were infected with this virus as well as the control virus (EGFP virus). Clear induction of these molecules in OCLs was demonstrated by green fluorescence and Western blot analysis. We examined the effects of $Rac1^{DN}$ expression on the cytoskeletal organization of OCLs using time-lapse video microscopy. M-CSF treatment caused rapid and prominent membrane ruffling and subsequent cytoplasmic spreading in OCLs, which was inhibited by $Rac1^{DN}$ overexpression to 70% of the control levels as quantified with image analysis softwares. On the other hand, actin ring formation in $Rac1^{DN}$-overexpressing cells as examined by rhodamine-labelled phalloidin staining was comparable to that in the control OCLs. OCLs expressing $Rac1^{DN}$ underwent apoptotic cell death even in the presence of M-CSF. We next examined bone-resorbing activity of these cells, and found that $Rac1^{DN}$ overexpression dose-dependently suppressed pit formation of OCLs on dentine slices. These data suggest that not only does Rac1 mediate survival signaling downstream of M-CSF in osteoclasts, but also plays an important role in the bone-resorbing activity of the cells by regulating membrane dynamics.

Disclosures: ***A. Fukuda****, None.*

F255

HSP70 Secreted during Osteoclast Differentiation Is Related to Cell Death of Osteoclast Precursor. E. Lee*, R. Hwang*, S. Li*, Y. Jin*, Y. Rhee*, S. Lim*. Division of Endocrinology, Department of Internal Medicine, College of Medicine, Brain Korea 21 Project for Medical Sciences, Yonsei University, Seoul, Republic of Korea.

Osteoclasts are highly specified multinucleated bone resorbing cells known as part of the mononuclear phagocyte system. RANKL is a type II membrane protein of the TNF family and plays a critical role in the regulation of osteoclastogenesis. Our aim was to identify and characterize the proteins induced by RANKL that are structurally and functionally undefined. In this study, we attempted to investigate the difference, using proteomics technique, of expression pattern between RANKL-induced cells and those not induced. Briefly, the cells were isolated from bone marrow of mouse femur and tibiae, and then were cultured in complete α-MEM media overnight. Next day, the non-adherent cells were removed, and only the adherent cells were further cultured in the presence of M-CSF. After 24 hr, the cells were treated with RANKL for 3 days. And the obtained cells were subjected to protein purification and subsequent two-dimensional electrophoresis. After visualization of protein spots, digital gel images obtained by scanner were analyzed by PDQuest software (Bio-rad, USA). Interest protein spots determined using peptide mass spectrometry method by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Among many spots induced by M-CSF or/and RANKL, the expressions of HSP60 and HSP70 were significantly upregulated in the differentiated osteoclasts. HSP60 and HSP70 proteins have been shown to be involved in diverse cellular responses of various cell types. Western blotting analyses also showed a remarkable increase in HSP70 during osteoclast formation in mouse bone marrow cell cultures in the presence of M-CSF and RANKL. HSP60 revealed increased, but not significant, during osteoclast differentiation. Surprisingly, HSP60 and HSP70 are secreted proteins during osteoclast differentiation. Secretions of HSP60 and HSP70 were increased in osteoclast precursor cells more than in mature osteoclast cells. To investigate the function of secreted HSP70 during osteoclastogenesis, the TRAP staining pattern of the cells treated with only M-CSF or/and RANKL was compared with those treated together with HSP70. The precursors of osteoclasts were all dead in the group treated with inducible HSP70. In conclusion, HSP70 might be one of natural self regulators of osteoclastogenesis. Our findings indicate that HSP60 and HSP70 have a role in regulation of osteoclast formation and function potentially by participating in osteoclastogenesis by M-CSF and RANKL.

Disclosures: ***E. Lee****, None.*

F257

Interactions between MITF and PU.1 Required for Osteoclast Differentiation. K. C. Mansky, R. Hu*, M. C. Ostrowski*. Molecular Genetics, Ohio State University, Columbus, OH, USA.

The microphthalmia associated transcription factor MITF is a basic helix-loop-helix zipper (bHLHzip) that is a critical regulator of terminal differentiation in osteoclasts. The severe osteopetrotic phenotype in mice harboring the MITF-mi allele is the result of a defect in the terminal differentiation of osteoclasts. Immature mononuclear osteoclasts are present in the mi/mi mice, but these do not fuse or form ruffled borders, or express genes necessary for osteoclast function such as TRAP and cathepsin K. Unlike MITF-mi mice, mice that are null for MITF expression (MITF-vga) do not exhibit a severe osteopetrotic phenotype. PU.1 is a hematopoietic transcription factor that is also necessary for osteoclast formation. Our lab has previously shown that mice that are heterozygote for both the mi mutation and for a PU.1 knockout allele (Pu.1+/-) have an age resolving osteopetrotic phenotype, suggesting that these factors collaborate during osteoclast formation. To better understand the role of MITF and PU.1 in osteoclast differentiation, we are analyzing the bone phenotype of the PU.1 (+/-), and the mi and vga mutations. We have determined that mice with genotype PU.1 (+/-);MITF-mi/MITF-vga have a severe osteopetrotic phenotype that does not resolve by 30 days of age. In addition, to genetic interactions, we have also demonstrated that MITF and PU.1 proteins physically and functionally interact. We have recently created mutations within the MITF HLH region that no longer interact with PU.1, and further characterization of these mutations is ongoing.

Because MTIF and PU.1 interactions are necessary for differentiation of osteoclasts, we are interested in understanding extracellular signals that regulate the activity of these transcription factors. The receptor activator of NF-kB ligand (RANKL) is a member of the tumor necrosis factor family and provides an essential signal to osteoclast progenitors for their differentiation into osteoclasts. We have previously demonstrated that MITF is a target of RANKL/p38 MAPK pathway required for osteoclast differentiation. Our preliminary evidence indicates that PU.1 is also a target of the RANKL/p38 MAPK pathway in osteoclasts. We have mapped two potential p38 MAPK sites in the PEST region of PU.1. Currently we are studying the role of these phosphorylation sites in regulating PU.1 activity. Our results indicate that interactions between MITF and PU.1 are critical for osteoclast differentiation and that RANKL/p38 MAP kinase activity may modulate the activity of the MITF/PU.1 complex.

Disclosures: ***K.C. Mansky****, None.*

F259

Reconstitution of DAP12 Expression Restores Osteoclast Multinucleation In Vitro. M. B. Humphrey[1], S. Spusta*[1], K. Ogasawara*[2], L. Lanier*[2], M. C. Nakamura[1]. [1]Dept. of Medicine, VA Medical Center and University of California, San Francisco, CA, USA, [2]Dept. of Microbiology and Immunology, University of California, San Francisco, CA, USA.

Deficiency of the signaling adapter protein DAP12 is associated with bony abnormalities in mice and humans. We and others have recently demonstrated that DAP12 deficient mice have increased bone mass and show abnormal osteoclast development in vitro. Under in vitro stimulation with M-CSF and RANK Ligand, DAP12 deficient osteoclast precursors form only mononuclear TRAP+ osteoclasts (OCs) able to resorb bone on dentine slices. Quantitative assessment of resorption on calcium phosphate discs shows that OCs derived in vitro from DAP12-/- mice resorb 3 fold less than those derived from C57BL/6 wild type mice.

To verify that lack of formation of in vitro multinucleated osteoclasts derived from DAP12 deficient mice is due to the absence of the DAP12 gene, we used retroviral transduction to express DAP12 in DAP12-/- osteoclast precursors. Retrovirus was generated using a PMX-pie vector cotransfected into 293T cells with a PCL-Ecotropic packaging plasmid. FLAG-tagged DAP12 cDNA was inserted in PMX-pie upstream of GFP cDNA located 3'of an IRES. Retroviral containing supernatants harvested at 48 hrs were used to spinoculate bone marrow macrophages cultured 24 hr in 20 ng/ml M-CSF. Retrovirus encoding FLAG-DAP12 and GFP or GFP alone (control) was used to transduce bone marrow macrophages isolated from DAP12 deficient mice. Following transduction, cells were treated with 10ng/ml M-CSF and 75ng/ml RANKL for 7-10 days. Efficiency of transduction by expression of GFP showed 30-50% transduction of cells at 7-10 days. DAP12-/- bone marrow macrophages transduced with control virus did not develop TRAP+ multinucleated osteoclasts following incubation with M-CSF and RANKL, instead developing only TRAP+ mononuclear OCs. Infection of DAP12-/- osteoclast precursors with DAP12 retrovirus led to formation of 17 +/- 2.4 multinucleated osteoclasts per well ($p<0.0001$). Thus expression of DAP12 in DAP12-/- osteoclast precursors restored formation of multinucleated osteoclasts.

Interestingly, DAP12-/- osteoclast precursors treated with extremely high amounts of M-CSF (100ng/ml) in combination with RANKL (100ng/ml) also formed multinucleated osteoclasts in vitro. Stimulation with extremely high levels of M-CSF was recently observed to rescue development of beta3 integrin -/- osteoclasts in vitro.

These findings verify a role for DAP12 in osteoclast multinucleation in vitro and suggest that DAP12 and M-CSF signal transduction pathways likely intersect and play overlapping roles during osteoclast formation.

Disclosures: ***M.C. Nakamura****, None.*

F262

Small GTP-binding Rab3D Modulates Intracellular Trafficking of a Subset of Post-TGN Vesicles Necessary for Osteoclastic Bone Resorption. M. H. Zheng[1], N. J. Pavlos*[1], J. Xu*[1], S. L. Teitelbaum[2], P. Rigby*[3], J. M. Papadimitriou*[4], F. P. Ross[2]. [1]Dept of Surgery and Pathology (Orthopaedics), University of Western Australia, Nedlands, Australia, [2]Department of Pathology, Washington University School of Medicine, St Louis, MO, USA, [3]Dept of Pharmacology, University of Western Australia, Nedlands, Australia, [4]Dept of Surgery and Pathology (Pathology), University of Western Australia, Nedlands, Australia.

Osteoclastic bone resorption is a highly dynamic process that requires the tight ordering of intracellular trafficking events in order to maintain the structural and functional polarization of the ruffled border and basolateral domains. Here we have identified the small Rab GTP-binding protein Rab3D as a putative regulator of secretory vesicle transport by a degenerative PCR-based approach. Using a combination of confocal immunofluorescence and time-lapse microscopy, Rab3D was found to localize and direct post-TGN vesicular transport and fusion events. Site direct mutagensis showed Rab3DQ81L mutant RAW264.7 precursor cells and derived osteoclasts displayed the enlargement and aggregation of Rab3D compartments in cytoplasm, where as Rab3DN135I mutant showed restricted Rab3D trafficking at the TGN and Rab3DΔCSC mutant displayed diffusely distribution of EYFP-signal in cytoplasm. Rab3DN135I mutant osteoclasts exhibited a typical defect of bone resorption capacity *in vitro*. Finally, we have shown that Rab3D knock out mice exhibit characteristics of osteosclerosis. The metaphyseal region in mice lacking Rab3D displays dense and irregular shaped trabecular bone. Histomorphometric analysis revealed that all of structure parameters of trabecular mass (BV/TV; Tb.N; Tb.Sp, ES) were significantly compromised in the Rab3D -/- mice. The volume of trabecular bone in Rab3D-/- mice was dramatically increased as compared to the wild type. In short, our results implicate a functional role for Rab3D in the early stages of post-TGN secretory vesicle transport in osteoclasts during bone resorption.

Disclosures: ***M.H. Zheng****, None.*

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Regulation of Cytochrome c Oxidase Activity by c-Src in Osteoclasts. T. Miyazaki[1], L. Neff[2], S. Tanaka[1], W. C. Horne[2], R. Baron[2]. [1]Department of Orthopaedic Surgery, The University of Tokyo, Tokyo, Japan, [2]Department of Cell Biology and Orthopaedics, Yale University, New Haven, CT, USA.

The non-receptor tyrosine kinase c-Src, which is highly conserved throughout evolution and widely expressed, is a member of a family of nine protein-tyrosine kinases that associate with the cytoplasmic surface of cellular membranes. It is generally thought that Src's regulation of cell adhesion, movement and proliferation involves its activity as a plasma membrane-associated molecular switch that links a variety of extracellular cues to specific intracellular signaling pathways. c-Src has also been reported to be present on late endosomes in fibroblasts, synaptic vesicles in PC12 cells, secretory vesicles in chromaffin cells, vesicular structures in osteoclasts and the Golgi apparatus in CHO cells. However, the functions of c-Src on intracellular membranes still remain to be determined. Recently, Lyn, another Src family kinase, was found in rat brain mitochondria. To examine whether or not c-Src is also located in mitochondria, we performed immunoelectron microscopy. c-Src was located within mitochondria where it appeared to be associated with the inner membrane. In addition, we found that c-Src phosphorylated cytochrome c oxidase (Cox) using blue native 2D gel analysis. To examine the functional consequences of the tyrosine phosphorylation of

Cox by Src, we investigated whether or not Src kinase activity affected Cox activity. Cox activity was reduced in Src family tyrosine kinase-deficient fibroblasts and Cox activity was restored by c-Src reintroduction, suggesting that c-Src regulates Cox activity. To examine the physiological significance of the up-regulation of Cox by c-Src, we selected osteoclasts as a model system. Down-regulation of Src kinase activity by kinase-dead Src or wild type Csk overexpression inhibited Cox activity in osteoclasts, while up-regulation of Src kinase activity by kinase-dead Csk expression induced higher Cox activity. Furthermore, bone-resorbing activity of osteoclasts is impaired by down-regulating Cox activity by Cox subunit IV (CoxIV) antisense, as well as by c-Src deficiency. Increasing Src kinase activity by expressing kinase-dead Csk does not reverse the inhibitory effect of CoxIV antisense on bone resorption, suggeting that Cox is a downsteam effector of c-Src. These results place c-Src's function in a new context and suggest a novel link between c-Src and Cox.

Disclosures: ***T. Miyazaki****, None.*

F268

Chk Controls Actin Organization and Bone Resorption through Regulating c-Src Activity in Osteoclasts. T. Matsubara*[1], F. Ikeda[1], K. Hata*[1], D. Tamura*[1], S. Tanaka[2], S. V. Reddy[3], G. Roodman[3], B. F. Boyce[4], G. R. Mundy[5], R. Nishimura[1], T. Yoneda[1]. [1]Osaka Univ, Osaka, Japan, [2]Facult Med, Univ Tokyo, Tokyo, Japan, [3]Cancer Inst, Univ Pittsburgh, Pittsburgh, PA, USA, [4]Univ Rochester Med Ctr, Rochester, NY, USA, [5]Univ TX Hlth Sci Ctr San Antonio, San Antonio, TX, USA.

Csk is a tyrosine kinase that selectively suppresses c-Src activity through phosphorylation of a tyrosine527 in the c-terminal portion of c-Src. Since Csk expression is ubiquitous but not osteoclast-specific and the phenotype of c-src-deficient mice indicates a specific role of c-src in osteoclast function, we hypothesized that osteoclasts had a unique Csk-like tyrosine kinase specifically regulating c-src activity and bone resorption in osteoclasts. Based on this hypothesis, we screened a cDNA library of human osteoclast-like cells and isolated a Csk homologue tyrosine kinase Chk. Western analysis demonstrated that Chk was restrictedly expressed in osteoclasts and brain, whereas Csk expression was observed in all tissues examined. Immunohistochemical examination showed that Chk was preferentially expressed in the ruffled borders in bone-resorbing osteoclasts in human bones. Biochemical studies showed that Chk phosphorylated tyrosine527 of c-Src and inhibited c-Src kinase activity. To further explore the interactions between Chk and c-Src in osteoclasts, we determined the subcellular localization of Chk and c-Src in TRAP-positive osteoclast-like cells that formed in spleen cells of wild-type (WT) or c-Src-deficient (src-/-) mice. In WT osteoclasts, Chk and c-Src were co-localized in the actin rings. In contrast, src-/- osteoclasts exhibited disrupted actin ring formation and diffuse Chk expression in the cytoplasm. Introduction of c-Src into src-/- osteoclasts using adenovirus system restored the actin ring formation and bone resorbing activity, indicating the importanceof c-Src in the regulation of actin organization and bone resorption. To investigate the role of Chk in the regulation of c-Src activity, actin organization and bone resorption, Chk was overexpressed in osteoclasts formed following the treatment with M-CSF and soluble RANKL in mouse spleen cells. We found that Chk overexpression profoundly disrupted actin ring formation and reduced c-Src activity. Moreover, an actin-binding protein vinculin that is normally localized in the actin ring dispersed in the cytoplasm following Chk overexpression. Most importantly, these cells demonstrated reduced bone resorption in the pit assays. In conclusion, our results suggest that Chk is a tyrosine kinase that is preferentially expressed in osteoclasts and inhibits the actin organization and bone resorption through negative-regulation of c-Src.

Disclosures: ***T. Matsubara****, None.*

F270

Regulation of Arp2/3 –WASp Complex Formation by Phosphoinositides in Osteoclasts. M. A. Chellaiah, R. S. Biswas. Department of OCBS, Dental School, University of Maryland, Baltimore, MD, USA.

Gelsolin is a critical protein in osteoclast podosome assembly. Transduction of mouse osteoclasts (OCs) with HIV-TAT peptides containing either full length gelsolin (FL-GSN) or phosphoinositide binding domains (PBDs) produced a dominant negative effect on actin ring organization, podosome assembly, and bone resorption of OCs. These OCs exhibited numerous podosome clusters in which each F-actin rich dot-like structure is larger than those observed in typical podosome structures. Translocation of podosome clusters to normal reorganization of podosomes at the clear zone area and formation of actin-ring was affected in these OCs. Also, the association of phosphoinositides (PIs) with the transduced gelsolin peptides containing PBDs affecting the function of PBDs in the endogenous gelsolin. Failure to form actin ring in these OCs may be due to decreased F-actin turnover, and increased PIs binding with the transduced peptides. Previously, we demonstrated transformation of podosome clusters to individual podosomes and translocation of these podosomes to the periphery in a time-dependent manner in OCs transduced with constitutively active, TAT-Rho^{Val14} (Chellaiah et al., 2000 JBC 275, 11993).

As an additional step to understand the molecular mechanisms on actin ring formation, we analyzed the effects of osteopontin (OPN) on PIs and Wiskott-Aldrich syndrome (WASp) protein distribution in mouse oseoclasts. WASp protein controls podosomes and dynamic actin-containing adhesion structures of primary human macrophages and actin ring formation in yeast cells. In osteoclasts, our observations have demonstrated colocalization of WASp with actin and PtdIns P2 (not PtdIns P3) in the actin ring structures of resorbing osteoclasts. Osteoclasts plated on glass coverslips exhibited colocalization of actin and WASp in podosomes as well as in the ring-like structure at the periphery. OPN stimulated PtdIns P2 and Arp3 association with WASp. Transduction of constitutively active Rho^{Val14} into OCs increases interactions of Arp2 or 3 protein and PtdIns P2 with WASp. The potent Rho inhibitor, C3 transferase decreased the OPN or Rho- induced cellular levels of PtdIns P2 as well as the Arp2/3-WASp complex formation, indicating that Rho regulates the synthesis of PtdIns P2. Moreover, transduction of OCs with TAT-PBDs of gelsolin decreased the association of PtdIns P2 with WASp as well as Arp2/3 interaction with WASp. Taken together, these results demonstrate that PIs and Rho GTPase are required for the WASp-Arp2/3 complex formation. Also, these observations identify the potential role of Arp2/3 and WASp complex in the signaling pathway that leads to OC actin ring formation.

Disclosures: ***M.A. Chellaiah****, None.*

F272

p62 ^{ZIP} - A New Member of RANKL Signaling Pathway in Osteoclasts (OCL) Formation and a Novel Target for Treating Bone Metastasis. N. Kurihara[1], S. V. Reddy[1], L. A. Ehrlich*[1], N. Kawanabe[1], M. T. Daiz-Meco*[2], J. Moscat*[2], G. D. Roodman[1]. [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University Complutense, Madrid, Spain.

Osteolytic bone destruction is extremely common in multiple myeloma and breast cancer, and occurs in up to 60 to 80% of patients and plays a critical role in promoting tumor growth. In particular, the cytokine RANK ligand (RANKL) is upregulated in the bone microenvironment in response to tumor products (IL-1, IL-11, prostaglandin, PTHrP). Adhesive interactions between tumor cells and marrow stromal cells result in production of IL-6, and TNF-α by marrow stromal cells, which further enhance tumor cell growth and survival, chemoresistance, and bone destruction. Therefore, agents that inhibit OCL formation and block the effects of tumor-stromal cell interactions should have a major impact on both tumor growth and osteolytic bone destruction.

Recently, a new member of the RANKL signaling pathway, sequestasome-1 (also known as p62 or ZIP for ζPKC interacting protein), was identified and implicated in NF-κB activation by RANKL. p62^{ZIP} plays a critical role in NF-κB activation induced by TNF-α, CD40 and IL-1 through its interactions with the atypical protein kinases, ζPKC or λPKC. Thus p62^{ZIP} is involved in multiple signaling pathways that result in enhanced tumor cell survival and bone destruction. It is our hypothesis that inhibiting p62^{ZIP} expression or that of the atypical PKC's will profoundly diminish osteolytic bone destruction and tumor growth in patients with bone metastases by blocking the effects of RANKL and IL-6 produced in the tumor-bone microenvironment in response to TNF-α.

To test this hypothesis, we have examined the role of p62^{ZIP} in RANKL and TNF-α induced human OCL formation. A retroviral construct containing the p62^{ZIP} gene or empty vector was transfected into human OCL precursors and the effects of overexpressing p62^{ZIP} on OCL formation were examined. Western blot analysis confirmed overexpression of p62^{ZIP} in these cells**.** OCL formation was significantly increased in cultures of OCL precursors overexpressing p62^{ZIP} and treated with RANKL or TNF-α compared to cultures of empty vector transduced cells. Furthermore, OCL precursors overexpressing p62^{ZIP} formed OCL at very low concentrations of RANK (1ng/ml) or TNF-α (10pg/ml) which did not induce normal OCL. The OCL formed by precursors transfected with p62^{ZIP} were larger than normal OCL. Importantly, RANKL induced OCL formation was blocked by transduction of normal OCL precursors with a p62^{ZIP} antisense construct. These results show that p62 plays an important role in OCL formation and suggest that blocking p62 signaling should have a profound effect on OCL formation induced by tumors.

Disclosures: ***N. Kurihara****, None.*

F274

NFATc1 Is a Critical Trancription Factor for RANKL-induced Differentiation of Osteoclasts. S. Kim*[1], H. Takayanagi[2], T. Koga*[2], T. Taniguchi*[1]. [1]Department of Immunology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan, [2]Department of Immunology, Graduate School of Medicine, University of Tokyo. PRESTO, Japan Science and Technology Corporation, Tokyo, Japan.

Receptor activator of NF-kB ligand (RANKL) is a key cytokine to induce the differentiation of osteoclast precursors into osteoclasts. The binding of RANKL to its receptor RANK induces the activation of various downstream molecules such as NF-kB and AP-1. Although other cytokines such as interleukin-1 (IL-1) also activate similar signal cascades, RANKL specifically induces the osteoclast differentiation.

We performed a genome-wide screening of RANKL-inducible genes using gene chip analysis. Nuclear factor of activated T cells c1 (NFATc1) was the most strongly induced transcription factor by RANKL. We confirmed the induction of mRNA and protein of NFATc1 by RANKL with northern blot analysis and immunofluorescence staining, respectively. We also examined the localization of NFATc1 protein in vivo and showed that it colocalized with osteoclasts stained for TRAP or cathepsin K. The activation and induction mechanism of NFATc1 has been well studied in T lymphocytes: Calcium signal induces the activation of calcineurin and calcineurin induces the dephosphorylation of NFATc1, resulting in its nuclear localization. Interestingly, we found out that the RANKL signal, but not the IL-1 signal, induces calcium influx in osteoclast precursors and the addition of BAPTA-AM, calcium specific chelator, strongly inhibits the induction of NFATc1 in mRNA level and blocks osteoclast differentiation. The osteoclast differentiation was also blocked by calcineurin inhibitors such as cyclosporinA and FK506. By using TRAF6- or Fos-deficient mice, we examined the induction mechanism of NFATc1. RANKL-induced NFATc1 expression was impaired in both mutant mice, suggesting that TRAF6 and c-Fos are involved in the induction of NFATc1. We also examined the activation of TRAP promoter by NFATc1. Using a reporter assay, we identified TRAP and calcitonin receptor genes as direct transcriptional targets of NFATc1, and revealed that c-Fos is one of the functional partners of NFATc1. Because NFATc1-deficient mice are embryonic lethal, we established an osteoclast formation system of embryonic stem cells (ES cells). NFATc1-deficient ES cells could not differentiate into osteoclasts while wild type cells differentiated into osteoclasts. Furthermore, overexpression of NFATc1 in the osteoclast precursors induced osteoclast differentiation without addition of RANKL. These results strongly suggest that NFATc1 is an essential transcription factor that integrates RANKL signaling for terminal differentiation of osteoclasts.

Disclosures: ***S. Kim****, None.*

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The Enzymatic Activity of a Unique Osteoclastic Protein-Tyrosine Phosphatase (PTP-oc) Is Essential for Activation of Osteoclast-Like Cells: Evidence for Activation via the c-src/NFκB Pathway. K. H. W. Lau[1], M. Amoui*[1], S. M. Suhr[2], D. J. Baylink[1]. [1]Musculoskeletal Disease Center, Jerry L. Pettis Mem VAMC, Loma Linda, CA, USA, [2]Biochemistry, Yonsei University, Seoul, Republic of Korea.

Our previous antisense studies have indicated that a structurally unique osteoclastic PTP, PTP-oc, has an essential role in osteoclast activity. This study investigated if the enzymatic activity of PTP-oc is required for osteoclast activation by determining the effects of overexpression of wild type (wt) and the inactive, dominant negative (dn) PTP-oc on bone resorption activity of osteoclast-like cells derived from two cell lines of osteoclast lineage (RAW264.7 and U937 cells). Several RAW264.7 and U937 stable clones overexpressing wt or dn PTP-oc were produced with our newly developed transposon-based pPC gene transfer vector. Each of the clones showed 4- to 6-fold overexpression of either wt or dn PTP-oc. The bone resorption activity of "osteoclast-like" cells derived from RAW264.7 clones overexpressing wt PTP-oc was significantly ($p<0.01$) greater by 2- to 3-fold, while that of "osteoclast-like" cells from RAW264.7 clones overexpressing dn PTP-oc was lower (by >50%, $p<0.05$) than that of "osteoclast-like" cells derived from control clones. Similar results were obtained with U937 clones. These findings are consistent with the interpretation that the enzymatic activity of PTP-oc is essential for osteoclast resorption. To determine the mechanism whereby PTP-oc activates osteoclast resorption, we determined the c-src activation [assessed by the decrease in tyr-527 phosphorylation level (PY-527)] and NFκB activation (monitored by IκBα degradation and NFκB nuclear translocation) in each clone, since activation of c-src and NFκB are each essential for osteoclast activation. RAW264.7 and U937 cell clones overexpressing wt PTP-oc each showed greater c-src activation (75% decrease in PY-527, $p<0.01$) and NFκB activation (70% decrease in cellular IκBα level and 2- to 3-fold increase in nuclear NFκB level, $p<0.01$ for each), whereas cells stably overexpressing dn PTP-oc had lower c-src activation (30% increase in PY-527, $p<0.05$) and NFκB activation (25% increase in cellular IκBα level and 75% decrease in nuclear NFκB level, $p<0.05$ for each) compared with corresponding control cells. The c-src activation ($r = -0.998$, $p<0.001$) and NFκB activation ($r = 0.989$, $p<0.05$) each correlated with the bone resorption activity of the "osteoclast-like" cells derived from these cell clones. In conclusion, we demonstrated for the first time that, 1) the PTP-oc enzymatic activity is essential for osteoclast activity, and 2) the PTP-oc-mediated osteoclast activation is mediated in part by activation of c-src and/or NFκB.

Disclosures: ***K.H.W. Lau****, None.*

F279

An Animal Model for the Isolation of Mammalian Osteocytes using a Membrane-Bound Enzyme, which in Bone Is Specifically Expressed in Osteocytes. K. E. de Rooij[1], E. de Wilt*[1], M. M. L. Deckers*[1], M. C. de Ruiter*[2], I. Que*[1], S. E. Papapoulos[1], C. W. G. Lowik[1]. [1]Endocrinology, Leiden University Medical Center, Leiden, Netherlands, [2]Anatomy, Leiden University Medical Center, Leiden, Netherlands.

Osteocytes are the most abundant cells in bone, which implies an important role for osteocytes in bone homeostasis. It has been generally accepted that osteocytes act as sensors of mechanical loading of the bone and convey these signals to the osteoblasts and osteoclasts to remodel the bone. At present, it is not known how osteocytes fulfil their mechanosensing function or which other functions they may perform in bone homeostasis. This is mainly due to the fact that osteocytes are very difficult to isolate from their surrounding matrix. In addition, little is known about the differentiation of osteocytes from osteoblasts.

In this study, we describe the identification of a membrane-bound protein that, within bone, is exclusively expressed in osteocytes. This protein is an enzyme involved in cell-cell interactions and belongs to a large family of receptor-like proteins. We have obtained transgenic mice in which one exon of this protein is replaced by the ß-galactosidase (LacZ) gene. Deficiency of the protein does not result in an aberrant phenotype in these mice. Long bones and calvariae of these transgenic mice were isolated and analyzed for ß-galactosidase activity by staining with X-gal. Histologic analysis showed that only osteocytes exhibit ß-galactosidase activity. The blue staining was present throughout the cells and the cellular processes could easily be distinguished.

To investigate the possibility of generating osteocytes *in vitro*, bone marrow cells of the transgenic mice were cultured under osteogenic conditions. After 21-28 days, blue stained cells could be observed associated with nodules and the number of LacZ positive cells could be increased by the addition of BMPs to the culture medium. Histologic analysis revealed that all positive cells were surrounded by extracellular matrix and cellular processes could be observed.

In conclusion, these mice represent an excellent model for the investigation of osteocyte biology. The formation of osteocytes *in vitro*, and the effect of different compounds on this process, can easily be monitored by analysis of LacZ activity by either staining with X-gal or enzymatic assays. Since the protein is present on the membrane, antibodies raised against this protein could provide the first tool for the isolation of mammalian osteocytes, allowing us to study differences in gene expression between osteocytes and osteoblasts. This will generate information on the specific properties of osteocytes and improve our knowledge about their function(s).

Disclosures: ***K.E. de Rooij****, None.*

F284

The Accuracy of Historical Height Loss for Detection of Prevalent Vertebral Fractures. K. Siminoski[1], K. Lee*[2], H. Jen*[2], R. Warshawski*[2]. [1]Radiology and Internal Medicine, University of Alberta, Edmonton, AB, Canada, [2]Radiology, University of Alberta, Edmonton, AB, Canada.

We have evaluated the accuracy of using historical height loss (HHL) to detect the presence of vertebral fractures. Subjects were women referred for specialist assessment of osteoporosis (n = 480; average age 56 yrs; range: 18 - 92 years). HHL was determined as the difference between the tallest recalled height and current height as measured using a stadiometer. Vertebral morphometry was performed from T4 to L4. Prevalent vertebral fracture was defined as a vertebral height ratio <0.80. One or more fractures were present in 34.3%; the average number of fractures among those with fractures was 2.3

Historical height loss was correlated with the number of vertebral fractures ($r = 0.53$; $p<0.001$). Height decreased by 1.2 cm per fracture; after adjustment for age and tallest recalled height, height loss per fracture was 1.0 cm ($r = 0.647$, $p < 0.0001$). In a multivariable model, adjusted height loss (adjusted for age and tallest recalled height) was 0.82 cm per thoracic fracture and 1.32 cm per lumbar fracture ($r = 0.66$, $p<0.0001$) .

The area under the receiver operating characteristic curve for the ability of HHL to detect vertebral fractures was 0.69 (95% confidence interval, 0.64-0.74; $p<0.001$). The risk of fracture increased substantially only when HHL >6 cm while the risk of fracture was reduced when HHL <2 cm. The odds ratio (shown in the first table) rose from 1 for HHL = none (likelihood ratio 0.5) to 20.7 when HHL >6 cm (likelihood ratio 10.5). Accuracy results are shown in the second table.

The following applications can be made from this data: HHL <2 cm rules out the presence of vertebral fracture with moderate accuracy. HHL >6 cm rules in the presence of vertebral fracture with moderate accuracy. This approach will correctly classify 54% of subjects; incorrectly classify 21% (mostly single low-grade fractures that are false negatives); and leave 25% unclassified.

Likelihood and Odds Ratios

Height Loss (cm)	Likelihood Ratio	Odds Ratio
None	0.5	1.0
0-2	0.5	1.0
2-4	1.5	3.0
4-6	1.1	2.2
6-8	4.8	9.4
>8	10.5	20.7

Historical Height Loss (HHL) Accuracy

Height Loss (cm)	Sensitivity	Specificity	PPV	NPV
None	100	0	34	INF
>0	87	25	38	79
>2	65	68	51	79
>4	37	86	58	72
>6	26	96	78	71
>8	13	99	85	69

PPV = positive predictive value. NPV = negative predictive value.

Disclosures: ***K. Siminoski****, None.*

F286

Postmenopausal Bilateral Oophorectomy and Hip Fractures in Older Women. D. M. Antoniucci*[1], D. E. Sellmeyer*[1], J. A. Cauley[2], K. E. Ensrud[3], J. L. Schneider*[1], S. R. Cummings[4], L. J. Melton[5]. [1]University of California, San Francisco, CA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA, [3]University of Minnesota, Minneapolis, MN, USA, [4]Coordinating Center and California Pacific Research Institute, San Francisco, CA, USA, [5]Mayo Clinic, Rochester, MN, USA.

Bilateral oophorectomy after natural menopause has been associated with an increased risk of osteoporotic fractures, raising questions about the advisability of incidental oophorectomy in older women. It has been postulated that the increased fracture risk is explained by a decline in serum estradiol and testosterone levels following the oophorectomy. We tested this hypothesis in a prospective study of a community-based cohort of 9704 Caucasian women aged 65 years or older (Study of Osteoporotic Fractures): we randomly selected 89 cases of hip fracture, 138 cases of new morphometric vertebral fracture and 247 controls. Serum samples collected at baseline were assayed for estradiol and free testosterone. There were no significant differences at baseline in age (72.8±5.6 vs. 72.9±5.5 years, p=0.9) or weight (64.2±10.8 vs. 65.8±13.2 kgs, p=0.40) between the women who underwent postmenopausal oophorectomy (n=51) and those who did not (n=423). Excluding the women on estrogen, total estradiol levels did not differ significantly in the two groups (0.59±0.3 vs. 0.66±0.3 pg/ml, p=0.23), but free testosterone levels were lower in the women who underwent oophorectomy than in those who did not (1.48±1.4 vs. 2.12±1.6 pg/ml, $p<0.001$). All analyses were adjusted for age and weight, and women on estrogen therapy were excluded. In a Cox-proportional hazards analysis that took account of the case cohort design, a history of postmenopausal oophorectomy was associated with an increased risk of hip fracture (HR=2.6; 95% CI=1.3–5.1), but not vertebral fracture (HR=1.1; 95% CI=0.3–5.1). The relationship between oophorectomy and hip fracture was not altered by adding serum estradiol level (HR=2.5; 95% CI=1.2–4.9), serum

free testosterone level (HR=2.7; 95% CI=1.3–5.4), or both (HR=2.6; 95% CI=1.3-5.3) to the model. These data confirm that postmenopausal oophorectomy is associated with an increased risk of hip fracture. In fact, postmenopausal oophorectomy had a larger effect in this cohort than surgical menopause prior to age 48 (HR=1.6; 95%CI=0.6-4.5). Importantly, the increased risk of hip fracture is not explained by decreases in estradiol or testosterone levels. Even though the mechanism by which oophorectomy after menopause increases the risk of hip fracture has not yet been fully elucidated, these findings suggest that incidental oophorectomy after menopause may not be innocuous, and should be considered carefully in each potential patient.

Disclosures: ***D.M. Antoniucci****, None.*

F288

One-Third of Osteoporotic Women Without Fractures will Experience Vertebral Fractures after Five Years if Untreated. R. Lindsay[1], S. Pack*[2], Z. Li*[3]. [1]Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Procter & Gamble Pharmaceuticals, Egham, United Kingdom, [3]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Vertebral fractures are a risk factor for further vertebral and nonvertebral fractures and 1–in–5 women will experience further vertebral fractures in the year following a vertebral fracture. Our objective was to complement this analysis by investigating longitudinal changes in a population of osteoporotic women who are initially fracture–free, modelling the fracture prevalence over time.

We estimate the 1–year risks of patients having increased numbers of vertebral fractures. We do this separately for different numbers of existing fractures to generate a matrix of risk estimates. Women were selected from the placebo arms of the risedronate fracture trials (VERT–MN, VERT–NA, HIP) with lumbar spine or femoral neck BMD T–score <-2.5 or prevalent vertebral fractures. A total of 2326 women met these criteria with a mean age of 73 years and 70% vertebral fracture prevalence. Mean lumbar spine and femoral neck T–scores were –2.7 and –2.6 respectively. Women received calcium and vitamin–D for up to 3 years. Using the 1–year risk estimates, we use a simple Markov model to model over time a hypothetical population of osteoporotic women, initially without vertebral fractures. Repeated application of the yearly risks simulates how the prevalence of fractures increases annually. Confidence intervals for the 5– and 10–year prevalence estimates were obtained using bootstrap computations.

If left untreated (only calcium and vitamin–D), after 1 year 8% (CI:6,10) of a population meeting the selection criteria would be expected to have one or more vertebral fractures, i.e. a 1–in–13 risk. After 5 years 33% (CI:25,41) will have vertebral fractures and 11% (CI:8,16) will have two or more. After 10 years the prevalence estimates are 55% (CI:44,65) and 29% (CI:22,37) respectively. Sensitivity analysis shows that the first fracture risk is highly influential and each 1% reduction in absolute risk translates to approximately a 4% reduction in 5–year prevalence. Correspondingly, a therapeutic agent reducing the 1–year relative risk by 75% would cut the 5–year prevalence estimate to 10%.Fracture, and multiple fracture, prevalence rapidly increases over time if an osteoporotic population is untreated. Treating to prevent the first fracture significantly reduces the future burden of the disease.

Disclosures: ***R. Lindsay****, None.*

F290

Effectiveness of Vertebral Morphometry Using DXA to Detect Prevalent Osteoporotic Vertebral Fracture. R. D. Chapurlat, F. Duboeuf*, O. H. Marion-Audibert*, P. D. Delmas. INSERM U403, Lyon, France.

Vertebral morphometry, which can be performed along with the BMD measurement using newest DXA machines, might save the time and cost of making radiographs to detect vertebral deformities. Diagnostic effectiveness of DXA morphometry compared to simple radiographs, however, has not been established so far.

We have performed vertebral morphometry using a Hologic Delphi machine (Instant Vertebral Assessment [IVA]) in 56 patients (30 ambulatory patients with a T score < -2.5 and 26 inpatients admitted for osteoporotic vertebral fracture) to detect prevalent vertebral deformities. Spine xrays were also made, as the gold standard for diagnosis of vertebral fracture. Both IVAs and xrays were read by 2 experts, without knowledge of results of the other technique, using the Genant semi-quantitative method. Discrepancies between readers were solved by consensus. First, we examined precision of vertebral deformity evaluation by calculating the agreement (kappa scores) between the 2 readers for reading of IVAs and xrays, and second, sensitivity and specificity of IVA for the diagnosis of vertebral deformity.

We identified 60 vertebral deformities by xrays in those 56 patients. Discrepancies between readers were greater with xrays for vertebrae T11 and above, whereas with IVA, frequency of discrepancies increased for T8 and above. With IVA, kappas varied from vertebra to vertebra, ranging from 1.00 to 0.41 for lumbar vertebrae, and from 0.84 to 0.31 for thoracic vertebrae. With xrays, kappas also varied with vertebrae, ranging from 1.00 to 0.85 for lumbar vertebrae, and from 0.76 to 0.31 for thoracic vertebrae. The agreement between the 2 techniques was good from L4 to T12 (kappas ranging from 0.79 to 0.65), whereas it was moderate or poor for other vertebrae (kappa ranging from 0.85 to 0.18 for T11 to T4). The mean sensitivity of the IVA assessment to detect vertebral deformities established using xray reading was 71% for lumbar vertebrae, 66% for thoracic vertebrae from T12 to T8 and 77% from T9 to 5. Specificity of the IVA reading to detect vertebral deformities was excellent, ranging from 97% for lumbar vertebrae to 96% for thoracic vertebrae from T12 to T8, and 93% for thoracic vertebrae from T7 to T5.

We conclude that when diagnostic accuracy of vertebral deformities is poor using xrays (for higher thoracic vertebrae), accuracy and precision of IVA parallel. Compared to xray assessment of vertebral deformities, IVA allows few false positives and a sizeable proportion of false negatives.

Disclosures: ***R.D. Chapurlat****, None.*

F292

Bone Mineral Density and Fracture Risk in Subjects with Type 2 Diabetes Mellitus: the Rotterdam Study. I. I. de Liefde*[1], M. van der Klift*[1], C. E. D. de Laet*[2], P. L. A. van Daele*[1], A. Hofman*[2], H. A. P. Pols[1]. [1]Department of Internal Medicine, Erasmus MC, Rotterdam, Netherlands, [2]Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, Netherlands.

Previous studies show conflicting results concerning the association between type 2 diabetes mellitus, bone mineral density (BMD) and fracture risk. Therefore, we looked into this association in participants of the Rotterdam Study, a large prospective population-based cohort study of 7983 men and women aged 55 years or older.

At baseline, between 1990 and 1993, blood samples were drawn both before and after a non-fasting glucose tolerance test. Subjects were classified as diabetics if they used anti-diabetic medication or when the pre- or post load serum glucose level was 11.1 mmol/L or more. Subjects reporting onset of diabetes at or before age 30 years (type 1 diabetes mellitus) were excluded from the analyses. BMD was measured using DEXA at both the femoral neck and lumbar spine. Follow-up of non-vertebral fractures occurred through an automated record system involving GP's and local hospitals. Two trained research physicians independently verified all reported fractures.

Complete baseline and follow-up data were available for 6650 individuals, including 792 subjects with diabetes. During follow-up (6.5 years on average), 771 subjects suffered at least one non-vertebral fracture. Adjusting for age, diabetics had a significantly higher BMD, both at the femoral neck and the lumbar spine, compared to non-diabetics (0.86 vs. 0.83 g/cm^2 and 1.12 vs. 1.09 g/cm^2, respectively). This remained unchanged after additional adjustment for other confounders. However, diabetics had an increased risk of non-vertebral fractures, both crude and after adjustment for age, gender, BMI, smoking, falling, visual site, renal function, lower limb disability, use of diuretics, and femoral neck BMD (HR 1.4 (95% CI 1.0-1.8)). For hip and wrist fractures, a similar but non-significant trend was observed. We repeated the analysis separately for diabetics treated with anti-diabetic medication before baseline and for newly diagnosed diabetics. The increased fracture risk was restricted to treated diabetics, with a significantly increased risk for non-vertebral and wrist fractures (HR 1.7 (1.2-2.5) and 2.1 (1.1-4.2) respectively), independent of potential confounders and BMD.

The results of this study show that subjects with type 2 diabetes mellitus have a higher BMD. However, especially those subjects with anti-diabetic medication, have an increased fracture risk compared to non-diabetics, suggesting that, independently of BMD, other factors such as polyneuropathy and general co-morbidity play an important role in the increased fracture risk.

Disclosures: ***I.I. de Liefde****, None.*

F294

Biochemical Markers of Bone Turnover Predict both Non-vertebral and Vertebral Fractures. J. Finigan, D. M. Greenfield*, A. Blumsohn, R. A. Hannon, R. Eastell. Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Biochemical markers of bone turnover have been shown, although not consistently, to have predictive value for fractures. We carried out a 10-year prospective study of a population-based group of women who were ages 50 to 85 years. At baseline we measured hip and spine bone mineral density (BMD) and markers of bone turnover: pyridinoline (PYD), deoxypyridinoline (DPD), N- and C-telopeptides of type I collagen (NTX, CTX), bone alkaline phosphatase (BAP, iBAP) and procollagen type I C-terminal propeptide (PICP), with urine markers corrected for creatinine. Incident vertebral fractures were determined by visual reading by a single radiologist of spinal radiographs at 0, 2, 5, 7 and 10 years, and non-vertebral fractures were confirmed by radiologist reports. The table shows the relative risks (RR) of fracture per standard deviation (log transformed) using a Cox Regression Model, based on time to first fracture.

Marker of bone turnover	Non-vertebral fracture (n=70) RR (95% CI)	Vertebral fracture (n=29) RR (95% CI)
PYD / Cr	1.47 (1.16, 1.86) ***	1.65 (1.16, 2.35) **
CTX	1.31 (0.98, 1.74) ^	1.88 (1.11, 3.18) **
DPD / Cr	1.30 (1.01, 1.67) *	NS
BAP	1.26 (1.01, 1.59) *	NS
iBAP	1.34 (1.04, 1.73) *	NS
PICP	NS	1.38 (0.95, 2.00) *
NTX / Cr	NS	1.70 (1.10, 2.60) *

* p<0.05, ** p<0.01, *** p<0.001, ^ p=0.052

PYD/Cr remained significant for both types of fracture when entered into multivariate analyses with BMD or age, while CTX remained significant for vertebral fractures only. With age only, DPD/Cr still predicted non-vertebral, and NTX/Cr predicted vertebral fractures.

The relative risks of extremity fractures (hands, feet and skull) for each of the markers are shown below.

Marker of bone turnover	Extremity fracture (n=22) RR (95% CI)
PYD / Cr	1.91 (1.26, 2.89) ***
DPD / Cr	1.61 (1.04, 2.50) *
BAP	1.59 (1.06, 2.38) **
iBAP	1.95 (1.24, 3.06) ***

Only PYD/Cr consistently predicts all types of fracture, independently of age and BMD. Some markers predict fractures of the hands, feet and skull at least as strongly as they predict non-vertebral fractures as a whole.

Disclosures: ***J. Finigan****, None.*

F295

Frailty, Debility and Poor Health Predict Vertebral Fractures but Not Non-vertebral Fractures. J. Finigan, D. M. Greenfield*, A. Blumsohn, R. A. Hannon, N. F. A. Peel*, R. Eastell. Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Many risk factors predict fractures overall, but it is less clear whether certain factors are specific to vertebral fractures. We carried out a 10-year prospective study of a population-based group of women who were ages 50 to 85 years. At baseline we measured bone mineral density (BMD) by dual-energy x-ray absorptiometry and collected fasting blood samples and 24-hour urine for biochemical measurements including serum calcium, alkaline phosphatase, parathyroid hormone, creatinine, phosphate, albumin and thyroid hormones, and urine calcium and creatinine. Medical and lifestyle data were obtained by standardized questionnaire. Incident vertebral fractures were determined by visual reading by a single radiologist of spinal radiographs at 0, 2, 5, 7 and 10 years, and non-vertebral fractures were confirmed by radiologist reports.

Risk factors that predicted both types of fracture included age, BMD at the lumbar spine, hip or total body, years of estrogen exposure and prevalent vertebral fracture. Relative risks (RR) that predicted vertebral fracture only, using a Cox Regression Model and survival time to first fracture, are shown in the table.

Risk factor	RR (95% CI) of Vertebral Fracture (n=29)
Height **	1.73 (1.20, 2.51) per SD reduction
Weight *	1.60 (1.02, 2.50) per SD reduction
Body fat (g) *	1.56 (1.02, 1.39) per SD reduction
Heavy smoking ^	2.30 (0.97, 5.41) upper quartile of pack-years v non-smokers
S calcium *	1.71 (1.12, 2.60) per SD reduction
S albumin ***	2.04 (1.33, 3.13) per SD reduction
S total T3 ***	1.88 (1.34, 2.64) per SD reduction
Grip strength **	2.78 (1.31, 5.92) lower quartile v the rest
Limited ability to:	
Carry a suitcase **	1.89 (1.22, 2.92) per difficulty level 1 - 3
Stand in a queue *	1.76 (1.07, 2.90) per level
Run for a bus **	2.46 (1.36, 4.45) per level

* p<0.05, ** p<0.01, ***p<0.001, ^ p=0.057

Serum albumin and T3, and self-reported ability to run for a bus, remained significant when entered into multivariate analyses with either lumbar spine or hip BMD or with age. When combined with age only, both heavy smoking and body fat were still at or close to significance.

Low levels of T3 and of albumin are each associated with sickness and chronic poor health. We conclude that overall frailty, which may comprise general poor health, small or thin body size and lack of strength and physical capability, predicts vertebral fractures but does not predict non-vertebral fractures.

Disclosures: ***J. Finigan****, None.*

F296

Relative Importance of BMD, Markers of Bone Turnover, Height and Body Weight as Predictors of Hip Fracture – A Prospective Case-Control Study. E. McCloskey[1], T. Jalava*[2], S. Vasireddy*[1], S. Bal[1], M. Beneton*[1], D. Charlesworth*[1], J. Kanis[1], A. Blumsohn[1]. [1]University of Sheffield, Sheffield, United Kingdom, [2]Schering Oy, Helsinki, Finland.

While it is accepted that combinations of risk factors are useful to identify patients at high risk of fracture, the role of biochemical markers alone or combined with BMD remains undefined. The aim of this study was to compare the ability of total hip BMD and biochemical markers to predict incident hip fracture in a large prospective case control study.

The study cohort comprised 5212 women aged 75 years or older (mean 80 years, range 75-100) enrolled to a double-blind placebo-controlled study of the bisphosphonate, clodronate (Bonefos®). Baseline BMD at the total hip was measured by DXA (Hologic QDR4500). Fasting serum was collected at entry between 9am and 1130am and was stored at -40C until analysis. In a case control analysis, baseline values of hip BMD, serum bone-specific alkaline phosphatase (Bone ALP, Beckman Ostase) and serum C-telopeptide of type I collagen (sβCTX, Roche Elecsys) were evaluated in 157 women who sustained incident hip fractures and in 544 randomly selected controls who remained free of hip fracture during a median follow-up of 4 years.

Cases with hip fracture had significantly lower hip BMD and higher sβCTX than controls, whereas serum BsALP was similar in the two groups. In univariate logistic regression analysis, the odds for hip fracture per 1SD increase in sβCTX was 1.3 (95%CI 1.1-1.5, P=0.01) while a decrease in hip BMD of 1SD demonstrated a larger gradient of risk (OR 2.5, CI 2.0-3.1, P<0.0001). Body weight alone was predictive of fracture (OR 1.85 per 1SD decrease, CI 1.48-2.32; P<0.001). Adjusting for treatment with clodronate did not influence results. Total hip BMD remained a significant predictor of hip fracture following adjustment for clinical predictors including age, height and body weight (OR 2.1, CI 1.6-2.6, P<0.0001). However, prediction of hip fracture by sβCTX was not independent of these clinical predictors (OR 1.1, CI 0.9-1.4, P=0.31) and was reduced still further following adjustment for hip BMD (OR 1.0, CI 0.8-1.2, P=0.63).

We conclude that these markers of bone turnover have limited incremental utility for prediction of hip fractures in an elderly female population. βCTX is predictive of fracture, but is not independent of age, body weight or hip BMD. Reduced body weight is an important predictor of hip fracture.

Disclosures: ***E. McCloskey****, None.*

F298

The Incidence of Hip and Colles' Fractures Correspond to Seasonal Changes in Serum Vitamin D and Parathyroid Hormone. J. A. Pasco*[1], M. J. Henry*[1], M. A. Kotowicz[1], K. M. Sanders*[1], E. Seeman[2], H. G. Schneider[3], G. C. Nicholson[1]. [1]Clinical & Biomedical Sciences: Barwon Health, The University of Melbourne, Geelong, Australia, [2]Medicine, The University of Melbourne, Melbourne, Australia, [3]Biochemistry, The Alfred Hospital, Melbourne, Australia.

Vitamin D insufficiency and secondary hyperparathyroidism are risk factors for fractures in institutionalized elderly populations. The aim of this study was to determine whether vitamin D insufficiency contributes to the population burden of fractures. Annual periodicities of fractures, circulating 25-hydroxyvitamin D (25OHD), parathyroid hormone (PTH), bone resorption markers (C-terminal telopeptide, CTx) and UV radiation were measured as part of the Geelong Osteoporosis Study, a population-based study set in south-eastern Australia (latitude 38-39S). Fractures were ascertained prospectively during three years using radiographs from the two radiological practices servicing a community of 27,203 women aged 55 years and over.

Cyclic variations in serum 25OHD lagged one month behind variations in UV radiation, with a peak following mid-summer and nadir following mid-winter (p<0.001). Periodicity of serum PTH was the inverse of serum 25OHD, with a phase shift of +1 month (p=0.058). The peak in serum CTx followed 1-2 months after the peak in serum PTH. Seasonal periodicity amongst 439 hip fractures and 307 Colles' fractures followed a simple harmonic model (p=0.078, 0.002, respectively) with the peak frequency occurring 0.5-2 months after peak serum PTH and about 1.5-3 months after the nadir in serum 25OHD. Periodicity of Colles' fractures and CTx were coincident.

The fall in serum vitamin D during winter is accompanied by secondary hyperparathyroidism, an increase in bone resorption markers and an increased frequency of hip and Colles' fractures. Whether vitamin D supplementation during winter will reduce the population burden of fractures requires investigation.

Disclosures: ***J.A. Pasco****, None.*

F300

Circulating Levels of Cytokine Soluble Receptors Predict Incident Fractures in Older Men and Women: The Health Aging and Body Composition Study (HABC). J. A. Cauley[1], J. M. Zmuda[1], A. B. Newman*[1], M. Pahor*[2], F. Tylavsky*[3], S. R. Cummings[4], T. Harris*[5]. [1]U of Pitt, Pittsburgh, PA, USA, [2]Wake Forest U, Winston Salem, NC, USA, [3]U of Tenn, Memphis, TN, USA, [4]UCSF, San Francisco, CA, USA, [5]NIA, Bethesda, MD, USA.

Cytokines play major roles in regulating bone remodeling in the bone microenvironment. Nevertheless, the relationship of circulating levels to fracture is uncertain. In the current analysis, we tested the hypothesis that greater concentrations of interleukin-6 (IL-6), tumor necrosis factor (TNF-α) IL-2-soluble receptor (sR), IL-6-sR, TNF sR1 and TNF sR2 are related to an increased risk of fracture. The study population included 2,971 well-functioning white and black women and men (42%, black; 51%, women) age 70-79 enrolled in HABC. Cytokines were measured in frozen plasma samples using standard protocols. Cytokine soluble receptors were measured in a subset (n=1430). Total hip BMD was measured at baseline and 4 years later with DXA (Hologic 4500A). During 3.9 years of 97% complete follow-up, we validated 156 non-traumatic fractures. We compared the top quartile of cytokine vs. the lower 3. Analyses were adjusted for race, gender, age, BMI, smoking, NSAIDS use, baseline BMD, health status and comorbidity. The Relative Risk (RR) of fracture was about 60-70% increased in subjects with the greatest cytokine soluble receptor levels compared to subjects with lower levels (Table). There was no association with IL-6 or TNF-α.

Table: RR of Fracture: Top Quartile of Cytokine vs. the lower 3.

Cytokine (pg/ml)	**RR**	**(95% CI)**
IL-6 (>2.78)	1.27	(0.68, 1.88)
()TNF-α >4.11)	1.20	(0.83, 1.74)
TNF sR1 (>1792)	1.64	(1.00, 2.67)
TNF sR2 (>4021)	1.76	(1.09, 2.85)
IL-6sR (>41,989)	1.66	(1.02, 2.70)
IL-2sR (>1,648)	1.79	(1.12, 2.85)

Of interest, the average annual percent loss in total hip BMD was greater among subjects with the highest IL-2sR levels (-0.48 %/yr) compared to lower levels (-0.30 %/yr) (p=0.0009). Similar results were observed for the other cytokine soluble receptors.

In conclusion, higher cytokine soluble receptors levels may identify subjects with an increased rate of bone loss and increased risk of fracture. The underlying mechanism is unknown but could reflect influences on bone turnover.

Disclosures: ***J.A. Cauley****, Merck 2, 8; Eli Lilly 2, 8; Pfizer 2; Novartis 2, 5.*

F302

Ten Year Fracture Prediction using QUS and Axial BMD Measurements in Peri-Menopausal Women: BUA Predicts Wrist Fractures Independently of BMD. A. Stewart*, D. M. Reid. Osteoporosis Research Unit, University of Aberdeen, Aberdeen, United Kingdom.

Quantitative ultrasound (QUS) has been shown to be predictive of fracture in the elderly, but few studies have measured women around the time of the menopause. The Aberdeen Prospective Osteoporosis Screening Study (APOSS) is a longitudinal study of bone mass studied throughout the peri-menopausal years. Initially over 5000 women aged 45 to 54 years were scanned using spine and hip DXA (Norland XR-26 scanner) and in a cohort of 1000 also by QUS (Walker Sonix 575, measuring broadband ultrasound attenuation (BUA) only). These women have now been followed up for over 10 years (mean = 10.9 yrs, SD 0.3 yrs) and incident fractures identified and validated. In the QUS cohort (n=1000) we have fracture data on 636 women. Of these 61 have had a validated fracture, while 23 indicated a fracture but these have not been been confirmed as yet. The remaining 552 women did not indicate an incident fracture. The fractures were mostly of the wrist (n = 14) and ankle (n = 15), with other fracture sites including rib (n = 3), foot/toes (n = 7), hands/fingers (n = 9) and miscellaneous (n = 13). Cox Regression Model was used to calculate relative risks (RR) with time to fracture/follow-up being the time dependent variable. All variables used were baseline measurements. Using BUA measurements the unadjusted RR is 1.37 (95%CI 1.06-1.76) for 1 SD change in BUA. When adjusted for age, height, weight and menopausal status the RR is 1.39 (1.08-1.79). However when also adjusting for neck BMD the RR is no longer significant (RR = 1.32, 95% CI 0.99-1.76). To compare with DXA measurements the RR (adjusted for age, height, weight and menopausal status) in this cohort for 1 SD is 1.64 (95%CI 1.22-2.21) for spine BMD and 1.38 (95%CI 1.03-1.84) for neck. BMD. If we just look at the wrist fractures (i.e. osteoporotic site) the unadjusted RR is 2.91 (95%CI 1.69-5.01) and adjusted for age, height, weight and menopausal status this remains highly significant (RR = 3.25 (95%CI 1.76-6.02)). After adjustment for the neck BMD in the wrist facture cohort the RR remained highly significant (RR = 3.20 (95%CI 1.67-6.12)) indicating BUA is an independent predictor of wrist fractures. To compare with DXA measurements the RR (adj. for age, height, weight and menopausal status) for 1 SD change is 2.07 (95%CI 1.12-3.86) for spine BMD and 2.23 (95%CI 1.17-4.25) for neck. BMD. In conclusion BUA is a predictor of fractures in early postmenopausal women. This is especially true of fractures at the wrist where BUA is independent of BMD measurement and a better predictor than BMD measurements. Therefore BUA may be a method of measuring future fracture risk at the time of the menopause to assist in decision making regarding prophylactic treatment.

Disclosures: ***A. Stewart****, None.*

F303

Prevalent Wrist Fracture After Age 50 is a Risk Factor for Incident Hip and Incident Morphometric Vertebral Fracture Independent of Bone Mineral Density (BMD). J. T. Schousboe[1], H. A. Fink[2], B. C. Taylor[2], K. L. Stone[3], T. A. Hillier[4], M. C. Nevitt[3], K. E. Ensrud[2]. [1]Park Nicollet Clinic, Mpls, MN, USA, [2]U of MN, Mpls, MN, USA, [3]UCSF, SF, CA, USA, [4]NW Kaiser Permanente, Portland, OR, USA.

Wrist fracture (WF) has been reported to be a risk factor for subsequent hip (HF) and vertebral fractures, but prior studies have not controlled for BMD.

We examined these associations with data from the Study of Osteoporotic Fractures (SOF), a prospective cohort study examining predictors and outcomes of osteoporosis in 9704 women aged ≥65 yrs. At SOF visit 1, 1100 women self-reported prior WF since age 50. 120 additional women reported WF occurring between visits 1 and 2 that were confirmed by review of radiographic reports. Calcaneal BMD was measured at visit 1and total Hip BMD was measured at visit 2. Incident morphometric vertebral fractures (MVF) were determined by comparison of digitalized lateral spine radiographs from visit 3 with those obtained at visit 1 (mean interval 3.8 years). Incident HF occurring after visit 2 were confirmed by review of radiographic reports.

Logistic regression was used to assess the age, age and calcaneal BMD, and multivariate adjusted odds ratios (OR) for incident MVF occurring after visit 1 in women with versus those without WF prior to visit 1.

Proportional hazards regression was used to assess the age, age and total hip BMD , and multivariate adjusted hazard ratios (HR) for incident HF occurring after visit 2 in women with versus those without WF prior to visit 2. As approximately half of HF occurred within the first 7 years of follow-up after visit 2, we also examined the HR for incident HF for the follow-up period from 0 to 7 years after visit 2, and separately during the follow-up period that began 7 years after visit 2.

Odds of Incident Morphometric Vertebral Fracture Associated with Prevalent Wrist Fracture

	Age adjusted OR (95%CI)	Age & BMD Adjusted OR (95%CI)	Multivariate Adjusted OR (95%CI)
All MVF	1.70 (1.28, 2.25)	1.38 (1.04, 1.83)	1.36 (1.02, 1.81)

Rate of Incident Hip Fracture Associated with Prevalent Wrist Fracture

	HR (95%CI)	HR (95%CI)	HR (95%CI)
All HF	1.41 (1.15, 1.73)	1.11 (0.90, 1.36)	1.10 (0.90, 1.36)
0-7 yrs follow-up period	1.83 (1.39, 1.44)	1.43 (1.09, (1.87)	1.45 (1.10, 1.91)
>7 yrs follow-up period	1.05 (0.76, 1.44)	0.83 (0.60, 1.15)	0.82 (0.59, 1.12)

Prior wrist fracture is a risk factor for morphometric vertebral fracture independent of age and BMD. Prior wrist fracture is also a risk factor for incident hip fracture; this association is largely explained by lower hip BMD among those with prior wrist fracture and wanes with increasing length of follow-up.

Disclosures: ***J.T. Schousboe****, None.*

F305

High Peak Bone Mass Associated with Physical Activity may Result in Fewer Fragility Fractures in Elderly Men. A. H. Gustavsson*[1], F. Nyquist*[2], C. Karlsson*[2], T. Olsson*[3], P. Nordström[1], M. K. Karlsson[2]. [1]Department of Surgical and Perioperative Sciences, Sports Medicine Unit, Umeå, Sweden, [2]Department of Orthopaedics, Malmö, Sweden, [3]Department of Public Health and Clinical Medicine, Umeå, Sweden.

Background: It has been suggested that increasing bone mass by intense physical activity during childhood and adolescence may decrease the risk of osteoporosis and subsequently the risk of fracture later in life. This implies that the gain in bone mass is not lost when physical activity is decreased.

Methods: In the longitudinal part of this study, 97 athletes (mean age 21.0±4.5 years) and 49 control subjects (mean age 22.4±6.3 years) were studied at baseline and after a mean period of 5 years. The groups did not differ in weight or height at baseline. Bone mineral density (BMD; g/cm2) was measured for total body, femoral neck, spine and arms using dual-energy X-ray absorptiometry. In a second cohort consisting of 400 former soccer and ice hockey players aged 60 years or more (mean age 71.0±6.0 years), and double age matched controls (mean age 71.0±5.9 years), the prevalence of fractures was evaluated using a questionnaire.

Results: At baseline, the athletes and controls differed significantly in bone density at all sites (P<0.001), but there were no other significant differences between the two groups considering anthropometric data. Between baseline and the follow up, 55 athletes stopped their active sports career. These men were found to have lost significantly more BMD at the femoral neck (0.09 vs. 0.02 g/cm2, P=0.002), and gained less total body BMD (0.02 vs. 0.05 g/cm2, P=0.02) than the 42 athletes that remained active throughout the study, but not compared to the controls. At follow up the former athletes still had higher BMD of the total body (1.33 vs. 1.27 g/cm2, P=0.001), neck (1.20 vs. 1.11 g/cm2, P=0.02), and arms (1.07 vs. 1.00 g/cm2, P<0.001) compared to the controls. In the second cohort the proportion of individuals with fragility fractures sustained after age 50 was reduced in the former athletes (2.0 % versus 4.2 %, P=0.02) and there was also a significant difference in wrist fractures alone (0.75 % versus 2.5 %, P=0.04).

Conclusion: In summary, the results of the present study suggest a sustained high BMD from previous physical activity may result in fewer fragility fractures in the elderly.

Disclosures: ***A.H. Gustavsson****, None.*

F307

Predictors Of Osteoporosis In Men: Data from the Canadian Multicentre Osteoporosis Study (CaMos). N. Diaz-Granados*[1], G. Tomlinson*[1], N. Kreiger*[1], T. M. Murray[1], W. P. Olszynski[2], A. M. Cheung[1], and the Camos Research Group*[3]. [1]University Of Toronto, Toronto, ON, Canada, [2]University Of Saskatchewan, Saskatoon, SK, Canada, [3]McGill University, Montreal, PQ, Canada.

In order to reduce the burden of illness associated with osteoporosis and its related fractures in men, we need to identify men who are at higher risk for osteoporosis. Baseline data from the Canadian Multicentre Osteoporosis Study (CaMos), a population-based prospective cohort study, were used to identify predictors of osteoporosis in men. Out of the 2884 men recruited into CaMos, we identified 1768 (61%) Caucasian men, aged 50 or older who had not taken oral steroids for more than 3 months and for whom we had measured femoral neck bone mineral density (FN BMD). T-scores were derived using the young male CaMos reference population and the WHO definition for Osteoporosis (T-score < -2.5) was the threshold used to dichotomize the outcome. Predictors were ranked *a priori* in order of importance by two clinical experts, an epidemiologist and a graduate student in epidemiology. Only the variables ranked in the top 50% were considered in these analyses and these included: age, education, height, weight, medical history, family medical history, reproductive history, smoking, physical activity, sunlight exposure, dietary habits (including calcium and caffeine intake). The mean age of men in this study was 65 years (range 50 to 96) and 5% (n = 89) had osteoporosis. Backwards stepwise logistic regression analysis using Akaike's Information Criterion (AIC) as the variable selection criterion demonstrated that lower weight (75 to 90 kgs: odds ratio (OR) = 0.2, 95% CI = 0.1 – 0.3, and > 90 kgs OR = 0.1, 95% CI =0.1 – 0.3, compared to < 75 kgs); current smoking (OR = 2.2, 95% CI =1.3 - 3.8); older age (OR = 1.8, 95% CI =1.4 – 2.4); a history of a minimal trauma fracture since age 50 (OR = 2.4, 95% CI =1.3 – 4.4); and a family history of osteoporosis (OR = 2.2, 95% CI =1.1 – 4.4) were all predictive of osteoporosis (the area under the Receiver Operating Characteristic curve for this model was 0.82, standard error = 0.02). The risk factors identified in this study are similar to those found for women and may be useful in identifying men who are at higher risk for osteoporosis and, by inference, osteoporotic fractures.

Disclosures: ***N. Diaz-Granados****, University Health Network/Merck Osteoporosis Scholarship 2.*

F309

ER-α Genotype Modulates the Relationship Between Bioavailable Estradiol Levels and Bone Loss and BMD in Elderly Men. S. Khosla[1], B. L. Riggs[1], E. J. Atkinson*[2], C. Mavilia*[3], F. Del Monte*[3], L. J. Melton[4], M. L. Brandi[3]. [1]Endocrinology, Mayo Clinic, Rochester, MN, USA, [2]Biostatistics, Mayo Clinic, Rochester, MN, USA, [3]Department of Clinical Physiopathology, University of Florence, Florence, Italy, [4]Health Sciences Research, Mayo Clinic, Rochester, MN, USA.

Bioavailable estradiol (bio E_2) levels are important determinants of BMD and of rates of bone loss in elderly men, and bio E_2 levels < 11 pg/ml may represent the threshold for skeletal estrogen (E) deficiency. Moreover, in women, E-deficiency or sufficiency has much less impact on rates of bone loss or fracture risk in subjects with the Pvu II pp genotype of the E receptor (ER)-α gene versus those with the P allele (PP or Pp) (JBMR 15:2479, 2000). To evaluate the interaction between E levels and ER-α genotype in men, we measured BMD and rates of bone loss (over 4 yrs) and related these to bio E_2 levels and the ER-α Xba I and Pvu II genotypes in 114 men age 60-90 yrs. The X/P and x/p alleles were in strong linkage dysequilibrium. As previously reported, in the overall group of subjects, the rate of bone loss at the mid-radius was related to bio E_2 levels for bio E_2 < 11 pg/ml (R = -0.36, P = 0.004), whereas there was no correlation between bio E_2 and the rate of bone loss for bio E_2 > 11 pg/ml (R = -0.09, P = NS). This relationship was, however, driven entirely by men carrying the X or P alleles; in men with the xx or pp genotypes, there was no correlation between bio E_2 and rate of bone loss, regardless of the bio E_2 level (Table). This was confirmed by statistical analysis testing for an interaction between bio E_2 (for values < 11 pg/ml) and genotype (P = 0.02 and 0.05 for the X and P alleles, respectively).

Bio E_2, pg/ml	Spearman correlation coefficient of bio E_2 to rate of mid-radius bone loss			
	+X (XX or Xx)	+P (PP or Pp)	-X (xx)	-P (pp)
< 11	-0.42*	-0.42*	-0.07	-0.15
> 11	-0.13	-0.17	-0.07	-0.15

*P < 0.005

In addition to longitudinal rates of bone loss, we also noted similar, statistically significant interactions between bio E_2 and Xba I and Pvu II genotypes for cross-sectional BMD at the radius and femur neck: BMD at these sites was higher (by 21-24%) in men with bio E_2 levels > 11 pg/ml versus those with bio E_2 < 11 pg/ml only if they were also carriers of the P or X alleles; BMD at these sites was not different in men with the pp or xx genotypes and high vs low bio E_2 levels. We conclude that the ER-α genotype modulates the relationship between bio E_2 levels and both bone loss and BMD in elderly men; as in women, men with the P or X alleles have rates of bone loss and BMD that depend much more on circulating E levels than those with the pp or xx genotypes.

Disclosures: ***S. Khosla****, None.*

F311

Older Men with Weight Loss Have Increased Rates of Hip Bone Loss, Irrespective of Adiposity or Intention to Lose Weight. K. Ensrud[1], R. Fullman*[2], M. Stefanick*[3], H. Fink[1], E. Barrett-Connor[4], C. Lewis[5], P. Bowman*[1], E. Orwoll[6], J. Cauley[7]. [1]VAMC & U of MN, Minneapolis, MN, USA, [2]UCSF, San Francisco, CA, USA, [3]Stanford U, Palo Alto, CA, USA, [4]UCSD, San Deigo, CA, USA, [5]U of AL, Birmingham, AL, USA, [6]OHSU, Portland, OR, USA, [7]U of Pittsburgh, Pittsburgh, PA, USA.

Older men with weight loss have higher rates of bone loss, but the effects of adiposity and intention to lose weight on this association are uncertain. To test the hypothesis that elderly men with recent weight loss have higher rates of hip bone loss irrespective of adiposity or intention to lose weight, we measured body mass index (BMI), body composition, and total hip bone mineral density (THBMD) at a baseline and subsequent exam (average 2.2 yrs between exams) in a cohort of 323 men aged ≥65 yrs at baseline. During this period, 15% of participants lost ≥5% of their baseline weight (weight loss), while the remaining 85% (stable weight or weight gain) had a <5% change from their baseline weight (76%) or gained ≥5% or more of their baseline weight (9%). The mean annual percent change in THBMD was calculated by category of weight change. The average age-adjusted rate of decline in THBMD increased from -0.26% /yr in men with stable weight or weight gain to -0.97%/yr in men with weight loss (p <0.0001). Further adjustment for baseline health status, body mass index, THBMD did not alter these results. Among both heavier and thinner men, higher rates of hip bone loss were observed in men with weight loss (p >0.09 for interaction between weight change and BMI). Our findings were unchanged when lean mass or fat mass was substituted for BMI in the analyses. In addition, higher rates of hip bone loss were observed in men with weight loss, irrespective of intention to lose weight (p > 0.39 for interaction).

	Mean Annual % Change in THBMD (95% CI)	
	Weight Loss	Stable Weight or Weight Gain
Overall Cohort	-0.97 (-1.28,-0.66)	-0.26 (-0.39,-0.13)
BMI ≥ 26.6*	-1.18 (-1.70,-0.66)	-0.14 (-0.31,0.04)
BMI < 26.6*	-0.85 (-1.24,-0.47)	-0.40 (-0.60,-0.21)
Trying to Lose Weight	-0.94 (-1.33,-0.55)	-0.18 (-0.38,0.02)
Not Trying to Lose Weight	-1.08 (-1.55,-0.61)	-0.28 (-0.48,-0.08)

*Median BMI=26.6 kg/m^2

Older men with weight loss in later years have increased rates of hip bone loss, even among overweight men and those trying to lose weight. Measurement of weight change should be included in the clinical assessment for osteoporosis risk in older men.

Disclosures: ***K. Ensrud****, Merck 2; Eli Lilly 2; Pfizer 2; Berlex 2.*

F313

How Many Fractures in Men Are Attributable to Low Bone Density? T. V. Nguyen, J. R. Center, J. A. Eisman. Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, Australia.

In the elderly population, approximately one third of osteoporotic fractures occur in men. Among many risk factors for fracture, low bone mineral density (BMD) is considered an important determinant. However, it is not known how many fractures are attributable to low BMD. This study was designed to estimate the proportion of fractures attributable to low BMD in a sample of 821 men (average age: 70 yr, range: 60 to 92 yr) who have been participating in the Dubbo Osteoporosis Epidemiology Study since mid-1989.

Approximately 11% and 21% of the men had femoral neck BMD T-scores<-2.5 and T-scores<-2.0, respectively. During the 13-year follow-up period, 118 men sustained a symptomatic low trauma fracture, including femoral neck (n=26), vertebrae (n=43), and distal radius and humerus (n=12). Approximately 23% and 34% of those with fracture had femoral neck BMD T-scores<-2.5 and T-scores<-2.0, respectively. The risk of fracture increased 3-fold (95% confidence interval [CI]: 1.8 - 5.0) among those with T-scores<-2.5, and 2.2-fold (95% CI: 1.4 - 3.4) among those with T-scores<-2.0. Between 18% and 20% of all fractures in elderly men were attributable to low BMD. The BMD-attributable risk was 38% for hip fractures, 41% for symptomatic vertebral fractures, and 34% for distal radius and humerus fractures.

In these older men, low BMD was a strong predictor of fracture risk, but still accounted for only a modest proportion of fractures. The majority of fractures occurred in men with BMD T-scores>-2.5. The data imply that current cut-off values of BMD for the diagnosis of osteoporosis in men are ina ppropriate.

Disclosures: ***T.V. Nguyen****, None.*

F316

Impaired Sleep Increases the Risk of Falls in Older Women: A Prospective Study with Objective Measurements of Sleep. K. L. Stone[1], J. L. Schneider*[1], T. Blackwell*[1], J. A. Cauley[2], K. E. Ensrud[3], T. A. Hillier[4], S. Ancoli-Israel*[5], D. Claman*[1], S. R. Cummings[6]. [1]University of California, San Francisco, San Francisco, CA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA, [3]University of Minnesota, Minneapolis, MN, USA, [4]Kaiser Permanente Center for Health Research, Portland, OR, USA, [5]University of California, San Diego, San Diego, CA, USA, [6]San Francisco Coordinating Center, San Francisco, CA, USA.

Sleep deprivation is associated with decreased cognitive and functional performance, both risk factors for falls. No study has used objective measurements of sleep to determine whether poor sleep increases the risk of falls, and perhaps fractures.

We assessed sleep patterns in 2,131 women aged 80 and older using the Sleep-Watch-O™ actigraph (Ambulatory Monitoring, Inc.), which utilizes a piezoelectric sensor to detect fine movements. An algorithm is applied to movement patterns to determine sleep-wake states. The actigraph was worn on the non-dominant wrist for a minimum of 72 hours. Incident falls were ascertained by postcard follow-up every 4 months. 'Fallers' were defined as those who experienced ≥ 1 fall per interval (on average) subsequent to the sleep assessment (mean follow-up 0.6 years).

240 women were classified as fallers. After controlling for age, body mass index, race, and use of sleep medications, women who self-reported excessive daytime sleepiness (score of ≥ 16 on the Epworth Sleepiness Scale) experienced 2.6-fold increased risk of being a faller (RR=2.6; 95% CI=1.3 – 5.4) compared to others. We found that objective measures of sleep were significantly associated with increased risk of falling. Relative risks (RR) and 95% confidence intervals (CI) are shown below (table).

Sleep Efficiency* (%)		# Long Wake Episodes**		Total Sleep Time (hours)	
Category	RR (95% CI)	Category	RR (95% CI)	Category	RR (95% CI)
< 60%	1.5 (1.1, 2.2)	< 5	1.0 (ref)	< 4	1.8 (1.2, 2.6)
60-<70%	1.8 (1.2, 2.7)	5 - <8	1.1 (0.8, 1.5)	4 - <6	1.3 (0.9, 1.7)
70-<80%	1.1 (0.7, 1.6)	≥ 8	1.5 (1.1, 2.2)	6 - <8	1.0 (ref)
≥ 80%	1.0 (ref)			≥ 8	1.2 (0.7, 2.1)

* % of time-in-bed scored as sleep ** wake episodes > 30 min

Further analysis suggests that these associations are only partly mediated by excessive daytime sleepiness. Other factors, such as decreased cognitive or physical function, may contribute to increased risk of falling among poor sleepers.

We conclude that sleep disruption and excessive daytime sleepiness significantly increase risk of falls. Improving sleep in older patients may significantly reduce risk of falls and fractures.

Disclosures: ***K.L. Stone****, None.*

F318

21 To 45 Year Old Premenopausal Women Suffering from Major Depression Are at Increased Risk for Osteoporosis. G. Cizza, F. Eskandari*, P. Martinez*, S. Torvik*, C. Kotila*, S. Mistry*, N. Sebring*, B. Drinkard*, D. Ronsaville*, J. Reynolds*, P. W. Gold*. National Institutes of Health, Bethesda, MD, USA.

Osteoporosis is a common and silent condition characterized by bone fragility and increased risk of fractures. Thus, the identification of unrecognized risk factors for osteoporosis is of scientific and clinical importance. Major depressive disorder (MDD) is also common, affecting 5-9% of women of all ages. Subjects with MDD often exhibit

hypercortisolism, increased levels of IL-6 and other endocrine changes that may predispose them to osteoporosis. To determine whether MDD is an unrecognized risk factor for low bone mass in premenopausal women, we assessed the proportion of subjects with osteopenia or osteoporosis (respectively defined as -1 and –2.5 SD below peak bone mass) by DEXA measurements, in 81 women with MDD (age=35.3±6.8) and in 44 healthy controls (age=34.7±6.9) matched for race.

Prevalence of osteopenia or osteoporosis at any skeletal site in women with MDD and matched controls.

Skeletal site	Osteopenia		Osteoporosis	
	MDD (n=81)	Controls (n=44)	MDD (n=81)	Controls (n=44)
AP Lumbar Spine	14%	16%	7%	0%
Femoral Neck	15	5	5	0
Trochanter	18	5	1	0
Total Femur	16	2	0	0

Women with MDD had a significantly greater prevalence of osteopenia or osteoporosis than controls at all hip sites (all p<.02, as assessed by Wilcoxon's rank sum test for ordinal data). The majority of these premenopausal subjects were unaware of having osteoporosis. Two of the 9 women with osteoporosis were younger than 30. Total calcium intake (dietary and supplements) was similar for women with osteopenia/osteoporosis (1085±814 mg/day), and women with normal BMD (above -1 SD) whether depressed (1357±657) or controls (1491±549). Reported caffeine and alcohol intake and level of physical conditioning (12 min Cooper test) were also similar in depressed and control subjects. However, mean 24 hr plasma cortisol levels (collected hourly) tended to be higher (p< .09) in subjects with osteoporosis than in women with normal BMD. In regression analysis there was a negative relationship between 24h mean plasma cortisol and spine BMD even after correcting for BMI (p<.003; r^2 .11). In conclusion, low bone mineral density is significantly more common in women with MDD than in matched controls, suggesting that MDD may be an unrecognized risk factor for osteopenia or osteoporosis. The prospective evaluation of these subjects being conducted in this study will help to establish whether there is a causal relationship between MDD and osteoporosis in premenopausal women.

Disclosures: ***G. Cizza****, None.*

F322

High Protein Diets Acutely Increase Intestinal Calcium Absorption but Not Kinetic Measures of Bone Resorption. J. E. Kerstetter[1], R. H. Raphael*[1], K. O. O'Brien*[2], D. M. Caseria*[3], D. E. Wall*[4], K. L. Insogna[3]. [1]School of Allied Health, University of Connecticut, Storrs, CT, USA, [2]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA, [3]School of Medicine, Yale University, New Haven, CT, USA, [4]School of Medicine, Yale University, New Haven, CT, USA.

High protein diets induce hypercalciuria in humans, but the source of the additional urinary calcium is unknown. It has been suggested that high protein diets increase bone resorption because the high endogenous acid production engendered by protein requires buffering, in part, from the skeleton. Whether this phenomenon explains the increase in urine calcium is unclear. Therefore, dual stable calcium isotopic methodology was used to evaluate the acute effect of a high protein diet on calcium kinetics in women. The study consisted of 2 weeks of a lead-in, well-balanced diet containing moderate calcium, sodium and protein followed by 10 days of an experimental diet. The experimental diet was controlled in calcium (20 mmol) and sodium (100 mmol) and contained one of two levels of protein (1.0 g/kg and 2.1 g/kg). Thirteen healthy women (10 young and 3 postmenopausal) received both levels of dietary protein in random order. On the morning of experimental day 5, subjects received an oral dose of ^{44}Ca followed by an IV dose of ^{42}Ca. Following isotope dosing, 8 serial blood collections, 36 hours of timed urine collections, and 5 days of spot urine samples were analyzed for calcium isotopes. By day 4, urinary calcium in every subject increased during the high protein in comparison to the moderate protein diet (3.57 ± 0.35 versus 5.23 ± 0.37 mmol/d, p < 0.0001, paired t-test). Intestinal calcium absorption increased from 18.5 ± 1.6 % during the moderate protein diet to 26.2 ± 1.9 % during the high diet (p < 0.0001, paired t-test). The increase in calcium absorption accounted for 90 % of the increase in urinary calcium. There were no differences in kinetic measures of bone formation (Vo+), bone resorption (Vo-) or bone balance between levels of dietary protein. All subjects were in negative bone balance regardless of the level of protein, suggesting 20 mmol of dietary calcium is insufficient to maintain bone health. These data directly demonstrate that the acute rise in urinary calcium in response to a high protein diet is explained, not by changes in bone homeostasis, but by an increase in intestinal calcium absorption.

Disclosures: ***J.E. Kerstetter****, None.*

F325

Chronic High-Dose Glucocorticoids in Children Induce Obesity with Minimal Bone Loss. M. B. Leonard[1], B. S. Zemel[1], V. A. Stallings*[1], H. I. Feldman*[2]. [1]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA, USA, [2]Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Steroid sensitive nephrotic syndrome (SSNS) is an excellent model of glucocorticoid (GC)-induced osteopenia in children. During treatment with GC, the underlying disease is inactive and does not recur as long as the GC therapy is continued. SSNS is frequently complicated by GC-induced obesity. The objective of this study was to assess bone density and bone dimensions in children with SSNS, relative to body size. Whole body and paired anterioposterior (AP) / lateral spine DXA (Hologic QDR 2000) measures of bone area, bone mineral content (BMC) and areal-bone mineral density (BMD, gm/cm^2) were obtained in the array mode in 188 healthy controls and 61 children with SSNS, ages 4 to 21 yrs. All SSNS subjects were in remission and 80% were on GC at evaluation. The paired spine scans were used to estimate vertebral volume assuming vertebrae approximate an elliptical cylinder; volumetric BMD was calculated using BMC from the lateral spine scan. The SSNS subjects had received a cumulative lifetime GC dose of (mean ± SD) 870 ± 760 mg/kg over a median interval of 18 mo (range 4 to 83). The average daily GC dose was 1.1±0.7 mg/kg/day. The SSNS subjects had significantly decreased height z-scores (Ht-Z) (-0.1±1.0 vs. 0.3±1.0, p < 0.01) and increased BMI z-scores (BMI-Z) (1.3±1.0 vs. 0.3±1.1; p < 0.0001) compared with controls, consistent with GC effects. Ht-Z was associated with cumulative GC exposure, p<0.001; BMI-Z was not. Bone measures were log transformed and multivariate regressions used to generate ratios (95% CI) for bone measures in SSNS compared with controls, adjusted for maturation, race and gender. All vertebral measures (AP and lateral area, BMC, areal-BMD and volumetric BMD for age, and AP and lateral BMC for bone area) were not different in SSNS compared with controls. However, when adjusted for BMI-Z, volumetric BMD (ratio 0.94 [95% CI 0.90-0.99]) and AP spine BMC for area (0.96 [0.92-0.99]) were marginally decreased in SSNS compared with controls; p < 0.05. Whole body bone area for height was increased in males with SSNS compared with controls (1.07 [1.02-1.12]), p=0.01; however, when adjusted for BMI-Z, the SSNS and controls were not different. Whole body BMC for age and for height were not different in SSNS, compared with controls. We have previously reported that obesity results in significantly increased vertebral volumetric density and increased whole body area and BMC for height. The GC-induced obesity in SSNS may protect the spine and whole body from bone loss relative to age and height. In conclusion, children with severe SSNS do not have significant bone deficits for age or height despite high-dose chronic GC exposure.

Disclosures: ***M.B. Leonard****, None.*

F327

Estrogen Receptor-Beta Plays a Regulatory Role in Cortical Bone but not in Trabecular Bone in Aged Male Mice. H. Z. Ke, H. Qi, D. T. Crawford*, H. A. Simmons, G. Xu*, T. A. Brown, D. D. Thompson. Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton, CT, USA.

It has been reported that lacking estrogen receptor-beta (ER-b) increases cortical bone and partially prevents age-related trabecular bone loss in female mice. However, the role of ER-b in skeletal growth, development and maintenance in males is not clearly understood. It has also been reported that ER-b does not play a role in bone growth and remodeling in males based on the data generated from ER-b knockout (KO) young male mice. The purpose of this study was to evaluate the effects of ER-b KO in male mice in older ages to determine whether ER-b plays a role in trabecular and cortical bone in males. Groups of KO male mice and wild-type (WT) littermates were autopsied at 6, 13 and 21 months of age. Body weight, seminal vesicle weight and femoral length did not differ at all age groups between KO and WT. Trabecular density by pQCT, trabecular bone volume and connectivity by micro-CT, and trabecular bone formation and resorption indices by histomorphometry at distal femoral metaphysis (DFM) did not differ significantly between KO and WT at all age groups, indicating that ER-b does not play a role in regulating trabecular bone. Trabecular density and trabecular bone volume gradually decreased with age between 6 and 21 months of age in both KO and WT, indicating the ER-b plays no role in age-related trabecular bone loss in males. At 6 months of age, KO mice did not differ from WT in total bone mineral content and density at DFM and femoral shaft (FS), illustrating that ER-b play minimal or no role in cortical bone during growth phase. Between 6 and 13 months of age, cortical bone area, cortical content and periosteal circumference of FS increased significantly with age in both KO and WT. However, all increments were significantly larger in KO than in WT. Therefore, these cortical bone parameters were significantly higher (+11% to +23%) in KO compared with WT at 13 months of age. At this age, KO male mice also had a larger marrow cavity, indicating a higher endocortical bone resorption compared with WT. Between 13 and 21 months of age, all cortical bone parameters of FS increased slightly with age in KO while did not change with age in WT. These data demonstrate that ER-b does not play a role in either trabecular or cortical bone during growth phase (prior to 6 months of age), and plays an inhibitory role in cortical bone formation and endocortical bone resorption but not in trabecular bone remodeling during the adult and aged phases (between 6 to 21 months of age) in male mice.

Disclosures: ***H.Z. Ke****, Pfizer 3.*

F328

Sex Hormone Binding Globulin, Bioavailable Estradiol and Testosterone and Vertebral Fractures in Men. E. R. Legrand, D. Chappard, C. Gibert*, P. Insalaco*, V. Simon*, M. Baslé*, M. Audran. Service de rhumatologie, CHU, Inserm, EMI 0335, Angers, France.

Background: The exact mechanism of bone loss remains unknown in primary osteoporosis in men. We have shown that serum concentration of Sex Hormone Binding Globulin (SHBG) was increased in men with osteoporosis and was correlated with bone markers and hip Bone Mineral Density *(Legrand, Bone 2001, 29: 90-95).*

Aim: To evaluate the relationships between SHBG, bioavailable testosterone and estradiol and vertebral fractures in men with low BMD.

Methods: 346 men (mean age 57.3 years) with a spine or hip low BMD (T-score < -1) were included in a cross sectionnal but prospective study. Age, BMI, clinical risk factors and BMD were checked. Fasting serum samples were assayed for total and free testosterone, total and bioavailable estradiol and SHBG. Spinal X-ray films were analyzed independently by two trained investigators who were unaware of the patient status. Vertebral fracture was defined as a reduction of at least 20 percent in the anterior, middle or posterior vertebral height.

Results: The 194 men (56%) with at least one vertebral fracture were older (62.0 vs 51.5 years, *p< 0.01*) and had a lower hip BMD (0.81 vs 0.89 gr/cm2, *p< 0.01*). After ajusting

for age and BMI, these men had a higher SHBG serum level (45.2 vs 30.7 nmol/l, *p< 0.01*), a lower serum free testosterone (11.6 vs 14.2 ng/l, *p< 0.01*), a lower serum bioavailable testosterone (12.1 vs 18.0 %, *p< 0.01*), a lower serum bioavailable estradiol (61.2 vs 89.5 %, *p<0.01*).
Logistic regression analysis showed that age (10 years increased : Odds Ratio=1.9, *CI 1.5-2.3*), hip BMD (1 SD decrease: O R=1.9, *CI 1.5-2.7*) were associated with the presence of at least one vertebral fracture. After adjusting for age and BMD, SHBG serum level (1 SD increase: OR= 1.8, *CI 1.2-2.6*), serum bioavailable testosterone(1 SD decrease: OR= 1.5, *CI 1.1-2.1*), serum bioavailable estradiol (1 SD increase: OR= 1.5, *CI 1.2-2.2*) were also strongly associated with the presence of vertebral fracture. The analysis was repeated for the presence of multiple fractures (at least 2 vertebral fractures) and the Odds Ratios were quite similar.
Conclusions: These results strongly suggest that serum SHBG and bioavailable sex steroids play a key role in pathogenesis of bone loss and vertebral fractures in men. Furthermore, these biological markers might be useful to evaluate the vertebral fracture risk in men, in addition to age and BMD.

Disclosures: ***E.R. Legrand****, None.*

F330

Acute Androgen Deprivation Causes Early, Sustained Bone Loss: A Longitudinal Study. P. S. Coates[1], J. M. Wagner[1], M. E. Shoup*[1], J. L. Ryan[1], S. M. Sereika*[1], J. B. Nelson*[1], D. L. Trump*[2], S. L. Greenspan[1]. [1]Univ. of Pittsburgh, Pittsburgh, PA, USA, [2]Roswell Cancer Center, Buffalo, NY, USA.

Androgen deprivation therapy (ADT) is a common, effective therapy for men with prostate cancer. However, the resultant hypogonadism is a risk factor for osteoporosis. To examine the relationship between the onset of therapy and bone loss, we studied 114 men prospectively over 12 months and assessed bone mineral density (BMD) and markers of bone turnover. We compared healthy age-matched controls (n=39) to men with stable prostate cancer and no metastatic disease who were: 1) not on ADT (n=26), 2) initiating ADT (<6 months, mean 3±1 months±SD, n=22), and 3) chronically on ADT (>6 months, mean 32±34 months, n=27). At baseline, there were no statistically significant differences in age, height, weight, BMI, PTH, or vitamin D. Table results are mean±SD, *p<0.05 across 4 groups.

BMD, Body Fat, and Markers at 12 Months

	No ADT	Acute ADT	Chronic ADT	Controls
Spine BMD (%/yr)	0.9±2.6	-2.1±2.7	0.1±5.9	-0.0±2.3
Total hip BMD (%/yr)	0.4±2.1	-2.6±2.5*	0.9±2.2	0.0±1.9
Total radius BMD (%/yr)	0.0±3.2	-2.6±2.8*	-1.8±3.1	-0.8±1.9
Total body BMD (%/yr)	0.1±2.5	-2.6±2.2*	-0.8±1.9	-0.2±1.3
Percent body fat (%/yr)	2.1±7.7	10.0±6.5*	1.2±6.2	1.0±5.6
NTx (nMBCE/nMCr)	28.8±19.7	64.0±36.4*	29.8±21.8	38.3±15.4
Osteocalcin (ng/mL)	4.3±1.1	7.8±2.1*	5.6±2.4	5.1±1.7

At 12 months, BMD at the total hip, radius, and total body were significantly reduced, percent body fat increased, and absolute urinary NTx and serum osteocalcin were significantly elevated in men on ADT compared to those in the other groups. At 6 months, absolute levels of urine NTx were inversely related to bone loss at the spine (r=-0.46, p<0.000), total hip (r=-0.34, p<0.01), total radius (r=-0.53, p<0.001), and total body (r=-0.34, p<0.01) at 12 months. Similarly, absolute levels of osteocalcin at 6 months were inversely related to bone loss at the spine (r=-0.36, p<0.01), total radius (r=-0.39, p<0.001), and total body (r=-0.35, p<0.01) at 12 months.
We conclude that initiation of acute ADT in men with prostate cancer results in significant decreases in BMD over 12 months, which can be predicted by early changes in biochemical markers. By approximately 3 years, the rate of bone loss slowed despite continued ADT. Further studies are needed to assess the long-term consequences of this early increase in bone turnover and bone loss in men with prostate cancer on ADT.

Disclosures: ***P.S. Coates****, None.*

F331

The Relative Roles of the Sex Steroids in Idiopathic Osteoporosis in Men. E. S. Kurland[1], S. Khosla[2], T. L. Colvin*[1], S. L. Heller*[1], J. Powell*[1], B. Seltzer*[1], D. McMahon*[1], J. P. Bilezikian[3]. [1]Medicine, Columbia University, College of Physicians and Surgeons, New York, NY, USA, [2]Endocrinology, Mayo Clinic, Rochester, MN, USA, [3]Medicine and Pharmacology, Columbia University, College of Physicians and Surgeons, New York, NY, USA.

The important role of estrogen in establishing peak bone mass in men has been established through observations in 1 ER-α and several aromatase deficient subjects. Direct studies in adult men have further confirmed an important role for estrogen in maintaining bone mass and controlling bone turnover. Little is known about the relative role sex steroids play in idiopathic osteoporosis (IOP) in young to middle aged men in whom all known secondary causes of osteoporosis including hypogonadism are ruled out and could not account for aberrations in estrogen physiology if present. To clarify a potential role of estrogen in IOP, we recruited 44 men with Z-scores of <-2.0 at the lumbar spine (LS), total hip and/or 1/3 radius, as well as 44 healthy non-osteoporotic men as controls with Z-scores >-1.0 at all sites. The 2 groups were matched for age, BMI, and ethnicity. Sex steroid measurements were made on both groups with results as follows:

Variable	Patients (n =44)	Controls (n =44)	Unadjusted p-value	Adjusted p-value *
BMI kg/m^2	24.1 ± 0.6	25.4 ± 0.3	0.06	---
Testosterone ng/dL	644.4 ± 24.4	722.9 ± 31.5	0.07	0.02
Estradiol, E_2 pg/mL	20.8 ± 0.9	25.2 ±0.9	0.0003	0.0006
Estrone pg/mL	35.9 ± 1.2	41.9 ± 1.9	NS	NS
Bioavailable T ng/mL	121.1 ± 7.8	154.5 ± 8.3	NS	0.007
Bioavailable E_2 pg/mL	9.7 ± 0.6	13.0 ± 0.7	0.0003	0.0006
SHBG nmol/L	41.0 ± 1.9	37.4 ± 2.1	NS	NS

* Adjusted for Age and BMI, all values ± SEM
To further clarify which of these indices best distinguished cases from controls, a step-wise discriminant analysis was performed. In order of importance, E_2, BMI, total T, and SHBG best explained differences between cases and controls accounting together for 30% of the variance between groups; E_2 accounted for 17% of the variance. Correlations between sex steroids and bone density (BMD) at all sites further demonstrated that in patients with IOP, E_2 was significantly correlated with BMD of the LS (r =0.3; p =0.05) with a trend for significance at the 1/3 radius for E_2 and total T. There was no correlation between sex steroids and BMD in controls (E_2 at LS, r= 0.03, p= 0.8). The results are consistent with a potentially important role for estrogen deficiency in the pathogenesis of IOP in men.

Disclosures: ***E.S. Kurland****, None.*

F333

Cytokines and Bone Loss in Pre-menopausal Women with Celiac Disease. M. L. Bianchi[1], M. T. Bardella*[2], S. Saraifogher*[1], E. Galbiati*[1], O. Borghi*[3], A. Dubini*[3]. [1]Bone Metabolic Unit, Istituto Auxologico Italiano IRCCS, Milano, Italy, [2]Cattedra di Gastroenterologia, Istituto di Scienze Mediche IRCCS, University of Milano, Milano, Italy, [3]Istituto Auxologico Italiano IRCCS, Milano, Italy.

Low bone mass and osteoporosis are frequently encountered in patients, especially post-menopausal women, affected by celiac disease (CD) and intestinal malabsorption.
We studied 64 women affected by CD (aged 35.3±4 yrs). All were pre-menopausal to avoid the influence of menopause on bone metabolism and bone mass. They were divided in two groups: (1) the GFD group, including patients in a stable condition, on a gluten-free diet (GFD) for at least 2 years, with no active signs of disease and no anti-gliadin and anti-endomisium antibodies; (2) the no-GFD group, including newly diagnosed patients evaluated before starting GFD.
All patients had regular menses, and had no other diseases besides CD, nor used drugs influencing calcium metabolism. The two groups were similar for age and clinical history.
A group of healthy women of similar age was also evaluated.
We measured calcium, phosphate, magnesium, calciotropic hormones, bone turnover markers, intestinal calcium absorption (calculating the area under the curve, AUC), lumbar spine BMD, cytokines IL-6, IL-1beta, TNFalpha, TNFbeta, IL-12, IL-18, RANKL, osteoprotegerin (OPG).
The main results were:
-- GFD group: moderate osteopenia (Z-score -1.4±.5), NTx slightly increased (53±3 nMBCE/mMcrea), intestinal calcium absorption decreased (AUC FAD% 4140±130 vs 5134±230 in controls, p<.02);
-- no-GFD group: osteopenia (Z-score -1.9±.5), NTx markedly increased (121±14 nMBCE/mMcrea, p<.03), PTH increased (51±8 pg/ml, p<.03), 25-OH D decreased (15.3±3.5 ng/ml, p<.03), intestinal calcium absorption decreased (AUC FAD% 3120±110, p<.01).
There was a slight increase in IL-6 only in the no-GFD group (1.3±.3 vs .6±.2 pg/ml in controls), while in both groups IL-12 and IL-18 were decreased (p<.05 vs controls), and RANKL and OPG were increased (RANKL: + 65% in the GFD group, + 418% in the no-GFD group, p<.02 vs controls; OPG: +53% and +121% respectively, p<.02).
It seems that also in pre-menopausal women, CD can cause derangement of bone metabolism and low bone mass, even if long-term gluten-free diet can induce some improvement. Reduced intestinal calcium absorption seems not to be the only mechanism affecting the calcium metabolism and the loss of bone mass. Various regulating cytokines appear to have a role in the bone loss in celiac patients, even during gluten free diet.

Disclosures: ***M.L. Bianchi****, None.*

F336

Ibandronate is Highly Efficacious in Postmenopausal Women with High Baseline Bone Turnover Rates. C. H. Chesnut[1], P. D. Delmas[2], C. Christiansen[3], P. Mahoney*[4], R. C. Schimmer[4]. [1]University of Washington, Seattle, WA, USA, [2]Claude Bernard University and INSERM Research Unit 403, Lyon, France, [3]Center for Clinical and Basic Research, Ballerup, Denmark, [4]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

Although there is still much discussion concerning the clinical utility of biochemical markers of bone turnover in postmenopausal osteoporosis (PMO), studies suggest that high levels of bone turnover are associated with faster, and possibly greater, bone mineral density (BMD) losses and increases in fracture risk. The efficacy of antiresorptive therapies in patients with high bone turnover at baseline remains to be fully established. Ibandronate is a highly potent, nitrogen-containing bisphosphonate that has recently demonstrated signif-

icant and substantial antifracture efficacy in postmenopausal women with osteoporosis.[1] In the overall population, oral daily (2.5mg; n=977) and intermittent (20mg every other day for 12 doses every 3 months; n=977) ibandronate regimens reduced vertebral fracture risk by 62% (p=0.0001) and 50% (p=0.0006), respectively, relative to placebo (n=975) after 3 years. Interestingly, a subgroup of patients who had high baseline levels of biochemical markers of bone resorption (CTX/creatinine or NTX/creatinine levels of more than 2 standard errors above the mean study values) achieved even greater relative fracture risk reduction than the overall population. After 3 years, the lifetable vertebral fracture rate in patients with high baseline bone turnover was 11.85% in the placebo arm (n=99) versus 3.87% and 2.78% in those taking daily (n=105) and intermittent (n=116) oral ibandronate, respectively, corresponding to relative risk reductions of 74% (p=0.0430) and 85% (p=0.0149), respectively. Thus, in patients with high baseline bone turnover, ibandronate demonstrates a substantial antifracture benefit, whether administered daily or with a between-dose interval of >2 months. These results confirm the robust and consistent antifracture effect with ibandronate.

1. Delmas PD, et al. Osteoporos Int 2002;13(Suppl 1):S15(Abstract O37).

Disclosures: ***C.H. Chesnut****, None.*

F338

Relationship of Early Changes in Bone Turnover to the Reduction in Vertebral Fracture Risk with Risedronate – The HIP Study. A. Blumsohn[1], I. P. Barton*[2], A. Chines[3], R. Eastell[1]. [1]University of Sheffield, Sheffield, United Kingdom, [2]Procter & Gamble Pharmaceuticals, Egham, United Kingdom, [3]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Anti-fracture efficacy of antiresorptive therapies is only partially explained by increases in bone mineral density (BMD). Early decreases in bone turnover may also play a role. We tested this hypothesis by measuring 2 bone turnover markers, the N-telopeptide of type I collagen (NTX) and procollagen type I N-propeptide (PINP), in osteoporotic patients in the risedronate HIP fracture trial. We studied 938 women with a femoral neck BMD T-score less than -3 (mean age, 74 years, SD 3) who received calcium (and vitamin D if required) and placebo or risedronate 2.5 or 5 mg daily for 3 years. The reductions in urinary NTX (median, 47 and 57%) and PINP (39 and 53%) at 3 to 6 months of risedronate 2.5 and 5 mg, respectively, were significantly associated (P<0.05) with the reduction in vertebral fracture risk (48% over 3 years). The changes in both NTX and PINP accounted for approximately one third (NTX, 33%, p<0.05; PINP, 30%, p<0.05) of risedronate's effect in reducing the risk of vertebral fractures over 3 years, compared with placebo. In keeping with our previous findings with the VERT study, the relationship between vertebral fracture risk and change from baseline in NTX was not linear (p<0.05 in the 5 mg group). There was little further improvement in fracture benefit below a decrease of 30 to 35% for NTX. In conclusion, the decrease in bone turnover in patients taking risedronate accounts for some of the reduction in vertebral fracture risk. There may be a level of bone resorption reduction below which there is no further fracture benefit.

Disclosures: ***A. Blumsohn****, None.*

F340

Intra-Venous Pamidronate Infusion Prevents Bone Loss and Renal Stone Formation During 90-Day Bed Rest Study. H. Ohshima*[1], Y. Watanabe*[2], C. Sekiguchi*[1], M. Fukunaga[3], A. Okada*[3], K. Kohri*[3], H. Ohta[3], Y. Seino[3], K. Takaoka[3], T. Esashi*[3], T. Matsumoto[3], T. Nakamura[3]. [1]Medical Research and Operations Office, National Space Development Agency of Japan, Tsukuba, Japan, [2]Advanced Engineering Services, Tsukuba, Japan, [3]Bone Loss Countermeasure Advisory Working Group, Tsukuba, Japan.

Introduction We have developed a bone loss countermeasure program for Japanese astronauts to reduce the risk of bone loss and renal stone formation for long-duration space flight. To validate the preventive pharmacological countermeasure in a ground-based study, NASDA participated in a 90-day bed rest study at the Space Clinic in Toulouse, sponsored by European, French and Japanese space agencies.

Methods Twenty-five male volunteers were split into three groups: control (CON; 9 subjects), pamidronate (PMD; 7), and exercise groups (EX; 9). PMD (60 mg in 500ml saline solution) was infused intravenously 14 days before the bed rest. BMD by DXA, urinary Ca excretion, and bone markers were measured before, during, and up to one year after bed rest. Abdominal radiograms were taken before and after bed rest.

Results DXA results showed the BMD of the total proximal femur decreased by 6.2 % in the CON group and 3.6 % in the EX group, while the PMD group exhibited no change after the three-month bed rest period. Urinary Ca excretion significantly increased in CON and EX groups during bed rest. In the PMD group, urinary Ca excretion decreased immediately after infusion, stayed low for one month, and gradually recovered to the baseline level towards the end of bed rest. Moreover, renal calcification was detected on the plain radiogram after bed rest, in two from the CON group and four from the EX group, but none from the PMD group. CTX, NTX and DPD increased in CON and EX groups, but were suppressed in the PMD group.

Conclusion PMD infusion effectively maintains the BMD of proximal femur under 90-day microgravity simulation by inhibiting bone resorption. It also reduces the incidence of renal calcification by inhibiting urinary Ca excretion.

Disclosures: ***H. Ohshima****, None.*

F342

Once-weekly Oral Ibandronate Prevents Bone Loss in Postmenopausal Women. L. B. Tankó[1], B. J. Riis[1], D. Felsenberg[2], E. Czerwinski*[3], A. Burdeska*[4], I. Jonkanski*[5], C. Hughes*[6], C. Christiansen[1]. [1]CCBR A/S, Ballerup, Denmark, [2]Radiology Department, University Hospital Benjamin Franklin, Berlin, Germany, [3]Polish Foundation of Osteoporosis, Krakow, Poland, [4]Hoffmann-La Roche Ltd, Basel, Switzerland, [5]Hoffman-La Roche Ltd, Basel, Switzerland, [6]Hoffman-La Roche Ltd, Welwyn, United Kingdom.

The aim of the present study was to investigate the efficacy, safety, and dose-response of once-weekly oral ibandronate in the prevention of postmenopausal bone loss. Six hundred and thirty women were randomized into four strata according to time since menopause (TSM, 1-3 years vs. >3 years) and baseline bone mineral density (BMD; normal: T-score > -1 vs. osteopenic: -2.5 ≤ T-score ≤ -1) of the lumbar spine. Within each stratum women were further randomized to receive once-weekly ibandronate (5, 10, or 20 mg/week) or placebo for 24 months. Efficacy parameters were the relative changes from baseline in spine (L1-4) and hip BMD, and biochemical markers of bone turnover (serum and urinary C-telopeptide of collagen type I (CTX), osteocalcin, and alkaline phospatase) measured by dual energy X-ray absorptiometry and ELISA, respectively. Once-weekly treatment with ibandronate induced dose-dependent increases in spine and hip BMD. At Month 24, differences between the relative changes in spine and hip BMD induced by 20 mg ibandronate and placebo was 4.0% and 2.7%, respectively. Similar or more pronounced differences were seen in osteopenic women of TSM 1-3 years (5.3% and 3.5%) and of TSM >3 years (3.5% and 2.9%), respectively. A dose-dependent suppression of all biochemical markers of bone turnover was observed with significant decreases in the 20 mg dose groups of all strata at Month 24. The overall safety results indicated that once-weekly oral ibandronate was well-tolerated at all three doses. Once-weekly oral treatment with 20 mg ibandronate provides an effective and safe therapy for the prevention of postmenopausal bone loss. This study provides further evidence of the effectiveness of ibandronate when administered, in a prevention setting, as a non-daily dosing regimen.

Disclosures: ***B.J. Riis****, None.*

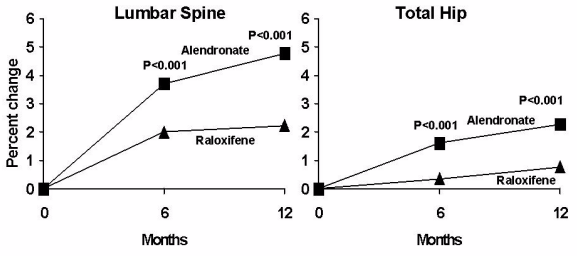
F344

Greater Increases in BMD with Alendronate Compared to Raloxifene: Results from EFFECT-International. P. Sambrook[1], P. Geusens[2], J. Ferrer-Barriendos*[3], C. Ribot[4], J. Angulo Solimano*[5], N. Verbruggen*[6], K. Gaines*[6], M. E. Melton[6]. [1]University of Sydney, Sydney, Australia, [2]Biomedical Research Institute-LUC, Diepenbeek, Belgium, [3]H-Central De Asthrias, Oviedo, Spain, [4]Hôpital Paule de Viguier, Toulouse, France, [5]Instituto Medico Miraflores, Lima, Peru, [6]Merck & Co., Inc., Whitehouse Station, NJ, USA.

This 12 month, multicenter, international, double-blind trial was designed to evaluate the effect on BMD of alendronate (ALN) as compared to raloxifene (RLX) when used to treat postmenopausal women with osteoporosis.

Patients with osteoporosis (T-score ≤-2.0 at either lumbar spine or total hip) were enrolled at 50 centers in 16 countries. The patients were randomized to treatment with either active ALN 70 mg once weekly + RLX placebo daily or active RLX 60 mg daily + ALN placebo once weekly. Treatment within the past year with estrogen, estrogen analogs, or bisphosphonates was an exclusion. Measurements included spine and hip BMD, markers of bone turnover (BSAP and urinary NTx), and adverse experience reporting for assessment of tolerability.

A total of 487 women were enrolled; mean age 62 years; 79% Caucasian. Alendronate produced a greater increase in BMD than did raloxifene at 12 months at the lumbar spine, total hip, hip trochanter, and femoral neck (P≤0.001). The increase in BMD with alendronate compared to raloxifene was also greater at 6 months at the spine and hip.



Greater reduction in bone turnover was seen with alendronate compared to raloxifene: NTx decreased by 68% with alendronate compared to 28% with raloxifene at 12 months (P<0.001); BSAP decreased by 51% with alendronate compared to 12% with raloxifene at 12 months (P<0.001). The difference between agents in reduction for both BSAP and NTx was noted at the earliest time point measured (6 months, P<0.001). Overall tolerability was similar for the 2 agents. Percentage of patients reporting upper GI AEs was similar between groups (15% ALN, 22% RLX). Reporting of vasomotor symptoms was greater with RLX (4% ALN, 10% RLX, P=0.01).

This study demonstrates that alendronate provides greater increases in BMD at both hip and spine sites than does raloxifene, and that alendronate has greater antiresorptive activity than does raloxifene. Although overall reporting of adverse events and upper GI events were similar, more patients reported vasomotor symptoms with raloxifene.

Disclosures: ***P. Sambrook****, Merck & Co., Inc. 2, 5, 8.*

F346

Sustained Anti-Fracture Efficacy of Risedronate Treatment Over 7 Years in Postmenopausal Women. S. Goemaere*[1], O. H. Sorensen[2], T. D. Johnson*[3], A. Chines[3], C. Roux[4]. [1]Ghent University Hospital, Ghent, Belgium, [2]Hvidovre University Hospital, Hvidovre, Denmark, [3]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [4]Hospital Cochin, Paris, France.

Risedronate (RIS) has been shown to significantly reduce vertebral and nonvertebral fracture incidence in randomized, placebo-controlled clinical trials.

In a 2-year extension of the multinational study (VERT-MN), RIS demonstrated a sustained antifracture effect. At the end of the 5 years, 220 women completed the study of which 164 participated in a further 2-year extension in which all patients were given RIS 5 mg daily. We now report the results for those women who either continued another 2 years on RIS (RIS group, n=83) or initiated RIS (PLBO/RIS group, n=81). Throughout the 7-year study, all patients received 1000 mg/d calcium and if baseline levels were low, up to 500 IU/day of vitamin D. The 2 groups had similar baseline characteristics in terms of age, BMD and prevalent vertebral fractures that were also similar to the original cohort.

Over 7 years, lumbar spine (LS) BMD increased from baseline by 11.5% in the RIS group and 6.1% in the PLBO/RIS group. The increase in LS BMD from year 5 to year 7 was 5.2% and 1.7%. for the PLBO/RIS and RIS groups, respectively.

Annualized incidence (%) of radiographic new vertebral fractures and clinical non-vertebral fractures is shown below

	Vertebral Fractures		Non-vertebral Fractures	
	PLBO / RIS	RIS	PLBO / RIS	RIS
Years 0-3	7.6	4.7	3.3	2.0
Years 4-5	12.3	5.2	4.3	3.0
Years 6-7	3.8	3.8	3.7	3.0

McNemar's analysis was performed to determine whether the vertebral fracture incidence in the RIS group has changed during years 6-7 as compared to years 4-5 . No significant change was observed suggesting a sustained antifracture effect of RIS. Similar analyses for the PLBO/RIS group showed a significant change, which is consistent with fracture risk reduction.

The cumulative incidence of clinical osteoporosis-related nonvertebral fractures over 7 years was 25.9% and 15.7% in the PLBO/RIS and RIS groups, respectively. Bone resorption marker urinary NTX decreased by 54% (median) at 3 months and remained decreased thereafter (range, 54-63%). In conclusion, the results of this study are supportive of a sustained antiresorptive effect of risedronate accompanied by a protective antifracture effect.

Disclosures: ***S. Goemaere****, None.*

F348

The Fracture Intervention Trial Long Term Extension (FLEX): 3 Year Interim Results. K. Ensrud[1], A. Santora[2], A. Schwartz[3], S. Suryawanshi*[2], E. Barrett-Connor[4], A. Feldstein*[5], W. Haskell*[6], M. Hochberg[7], J. Torner*[8], D. Black[3]. [1]VAMC & U of MN, Minneapolis, MN, USA, [2]Merck, Rahway, NJ, USA, [3]UCSF, San Francisco, CA, USA, [4]UCSD, San Deigo, CA, USA, [5]Kaiser Center for Health Research, Portland, OR, USA, [6]Stanford U, Stanford, CA, USA, [7]U of MD, Baltimore, MD, USA, [8]U of IA, Iowa City, IA, USA.

Prior trials including the Fracture Intervention Trial (FIT) have shown that therapy with alendronate (ALN) increases bone mineral density (BMD) and decreases fracture risk for at least 4 yrs. However, it is unknown if continued therapy with ALN will result in preservation or further gains in BMD. To determine whether further therapy with ALN increases BMD relative to placebo (PBO), FLEX enrolled 1099 women aged 60-86 yrs who were assigned to ALN in FIT and received 3-6 yrs of ALN (mean 5 yrs) and re-randomized them to ALN 10 mg/d (30%), ALN 5 mg/d (30%), or PBO (40%) for 5 additional years. All women receive calcium/ vit D supplements. Primary endpoint is change in total hip BMD. We report here results from a pre-planned interim analysis after 3 yrs.

Among these women who had prior ALN therapy in FIT, further therapy with ALN (5 & 10 mg groups pooled) for 3 yrs maintained or increased mean BMD compared with PBO at the total hip (2.0% difference) and lumbar spine (2.6%) (Table). Cumulative increases in total hip BMD from FIT baseline were higher for pooled ALN groups (3.0%) compared with PBO (0.9%) (Table). Compared with the 5 mg group, maintenance of hip BMD during FLEX was slightly greater in the 10 mg group, though gains in spine BMD did not significantly differ. Evaluation of change in bone turnover markers showed partial resolution of prior treatment effect in the PBO group (mean 25% increase in urinary N-telopeptide/Cr and 17% increase in bone alkaline phosphatase from FLEX baseline) compared with relatively stable levels in the pooled ALN groups (p<0.001 for both comparisons). Safety and tolerability profile of ALN was similar to that of PBO, including the incidence of upper GI adverse events (30% for pooled ALN groups & 36% for PBO). In conclusion, compared with women who stop therapy after an average of 5 yrs, those who continue ALN maintain a higher BMD.

Table. Mean % Change (SE) in BMD from FLEX Baseline to FLEX Month 36 & from FIT Baseline to FLEX Month 36 (note: all patients received 3-6 yrs of ALN use prior to FLEX)

Site	% Change from FLEX Baseline to FLEX Month 36 (mean 2.9 yrs) Mean (SE)		% Change from FIT Baseline to FLEX Month 36 (mean 8.6 yrs) Mean (SE)	
	PBO	ALN	ALN / PBO	ALN / ALN
Total Hip	-2.39* (0.18)	-0.42*† (0.15)	0.85* (0.27)	3.00*† (0.23)
Lumbar Spine	0.94* (0.24)	3.49*† (0.20)	10.41* (0.42)	12.93*† (0.35)

* p < .01 within-treatment test of mean = 0

† p < .001 for comparison between ALN vs. PBO

Disclosures: ***K. Ensrud****, Merck 2; Eli Lilly 2; Pfizer 2; Berlex 2.*

F350

Consistency of Response to 6-Year Treatment with Alendronate among Subgroups. D. J. Hosking[1], C. Christiansen[2], R. D. Wasnich[3], M. Wu*[4], P. D. Ross[4], A. Mantz*[4], C. Sisk*[4], A. Santora[4]. [1]Mineral Metabolism, Nottingham City Hospital, Nottingham, United Kingdom, [2]Center for Clinical and Basic Research, Ballerup, Denmark, [3]Hawaii Osteoporosis Center, Honolulu, HI, USA, [4]Merck & Co., Inc., Rahway, NJ, USA.

The bisphosphonate alendronate is effective for both the treatment and prevention of osteoporosis. The purpose of the present investigation was to evaluate changes in lumbar (L1-L4) spine bone mineral density (BMD) after 6 years of treatment with alendronate in healthy postmenopausal women as a function of age, years since menopause, baseline BMD, or body mass index (BMI). A separate analysis was conducted for each variable using tertile subgroups. In the Early Postmenopausal Intervention Cohort (EPIC) study, 1609 healthy postmenopausal women were randomized to placebo, alendronate 2.5 mg, alendronate 5 mg, or open-label estrogen/progestin. BMD was measured annually using a Hologic QDR 2000 densitometer. Relative to placebo, there was a consistent, dose-related benefit of alendronate treatment on lumbar spine BMD for all subgroups, although the magnitude of BMD changes varied among certain subgroups. Percent changes in BMD at Year 6 are presented for the placebo and 5 mg groups in the following table:

AGE (yr)	**≤52**		**53 to 55**		**>55**	
Treatment	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**
Placebo	95	-4.38 (4.44)	56	-3.55 (4.49)	79	-0.86 (3.92)
Alendronate 5 mg	67	2.55 (4.69)	38	2.60 (4.66)	44	6.16 (4.65)

YSM	**≤2**		**3 to 7**		**>7**	
Treatment	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**
Placebo	85	-5.53 (4.24)	83	-2.09 (4.03)	62	-0.64 (3.87)
Alendronate 5 mg	44	2.41 (5.49)	53	4.67 (4.08)	51	3.68 (5.06)

BASELINE SPINE BMD	**T≤-1.5**		**T= -1.4 to -0.6**		**T>-0.6**	
Treatment	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**
Placebo	73	-2.24 (5.08)	83	-3.01 (3.94)	74	-3.64 (4.55)
Alendronate 5 mg	39	3.48 (4.47)	51	4.21 (5.20)	59	3.22 (5.00)

BMI (kg/m²)	**≤23.41**		**23.42 to 26.82**		**>26.82**	
Treatment	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**
Placebo	78	-4.48 (4.43)	84	-2.86 (4.48)	68	-1.37 (4.21)
Alendronate 5 mg	63	2.59 (4.10)	44	3.99 (4.61)	42	4.81 (6.04)

Women in the placebo group who were younger, were less than 7 years postmenopausal, had higher baseline spine BMD, or had lower BMI values had greater BMD declines compared with other women, but alendronate treatment exhibited a positive benefit in every situation. Statistical tests for interaction did not detect any significant treatment-by-subgroup interactions. In conclusion, postmenopausal women treated with placebo for 6 years experienced significant progressive declines in BMD. Conversely, treatment with alendronate for 6 years produced dose-dependent increases in bone mass irrespective of years since menopause, baseline lumbar spine BMD, and body mass index (BMI).

Disclosures: ***D.J. Hosking****, Merck & Co., Inc. 2, 8; Proctor & Gamble 5; Novartis 5; Lilly 5.*

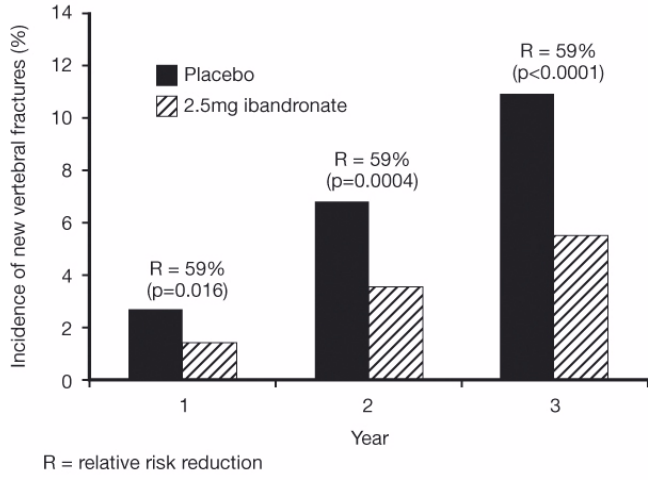
F352

Oral Daily Ibandronate Rapidly Reduces the Risk of New Mild, Moderate and Severe Vertebral Fractures after 1, 2 and 3 Years. D. Felsenberg[1], P. D. Miller[2], R. C. Schimmer[3], S. E. Papapoulos[4]. [1]Universitätsklinikum Benjamin Franklin, Berlin, Germany, [2]CCBR, Lakewood, CO, USA, [3]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [4]Leiden University Medical Center, Leiden, Netherlands.

Ibandronate, a potent, nitrogen-containing bisphosphonate, is the subject of an extensive clinical development program. A recent multicenter, randomized, placebo-controlled phase III study in postmenopausal women with osteoporosis (oral iBandronate Osteoporosis vertebral fracture trial in North America and Europe: BONE) demonstrated that oral daily (2.5mg) ibandronate significantly and substantially reduces the risk of new morphometric vertebral fractures (VFs) by 62% (p=0.0001) versus placebo after 3 years.[1] In addition, daily ibandronate normalized the rate of bone turnover and significantly increased spinal and hip BMD. To investigate the ability of oral daily ibandronate to prevent new incident VFs of varying degrees of severity, a retrospective exploratory analysis was conducted using established criteria.[2] Definitions used to diagnose mild, moderate and severe incident VFs were as follows: relative height reduction in a vertebral body (anterior, middle or posterior heights) of >20%–25% for mild fracture, >25%–40% for moderate fracture and >40% for severe fracture. Oral daily ibandronate consistently and significantly reduced risk of combined new mild, moderate and severe VFs after 1 (50%; p=0.03), 2 (52%; p=0.001) and 3 (49%; p=0.0002) years of treatment. For the combined analysis of VFs of a higher degree of severity (moderate and severe), an even more pronounced effect was seen: daily ibandronate significantly and consistently reduced the risk of debilitating moderate and severe VFs by 59% after 1, 2 and 3 years (see figure). These findings corroborate the

results from the analysis of new morphometric VFs. In summary, oral daily ibandronate significantly reduces risk of new mild, moderate and severe VFs within 1 year of treatment. This magnitude of risk reduction is sustained throughout 2 and 3 years of therapy. The proven oral daily ibandronate (2.5mg) regimen is the active comparator in ongoing studies to evaluate two novel treatment approaches: once monthly oral and intermittent i.v. injections.

1. Delmas P, et al. Osteoporos Int 2002;13(Suppl. 1):S15.
2. Genant HK, et al. JBMR 1993;8:1137–48.



Disclosures: ***D. Felsenberg****, None.*

F354

A Multinational Study Demonstrating Significant Reduction in the Incidence of New Vertebral Fractures with Oral Daily and Intermittent Ibandronate. R. Wasnich[1], D. Felsenberg[2], R. Lorenc[3], C. Hughes*[4], R. C. Schimmer[4]. [1]Radiant Research, Honolulu, HI, USA, [2]Universitätsklinikum Benjamin Franklin, Berlin, Germany, [3]Osteoporotic Center, The Children's Memorial Health Institute, Warsaw, Poland, [4]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

Less frequent bisphosphonate dosing is predicted to enhance patient management in postmenopausal osteoporosis. Ibandronate is a potent, nitrogen-containing bisphosphonate that can be given with extended between-dose intervals. The fracture efficacy of oral daily (2.5mg) and intermittent (20mg every other day for 12 doses every 3 months) ibandronate was recently demonstrated in a multinational, placebo-controlled, double-blind, randomized study (oral iBandronate Osteoporosis vertebral fracture study in North America and Europe: BONE), involving 2,946 women in North America and Europe.[1] After 3 years, daily and intermittent ibandronate significantly reduced the risk of new incident vertebral fractures in the overall population by 62% [p=0.0001] and 50% [p=0.0006], respectively, relative to placebo. Substantial increases in lumbar spine and hip BMD and decreases in biochemical markers of bone turnover were also observed. Both regimens were well tolerated, with a safety profile similar to placebo. The frequency of patients with new incident vertebral fractures, and the corresponding reduction in the relative risk, was also analyzed by continent. A significant reduction in the incidence of new vertebral fractures was observed in both European and North American patient subgroups. In the North American subgroup (n=995), the incidence of new vertebral fractures was significantly lower in the daily (3.2%; 95% CI, 1.0–5.4; p=0.0242; n=331) and intermittent (3.7%; 95% CI, 1.3–6.1; p=0.0372; n=333) arms compared with the placebo arm (8.0%; 95% CI, 4.6–11.3; n=331) after 3 years. At this time point, the relative-risk reductions compared with placebo were 75% and 54%, respectively. A similar finding was observed in the European subgroup (n=1,933). After 3 years, the incidence of new vertebral fractures was significantly lower in the daily (5.4%; 95% CI, 3.5–7.3; p=0.0028; n=646) and intermittent (5.5%; 95% CI, 3.6–7.4; p=0.0051; n=644) arms compared with the placebo arm (10.3%; 95% CI, 7.7–13.0; n=643). At this time point, the relative-risk reductions compared with placebo were 57% and 48%, respectively. This sub-analysis confirms the robust and consistent fracture efficacy of oral daily and intermittent ibandronate in postmenopausal osteoporosis. Ongoing studies are evaluating the efficacy and safety of oral monthly ibandronate regimens in postmenopausal osteoporosis.

1. Delmas PD, et al. Osteoporos Int 2002;13:S15(Abstract O37).

Disclosures: ***R. Wasnich****, None.*

F356

The Effect of Hormone Replacement Therapy, Alendronate, or the Combination of Both on Hip Structural Geometry: A 3-Year, Randomized, Double-Blind, Placebo-Controlled, NIH-Funded Clinical Trial. S. L. Greenspan[1], R. Bhattacharya*[1], T. J. Beck[2], N. M. Resnick*[1], R. A. Parker*[3]. [1]Univ. of Pittsburgh, Pittsburgh, PA, USA, [2]Johns Hopkins School of Medicine, Baltimore, MD, USA, [3]Harvard School of Public Health, Boston, MA, USA.

Fracture reduction by hormone replacement therapy (HRT) and alendronate (ALN) is only partially explained by increased bone mineral density (BMD). These agents may improve bone strength by alterations in structural geometry, which are not depicted by BMD. To examine bone structural geometry following treatment with HRT, ALN, and combination therapy (HRT+ALN) vs. placebo, we examined femoral neck geometric parameters at the narrow neck (NN) and intertrochanter (IT) during a 3-year, blinded, prospective clinical trial. Cross-sectional moments of inertia (CSMI), bone cross-sectional area (CSA), section modulus (SM, a measure of bending strength), cortical thickness (CT), and buckling ratio (BR, an index of cortical stability) were assessed from Hologic BMD DXA data using "Hip Structure Analysis" by Beck. We randomized 373 community-dwelling postmenopausal women to ALN 10 mg/d, HRT (conjugated estrogen 0.625 mg/d ± progesterone), ALN+HRT, or placebo.

Intertrochanteric Mean Percent Change

	PLB (n=81)	HRT (n=81)	ALN (n=84)	HRT+ALN (n=83)
BMD, intertrochanter*	-0.6	3.0#^	4.2#	5.5#
IT CSMI*	5.9#	5.2#^	9.3#	12.0#
IT CSA*	2.5	3.3#^	7.1#**	8.2#
IT SM*	3.3#	3.7#^	7.4#	9.9#
IT CT*	-0.0	1.5^	5.3#	6.4#
IT BR*	4.2	0.6	-2.7#	-3.6#

Femoral Neck Mean Percent Change

	PLB (n=81)	HRT (n=81)	ALN (n=84)	HRT+ALN (n=83)
BMD, femoral neck*	-0.6	1.8#^	2.8#	4.1#
NN CSMI*	-3.5	4.6	4.9	10.0#
NN CSA*	-6.4#	-0.4	-0.1	3.7
NN SM*	-6.0#	1.2	2.0	5.9
NN CT*	-7.1#	-2.4#	-1.6	1.1
NN BR*	12.1#	8.0#	5.9#	5.9#

Table Legends: *p<0.05 across 4 groups, #p<0.05 from baseline, ^p<0.05 HRT vs. HRT+ALN, **p<0.05 HRT vs. ALN

All BMD and structural parameters improved on active treatment. Only one structural parameter improved more on ALN than HRT, but nearly all IT parameters improved more on combination therapy than with HRT alone. Because of less precision for structural parameters than BMD, we cannot determine whether combination therapy is superior to ALN alone, although the trends all support this conclusion.

Disclosures: ***S.L. Greenspan****, Merck & Co., Inc. 2, 5, 8.*

F358

The Efficacy of Clodronate (Bonefos®) to Reduce the Incidence of Osteoporotic Fractures in Elderly Women Is Independent of the Underlying BMD. E. McCloskey[1], T. Jalava*[2], A. Oden*[3], H. Johansson*[3], K. Kayan*[1], M. Beneton*[1], J. Brazier*[1], J. Nicholl*[1], A. Richards*[1], J. Cliffe*[1], L. Reaney*[1], J. Nevalainen*[2], J. Kanis[1]. [1]University of Sheffield, Sheffield, United Kingdom, [2]Schering Oy, Helsinki, Finland, [3]University of Stockholm, Stockholm, Sweden.

Bisphosphonates significantly reduce the incidence of fractures in women with low BMD, but their efficacy in women with normal or osteopaenic BMD is less well studied. The aim of this study was to quantify the effect of oral clodronate on the incidence of osteoporotic fractures in an elderly cohort of women unselected for osteoporosis.

We studied 5592 women aged 75 years and older, randomly selected from the local population, in a 3 year double-blind, placebo controlled study of clodronate 800mg daily. Women were randomised regardless of the presence or absence of risk factors such as low BMD or prior fracture. At baseline, the two treatment groups were well-matched for the prevalence of risk factors except for a small but statistically significant higher prevalence of prior non-vertebral fractures in the clodronate group (24.4 vs. 21.1%, p=0.004). The efficacy of clodronate was determined using Cox regression analysis and the interaction between BMD and other factors on the effect of clodronate to reduce fracture risk was examined using a Poisson model.

At entry to the study, only 19.3% of women had osteoporosis defined as a total hip BMD<0.64g/cm^2 (Hologic QDR4500). Treatment with clodronate was associated with a significant decrease in the incidence of any clinical fracture (HR 0.80, 95%CI 0.68-0.94, p=0.005) or any osteoporotic fracture during the 3 years of treatment (HR 0.77, 95%CI 0.64-0.93) though the incidence of hip fractures was similar in the two groups (2.0 vs. 2.1%, p=0.92). The reduction in risk remained unchanged in models adjusting for age, BMI, total hip BMD and prior fracture. There was no significant interaction between the reduction in risk and total hip BMD at entry to the study when examined as a continuous variable (p=0.22) or as a categorical variable (osteoporosis, osteopenia or normal, p=0.71). The number needed to treat to prevent an osteoporotic fracture varied from 62 in those without hip osteoporosis to 25 in those with osteoporosis.

We conclude that oral clodronate 800mg daily reduces the rate of osteoporotic fractures by a mean of 23% during 3 years of treatment in elderly women unselected for osteoporosis. This effect occurs in women with BMD values above or below the osteoporotic threshold. However, strategies that target treatment to women at increased risk of fracture, including those with low BMD, should be used to direct clodronate therapy.

Disclosures: ***E. McCloskey****, Schering OY 2, 5, 8.*

F361

Salmon Calcitonin Nasal Spray (CT-NS) May Preserve/Improve Trabecular Microarchitecture (TMA) in the Distal Radius through 2 Years in Postmenopausal Osteoporotic Women (PM-OP): High Resolution MRI (HR-MRI) Results from the QUEST Study. C. Chesnut[1], S. Majumdar[2], A. Shields[1], D. Newitt[2], E. Laschansky*[1], A. Kriegman*[3], M. Olson*[4], F. Hornig*[4], M. Azria[4], P. Richardson[3], L. Mindeholm*[4]. [1]University of Washington, Seattle, WA, USA, [2]UCSF, San Francisco, CA, USA, [3]Novartis Pharma, East Hanover, NJ, USA, [4]Novartis Pharma, Basel, Switzerland.

CT-NS reduces spine fracture (fx) frequency in PM-OP♀, but with modest effects on bone quantity (BMD) and bone turnover (BT: markers of bone resorption); to explain this apparent paradox a primary CT-NS effect on bone quality (specifically TMA) has been postulated (JBMR, 2001). The 2 yr double blind placebo (P) controlled randomized QUEST trial in 91 PM-OP♀ examines the effects of CT-NS 200 I.U. q.d. on TMA utilizing HR-MRI.

Assessment of QUEST HR–MRI results (axial images [resolution 156x156x500 um] at 1.5 Tesla using a dedicated wrist coil; 4 regions of interest, 5 mm volume each, extending 12 mm from joint line into distal shaft):

HR-MRI at the distal radius (DR) after 2 years of 200 IU CT-NS or placebo

Parameter	Region of distal radius	N(CT-NS/P)	% change CT-NS/P	p within group CT-NS/P	p between groups
Bone volume/ total volume	I	32/33	+2.49/+0.13	p=.02**/NS	p=.10
	II	33/35	+1.04/-1.83	p=NS/NS	p=NS
	III	34/36	-1.45/-5.91	p=NS/.001**	p=.08*
	IV	33/30	-1.23/-5.78	p=NS/.003**	p=NS
Trabecular Number † (#)	I	32/33	+1.83/+0.34	p=.003**/NS	p=.07*
	II	33/35	+0.53/-0.85	p=NS/NS	p=NS
	III	34/36	+0.47/-3.76	p=NS/.004**	p=.03**
	IV	33/30	+0.46/-4.92	p=NS/.01**	p=.057*
Trabecular Spacing†	I	32/33	-2.63/+0.11	p=.02**/NS	p=.06*
	II	33/35	-0.39/+2.67	p=NS/NS	p=.NS
	III	34/36	+1.47/+8.09	p=NS/.001**	p=.051*
	IV	33/30	+1.61/+8.85	p=NS/.004**	p=NS
Trabecular Thickness †	I	32/33	0.72/-0.29	P=NS/NS	P=NS
	II	33/35	0.50/-1.05	P=NS/NS	P=NS
	III	34/36	-1.96/-2.57	p=.04**/.02**	p=NS
	IV	33/30	-1.76/-1.45	p=NS/NS	p=NS

†apparent, as measured by HR-MRI **pψ0.05 *0.05<pψ0.10

As expected, trab# and trab spacing varied inversely (r=-.83–-.97, p<.0001). 2 years of CT-NS (last available value, means adjusted for baseline values) increased or preserved bone vol, trab # and thickness at DR, while preserving or decreasing trab spacing; P resulted in significant losses of bone vol, trab # and thickness. The difference between CT-NS and P approached or reached significance in at least one region of DR for all parameters measured except trab thickness; intrasite differences in therapeutic response were noted at DR.

In conclusion, 200 IU CT NS, as compared to P, may preserve/improve TMA through 2 years in PMOP♀, as measured by the unique non-invasive HR-MRI technique at the DR. Such findings, from the first study designed a priori to examine the effects of antiresorptive Rx on TMA, may provide a partial explanation of CT-NS's mechanism of action in reducing OP fx.

Disclosures: ***C. Chesnut****, Novartis Pharmaceuticals 2, 5.*

F364

Effect of Octreotide on the Skeletal Response to Oral Calcium Supplementation. D. M. Prentis*, J. A. Clowes, N. Nazarina*, H. C. Allen*, R. Eastell, A. Blumsohn. Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Oral intake of several nutrients, including calcium and glucose, results in acute suppression of bone turnover markers. We have previously shown that response of serum β C-terminal telopeptide of type I collagen to glucose is not mediated by insulin and can be abolished by octreotide, a somatostatin analogue. This suggests that another somatostatin inhibitable gut peptide may mediate this response. The acute skeletal response to calcium could be mediated by PTH or by a direct effect of ionized calcium on osteoclast function. We have examined an alternative possibility that an octreotide-inhibitable factor could mediate the response to oral calcium.

Nine subjects (6M, 3F, age 29.2±6.4 SEM) were each studied on 4 occasions in a randomised single blind crossover study. On each occasion each participant received either 1) oral water + iv saline, 2) oral calcium carbonate (1000 mg) + iv saline, 3) oral calcium + iv octreotide (50 μ g/hr) and 4) oral water + iv octreotide. The infusion was started at 09:00 (time -30 min) and oral calcium was given at 09:30 (time 0 min) following an overnight fast. We measured serum β C-terminal telopeptide of type I collagen (sβ CTX), osteocalcin (OC), procollagen type I N-terminal propeptide (PINP), ionised calcium, and PTH over 4 hours following the oral load.

Octreotide completely abolished the decrease in sβ CTX in response to calcium. At 240 minutes sβ CTX decreased 35.65%± 5.39SEM (P<0.001) in response to calcium alone, compared to an increase of 17.44%± 6.61SEM (P>0.05) for calcium and octreotide. Calcium inhibited PTH secretion with a maximum decrease of 42.24 ± 7.01 (P<0.01) at 90min. Pre-treatment with octreotide delayed the decrease in PTH with a maximum response of -30.83± 7.54 (P<0.01) at 240min. Octreotide alone, without calcium, resulted in a 69.64%± 15.47 increase in PTH from baseline (-30min). Octreotide alone increased sβ CTX by 27.15%± 4.75 compared to a 10.79%± 4.74 increase with placebo at 240 minutes (P<0.01). Both response to calcium and inhibition of response with octreotide were less consistent with bone formation markers (OC, PINP).

We conclude that the acute response of serum β CTX to oral calcium is abolished during octreotide infusion, despite a similar PTH response. However, baseline PTH secretion is higher during octreotide infusion, and octreotide may also have a direct effect on bone resorption, skeletal blood flow, or metabolic clearance of collagen fragments. The acute antiresorptive response to oral calcium could be mediated, at least in part, by a somatostatin inhibitable endocrine factor.

Disclosures: ***D.M. Prentis****, None.*

F367

Vertebroplasty : Insufficient Diagnosis and Treatment of Osteoporosis Post Procedure. C. Recknor[1], A. B. Klemes[2]. [1]United Osteoporosis Centers, Gainseville, GA, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Vertebroplasty is being used more frequently for pain relief in vertebral compression fractures. Ideally osteoporosis diagnosis and treatment should occur long before these procedures. It is not known if this is truly what is happening in the centers doing vertebroplasty. We wanted to look at how often DXAs are being done on these patients and how often treatment is being initiated post procedure.

We did a retrospective review of 156 patients who had such procedures between 1999 and 2001. Thirty-nine of these patients (25%) had received follow-up DXAs after the procedure. 35 of the 39 (90%) had T scores below -2. 29 of the 39 (74%) were osteoporotic according to WHO criteria.

We then looked at a subset of these patients on whom DXAs had been performed. A total of four patients had been on therapy prior to the procedure. Their treatment consisted of Salmon Calcitonin nasal spray.

Eight patients commenced therapy after the procedure. Six of these patients were started on oral bisphosphonates, one was started on IV Pamidronate therapy and one was started on Raloxifene. One of the original four on Salmon Calcitonin was switched to a bisphosphonate after the procedure.

Despite the aggressive intervention with vertebroplasty, the underlying diagnosis of osteoporosis was frequently not addressed. Post procedure only 25% of patients had DXA scans performed. In this study even after the diagnosis was made only eight patients were treated.

Disclosures: ***C. Recknor****, None.*

F370

Estrogen + Progestin Reduces the Risk of Fractures Regardless of Risk Factors: The Women's Health Initiative. N. B. Watts, J. Robbins, Z. Chen, S. R. Cummings, R. Jackson, A. LaCroix, M. LeBoff, C. E. Lewis, J. McGowan, J. Neuner*, J. Pettinger*, M. Stefanick*, J. Wactawski-Wende, J. A. Cauley. University of Cincinnati College of Medicine, Cincinnati, OH, USA.

In the Women's Health Initiative (WHI) Estrogen plus Progestin trial, 16,608 postmenopausal non-hysterectomized women, age 50 to 79 at baseline, were randomized to conjugated equine estrogen 0.625 mg + medroxyprogesterone acetate 2.5 mg/day or placebo and followed for an average of 5.2 years. Hip and vertebral fractures were reduced by 34% and total fractures by 24%. Risk factors for fractures were assessed by questionnaire, interview and clinical examination. To see if clinical risk factors could be used to identify women who experienced greater fracture reduction, we explored over 100 subgroups for potential interactions between baseline characteristics and the effect of therapy. We formed a summary score using 9 risk factors: age (≥70), race (White/Asian), weight (<127 lbs), current smoking, family history of fractures, personal history of fractures; low calcium intake (<600 mg/day), self report of ≥2 falls in the past year; and never use of HRT before the trial. We divided the women into approximate tertiles based on the number of risk factors (0-2, 3, 4-9). The annual incidence of all fractures in control subjects from tertile I (lower risk) through tertile III (higher risk) was 1.4%, 1.8%, and 2.5%,demonstrating the validity of the scores. Estrogen plus progestin on fractures reduced the risk of fractures similarly, regardless of the number of risk factors (table).

Number of risk factors	0-2	3	4-9
Number of subjects	2,748	2,657	3,094
Percent (per year) with any fracture			
Placebo	1.4%	1.8%	2.6%
E+P	1.1%	1.4%	2.0%
Hazard ratio	0.75	0.77	0.75
95% CI	0.62,0.92	0.62,0.92	0.65,0.87

Thus, risk factors for fracture do not permit identification a group of women who might derive a greater anti-fracture benefit from estrogen plus progestin therapy. The overall risks of this therapy must be considered when evaluating its use for prevention of fractures.

Disclosures: ***N.B. Watts****, None.*

F372

Long Term Treatment of Aging Hypogonadal Men with a Unique Testosterone Gel (Testim™) Reduces Osteoporotic Changes and Improves Bone Mineral Density. R. T. Bachand*[1], T. M. Smith*[2], A. R. Secrest*[1], J. P. Rodzvilla*[2]. [1]Auxilium Pharmaceuticals, Inc, Libertyville, IL, USA, [2]Auxilium Pharmaceuticals, Inc, Norristown, PA, USA.

Aging hypogonadal men are at increased risk for osteoporotic fractures. Hypogonadism has been correlated with decreases in bone mineral density (BMD) and has been defined as a risk factor for falls (i.e., as a result of decreased muscular strength and impaired balance). Since a large proportion of elderly men have serum T levels below the normal adult reference range [300 – 1000 ng/dL (10.4 – 34.7 nmol/L)], the potential for improving BMD with testosterone (T) replacement therapy warrants further investigation. In the present study, 291 hypogonadal males (mean age of 58 years) previously enrolled in a 3 month active- and placebo-controlled study were followed in a 12 month, open-label, multi-dose, multi-center study to determine the effects of T replacement therapy on T level maintenance, body composition, sexual functioning, mood, and BMD. Baseline serum T was 258 ± 92 ng/dL (± SD; 9.0 ± 3.2 nmol/L). By month 15, following treatment with 100 mg/d of Testim™, serum T increased to 455 ± 365 ng/dL (15.8 ± 12.7 nmol/L). The mean BMD of the lumbar spine (L1 – L4), measured by dual x-ray absorptiometry, increased significantly ($P < 0.001$) from 1.177 before treatment to 1.214 gm/cm^2 (+ 2.75%) at month 15. Additionally, Testim™ therapy was well tolerated with only the known class effects of T therapy being noted (e.g., mild increases in hemoglobin, hematocrit, RBCs, and prostate specific antigen levels). The results of this study confirm the ability of T replacement therapy utilizing Testim™ to produce significant increases in BMD. T replacement therapy may offer a promising therapeutic option in aging males by preventing future bone loss and the associated risks of fractures. T replacement therapy may also offer additive protection from fractures by reducing the propensity for falls.

Disclosures: ***R.T. Bachand****, Auxilium Pharmaceuticals, Inc. 3.*

F374

PSK3471, a New Anabolic Designer Estrogen Fully Restores Bone Mass in Ovariectomized Mice with Established Osteopenia. P. Clément-Lacroix, D. Minet*, C. Belleville*, L. Lepescheux, F. Nique*, N. Bruyniks, M. Resche-Rigon, R. Baron. ProSkelia Pharmaceuticals, Romainville, France.

Raloxifene (RAL), an early SERM, has proven efficacy in the management of osteoporosis in postmenopausal women, increasing bone mass and reducing the risk of vertebral, but not non-vertebral, fractures. PSK3471 is a novel selective designer estrogen with good safety profile on breast and uterus, which efficiently prevents bone loss in OVX mice. In the present study we have evaluated the potential anabolic effects of PSK3471 by testing its ability to restore bone mass to normal levels in ovariectomized (OVX) mice with established osteopenia.

Eight weeks after ovx, mice displaying a clear osteopenia, were treated with PSK3471 (0.03, 0.1, 0.3 mg/kg/d) or RAL (10 mg/kg/d) p.o. for 8 weeks or with E2 0.25 mg/kg/wk s.c. As expected, significant bone loss was observed in the proximal tibia two months after ovariectomy (-13% and –22% compared to sham controls, for total and trabecular BMD respectively) and was associated with increased bone turn over, as measured by bone markers. Two months later, i.e. 4 months after ovx, no further bone loss was observed and the bone turnover was normalized compared to age-matched sham-operated mice.

After 8 weeks, total and trabecular BMD were similar in sham-operated mice and in mice treated with estradiol, or receiving the lowest dose of PSK3471. Furthermore, increasing the dose of PSK3471 not only fully restored OVX-associated bone loss, but also elicited an increase in trabecular bone mass versus sham-operated mice (+15% vs. sham $p<0.001$, for PSK3471 0.1 and 0.3 mg/kg/d). In contrast to PSK3471, RAL (10 mg/kg/d) only partially restored OVX-induced bone loss (+7.9% vs. OVX; ns). MicroCT analysis confirmed that PSK3471 increased cancellous bone volume (+52% to +68% vs. OVX; $p<0.01$), whereas RAL exhibited only a partial effect (+14% vs. OVX, ns). Most interestingly, and in contrast to RAL, PSK3471 also dose-dependently increased cortical BMD in the tibia (+2.7% to +3.8% vs. OVX; $p=0.03$, at 0.1 and 0.3 mg/kg). Finally, and although E2 treatment significantly increased serum OCN (+20%: $p<0.05$), only trends towards increases were observed in groups treated with PSK3471 as compared to OVX (+9.7%, +19% and +9.2% for 0.03, 0.1, and 0.3mg/kg/d respectively).

Thus, PSK3471 not only protects against ovx-induced bone loss but it is also able to restore bone mass in mice with established osteopenia, acting at both trabecular and cortical bone, whereas RAL was less efficient and acting only at the trabecular bone level. PSK3471 therefore acts as a tissue selective bone anabolic designer estrogen and may provide an advantage in the prevention of non-vertebral fractures.

Disclosures: ***P. Clément-Lacroix****, None.*

F376

Response to Treatment with Estrogen Is Related to Endogenous Levels of Serum Testosterone and SHBG. P. B. Rapuri[1], J. C. Gallagher[1], G. Haynatzki*[2]. [1]Bone Metabolism Unit, Creighton University, Omaha, NE, USA, [2]Department of Medicine, Creighton University, Omaha, NE, USA.

Recent evidence suggests that in postmenopausal women, low serum concentrations of endogenous hormones are related to bone mineral density (BMD) and fracture risk. The aim of the present study was to see if the response in BMD to estrogen/hormone therapy (ET/HT) was related to baseline serum testosterone and sex hormone binding globulin (SHBG) levels. 489 elderly women aged 65-77 years were randomly assigned to one of the following regimens, placebo, calcitriol, ET/HT and ET/HT+calcitriol.The endogenous serum total testosterone and SHBG levels were determined by a sensitive RIA. Serum bioavailable testosterone levels were calculated as the difference between total and SHBG bound testosterone. BMD was measured by DEXA and rate of change in BMD in spine and proximal femur after 3 years of treatment was compared between the tertiles of serum total and bioavailable testosterone. The data were analyzed by procedure Mixed from the SAS statistical package. Adjustments were made for appropriate covariates like age, weight etc. There was no relation between the effect of calcitriol treatment and serum total, bioavailable testosterone and SHBG levels. In women receiving ET/HT alone, the lowest tertile of total and bioavailable testosterone had significantly ($p<0.05$) higher increases in BMD at femoral neck, trochanter and total femur compared to the highest tertile. Although, there was a similar trend for spine BMD, the differences were not statistically significant. On the other hand, the highest tertile of SHBG was associated with significantly ($p<0.05$) higher increases in BMD at spine and proximal femur. In women receiving combination treatment with ET/HT+calcitriol, there was no relation between total, bioavailable testosterone levels and rate of change in BMD. However, the highest tertile of SHBG was associated with higher increases in BMD in these women though not statistically significant. These results show that the increase in BMD on ET/HT treatment is significantly higher in women whose pretreatment serum levels of total and bioavailable testosterone are low and SHBG levels are high. Whether testosterone independently has an effect on BMD or through its conversion to estradiol is not known.

	spine			femoral neck		
Bio T(tertiles)	1	2	3	1	2	3
Placebo	-1.9±1.0	-0.1±0.9	-0.3±0.8	-1.5±0.8	0.6±0.8	-0.8±1.0
HRT	7.5±1.6	5.4±0.8	4.3±1.0	6.8±1.1	3.1±0.8*	1.4±0.8*
HRT+Calcitriol	8.4±0.8	6.2±1.0	6.5±1.1	6.0±0.8	4.6±0.8	4.3±1.0
SHBG (tertiles)	1	2	3	1	2	3
Placebo	-1.2±0.8	-0.4±1.0	-0.6±0.9	-0.6±1.0	0.2±0.9	-1.2±0.8
HRT	4.9±1.0	3.3±0.8	8.2±1.2	1.9±0.7	1.9±1.0	6.7±0.9*†
HRT+Calcitriol	5.7±0.9	7.7±1.0	7.0±1.1	4.1±0.9	4.6±0.7	6.4±0.9

Values are mean ±SEM. *$p<0.05$ compared to tertile 1.
† $p<0.05$ compared to tertile 2

Disclosures: ***P.B. Rapuri****, None.*

F378

The Effects of Estrogen Plus Progestin, on Bone Mineral Density (BMD) in Postmenopausal Women of Different Ages and Ethnic Groups Enrolled in the Women's Health Initiative (WHI). M. S. LeBoff[1], J. Cauley[2], Z. Chen[3], A. LaCroix[4], M. Pettinger*[5], S. Cummings*[6], N. B. Watts[7], C. Lewis[8], J. McGowan[9], M. Stefanick[10], J. Wactawski-Wende[11], J. Robbins[12], R. Jackson[13], J. Neuner*[14]. [1]Endocrine, Diabetes, Hypertension Division, Brigham and Women's Hospital, Boston, MA, USA, [2]U Pitt, Pittsburgh, PA, USA, [3]U AZ, Tucson, AZ, USA, [4]FHCPC, Seattle, WA, USA, [5]FHCRC, Seattle, WA, USA, [6]UCSF, San Francisco, CA, USA, [7]U Cincinnati, Cincinnati, OH, USA, [8]UAB, Birmingham, AL, USA, [9]NIAMS, Bethesda, MD, USA, [10]Stanford, Palo Alto, CA, USA, [11]University at Buffalo, Buffalo, NY, USA, [12]UC Davis, Sacramento, CA, USA, [13]Ohio State University, Columbus, OH, USA, [14]U WI, Milwaukee, WI, USA.

In most longitudinal randomized clinical trials (RCT), estrogen and progestin (E+P) therapy increases BMD. However, many of these RCT are limited by small sample size and lack of inclusion of women over wide age ranges or from different ethnic backgrounds. Data from the WHI, RCT recently showed that E+P (0.625 mg of conjugated equine estrogen plus 2.5 mg medroxyprogesterone acetate) vs placebo for an average of 5.2 years lead to a 34% reduction in hip and clinical spine fractures in a racially diverse group of 16,608 healthy postmenopausal women enrolled between ages 50 to 79 years (JAMA 20002; 288:321). BMD was measured in 3 of the 40 clinical centers (n=854) at baseline and year 1 and year 3 using DXA (Hologic QDR-2000 or 4500). At baseline, 1.55% of the women randomized to receive E+P and 3.25% assigned to the placebo group were osteoporotic (T-score below -2.5), respectively. From baseline to year 3, the increase in spinal BMD was 6.2±5.8%** in women randomized to E+P vs 1.7±5.4 % in those randomized to placebo. In women treated with E +P, BMD of the, hip, and total body also increased significantly from baseline to year 3.

Change (%) in Total Hip BMD (Baseline to year 3)

	N	E+P	N	Placebo
		Mean ±SD (%)		Mean ± SD (%)
Entire Subsample Age	446	3.70 ±4.07**	408	0.14 ± 4.4
50-59	166	3.75 ±4.1**	131	0.05 ±3.7
60 – 69	188	3.68 ±3.9**	179	0.14 ±4.4
70+	92	3.63 ±4.2**	98	0.26 ±5.1
Race/Ethnicity				
White	371	3.68 ±4.0**	335	0.09 ±4.5
Black	36	2.96 ±3.7**	51	0.15 ±3.6
Hispanic	29	4.91 ±5.4**	15	-0.10 ±4.1

There were no significant differences in the change in lumbar spine, total hip (shown in Table), or total body bone density in women according to age or race. In summary, WHI results showed significant increases in BMD in women randomized to E+P from baseline to year 3 and that the effects of estrogen plus progestin on BMD increments were similar across all age ranges and ethnic groups.
E+P vs. Placebo** $p<0.0001$

Disclosures: ***M.S. LeBoff****, None.*

F380

Bone Response to Treatment with Lower Doses of Conjugated Equine Estrogens With and Without Medroxyprogesterone Acetate in Early Postmenopausal Women. R. Lindsay[1], M. Kleerekoper[2], J. Gallagher[3], J. Pickar*[4]. [1]Internal Medicine, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Medicine, Wayne State University School of Medicine, Detroit, MI, USA, [3]Medicine, Creighton University Medical Center, Omaha, NE, USA, [4]Clinical Research and Development, Wyeth Research, Collegeville, PA, USA.

Lower doses of conjugated equine estrogens (CEE) alone or combined with lower doses of medroxyprogesterone acetate (MPA) increase mean bone mineral density (BMD) from baseline at the spine and hip in early postmenopausal women. The incidence of continued bone loss (loss of BMD >2% from baseline) among these women using lower doses of CEE and CEE/MPA is unknown. Thus, we assessed the incidence of continued bone loss with lower doses of CEE with and without MPA.

This randomized, double-blind, placebo-controlled, multicenter substudy of the Women's Health, Osteoporosis, Progestin, Estrogen (Women's HOPE) trial included 822 healthy, postmenopausal women with an intact uterus. Patients received CEE 0.625, CEE 0.625/MPA 2.5, CEE 0.45, CEE 0.45/MPA 2.5, CEE 0.45/MPA 1.5, CEE 0.3, CEE 0.3/MPA 1.5 (all doses in mg/d), or placebo for 2 years.

Changes from baseline in spine and total hip BMD were assessed annually and compared among treatment groups in an intent-to-treat analysis. Continued bone loss was defined as a loss in spine BMD or hip BMD of >2% compared to baseline values.

Less than 10% of patients in each active treatment group lost >2% of spinal BMD at 12 months, with the exception of patients on CEE 0.3/MPA 1.5 (15.6%), compared to 41.2% of patients on placebo. At 24 months, the percentages of patients in the active groups who lost >2% of spine BMD ranged from 4.5% with CEE 0.45/MPA 1.5 to 15.6% with CEE 0.3/MPA 1.5, compared to 55.3% of patients on placebo. Over 85% of patients on each of the active treatments did not experience losses of BMD that were >2% at the hip at 12 and 24 months. In contrast, BMD decreased by >2% in 30.6% of patients on placebo at 12 months and 36.5% at 24 months.

These results indicate that continued bone loss among early postmenopausal women treated with lower doses of CEE or CEE/MPA is uncommon.

Disclosures: ***R. Lindsay****, Wyeth Pharmaceuticals 2, 8; Eli Lilly 8; Proctor and Gamble 8; Aventis Pharma 8.*

F382

Risk-Benefit Assessment of Raloxifene: Influence of Baseline Cardiovascular Risk. E. Barrett-Connor[1], J. A. Cauley[2], A. Sashegyi*[3], P. M. Kulkarni*[3], D. A. Cox*[3], M. J. Geiger*[3]. [1]UCSD School of Medicine, La Jolla, CA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA, [3]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.

Raloxifene is a selective estrogen receptor modulator (SERM) indicated for the prevention and treatment of osteoporosis in postmenopausal women. We used the global risk index defined in the Women's Health Initiative (WHI) estrogen-progestin trial to assess the overall risk-benefit profile of raloxifene (RLX) and assessed whether this profile is influenced by cardiovascular (CV) risk.

The Multiple Outcomes of Raloxifene Evaluation (MORE) 4-year osteoporosis treatment trial randomized 5133 postmenopausal women (mean age, 67 yrs) to placebo (N=2576) or RLX 60 mg/d (N=2557). Global index events included coronary heart disease (non-fatal MI, coronary death, or silent MI determined by ECG), stroke, pulmonary embolism, invasive breast cancer, endometrial cancer, colorectal cancer, hip fracture, and total mortality. Events were adjudicated by physicians blind to treatment assignment. Cox proportional hazard models were used to analyze the first occurrence of any global index event. Baseline CV risk was assessed by a quantitative risk score based on prior CV event history or CV risk factors.

Women treated with RLX 60 mg/d compared with placebo had a significantly lower risk of experiencing a global index event (HR, 0.75; 95% CI, 0.60-0.96); the risk tended to decrease further as CV risk increased (Table). Contributing to this effect was a lower risk of major CV events (MI, stroke, and coronary death) among women treated with RLX 60 mg/d that also tended to decrease further with increasing CV risk (Table).

The significant reduction in global index with RLX suggests a favorable risk-benefit profile for prevention and treatment of osteoporosis in postmenopausal women overall and in those at increased CV risk.

CV Risk Points	N*	Global Index HR (95% CI)	CV Events† HR (95% CI)
Total Cohort	5133	0.75 (0.60-0.96)	0.71 (0.48-1.06)
≥ 2	3106	0.76 (0.58-1.00)	0.67 (0.44-1.02)
≥ 3	1726	0.66 (0.47-0.92)	0.62 (0.38-1.01)
≥4	676	0.50 (0.30-0.81)	0.41 (0.21-0.82)
≥5	252	0.32 (0.15-0.69)	0.16 (0.05-0.53)
≥6	186	0.39 (0.16-0.95)	0.17 (0.04-0.74)

*Data represent hazard ratio (95%CI) for RLX 60 mg/d vs placebo†MI, coronary death, and stroke

Disclosures: ***E. Barrett-Connor****, Eli Lilly and Company 2, 5.*

F385

Both hPTH (1-34) and bFGF Increase Bone Mass in Osteopenic Animals with Different Effects on Trabecular Bone Architecture. N. E. Lane[1], W. Yao[1], M. Balooch*[1], J. H. Kinney[2], T. J. Wronski[3]. [1]Dept. Medicine, UCSF, San Francisco, CA, USA, [2]Dept. Mechanical Engineering, Lawrence Livermore National Lab., Livermore, CA, USA, [3]Dept. Physiological Sciences, University of Florida, Gainesville, FL, USA.

Osteoporosis is a syndrome of excessive skeletal fragility that results from both the loss of trabecular bone mass and trabecular bone connectivity. Recently, basic fibroblast growth factor (bFGF) has been found to increase trabecular bone mass in osteopenic rats (Lane, Ost. Int 2003, Wronski, JBMR 2002). The purpose of this study was to compare how trabecular bone architecture and strength are altered by two different bone anabolic agents, bFGF and hPTH (1-34), in an osteopenic rat model. METHODS: Six-month-old female Sprague Dawley rats were ovariectomized (OVX) or sham-operated (sham) and maintained untreated for 60 days. Then OVX rats were injected sc with 1mg/kg bFGF , 40ug/kg hPTH (1-34), or vehicle 5d/wk for 60 days. At sacrifice, the right proximal tibial metaphysis (PTM) was used for micro-CT scanning and then processed for histomorphometry to assess bone architecture and turnover. The left PTM was used for nanoindentation/mechanical testing of individual trabeculae (Balooch, J Biomed Mat 1998). The data were analyzed with Kruskal Wallis and post-hoc testing. RESULTS: Ovariectomy at day 120 resulted in decreased trabecular bone volume, connectivity and number compared to sham, hPTH(1-34) and bFGF treated groups. Treatment of OVX animals with bFGF and hPTH(1-34) both increased trabecular bone mass compared to OVX but hPTH (1-34) increased trabecular thickness and bFGF increased trabecular number and connectivity. Histomorphometry revealed an increased osteoid volume in bFGF-treated animals compared to both the hPTH(1-34) and OVX animals. Nanoindentation demonstrated that Elastic Modulus and Hardness of the trabeculae in bFGF-treated animals were similar to that of the PTH-treated, OVX and sham animals (see table).

SUMMARY. Both hPTH (1-34) and bFGF are anabolic agents in the osteopenic female rat. However, hPTH (1-34) increases trabecular bone volume primarily by thickening existing trabeculae while bFGF adds trabecular bone mass through increasing trabecular number and trabecular connectivity. These results suggest the possibility of sequential treatment paradigms for severe osteoporosis.

Groups / Treatment	N	Trabecular Bone Volume(%)	Trabecular Connectivity ($1/mm^3$)	Trabecular Number (1/mm)	Osteoid Volume (%)	BFR/BS ($\mu m^3/\mu m^2/d$)	Elastic Modulus (Gpa)
Sham + Vehicle	10	17.6 ± 4.5	44.2 ± 10.3	3.0 ± 0.8	0.13±0.05	0.12±0.04	24.1±3.6
OVX + Vehicle	14	7.1 ± 3.6[a]	10.9 ± 4.4[a]	2.2 ± 0.5[a]	0.19±0.06	0.30±0.06	25.7±3.7
OVX + PTH	14	20.4 ± 7.1[b]	16.9 ± 6.7[a]	2.3 ± 0.4[a]	0.30±0.09	0.44±0.1[a]	26.6±3.8
OVX +FGF	14	19.1 ± 5.3[b]	28.9 ± 12.1[ab]	2.9 ± 0.7[b]	4.54±2.51[ab]	0.42±0.08[a]	26.7±3.3

a = p<0.05 from Sham; b = p<0.05 from OVX

Disclosures: ***W. Yao****, None.*

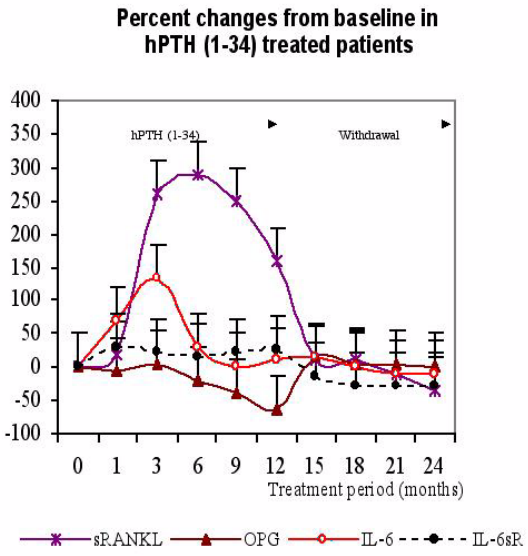
F387

Changes in Serum RANKL, OPG and IL-6 During hPTH (1-34) Administration in Patients with Glucocorticoid Induced Osteoporosis. N. E. Lane, W. Yao, C. D. Arnaud. Dept. Medicine, UCSF, San Francisco, CA, USA.

In vitro and *in vivo* animal studies report both RANKL and IL-6 are produced by osteoblasts in response to PTH stimulation. Since osteoclast maturation and activity are influenced by RANKL, OPG and IL-6, we determined the association of serum levels of sRANKL, OPG, IL-6 and IL-6sR and BMD at multiple time points in patients treated with hPTH (1-34) for 1 yr. and 1yr. follow-up. All subjects had post-menopausal osteoporosis, were treated chronically with glucocorticoid + HRT and were randomized to hPTH (1-34) 40 μg/d (n=28) or control (n=23). Serum sRANKL, OPG, IL-6 and IL-6sR were monitored at baseline (BSL), 1 mo, and then every 3 mos. thereafter. BMD of lumbar spine (SP), total hip region (HIP) and neck of hip region (NKHIP) were obtained at baseline and then every 6 mos thereafter. Differences between and within the hPTH (1-34) and the control groups were analyzed by repeated measurements ANOVA. The relationships between serum sRANKL, OPG and BMD were analyzed by Spearman's correlation.

RESULTS In control group, no significant changes were observed in sRANKL, OPG, IL-6 or IL-6sR levels. However, positive correlations were found between OPG and SP-BMD or NKHIP-BMD at all time points (r = 0.261, p = 0.04 and r = 0.203, p = 0.05), respectively. Negative correlations were found between sRANKL and SP-BMD or HIP-BMD or NKHIP-BMD (r = -0.34, p = 0.02 and r = -0.39, p = 0.01 or r= -0.491, p = 0.0008), respectively. In the hPTH (1-34) group, serum sRANKL increased more than 250% over the BSL level at 3 mos. and was maintained at 150% above BSL for the next 9 mos. The serum OPG level was maintained at the BSL level for the first 3 mos. but decreased by 22% at 6 mos., 38% at 9 mos. and 64% at 12 mos. Serum IL-6 levels increased more than 130% over the BSL level at 3 mo. but returned to BSL level by 9 mos. Serum IL-6sR levels increased about 20% above baseline level by 1 mo. and maintained in this increased level for the next 11 mos. After hPTH (1-34) treatment was discontinued at 12 mos, sRANKL, OPG, IL-6 an IL-6sR returned towards BSL values by 15 mos. (Figure).

SUMMARY Daily hPTH (1-34) injections increased sRANKL and IL-6 production by osteoblasts. Our results support the hypothesis that hPTH (1-34) increases osteoclast activity by stimulating osteoblast production of RANKL and IL-6. Furthermore, serum OPG had a positive while sRANKL had a negative correlation with BMD in the controls.



Disclosures: ***N.E. Lane****, None.*

F392

IkB Kinase Beta (IKKb) Inhibition Prevents Ovariectomy-Induced Bone Loss in Mice. G. Hattersley[1], D. Garnet*[2], R. Fajardo*[3], D. von Stechow*[3], J. Adams*[1], M. Rosenblatt[2], J. M. Alexander[2]. [1]Millennium Pharmaceuticals, Inc, Cambridge, MA, USA, [2]Division of Bone and Mineral Metabolism Research, BIDMC-Harvard Medical School, Boston, MA, USA, [3]Orthopedic Biomechanics Laboratory, BIDMC-Harvard Medical School, Boston, MA, USA.

Nuclear factor (NF)-κB is one of several transcription factors that play a critical role in regulating osteoclast differentiation from hematopoietic progenitor cells. Nuclear translocation and transcriptional activity of NF-κB requires release from an inhibitory protein (IκB) that sequesters NF-κB in the cytoplasm. This process is partly controlled by the activity of IκB kinases that phosphorylate IκB, leading to ubiquitination and subsequent proteolysis, exposing a nuclear localization sequence on NF-κB permitting its translocation to the nucleus and binding to target DNA elements. We explored the hypothesis that a novel non-peptide IκB kinase beta inhibitor would disrupt NF-κB signaling and protect mice from developing ovariectomy (OVX) induced osteopenia by impairing osteoclast differentiation and function. In cultures of mouse bone marrow cells the IKKb inhibitor potently suppressed RANKL/1,25-dihydroxyvitamin D_3 induced osteoclast formation. In addition we also observed significant inhibition of bone resorption by mature osteoclasts isolated from neonatal mouse bone. We next assessed the in vivo protective effects of the IKKb inhibitor on bone mineral density and trabecular architecture in 12-week old OVX Swiss-Webster mice after 5 weeks of treatment initiated immediately after OVX using micro-computed tomography. Vehicle-treated OVX mice showed an 83% loss of distal metaphyseal trabecular bone compared to sham animals. This loss was associated with a marked decrease in trabecular number, thickness, and connectivity. In contrast, daily oral dosing with the IKKb inhibitor fully protected bone density and trabecular architecture, with all trabecular indices not significantly different from sham mice. At the diaphysis, the vehicle-treated OVX mice showed a significant decrease in cortical thickness (Ct.Th) (9%), together with an increased marrow volume (%MV/TV), indicating that bone loss occurs at the endosteal surface of the diaphysis. IKKb inhibitor-treated mice exhibited no loss of cortical bone, with Ct.Th and %MV/TV measurements that were not significantly different from sham mice. In conclusion, we have demonstrated the inhibition of osteoclast formation and function by an IKKb inhibitor in vitro and its efficacy in preventing OVX-induced osteopenia in vivo. Inhibition of IKKb may offer a new strategy to prevent bone resorption in diseases associated with bone loss.

Disclosures: ***G. Hattersley****, Millennium Pharmaceuticals Inc 1, 3.*

F395

Bone Mineral Density Is Increased Following Monthly Administration of AMG 162 in Cynomolgus Monkeys. J. E. Atkinson[1], P. Cranmer*[1], S. Mohr*[2], M. Niehaus*[2], C. P. Jerome[3], M. E. Cosenza*[1], C. R. Dunstan[4], A. M. DePaoli[1]. [1]Amgen Inc, Thousand Oaks, CA, USA, [2]Covance Laboratories GmBH, Muenster, Germany, [3]SKELETECH, Inc., Bothell, WA, USA, [4]ANZAC Research Institute, Concord, Australia.

AMG 162 is a fully human monoclonal antibody that binds with high affinity and specificity to RANKL (Receptor Activator of NF kappa B Ligand). We have previously demonstrated in cynomologus monkeys that a single subcutaneous dose of AMG 162 was associated with prolonged blood levels, as well as marked and sustained reduction in bone turnover markers. This study determined the effect of monthly dosing on bone turnover markers and bone-mineral density (BMD). Sixty-four cynomolgus monkeys (3-4 yrs old) were assigned to 4 treatment groups (8/sex/group) and received once monthly subcutaneous doses of placebo or 1, 10 or 50-mg/kg body weight of AMG 162. Serum and urine samples were collected at pretreatment, months 3 and 6 for markers of bone turnover [Osteocalcin (OSCL- Metra Osteocalcin™), serum crosslaps (SCRL- Serum Crosslaps ™) and urine N-telopeptide (UNTx- Osteomark®)]. Peripheral QCT(pQCT) was used to measure BMD at the distal radius and proximal tibia.

Treatment with AMG 162 at 10 and 50 mg/kg for 6 months caused highly significant dose dependent reductions in serum and urine markers of bone resorption and formation. pQCT measurements revealed dose-related statistically significant increases in BMD at 3 and 6 months at the 10 and 50 mg/kg doses as noted in the table below.

Treatment Effects on Bone Markers (% of control) and Bone Mineral Density (% change from baseline)

AMG 162 Dose	Month	SCRL	OSCL	UNTx	Total BMD	
					Radius	Tibia
Control	3	-	-	-	-1.23	-5.6
	6	-	-	-	-4.42	-7.9
10 mg/kg	3	-43.9	-59.0	-66.3	+9.4*	+5.0*
	6	-30.5	-30.7	-47.8	+6.4*	+5.1*
50 mg/kg	3	-64.7	-80.7	-91.3	+10.9*	+9.0*
	6	-63.6	-77.5	-79.9	+14.6*	+11.2*

*p<0.05 vs. control

In summary, monthly SC dosing of AMG 162 resulted in a dose-dependent rapid and sustained decrease from baseline in bone turnover and an increase in BMD. AMG 162 has potent anti-resorptive activity and could be a convenient treatment for osteoporosis and/or metastatic bone disease.

Disclosures: ***J.E. Atkinson****, None.*

F402

Nongenotropic, Anti-Apoptotic Signaling of 1a,25$(OH)_2D_3$ Through the Ligand Binding Domain (LBD) of the Vitamin D Receptor (VDR) in Osteoblasts and Osteocytes: Mediation by Src, PI3 and JNK Kinsases. A. M. Vertino[1], J. Chen[1], S. Kousteni[1], L. Han[1], H. Peng*[1], T. Bellido[1], C. Bula*[2], A. W. Norman[2], S. C. Manolagas[1]. [1]Div Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, CA Veterans Healthcare System, Univ of Arkansas for Med Sciences, Little Rock, AR, USA, [2]Dep of Biochem, Univ of California, Riverside, CA, USA.

Because sex steroids regulate the lifespan of bone cells by modulating the activity of cytoplasmic kinases via a nongenotropic action of their classical receptors, we have explored the possibility that other members of the "nuclear receptor" superfamily might exhibit similar nongenotropic actions. We report that 1α,25(OH)2D3 and synthetic analogs like 1α,25(OH)2-lumisterol3, which exhibit low binding affinity for the VDR in standard equilibrium assays and lack transcriptional activity, protected murine osteoblasts, OB-6 osteoblastic cells or MLO-Y4 osteocytic cells from apoptosis induced by the topoisomerase II inhibitor etoposide. Consistent with a nongenotropic mechanism of VDR action, this effect was reproduced in HeLa cells transiently-transfected with either the wild type VDR or a mutant consisting only of the LBD of the VDR. The LBD of the VDR bound 1α,25(OH)2D3 as effectively as the wt VDR, but unlike the full length receptor, was incapable of inducing VDRE-mediated transcription in cells transfected with a osteocalcin promoter containing reporter construct. The anti-apoptotic effect of 1α,25(OH)2D3 and analogs in calvaria cells could be blocked by inhibitors of each one of 3 different cytoplasmic kinases: the Src kinase inhibitor PP1, the PI3 kinase inhibitor Wortmannin, and the JNK kinase inhibitor SP600125. However, inhibition of p38 with SB203580 or ERK with either U0126 or transfection of a dominant negative MEK in VDR-containing HeLa cells, the kinase that phosphorylates ERKs, did not interfere with these anti-apoptotic actions. Further, 1α,25(OH)2D3 induced a rapid (5 min) association of the VDR with the Src kinase in OB-6 cells, as determined by immunoprecipitation of cell extracts with an anti-VDR specific antibody followed by Western blot analysis with an anti-Src antibody. Finally, either actinomycin D or cyclohexamide prevented the anti-apoptotic effect of 1α,25(OH)2D3, indicating that transcriptional events downstream from kinase activation are also required for these phenomena. These findings suggest that nongenotropic modulation of kinase activity is a general property of the nuclear receptor superfamily; and that vitamin D analogs that activate nongenotropic signals, but lack classical transcriptional activity, may exhibit distinct biological profiles compared to those exhibited by the natural ligands of the VDR.

Disclosures: ***A.M. Vertino****, None.*

F407

Actinobacillus actinomycetemcomitans' **Cytolethal Distending Toxin Induces Receptor-Activator of NF-kB Ligand Expression by Periodontal Ligament Cells and Gingival Fibroblasts.** G. N. Belibasakis[1], A. Johansson*[2], Y. Wang*[3], C. Chen*[3], U. H. Lerner[4], S. Kalfas*[5]. [1]Divisions of Oral Microbiology and Oral Cell Biology, Umeå University, Faculty of Medicine and Odontology, Umeå, Sweden, [2]Division of Periodontology, Umeå University, Faculty of Medicine and Odontology, Umeå, Sweden, [3]Division of Primary Oral Health Care, University of Southern California, School of Dentistry, Los Angeles, CA, USA, [4]Division of Oral Cell Biology, Umeå University, Faculty of Medicine and Odontology, Umeå, Sweden, [5]Department of Preventive Dentistry and Periodontology, Aristotle University of Thessaloniki, Thessaloniki, Greece.

The Gram-negative, facultative anaerobe *Actinobacillus actinomycetemcomitans* is implicated in the pathogenesis of aggressive periodontitis, a disease entity characterized by

rapid alveolar bone loss in the presence of limited inflammation. The interactions of this bacterium with the resident connective tissue cells of the periodontium, which are anatomically associated with the alveolar bone, are poorly understood. Therefore, the purpose of this study was to investigate the effect of *A. actinomycetemcomitans*' surface components on the expression of receptor-activator of NF-kB ligand (RANKL) and osteoprotegerin (OPG), in periodontal ligament (PDL) cells and gingival fibroblasts (GF). Human primary GF and PDL cell cultures were challenged with the bacterial extract for up to 48 h. The mRNA expression of RANKL and OPG were investigated by semi-quantitative RT-PCR, whereas protein expression of RANKL on the surface of the cells was investigated by flow cytometric analysis, using either anti-RANKL, or rh OPG-Fc chimeric protein. The results showed that RANKL mRNA expression was induced in the bacterially challenged PDL cells and GF, in a time- and concentration-dependent manner. The expression of OPG remained unaffected under the concentrations required for RANKL induction. Up-regulation of RANKL on the cell surface was apparent after 48h of bacterial challenge. A cytolethal distending toxin (CDT) knockout strain of *A. actinomycetemcomitans* did not induce RANKL expression, in contrast to its wild-type strain. In addition, *A. actinomycetemcomitans*' lipopolysaccharide had only limited effect on RANKL expression. In conclusion, these results indicate that *A. actinomycetemcomitans* induces RANKL expression by periodontal connective tissue cells, an event that may be associated to osteoclastogenesis occurring during the progress of aggressive periodontitis. The CDT appeared to play a crucial role in this process, and may therefore be the surface-associated component of *A. actinomycetemcomitans* previously reported to exhibit bone-resorbing activity.

Disclosures: ***G.N. Belibasakis****, None.*

F409

Surface Immobilized Bisphosphonate Improves Implant Fixation. B. Skoglund*[1], P. Tengvall*[2], P. Aspenberg*[1]. [1]Neuroscience and Locomotion/ Section of Orthopaedics and Sports Medicine, Faculty of Health Sciences, Linkoping University, Sweden, Linkoping, Sweden, [2]Department of Physics, Division of Applied Physics, Institute of Technology, Linkoping University, Linkoping, Sweden.

The trauma involved in inserting implants into bone leads to resorption at the interface. The purpose of this study was threefold. First, to establish whether initial resorption around an implant is important for later fixation. Secondly, to improve fixation with the use of a bisphosphonate. Thirdly, to evaluate a new approach for administering drugs locally, via surface immobilization on the implant.

Stainless steel screws were inserted medially in the tibiae of 94 male Sprague-Dawley rats. 20 rats received systemic ibandronate (3 µg) or saline. 58 rats received ibandronate or saline solution directly applied into the screw hole before insertion. Finally, 16 rats received screws with surface immobilized ibandronate or controls. These screws had been roughened and coated with immobilized and cross-linked fibrinogen. Pamidronate was then immobilized to fibrinogen, and finally ibandronate was adsorbed overnight on top of this. Tibiae were harvested at 14 days. Biomechanical tests were performed on 66 tibiae. Systemically treated tibiae were tested for pullout strength. Locally treated tibiae were tested for either pullout or removal torque. 20 tibiae were prepared for histology.

Data was analysed with Student's unpaired t-test. Institutional guidelines for the care and treatment of laboratory animals were adhered to.

Systemic ibandronate increased the pullout force by 30 % (p=0.04). Local treatment increased the pullout force by 15 % (p=0.02) and stiffness by 28 % (p= 0.01). Removal torque was increased by 60 % (p=0.04), and the maximum friction moment by 51 % (p=0.04). Energy was increased by 68 % (p= 0.02). Surface immobilized ibandronate increased pullout force by 28 % (p=0.0009) and energy by 90 % (p=0.0008).

The present study shows that early resorption is important for implant fixation. Further, systemic and local administration of bisphosphonates can counter this initial resorption. Finally, our method of surface immobilisation is a useful approach for administration of drugs at a bone-implant interface.

Surface Immobilization of Ibandronate - Effects on Pullout Strength

	Biomechanical data				Percent Increase by Ibandronate 95 % CI			
	Treatment	n	m	sd	min	mean	max	p
Force at Failure (N)	Control	8	46	9	15	28	42	0.0009
	Ibandronate	8	60	3				
Stiffness (N / mm)	Control	8	68	13	- 29	- 8	13	0.45
	Ibandronate	8	62	17				
Energy (Nmm)	Control	8	15	5	49	90	132	0.0008
	Ibandronate	8	29	8				

Disclosures: ***P. Aspenberg****, None.*

F411

Bisphosphonate Treatment (Risedronate) with Conventional Periodontal Treatment Prevents Periodontal Bone Loss: Preliminary Results of a Randomized Placebo Controlled Trial. N. E. Lane, G. C. Armitage*, P. Loomer, S. Hseih*, S. Majumdar, H. Y. Wang*, T. Munoz*. Dept. Medicine, UCSF, San Francisco, CA, USA.

Bone loss in periodontitis results from inflammatory reactions to bacterial plaque resulting in release of cytokines that stimulates osteoclastic bone resorption. Since bisphosphonates inhibit osteoclastic bone resorption, we performed a randomized placebo-controlled trial to determine if 1 yr. of bisphosphonate treatment when used in conjunction with conventional nonsurgical periodontal treatment in subjects with moderate to severe periodontitis will reduce periodontal bone loss compared to subjects receiving conventional therapy and placebo. 73 subjects with moderate to severe periodontitis (mean full-mouth clinical attachment loss [CAL] ≥ 1.5mm) were randomized (2:1) to risedronate (Ris, 5mg/d) or placebo(PL) tablets. Bitewing dental radiographs were obtained at baseline (BSL) and 12 mos. Biochemical markers of bone turnover (deoxypyridinoline crosslinks DPD/Cr) were determined every 3 mos. Conventional periodontal therapy consisted of oral hygiene instructions, scaling and root planing, and routine periodontal maintenance care at 3 mo. intervals. Full-month clinical assessments were done every 6 mos., included CAL, probing depth (PD), bleeding on probing (BOP), and plaque index (PI). Trabecular bone mass in the periodontal bone was assessed by fractal analysis using at least 1 paired site per subject (BSL and 12 mos.) Paired t-tests between treatment and placebo groups were used to assess treatment response. Correlations between different measures were also obtained. RESULTS: The mean age and mean full-mouth CAL measurements of the study subjects in the Ris and PL groups were similar. Improvements in CAL, BOP, and PD were significantly greater in the Ris group compared to the PL. DPD/Cr excretion was significantly lower in Ris compared to the PL group. Significant correlations were found at all time points for low fractal readings and BOP [BSL ($p<0.04$), at 12 mos. ($p<0.0043$)]; and high fractal readings and PD [BSL $p<0.002$, at 12 mos. $P<0.02$]. CAL at BSL was correlated with high fractal readings at BSL and 12 mos. [BSL $p<0.02$, at 12 mos. $P<0.005$]. Bisphosphonate administration improves the clinical outcome of nonsurgical periodontal therapy and may be an appropriate adjunctive treatment to preserve periodontal bone mass.

	Δ in CAL	Δ BOP*	Δ Fractals	PD**	ΔDPD / Cr
Risedronate	1.03±0.9	0.27±0.31	0.004±0.03	0.79±1.0	-2.80±3.6
Placebo	0.61±0.7	0.13±0.24	0.01±0.04	0.45±0.8	-0.60±2.1
P Value	0.008	0.05	NS	0.014	0.01

* = % improved, **= change in probing depth in mm.

Disclosures: ***N.E. Lane****, NIH 2.*

F414

Neutralization of Intrinsic FGF-23 Action by Antibodies Reveals the Essential Role of FGF-23 in Physiological Phosphate and Vitamin D Metabolism. T. Shimada*[1], Y. Yamazaki*[1], Y. Aono*[2], H. Hasegawa*[1], R. Hino*[1], Y. Takeuchi[3], T. Fujita[3], S. Fukumoto[4], T. Yamashita[1]. [1]Parmaceutical Research Laboratories, KIRIN Brewery, Takasaki, Japan, [2]Parmaceutical Development Laboratories, KIRIN Brewery, Takasaki, Japan, [3]Department of Medicine, University of Tokyo School of Medicine, Tokyo, Japan, [4]Department of Laboratory Medicine, University of Tokyo Hospital, Tokyo, Japan.

FGF-23 is the latest fibroblast growth factor that has been shown to play pathogenetic roles in mineral disorders such as hypophosphatemic rickets/osteomalacia. Recent report concerning FGF-23 null mice demonstrated that lack of FGF-23 resulted in severe hyperphosphatemia and elevated serum 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] level, suggesting the important roles of FGF-23 in physiological homeostasis of phosphate and vitamin D. However, such abnormal phenotypes were outcomes derived from the congenital absence of FGF-23. In addition, FGF-23 null mice demonstrated short life span. Therefore it is still unclear whether FGF-23 is indispensable for mineral homeostasis during adulthood. To address this issue, we neutralized intrinsic FGF-23 activity by anti-FGF-23 antibodies and clarified physiological importance of FGF-23 *in vivo*. We developed three monoclonal antibodies recognizing different part of human FGF-23, termed FN1, FC1 and FC2. FN1 and FC1 could capture mouse recombinant FGF-23 as well as human FGF-23, whereas FC2 could not. To evaluate the neutralizing activity of these antibodies, we administered each antibody into BALB/c mice (8-10w). As a result, FN1- or FC1-treated mice showed significant elevation of serum phosphate and $1,25(OH)_2D$ levels, although administration of FC2 antibodies did not change them at all. Moreover, combinatory use of FN1 and FC1 demonstrated more significant effects to induce hyperphosphatemia and high $1,25(OH)_2D$ levels with enhanced expression of renal sodium dependent phosphate cotransporter (NaPi-2a) protein and 25-hydroxyvitamin D-1alpha-hydroxylase mRNA. These phenomena are consistent with those observed in FGF-23 null mice. Therefore, considering previous studies that recombinant FGF-23 could decrease these serum parameters, it is indicated that FN1 and FC1 neutralized the action of intrinsic mouse FGF-23. Taken together, neutralization of FGF-23 significantly affected phosphate and vitamin D metabolism in normal adult mice, indicating that FGF-23 physiologically plays key roles in regulating these parameters. In addition, present study provides the possibility of neutralizing antibodies against FGF-23 as therapeutic tools for disorders caused by inappropriately excess FGF-23 actions.

Disclosures: ***T. Shimada****, None.*

F416

Correlated Changes in PPi and Osteopontin Levels in Hypo- and Hypermineralization Disorders: Novel Therapeutic Targets for Hypophosphatasia. L. Hessle*[1], D. Harmey[1], S. Narisawa*[1], K. A. Johnson*[2], R. Terkeltaub[2], J. L. Millan[1]. [1]The Burnham Institute, La Jolla, CA, USA, [2]VAMC/UCSD, San Diego, CA, USA.

Inorganic pyrophosphate (PP_i) is an important inhibitor of hyroxyapaptite deposition and propagation. Tight regulation of PP_i levels is required for controlled deposition of mineral. A key enzyme in the depletion of the extracellular PP_i pool is tissue non-specific alkaline phosphatase (TNAP), which hydrolyzes PP_i thereby promoting mineralization. Deletion of the TNAP gene (*Akp2*) in mice results in hypophosphatasia characterized by elevated levels of PP_i, poorly mineralized bones and spontaneous fractures. In contrast, nucleosidetriphosphate pyrophosphohydrolase (NPP1) and the transmembrane PP_i-channeling protein ANK, elevate the extracellular PP_i pool. Mice lacking either the NPP1 gene

(*Enpp1*), or deficient in the ANK gene (*ank/ank*), show decreased levels of PP_i, and exhibit soft-tissue ossification and osteoarthritis. We examined the expression of osteoblastic genes in primary osteoblasts (MOBs) from NPP1- and ANK-deficient mice, and found expression of the mineralization inhibitor osteopontin (OPN) was decreased. RT-PCR analyses of wild-type MOBs treated with exogenous PP_i revealed an increase in OPN expression and decreased NPP1 and ANK expression. This supports a direct regulation of OPN expression by NPP1 and ANK mediated by PP_i. Further, these data support a negative feedback mechanism by PP_i on NPP1 and ANK expression. As TNAP has a crucial role in regulating PP_i levels, we hypothesized that OPN levels may also be altered in TNAP null (*Akp2*$^{-/-}$) mice. Accordingly, we found that *Akp2*$^{-/-}$ mice demonstrated significant elevations in serum OPN levels compared to wild-type mice, as measured by ELISA. Interestingly, PP_i and OPN levels appeared normal in *Akp2*/*Enpp1* and *Akp2*/*ank/ank* double homozygote mice, suggesting that TNAP, NPP1 and ANK act antagonistically in regulating PP_i and OPN levels. We conclude that under normal conditions the concerted action of TNAP, NPP1 and ANK regulate PP_i levels. Hypophosphatasia arises from deficits in TNAP activity, resulting in an increase in PP_i levels and a concomitant increase in OPN levels; the combined inhibitory effect of these molecules leads to hypomineralization. In contrast, an NPP1 or ANK deficiency lead to a decrease in the extracellular PP_i and OPN pools, thereby promoting hypermineralization. The mineralization defects in Akp2$^{-/-}$ mice, along with elevated PP_i and OPN levels are normalized by ablation of either the NPP1 or ANK gene. Thus, NPP1 and ANK represent rational therapeutic targets for hypophosphatasia, a disease for which to-date there is no treatment.

Disclosures: ***D. Harmey****, None.*

F418

Association between Vitamin D levels, Activity, Muscle Strength, Falls and Fractures in the Prospective Population-based OPRA Study of Aged Women. P. Gerdhem*, K. A. M. Ringsberg*, K. J. Obrant, K. Akesson. Department of Orthopaedics, Malmo University Hospital, Malmo, Sweden.

Introduction: Vitamin D supplements have been used to prevent falls and fractures. The effect may be mediated through increased bone mass, but also through increased muscle strength. Most studies have involved high-risk individuals with low activity. The aim of this study was to evaluate the association between vitamin D levels, muscle function, falls and fracture in ambulatory, independently living women.

Methods: We investigated the prevalence of 25-OH vitamin D deficiency and the predictivity on falls and fractures in a population-based sample of 1044 exactly 75-year old women (range 75.01-75.99 yrs) in the Osteoporosis Prospective Risk Assessment study in Malmo (latitude 56^0 North) followed for mean 3.0 yrs (range 2.9-3.4 yrs). Baseline assessments occurred all year round.

Results: Only 4% of the women were vitamin D deficient with 25-OH vitamin D levels below 20 ng/mL. Mean levels of vitamin D were highest in September (40.4 ng/mL) and lowest in February (35.9 ng/mL). Vitamin D levels correlated with the fall associated variables gait (r=-0.15, p<0.001), Romberg balance test (r=0.15, p<0.001), self-estimated activity level (r=0.16, p<0.001), but not with thigh muscle strength. After 3 years, 44% had fallen at least once. Vitamin D deficient women were at increased risk of falling during the first year (odds ratio (OR) 2.09; 95%CI 1.03-4.25) but not for the remaining 2 yrs of follow-up (OR 1.14; 0.46-2.79). After 3 years, 12% (126 of 1044 women) had sustained a total of 165 low-energy fractures (30 hip, 29 wrist, 13 proximal humerus, 44 vertebral and 49 other fractures). Vitamin D deficiency predicted hip fractures (OR 4.10; 1.35- 12.46) and having more than 2 low-energy fractures (OR 3.85; 1.09-13.64). The fall and fracture prediction of vitamin D deficiency was independent of season of baseline assessment. Vitamin D deficiency was not independently predictive of falls or fractures when balance, gait or activity level were included in a logistic regression model. Data was comparable when women put on vitamin D supplements during the follow-up period were excluded from the calculations.

Conclusion: 25-OH vitamin D deficiency predicted falls in aged women during the first year of follow-up and predicted hip and multiple fractures during the 3-year follow-up. The predictive ability was related to other fall associated variables. The prevalence of vitamin D deficiency was lower than expected, even though only 5% used vitamin D supplementation. This may be explained by the fortification of dairy products in Sweden and that 98% of the women spent 30 minutes or more outside per day.

Disclosures: ***P. Gerdhem****, None.*

F421

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], A. Shaikh*[1], M. Rubin*[2], S. J. Silverberg*[2], T. I. Standish*[1], Z. A. Syed*[1]. [1]Medicine, McMaster University, Hamilton, ON, Canada, [2]Columbia University College of Physicians and Surgeons, NY, NY, USA, [3]University of Hong Kong, Hong Kong, China.

A randomized, double blind, placebo-controlled trial was conducted to determine if alendronate 10mg daily (ALN) maintains or improves bone mineral density (BMD) in patients (pts) with primary hyperparathyroidism (PHPT). ALN's effects on serum and urine calcium, PTH and biochemical markers of bone turnover were also evaluated. We now update and complete our results of this 2 year study with biochemical data. Eligible pts were asymptomatic and did not meet surgical guidelines. 44 pts were randomized to placebo (PBO) or ALN for months 1-12 then all received ALN for months 13-24. BMD was measured every 6 months by dual energy X-ray absorptiometry (DEXA). Total and ionized serum calcium, phosphorus, PTH, 25-hydroxy vitamin D (25D), 1,25-dihydroxy vitamin D (1,25D), bone- specific alkaline phosphatase (BSAP), 24-hour urine calcium (UCa) and urine N-telopeptide (NTX) were measured on a three-monthly basis. Primary endpoints were BMD at the lumbar spine (LS), total hip (TH) and distal one-third radius (1/3r), bone markers and serum calcium. 37 pts completed 24 months of the protocol. Paired t-tests were conducted for groups A (ALN years 1 and 2) and B (PBO year 1, ALN year 2). Second year mean differences for primary and repeated measures analysis of variance for secondary outcome indices were utilized. Treatment with ALN rapidly decreased NTX by 66% (p<0.001) at 3 months; it remained suppressed for the two-year period. In the PBO arm, NTX remained elevated until pts were crossed over to ALN. BSAP decreased in the ALN group by 49% at 6 months (p<0.001) and by 54% at 9 and 12 months (p<0.001). bone specific alkaline phosphatase (In the PBO group, BSAP did not decrease until pts were crossed over to ALN when levels declined and remained suppressed. Total serum calcium and ionized calcium did not change for the 2 year period in both ALN and PBO groups. The 24-hour UCa did not change and PTH levels remained stable in both groups. No changes were seen in 25-D or 1,25-D or in serum phosphorus. ALN treatment over two years was associated with a significant increase in the LS BMD by 6.85% (0.05 g/cm^2, p<0.001) and a 2.9% increase in the TH BMD (0.02 g/cm^2, p=0.09). The BMD at the 1/3r site remained stable (0.007g/cm^2, p=0.12) compared to baseline. ALN significantly decreases bone turnover and increases BMD at the lumbar spine after 12 and 24 months of therapy from baseline. Serum and urine calcium, PTH and vitamin D levels remain stable with ALN in PHPT. ALN may be an alternative to surgery in some patients with PHPT.

Disclosures: ***A.A. Khan****, Merck and Company 2.*

F423

Pseudohypoparathyroidism without Albright's Hereditary Osteodystrophy Features: A Novel, Autosomal Recessive Form in a Kuwaiti Kindred. L. M. Ward[1], M. Bastepe[2], I. French*[1], S. E. Lawrence*[1], F. H. Glorieux[3], H. Jueppner[2], M. L. Lawson*[1]. [1]Division of Endocrinology and Metabolism, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada, [2]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [3]Genetics Unit, Shriners Hospital for Children, Montreal, PQ, Canada.

Pseudohypoparathyroidism (PHP) is a form of PTH resistance characterized by hypocalcemia and hyperphosphatemia despite elevated PTH levels. We have recently identified a novel form of PHP in a Kuwaiti kindred, where the inheritance follows an autosomal recessive (AR) pattern. The aim of this study was to characterize the phenotype in this novel form through detailed clinical, biochemical and genetic studies. PHP was identified in 4 children (3 girls) of the same generation, from three related, consanguineous Kuwaiti families (referred to as F1, F2 and F3). The parents showed no clinical or biochemical evidence of the disease. All the affected children presented with symptoms of hypocalcemia (including seizures in 1 patient) at mid-puberty (mean age at diagnosis 13.22 ± 1.74 years). At diagnosis, mean ionized calcium, phosphate, and iPTH levels were 0.84± 0.05 mmol/L (N:1.1-1.3), 1.90± 0.36 mmol/L (N:1.1-1.6) and 18.53± 7.07 pmol/L (N:1.1-6.8), respectively. Features of Albright's Hereditary Osteodystrophy (AHO) were absent. PTH infusions tests (Parathar© 3 units/kg), performed in one adolescent from F1 and in one from F2, demonstrated a < 4-fold increase in $_u$cAMP (N: > 10-fold increase) and a 5-6% reduction in percent tubular reabsorption of phosphate (N: 5-15% reduction). There was no clinical or laboratory evidence of additional hormone resistance. Genetic studies were undertaken to determine whether the affected members of the kindred showed the same loss of *GNAS1* exon A/B methylation that has been observed in patients with PHP-1b, another inherited PHP sub-type without AHO features. Southern blot analyses using gDNA from two affected girls (one from F2 and one from F3) showed no loss of methylation at the A/B exon. Analysis of several informative microsatellite markers further demonstrated that two affected and two unaffected members from F3 had inherited the same allele from their mother, thus arguing against linkage to the region on chromosome 20q13.3 which comprises the *GNAS1* gene. In conclusion, we have identified a novel form of PHP characterized by AR inheritance, absent AHO features, a sub-normal $_u$cAMP response and a borderline $_u$phosphate response to PTH. The disease appears to be unlinked to the *GNAS1* region. Further studies to determine the disease locus and to characterize the skeletal phenotype at the bone tissue level are presently underway.

Disclosures: ***L.M. Ward****, None.*

F426

Intrauterine Programming of Urinary Calcium and Magnesium Excretion in Children Born to Mothers with Insulin Dependent Diabetes Mellitus. M. Z. Mughal, J. Eelloo*, S. Sibartie*, S. A. Roberts*, M. Maresh*, C. P. Sibley*, J. E. Adams. Central Manchester & Manchester Children's Hospitals and the University of Manchester, Manchester, United Kingdom.

We have previously shown that offspring of diabetic rats have reduced urinary calcium and magnesium excretion compared to offspring of controls, and these differences persisted up to16 weeks after birth, a time equivalent to young adulthood in humans. In this cross-sectional study, we studied the urinary excretion of calcium & magnesium in 5 to 18 year old children born to mothers with Insulin Dependent Diabetes Mellitus (ChIDDM). Concentrations of calcium (Ca), magnesium (Mg), sodium (Na) and creatinine (Cr) were estimated using the Hitachi 917 analyser in the first urine passed in the morning in 44 (28 male) ChIDDM and 127 (58 male) healthy controls. The ratios of urinary of Ca & Mg to Cr were used in the analysis. Dietary intake of Ca, Mg, phosphate (P), sodium (Na) and protein (Pr) were estimated from 3-day food diaries.

	CONTROLS		ChIDDM		
	Median	CI	Median	CI	P*
Age (yrs)	11.3	10.3 to 12.8	10.9	9.9 to 12.5	0.57
Ca/Cr	0.31	0.23 to 0.40	0.23	0.18 to 0.32	0.20
Mg/Cr	0.56	0.52 to 0.61	0.46	0.42 to 0.55	0.012
Ca intake (mg/day)	810	670 to 900	950	680 to 1180	0.12
Mg intake (mg/day)	230	210 to 250	260	220 to 270	0.034
P intake (mg/day)	1140	1020 to 1240	1400	1080 to 1480	0.007
Na intake (mg/day)	2560	2350 to 2800	2830	2530 to 3020	0.031
Pr intake(mg/day)	61	59 to 64	75	65 to 81	0.001

* Mann-Whitney U-test

Analysis of covariance was used to test for differences in Ca/Cr and Mg/Cr between controls and ChIDDM after allowing for the effects of gender and age (using a cubic spline representation). This showed that Ca/Cr was significantly lower in ChIDDM compared with controls (p=0.041, difference in Ca/Cr 0.099, 95% CI 0.004 to 0.19). Mg/Cr was highly significantly lower in ChIDDM compared with controls (p<0.0001, difference in Mg/Cr 0.15, 95% CI 0.08 to 0.23).

As in the animal studies, urinary excretion of Mg and Ca were lower in ChIDDM compared with controls. These results are not explained by dietary intake of Ca & Mg or other nutrients that are known to influence their urinary excretion. Thus, these results provide a compelling evidence for intrauterine programming of renal Ca and Mg handling in children born to mothers with insulin dependent diabetes mellitus.

Disclosures: ***M.Z. Mughal****, None.*

F428

Overgrowth in Sotos Syndrome Is Caused by Missense Mutations in the Nsd1-gene, which Is Expressed in Growth Plate Chondrocytes. M. Karperien[1], L. de Boer*[1], J. Tjon*[1], S. G. Kant*[2], M. H. Breuning*[2], J. M. Wit*[1]. [1]Pediatrics, LUMC, Leiden, Netherlands, [2]Clinical Genetics, LUMC, Leiden, Netherlands.

Sotos syndrome is characterized by typical facial features, overgrowth, advanced bone age, macrocephaly, and psychomotor retardation. It is associated with haploinsufficiency of NSD1, a cofactor for Nuclear Hormone Receptor signalling, localized on chromosome 5q35. In the majority of cases in Japan, this is caused by a microdeletion. In order to elucidate the molecular basis of the overgrowth in more detail, we have studied the occurrence of deletions and mutations in the NSD1 gene in a panel of 26 Dutch patients with suspected Sotos syndrome. In addition, we have studied NSD1 expression in human growth plate specimens.

Based on a clinical score, the patients were catagorized in 3 groups: typical (n=8), dubious (n=11) and atypical (n=7) Sotos syndrome by a panel of clinical geneticists and pediatric endocrinologists. Only one patient (of the typical group) contained a chromosomal deletion as determined by fluorescent in situ hybridization. Subsequently, all coding exons of the NSD1-gene were sequenced. We detected heterozygous missense mutations resulting in inactivation of the protein in 5/8 (62,5%) patients in the typical group, 2/11 (18%) in the dubious group and 0/7 (0%) in the atypical group. From 4 patients, DNA of the parents could be analysed. In all cases, the mutations were *de novo* and each patient has its own nucleotide alteration. Of particular interest was a family with two affected children (catagorized in group 1 and 2), who have a similar *de novo* mutation. This was explained by mosaicism of the mother, who had various characteristics typical of Sotos syndrome and contained the mutation in DNA isolated from dermal fibroblasts but not from whole blood. Immunohistochemistry using two NSD1-specific antibodies demonstrated the protein in 3 pubertal growth plate specimens, predominantly in hypertrophic chondrocytes. Identical staining patterns were observed using both antibodies.

In conclusion, in the majority of patients with typical Sotos syndrome (75%) deletions or mutations were detected in the NSD1 gene. In contrast to Japanese Sotos patients, predominantly missense mutations instead of chromosomal deletions were detected. Mutations in NSD1 were rare in the dubious group (18%), and absent in the atypical group. Mosaicism, previously not recognized in Sotos syndrome, may contribute to the heterogenic presentation. The growth fenotype in Sotos syndrome may be explained by disturbed regulation of nuclear hormone receptor signalling, e.g. for estrogen, thyroid hormone and retinoids, in growth plate chondrocytes.

Disclosures: ***M. Karperien****, None.*

F431

The Calcimimetic Compound, Cinacalcet HCl, Ameliorates Osteitis Fibrosa in Rats with Chronic Renal Insufficiency. M. Wada*, Y. Furuya*, N. Kobayashi*, S. Obana*, M. Ozai*, S. Miyata*, N. Nagano. Pharmacology, Pharmaceutical Development Labs, Kirin Brewery Co., Ltd, Takasaki-shi, Japan.

Osteitis fibrosa is a common complication of chronic renal insufficiency and is characterized by increased bone turnover, bone marrow fibrosis and cortical defects. In this study, we evaluated the effects of the calcimimetic compound AMG 073 (cinacalcet HCl) on osteitis fibrosa in rats with chronic renal insufficiency. Rats were subjected to partial nephrectomy (NX) and orally treated with cinacalcet HCl for consecutive 41 days. After treatment, tibia and femurs were dissected for bone analyses. Tibial fibrosis volume (FbV/TV) and cortical porosity (Ct.Po) were evaluated by a bone histomorphometrical technique, and femoral cortical bone mineral density (Ct.BMD) and bone energy absorption were evaluated by pQCT and a three-point bending test, respectively. At the end of treatment, the serum PTH levels of Nx + Vehicle rats were about more than 1,500 pg/mL. Treatment with cinacalcet HCl reduced serum PTH level by 66% compared to that of the Nx+Vehicle control 90 min after dosing ($P < 0.05$). The FbV/TV of the Nx+Vehicle control was 5.8%, indicating the development of osteitis fibrosa. Moreover, the tibial Ct.Po of the group was about 7-fold greater than that of Sham+Vehicle group. Treatment with cinacalcet HCl reduced tibial FbV/TV and Ct.Po by 78% and 65, respectively, compared to the Nx + Vehicle group. Furthermore, analyses by pQCT and a three-point bending test showed that cinacalcet HCl reversed the reduction in Ct.BMD and cortical bone strength (energy absorption) toward normal. Finally, serum PTH levels were correlated with FbV/TV (r = 0.78) Ct.Po (r = 0.84) Ct.BMD (r = -0.82) and energy absorption (r = -0.48). These results indicate that cinacalcet HCl ameliorates osteitis fibrosa in rats with secondary hyperparathyroidism primarily by reducing PTH levels.

Effects of cinacalcet HCl on bone indices

	Tibial Fb.V / TV (%)	Tibial Ct.Po (%)	Femoral Ct.BMD (mg / cm^3)
Sham + Vehicle	0.0 ± 0.0*	0.8 ± 0.1*	1398 ± 4**
Nx + Vehicle	5.8 ± 1.9	5.4 ± 1.5	1268 ± 29
Nx + 3 mg / kg	3.7 ± 1.0	3.9 ± 1.2	1287 ± 31
Nx + 15 mg / kg	1.3 ± 0.3*	1.9 ± 0.5*	1347 ± 14*

*P < 0.05, **P < 0.01, vs. Nx + Vehicle

Disclosures: ***M. Wada****, Kirin Brewery Co.,Ltd. E.*

F434

NIK Is a Potential Target for Treatment of Arthritis, Controlling Osteoclast and Lymphocyte Activity. K. Aya, O. Kanagawa*, D. V. Novack. Medicine and Pathology, Washington University, St. Louis, MO, USA.

NF-κB inducing kinase (NIK) is a serine/threonine kinase in the MAP3K family. Mice with NIK deficiency have dysfunctional B and T cells, and lack peripheral lymph nodes. We have previously shown that unmanipulated NIK-/- mice have normal osteoclast (OC) numbers and bone architecture, but show resistance to RANKL-stimulated osteoclastogenesis in vivo and in vitro. To determine whether NIK might be involved in pathological bone destruction, we turned to models of arthritis, in which joint inflammation is associated with local bone erosion. Serum transfer arthritis is induced by injection of arthritigenic serum (from KRNxNOD mice) containing pre-formed antibodies, and does not require lymphocyte function in the host. NIK+/+ mice and NIK-/- mice were injected with arthritigenic serum on day 0, day 2 and day 7, with LPS on day 2. At day 14, the clinical score and thickness of hind paws of NIK-/- mice were similar to NIK+/+ mice, as was histological evaluation of inflammation. In contrast, bone erosion was significantly lower in NIK-/- mice, and, histologically, the surface of bone remained flat, suggesting a defect in NIK-/- OC function. Cells from arthritic paws cultured without additional cytokines, yielded TRAP positive multinucleated cells in NIK+/+, and to a lesser extent in NIK-/-, wells. On artificial matrix, NIK-/- cultures showed less resorptive activity than NIK+/+ cultures. Having demonstrated a critical role for NIK in the effector phase of inflammatory arthritis, we asked if NIK is involved in the induction phase, which depends on lymphocyte function. Antigen-induced arthritis (AIA) was induced in NIK+/- and NIK-/- mice. All NIK+/- mice had severe arthritis with bone erosion, but NIK-/- mice had little joint inflammation, and no bone erosion, indicating that NIK is essential for induction of AIA. To determine if the lack of lymph nodes (LN) was responsible for this resistance to arthritis, AIA was induced in lymphotoxin-α -/- mice, which lack LN but have mature lymphocytes, and 3 of 4 had severe arthritis. In contrast, RAG2-/- mice, which have LN but lack mature lymphocytes, were resistant to AIA. We were therefore able to use RAG2-/- mice as recipients in lymphocyte transfer experiments in the context of AIA. RAG2 -/- mice receiving NIK+/+ splenocytes had severe AIA, but mice given NIK-/- splenocytes had little arthritis, indicating that NIK expression in lymphocytes is required for initiation of inflammatory arthritis. Thus, NIK expression is important in both the induction and effector phases of inflammatory arthritis, and might represent an attractive target for the treatment of rheumatoid arthritis. NIK expression in OCs may also be critical in other conditions of pathological bone loss.

Disclosures: ***K. Aya****, None.*

F436

Predictive Value of a Cartilage Degradation Marker for Radiologic Osteoarthritis: -The Rotterdam Study. M. Reijman*[1], J. M. W. Hazes*[2], S. M. A. Bierma-Zeinstra*[3], B. W. Koes*[3], S. Christgau*[4], C. Christiansen*[5], A. G. Uitterlinden[6], H. A. P. Pols[6]. [1]Epidemiology and Biostatistics, Erasmus MC, Rotterdam, Netherlands, [2]Rheumatology, Erasmus MC, Rotterdam, Netherlands, [3]General Practice, Erasmus MC, Rotterdam, Netherlands, [4]Osteometer BioTech A/S, Herlev, Denmark, [5]Centre for Clinical and Basic Research A/S, Ballerup, Denmark, [6]Internal Medicine, Erasmus MC, Rotterdam, Netherlands.

Osteoarthritis is a common locomotor disease characterized by degradation of articular cartilage, but accurate prognostic markers are lacking. Recently a specific marker of cartilage degradation, measured as the urinary concentration of C-telopeptide fragments of collagen type II (CTX-II), corrected for creatinine (ng/mmole creatinine), was developed.

The aim of the present study was to investigate the predictive value of CTX-II, for progression of radiological osteoarthritis of the hip and knee.

From all subjects of the Rotterdam study (N = 1238 participants) with available urine samples and X-rays, the radiographs at baseline and follow-up, with a mean follow-up time of 6.6 years, were evaluated for joint space narrowing (JSN) of the knee and hip joints. We used three cut off points for JSN, namely 1.0, 1.5 and 2.0 mm decrease of the joint space width between baseline and follow-up. JSN was evaluated per compartment (knee: medial and lateral, hip: lateral, superior and axial), and a JSN of minimal 1 (out of 5) compartment was defined as positive progression. The association between quartiles CTX-II at baseline and radiological progression of OA was measured by logistic regression analysis to calculate odds ratios as estimation for relative progression risk (predictive value). CTX-II concentrations at baseline differed between sexes (P-value = 0.000), increased with age (P-value for change = 0.033) and increased with BMI (P-value = 0.03). We therefore adjusted the crude odds ratios for age, gender and BMI.
The more JSN at follow-up, the stronger the association we found with CTX-II concentration. Furthermore, the highest quartile CTX-II showed the strongest association with JSN. Increased baseline concentration of CTX-II appears to be a strong predictive factor for radiological progression of osteoarthritis of the knee and hip, suggesting that it could be useful for identifying patients at high risk for cartilage destruction.

Predictive value of quartiles CTX-II for radiological progression of OA

	JSN > 1.5 mm (96 cases)		JSN > 2.0 mm (37 cases)	
	crude	adj	crude	adj
1st (reference)	1	1	1	1
2nd (128-177)	1.8	1.6	4.2	4.4
3rd (178-246)	2.6	2.2	5.9	6.3
4th (247-1277)	3.5	2.7	9.0	8.8

Disclosures: ***M. Reijman****, None.*

F443

Parathyroid Ablation Upregulates Placental Calcium Transfer whereas Absence of PTH does not. C. S. Noseworthy*[1], J. P. Woodrow*[1], N. J. Fudge*[1], G. Karsenty[2], C. S. Kovacs[1]. [1]Memorial University of Newfoundland, St. John's, NF, Canada, [2]Baylor College of Medicine, Houston, TX, USA.

Fetal calcium metabolism is regulated differently than in the adult, with parathyroid hormone related protein (PTHrP) stimulating placental calcium transfer, and PTHrP and parathyroid hormone (PTH) sharing in the regulation of the fetal blood calcium and skeletal mineral accretion. Studies in fetal lambs have suggested that the parathyroids directly regulate placental calcium transfer through the production of PTHrP, whereas aparathyroid (*Hoxa3* null) fetal mice have no disturbance in the rate of placental calcium transfer or the production of PTHrP.
We studied *Gcm2* null fetuses to determine the effects of parathyroid ablation with persistence of a thymic source of PTH on fetal mineral and bone homeostasis.
Gcm2 mice were placed into the *Black Swiss* (Taconic) background in order to be compared to *Hoxa3* null mice. Studies included ionized Ca (*i*Ca), Mg and P on maternal and fetal blood; placental transfer of ^{45}Ca at 5 min using standard technique; and assessment of the mineral content of the long bones by von Kossa.
Aparathyroid (*Hoxa3* null) fetuses have *i*Ca 1.07 ± 0.07 mmol/l, reduced well below the maternal levels; low Mg and increased P; normal placental calcium transfer; and reduced skeletal mineralization as confirmed by von Kossa and atomic absorption spectroscopy on ashed skeletal residue. In contrast, *i*Ca of *Gcm2* null fetuses was 1.34 ± 0.01 mmol/l, equal to the maternal level but significantly lower than heterozygous (het) and wild-type (wt) siblings (1.82 ± 0.01 and 1.77 ± 0.02 mmol/l respectively, $p < 0.001$). Mg was 0.87 ± 0.01 mmol/l in *Gcm2* null fetuses, modestly reduced below maternal, wt and het levels ($p<0.01$), while P was no different among fetuses. Placental calcium transfer (normalized to het mean value in each litter) was upregulated in *Gcm2* null fetuses (112 ± 7.2 % in null vs 92.4 ± 6.0 % in wt, $p < 0.04$). Skeletal mineral content was not visibly altered among wt, het and null, and the growth plates were of normal length and morphology.
In summary, parathyroid ablation with auxiliary thymic production of PTH in *Gcm2* null leads to modest fetal hypocalcemia and hypomagnesemia, upregulated placental calcium transfer, and no disturbance in skeletal calcium content. These results confirm findings in other murine models that the fetus requires a blood calcium equal to the mother's but no higher in order to sufficiently mineralize the skeleton before birth. These results also suggest that PTH partly regulates placental calcium transfer, because *Gcm2* null mice (which produce PTH) were able to upregulate placental calcium transfer in response to modest hypocalcemia, while *Hoxa3* null mice (which do not produce PTH) were not.

Disclosures: ***C.S. Noseworthy****, None.*

F445

The Anabolic Response to Intermittent PTH (1-34) Requires Connexin43 (Cx43) Mediated Gap Junctional Communication. C. H. M. Castro, J. P. Stains, R. Civitelli. Bone and Mineral Diseases, Washington University, St. Louis, MO, USA.

Recessive (Cx43 gene deletion) or dominant (connexin45 [Cx45] expression) interference with Cx43 function alters osteoblast gene expression and inhibits PTH induced cAMP accumulation and osteoblasts differentiation, whereas PTH up-regulates Cx43. We tested whether Cx43 is involved in the anabolic response to PTH *in vivo* using heterozygous Cx43 null mice ($Cx43^{+/-}$), whose Cx43 abundance and gap junctional communication are reduced relative to wild type ($Cx43^{+/+}$) animals (the homozygous null mutation is perinatally lethal). We administered subcutaneously either 200 or 400 ng/g b.w. of human PTH (1-34) (PTH-200 and PTH-400), or vehicle to 4-months-old $Cx43^{+/-}$ and $Cx43^{+/+}$ mice (n=7 per group) daily (5 days/week). After 4 weeks of treatment, whole body bone mineral density (BMD) measured by DEXA (PIXImus, Lunar-GE) increased 6.4±1.0% and 11.4±1.9% in the PTH-200 and PTH-400 groups, respectively. This response was significantly smaller in $Cx43^{+/-}$ animals (whose baseline BMD was not different than that of $Cx43^{+/+}$ littermates), with 3.6±1.2% and 3.1±0.5% increments from baseline, respectively. No significant changes occurred in vehicle treated animals. Histomorphometric analysis of PTH-400 mice also showed a 2-fold increase of bone volume/total volume in $Cx43^{+/+}$ mice and no significant changes in $Cx43^{+/-}$ littermates. While vigorous activation of bone formation was demonstrated in wild type animals, with increases of osteoblast number (~70%) and all static and dynamic parameters of bone formation, changes in heterozygous animals were marginal at best. Likewise, PTH-400 significantly increased osteoclast number in $Cx43^{+/+}$ but not in $Cx43^{+/-}$ animals. *In vitro*, PTH activation of the rat osteocalcin promoter was significantly reduced in ROS 17/2.8 cells transfected with Cx45, which decreases endogenous Cx43 mediated gap junctional communication, relative to wild type cells. We mapped this connexin sensitivity of PTH response to a proximal region (-199 to +32) of the promoter, but excluded the participation of the CT-element, previously identified as a connexin response element (CxRE). In fact, Cx45 overexpression in these cells reversed PTH response from activation (1.4-fold in ROS 17/2.8 cells) to repression (0.89-fold in ROS/Cx45 cells), when a –199 to +32 promoter sequence was used. Thus, the anabolic response to intermittent PTH administration is curtailed in conditions of Cx43 deficiency, the consequence of an osteoblastic failure to mount a full response to the hormone's anabolic action. Cx43 mediated intercellular signals control response to PTH in part by modulating osteoblast gene transcription via alternative CxREs.

Disclosures: ***R. Civitelli****, None.*

F447

Comparison of Intact and Whole Parathyroid Hormone Levels After Parathyroidectomy Between Patients with Primary and Secondary Hyperparathyroidism. H. Yamashita[1], P. Gao[2], S. Noguchi*[1], S. Uchino*[1], S. Watanabe*[1], T. Ogawa*[1], M. Fukagawa[3]. [1]Surgery, Noguchi Thyroid Clinic, Beppu, Oita, Japan, [2]Scantibodies Laboratory Inc, Santee, CA, USA, [3]Dialysis and Metabolism, Kobe University School of Medicine, Kobe, Japan.

Most current commercial 2nd generation intact PTH (iPTH) assays cross-react with 7-84 PTH and this interference accounts for up to 90% of immunoreactivity in samples obtained from uremic patients. It is reported that 7-84 PTH has an antagonistic action to 1-84 PTH, however, very little is known about the production and metabolism of 7-84 PTH. A new 3rd generation cyclase activating PTH (CAP) novel immunoradiometric assay, was shown to specifically measure 1-84 PTH without any cross reactivity to 7-84 PTH. Using this CAP assay, we studied the production and metabolism of 7-84 PTH in primary hyperparathyroidism (pHPT).
This study comprised 63 patients with pHPT caused by a single adenoma who underwent parathyroidectomy. Preoperatively, blood samples were drawn from the bilateral internal jugular vein by ultrasonographic guidance and from the peripheral vein. During surgery, blood samples were drawn after anesthesia (basal level), before excision (pre-excision level) of one enlarged parathyroid gland, and at 5, 10, and 15 minutes post excision. The 7-84 PTH level was calculated by subtraction of the 3rd generation CAP assay value from the 2nd generation iPTH assay value.
There were 34 patients whose iPTH assay levels differed by more than 10% between the right and left internal jugular. In those 34 patients, the level of 7-84 PTH levels obtained from the adenoma side was significantly higher than those from the contralateral side (124±18 vs. 44±18 pg/ml, $p<0.05$).The plasma CAP and iPTH value had dropped to 25±10 and 32±11% at 5min, 14±7 and 21±7% at 10 min, and 10±5 and 17±7% 15 min after removal of an enlarged gland of pre-excision value, respectively ($p<0.001$). Interestingly, the 7-84 PTH level after 5 min was higher than that at pre-excision in seven of 63 patients (11%). Further, the level at 10 min was higher than that at 5 min 1n 10 (16%) and the level at 15 min was higher than that at 10 min 1n 15 (24%).
Our results suggest that 7-84 PTH may not only be produced by parathyroid adenomas but also may be produced by the metabolism of 1-84 PTH during circulation and the new 3rd generation CAP assay may be a more useful adjunct to parathyroidectomy than the currently used 2nd generation iPTH assay.

Disclosures: ***H. Yamashita****, None.*

F449

Amphiregulin: A Possible Mediator of Parathyroid Hormone's Anabolic Actions in Bone. L. Qin[1], L. Raggatt[1], X. Li*[1], J. Tamasi[2], J. H. M. Feyen[2], N. C. Partridge[1]. [1]Physiology and Biophysics, University of Medicine and Dentistry of NJ, Robert Wood Johnson Medical School, Piscataway, NJ, USA, [2]Bristol-Myers Squibb Pharmaceutical Research Institute, Pennington, NJ, USA.

Parathyroid hormone (PTH) is the major mediator of calcium homeostasis and bone remodeling, having both bone resorption and bone formation actions. It is well documented that intermittent injection of PTH increases bone formation. A number of genes have been found to be regulated by PTH and mediate PTH's functions. Recently we have found that the expression of amphiregulin, a member of the epidermal growth factor (EGF) family, is rapidly and highly up-regulated by PTH in several osteoblastic cells in a PKA-dependent manner. In addition, we report that *in vivo* hPTH (1-38; 8 μg/100g) injection into 28-day old male rats dramatically elevated the level of amphiregulin mRNA in the femoral metaphyses to about 12-fold and 2-fold after 1 h and 4 h, respectively. The up-regulation of amphregulin by PTH in osteoblastic cells is a primary response since it does not require *de novo* protein synthesis. We performed a complete study of the expression patterns of all the EGF-like ligands (EGF, TGF-α, amphiregulin, epiregulin, HB-EGF and betacellulin) and their receptors (EGFR and ErbB2) in UMR 106-01 cells using real-time RT-PCR and found that all of these proteins are expressed but amphiregulin is the only member that is highly regulated by PTH. Functional studies using cell numbers, [^{3}H]thymi-

dine incorporation into DNA and cell cycle analysis demonstrated that amphiregulin is a potent growth factor for rat primary calvarial osteoblastic cells. Furthermore, Western blot analysis showed that amphiregulin strongly and quickly stimulated Akt and ERK phosphorylation, c-fos and c-jun expression in osteoblastic cells (UMR 106-01 and rat primary calvarial osteoblastic cells). All of these functions require the EGFR, since specific inhibitors (compound 32 and compound 56) for the EGFR completely abolished amphiregulin's actions. Finally, μCT analysis of femurs and tibiae from amphiregulin knockout mice revealed that those mice have significantly less tibial trabecular bone than wild-type siblings at 4-weeks while femoral cortical bone is unaffected. Taken together, our results suggest that amphiregulin is a possible mediator of PTH's anabolic functions in bone.

Disclosures: ***L. Qin****, None.*

F451

Wnt Signaling Pathway: A Target for PTH Action in Bone and Bone Cells. N. H. Kulkarni*[1], D. H. Halladay*[1], R. R. Miles*[1], C. A. Frolik*[1], R. J. S. Galvin*[1], T. R. Fuson*[1], T. J. Martin*[2], J. E. Onyia*[1]. [1]Gene Regulation, Bone Research & Enabling Biology, Eli Lilly & Company, Indianapolis, IN, USA, [2]St. Vincent's Institute for Medical Research, Fitzroy, Victoria, Australia.

Parathyroid hormone (PTH), a potent modulator of bone metabolism has been shown to increase bone formation in animals and humans. The Wnt signaling pathway has recently been demonstrated to play an important role in bone formation and regulation of bone density. In humans, activating mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) causes a high bone density phenotype. These mutations are thought to inhibit the binding of the Wnt antagonist Dickkopf (DKK-1) to LRP5, preventing formation of endocytic ternary complex with Kremen 1, thus stimulating the Wnt pathway. Since, stimulation of Wnt signaling pathway and PTH treatment both lead to increased bone formation, the anabolic effects of PTH on bone might be mediated in part by the Wnt pathway. To test this hypothesis, we investigated the expression and effects of PTH on components of the Wnt signaling pathway in rat metaphyseal bone, *in vivo;* and primary osteoblast cultures, osteoblastic cells (UMR106) and cultured murine osteoclasts. Northern analysis revealed the expression of Wnt components LRP5, LRP6, frizzled-1 (FZD-1), DKK-1 and Kremen-1 *in vivo* and *in vitro*. PTH upregulated the mRNA expression of LRP6 and FZD-1, while decreasing LRP5 and DKK-1 in rat metaphyseal bone in vivo and in UMR 106 cells in vitro. Since, stimulation of the canonical Wnt pathway results in the accumulation of β-catenin, we analyzed the stabilization of β-catenin using a monoclonal antibody. Western blot analysis showed that PTH (10^{-6} to 10^{-10}M) stabilized β-catenin at (1 to 24h) in UMR 106 cells. Futhermore, the ability of PTH to induce downstream Wnt signaling response was measured by TCF-luciferase reporter gene activity. There was a 6 and 3 fold increase in the reporter gene activity at 8 and 24h respectively, after PTH treatment of UMR 106 cells. These findings suggest that stimulation of canonical Wnt signaling pathway by PTH may occur via differential expression of the receptor complex FZD-1/LRP5 or LRP6 accompanied by down regulation of the antagonist DKK1, resulting in stabilization of β-catenin and activation of the TCF family of transcription factors. Taken together, these results indicate a possible role of the Wnt signaling pathway in PTH anabolic actions in bone.

Disclosures: ***N.H. Kulkarni****, None.*

F453

Tuberoinfundibular Peptide of 39 Residues (TIP39), a Ligand of the PTH Receptor Family, may Be Involved in Early Brain Development in Zebrafish. E. Blind[1], S. Wortmann*[1], I. A. Hansen*[1], S. Meyer*[1], C. Neuner*[2], C. Winkler*[2]. [1]Medicine - Endocrinology, University of Wuerzburg, Wuerzburg, Germany, [2]Physiological Chemistry 1, University of Wuerzburg, Wuerzburg, Germany.

The polypeptide TIP39 (tuberoinfundibular peptide of 39 residues), which has been isolated initially from bovine hypothalamus, is a potent activator of the PTH-2 receptor, but acts as an antagonist on the PTH-1 receptor. Despite the known interaction with the PTH receptor family, the function of this new peptide is largely unknown. Starting from database search results we cloned the cDNA coding for the TIP39 protein precursors from zebrafish (Danio rerio) by RT-PCR and RACE-PCR from total RNA extracted from total adult zebrafish tissue. To study the zebrafish TIP39 expression pattern during embryogenesis, we performed in-situ hybridization with digoxygenin-labeled antisense cRNA probes. To manipulate functional expression levels of TIP39 during the early development of the zebrafish, we used a morpholino-based antisense oligonucleotide strategy. The deduced, processed zebrafish TIP39 has an identical length compared to human TIP39 and is highly conserved with 59% aa identity. In man, TIP39 was found to be expressed (as detected by RT-PCR) in adult brain, kidney, and trachea, as well as in fetal brain and liver (Hansen et al., J Endocrinol 2002, 174: 95-102), with the expression pattern in mouse brain (by in-situ hybridization) being localized in many but not all neuron groups. Similarly, in zebrafish embryos at 23 hours after fertilization, TIP39 expression was detected in restricted domains of the developing brain. The inhibition of TIP39 expression at this stage of development using a morpholino-induced knockdown of TIP39 translation resulted in a severely altered phenotype with abnormal development of anterior head structures. In summary, an undisturbed TIP39 expression seems to be essential for normal brain development during early zebrafish ontogenesis.

Disclosures: ***E. Blind****, None.*

F456

Interactions between the Mid-Region of PTH-(1–34) and the Receptor Orient the Hormone for Receptor Activation. A. Wittelsberger[1], M. Corich*[1], B. Lee*[1], A. Barazza*[1], R. Yacobi*[1], P. Czodrowski*[2], D. F. Mierke*[2], J. M. Alexander[1], M. Rosenblatt[1], M. Chorev[1]. [1]Bone and Mineral Metabolism, BIDMC-Harvard Medical School, Boston, MA, USA, [2]Chemistry and Molecular Pharmacology, Brown University, Providence, RI, USA.

Based on structure-activity studies, PTH-(1–34) is comprised of two functional α-helical domains: the N-terminal helix required for activation of the receptor, and the C-terminal helix responsible for binding. Based on our studies, this model now must be re-examined. We used photoaffinity scanning (PAS) to identify key ligand–receptor interactions for residues from the mid-region of PTH-(1–34), an unstructured domain subtended by the two α-helices [1,2]. Four PTH analogs, containing a single photoreactive *p*-benzoylphenylalanine (Bpa) residue in position 11, 15, 18, or 21, all photocrosslinked to the juxtamembrane part of the N-terminal extracellular domain (N-ECD) of the PTH1-Rc, i.e. in the regions [165-174], [183-189], [190-224], and [165-177], respectively. Addition of these mid-region contacts as constraints to our previously proposed model of the PTH–PTH1-Rc complex compels substantial refinement of the model. After extensive molecular simulation experiments, we find: 1) The overall receptor-bound conformation of the hormone is not extended, but bent, with the mid-region more compact than previously thought. 2) Region [165-177] of the N-ECD of the receptor, shown previously to have a propensity to form an amphipatic helix, is re-directed towards the extracellular surface of the helix bundle. 3) The activation domain of the hormone traverses the external surface of the receptor between the top of transmembrane helices (TM) 1 and 2, rather than between TM–7 and TM–1. Elucidation of the novel contact points for the mid-region of PTH-(1–34) significantly alters the view of both the receptor-bound tertiary structure of the hormone as well as the topological orientation of the C-terminal part of the N-ECD in the hormone–receptor bimolecular complex. We conclude that the mid-region of PTH-(1–34) has a critical role in fixing the entry of the N-terminal helical part of the hormone into the heptahelical bundle between TM–1 and TM–2, by extensive contacts with the receptor. This anchorage orients the amino terminus into position to activate the receptor.

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Disclosures: ***A. Wittelsberger****, None.*

F459

NFκB Suppression of VDR Transactivation Requires the Rel Homology Domain of p65. X. Lu*, P. Fiester*, J. Rubin, M. S. Nanes. Division of Endocrinology, Department of Medicine, Emory University, VA Medical Center, Decatur, GA, USA.

Gene transcription stimulated by vitamin D requires binding of the liganded VDR to DNA, assembly of the coactivator complex with pre-initiation factors, and activation of RNA polymerase II. We have shown that suppression of VDR activity by the inflammatory cytokine TNF-α is due to activation of the transcription factor NFκB. Furthermore, suppression of VDR is conferred by the p65 subunit of NFκB. To define active region(s) of p65 that mediate inhibition of VDR function, several deletion and single amino acid mutants of p65 were designed in an expression construct bearing an independent nuclear localization signal and a c-myc tag. Nuclear compartmentalization of the mutant p65 or wild-type proteins was confirmed by western analysis for the myc tag and/or p65. ROS 17/2.8 cells were transiently transfected with a VDREx2-luciferase reporter alone or with the p65 expression vectors. Cells were treated with 10^{-8}M $1,25(OH)_2D_3$ (vitamin D) and luciferase expression was measured as an index of transcription. Forced expressed of p65 or its mutants revealed that the RHD domain of p65 is critical for the inhibition of VDR transactivation. Additionally, neutral amino acid substitution of potentially phosphorylated sites S276, S281, Y257, and Y288, within the RHD domain, prevented the p65 inhibition of VDR transactivation. To determine if p65 competes with the VDR for binding to the transcriptional coactivators CBP or SRC-1, GST-VDR-LBD fusion proteins were used in pull-down assay with in vitro translated [^{35}S]CBP or [^{35}S]SRC-1. CBP failed to bind the VDR-LBD, as reported previously, but increased binding of the liganded VDR-LBD to SRC-1. Addition of in vitro translated wild-type p65 decreased VDR-LBD interaction with SRC-1 in a dose dependent manner. In contrast, RHD-spanning or RHD point mutants of p65 failed to disrupt VDR-SRC-1 binding, suggesting a role for the p65 RHD in the inhibition of VDR transactivation. Additional experiments revealed that p65, which binds CBP directly, interacts with SRC-1 only very weakly, and transient forced expression of SRC-1 in ROS 17/2.8 cells did not, by itself, reverse the p65 inhibition of VDR trans-activation. These results suggest that NfkB p65 inhibits the VDR transcriptional response by weakening the formation of the multiprotein nuclear coactivator complex. p65 may inhibit formation of the complex with VDR, in part, by competitively disrupting VDR-SRC-1 interaction, and also by directly binding to CBP.

Disclosures: ***X. Lu****, None.*

F461

Lithocholic Acid Is a New Vitamin D Receptor Ligand that Stimulates Transcription via Novel VDRE Motifs and a Unique Pattern of Coregulator Recruitment. P. W. Jurutka[1], P. D. Thompson*[1], K. Eichhorst*[1], N. Hall*[1], C. Encinas Dominguez*[1], G. K. Whitfield[1], J. C. Hsieh[1], C. A. Haussler*[1], D. J. Mangelsdorf*[2], M. R. Haussler[1]. [1]Biochemistry, University of Arizona, Tucson, AZ, USA, [2]Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX, USA.

The nuclear vitamin D receptor (VDR) mediates the effects of 1,25-dihydroxyvitamin D3 (1,25D) to alter gene transcription and achieve bone mineral homeostasis. In addition, extraosseous actions of 1,25D-VDR are observed in many epithelial cell types, and the cytotoxic bile acid, lithocholic acid (LCA), has been identified as a novel VDR ligand. Both 1,25D and LCA have been reported to induce genes coding for cytochrome P450 (CYP) enzymes involved in cellular detoxification. Herein, we demonstrate that VDR liganded with LCA stimulates transcription of several vitamin D responsive element (VDRE)-linked reporter genes in intestinal, kidney and bone cells. Studies employing human cell lines (HT-29 colon cancer and IN407 embryonic intestine) revealed that both 1,25D- and LCA-VDR elicit transcriptional activation from classical DR3 VDREs as well as from a novel ER6 VDRE located in the promoter region of the human CYP3A4 gene. Each ligand can also upregulate CYP3A4 mRNA and protein levels. Gel mobility shift assays demonstrated that, like 1,25D, LCA enhances retinoid X receptor (RXR) heterodimerization with VDR and subsequent VDRE binding, in vitro. In addition to VDRE binding, the VDR-RXR heterodimer recruits a p160-type coactivator such as SRC-1, subsequently interacting with the DRIP205 mediator to stimulate transcription. We tested the ability of LCA-VDR to associate with these proteins in gel mobility shift and pull-down assays. Whereas both 1,25D and LCA enhanced the association of VDR with DRIP205, LCA-VDR did not interact with SRC-1. This surprising result was also observed in a mammalian two-hybrid system which further revealed that SRC-2 and SRC-3 were ineffective in their interaction with LCA-VDR. Finally, selected residues in the ligand binding domain were altered to create VDR mutants that retain 1,25D binding and transactivation but do not stimulate transcription in response to LCA. Thus, VDR serves as a high affinity "endocrine receptor" for 1,25D and also as an "adopted orphan" nuclear receptor functioning as a lower affinity bile acid sensor that likely employs an alternative set of coregulators for control of gene expression. In conclusion, we propose that VDR is a bifunctional regulator with the 1,25D-liganded conformation facilitating primarily bone mineral homeostasis, and the LCA-liganded configuration mediating cellular detoxification by upregulation of CYP3A4.

Disclosures: ***P.W. Jurutka****, None.*

F463

Coactivator LxxLL Peptides Derived From VDR-Phage Display Screens Exhibit Ligand-Dependent Characteristics in Cultured Cells. L. A. Zella*, H. Yamamoto, M. Yamazaki*, N. K. Shevde, J. W. Pike. Biochemistry, University of Wisconsin-Madison, Madison, WI, USA.

The vitamin D receptor (VDR) mediates the biological actions of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) through its capacity to regulate transcription. The VDR is known to bind to specific regulatory regions on numerous target genes in association with the retinoid X receptor (RXR) and to recruit coactivator complexes such as SRC/CBP/p300 and DRIP/Mediator necessary for various facets of transcriptional control. The transactivation domains of both VDR and RXR interact directly with the p160 coactivators and DRIP205 via an LxxLL motif located within the coactivator. In our previous studies, we have shown that small 19-mer peptides containing this LxxLL motif which bind both VDR and RXR can inhibit vitamin D response. In addition, LxxLL peptides selective for RXR can also inhibit vitamin D response. In this report, we screened several phage libraries directly with purified human VDR to identify peptide sequences that displayed higher affinity and selectivity for the VDR. Two classes of LxxLL peptides were obtained: those screened in the presence of VDR agonists and those screened in the presence of the VDR antagonist ZK159222. Agonist-derived peptides contained the consensus sequence Lx E/H x H/F P L/M/I LxxLL and exhibited significant homology to the active LxxLL box found in DRIP205. Antagonist-derived sequences were restricted to the central LxxLL motif. Peptides sequences were then fused in-frame downstream of the Gal4 DNA binding domain, transfected into MC3T3-E1 cells and their activities assessed in a mammalian two-hybrid system for selectivity and ligand dependence using VDR-VP16 and RXR-VP16. While the bulk of the LxxLL peptides interacted with both VDR and RXR, selectivity for VDR was observed with at least one of the peptides. It was also clear that the relative affinity of the peptides differed between the two receptors. The transcriptional activities of the vitamin D agonist-derived peptides were dependent upon 1,25(OH)2D3, but were not induced by the antagonist ZK159222. Analogs of vitamin D3 promoted unique patterns of both potency and efficacy with these peptides. Conversely, the activities of the peptides that were derived from the antagonist screen were largely ligand-independent. We next assessed the ability of these LxxLL peptides to inhibit vitamin D response using activation of a transfected reporter gene fused downstream of the vitamin D-sensitive osteocalcin gene promoter. All agonist peptides inhibited vitamin D response in a dose-dependent manner. Current studies are focused on the use of these peptides to suppress endogenous vitamin D3 responses in osteoblasts and other cell types.

Disclosures: ***L.A. Zella****, None.*

F466

The Intracellular Estradiol Binding Protein: An hsp27-Related Estrogen-Binding, Estrogen Receptor-Interacting Protein that Squelches Estrogen-Directed Transactivation. H. Chen[1], B. Hu[2], M. Hewison[3], L. Nguyen*[1], C. Encinas*[1], J. S. Adams[1]. [1]Division of Endocrinology, Diabetes and Metabolism, Cedars-Sinai Burns and Allen Research Institute, Los Angeles, CA, USA, [2]Pathology, Cedars-Sinai Medical Center, Los Angeles, CA, USA, [3]Division of Medical Sciences, The University of Birmingham, Queen Elizabeth Medical Centre, Birmingham, United Kingdom.

Using estrogen receptor a (ER alpha)-bearing cells from the gonadal steroid-resistant New World primate (NWP), cotton-top marmoset , as an illustrative “experiment of nature”, we have recently identified a novel means of steroid hormone-receptor transregulation embodied by the heat shock protein 27 (hsp27)-related intracellular estradiol (E2) binding protein (IEBP). The aim of these studies was to determine how IEBP influences ER-ERE-directed transactivation. A line of estrogen-responsive primate breast cells were stably transfected with IEBP and overexpression of the transfected protein was confirmed by Western blot. In breast cells stably overexpressing IEBP, E2-stimulated ERE-luciferase reporter activity was significantly reduced 50-70% ($p<0.001$) with no accompanying change in endogenous ER expression; by comparison transfection of the ER alpha significantly increased reporter activity 0.5-fold ($p<0.05$). IEBP transfection increased extractable, specific E2 binding in postnuclear extracts 2-4-fold ($p<0.001$). The increase and decreased of ERE-directed luciferase expression under the influence of ER alpha and IEBP, respectively, was confirmed with fluorescent microscopy of MCF-7 cells stably-transfected with the ERE-luciferase reporter construct. Using yeast two hybrid screening for IEBP-protein interaction partners, 1] IEBP was shown to interact with ERalpha and 2] the IEBP-ER interaction was E2-dependent and reversed with the selective estrogen receptor modulator, tamoxifen. GST pull-down assay confirmed the interaction between ER alpha and IEBP and showed that the IEBP interaction domain resided in the ligand binding domain of the ER alpha (residues 246-595). Co-immunostaining of the ER and IEBP demonstrated 1] co-localization of the two proteins in the cytoplasm in the absence of E2 and 2] translocation and co-localization of the ER and IEBP to the nucleus after incubation with E2. In summary, we have functionally characterized a novel regulator of estrogen action. IEBP is an hsp27-related estrogen-binding, ER-interacting protein which acts to squelch E2-ER-ERE-directed transactivation.

Disclosures: ***H. Chen****, None.*

F468

Osteoblast Kinetics In Vivo. T. Kobayashi, E. Hinoi, H. M. Kronenberg. Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

During bone modeling and re-modeling, cells of the osteoblast lineage replicate, become bone-forming osteoblasts, and then, in a regulated fashion, die or become lining cells or osteocytes. To understand better the kinetics of these processes, we have developed a method for marking cells of the osteoblast lineage and following them over time. We genetically marked osteoblasts at particular times using the Cre/loxP recombination system in combination with the Rosa 26R (R26R) Cre reporter system. In order to induce Cre activity in osteoblasts at desired times, tamoxifen (TAM)-inducible Cre (Cre-ERt) was expressed under the control of the mouse 3.2kb-long type I (a1) collagen gene promoter. The R26R mice express LacZ in any cells in which Cre removes engineered “stop” sequences. Cre-transgenic mice were crossed with R26R LacZ mice to obtain doubly mutant male mice (Studs). Studs were crossed to 8 week old wild-type CD-1 female mice to obtain doubly mutant pups. To study osteoblasts in the perinatal period, pregnant mothers were injected with 2 mg TAM intra-peritoneally at 18.5 dpc. After vaginal delivery, pups were sacrificed on consecutive days, X-gal stained, and histologically analyzed. To study mice at one months of age, mice doubly heterozygous for Cre and R26R genes were injected with 100mg/kg TAM subcutaneously and sacrificed on consecutive days. Littermates of the same sex were compared. TAM itself had no effect on bone morphology or on TUNEL positivity determined using perinatal mice 2days after TAM injection. At the perinatal stages, basal X-gal staining due to endogenous beta galactosidase activity was minimal. TAM injection at E18.5 induced efficient X-gal staining postnatally, and the maximal staining intensity was obtained at P1.5 in approximately 70% of more than 25 TAM-injected dams. (In the rest of the cases, Xgal staining was not efficiently induced at P1.5 and showed a gradual increase after P2.5.) After the peak number of cells stained at P1.5, the number of stained cells decreased dramatically at P2.5 in both calvaria and tibia. Histological examination showed that X-gal positive cells at P1.5 were matrix-attached osteoblasts, some osteocytes, and cells of tendons and ligaments. At P2.5, numbers of not only of Xgal-stained osteoblasts but also of osteocytes were greatly reduced. In control studies, TAM injection to P0.5 pups produced large amounts of LacZ positive cells at P2.5, suggesting that the decrease in LacZ positive cells at P2.5 was not due to poor LacZ activity. Study using one month old mice also showed patterns similar to those in the perinatal study. Lineage tracing of osteoblasts using this method suggests that osteoblast turnover is surprisingly rapid both in perinatal stages and at the age of one month in the mouse.

Disclosures: ***T. Kobayashi****, None.*

F470

Classical Genotropic versus Nongenotropic (Kinase-Initiated) Regulation of Gene Transcription by the Estrogen Receptor (ER): Evidence for Extensive Divergence of Target Gene Population Controlled via the Two Mechanisms. M. Almeida[1], J. D. Shaughnessy[2], F. G. Zhan[2], L. Han[1], H. Peng*[1], S. A. Stewart*[1], C. A. O'Brien[1], R. L. Jilka[1], S. Kousteni[1], S. C. Manolagas[1]. [1]Div Endo/Metab, Center for Osteoporosis and Metabolic Bone Diseases, CA Veterans Healthcare System, Univ of Arkansas for Med Sciences, Little Rock, AR, USA, [2]Myeloma Institute, Univ of Arkansas for Med Sciences, Little Rock, AR, USA.

Elucidation of kinase-initiated routes by which the ER controls gene transcription, along with evidence of distinct biologic outcomes in response to ligands that can selectively activate nongenotropic estrogen-like signaling (ANGELS), suggest that the ER controls a range of genes wider than that regulated by its trans/cis interactions with DNA. To ascertain the extent and significance of nongenotropic ER-mediated transcription, we employed stable transfectants of HeLa cells carrying either the wild type ERα or the ligand binding domain of the ERα localized to the cell membrane (E-Mem), MCF-7 breast carcinoma cells, as well as murine tibiae and uteri from mice treated with 17β-estradiol (E2) or the nongenotropic signaling activator 4-estren-3α,17β-diol (estren) and then subjected them to: a) real time PCR analysis b) an array analysis of 96 genes associated with signal transduction pathways c) an array analysis of 54 selected transcription factors (TFs) and d) a comprehensive array analysis of ~ 40.000 genes and ESTs using the Affymetrix U133 chip. We report that in all 4 specimens nongenotropic ER actions regulated a larger number of genes and TFs than genotropic ER actions, irrespective of the method of analysis. Specifically, the expression of Wnt2, Frizzled10, Egr-1 and c-fos was strongly upregulated in E-Mem-containing HeLa cells treated with E2 or estren, or in ERα-containing HeLa cells treated with estren. Upregulation of Frizzled10 by estren was reproduced in MCF-7 cells. Egr-1 and c-fos were only modestly, if at all, upregulated by activation of ERα with E2. Egr-1 was upregulated by both estren and E2; but complement 3 (C3), only by E2 in the uteri. Estren had no effect on C3, cathepsin D, progesterone receptor, bcl-2 and cyclin D1 in MCF-7 cells, whereas E2 upregulated all these ERE-containing genes. On the other hand, estren selectively activated pten in the tibia whereas the activity of gadd45a, mdm2 and trfr was modulated by both estren and E2. Finally, in tibia estren, but not E2, modulated the activity of AP-1, AP-2, GAS/ISRE, and SP1-binding TFs; and both estren and E2 modulated the activity of MEF-2, Myc-Max, PPAR, Stat5, Stat6 and TFIID. This and interim analysis of the Affymetrix data support an extensive divergence in gene expression depending on the mode of ER activation.

Disclosures: ***M. Almeida****, None.*

F472

Apparent Pubertal Effects on Mechanosensitivity in Girls but Not Boys: An Estrogen Effect? N. J. Crabtree[1], T. J. Beck[2], K. Uusi-Rasi[2], M. S. Kibirige*[3], J. N. Fordham[3], N. J. Shaw[1]. [1]Birmingham Children's Hospital, Birmingham, United Kingdom, [2]Johns Hopkins University, Baltimore, MD, USA, [3]South Cleveland Hospital, Middlesbrough, United Kingdom.

The skeleton is believed to adapt its strength throughout life to changing mechanical demands. It has been hypothesized that sensitivity to load stimuli might be influenced by hormonal changes. Load sensitivity should be apparent in the relationship between bone strength and load magnitude; pubertal changes in that relationship may signify a hormonal effect. According to the muscle-bone mechanostat theory, skeletal loads mainly arise from muscle forces actuating bony levers and limb muscle mass should be a reasonable measure of skeletal load. The aim of this study was to examine the possible effects of puberty & gender on the strength-load relationship in a group of healthy pre- & post pubertal children.

260 healthy white children aged 5-18 years had whole body DXA. From these, section modulus (an index of bending strength) was derived from manual region analysis at the mid femoral shaft & combined with measurements of femur length & leg lean mass. Section Modulus (Z), Femoral Strength (FS), defined as Z/femoral length, & the ratio of femoral strength to load (FS/lean leg mass) were compared between gender & pubertal groups using MANOVA, including lean body mass, fat body mass, age and height in the model.

After adjustment for body size, there were no significant gender differences in the prepubertal children for Z, FS or FS/lean leg mass. No significant increases in these size-adjusted parameters were observed in post-pubertal boys. However post-pubertal girls showed significant increases for all three parameters. Additionally a significant gender & puberty interaction was seen for the ratio of strength to load with girls having significantly higher post-pubertal values than their male counterparts.

	Girls		Boys	
	Pre-	Post-	Pre-	Post-
$Z(cm^3)$	*1.56 (0.07)	*2.01 (0.09)	1.55 (0.6)	1.66 (0.11)
FS (cm^2) (*100)	*4.1 (0.2)	*5.2 (0.2)	4.1 (0.2)	4.2 (0.3)
FS / Lean (cm^2/kg) (*1000)	*3.6 (0.1)	*‡4.9 (0.2)	3.7 (0.1)	‡4.0 (0.2)

P<0.05 Diff between *pre- & post- puberty, ‡genders

During puberty, both boys & girls show dramatic increases in circulatory sex hormone levels. Post-pubertal differences in girls may be related to body size independent estrogenic effects on bone, while it would appear that androgens do not have a similar effect in boys. Estrogen may increase bone mechanosensitivity, resulting as this data suggests, in relatively greater bone strength for equivalent muscle loads.

Disclosures: ***N.J. Crabtree****, None.*

F475

Association between 25-OH Vitamin D3 and Periodontal Disease. T. Dietrich*[1], K. J. Joshipura*[2], B. Dawson-Hughes[3], H. A. Bischoff[4]. [1]Periodontology, Charité, HU Berlin, Berlin, Germany, [2]Epidemiology, Harvard School of Public Health, Boston, MA, USA, [3]Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA, [4]Arthritis Research Center, Brigham and Women's Hospital, Boston, MA, USA.

Periodontal disease (PD) is a chronic inflammatory disease that is characterized by the loss of periodontal attachment and alveolar bone. It is a major cause of tooth loss, particularly in the elderly. Osteoporosis and low bone mineral density have been associated with an increased risk of PD. Vitamin D has been shown to improve bone mineral density and a randomized controlled trial with vitamin D and calcium decreased tooth loss in the elderly. The purpose of this analysis was to evaluate the association between serum levels of 25-hydroxyvitamin D3 (25-OHD) and PD in a representative sample of the US population (NHANES III).

We analyzed data of 10,899 subjects aged 20+ years who had data on periodontal attachment loss (AL) and serum 25-OHD levels as well as complete data on all covariates. PD was modeled as the dependent variable in a weighted multiple logistic regression (defined as mean AL ≥ 1.5 mm) and in a weighted multiple linear regression (mean AL). Serum OHD levels were categorized as previously published (<37.5, 37.5-<50, 50-<62.5, 62.5-<75, ≥75 nmol/l). The models adjusted for age, gender, race/ethnicity, smoking, gingival bleeding, diabetes, calcium intake, poverty-income ratio, body mass index and estrogen use. Age, gender, race/ethnicity, calcium intake and number of missing teeth were considered as potential effect-modifiers. There was a significant independent association between 25-OHD and PD in both models. Compared to the highest 25-OHD category, subjects with low 25-OHD levels (<37.5 nmol/l) were more likely to have PD (Odds-Ratio: 1.54, 95% CI 1.13-2.11). There was a significant trend across 25-OHD categories (p=0.02). In the linear model, the mean difference in AL was 0.1 (0.02-0.18) mm between the lowest and highest 25-OHD category. No interaction was found with age, gender, race/ethnicity, calcium intake or number of missing teeth. When bone mineral density (BMD) of the total femoral region was entered into the model in a subgroup (n=9,922), BMD was not a significant predictor of PD and did not change the OR estimate for the 25-OHD PD association.

This analysis suggests that low serum levels of 25-OHD are associated with an increased risk for PD. This association could not be fully explained by BMD and was independent of gender, age and race/ethnicity. Given the high prevalence of both vitamin D deficiency and PD, vitamin D supplementation might be a useful prevention strategy for PD.

Disclosures: ***T. Dietrich****, None.*

F479

Antagonistic Actions of TEI-9647 Depend on the Primary Structure of AF-2 domain of Vitamin D Receptors. E. Ochiai*, D. Miura*, H. Eguchi*, K. Takenouchi*, Y. Harada*, Y. Azuma, T. Kamimura*, S. Ishizuka. Teijin Institute for Bio-Medical Research, Tokyo, Japan.

We had reported that the vitamin D analog, (23S)-25-dehydro-1α-hydroxyvitamin D3-26,23-lactone (TEI-9647), functions as an antagonist of the vitamin D receptor (VDR)-mediated genomic actions of 1,25-(OH)2D3. Intriguingly, we recently found that TEI-9647 functions as an agonist, not antagonist in rodent cells, whereas it acts as an antagonist in human cells. The primary structures of the human and rodent ligand binding domain (LBD) of VDR are highly conserved, but there are a few differences. Especially, the difference in AF-2 domain was considered to be important for gene transactivation. These findings lead to us to a hypothesis that differences in VDR sequence between species, especially in AF-2 domain, might be decisive for agonistic/antagonistic effect of TEI-9647 and suggesting important role of interaction between TEI-9647 and AF-2 domain. To elucidate this hypothesis, we examined the effect of TEI-9647 on transactivation by luciferase reporter assay using human osteosarcoma cell line (SaOS-2 cells) or ROS 24/1 cells rat osteosarcoma cell line (ROS 24/1 cells) cotransfected with VDR and human vitamin D 24-hydroxylase promoter-driven luciferase reporter. TEI-9647 acts as an antagonist in SaOS-2 cells and ROS 24/1 cells overexpressed wild type human VDR (WT hVDR). On the contrary, TEI-9647 acts as an agonist in both cell lines overexpressed WT rat VDR (WT rVDR), does not show any antagonistic effect even in 10^{-7} M. And we also investigated using the VDR chimeras composed of the AF-2 domain of hVDR and corresponding residues of rVDR. Each chimeric VDRs had normal transcriptional potency activated by 1,25-(OH)2D3. Displacement of the AF-2 domain of hVDR to corresponding domain of rVDR diminished the antagonistic effect of TEI-9647 and TEI-9647 exhibited agonistic effect. On the contrary, displacement of the AF-2 domain of rVDR (the 25 C-terminal residues) to corresponding residues of hVDR diminished the agonistic effect of TEI-9647 and TEI-9647 exhibited antagonistic effect. Investigation using mutated human VDR (C403S, C410N) revealed that Cys 403 and/or 410 was necessary for antagonistic effect of TEI-9647. These results suggested that the species specificity of VDR, especially in AF-2 doamin, might be decisive for agonistic/antagonistic effect of TEI-9647 and important role of interaction between TEI-9647 and two Cys residues in AF-2 domain of human VDR for antagonistic effect of TEI-9647. This is the first report that not only the identification of critical residues in hVDR for TEI-9647 function but also the species specificity of VDR affects the ligand's activity were discovered.

Disclosures: ***E. Ochiai****, None.*

F482

Assay Variation Confounds Hypovitaminosis D Diagnosis: A Call for Standardization. N. Binkley, D. Krueger, C. Cowgill, K. E. Hansen, M. K. Drezner. UW Osteoporosis Clinical Center and Research Program, University of Wisconsin-Madison, Madison, WI, USA.

Endemic hypovitaminosis D contributes to osteoporosis development. However, variation in 25 hydroxyvitamin D (25OHD) measurement is reported, which likely confounds the diagnosis of deficiency. The purpose of this report is to emphasize the extreme variability observed in serum 25OHD measurement between laboratories.

Initially, marked serum 25OHD variability was noted in subjects screening for osteoporosis clinical trials. Subsequently 10 sera were sent for 25OHD measurement in three different laboratories. Finally, 10 specimens were measured in one laboratory before and after adding a known amount of 25OHD.

In the initial observation, 61 osteoporotic women screened for clinical trials had serum 25OHD determinations (41 lab A, 20 lab B.) Their mean (SEM) serum 25OHD concentration was 46 (2.1) and 21 (2.3) ng/ml in laboratory A and B respectively. Furthermore, there was little overlap in serum 25OHD among these clinically similar individuals. Specifically, using 32 ng/ml as an arbitrary cutoff, 17% of those measured in lab A but 90% in lab B were below this value. Finally, 3 individuals were measured in both labs; their 25OHD values (A/B) were 68/48, 50/13 and 43/16 ng/ml.

Subsequently, 25OHD concentration in 10 sera from nursing home residents were evaluated in three different laboratories; a university research lab and two national referral labs; C, D and E respectively. Laboratories A-D utilized various RIA methodologies, lab E used a chemiluminescent immunoassay. Results (ng/ml) from these 10 specimens are reported below.

ID #	Lab C	Lab D	Lab E	ID #	Lab C	Lab D	Lab E
1	14	20	43	6	17	27	30
2	17	20	29	7	23	27	40
3	17	20	29	8	11	23	24
4	19	21	25	9	16	23	20
5	14	20	27	10	8	12	13

Finally, sera were obtained from 10 healthy adults. Two aliquots from each individual were sent to laboratory D; one aliquot had 20 ng/ml of 25OHD added. The measured 25OHD concentration in all spiked sera was higher; however, this increase varied from 9-27 ng/ml between individuals.

In conclusion, 25OHD assays yield markedly differing results; whether an individual is found to have low or normal vitamin D status may be a function of the laboratory utilized. If progress is to be made in correcting widespread hypovitaminosis D, the bone community must accept the challenge of standardizing 25OHD measurement.

Disclosures: ***N. Binkley****, Merck 2, 5, 8; Eli Lilly 2, 5; Novartis 2; Aventis 2.*

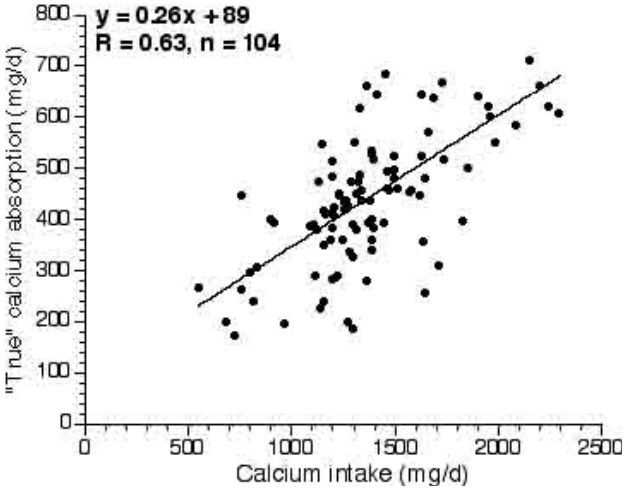
SA001

Calcium Absorption Is Similar from Milk and Two Types of Fortified Orange Juice at High Intake Levels. S. A. Abrams, I. J. Griffin*, P. M. Davila*, K. M. Hawthorne*. Pediatrics, Baylor College of Medicine, Houston, TX, USA.

We measured calcium absorption in groups of 9 to 12 year-old children who were given diets including at least 240 ml/day of milk or one of two types of calcium-fortified orange juice. One orange juice (OJ-1) contained calcium citrate malate as the calcium source and the other contained a mixture of calcium phosphate and calcium lactate as the calcium source (OJ-2). Drinks were extrinsically tagged with a calcium stable isotope (46Ca). Beverages were given with a breakfast meal including a total of 350-550 mg of calcium. Complete 24-hour dietary calcium intake was determined using weighed dietary records. Fractional absorption was very similar between groups and total absorption was not significantly different (p=0.31 by ANOVA) despite differences in intake (see Table below). Calcium intake was linearly correlated with total daily absorption (r = 0.63) suggesting a benefit to increased calcium intake on total absorption up to as much as 2.2 g/day (see figure below). These findings suggest that these three sources of calcium provide similar bioavailability and lead to comparable levels of daily calcium absorption when included as part of a mixed diet containing calcium intakes comparable to recent dietary recommendations. Calcium intake up to a gram over current recommendations may lead to increased calcium absorption using bioavailable calcium sources. Funded in part by NIAMS (AR43740). Also funded in part by The Minute Maid Company (Houston, TX).

Calcium absorption

Group	Number of subjects	Calcium intake (mg/d)	Percent Absorption	Total absorption (mg/d)
Milk	40	1268±172	33.7±7.8	429±124
OJ-1	30	1510±255	32.5±10.0	492±157
OJ-2	39	1413±503	33.1±10.3	455±198



Disclosures: ***S.A. Abrams****, Minute Maid Company R, C.*

SA002

See Friday Plenary number F002

SA003

Assessment of Muscle Strength and the Bone Muscle Unit in the Tibia in Children. K. M. Howard*, M. DiIenno*, R. M. Herskovitz*, B. S. Zemel, M. B. Leonard. Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA, USA.

Bone expands in response to biomechanical forces. Therefore, the evaluation of the bone muscle unit should play a significant role in the assessment of skeletal disorders in childhood. The purpose of this study was to compare measures of muscle cross-sectional area and functional muscle strength as they relate to bone mass and dimensions in children and adolescents. Measurements were completed in 20 healthy controls, 38 nephrotic syndrome patients, and 19 Crohn's disease patients, 5-20 years of age (mean 12.0 ± 3.7). Peripheral quantitative computed tomography (pQCT, Stratec XCT-2000) measures of cortical bone dimensions and content were obtained in the left tibia, 38% proximal to the growth plate. Muscle cross-sectional area (CSA, cm^2) was measured at the 66% tibia site. Isometric muscle strength (peak torque, Ft-lbs.) was measured in plantarflexion and in dorsiflexion in the left ankle at 4 positions (10° of dorsiflexion, neutral, 10° of plantarflexion, and 20° of plantarflexion) using the Biodex Multi-Joint System 3 Pro dynamometer. The correlations between bone and muscle measures were assessed with Pearson product-moment estimates. All 8 Biodex strength measures were significantly correlated with muscle CSA on pQCT: $R^2 \geq 0.62$, $p < 0.001$. The correlation with muscle CSA was greatest for dorsiflexion in the 20° position (Dorsi-20): $R^2 = 0.81$, $p < 0.001$. Comparisons of bone and muscle measures demonstrated that Dorsi-20 explained 87% and 84% of the variability in cortical bone content and cross-sectional area, respectively. In contrast, muscle CSA explained 82% and 75% of the variability in these bone measures. The relationship between muscle strength and muscle CSA (i.e. muscle efficiency) relative to tibia length was compared across the 3 groups; no differences were detected. In conclusion: the correlations between functional measures of isometric muscle strength and bone are greater than the correlations between muscle CSA and bone. These functional strength measures may provide additional insight into the impact of chronic diseases on bone mineral accretion.

Disclosures: ***M.B. Leonard****, None.*

SA004

See Friday Plenary number F004

SA005

Vitamin D Status as a Determinant of Peak Bone Mass in Young Finnish Men. V. V. Välimäki*[1], H. Alfthan*[2], E. Löyttyniemi*[3], H. Suominen*[4], M. J. Välimäki*[1]. [1]Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland, [2]Department of Clinical Chemistry, Helsinki University Central Hospital, Helsinki, Finland, [3]Department of Statistics, University of Turku, Turku, Finland, [4]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland.

Severe vitamin D deficiency causes rickets, but scarce data is available on to what extent a milder hypovitaminosis D determines the development of the peak bone mass in young adults. Our aim was to evaluate the prevalence of hypovitaminosis D {serum 25-hydroxyvitamin D [25(OH)D] ≤ 37.5 nmol/l} and the relationship between vitamin D status and peak bone mass among young Finnish men.

A cross-sectional study of determinants of peak bone mass with data on lifestyle factors

collected retrospectively was performed in 220 young men, aged 18.3 to 20.6 years. 170 men were recruits of the Finnish Army, and 50 were men of similar age, who had postponed their military service for reasons not related to health. Bone mineral density (BMD) was measured in lumbar spine and upper femur by dual-energy X-ray absorptiometry (DXA). Serum 25(OH)D concentrations were followed prospectively for one year.
In July 2000 26.8% of the men had hypovitaminosis D. Six months later in wintertime the respective percentage was 94.6% and next summer 29.8%. After adjusting for age, height, weight, exercise, smoking, calcium and alcohol intake there existed a positive correlation between serum 25(OH)D and lumbar spine (p=0.035), femoral neck (p=0.061), trochanter (p=0.056), and total hip (p=0.068) BMD. Of the two determinants of areal BMD, bone mineral content (BMC) but not scan area correlated with vitamin D status.
Hypovitaminosis D is very common in Finnish young men, especially during wintertime, and it may have detrimental effects on the acquisition of maximal peak bone mass. Since in Finland vitamin D supplementation to infants is now stopped at the age of three years, it can be asked, whether it at our latitude should be continued from that age onwards, no more for the prevention of rickets but as a prophylaxis of osteoporosis.

Disclosures: ***V.V. Välimäki****, None.*

SA006

Relationship of Calcium Intake and Calcium Retention in Adolescent Boys. M. Braun*[1], B. R. Martin[1], M. Kern*[2], G. P. McCabe*[3], M. Peacock[4], A. Machtan*[1], J. Liesmann*[1], A. Kempa-Steczko*[1], C. M. Weaver[1]. [1]Foods & Nutrition, Purdue University, West Lafayette, IN, USA, [2]San Diego State University, San Diego, CA, USA, [3]Statistics, Purdue University, West Lafayette, IN, USA, [4]Indiana University School of Medicine, Indianapolis, IN, USA.

We previously determined the relationship between calcium (Ca) intake and Ca retention in adolescent girls. The intake where maximal Ca retention occurred was determined by a non-linear regression model. It is not known whether the larger skeletal mass of boys requires more dietary Ca to optimize Ca retention or if boys utilize Ca more efficiently than girls.
Forty-four adolescent males, aged 13-15 were tested on two Ca intakes each that ranged from 800-2100 mg/day in a random order, crossover design. All procedures were approved by the Purdue University and Indiana University School of Medicine Institutional Review Boards. Two 3-week metabolic balance sessions were separated by a 2-week wash-out period. Subjects consumed a controlled diet and Ca intake was varied via fortified beverage. After one week equilibration period, calcium retention was determined as intake minus excreta over the next two weeks.
Compared to girls studied during their period of pubertal growth, boys had higher Ca retention at all intakes. This higher Ca retention was achieved through lower urinary output and higher Ca absorption. Thus, boys attain a higher bone mineral content by increased utilization efficiency. Adding measures of body size into the model did not eliminate gender differences.

Disclosures: ***M. Braun****, None.*

SA007

Heterodimeric Bone Morphogenic Protein 2/7 Enhances Osteoblastic Differentiation *in Vitro* and Bone Formation *in Vivo*. W. Zhu*[1], J. Arimizu*[1], E. A. Ford*[1], J. R. Lieberman*[2], B. A. Rawlins*[1], O. Boachie-Adjei*[1], R. G. Crystal*[3], C. Hidaka*[1]. [1]Laboratory for Soft Tissue Research, Hospital for Special Surgery, NY, NY, USA, [2]University of California, Los Angeles, CA, USA, [3]Weill Medical College of Cornell University, NY, NY, USA.

While recombinant bone morphogenic proteins (rBMPs) are homodimers, BMP heterodimers resulting from the co-expression of two different BMP genes may be more potent in supporting bone formation. In this study, we tested whether BMP2/7 heterodimer generated by combination gene therapy with adenovirus vectors encoding BMP2 (AdBMP2) and BMP7 (AdBMP7) would be more potent in supporting osteoblastic differentiation and bone formation than either homodimer alone. A549 epithelial cells were transfected with AdBMP2, AdBMP7, or a combination of the two. The secretion of BMP2/7 heterodimer in the supernatant of co-transfected cells was verified by immunoprecipitation with anti-BMP7 antibody followed by enzyme linked immunosorbant assay for BMP2. To test the potency of BMP2/7 *in vitro*, C2C12 myoblastic cells were stimulated with cell supernatants containing BMP2 or BMP7 homodimer, or BMP2/7 heterodimer. Time course studies showed that BMP2/7 increased the alkaline phosphatase (ALP) activity and osteocalcin (OCN) expression at earlier times than either homodimer, indicating an acceleration of osteoblastic differentiation. After 7d, BMP2/7 induced a 200-fold higher ALP activity and 10-fold higher OCN expression than either homodimer ($p<0.05$, all comparisons). Dose response studies showed that a maximal OCN expression and ALP activity could be induced with <7.5 ng/ml of BMP2/7 after 7d. These levels were 3-fold (OCN) and 20-fold (ALP) higher than those which could be induced by maximal stimulation with up to 1 μg/ml of rBMP2 or rBMP7 ($p<0.05$, all comparisons). To test *in vivo* bone formation, we performed single level posterior spine fusion in rats by implantation of bone graft and cellulose sponge soaked with AdBMP2 (n=13, $1.5x10^{10}$ particle units [pu]) or AdBMP7 (n=19, same dose) or a combination of the two (n=15, $0.75x10^{10}$ pu each vector). Fusions were assessed 8 wk post-surgery by x-rays in a blinded fashion. Treatment with both AdBMP2 and AdBMP7 resulted in fusion in 14 out of 15 spines (93%). This was 2-fold higher than that achieved with AdBMP2 alone ($p<0.02$) and 3-fold higher than AdBMP7 alone ($p<0.001$). No bilateral fusion occurred in control spines treated with AdNull (vector with no transgene) or saline. Our findings suggest that BMP2/7 heterodimers have increased osteogenic potency than either BMP2 or BMP7 homodimer. Use of heterodimeric BMPs may be an attractive strategy for improving the clinical efficacy of BMP therapy.

Disclosures: ***W. Zhu****, None.*

SA008

See Friday Plenary number F008

SA009

Effects of Cartilage-Derived Morphogenic Proteins (CDMPs) on the Pluripotent Mesenchymal Cell Line C2C12. L. C. Yeh*, A. D. Tsai*, J. C. Lee. Biochemistry, Univ. Texas Health Sci. Ctr. at San Antonio, San Antonio, TX, USA.

The pluripotent mescenchymal cell line C2C12 was used as a model of osteogenesis to examine the effects of cartilage-derived morphogenetic protein (CDMP) -1, -2 and –3. Cells cultured in the presence of CDMPs no longer exhibited the elongated shape characteristic of myoblasts. The patterns of gene expression in C2C12 cells treated with the CDMPs were also altered. In contrast to OP-1, none of the CDMPs affected significantly mRNA expression of MyoD, a known regulator of myogenic differentiation. CDMP-1, -2, and -3 stimulated osteocalcin mRNA by 2.5-, 1.5- and 1.8-fold, respectively. All three CDMPs down-regulated significantly the expression of Scleraxis, a recently identified transcription factor reportedly specific for tendons and ligaments and may be involved in restricting progenitor cells for connective tissues. The three CDMPs regulated differentially the spatial and temporal pattern of expression of different members of the BMP family members as well as the BMP receptors in a dose-dependent manner. At 300 ng/ml, all three CDMPs stimulated significantly alkaline phosphatase (AP) activity and moderately (20-25%) extracellular matrix synthesis as measured by quantitative Alcian Blue staining. A significant synergy (up to about 5-fold) was observed between OP-1 and the individual CDMPs in stimulating AP activity. These data contribute important new basic knowledge on these protein factors in bone biology.

Disclosures: ***J.C. Lee****, Stryker Biotech 2, 5.*

SA010

See Friday Plenary number F010

SA011

Human Neuralin-2, a Novel BMP Binding Protein, Acts as a BMP Antagonist *In Vitro* and *In Vivo*. J. K. McEntire*[1], E. Su*[2], J. K. Cecil*[2], S. Biolsi*[1], P. Grealish*[1], G. Krishnan[2], M. White*[1], H. Bullock*[2], R. Miles*[2], R. C. Smith*[1]. [1]BioTechnolgies Discovery Research, Eli Lilly and Company, Indianapolis, IN, USA, [2]Gene Regulation and Bone, Eli Lilly and Company, Indianapolis, IN, USA.

The Bone Morphogenetic Proteins (BMPs) have been characterized as essential molecules in embryonic development, bone biology, cartilage biology, and an ever-growing variety of other systems. Modulation of BMPs and other members of the TGF super family are important for differentiation and homeostasis of bone and cartilage tissue. A large family of BMP modulating proteins has been identified including chordin, gremlin, DAN, neuralin-1, CRIM-1, and procollagen IIA. These molecules act as potent BMP antagonists by directly binding BMPs and inhibiting their receptor interaction. We have mined a human cDNA, which we call neuralin-2 due to its high sequence conservation with a mouse Genbank cDNA sequence annotated as neuralin-2. Human neuralin-2 is structurally related to neuralin-1 [(identified as a molecule expressed in the mouse neural plate) Coffinier *et al*. Mech of Dev., 2001, 100:119-122]. Both neuralin-1 and neuralin-2 contain three of the conserved cysteine-rich regions shown in other BMP antagonists to bind various members of the TGF-beta superfamily. Neuralin-2 has a restricted expression pattern in mouse and human tissues. Tissues of expression include heart, bone, and skeletal muscle. *In vitro* binding studies demonstrate that neuralin-2 is able to bind BMP-2 and -4. As predicted for a BMP antagonist, microinjection of neuralin-2 RNA into the ventral side of developing Xenopus embryos causes twinning and hyperdorsalization. Neuralin-2 is able to inhibit the ability of BMP-4 to induce osteoblast differentiation in mouse mesenchymal stem cells *in vitro*. In summary, neuralin-2 is a novel member of a growing family of proteins containing cysteine-rich regions, that function to modulate various members of the TGF-beta superfamily. Neuralin-2 as well as other BMP modulating proteins may be potential therapeutic targets for various bone and cartilage disorders.

Disclosures: ***J.K. McEntire****, Eli Lilly and Company 3.*

SA012

See Friday Plenary number F012

SA013

Brief rhBMP-2 Treatment of Glucocorticoid-inhibited MC3T3-E1 Osteoblasts Rescues Cell Cycle Progression and Mineralization in a Cbfa1-independent Manner. C. A. Luppen*[1], N. Leclerc*[1], T. Noh*[1], A. L. Boskey[2], E. Smith*[3], B. Frenkel[3]. [1]Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA, USA, [2]Mineralized Tissues Research Section, Hospital for Special Surgery, New York, NY, USA, [3]Orthopaedic Surgery, University of Southern California, Los Angeles, CA, USA.

Glucocorticoids (GC) inhibit bone formation in vivo. In MC3T3-E1 osteoblasts, chronic administration of 1 μM dexamethasone (DEX) starting at confluency (day 3) results in >98% inhibition of BMP-2 gene expression and apatite mineral deposition. The GC-inhibition of mineralization is counteracted by chronic co-treatment with rhBMP-2, starting on either day 3 or 5. We show here that brief exposure to rhBMP-2 is sufficient to induce irreversible commitment to mineralization. MC3T3-E1 cells were treated with DEX on days 3-14 and rhBMP-2 was administered on day 5 for 15 minutes to 12 hours. Remarkably, 6 hour exposure to 100 (but not 10) ng/ml rhBMP-2 completely rescued mineralization as demonstrated by Alizarin Red staining. Calcium accumulation was accelerated in DEX/rhBMP-2 co-treated cultures even compared to control. The rhBMP-2 rescue of DEX treated cultures was further characterized in terms of collagen accumulation; mineral-to-matrix ratio (FTIR); alkaline phosphatase (ALP) enzymatic activity; Cbfa1 DNA binding (gel shift); Cbfa1 transcriptional activity (luciferase assay); and cell cycle progression (flow cytometry). DEX inhibited collagen accumulation by >80% on days 6-14. Unlike mineralization, rhBMP-2 did not rescue collagen in DEX treated cultures. Rescued cultures had higher mineral-to-matrix ratios compared to control, consistent with the selective rescue of mineralization. The rhBMP-2 rescue did not correlate with ALP activity. Surprisingly, neither DEX nor rhBMP-2 altered Cbfa1 DNA binding activity; and, neither affected Cbfa1 transcriptional activity. Finally, we studied the differentiation-related cell cycle, which persists during commitment to mineralization in untreated, but not DEX treated, cultures. Although rhBMP-2 alone, like DEX, was antimitogenic, rhBMP-2 rescued the differentiation-related cell cycle in DEX inhibited cultures. In conclusion, rhBMP-2 rescues mineralization in DEX inhibited MC3T3-E1 osteoblasts via a mechanism other than Cbfa1 stimulation. The concomitant rescue of the differentiation-related cell cycle offers promising avenues in the study of Cbfa1-independent mechanisms of GC and BMP action in osteoblasts. The efficacy of short term BMP-2 treatment in GC inhibited osteoblasts supports the idea of using short lived BMP vectors for both traditional and gene therapeutic approaches.

Disclosures: ***C.A. Luppen****, None.*

SA014

See Friday Plenary number F014

SA015

Bone Formation Following OP-1 Implantation is Improved by Autogenous Bone Marrow. H. Takigami*[1], L. Latson*[1], D. Togawa*[2], T. W. Bauer*[3], K. Powell*[1], G. F. Muschler[2]. [1]Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH, USA, [2]Department of Orthopaedic Surgery, The Cleveland Clinic Foundation, Cleveland, OH, USA, [3]Departments of Orthopaedic Surgery and Pathology, The Cleveland Clinic Foundation, Cleveland, OH, USA.

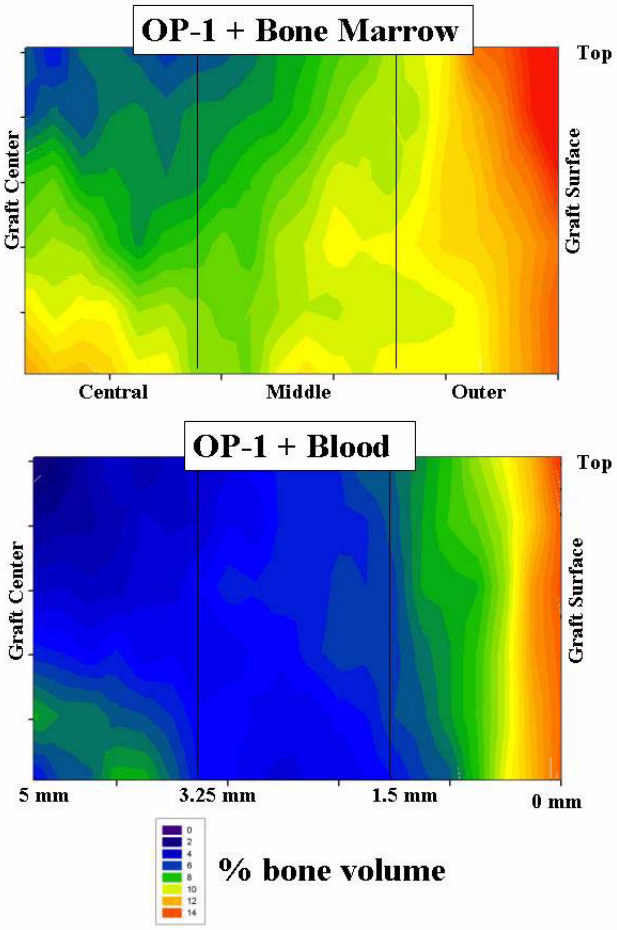
INTRODUCTION: Implantation of Osteogenic Protein-1 (OP-1, aka BMP-7) induces bone formation due to OP-1 activation of local cells. This study was designed to test the hypothesis that increasing the target cell population for OP-1 by addition of autogenous bone marrow (BM) containing osteogenic progenitors will increase in bone formation when OP-1 is used as a graft material.

METHODS: Seven beagle dogs underwent grafting. Four 1.0 cm diameter x 1.0 cm long cylindrical drill defects were made in the left proximal femur extending through the lateral femoral cortex into the intramedullary canal. Each site was separated by 1.5 cm of normal bone and intramedullary marrow tissue. OP-1 implants were mixed with an equal volume of clotted blood or with BM aspirated from the proximal humerus. MicroCT images were obtained (~18 micron resolution) at 4 wks. The percent bone volume (%BV) was measured within each defect in 3 dimensions. The %BV was compared in the outer, middle, and central thirds of the grafted cylinders.

RESULT: In the two most proximal defect sites, OP-1+BM (Fig.1) had significantly greater %BV than OP-1+Blood (Fig. 2) (mean 10.5 % vs 7.5 %, p=0.03). This difference was most evident in the middle third of the defect, between 1.5 and 3.25 mm from the graft surface ($p<0.01$). In the two distal sites, where cancellous bone is not normally found, little bone was formed in either group and there were no differences between groups.

DISCUSSION: Addition of BM containing osteogenic cells significantly increased bone formation at the site of OP-1 implantation, especially in the region 1.5 to 3.25 mm from the surface of the defect.

CONCLUSIONS: These data support further investigation of the clinical use of bone marrow aspirates as a means of optimizing the efficacy of OP-1 device implantation. These also support the concept that bone formation in response to OP-1 implantation is site dependent and influenced by the local cell environment.



Disclosures: ***H. Takigami****, None.*

SA016

See Friday Plenary number F016

SA017

Conditional Alk-3 Deletion Enhances Cancellous Bone Mass in Long bone and Hypertelorism in the Skull of Adult Mice. C. Chung*[1], T. Maruyama*[2], T. Manabe*[2], T. Miwa*[3], Y. Mishina*[4], K. Nishimori*[2], M. Noda[1]. [1]Molecular pharmachology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Molecular and Cellular Biology, Tohoku University, Sendai, Japan, [3]Genome Information Research Center, Oosaka University, Oosaka, Japan, [4]Laboratory of Reproductive & Developmental Toxicology, NIEHS, Research Triangle Park, NC, USA.

BMP signaling is mediated by 2-types of Ser/Thr kinase receptor(type I and type II), while the specificity of signaling is primarily determined by type I receptor. Alk 3(BMPR-IA) is classified as a BMPR-I receptor, however it's functions for BMP signaling, especially in the adult mice is not fully understood because of the lethal phenotype of the null mice. To investigate the role of BMP/BMPR signaling, conditional knock out mice were created. In these mice, Alk3 was deleted by crossing with Cre-expressing mice using the Cre/lox p system. The Alk3 (fx/) mice were viable and survived normally as it's control littermates. However the conditional knock out mice revealed shorter limbs, smaller body size, and shortening of the midface with hypertelorism suggesting a defect in the cranial sutures. Even though the sagittal suture was still patent when examined by micro CT, the bone morphology around the suture area was less bulky compared to the WT control. Further examination on the bone using soft-x-ray and micro CT revealed significant increase ($p<0.05$) in cancellous bone volume (Bone volume/Tissue volume) in the metaphyseal regions of long bone. Calvarial sutures were less distinct compared to the wild type. To investigate the mechanism of increase in bone volume (BV/TV) in the conditional knockout mice, bone marrow cells were harvested from femur and tibia, and *in vitro* ostoeclastogesis as well as mineralized nodule formation were conducted to evaluate the osteoblastic activity of the bone marrow cells. No major alteration was observed in the cells from conditional knockout mice in terms of osteoclast development and mineralized nodule formation compared to those from wild type. These observations revealed that Alk3 paradoxically acts as an inhibitor for the levels of cancellous bone mass in adult mice.

Disclosures: ***C. Chung****, None.*

SA018

Bone Formation During Distraction Osteogenesis is Enhanced by Thrombin Peptide (TP508). G. Li[1], J. T. Ryaby[2], D. H. Carney*[3], H. Wang[2]. [1]Department of Trauma and Orthopaedic Surgery, Queen's University of Belfast, Belfast, United Kingdom, [2]Research Department, OrthoLogic Corp., 1275 West Washington Street, Tempe, AZ, USA, [3]R&D Department, Chrysalis BioTechnology, 2200 Market Street, Suite 600, Galveaton, TX, USA.

The thrombin-related peptide, TP508 is a synthetic 23 amino acid peptide, which represents the receptor-binding domain of thrombin. TP508 interacts with specific thrombin receptors on cells to initiate tissue repair. It has been demonstrated that TP508 enhances revascularization of injured tissue and promotes wound healing, cartilage and fracture repair. The long bone consolidation phase of distraction osteogenesis causes considerable morbidity for the patients. The aim of this study was to examine the effect of TP508 on bone regeneration during distraction osteogenesis in a rabbit leg-lengthening model.

Unilateral tibial osteoectomies were performed and stabilised with MX100 Orthofix lengthener in male adult NZW rabbits. Following a latency period of 7 days, distraction was initiated at rate of 1.4 mm / day (in one step) for 6 days. TP508 (100 µg/kg and 10 µg/kg) or saline (300 µl) were injected into the osteotomy / lengthening gap at day 1, day 8 and day 13 post-surgery. Radiography was taken regularly and animals were sacrificed at day 27 post-surgery. The regenerates were assessed by pQCT and paraffin histology.

The radiographic evaluation indicated a significant increase in new bone formation in the distraction regenerates in the TP508 treated groups, compared to the controls post-lengthening. pQCT images at 2 weeks post-lengthening demonstrated a greater bone formation in the TP508 treated groups compared to the controls. The mean bone mineral density (BMD) of the regenerate was significantly greater ($p<0.01$, t-test) in the TP508 treated groups (100 µg/kg, BMD = 498.15 ±19.39 mg/ccm; 10 µg/kg, BMD= 448.65 ±10.39 mg /ccm) than that in the controls (BMD = 355 +/- 2.83 mg/ccm). There was no statistical difference in the BMD between the two treated groups ($p = 0.086$, t-test). For histological evaluation, in the 100 µg/kg treated group, the regenerates were mainly filled with well-vascularized woven bone. In the10 µg/kg treated group, 60-70% of the regenerate was woven bone, and the fibrous tissues and cartilage were seen in the centre of the regenerates. While in the saline control group, the regenerates were mainly fibrous tissues and cartilage with small amount of woven bone present.

This study has demonstrated that TP508 has enhanced bone formation during distraction osteogenesis in the rabbit. The findings indicate that TP508 can be used in accelerating osteogenesis as well as angiogenesis in clinical procedures such as bone lengthening and high-energy fracture treatment.

Disclosures: ***G. Li****, OrthoLogic, 1275 West Washington Street, Tempe, USA 2.*

SA019

Biomineralisation Markers During a Phase of Active Growth in Pinctada Margaritifera. M. Rousseau*[1], C. Milet*[1], E. Plouguerné*[2], E. Lopez*[1], M. Fouchereau-Peron*[2]. [1]Department of Aquatic Environments and Populations, Museum National Histoire Naturelle, Paris, France, [2]Department of Aquatic Environments and Populations, Museum National Histoire Naturelle, Concarneau, France.

In order to search for the biochemical parameters involved in calcium and carbonate transport during crystal formation and biomineralisation in nacreous mollusca, the carbonic anhydrase activity , the levels of calciotropic hormones (CT and CGRP) in hemolymph and in tissues and the circulating concentration of calcium was measured in *Pinctada margaritifera* during a phase of active growth.

When carbonic anhydrase activity was measured both in mantle and gill tissue, an increased linear relationship was observed between this enzyme activity and the age of the animals. No variation of this enzyme activity appeared in the mantle.

Age, months	Gill carbonic anhydrase activity, U / mg of proteins	Mantle CGRP-LI, pg / mg of proteins	Circulating CGRP-LI, pg / ml	Total calcium, mM
7	4.5 ± 0.7			
12	7.5 ± 0.5*			
18	6.7 ± 0.2*			
24	11.6 ± 1.5*	189 ± 20	394 ±25	11.5 ± 0.2
36	17.3 ± 3.8*	158 ± 42	503 ± 42	11.3 ± 0.1
48	15.8 ± 2.4*	111± 20*	265 ± 93*	12.3 ± 0.3*

• $p< 0.05$ when compared to the 7 (carbonic anhydrase activity) or 24 (CGRP and calcium concentrations) months group.

Similarly, the circulating level of total calcium increased during the growth of the animals.

Calciotropic hormones were radioimmunoassayed (using an anti salmon CT and an anti human CGRP antibody) in gill, mantle and hemolymph. Only a CGRP-related molecule could be detected. The concentration of this molecule decreased as a function of growth both in hemolymph and in mantle. No variation of this parameter was observed in gill tissue. These immunologically CGRP related molecules were able to interact with the specific CGRP receptors present in rat liver membranes and represent biologically CGRP related molecules.

These data demonstrate that carbonic anhydrase and a molecule biologically and immunologically related to CGRP are involved during the growth of the animals. In addition, this study shows the presence of three main calcium compartments, gill, hemolymph and mantle, involved in the biomineralisation process.

Disclosures: ***C. Milet****, None.*

SA020

See Friday Plenary number F020

SA021

Roles of Lysyl Hydroxylase 2 in Collagen Cross-linking and Mineralization. S. Pornprasertsuk*, W. R. Duarte, Y. Mochida*, M. Yamauchi. Collagen Biochemistry Laboratory, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

The hydroxylation of specific lysine (Lys) residues is a post-translational modification critical for glycosylation and cross-linking of collagen. At present, three isoforms of lysyl hydroxylase (LH1, 2, 3), the enzyme that catalyzes this modification, have been identified but their precise roles are still unclear. Based on our previous findings (J Bone Min Res 14:1272-1280, 1999), we hypothesized that *LH2* may regulate the telopeptidyl Lys hydroxylation of collagen, i.e. cross-linking, and collagen mineralization. In order to test this hypothesis, several osteoblastic cell clones constitutively overexpressing *LH2* were generated and characterized. The full-length mouse *LH2* cDNA was ligated into pcDNA3.1/V5-His TOPO vector, and then transfected into MC3T3-E1 cells to generate stable clones overexpressing *LH2*. An empty pcDNA3.1/V5-His vector (Ev) was also transfected into MC3T3-E1 cells and used as a control. The levels of *LH2* in several clones were determined by immunoprecipitation followed by Western blot analysis using antiV5 antibodies. MC3T3-E1 cells, clones overexpressing *LH2* and Ev clones were cultured in the presence of 50 µg/ml ascorbic acid and 2 mM βglycerophosphate for up to 4 weeks and, at several time points, analyzed for the mineralized nodule formation, Lys hydroxylation, and cross-linking of collagen. The expression of type I collagen (COLI), osteocalcin (OCN) and bone sialoprotein (BSP) mRNAs was evaluated by RT-PCR. In both MC3T3-E1 cells and Ev clones, mineralized nodules were formed at 2 weeks and their number increased thereafter. The ratio of two major immature reduced collagen cross-links, dihydroxylysinonorleucine (DHLNL) to hydroxylysinonorleucine (HLNL), from the control cells was 3.4 and 2.7 at 2 and 4 weeks, respectively, similar to murine bone collagen. In comparison with the control groups, the formation of mineralized nodules by *LH2*-overexpressing clones was significantly delayed and the number of nodules formed was fewer. In the clone expressing the highest level of *LH2*, the mineralized nodules were not formed even after 4 weeks of cell culture. Furthermore, the ratio of DHLNL to HLNL in the overexpressing clones was markedly higher (6.3-9.5 and 7.2-10.6 at 2 and 4 weeks, respectively) than that of the control cells. The Lys hydroxylation in the helical domains of the collagen molecules (both α1 and α2 chains) appears to be unaffected. The expression of COLI, OCN, and BSP mRNAs was comparable among clones and control groups. These results indicate that *LH2* may regulate the telopeptidyl Lys hydroxylation of collagen, cross-linking and subsequent matrix mineralization *in vitro*.

Disclosures: ***S. Pornprasertsuk****, None.*

SA022

Identification and Characterization of Spherical Structures Associated with Mineralization in MLO-A5 Cells. C. Barragan-Adjemian*[1], D. Eick*[1], V. Dusevich*[1], M. Dallas*[1], D. Nicolella[2], L. Bonewald[1]. [1]Oral Biology, University Missouri Kansas City, Kansas city, MO, USA, [2]Southwest Research Institute, San Antonio, TX, USA.

There has been a long-standing controversy as to whether mineralization of bone occurs through extracellular matrix vesicles, exclusively through collagen type 1 and more recently this controversy has expanded to include non-collagenous proteins. The role of matrix vesicles during initiation of calcification has been well characterized in the growth plate. Matrix vesicles are submicroscopic extracellular membrane particles that initiate formation of crystals through the action of phosphatases, calcium binding phospholipids and other proteins. These preformed crystals are exposed to the extracellular matrix, where they grow and expand. However, the presence and function of matrix vesicles in bone mineralization is inconclusive.

The goal of the present study was to determine the mechanism of mineralization used by MLO-A5 cells. These cells mineralize in 3-6 days in culture and are considered to be a good model for the study of mineralization *in vitro*. These cells express large amounts of alkaline phosphatase, approximately 7 to 9 times more than primary osteoblasts, and form a mineralized honey-combed structure in sheets, not nodules. Between 3-6 days in culture, spherical structures (30 nm) were observed by scanning electron microscopy localized on the cytoplasmic membrane of cells that at 6-9 days become associated with collagen fibers. These spherical structures grow in size (up to 1 um) and coalesce following the pattern of collagen type 1 fibers. The spherical structures are of high density as determined by backscatter detection. The content of these spherules was determined to be phosphate and calcium in ratios similar to hydroxyapatite as determined by X-ray energy dispersive analysis. Using transmitted electron microscopy, the spherical structures show needle-like crystal extensions. By atomic force microscopy, again spherical structures were shown to be associated with the collagen fibrils. Collagen mediated mineralization was also observed at later time points in culture. In conclusion, MLO-A5 cells mineralize by three mechanisms. In addition to collagen-mediated mineralization and a small amount of dystrophic mineralization, a major mechanism whereby MLO-A5 cells mineralize is through the production of spherical structures that may be matrix vesicles. This data supports the hypothesis that bone can mineralize through other mechanisms than through collagen.

Disclosures: ***C. Barragan-Adjemian****, None.*

SA023

Bone Mineral Concentration and Appositional Rate in Rapidly Growing Chick Models of Homocystinuria and Vitamin B6 Deficiency (VB6D). P. G. Masse[1], P. G. T. Howell[2], A. Boyde[3], D. E. C. Cole[4], D. S. Howell[5]. [1]Nutrition, Université de Moncton, Moncton, NB, Canada, [2]Eastman Dental Institute, University College London, London, United Kingdom, [3]Anatomy, University College London, London, United Kingdom, [4]Pediatrics (Genetics), University of Toronto, Toronto, ON, Canada, [5]Medecine, University of Miami/VA Medical Center, Miami, FL, USA.

Collagen (Coll) molecular defect associated to hyperhomocysteinemia (0.6% dl-homocysteine-rich diet) and VB6D has been evidenced by TEM/SEM. Changes in coll solubility and coll cross-linking were hallmarks of connective tissue abnormality in both cases. The coll defect has been attributed to the formation of a thiazine ring complex formed from the sulhydryl groups in excess (due to elevations of sulfur amino acids) with the aldehydes generated by lysyl oxidase deamination of lysine (lys) and OH-lys residues on coll molecules. Experimentally-induced homocystinuria was characterized by distinctive alterations of bone growth and skeletal development, including a reduction in bone density and greater radial and longitudinal bone growth. Bone length and diaphyseal diameter of VB6D-animals were not different from controls but dry weight and bone density of tibias were reduced. In the present study, the effect of homocysteine and VB6D on the mineral apposition rate (MAR) and degree of mineralisation of bone was examined. 70% ethanol fixed tibias were cut as 2-3mm thick slices and embedded in PMMA, trimmed in blocks and micromilled to extreme flatness. Block surfaces were imaged using confocal fluorescence microscopy to visualize tetracycline and alizarin labels given at a 48 hour interval. After carbon coating, they were studied by quantitative backscattered electron imaging (qBSE, 20kV, 0.5nA, 17mm working distance, annular solid state BSE detector) using monobrominated and monoiodinated dimethacrylates as standards. Calcified cartilage residues in bone were excluded from this analysis. MAR in trabecular bone of the metaphysis was lower in the VB6D group (4.15 ± 0.72µm/day) compared with control (4.65 ± 0.64 µm/day) and hyperhomocysteinemic groups (4.72 ± 0.53µm/day), but on a per animal basis, this was not significant. The degree of mineralisation of metaphyseal trabecular bone was significantly different between groups, being significantly lower in hyperhomocysteinemic and higher in VB6D than controls ($p < 0.001$). Bone in hyperhomocysteinemia is formed more rapidly and is less well mineralised than controls. Conversely, bone in VB6D is formed more slowly and is better mineralised than controls.

Disclosures: ***P.G. Masse****, None.*

SA024

Multi-mode X-ray Investigation of Bone in Regenerated Newt Limbs. S. R. Stock[1], K. Ignatiev*[1], H. G. Simon*[2], F. De Carlo*[3], J. D. Almer*[3]. [1]Institute of Bioengineering and Nanoscience in Advanced Medicine, Northwestern University, Chicago, IL, USA, [2]Dept. of Pediatrics and CMIER, Northwestern University, Chicago, IL, USA, [3]Xor-aps, Argonne National Laboratory, Argonne, IL, USA.

Limbs regenerate in adult newts and salamanders; uncovering how regeneration can be "turned back on" in mammals is of great interest. One aspect of which has been largely neglected is the study of bone formation, specifically the amount and organization of the mineral phase and its spatial distribution with time since the onset of regeneration. This study applies lab and synchrotron microCT and synchrotron microbeam diffraction/scattering to map the mineral and its spatial variation in newt limbs after regeneration (periods between 37 and 85 days) and in controls (contralateral limb from the same animal). We find that the missing limb skeletal elements are restored in a proximal to distal direction, which reiterates the developmental patterning program, except that ossification of the humerus distal to the amputation site lags behind that of the regenerating radius and ulna, metacarpals and phalanges and that carpal bone ossification is still further delayed. Local mineral levels track the macroscopic pattern of mineral onset. Synchrotron microCT of newt hand bones (control limbs) reveals an unexpectedly rich structure and indicates bone in this amphibian is more complex than generally thought. Microbeam diffraction/scattering mapping of the mineral constituent is demonstrated and offers the promise of in vivo mapping of mineral acquisition in individual newts as a function of regeneration time.

Disclosures: ***S.R. Stock****, None.*

SA025

Atorvastatin Inhibits Hypercholesterolemia Activation of Cell-Cell Interactions in the Aortic Valve to Induce Cellular Proliferation and Bone Matrix Production. N. M. Rajamannan[1], M. Subramaniam[2], R. S. Bhattacharyya*[3], T. C. Spelsberg[2], P. H. Stern[3]. [1]Cardiology, Northwestern University, Chicago, IL, USA, [2]Molecular Biology and Biochemistry, Mayo Clinic, Rochester, MN, USA, [3]Dept of Pharmacology, Northwestern University, Chicago, IL, USA.

Calcific aortic stenosis involves proliferation and bone matrix expression of the aortic valve. Hypercholesterolemia (chol) is a risk factor for aortic stenosis, but the signaling steps in this process are not known. We postulate that LDL chol inhibits nitric oxide synthesis in the aortic valve endothelial cells (AEC) and that the subendothelial myofibroblast cells (SEC) respond with proliferation and bone matrix production via the MAP kinase pathway. Previously we have shown that PDGF is produced from AEC. We developed an in vitro AEC/SEC cell/cell model to test this hypothesis. Methods: Conditioned media (CM) was collected from AEC treated with serum free (SF) media, LDL chol (10ug/ml), and/or atorvastatin 10-5 M to produce conditioned media (CM). The AEC and the CM were tested for endothelial nitric oxide synthase (eNOS) expression by Western immunoblotting. The CM effects on proliferation and osteopontin (OPN) expression of the SEC were assessed by measurement of 3H-thymidine (3H) incorporation and Western blot. To determine the pathway for the proliferation by CM, effects of PDGF on the SEC on the (3H) incorporation and expression of p42/44 MAP kinase (MK) and was tested by Western blot. The effects of a MK inhibitor PD98059 (PD) on the (3H) and p42/44 expression were assessed. Results: eNOS protein expression were decreased by LDL cholesterol (2-fold) and increased by atorvastatin in the AEC (2-fold). CM from AEC exposed to LDL cholesterol markedly increased (3H) and OPN expression 150%($p<0.05$) whereas CM from the Atorvastatin+LDL treated cells inhibited proliferation and OPN expression by 50% ($p<0.001$). Addition of PDGF stimulated proliferation of the SEC (300% $p<0.001$). PD inhibited proliferation elicited by the PDGF (100%) and p42/44 protein expression (3-fold) ($p<0.001$). Conclusions: These results suggest the AEC cell signals the mitogenic activity and bone matrix expression of aortic valve SEC cells via 2 signaling mechanisms: 1) LDL cholesterol eNOS expression in the AEC, resulting in proliferation and bone matrix production and atorv inhibited this process 2) growth factors produced by the AEC, including PDGF, stimulate proliferation via the MAP kinase pathway.

Disclosures: ***N.M. Rajamannan****, None.*

SA026

Involvement of the Nuclear Factor I (NFI) Family of Trascription-Replication Factors in Chondrogenic Differentiation of ATDC5 Cells: Application of Gene-Trap Mutagenesis. T. Uchihashi*[1], M. Watanabe*[1], M. Yamagata*[1], A. Suzuki*[1], H. Kondou*[1], K. Ozono[2], T. Michigami[1]. [1]Department of Environmental Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan, [2]Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan.

Gene-trap mutagenesis is a genome-wide approach utilized to clarify the gene function based on an idea that random insertion of a trapping vector may disturb the function of inserted genes. In the current study, to identify the molecules involved in chondrocyte differentiation, we applied this approach to the murine mesenchymal cell line ATDC5 cells, which differentiate into mature chondrocytes in the presence of insulin. As the trap vector, we used pPT1-geo, which lacks its own promoter and enhancer, but contains lacZ-neo fusion gene as the reporter and the selection marker. After introducing pPT1-geo into ATDC5 cells by electroporation, the neomycin-resistant clones were screened for high beta-galactosidase activity, and the selected clones were then cultured in the differentiation medium to evaluate the chondrogenic phenotype by alcian blue and alizarin red staining. One of the clones, named UT7-57, exhibited accelerated mineralization compared with parental ATDC5 cells. The expression of type II collagen reached the maximum level earlier in this clone compared with the parental cells. In addition, the expression of chondromodulin-I was decreased in the clone. 5'RACE analysis revealed that pPT1-geo was inserted in the intron 6 of the nuclear factor I-B (NFI-B) gene in the clone. NFI-B belongs to the nuclear factor I (NFI) family of transcription-replication factors, and has been reported to express around developing cartilage. We detected the expression of all the members of the family (NFI-A, -B, -C and -X) in parental ATDC5 cells by RT-PCR. In the clone UT7-57, the trapped allele of NFI-B was assumed to express a fusion protein containing the N-terminal region of NFI-B and lacZ-neo product instead of the C-terminal functional domain of NFI-B, which was confirmed by Western blotting using the antibody against beta-galactosidase. It was speculated that the resulted mutant protein might act as a dominant-negative form against NFI family members. Northern blot analysis demonstrated that the expression of wild -type NFI-B transcript diminished in the process of differentiation of the clone UT7-57, while it persisted in the parental ATDC5 cells. These data suggested the involvement of NFI family transcription factor(s) in chondrogenic differentiation of ATDC5 cells.

Disclosures: ***T. Uchihashi****, None.*

SA027

See Friday Plenary number F027

SA028

CTGF/Hcs24/CCN2, Hypertrophic Chondrocyte-Specific Gene Product, Interacts with Perlecan in Regulating the Proliferation and Differentiation of Chondrocytes. T. Nishida[1], S. Kubota*[1], T. Fukunaga*[2], S. Kondo*[1], G. Yosimichi*[1], T. Nakanishi*[1], T. Takano-Yamamoto*[2], M. Takigawa[1]. [1]Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan, [2]Department of Orthodontics, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan.

Connective tissue growth factor/hypertrophic chondrocyte-specific gene product 24/CCN family member 2 (CTGF/Hcs24/CCN2) plays important roles in the control of the proliferation and differentiation of chondrocytes *in vitro*. To clarify the mechanisms of regulation by CTGF/Hcs24 with respect to cartilage metabolism, we investigated the interaction between CTGF/Hcs24 and heparan sulfate proteoglycan perlecan. An immunofluorescence study showed that CTGF/Hcs24 was colocalized with heparan sulfate and perlecan in human chondrosarcoma-derived chondrocytic cell line HCS-2/8 *in vitro*. Northern blot analysis showed that perlecan, syndecan-1, -2, and -4 transcripts were detected in HCS-2/8 cells. Particularly, expression of the perlecan gene increased markedly in HCS-2/8 cells by rCTGF/Hcs24 treatment. We also found that CTGF/Hcs24 interacted with perlecan from HCS-2/8 cells *in vitro*. Furthermore, CTGF/Hcs24-stimulated gene

expression of the aggrecan gene, as well as DNA/proteoglycan synthesis, was diminished when HCS-2/8 cells were pretreated with heparinase, indicating that the effects of CTGF/Hcs24 on chondrocytes occurred through the interaction between CTGF/Hcs24 and heparan sulfate on the cells. An *in vivo* study using mouse growth plate revealed that CTGF/Hcs24 produced by hypertrophic chondrocytes was localized from the proliferative to the hypertrophic zone, whereas perlecan was predominantly localized in the prehyphertrophic zone. Consistent with such findings *in vivo*, the binding of ^{125}I-rCTGF/Hcs24 to maturing chondrocytes was at higher levels than that to chondrocytes in hypertrophic stages. These findings suggest that CTGF/Hcs24 produced in the hypertrophic region may act on chondrocytes in the proliferative and maturative zone via some heparan sulfate proteoglycan, such as perlecan.

Disclosures: ***T. Nishida****, None.*

SA029

See Friday Plenary number F029

SA030

LIMBIN, a Gene Required for Normal Lengthening of Limbs, Is Expressed in Chondrocytes in Culture and its Levels Are Down-Regulated by BMP2. Y. Maeda[1], H. Takeda*[2], K. Tsuji[1], A. Nifuji*[1], T. Tsuji*[3], T. Kunieda*[4], M. Noda[1]. [1]Dept of Molecular pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Shirakawa Institute of Animal Genetics, Fukushima, Japan, [3]Department of Oral Morphology, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan, [4]Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan.

LIMBIN (LBN) is a newly identified molecule whose truncation mutation results in short limb phenotype in bovine chondrodysplastic dwarfism (BCD). Structural analysis of LBN revealed the presence of the putative trans-membrane domain and two coiled- coil domain as well as nuclear localization signals. However, how Lbn regulates cartilage and bone metabolism is not known. This paper examines whether bone morphogenetic protein (BMP) regulates expression of this novel gene in chondrocytes. In situ hybridization revealed strong Lbn expression in calvaria and the perichondrium of the developmental vertebrae in day 14.5 mouse embryos. Lbn mRNA expression was detected in the primary cultures of chondrocytes obtained from newborn mice rib and BMP treatment suppressed its expression by about 50% within 24 hours. This effect lasted up to 48hours. Cyclohexamide alone did not significantly suppressed Lbn mRNA expression. However, in the presence of cyclohexamide, BMP no longer suppressed Lbn gene expression suggesting the requirement for new protein synthesis. The basal levels of Lbn gene expression were not altered by the presence of DRB, a transcription inhibitor, while BMP treatment failed to suppress Lbn gene expression in the presence of DRB indicating that BMP suppression of Lbn gene mRNA is not due to the reduction in the stabilization of the transcript. TGFβ treatment similarly suppressed Lbn gene expression in the chondrocyte cultures. Co-treatment with BMP and TGFβ did not further suppressed the levels of the Lbn revealing that common pathways for these two cytokines may exist in terms of their suppression of the Lbn gene expression. These data indicated that this novel regulator, Lbn, is under the control of BMP and TGFβ.

Disclosures: ***Y. Maeda****, None.*

SA031

Differential Gene Expression Analysis using Paraffin Embedded Tissues after Laser Capture Microdissection. J. Kim*[1], M. Hwang*[1], Y. Kim*[1], H. Kim*[1], E. Park[1], S. Kim[1], R. Park*[1], H. Shin[2], I. Kim*[1], A. J. van Wijnen[3], J. B. Lian[3], G. S. Stein[3], J. Choi[1]. [1]Biochemistry & SDGRC, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, [2]Pathology & SDGRC, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [3]Cell Biology, UMASS Medical School, Worcester, MA, USA.

Recent advance in laser capture microdissection allows to precise removal of pure cell populations from morphologically preserved tissue sections. However, the RNA from the paraffin embedded sample after microdissection is usually degraded. The purpose of this study was to determine the optimal fixative for RNA extraction from the paraffin-embedded sample after the laser microdissection. The integrity of RNA was evaluated with the intactness of 18S and 28S ribosomal RNA by electrophoresis and the length of individual gene transcript by RT-PCR. The various fixatives were ethanol, isopropanol with several concentration and methacarn that was combination of methanol, chloroform and acetic acid. Among fixatives, methacarn was the best for RNA preservation from paraffin-embedded tissues including liver, lung, kidney, muscle, and limb. In RT-PCR analysis, methacarn showed a comparable length of RNA for individual genes such as glyceraldehyde-3-phosphate-dehydrogenase and bone related genes, alkaline phosphatase and osteonectin. The laser microdissection with methacarn fixation technique was applied to analyze the differential gene expression between hypertropic and proliferative chondrocytes in mouse growth plate. The expression of type X collagen, a specific gene for hypertrophic chondrocyte, was observed only in hypertrophic chondrocytes while type II collagen was observed more broadly in growth plate. The laser microdissection combined with the methacarn fixation technique will facilitate the examination of differentially expressed genes from various tissues.

Disclosures: ***J. Choi****, None.*

SA032

See Friday Plenary number F032

SA033

Determining Gene Expression Patterns in Biglycan Deficient Pre-Osteoblasts Using Oligonucleotide Microarrays. X. Chen[1], X. Bian*[2], L. Yang*[3], T. Teslovich*[4], D. Stephan*[4]. [1]Csdb, NIDCR, NIH, Bethesda, MD, USA, [2]Ccr, NCI, NIH, Bethesda, MD, USA, [3]Cbel, CIT, NIH, Bethesda, MD, USA, [4]Rcgm, Children's Hospital, Washington, DC, USA.

Mice deficient in biglycan, a small leucine-rich proteoglycan (SLRP), have a defect in the proliferation and differentiation of osteoblast precursors. In order to identify a set of genes that plays a key role in this abnormality, we determined the global gene expression patterns in bgn-deficient (bgn-KO) pre-osteoblasts using microarray technology. Calvarial cells were harvested from newborn mice and cultured in the presence or absence of BMP-4 for 7 days. The total RNA was labeled and hybridized to AffymetrixTM chips containing ~ 6000 genes, and analyzed with a software GeneSpringTM. Seventy-nine genes were selected, and applied hierarchical clustering to classify these genes according to the similarity of expression levels. Nine of those genes that had expression of at least a 1.5 fold difference between bgn-KO and wild type (Wt), and were grouped as two clusters. One cluster consisted of AU046135 (EST), osteoglycin (Ogn), preproenkephalin (Penk1), and aldehyde dehydrogenase (Aldh1), were expressed significantly lower in bgn-KO than in Wt, and did not respond to BMP-4 in either bgn-KO or Wt cells. Interestingly, mice deficient in Ogn (another SLRP family member) are very similar to the bgn-KO mice, having fragile skin due to defective collagen fibrillogenesis. Penk1 and Aldh1 are important for the differentiation of neonatal cardiac muscle cells, and the development of kidney, stomach and intestine, respectively. A second cluster consisted of osteoblast-specific factor 2 (Osf2), haptoglobin (Hp), secretory leukocyte protease inhibitor (Slpi) and protein synthesis initiation factor 4A (Eisf4a2), were low in bgn-KO cells, but highly up-regulated by BMP-4 compared to Wt cells. Osf2 is a cell adhesion molecule made by pre-osteoblasts thought to be involved in osteoblast recruitment, attachment and spreading, while Slpi is known to suppress inflammation and prevent joint damage. Eifa4 is highly expressed in brain, kidney, lung and heart, while the function of Hp is not clear. In addition, many more genes were stimulated by BMP-4 in bgn-KO cells compared to those in Wt (63 vs. 8). In conclusion, the display of global gene expression patterns presents a broad picture of gene regulation that may control the skeletal defects caused by bgn deficiency. The data also provides valuable new information for selecting novel relevant genes for further investigation. From a global standpoint, our data indicates that bgn deficiency may be involved in multiple organ defects that could partially be rescued by BMP-4.

Disclosures: ***X. Chen****, None.*

SA034

See Friday Plenary number F034

SA035

Up-Regulation of Vimentin, Ribosomal Protein L6 (RPL6) and Type I PTH Receptor (PTH1R) Genes by Intermittent PTH Treatment in MC3T3-E1 Osteoblast-like Cells: Use of Real-Time RT-PCR in Combination with Genearray Analysis. A. Iida-Klein[1], S. S. Lu[1], D. Bischoff[2], D. T. Yamaguchi[2], R. Lindsay[1]. [1]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Research and Development, Greater Los Angeles Healthcare System, Los Angeles, CA, USA.

We previously demonstrated that the intermittent but not continuous treatment with human parathyroid hormone 1-34 fragment (hPTH1-34) up-regulated expression of bone forming genes including type I procollagen alpha 1 (Col1A1), alkaline phosphatase (ALP) and type I PTH receptor (PTH1R) in mouse osteoblast-like MC3T3-E1 cells, assessed by RT-PCR (ASBMR, 2002). In order to further investigate what other genes are regulated by intermittent PTH treatment, we treated MC3T3-E1 cells with 10 nM hPTH1-34 by pulsing with hPTH1-34 for 6 hours, followed by 2x wash and an additional 42 hr incubation in serum containing, PTH-free medium. This treatment protocol was repeated for 6 cycles. Total RNA was extracted and purified (Qiagen, CA). After the quality and quantity of the purified RNA was determined by optical density and gel electrophoresis, RNA samples were subjected to the mouse Atlas array system (Clontech, CA), and the results analyzed using ImaGene software (4.1 version, BioDiscovery Inc., CA). Out of 1176 genes, vimentin and ribosomal protein L6 (RPL6) genes were highly up-regulated by intermittent PTH treatment in addition to several known bone-related genes such as Col1A1, Col1A2, cathepsin D and fibronectin in two independent experiments. Moreover, the PTH1R gene, whose expression is relatively low in the absence of exogenous PTH, appeared to be up-regulated by intermittent PTH but down-regulated by continuous PTH treatment. To confirm the gene array findings, real-time RT-PCR (rtRT-PCR) was employed using the same RNA samples used for gene array analysis in triplicates. Intermittent PTH tended to up-regulate (24%) but continuous PTH down-regulated (39%) expression of the PTH1R gene. Confirmation of the results using different primer pairs is currently being processed. Thus, use of real-time RT-PCR in combination with gene array analysis may be a useful tool to detect a subtle regulation of the low abundant genes as well as highly inducible genes.

Disclosures: ***A. Iida-Klein****, None.*

SA036

See Friday Plenary number F036

SA037

The Transient Receptor Potential Channels TRPV5 and 6 Are Differentially Expressed and Regulated in Osteoblasts and Osteoclasts. B. C. J. van der Eerden[1], H. Charif*[1], T. J. de Vries[2], H. A. P. Pols[1], R. J. M. Bindels*[3], J. P. T. van Leeuwen[1]. [1]Internal Medicine, Erasmus MC, Rotterdam, Netherlands, [2]Periodontology, ACDA, Amsterdam, Netherlands, [3]Cell Physiology, UMC, Nijmegen, Netherlands.

Recently, two new members of the transient receptor potential channel (TRP) family were identified, named TRPV5 and TRPV6, which were recognized as the gatekeepers of active Ca^{2+} transport in kidney and intestine. In analogy, Ca^{2+} transport is also crucial for bone (re)modeling. In this study, we investigated the expression of TRPV5 and 6 along with the known Ca^{2+} transport proteins calbindin (CaBP)-D_{9K} and -D_{28K}, plasma membrane Ca^{2+}-ATPase 1 (PMCA1) and the Na^{+}/Ca^{2+} exchanger 1 (NCX1) in osteoblasts and osteoclasts.

Human osteoblasts (SV-HFO) were cultured for 23 days with β-glycerophosphate (β-GP) in the absence or presence of 1 μM dexamethasone (DEX). Mouse osteoblasts (KS483) supplemented with ascorbic acid and β-GP, were cultured towards mineralization during 21 days. Human and mouse osteoclasts were derived from peripheral blood monocytes and primary bone marrow cultures, in the presence of M-CSF and RANKL during 5 and 21 days, respectively. At various timepoints during osteoblast cultures as well as at the end of the osteoclast cultures, total RNA was isolated, reverse-transcribed into cDNA and expression was studied using real-time PCR.

TRPV5 mRNA was undetectable in human and mouse osteoblasts. In contrast, TRPV6 mRNA was detected throughout differentiation of osteoblasts from both species. TRPV6 expression in human osteoblasts was confirmed by immunostaining. Moreover, TRPV6 mRNA expression in SV-HFO cells increased approximately 5-fold during the DEX-induced mineralization, while no change was observed in cultures without DEX. In analogy to TRPV6, CaBP-D_{28K} and NCX1 mRNA were upregulated in the presence of DEX at all stages but most prominently during mineralization (about 3-fold). In mouse osteoblasts, similar expression patterns were observed in relation to differentiation and mineralization. In human and mouse osteoclasts both TRPV5 and 6, NCX1 and PMCA1 mRNA were detected. In addition, both calbindins were detected in the mouse osteoclasts.

In conclusion, this is the first report to demonstrate mRNA expression of TRPV5 and 6 in bone cells. Interestingly, TRPV5 was only detected in osteoclasts and not in osteoblasts, while TRPV6 was expressed in both cell types, suggesting a cell type specific function. In summary, all intestinal and renal Ca^{2+} transporting proteins are present in osteoclasts and osteoblasts, supporting a role in the movement of Ca^{2+} to and from the bone. The expression of several of the genes indeed correlates with the mineralization of extracellular matrix.

Disclosures: ***B.C.J. van der Eerden****, None.*

SA038

See Friday Plenary number F038

SA039

Autoregulation of Bone Sialoprotine (BSP) in Pre-osteoblastic and Non-osteoblastic Cells. Q. Tu, J. Chen. Division of Oral Biology, Tufts School of Dental Medicine, Boston, MA, USA.

Bone sialoprotein (BSP), a phosphorylated and sulfated glycoprotein, represents one of the major non-collagenous, extracellular matrix proteins in bone and other mineralized tissues. BSP is believed to function in the formation and remodeling of the mineralized connective tissue matrix. Matrix components often affect cell function by interacting with members of the intergrin family of cell surface receptors. BSP has RGD sequences that mediate cell attachment through αvβ3 and αvβ5 integrins. Uncertainty, however, exists about the BSP coupled signaling pathway. It has been shown that Runx2/Cbfa1, a transcription factor in osteogenesis, may regulate BSP expression through multiple signal transduction pathways. In this study we report that BSP regulates its own expression through an autoregulative mechanism. We examined the expression of mouse BSP using a 9.0 kb murine BSP promoter linked to a luciferase reporter gene co-transfected with a murine BSP expressing vector, and the effect on transcription was analyzed in transient transfection assays. We found that the forced overexpression of BSP stimulated mouse BSP promoter activity in a dose-dependent manner in both MC3T3-E1 preosteoblast and HEK293 cell lines. The absence of endogenous Cbfa1 expression in HEK293 cells suggested that this stimulation and autoregulation was Cbfa1 independent. To further determine the signaling pathway of BSP autoregulation cells were cultured in the presence of PMA for 20 hours, which reduced PKC activity and almost abolished the luciferase reporter expression in HEK 293 cell line, but had little effect in MC3T3-E1 preosteoblasts. The results indicated that a PKC signaling pathway mediated the BSP autoregualtion. The different affect of reduced PKC activity in MC3T3 cells suggested that other regulatory factor(s) might be operative in MC3T3-E1 preosteoblasts. When HEK 293 and MC3T3-E1 cells were co-transfected with a CMV-Cbfa1 cDNA expression vector, the expression of Cbfa1 gene was increased together with the promoter activity of the BSP reporter construct. Notably, these changes were not inhibited by reduced PKC activity. These results suggest that endogenous expression of Runx2/Cbfa1 might exert a compensative effect since BSP-mediated regulation was not blocked by the reduced PKC levels in MC3T3-E1 cells. Taken together our results indicate that expression of BSP gene can be autoregulated by a positive feedback in a cell-dependent manner and this autoregulation is mediated by a PKC signaling pathway. Further investigation is underway to identify the promoter element(s) involved in this autoregulation. Supported by NIH/NIDCR Grant RO1 DE11088 and partially by PO1 DE13221.

Disclosures: ***Q. Tu****, None.*

SA040

Biglycan Reduces Osteoclast Differentiation. Y. Bi*[1], T. M. Kilts*[1], X. Chen[1], E. M. Greenfield[2], M. F. Young[1]. [1]NIDCR, NIH, Bethesda, MD, USA, [2]Department of Orthopaedics, CWRU, Cleveland, OH, USA.

Small leucine-rich proteoglycans (SLRPs) are extracellular molecules that bind to collagens and growth factors to regulate cell growth and matrix assembly. It has been previously shown that mice deficient in biglycan (bgn), a class I SLRP, develop an age-related osteopenia. This phenotype could be due to impaired bone formation and/or increased bone resorption. Bgn has been shown to play an important role in the proliferation, survival and differentiation of osteoblast precursors. Since osteoblasts and their precursors regulate the differentiation of osteoclasts through cell-cell and cell-matrix contact, we hypothesized that bgn is involved in regulating osteoclast differentiation. To test this, we used a murine calvarial model to compare the induction of osteolysis by LPS in wildtype (WT) and bgn knockout (KO) mice. Titanium particles, as a LPS-carrier, were implanted onto the parietal bones of each mouse. After the indicated periods of time, parietal bones were harvested and processed for X-ray using a Faxitron. The extent of osteolysis in each bone was determined from the X-ray image by computer-assisted histomorphometry. Time course experiments showed that LPS-induced osteolysis occurred more rapidly and extensively in bgn KO mice compared to WT mice. There was 55% less bone resorption in WT mouse parietal bones than that in bgn KO mouse parietal bones when osteolysis reached its peak at day 7. To further understand the mechanism, we determined the effects of bgn on osteoclast differentiation in vitro. For this purpose, we co-cultured spleen-derived osteoclast precursors with primary calvarial cells from either WT or bgn KO mice in the presence of 1a, 25-dihydroxyvitamin D3 (1,25-D3). Time course and dose response experiments showed that TRAP+ multinuclear cells (MNCs) appeared earlier and more extensively in the co-cultures containing calvarial cells from bgn KO mice than WT mice, regardless of the source of spleen cells. Thus, the effect of bgn on osteoclast differentiation is mediated by calvarial cells. Further studies using RT-PCR, northern blot, western blot and ELISA analyses showed that the expression of RANKL and OPG in WT calverial cells was not significantly different from bgn KO cells in the presence of 10 nM 1,25-D3. These data suggest that the increased osteoclast differentiation in the bgn deficient cell culture might occur via a novel RANKL/OPG-independent mechanism. Bgn has been shown to bind and regulate cytokines and growth factors in bone matrix. We propose that biglycan might down-regulate osteoclast differentiation and reduce bone resorption indirectly by modulating the production, storage and/or activity of cytokines that regulate osteoclasts and their precursors.

Disclosures: ***Y. Bi****, None.*

SA041

See Friday Plenary number F041

SA042

Bone Acidic Glycoprotein-75 Delineates the Extracellular Sites of Future Bone Sialoprotein Accumulation and Apatite Nucleation in UMR 106-01 BSP Cultures. R. J. Midura*[1], A. Wang*[1], D. Lovitz[2], D. Law*[2], K. Powell*[1], J. P. Gorski[2]. [1]Biomedical Engineering, ND20, The Cleveland Clinic Foundation, Cleveland, OH, USA, [2]School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO, USA.

The present study was undertaken to determine the kinetic and temporal relationship of bone acidic glycoprotein-75 (BAG-75) deposition as compared to that for bone sialoprotein (BSP) and for hydroxyapatite crystals during de novo biomineralization of UMR 106-01 BSP osteoblastic cultures. Addition of a phosphate source to UMR cultures activates a mineralization program in which BSP localizes to extracellular matrix sites where hydroxyapatite crystals are subsequently nucleated [Wang, A., et al., J. Biol. Chem., 275:11082-11091, 2000]. Using highly specific antibodies for BAG-75 and BSP together with confocal microscopy and electron microscopy, we show for the first time that, prior to phosphate addition in UMR cultures, BAG-75 has already been deposited within these very same sites in two size populations (approximately 15-25 and 150-200 microns in diameter). We refer to these previously unrecognized extracellular structures as biomineralization foci (BMF) precursors. TUNEL data are presented to indicate that these BMF are not the result of a dystrophic process involving dead or dying cells. The shape and size of the smaller population of BMF precursors are similar to structures assembled through self-association of purified BAG-75 protein. Transmission electron microscopy showed the ample presence of 10-nm thin-filaments in BMF, which are similar in size to purified BAG-75 self-assembled filaments. Immediately after phosphate addition, BSP quantitatively accumulates within these BAG-75 defined BMF associated with both thin-filaments and 300-800 nm diameter translucent particles, but not with matrix vesicles. By 8 hours after phosphate addition, calcium phosphate crystals are observed in BMF by EDAX and atomic adsorption spectroscopy. In summary, BAG-75 is the earliest detectable matrix protein biomarker that accurately predicts the sites for de novo biomineralization in UMR cultures. We hypothesize that BAG-75 may perform a key structural role in the assembly of BMF precursors, and the recruitment of BSP necessary for subsequent apatite nucleation.

Disclosures: ***R.J. Midura****, None.*

SA043

See Friday Plenary number F043

SA044

Osterix (Osx) Regulates Bone Sialoprotein (BSP) Expression in a Novel *in vivo* Model. L. Li*, J. Tang*, J. Chen. Division of Oral Biology, Tufts School of Dental Medicine, Boston, MA, USA.

Osx is a zinc finger-containing transcription factor that is required for osteoblast differentiation and bone formation. It has been suggested that Osx acts downstream of Runx2/Cbfa1, a critical transcriptional factor in osteogenesis. A lack of bone formation is seen in both Osx null and Runx2/Cbfa1 null mice indicating a failure in osteogenic differentiation. It is speculated that Runx2/Cbfa1 regulates osteoblast differentiation and induces the production of extracellular matrix proteins including BSP through its regulation on Osx expression. This study was designed to specifically determine the Osx regulation on BSP gene expression using an in vivo animal model we have recently established. Through microinjection of a construct containing 800 bp avian retroviral receptor gene (TVA) driven by a 4.9 kb mouse BSP promoter a transgenic mouse line BSP/TVA was generated. The distribution of expression of TVA gene expression was found to be consistent with that of endogenous BSP in in situ hybridization of the bone tissues from BSP/TVA mice. A 1.2 kb fragment of mouse Osx was cloned into a RCASBP (A) vector (gifts from Drs. de Crombrugghe and Hughes, respectively). The construct was subsequently used to transfect chicken fibroblasts (DF-1) from which the stock virus was collected. The viral construct RCASBP)/Osx was then introduced into nine 5-day-old BSP/TVA mice by introperitoneal injection. An empty RCASBP vector was also used in the entire experiment serving as a control. The effect of Osx on BSP gene expression was determined by RT-PCR using the bone tissues from the infected mice sacrificed at 9, 14 and 28 days of age, respectively. It was found that, after systemic viral infection, the expression of BSP was increased by 2.3-ad 1.3-folds in mandibular and calvarial bones, respectively, in 9-day-old animals. A 2.1- and 1.4-folds increase was detected in the same bone tissues in 14-day-old mice. However, the increase rate was lower in older BSP/TVA mice (28-day-old) in which only 1.4- and 1.1-folds elevations were observed in their bones. While Osx also stimulated the BSP expression in tibia bone the increase level was not as significant as that in mandibular or calvarial bones. These results indicate that Osx acting downstream of Runx2/Cbfa1 up-regulates BSP expression in bone tissues. This up-regulation is tissue specific and developmental stage dependent. The BSP/TVA transgenic mouse is a unique and versatile model in which various gene expressions and their regulation can be studied in vivo. Supported by NIH/NIDCR Grant RO1 DE11088 and partially by PO1 DE13221.

Disclosures: ***J. Chen****, None.*

SA045

Bone Morphogenetic Protein-1 Expression and Carboxyl of Pro-Collagen Type I Production During Osteogenesis. P. Manduca, S. Zanotti*, C. Volta*, A. Favre*, O. Barbieri*, S. Marchisio*, S. Astigiano*. Dobig, University of Genova, Genova, Italy.

We previously reported of the chemoattractant role for endothelial cells of the trimeric fragment of procollagen type I (C3), and that C3 is found in the conditioned medium of osteogenic cultures maximally during the phase of highest expression of Alkaline Phospahtase, its detaction decreasing upon nodule formation, before decline of pro-collagen type I synthesis in later osteogenesis (1,2). We here report investigations directed to understand what causes the accumulation of secreted C3 in the conditioned medium., with particular regard to the expression during osteogenesis of the deputate gene for the proteolysis of pro-collagens generating trimeric C3 fragments: the gene codifying for BMP-1 and mTld, two products of differential splicing, differing in their affinity for type I pro-collagen. By RT-PRC, with primers that identify BMP1 and mTld we show that these are produced in osteoblasts cultures differentiating in vitro in developmentally regulated fashion, corresponding to the increase of the concentration of C3 detected in the conditioned medium. In ossifying rats tibiae (from 18 days of fetal life to 10 days after birth) by in situ hybridization, with a probe identifying both BMP1 and mTld transcripts, we show that the gene is expressed in specific times and places both in the developing cartilage model and in forming tibial bone. Gene expression by ISH is accompained by positive immunoperoxidase stain for the protein BMP-1, whose maximal intensity of stain follows in time that of maximal gene expression. In the cartilage bone model the expression of BMP-1 gene and protein is diffuse in proliferating chondrocytes, lost at their hypertrophy, and occurs again in the secondary ossification center. In bone they are expressed by lining osteoblast and osteoblast embedded in recently deposed bone matrix, and undetected at later time of bone growth and in osteocytes. Expression of the BMP1 protein correlates with detection by immunoperoxidase staining of the a1 and a2 chains of C3. In summary, BMP-1 expression is modulated during osteogenesis in vitro and is a developmental feature of the osteogenic phenotype. The expression of BMP-1 in the cells of the bone compartment is compatible with the fact that the production of C3 I could enhance endothelial cells mobility towards and within the forming bone. 1-Stringa E. et al., Bone (1995) 16, 663-670 2-Palmieri D, et al.J: Biol.Chem. (2000) 275, 32658-32663

Disclosures: ***P. Manduca****, None.*

SA046

See Friday Plenary number F046

SA047

Bone Sialoprotein Promotes Invasion Through Basement Membrane by Forming a Complex with Both MMP-2 and Integrin-$\alpha_v\beta_3$. A. Karadag[1], N. S. Fedarko*[2], L. W. Fisher[1]. [1]Nidcr, NIH, Bethesda, MD, USA, [2]Deptartment of Medicine, Johns Hopkins University, Baltimore, MD, USA.

It has been known since the mid-1990's that the bone matrix protein, bone sialoprotein (BSP), is strongly up-regulated in breast, prostate, lung, thyroid and a number of other osteotropic cancers. However, the mechanism by which this SIBLING (Small Integrin-Binding LIgand, N-linked Glycoprotein) protein may enhance the metastatic properties of tumors is unknown. In this study we have used a modified Boyden chamber assay of invasion as a model system to study the effects of recombinant human BSP on the invasiveness of several cancer cell lines through an artificial basement membrane (Matrigel).

BSP greatly enhanced (3-10 fold) the invasiveness of many cancer cell lines (e.g. SW-579, PC-3, MDA-MB-231 and NCI-H520) through Matrigel in a dose-dependent manner. Integrins clearly were involved in this enhanced invasion because cells treated with a mutant BSP whose integrin-binding tripeptide, RGD, was modified to the inactive KAE, invaded only as well as untreated controls. Similarly, addition of a blocking antibody for $\alpha_v\beta_3$ integrin negated the BSP effect. Because our previous work had shown that BSP could bind and activate MMP-2, we next hypothesized that this matrix metalloproteinase could also be playing a role in the BSP effect. This was shown to be true for the two cell lines tested because 1) a general MMP inhibitor, 2) a MMP-2 specific inhibitor, and 3) a MMP-2 blocking monoclonal antibody, all completely negated the BSP-enhanced invasion.

To test if both the integrin and MMP-2 observation were due to a single trimeric complex, a pull-down experiment was performed. An $\alpha_v\beta_3$ integrin monoclonal antibody immobilized on immunoaffinity gel matrix was complexed first with recombinant $\alpha_v\beta_3$ integrin, then with BSP (or BSP-KAE), and finally with pro or active MMP-2. The beads were washed after each step and finally analyzed by gelatin zymography. Complexes containing the BSP showed strong bands for both pro and active MMP-2 while untreated or BSP-KAE treated beads showed little or no MMP-2 activity bound. The presence of the same MMP-2-BSP-integrin complex was also verified by FACS analysis using the recombinant BSP and fluorescently labeled MMP-2. Controls for the FACS experiments included BSP-KAE and a blocking antibody for $\alpha_v\beta_3$ integrin.

In conclusion, BSP, MMP-2 and $\alpha_v\beta_3$ integrin are known to be expressed in many osteotropic cancers. We have shown that BSP greatly enhanced the ability of several breast, prostate, lung and thyroid cell lines to invade through an artificial basement membrane by bridging active MMP-2 molecules to the $\alpha_v\beta_3$ integrin on the surface of the cells, presumably at the leading edge of the invasionary processes.

Disclosures: ***A. Karadag****, None.*

SA048

See Friday Plenary number F048

SA049

CSF-1 Treatment Corrects Septoclast Abnormalities at the Chondroosseous Junction in the *Toothless* Rat. A. Gartland[1], A. Mason-Savas*[1], C. MacKay*[1], E. R. Lee[2], J. Mort*[2], P. R. Odgren[1], S. C. Marks[1]. [1]Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA, [2]Electron Microscopy Unit, Shriner's Hospital for Children, McGill University, Montreal, PQ, Canada.

The *toothless (tl)* mutation in the rat is a naturally occurring, autosomal recessive mutation in the *CSF*-1 gene resulting in profound deficiencies of bone-resorbing osteoclasts. The *tl* rats have severe, unremitting osteopetrosis with a highly sclerotic skeleton, lack of marrow spaces, the failure of tooth eruption and a progressive, severe growth plate chondrodystrophy. Injections of CSF-1 restore osteoclast populations, bone resorption, and tooth eruption in *tl* rats but do not improve the growth plate phenotype. A variety of cells participate in the advancing osseous front at the chondroosseous junction (COJ) through activities that contribute to the longitudinal growth of long bones. A recently-identified cell, the septoclast, is a unique cell specifically differentiated to facilitate angiogenesis at the chondroosseous junction. This cell is long and slender, lies just outside the invasive capillary sprouts, and stains intensely for the cysteine proteinase cathepsin B. The apex of the septoclast ends on the transverse septum in a structure that resembles the ruffled border of osteoclasts, yet these two cell types are from different cell lineages. Given that the *tl* rats have retarded bone growth at the COJ, accompanied by suppressed angiogenesis and an absence of osteoclasts, we decided to determine if septoclasts were also absent in the *tl* rat. We compared the COJ in the proximal tibia of 2-week old *tl* rats and normal littermates, +/- 1 week CSF-1 treatment, with respect to number, distribution and cathepsin B activity of septoclasts. In normal rats, COJ septoclasts were present as thin cells regularly aligned along the axis of the bone and exhibited strong staining for cathepsin B. *tl* rats had a reduced number of cathepsin B positive cells with morphology similar to septoclasts. These were found somewhat further below the COJ than normal. Treatment of *tl* rats with injections of CSF-1 improved the number and distribution of cathepsin B-positive septoclasts at the COJ. Ultrastructural evaluations of the COJ from the normal and *tl* rats confirmed these cells to be septoclasts. The presence of septoclasts in an osteoclast-free region of the *tl* skeleton confirms that these two catabolic cells are not under identical developmental controls. The reduced number and alignment of septoclasts in *tl* rats and their restoration with CSF-1 treatment implies a role for CSF-1 in the normal differentiation of septoclasts. These data confirm that septoclasts play a significant role in endochondral ossification.

Disclosures: ***A. Gartland****, None.*

SA050

Relation between Bone Morphometric Changes and Pain Perception in Different *in vivo* Models for Bone Cancer-Related Pain. H. Vermeirsch*[1], R. Nuydens*[1], M. Janicot*[2], P. Salmon*[3], L. Andries*[4], T. Meert*[1]. [1]CNS department, Pain & Neurology, Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica NV, B-2340 Beerse, Belgium, [2]Oncology, J&J PRD, B-2340 Beerse, Belgium, [3]Skyscan, B-2630 Aartselaar, Belgium, [4]HistoGeneX, B-2650 Edegem, Belgium.

For many patients, pain is the first sign of cancer and 30 to 50 % of all cancer patients will experience moderate to severe pain, this number augments to 75 - 95% when patients with metastatic or advanced cancer are considered. Therefore, to significantly increase patients' quality of life, there is a need for the development of drugs that effectively treat bone cancer pain. The mechanisms of bone cancer pain are poorly understood but osteolysis (bone breakdown) seems to play a major role. Osteoclasts induce an acidic pH that might activate sensory neurons. Also growth factors released during bone resorption may act on sensory neurons. Other mechanisms involve the release of pronociceptive agents from tumor and inflammatory cells.
Several animal models of cancer pain were developed to study the basic neurobiological mechanisms of cancer pain.
We evaluated 3 animal models for bone cancer pain using classical histology and micro CT technology in order to determine the precise location of the tumor and the degree of bone destruction. In vivo testing, to evaluate the development of hyperalgesia and allodynia in the affected hind limb, included different nociceptive tests such as the hot-plate and cold-plate (thermal nociception), the von Frey assay and the pin prick (mechanical nociception). Alterations in the pain measurements correlated with the degree of bone destruction. The increased pain sensation in the bone cancer models could be modulated by morphine treatment. On the other hand subcutaneous tumors, LoVo cells grafted in nude mice, did not induce any signs of increased pain sensation.
The used models and the multidisciplinary approach of this study yield new insights on the development of bone cancer pain and allows the evaluation of potential effects of analgesics.

Disclosures: ***H. Vermeirsch****, Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutica NV 3.*

SA051

Calcium-Sensing Receptor Deduces mRNA of Human Securin, Pituitary Tumor Transforming Gene, in Rat Testicular Cancer. J. Tfelt- Hansen*[1], N. Chattopadhyay*[1], P. Scwarz[2], E. Brown[1]. [1]Endocrinology, Brigham and women's hospital, Boston, MA, USA, [2]Clinical Biochemistry and Endocrinology, Copenhagen University Hospital Hvidovre, Copenhagen, Denmark.

Pituitary tumor transforming gene (PTTG), the human securin, is an oncogene. Few normal tissues express PTTG, though in the testis it is abundantly expressed. In cancer, however, its wide expression has been directly correlated to tumor invasiveness. Besides changes during the rat spermatogenic cycle, very little has been described about the regulation of PTTG in testis. Here we investigate the role of the calcium-sensing receptor (CaR) in regulating PTTG in a widely used model of rat testicular cancer (H-500 Leydig cells) for humoral hypercalcemia of malignancy (HHM). In this report we show for the first time that calcium upregulates PTTG mRNA, as assessed by northern blotting and real-time PCR. PTTG mRNA upregulation by calcium has a rapid onset at 0.5 h and remains upregulated until 40 h. A dose-response curve shows the upregulation of PTTG mRNA with an increase of 4.22 ± 1.61, 5.11 ± 1.11, and 5.64± 1.92 (mean ± S.E.) fold at 5, 7.5, and 10 mM calcium, respectively, compared with 0.5 mM Ca^{2+}_{o}. The effect of calcium on PTTG expression was abolished by using a dominant-negative CaR (R185Q) construct, thus showing that the CaR is the main mediator for calcium's effect on PTTG. Another G-protein-coupled receptor (GPCR) agonist, ADP, showed no effect on PTTG expression. In conclusion, we show for the first time that a GPCR, the CaR, stimulates the synthesis of PTTG in a model for HHM.

Disclosures: ***J. Tfelt- Hansen****, None.*

SA052

See Friday Plenary number F052

SA053

Immunohistochemical Profile of Osteosarcoma in Adults. M. P. Roudier[1], B. P. Rubin*[2]. [1]Urology, University of Washington, Seattle, WA, USA, [2]Pathology, University of Washington, Seattle, WA, USA.

Osteosarcoma (OS) is rare in adults and many cases of OS present with an extensive soft tissue component with a paucity of osteoid, which can appear as bland spindle cell lesions, small round cell neoplasms, or malignant pleomorphic neoplasms. Limited information regarding the immunohistochemical (IHC) profile of OS is available, thus, IHC studies could be misleading, especially on needle core biopsies.
A 324-core tissue array was constructed with triplicate cores of 94 OS including 40 osteoblastic, 33 fibroblastic, 8 parosteal, 7 chondroblastic, 5 giant cell rich and 1 small cell OS subtypes using a MTA1 tissue arrayer (Beecher Instruments, Sun Prairie, WI). Control tissues (20) included carcinomas, lymphoma and a wide variety of soft tissue neoplasms. Fifty-eight (58) antibodies commonly used in the differential diagnosis of these tissues were studied. Array sections were used when the yield of cores reached 87%. Antibodies were scored on a semi-quantitative scale as follows: 1+ = 0-25% of cells, 2+ = 26-50% of cells, 3+ = >51% of cells.
Vimentin was positive in 100% and osteonectin in 73% of OS cases. Epithelial markers revealed positivity for pan-cytokeratin (AE1/AE3) (20%) and EMA (20%). Other cytokeratins were negative except one OS case with focal positivity for 35βH11. S-100 protein was positive in 20%, predominantly in chondroblastic areas. MelanA was negative in all OS. Microphtalmia transcription factor and tyrosinase were positive in 1% and 2%, respectively. Smooth muscle markers revealed OS positivity for: calponin (57%), smooth muscle actin (43%), smooth muscle myosin (10%), desmin (10%), HHF35 (4%), and caldesmon (1%). Skeletal muscle markers revealed: myogenin (3%) and MyoD1 (0%). Calcitonin and thyroglobulin were positive in 37% and 30% of OS cases respectively. Progesterone receptor was positive in 20% of OS cases, but estrogen receptor was negative in all OS cases. Other positive antibodies included CD99 (100%), NSE (57%), PLAP (24%), WT-1 N terminus (16%), Fli-1 (15%), neurofilaments (11%), GFAP (10%) and AlK-1 (1%). C-KIT (CD117) was negative in all OS cases.
OSs in adults display immunoreactivity for a variety of commonly used diagnostic antibodies, without a characteristic immunohistochemical profile. Therefore great care should be used in interpreting immunohistochemical results in soft tissue tumors in adults, which may represent occult osteosarcomas with a minor, unsampled component of osteoid. Clinical correlation including radiologic examination for signs of bone involvement and calcification can be helpful in excluding the possibility of OS.

Disclosures: ***M.P. Roudier****, None.*

SA054

Kyphoplasty Enhances Function and Structural Alignment in Multiple Myeloma. J. M. Lane[1], R. E. Hong*[1], T. Kiechle*[1], R. Niesvizky*[2], R. Pearse*[2], D. Siegel*[2]. [1]Orthopaedics, Hospital for Special Surgery, New York, NY, USA, [2]Hematology / Oncology, New York Presbyterian Hospital, New York, NY, USA.

Purpose: Multiple myeloma is associated with vertebral compression fractures and secondary kyphosis. These pathological fractures result in pain and functional disability. Kyphoplasty that utilizes minimal invasive balloon tamps to reduce osteoporotic vertebral compression fractures has been applied to multiple myeloma. This investigation tests the hypothesis that kyphoplasty can significantly restore vertebral height and spinal function.
Materials: 18 consectutive patients with vertebral fractures secondary to multiple myeloma underwent kyphoplasty balloon reduction and stabilization with PMMA. Patients were evaluated prior to treatment and post-operatively with the validated general Oswestry back quesstionnaire. 7 patients with 20 fractures were measure for height restoration on long kyphosis (36 inch) standing films. Over 90% of the fractures were older than 3 months by history and MRI.
Results: By Oswestry analysis the average pre-op score was 48.94 and the average post-op score was 32.70 with an average improvement of 16.23 ($p < .002$). The enhanced function was greatest for patients with the poorest pre-op Oswestry scores and of little improvement for patients with initial scores better than 28. An average height restoration of 25.3% ($p < .001$) and 34.9% ($p < .001$) was achieved in the anterior and medial vertebral body respectively. No patients underwent a major complication. These results will be correlated with stage of disease, cytogenetics, B2M, LDH, proliferation index, treatment with bisphosphonates, and response to systemic therapy.
Conclusions: Kyphoplasty has proven safe and efficacious in treating both acute and chronic vertebral compression fractures secondary to myeloma. This balloon tamp methodology results in vertebral height restoration and enhanced function with no major complications. This prospective cohort study established the functional and structural benefits of kyphoplasty for patients with pathological vertebral fractures related to multiple myeloma.

Disclosures: ***J.M. Lane****, Kyphon, Inc. 2, 6.*

SA055

See Friday Plenary number F055

SA056

Radiation Treatment Decreases Bone Cancer Pain, Osteolysis, and Tumor Size. M. Goblirsch*[1], W. Matthews*[1], C. Lynch*[1], P. Alaei*[2], B. J. Gerbi*[2], P. W. Mantyh[3], D. R. Clohisy[1]. [1]Orthopaedic Surgery, University of Minnesota, Minneapolis, MN, USA, [2]Therapeutic Radiology, University of Minnesota, Minneapolis, MN, USA, [3]Preventive Sciences, University of Minnesota, Minneapolis, MN, USA.

Radiotherapy is the most widely used palliative treatment for primary bone cancer in animals and metastatic bone cancer in humans; however, the mechanism(s) responsible for pain relief following radiation are unknown. The effects of radiation on mice with painful bone cancer has been studied to elucidate the mechanism by which said treatment decreases bone cancer pain. Male C3H/HeJ mice received intramedullary injection of 10^5 2472 osteolytic sarcoma cells. Seven days after tumor inoculation, mice were administered a single, localized 20 Gy dose of radiation to the left femur. Radiation was performed in a custom-designed, lead-shrouded restraining apparatus, permitting only the tumor-injected femur to receive radiation. Lithium fluoride thermoluminescent dosimetery was used to verify the efficacy of the delivered dose and lead shielding. Analysis of the effects of 20 Gy treatment was performed through assessment of previously validated behavioral measures, Faxitron radiographs, and tumor areas. Pain behaviors were recorded every third day, post-treatment, for 17 days. Comparison of groups was analyzed by one-way ANOVA and Fisher's PLSD statistical tests. A single 20 Gy treatment dramatically decreased bone cancer pain. Assessment of pre-tumor injection and post-tumor inoculation behaviors (limb

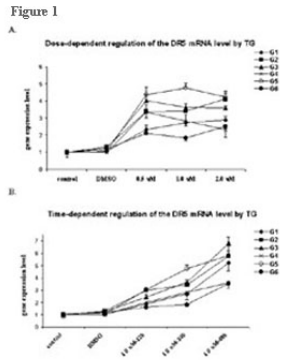
use, Rota-rod, and spontaneous guarding) revealed steady improvement in radiation-treated mice ($p<0.05$) and showed no difference in pain measures with sham-injected mice 8 days post radiation. Evaluation of bone destruction, via Faxitron images, revealed significant reduction in the progression of cancer-induced osteolysis ($p<0.001$). Tumor area was significantly less in sarcoma-injected, radiation-treated mice ($2.8mm^2 \pm 2.4$ vs. $10.0\ mm^2 \pm 1.4$ for the untreated group; $p<0.02$). This investigation describes the initial experimental model in which radiation therapy decreases bone cancer pain. We demonstrate that the administration of a localized, single dose of radiation to the tumorous limb decreases painful behavior and increases limb mobility. Also, decreased cancer-induced osteolysis and decreased tumor burden was evident. Future use of this experimental system should promote ongoing discoveries regarding the cellular and molecular mechanisms responsible for decreasing bone cancer pain after radiation treatment.

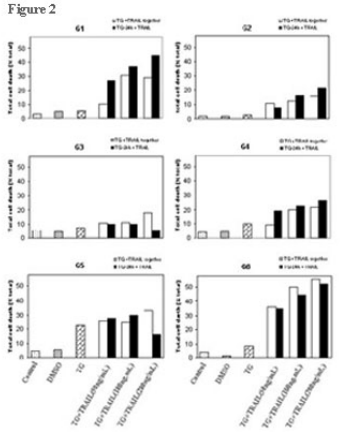
Disclosures: ***D.R. Clohisy****, None.*

SA057

Thapsigargin Sensitizes TRAIL-induced Apoptosis in Giant Cell Tumor of Bone. L. Huang*[1], J. K. Xu*[2], Y. Y. Cheng*[1], M. H. Zheng[2], S. M. Kumta*[1]. [1]Dept. of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong Special Administrative Region of China, [2]Dept. of Orthopaedic Surgery, The University of Western Australia, Perth, Australia.

Although TNF-related apoptosis-inducing ligand (TRAIL) has attracted great attention in recent years as a promising anti-cancer reagent, several studies showed some tumor cells somehow remain insensitive to TRAIL in *in-vitro* cultures. In this study we examined the sensitization of TRAIL-induced apoptosis in tumour cell of giant cell tumour of bone (GCT). Thirteen GCT specimens and six GCT primary cultures were used to analyze the expression of death signal transducing receptors DR4 and DR5 and decoy receptors DcR1 and DcR2. Thapsigargin (TG), an agent known to cause perturbations in intracellular Ca2+ homeostasis was used to sensitize GCT tumor cells to TRAIL-induced apoptosis. At moderate concentrations (100ng/ml TRAIL and 1uM TG), neither of the two agents alone promotes significant cell death (< 10%) in GCT tumor cells as measured by flow cytometry using Annexin-V-Fluos and propidium iodid (PI) staining. Interestingly, combined treatment markedly enhanced cell death by 4~6 folds. Most noteworthy, in four out of six GCT cultures, pre-treatment of TG for 24 hours further enhanced TRAIL-induced cell death when compared to the co-treatment together. In addition, TG was shown to up-regulate DR5 and DR4 gene expression in time- and dose-dependent manners as evidenced by real-time quantitative RT-PCR analysis. In short, our findings suggest that TG is able to sensitize GCT tumor cells to TRAIL-induced cell death through up-regulation of the expression of death signal transducing receptors, DR5 and DR4. Our findings would be valuable for design of new treatment modalities for patients with GCT.





Disclosures: ***L. Huang****, None.*

SA058

See Friday Plenary number F058

SA059

Parathyroid Hormone-related Protein Promotes Lung Cancer Cell Survival. R. H. Hastings[1], F. Araiza*[2], D. W. Burton[2], L. J. Deftos[3]. [1]Anesthesiology, VA San Diego Healthcare System, San Diego, CA, USA, [2]Research, VA San Diego Healthcare System, San Diego, CA, USA, [3]Medicine, VA San Diego Healthcare System, San Diego, CA, USA.

Parathyroid hormone-related protein (PTHrP) has been studied extensively in lung cancer because of its relation to humoral hypercalcemia of malignancy. Little work has focused on its action as a lung carcinoma growth factor, even though PTHrP is expressed in more advanced, aggressive tumors, and may play an active role in cancer progression. PTHrP has effects on apoptosis in many malignant and non-malignant cells. We investigated the effects of PTHrP on caspase activity after UV irradiation in BEN cells, a human squamous lung carcinoma line. Cells at 70% confluency were treated 24 h with 100 nM PTHrP 1-34, PTHrP 38-64, PTHrP 67-86, PTHrP 107-139, or PTHrP 140-173 in DMEM/FBS. Cells were then exposed 30 min to 50 mJ/cm2 UV-B. Caspase 3, 8, and 9 activities increased 5-fold 24 h after UV. PTHrP 1-34 and 140-173 reduced caspase 3 activity after UV 19 ± 4% and 29 ± 7%, respectively, and increased cell protein 36 ± 12% (mean ± SEM, N = 7), suggesting improved survival. Reductions of caspase 8 and 9 were similar. Other peptides had no effect. Treating cells with caspase 9 inhibitor reduced caspase 3, 8 and 9 activities after UV, but caspase 8 inhibitor affected only caspase 8. These data indicate that caspase 9 acts upstream of caspase 8 and caspase 3 after UV-B exposure in BEN cells, and that apoptosis occurs through a mitochondrial-dependent pathway. PTHrP 1-34 and PTHrP 140-173 both protect against apoptosis, as evidenced by reductions in caspase activities and increases in cell survival. PTHrP protects against mitochondria-mediated apoptosis by increases in Bcl-2 or activation of the Akt/PI3 kinase in other cells (J Cell Biol 136:205 1997, JBC 277:19374 2003), but the mechanism for the protective effect in BEN cells remains to be determined. Protective effects against apoptosis support the hypothesis that PTHrP may contribute to lung cancer cell survival and promote cancer progression.

Disclosures: ***D.W. Burton****, None.*

SA060

The Bisphosphonate Risedronate Inhibits Angiogenesis and Tumor Growth In Vivo. P. Fournier*[1], S. Boissier*[1], C. Serre*[1], M. Colombel*[1], F. H. Ebetino[2], P. A. R. Clezardin[1]. [1]INSERM, Lyon, France, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Bisphosphonates inhibit tumor cell invasion and adhesion to bone in vitro. In the present study, we have investigated the effects of the nitrogen-containing bisphosphonate risedronate (RIS) on angiogenesis and tumor growth in vivo.

RIS dose-dependently reduced endothelial cell survival and inhibited capillary-like tube formation in vitro. We have previously shown that bisphosphonates accumulate in rat kidney and prostate tissues but not in other nonmineralized tissues. Castration induces the regression of the vasculature in the prostate and testosterone treatment of castrated rats causes a rapid vascular regrowth in the prostate. The effect of RIS on the re-vascularization of the prostate gland induced by testosterone in castrated rats was therefore tested. A daily s.c. treatment of castrated rats with testosterone + RIS (20 and 100 microg/kg/day) for 6 days reduced the prostate weight (20% inhibition) compared to that observed with testosterone alone. Morphometric analysis of immunostained blood vessels in prostate tissue sections demonstrated that RIS treatment induced a 45% reduction of the mean vessel area (testosterone + RIS: 865 ± 122 microm2 versus testosterone: 1561 ± 174 microm2). In sharp contrast, clodronate (a bisphosphonate that lack nitrogen in its structure) did not inhibit testosterone-induced prostate regrowth and revascularization when used at a dose up to 20 mg/kg/day.

Angiogenesis is associated with tumor growth and metastasis progression. The effects of RIS were therefore studied on the growth of s.c. tumor xenografts and the formation of bone metastases induced by human MDA-MB-231/B02 breast cancer cells stably transfected to express the green fluorescent protein. Continuous treatment of nude mice with RIS (150 microg/kg/day) did not inhibit the growth of s.c. tumors. In contrast, treatment of animals bearing bone metastases with RIS at a similar dose almost completely inhibited the formation of osteolytic lesions as judged by radiography (RIS: 0 mm2 versus 4.4 ± 1.6 mm2) and histomorphometric analysis using the bone volume (BV) to tissue volume (TV) ratio (RIS: 69.1 ± 10.1% versus placebo: 7.7 ± 4.1%). For comparison, the BV/TV ratio of normal age-matched mice was 12.9 ± 2.3 %. The extent of skeletal tumor burden was also substantially reduced by RIS as judged by fluorescence imaging (RIS: 6.3 ± 6.2 mm2 versus placebo: 20.8 ± 10.3 mm2) and by histomorphometry analysis using the tumor burden (TB) to total soft tissue volume (TV) ratio (RIS: 4.1 ± 6.9 % versus 20.5 ± 10.2 %).

Overall, our results indicate that RIS inhibits angiogenesis and tumor growth in tissues where it accumulates.

Disclosures: ***P.A.R. Clezardin****, Procter & Gamble Pharmaceuticals 2.*

SA061

Withdrdawn

SA062

Clodronate Prevents Osteolytic Breast Cancer Bone Metastases in a Mouse Model: Effects on Bone Mineral Density. S. M. Käkönen, P. Isaksson*, P. T. Lakkakorpi, T. Österman*, C. Malmström*, M. Suominen*, E. Aho*, B. Sjöholm*, S. Hokkanen*, R. Hannuniemi*. Preclinical R&D, Schering Oy, Turku, Finland.

Oral clodronate has recently been shown to prevent the occurrence of skeletal metastases in primary breast cancer. The aim of this study was to investigate the preventive effects of clodronate in a preclinical mouse model that resembles the characteristics of osteolytic bone metastasis in human breast cancer. Furthermore, the effect of osteolysis and clodronate treatment on bone mineral density (BMD) in tumor bearing mice was examined.

MDA-MB-231 human breast cancer cells were inoculated into the left cardiac ventricle of 5-week-old nude mice and clodronate (10, 20, and 40 mg/kg/d, N=20-23/group) was administered s.c. for 21 days after the inoculation. On day 22, the mice were sacrificed and radiographed, followed by densitometric measurements by pQCT at proximal and diaphyseal (at 30% and 50% of proximal end) sites of tibia. Clodronate-treated groups were compared to untreated positive (cancer cell inoculation) and negative (saline inoculation) control groups.

In the positive control group, 30% of the mice were sacrificed before the day 22 due to paraplegia or cachexia, whereas 22%, 9%, and 4% of the mice in the clodronate groups (10, 20, and 40 mg/kg/d, respectively) were sacrificed before the end of the study. Clodr-

onate significantly reduced the area and number of osteolytic lesions in all treatment groups and totally prevented the occurrence of lesions in mice receiving 20 or 40 mg/kg/d of clodronate as determined by radiography. At proximal tibia, positive control group had significantly lower total and trabecular bone mineral content (BMC) and BMD compared to the negative controls, whereas clodronate groups had significantly higher total and trabecular densitometric parameters compared to both positive and negative control groups. In the positive control group, cortical BMC was lower at proximal and 30% diaphyseal sites compared to the negative controls, whereas no changes were observed in cortical BMD at any of the sites measured. In addition, no differences in the values of densitometric parameters were observed at the 50% diaphyseal site where only few osteolytic lesions were observed.
In conclusion, our data indicate that clodronate prevents tumor-induced osteolysis when determined by radiography and densitometry. In addition, densitometric analyses support the hypotheses that active bone remodeling in trabecular bone areas is favorable for the osteolytic tumor growth and that the tumor-induced osteolytic effects are local. The data support clinical findings and suggest that clodronate offers a potent treatment for the prevention of bone destruction in breast cancer.

Disclosures: ***S.M. Käkönen****, Schering Oy 3.*

SA063

See Friday Plenary number F063

SA064

A Bone Marrow Stromal Cell Ossicle Model Provides Insight into the Pathophysiology of Prostate Cancer Metastasis. A. Schneider, L. M. Kalikin*, A. C. Mattos*, P. H. Krebsbach, K. J. Pienta*, L. K. McCauley. University of Michigan, Ann Arbor, MI, USA.

One of the most common yet untreatable complications associated with advanced prostate cancer (CaP) is the development of skeletal metastases. Recent studies suggest that interactions between CaP-derived factors and bone cells in the metastatic foci favors the formation of lesions characterized by the presence of immature, woven-like bone. Parathyroid hormone-related protein (PTHrP) is an autocrine, paracrine and intracrine factor that plays a major role in bone remodeling through its anabolic and catabolic actions. In CaP, PTHrP is highly expressed in the primary tumor as well as in the bone metastatic lesion. Therefore, we hypothesized that in the bone microenvironment, CaP-derived PTHrP acts as a key factor responsible for the increased aberrant bone remodeling by altering osteoblast function. A novel in vivo strategy was developed where ectopic ossicles were generated in immunocompromised mice by the coimplantation of murine bone marrow stromal cells (BMSCs) and human CaP cells seeded in gelatin scaffolds. BMSCs were coimplanted with one of the following human CaP cell lines: VCaP, LNCaP (parental), LNCaP stably transfected with full-length PTHrP (LNCaP-PTHrP) or LNCaP with empty vector (LNCaP-pcDNA). BMSCs-only implants served as controls. Six weeks following surgical implantation, ossicles were harvested and analyzed by microradiographic and histomorphometric analyses. Results indicated that human CaP cells form tumor foci in the ectopic ossicle microenvironment. The presence of CaP cells adjacent to the endocortical and trabecular bone was confirmed by PSA+ immunoreactivity. Ossicles generated from the implantation of LNCaP-PTHrP/BMSCs had nearly a six-fold increase in radiopacity, suggesting a higher degree of mineralization as compared to LNCaP-pcDNA/BMSCs implants. In another set of experiments, a non-invasive in vivo bioluminescence imaging system (Xenogen IVIS) was applied to track tumor colonization and growth in mice bearing ossicles. Intracardiac inoculation of PC-3 cells stably expressing luciferase (PC-3Luc) localized and progressively grew in close proximity to the ossicles. These observations suggest that developing osseous structures like the ossicles may provide a suitable environment to attract and support metastatic tumor growth. Similarly, bioluminescence imaging of PC-3Luc/BMSCs coimplants resulted in an effective real-time approach to monitor CaP tumor growth and burden in the bone microenvironment. In summary, the ectopic ossicle model system offers a powerful and versatile strategy to improve our understanding of the pathophysiology of prostate cancer bone metastasis.

Disclosures: ***A. Schneider****, None.*

SA065

The Wnt/Beta-Catenin Pathway Is Expressed in Prostate Cancer and in Bone Metastases. G. Chen*[1], N. Shukeir*[1], A. Potti*[2], K. Sircar*[3], D. Goltzman[1], S. A. Rabbani[1]. [1]Calcium Research Lab, McGill University, Montreal, PQ, Canada, [2]Department of Medicine, Division of Oncology, University of North Dakota School of Medicine, Fargo, ND, USA, [3]Department of Pathology, McGill University Health Centre, Montreal, PQ, Canada.

Prostate carcinoma is one of the most common cancers affecting men and has a high propensity to metastasize to bone where both osteoblastic new bone formation and osteoclastic osteolysis may occur. Recently the Wnt family of growth factors and its effector signaling molecule beta-catenin have been described in several types of malignancy. We assessed protein expression of Wnt1 and beta-catenin by immunohistochemistry and Western blotting in normal and malignant human prostate cancer cell lines, in human samples of normal, hyperplastic and malignant prostate tumors and in human samples of bone with prostate cancer metastases. The majority of beta-catenin is normally located in the cell membrane. Wnt signaling inhibits the multicomponent destruction complex which targets beta-catenin for degradation and leads to nuclear accumulation of this protein where it promotes the formation of transcriptionally active complexes. Compared with expression in normal prostate epithelium cell line, Wnt1 and beta-catenin were both more highly expressed in DU145, LNCap and PC3 prostate cancer cell lines, and especially in the most malignant line (PC3). In five normal prostate tissues Wnt1 was not detected and only membrane staining of beta-catenin was observed. Moderate Wnt1 and beta-catenin expression were observed in benign prostate hyperplasia. In 44 cases of human prostate cancer, positive cytoplasmic and nuclear staining for beta-catenin was observed in 24 (55%). In 23 of these 44 cases (52%) staining of Wnt-1 was also seen. Samples were more likely to be positive and stained more intensely for Wnt1 and for beta-catenin in more aggressive cancers as determined by high Gleason score and serum PSA levels. In bone samples, metastatic cancer cells revealed higher expression of Wnt-1 and cytoplasmic/nuclear beta-catenin than in specimens of primary tumor or lymph node metastases. These studies show that the Wnt/beta-catenin pathway is expressed in malignant but not normal prostate tissue and at higher levels in more aggressive and metastatic cancer. Since components of the Wnt signaling pathway are also present in osteoblasts, Wnt released from prostate cancer metastases may contribute to the stimulation of osteoblastic lesions in bone harboring such metastases.

Disclosures: ***G. Chen****, None.*

SA066

See Friday Plenary number F066

SA067

PTHrP Is Responsible for DNA Repair. N. Ilievska*, T. J. Martin, M. T. Gillespie. St. Vincent's Institute of Medical Research, Fitzroy, Australia.

Parathyroid hormone-related protein (PTHrP) is commonly expressed in breast cancers and its production by cancer cells in bone promotes osteolysis and metastasis establishment and growth. In addition, PTHrP affects cellular functions including differentiation, proliferation and apoptosis. In a clinical study PTHrP expression in primary breast cancers was an independent prognostic indicator for patient survival. To determine whether PTHrP might influence the behaviour of breast cancer, we have examined the effect of PTHrP expression by and upon MCF-7 and MCF-10A cell lines. When PTHrP was overexpressed in these cell lines, several DNA repair (XPG, BRCA1, BRCA2 and Rad51), cell cycle (p21 and p53), and apoptosis-related genes such as Bcl-2 were regulated, both at the mRNA and protein level: the DNA repair genes were elevated. To determine whether this was a result of an intracrine or autocrine action of PTHrP, a range of PTHrP peptides were assessed for their ability to alter gene expression in MCF-7 and MCF-10A cells. PTHrP peptides 1-34, 1-108, 106-139 or 122-139 did not affect mRNA or protein levels for these target genes, whilst PTHrP peptides (107-139 or 107-111 at 100 nM) encompassing osteostatin (TRSAW: 107-111) upregulated the expression of mRNA levels for these genes within 4 to 24 hrs, with parallel increases in protein levels. Mutant peptides of osteostatin (TRSPW, TRGAW, PRSAW, YRSAW, TKSAW and TASAW) identified a requirement for Thr107 and Ser 109 for activity on DNA repair genes.
To determine the signal transduction pathway(s) involved in osteostatin-induced regulation of DNA repair genes, MCF-10A cells were treated with the NFkB inhibitor (N-acetyl-L-cystine), PKA inhibitor (H89), MAPK inhibitor (PD098059) or PKC inhibitor (chelerythrine chloride) with and without TRSAW (100nM). These inhibitors implicated the involvement of PKA, PKC and MAPK pathways in the effects of TRSAW on DNA repair genes.
Finally, to determine if PTHrP was indeed responsible for DNA repair, the ability of osteostatin or mutants peptides to protect against etoposide induced DNA damage was assessed by COMET assays. TRSAW and peptides that elevated DNA repair gene expression protected against etoposide-induced DNA damage, whilst peptides that had no effect upon DNA repair gene expression were ineffective in the COMET assay.
Combined, these data indicate a new function for PTHrP to repair DNA damage, and this activity could account for the widespread distribution of PTHrP, and its association as a prognostic indicator of survival in patients with breast cancer.

Disclosures: ***M.T. Gillespie****, None.*

SA068

Antisense Parathyroid Hormone-related Peptide (PTHrP) Inhibits Invasion and Metastasis and Improves Survival in a Model of Metastatic Prostate Cancer. D. C. Huang[1], J. S. Rhim*[2], R. Kremer[1]. [1]Medicine, Royal Victoria Hospital, McGill University, Montreal, PQ, Canada, [2]Center for Prostate Disease Research, Uniformed Services, University of the Health Sciences, Bethesda, MD, USA.

Previous studies have demonstrated that the Ki-*ras* oncogene induces overexpression of PTHrP in human prostate cancer cell lines (FNC267B1-*ras*) established from normal human neonatal prostate epithelial cells. This cell line is metastatic to liver and lungs. Here we hypothesized that Ki-*ras* induced overexpression of PTHrP may promote prostate cancer metastasis. To test this hypothesis, we first neutralized PTHrP production in FNC267B1-*ras* cells by stable transfection of a CMV promoter driven antisense PTHrP cDNA plasmid vector. Several stable FNC267B1-*ras*/Antisense PTHrP cell lines were established and PTHrP production examined. Cell lines in which PTHrP was inhibited by over 90% were then examined both *in vitro* and *in vivo* for their invasion and metastatic abilities. These FNC267B1-*ras*/Antisense PTHrP cell lines displayed a highly significant inhibition of cell adhesion to Matrigel basement membrane matrix by over 35% ($p<0.005$) as compared to wild type cells transfected with the vector alone. There was also a significant reduction of colony formation in soft agar culture in FNC267B1-*ras*/Antisense PTHrP cells. In order to determine the role of PTHrP in prostate cancer metastasis *in vivo*, we injected wild type FNC267B1-*ras* or FNC267B1-*ras*/Antisense PTHrP cells into the left cardiac ventricle of female Balb/C nude mice. Kaplan-Meier analysis revealed a highly significant survival benefit in mice injected with FNC267B1-*ras*/Antisense PTHrP cells as compared to wild type FNC267B1-*ras* cells ($p<0.005$). Wild type FNC267B1-*ras* cells

injected nude mice displayed significant weight loss and circulating calcium elevations as compared to FNC267B1-*ras*/Antisense PTHrP cell injected nude mice. Macroscopic and microscopic histological analysis demonstrated that PTHrP inhibition resulted in a significant reduction of size and number of metastatic lesions of lungs and liver ($p<0.001$). Our *in vitro* and *in vivo* data therefore demonstrate that PTHrP plays an important role for invasion, metastasis and calcium homeostasis in a metastatic prostate cancer model, and suggest that antisense PTHrP should be further evaluated as a therapeutic agent in human prostate cancer.

Disclosures: ***D.C. Huang****, None.*

SA069

See Friday Plenary number F069

SA070

In Vivo Imaging of TGF-β-induced RANK Ligand Transcriptional Activation in Prostate Cancer. J. Zhang[1], M. Liu*[1], A. Schneider[2], J. Dai[1], Y. Lu*[1], R. Kitazawa*[3], S. Kitazawa*[3], L. K. McCauley[2], E. T. Keller[1]. [1]Department of Pathology and ULAM, University of Michigan, Ann Arbor, MI, USA, [2]Department of Perio/Prev/Geriatrics, University of Michigan, Ann Arbor, MI, USA, [3]Division of Molecular Pathology, Kobe University, Kobe, Japan.

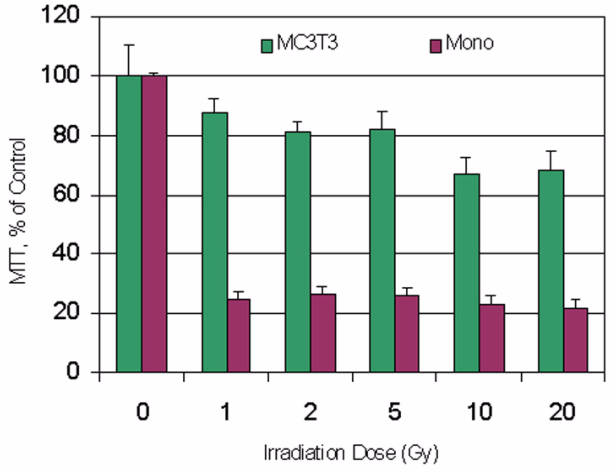
Prostate cancer (CaP) metastasizes to bone resulting in a mixture of osteoblastic and osteolytic lesions. Receptor activator of NFkB ligand (RANKL) mediates CaP-induced osteoclast activity. The mechanism of RANKL upregulation in CaP cells at bone sites is unknown. Transforming growth factor-β (TGF-β), a protein found in bone, has been reported to increase RANKL mRNA expression in vitro. The goal of this study was to determine if TGF-β modulates RANKL mRNA expression through transcription in vivo. Accordingly, we stably transfected PC3 human CaP cells with the human RANKL 5'-promoter (1.97kb) driving luciferase (lux) and selected several clones for evaluation (PC3-Rp). We initially evaluated RANKL promoter activation in vitro. TGF-β (0.1-10 ng/ml) induced RANKL promoter activity in a dose-dependent manner based on quantitative bioluminescent imaging (BLI). BLI correlated with conventional lux enzyme measurement and both RANKL protein and mRNA expression. To determine if transcriptional activation occurs in vivo we used an ectopic ossicle model. Specifically, gelatin sponges that inoculated with either murine bone marrow stromal cells (BMSCs) alone or with BMSCs and PC3-Rp cells were implanted subcutaneously into 4-6-wk-old male nude mice. Mineralized ossicles (with tumors in co-implanted sponges) were allowed to develop over 6 wks. Vehicle, TGF-β (200ng/mouse), or VitD (100ng/mouse, as positive control for RANKL promoter activation), were administered via i.p injection. Pre-, 1, 6, and 24 hr after administration, the mice were anesthetized for BLI of the whole body and of the excised ossicles at the end of 24 hr. TGF-β and Vit D increased lux activity by 54% and 190% compared to vehicle. Implants were homogenized for RANKL lux activity measurement that paralleled the BLI results. The BLI data were further validated using radiographs and histology to confirm tumor presence and immohistochemistry for the lux enzyme. We conclude that TGF-β induces RANKL mRNA expression through transcriptional activation in CaP cells in vivo. These data support the idea that cross-talk between bone and tumor is an important aspect of bone metastasis development, specifically as TGF-β is released from the resorbing bone matrix it induces RANKL expression, which in turn induces further bone resorption through induction of osteoclastogenesis. Additionally, these data demonstrate that the ossicle model is a useful tool for studying tumor and bone stromal interaction.

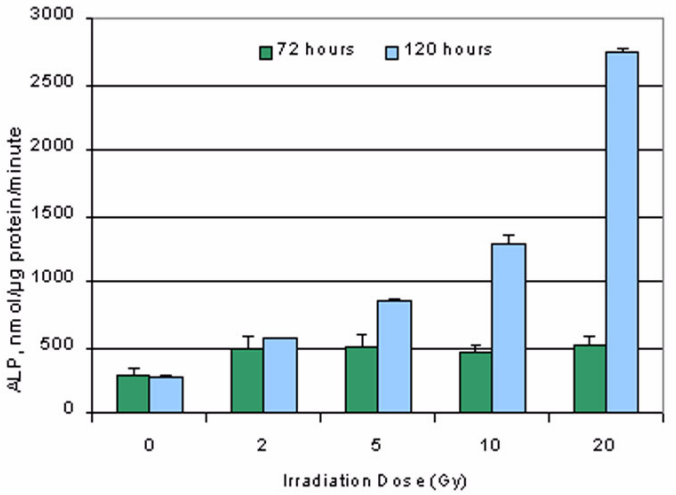
Disclosures: ***J. Zhang****, None.*

SA071

Effects of Radiation Therapy on Osteoblasts and Osteoclast Precursors. M. J. Allen*, B. S. Margulies*, T. A. Damron*. Orthopedic Surgery, SUNY Upstate Medical University, Syracuse, NY, USA.

Radiation therapy is generally considered to inhibit new bone formation, yet an "osteoblastic flare response" is commonly seen after radiation therapy in patients with osteolytic bone tumors. The specific aim of this study was to determine whether this response could be the result of differences in the sensitivity of osteoblasts and osteoclast precursor to ionizing radiation. To determine this, we used a cell culture model in which murine MC3T3 osteoblasts and primary human bone marrow monocytes (collected under an IRB approved protocol) were exposed to 0, 1, 2, 5, 10, and 20 Gy of X-irradiation. Measurements of cell number (MTT assay), cytotoxicity (LDH assay), apoptosis (caspase assay, flow cytometry), ALP and osteocalcin were made at intervals up to 120 hours. Data were analyzed using ANOVA (with post-hoc testing where appropriate) at a significance level of $p<0.05$. Monocytes were found to be extremely radiosensitive (>75% decreases in cell number at doses of 1 Gy or more, $p<0.001$). In contrast, osteoblasts were extremely radioresistant (only 30-35% inhibition at 10 and 20 Gy) (Figure 1). Apoptosis was seen in osteoblasts at 24 hours post-irradiation but evidence of cell death was not seen until 72 hours. Interestingly, osteoblast metabolic activity was stimulated by irradiation, with statistically significant, dose-dependent increases in both osteocalcin and ALP at 120 hours (Figure 2).
These data indicate that the two pools of bone cells display profound differences in radiosensitivity. By reducing the pool of available osteoclast precursors, radiation causes significant inhibition of osteoclast formation under M-CSF/RANKL stimulation (data not shown). Since osteoblast activity is stimulated by radiation, the net effect of these changes would likely be a transient increase in net bone mass, thereby explaining the clinical observation that prompted this study. Additional work is now needed to explore the molecular mechanisms by which radiation modulates these changes in osteoblastic activity.





Disclosures: ***M.J. Allen****, None.*

SA072

Validation of Dental Panoramic Radiographic Measures to Identify Women with an Increased Risk of Osteoporosis. A. Taguchi[1], Y. Suei*[1], M. Sanada*[2], T. Nakamoto*[1], M. Ohtsuka*[1], H. Sumida*[1], K. Tanimoto*[1], I. Kodama*[2], M. Tsuda*[2], K. Ohama*[2]. [1]Oral & Maxillofacial Radiology, Hiroshima University, Hiroshima, Japan, [2]Obstetrics & Gynecology, Hiroshima University, Hiroshima, Japan.

Recent studies suggest that mandibular inferior cortical shape and width on dental panoramic radiographs may be useful screening tools for low spinal bone mineral density (BMD) in postmenopausal women. However, little is known as to whether these measures are validated in comparison with other risk indices such as the Osteoporosis Self-assessment Tool (OST) developed for postmenopausal Asian women (Koh et al., 2001), and as to whether these measures can be used in postmenopausal women with hysterectomy, oophorectomy and/or estrogen use as well as in normal postmenopausal women. In this study, we calculated sensitivity, specificity, positive predictive value, negative predictive value and accuracy for dental panoramic measures (cortical shape and width) and OST (=integer (0.2*weight)-integer (0.2*age)) using data from 157 normal postmenopausal (group A) (mean age [SD], 57.4 [7.5] years) and 159 postmenopausal with hysterectomy, oophorectomy and/or estrogen use (group B) (56.3 [8.0] years). Spinal BMD was measured by dual x-ray abosrptiometry (DXA, Lunar DPX-alpha). Spinal fracture was not found in all subjects. Osteoporosis (OP) was defined as a BMD T-score of -2.5 or less at the lumbar spine (L2-L4). There were 38 women with OP in group A and 40 in group B. Cortical shape (normal or any cortical erosion) and width were evaluated on dental panoramic radiographs. Reciever operating characteristics (ROC) curve analyses were used to determine the cutpoints of cortical width and OST for the identification of spinal OP. Area under ROC curves to identify women with OP in group A were smaller in cortical width (0.771, SE=0.05) than OST (0.829, SE=0.04). At the thresholds of < 4.5 mm (cortical width) and =< -1.0 (OST), and selecting any cortical erosion, the sensitivity and specificity to identify women with OP were 89.5% and 33.9% for cortical width, 86.8% and 57.8% for OST, and 86.8% and 63.6% for cortical shape in group A. In group B, those were 92.5% and 35.0% for cortical width, 72.5% and 58.1% for OST, and 80.0% and 64.1% for cortical shape. Accuracy was somewhat higher for cortical shape (69.2% in group A, 68.2% in group B) than that for cortical width (47.1%, 49.7%) and OST (64.8%, 61.8%). Our results suggest that women with OP may be identified by cortical shape on dental panoramic radiographs as well as OST. Cortical shape may also be useful in postmenopausal women with hysterectomy, oohorectomy and/or estrogen use.

Disclosures: ***A. Taguchi****, None.*

SA073

Bone Mass and Body Composition of Neonates From Fan- and Pencil-Beam Dual Energy X-ray Absorptiometry. M. Hammami*, W. Koo. Pediatrics, Wayne State University School of Medicine, Detroit, MI, USA.

This study aims to determine the ability of physiologic parameters (weight, length, race and gender) to predict dual energy x-ray absorptiometry (DXA) measured bone mass and body composition in human neonates. In addition, we aim to compare the DXA measurements using the fan beam (FB) and pencil beam (PB) DXA techniques.
Bone mineral content (BMC), fat mass (FM) and lean mass (LM) were measured with FB DXA in 73 neonates and were measured with the older PB DXA technique in another cohort of 67 neonates. All subjects were healthy singletons and both cohorts have comparable birth weights and gestational ages. The predictive ability of physiologic parameters including weight, length, race and gender on DXA measurements was determined separately for each cohort with regression analysis. The instrument effect on each body composition measure was determined by univariate analysis of covariance that controlled for weight if there was significant interaction between instrument and profile of body composition measurements. Regression equations based on infants who had PB DXA measurements were used to predict the body composition of infants who had FB DXA measurements. The data in turn were compared to the measured values by paired t test.
Results showed that for each cohort of infants, weight was the dominant predictor of bone mass and body composition. FB and PB measured BMC, FM and LM were different whether controlled for weight or as a fraction of weight ($p<0.01$ for all comparisons). The predicted and measured FB derived BMC, FM and LM were correlated ($r=0.86$, 0.67 and 0.93, $p<0.001$ for all comparisons) although at different mean levels ($p<0.001$).
We conclude that body weight is the best physiologic predictor of bone mass and body composition in human neonates regardless of DXA technique. FB DXA measurements were correlated although differed to that from PB DXA. Thus, group comparison of data from either technique is possible after transformation of one set of data.

Disclosures: ***M. Hammami****, None.*

SA074

See Friday Plenary number F074

SA075

Osteoporosis in Chinese-American Women: Risk Factors for Fracture and Development of a Database for Bone Densitometry. M. A. Donovan[1], A. R. Opotowsky*[1], R. B. Babbar*[2], A. L. Rohira*[2], M. Della Badia*[1], G. Liu*[2], J. P. Bilezikian[1]. [1]Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA, [2]Medicine, New York University Downtown Hosptial, New York, NY, USA.

Asian women have consistently been shown to have lower BMD than Caucasian women. Yet, studies in Asia and the USA suggest that Chinese women may have a lower rate of hip fracture than Caucasian women. This apparent paradox is rooted in the idea that the relationship between BMD and fracture risk is equivalent for Caucasian and Asian women. But, it is not known whether a given T-score or absolute bone density carries the same fracture risk among Chinese-American women as it does for Caucasian women. The purpose of this study is to develop a clinically useful database for BMD among Chinese-American women in order to establish a relationship between BMD and fracture risk in this population. This information will be compared to databases established for Caucasian women in the USA and to Chinese women in Hong Kong and Beijing, China. Vertebral fracture risk as a function of BMD will also be determined. In addition, hip geometry, and relationships between calcium intake, BMD, country of origin, and years in the USA will be evaluated as other potentially important variables. 280 Chinese-American women between the ages of 20 and 90 (40 women per age decade) are being recruited. Along with DXA of the hip and spine, the study includes completion of a customized bilingual questionnaire that covers numerous demographic, familial, nutritional, behavioral, and attitudinal points. Women over 50 will also be evaluated for morphometric vertebral fracture with IVA and a subset will undergo digital x-ray of the hip for pelvimetry measurements. The project also includes a detailed assessment and case-control comparison of 35 patients who will have sustained a hip fracture. Between 11/02 and 4/03, 140 women have been recruited and their demographic, dietary and DXA results have been incorporated into the database. Preliminary data suggest that bone density rises by decade through the fifth decade (ages 40-49) and then falls progressively at the lumbar spine and hip. These results are consistent with previously published data in an indigenous Chinese population, but are different from databases constructed from Caucasian populations. The results suggest that Chinese-American women may achieve adult peak bone mass later than their Caucasian counterparts, which may have significant consequences for risk assessment and treatment. As this work continues, we expect to have generated, for the first time, a database that has direct relevance to the Chinese population in the United States.

Disclosures: ***M.A. Donovan****, Procter and Gamble 2; Merck 2.*

SA076

See Friday Plenary number F076

SA077

A Pilot Study of the Use of Radiographic Absorptiometry in the Measurement of Cortical and Trabecular Phalangeal Bone Mineral Density in Chinese Men and Women. Z. H. Liu*[1], X. Bi*[2], L. Aldayeh*[2], s. Silverman[3]. [1]Osteoporosis Committee of China Gerontological Society, Beijing, China, [2]CompuMed, Inc., Los Angeles, CA, USA, [3]OMC Clin Res Ctr, Beverly Hills, CA, USA.

Radiographic Absorptiometry (RA) automatically measures volumetric BMD in the 2nd, 3rd, and 4th middle phalanges. A study was conducted to investigate the ability of RA to separate cortical and trabecular phalangeal BMD, offering an inexpensive and safer alternative to QCT. The study was conducted using X-rays from RA tests of 556 patients. These x-rays were analyzed to compare the rate of decline of cortical, trabecular and total phalangeal BMD.
A normal Chinese population with no known osteoporosis or arthritis, of ages 10 to 89, with 277 male and 279 female were considered for the study. A two view standard AP x-ray was acquired for the non-dominant hand of each volunteer with an aluminum reference wedge placed near the hand. Based on the OsteoGram R technology (CompuMed Inc, Los Angeles, CA), which applies RA, a customized module has been developed to perform automated bone tissue segmentation isolating three distinct areas in each phalanx:
1. An axial cylinder, 2.2x2.2 m.m. in size, in the middle of the distal trabecular zone
2. An axial cylinder, 2.2x2.2 m.m. in size, in the middle of the proximal trabecular zone
3. A tube segment in middle of the phalanx that includes the cortical tissue and excludes the central non-cortical tissue.

The module performed BMD assessments in the cortical and the two trabecular areas in addition to the standard total phalangeal BMD. Considering the two radiographic views for each volunteer's hand and the three bones in each view, the unsupervised automated algorithm properly segmented the trabecular and cortical areas with a success rate of 90% for male and 92% for female.
Time courses of unadjusted BMD results showed peaks in the age range of 25-35. Using linear regression analysis the rate of BMD loss was calculated in BMD-AU (Arbitrary Units) per year for all subjects 35 years and older.For males, BMD decreased at the same rate in both trabecular and cortical tissues (~ -0.3), where as total BMD decreased at a higher rate (~ -0.5). For females, trabecular BMD decreased at a slightly higher rate than male (~ -0.4). However, cortical BMD decrease was much higher and close to total BMD decrease at the rate of -1.0. Further development of the module will investigate applying cortical thickness measurements. We believe that adding cortical and trabecular measurements to the RA BMD report could provide useful information to assist clinicians in providing better osteoporosis care.

Disclosures: ***Z.H. Liu****, None.*

SA078

PIXI Forearm Scan Prescreen for Osteoporosis Case Finding. P. J. Ryan, G. Worcester*. Osteoporosis unit, Medway Maritime Hospital, Gillingham, kent, United Kingdom.

We investigated the PIXI scanner in forearm mode for Osteoporosis case finding, used as a prescreen for axial scanning. A reference population was established from 165 patients attending the Fracture Liaison service who had bone density measured with the PIXI scan and Spine and Hip DXA (Hologic QD4500C). Comparison was made of T scores using the PIXI and axial measurements (References range NHanes 111 female hip and Hologic female spine and men). Patients were of average age 63 years, and 20 male, 143 female. Average T scores were –2.16 PIXI, -1.55 Spine and –1.43 Femoral neck. Regression comparison shows PIXI T scores about –1 SD below Spine and Hip T scores. Threshold values for PIXI T scores were calculated with an attempt to detect and exclude patients with spine or hip T scores <-1.5 (Steroid users), <2.0 (Fractures), <-2.5 (No steroids, No fractures). A lower PIXI threshold to determine those who required treatment without axial scanning could not be established as only very small numbers would be selected. Upper thresholds to determine those who did not require therapy could be established for the T <-2.5 spine and hip threshold. An upper PIXI threshold of T - 1.9 would result in reassurance of 40 % patients (with a misclassification of 1/16) on PIXI scan alone that their Spine and Hip T scores were greater than –2.5. For Axial T scores of –2.0 and -1.5 PIXI thresholds to identify a population that could be reassured was less reliable. The thresholds above were used to screen patients > 65 years in 3 general practices with clinical risk factors. PIXI and axial scans were acquired for those below the thresholds identified above (average age 73 years, 160 pts) and in this older group results supported the notion that a treatment threshold cannot be established. We conclude that PIXI forearm can be used as a prescreen in the elderly for a Spine and Hip –2.5 T score threshold. However, forearm T scores are set too low with respect to spine and hip T scores, and the majority of patients will still require an axial scan.

Disclosures: ***P.J. Ryan****, None.*

SA079

See Friday Plenary number F079

SA080

Does Radius Bone Density Measurement Over-Diagnosis Osteoporosis in Men? N. L. Vallarta-Ast[1], D. Krueger[2], N. Binkley[2]. [1]Radiology, Wm S Middleton VAMC, Madison, WI, USA, [2]Osteoporosis Clinical Center and Research Program, University of Wisconsin, Madison, WI, USA.

Radius bone mineral density (BMD) measurement increases the number of men diagnosed as osteoporotic. However, controversy exists whether this is improving DXA sensi-

tivity or leading to over-diagnosis. This possibility was evaluated by retrospective review in a large densitometric dataset.

Men referred for BMD measurement at the Wm. S. Middleton VAMC from 1997 to 2003 were considered; the 1197 who had spine, femur and radius measurements were included. Patient age ranged from 24 to 93 years (mean 67.2). Previous low trauma fracture was reported to the technologist in 507. All scans were performed using a GE Lunar Expert densitometer; T-scores were obtained using the manufacturer's male normative database.

Osteoporosis, defined as a T-score ≤-2.5 at the spine, total femur, femur neck, trochanter, .3 or ultradistal radius, was identified at one or more sites in 521 men. Of these men, osteoporosis was present at the spine in 201, femur in 389 and radius in 294. The prevalence of prior low-trauma fracture in those with "densitometric osteoporosis" at the spine, femur and radius was 58%, 52% and 53% respectively. Similarly, in men classified as osteoporotic only at the spine, femur or radius, the prior fracture prevalence was 49%, 49% and 53%. Thus, the prevalence of prior low-trauma fracture did not differ in either the entire osteoporotic group or in those uniquely identified at any single site. Furthermore, the percentage uniquely diagnosed as osteoporotic at the radius is very similar in the entire group, those with prior low-trauma fracture, vertebral fracture or hip fracture (Table). Finally, of the 5 men with normal femur and spine BMD but osteoporosis at the radius, 4 had a prior low-trauma fracture.

In conclusion, men diagnosed with osteoporosis at any site have a comparable prevalence of prior low trauma fracture. Similarly, fracture prevalence does not differ in those uniquely diagnosed as osteoporotic at the radius. Furthermore, the percentage of men uniquely diagnosed as osteoporotic at the radius is remarkably similar in those with prior fracture as in the entire group. Thus, these data suggest that radius measurement does not overdiagnose osteoporosis. Until prospective fracture risk data becomes available, use of radial sites for osteoporosis diagnosis in men seems appropriate.

Percentage of men uniquely diagnosed at each site as osteoporotic

Group	n	%≤-2.5@spine	%≤-2.5@femur	%≤-2.5@radius
Entire	1197	3	12	7
Prior Low trauma Fx	507	4	13	7
Prior vertebral Fx	167	5	11	7
Prior hip Fx	48	0	21	4

Disclosures: ***N.L. Vallarta-Ast****, None.*

SA081

A Comparison of Lumbar Vertebral T-scores by Three Methods: Average L1-L4 BMD, Lumbar BMD by ISCD Criteria, and Lowest Vertebral Body T-score. K. E. Hansen[1], N. Vallarta-Ast[2], D. Krueger[1], M. Drezner[1], N. Binkley[1]. [1]Osteoprososis Clinical Center and Research Program, Univeristy of Wisconsin, Madison, WI, USA, [2]Radiology, Wm. S. Middleton VAMC, Madison, WI, USA.

The ISCD recommends exclusion of abnormal vertebrae from analysis to allow more accurate determination of lumbar bone mass, although in clinical practice the L1-4 T-score is often reported. Herein we compare three different methods of lumbar spine T-score interpretation, and how these results might influence therapy in men. Scans obtained with a GE Lunar Expert-XL densitometer were reviewed on 211 male veterans (mean age 67.6 years) undergoing bone densitometry at the Wm. S. Middleton VAMC. Prior low-trauma fracture was reported in 84 men. Spine T-scores were obtained using the L1-L4 average, the lowest vertebral T-score and after vertebra exclusion based on ISCD recommendations. Resulting diagnoses are summarized in the table. No men had osteoporosis using the L1-4 method; conversely, no men had normal bone mass utilizing the lowest vertebral T-score. Furthermore, 3 scans were unevaluable by ISCD criteria due to severe scoliosis. A focal structural defect led to exclusion of one or more vertebra in 164 (78%) of 211 men, 80 (38%) due to a lack of increase in bone mineral content or area and 82 (39%) secondary to unusual discrepancy in T-score between adjacent vertebra. The fourth lumbar vertebra was most often excluded (164 men or 78%), followed by the L3, L2 and L1 vertebra (48%, 34%, and 33% respectively). An arbitrary threshold for pharmacological therapy (T-score < -2) was met less frequently, using the average L1-L4 T-score (40/19%), than using the ISCD criteria (91/43%), and the lowest vertebral body T-score (112/53%) ($p < 0.0001$). Additionally, more men with prior fracture were identified for treatment using the latter methods (49% and 60% respectively). The lowest lumbar T-score method was most likely to diagnose osteoporosis ($p<0.0001$).

In these male veterans, implementing ISCD criteria or lowest T-score methods resulted in a greater percent of men, with or without prior fracture, being diagnosed with osteoporosis and meeting therapy guidelines. Further study evaluating prospective fracture rates using these two approaches is appropriate to determine the ideal interpretation method.

Diagnosis and treatment categorized by method

	L1-L4 T-Score	Lumbar T-score by ISCD Criteria	Lowest Lumbar T-score
Osteoporosis	0	59 including 27 with prior fracture*	89 including 36 with prior fracture*
Osteopenia	75	101	122
Normal	136	48	0
Met Threshold for Therapy	40 including 18 with fracture	91 including 41 with fracture*	112 including 50 with fracture*

*$p < 0.0001$ comparing ISCD to either L1-L4 or Lowest Lumbar T-score.

Disclosures: ***K.E. Hansen****, None.*

SA082

See Friday Plenary number F082

SA083

Spinal Bone Mineral Density in Healthy Urban Asian Indian Women Presenting for a Preventive Health Check-Up. A. Keramet*[1], R. Bhambri*[1], D. Chakravarty*[2], A. Mithal[1]. [1]Endocrinology, Max MedCentres, New Delhi, India, [2]Radiology, Max MedCentres, New Delhi, India.

Bone mineral density (BMD) measurement is an important tool in the diagnosis and management of osteoporosis. Ethnic differences in BMD are well documented and may be due to factors such as genetic traits, body composition, calcium and vitamin D nutrition and metabolism. There is a lack of normative BMD reference values for the Asian Indian population and the guidelines for the diagnosis of osteoporosis in Caucasians may not apply to this population.

In this cross-sectional study we report the lumbar spine (L1-L4) BMD values of 974 female subjects in the New Delhi area (urban, north India). The subjects, who were otherwise healthy, had spinal BMD measurements as part of a preventive health check-up. The anterio-posterior lumbar spine (L1-L4) BMD was measured by axial dual energy X-ray absorptiometry (DXA) using a Lunar Prodigy machine.

The spinal BMD measurements are shown in the table along with the normal Caucasian female reference values and the percent difference between these two groups.

Available data, although limited, suggests the presence of lower BMD values (by 5-15%) in Asian Indian women compared to their Caucasian counterparts. However, in this study population, comprising exclusively economically well to do urban Asian Indian women, the difference in the mean spinal BMD with the normal Caucasian reference values was marginal across all the age groups studied, particularly in women over 40. This information highlights the urgent need for large multicentric population based studies in India, incorporating fracture-BMD correlations, to determine whether we need a different BMD reference range for Asian Indians.

Keywords: bone mineral density, Asian Indian women

Spinal BMD of Asian Indian women compared to manufacturer's database

Age Group (years)	N	Lumbar Spine BMD (g/cm^{2}) +/-1 sd	Reference mean BMD (Caucasian)	Percent difference
20-29	22	1.148+/-0.17	1.188	-3.4%
30-39	47	1.144+/-0.13	1.207	-5.2%
40-49	353	1.163+/-0.16	1.170	-0.6%
50-59	350	1.072+/-0.16	1.081	-0.8%
60-69	156	0.989+/-0.19	0.995	-0.6%
70-79	46	1.011+/-0.17	0.960	+5.3%

Disclosures: ***A. Mithal****, None.*

SA084

See Friday Plenary number F084

SA085

Screening Tools and Bone Mineral Density in the Evaluation of Osteoporosis in a Family Medicine Office. C. M. Jachna[1], S. Agrawal*[2]. [1]Internal Medicine, KU Medical Center, Kansas City, KS, USA, [2]Family Medicine, KU Medical Center, Kansas City, KS, USA.

To demonstrate the potential use of the Osteoporosis Self-Assessment Tool (OST) and the Osteoporosis Risk Assessment Instrument (ORAI) to identify women under age 65 for bone mineral density testing who are at risk for developing osteoporosis.

This study was retrospective chart review of 119 women ages 45-65 that presented for an annual well woman visit at a family medicine clinic. Demographic data and identifiable National Osteoporosis Foundation (NOF) risk factors for osteoporosis were collected. Age, weight, and hormone replacement therapy status were used to calculate each woman's individual score on the OST and the ORAI and to identify women who met criteria, using the screening tools, for bone mineral density (BMD) testing. In addition, BMD results of the women who had received testing at the discretion of the physician were collected. Recommendations for osteoporosis prevention or treatment were identified. Descriptive statistics were used to characterize the study population. Using Fisher's exact test the woman who received BMD testing were compared to the women who met criteria for screening by the OST and ORAI.

On average, women were 53 years old with an average BMI of 27. Two percent had a diagnosis of osteoporosis. The most common identified NOF risk factors for osteoporosis were tobacco abuse (17%), premature menopause (16%) and low body weight (14%). Sixty (50%) of women had osteoporosis prevention documented during their well woman visit. Twenty percent of women were on calcium, 51% were on hormone replacement therapy, and 2% were on a bisphosphonate. Nineteen percent and 15% of women, respectively, met criteria for BMD testing by ORAI and OST screening tool scores. Seventeen percent of women had previously received BMD testing and of these 11 (55%) were normal, 7 (35%) had osteopenia, and one had osteoporosis. The majority of women (70%) who had received BMD testing did not meet criteria for screening by the ORAI or OST. Those who received BMD testing did not have significantly more identified NOF risk factors compared with those who were not screened. Women who met OST criteria for BMD screening, were significantly more likely to have received BMD testing compared with women who did not

meet OST criteria. (p=0.013) There was no significant association between those who met screening criteria by ORAI and those who received BMD testing. Eleven (9%) women and 18 (15%) of women who potentially could have been identified for BMD testing with the use of the OST and ORAI screening tools respectively did not receive screening.
The OST and ORAI can be used to efficiently identify a group of women at risk for osteoporosis that are currently not being screened.

Disclosures: ***C.M. Jachna****, None.*

SA086

Hip Axis Length Assessed by the Norland DXA Scanner in Normal Chinese. J. M. Wang*[1], H. M. Ju*[2], T. V. Sanchez[3]. [1]Research and Development, Norland--a CooperSurgical Company, Beijing, China, [2]Harbin Orthopedic and Trauma Hospital, Harbin, China, [3]Research and Development, Norland--a CooperSurgical Company, Socorro, NM, USA.

Hip Axis Length (HAL) as assessed by DXA is known to be significantly related to hip fracture risk. This study examines HAL in a normal Chinese population using standard procedures.
A population of 50 females, without a history of hip fractures, over 22 years of age from the Harbin area of China underwent examination of the proximal femur area using the Norland XR-36. All scans were performed using Research Scan Software with a point resolution of 1.0 x 1.0 mm and a measurement scan speed of 90 mm/s with subjects positioned with the Hip Positioning Sling. Analysis of the HAL was performed on-screen using the Ruler Tool system. Repeatability of HAL studies, with repositioning, was 1.8%. Analysis repeatability of HAL studies was 0.9%. All studies in this report were audited and analyzed by the same factory trained operator (JMW).
As expected, HAL was not significantly related to age, body height, body weight or body mass index. HAL in this Chinese population averaged 9.18 cm (SD = 0.44 cm) which is substantially shorter than the reported average HAL of 10.67 cm (SD = 0.67 cm), assessed on Norland equipment, in Caucasian subjects.
In conclusion, it would seem that Chinese women have a significantly shorter HAL than is seen in the Caucasian population. Given this substantial difference in HAL and given the reported relationship between HAL and fracture risk (Faulkner, KG, et al. J Bone Mineral Res. 9:1065-1070, 1994), we might expect this Chinese population has a reduced hip fracture risk.

Disclosures: ***J.M. Wang****, Norland--a CooperSurgical Company 3.*

SA087

See Friday Plenary number F087

SA088

Human Lactation: Changes in Hip Bone Geometry but Not Bone Density Depend on Calcium Intake. B. C. C. Khoo*[1], R. I. Price[1], T. J. Beck[2], G. N. Kent[3], D. H. Gutteridge[1], J. Allen*[1], K. P. Singer*[4], S. S. Dhaliwal*[1]. [1]Charles Gairdner Hospital, Perth, Australia, [2]Johns Hopkins University, Baltimore, MD, USA, [3]Path Centre, Perth, Australia, [4]University of WA, Perth, Australia.

Human lactation (lac) delivers 5-8 mmol/d of milk calcium (Ca). Maternal Ca homeostasis taps (i) increased dietary Ca (+/- increased Ca gut absorption efficiency [Fα; ~0.25-0.3]); (ii) decreased urinary Ca excretion; (iii) bone mass loss. We and others showed (ii) & (iii) are relevant; maternal DXA areal (a) BMD decreases (eg; 0-6% in hip) at peak lac (PL) (~6mo postpartum [PP]), with postweaning (PW) recovery (1), but dietary Ca affects neither bone loss nor Fα (2). Unlike aBMD, Hip Structural Analysis (HSA, 3) describes bone structural geometry, including section modulus (Z; a strength index). This study applied HSA to human lactation.
From 100 pregnant women at 36 wk, randomised to Ca (25mmol/d) and studied through lac to 12mo PW, those N=47 with Fα measured (stable Ca isotopes; 2) at PL were triaged into tertiles of "Abs Ca" = {(Dietary Ca +/- 25mmol Ca x [Ca compliance]) x Fα} (tertile means 6.6, 11.8 & 15.8 mmol/d). % changes (Δ%; = PL minus 2wk PP) in aBMD & HSA, at the femoral neck, inter-troch. (IT) and shaft (Sh) were compared between tertiles. %Δ for 6-12 mo PW minus PL (N=29) were also analysed by tertiles of "Abs Ca".
Main findings; (i) Δ%"narrow neck" Z=6.3% loss ($p<0.01$) by PL, with NS between tertiles & no recovery PW; (ii) Δ%IT Z unchanged at PL but increased by PW (1.9-5.3%, $p<0.05$) (iii) mid-tertile Sh Z decreased (2.4%, $p<0.05$) at PL (flanking tertiles NS), tending towards recovery, PW. The "U-shaped" response of Sh Z by PL arose from a combined thresholded increase in Sh bone width, only at high Ca intake (1.4%, $p<0.05$), and a linear increase in Sh endosteal width with Ca intake (1.2 – 3.9%, $p<0.05$) (iv) aBMD fell by PL at all sites except troch., with part recovery by PW. There were no Ca-dependent differences in Δ%aBMD (v) For changes in Sh Z at PL, ANCOVA with shaft aBMD, treatment (Ca or placebo), height & weight as covariates showed only height ($p<0.01$) as a predictor of "change in Sh Z", in addition to the latter's quadratic dependence on Abs Ca ($R^2 = 0.25$, $p=0.005$). The nadir in Δ%Sh Z was at 10.4 mmol/d Abs Ca (~1.4 g/d Ca dietary intake).
We conclude that lactation-induced changes in Z, but not aBMD, are Ca-intake dependent. In the femoral shaft these changes show a "U-shaped" dependence on Ca intake. The IT Z increase by postweaning suggests a possible site-selective long term lactation-induced protection of bone strength. 1. Kent GN etal 1993 Osteo Int S1:44-7; 2. Kent GN etal 1991 Calc Tiss Int 48:293-5: 3. Beck TJ etal 2000 JBMR 15:2297-2304

Disclosures: ***B.C.C. Khoo****, None.*

SA089

See Friday Plenary number F089

SA090

Femoral Neck Strength Comparison between Subjects with and without Down Syndrome. F. Baptista*, P. Nogueira*, A. Varela*, L. B. Sardinha*. Exercise & Health Laboratory, Faculty of Human Movement, Lisbon, Portugal.

It has been reported that children and adults with Down syndrome (DS) have lower bone mass in the lumbar spine, compared to their peers without mental retardation or with mental retardation but without DS. However, regarding femoral neck there is no published data. This study was designed to compare structural measures of femoral neck strength, as suggested by Karlamangla et al. (2002), between males and females with and without DS. Due to the disproportionate shortening of the long bones of the legs in DS, it is necessary to avoid size related artifacts in the analysis of bone data when comparison between different groups are made on this skeletal region. Subjects were 51 females (20 with DS) and 56 males (23 with DS), 14-40 yr. Assessment of femoral neck bone mineral density (BMD), femoral neck width (FNW), and hip axis length (HAL) was performed with dual x-ray absorptiomatry (QDR 1500). ANCOVA was used to analyze the data. For this purpose, compressive, bending and impact strengths were used as dependent variables adjusted for age. Compressive strength (BMD*FNW/weight) and bending strength (BMD* $(FNW)^2$/HAL*weight) express the forces that femoral neck has to withstand in weight bearing, while impact strength (BMD*FNW*HAL/(height*weight) expresses the energy that femoral neck has to absorb in an impact from standing height. Mean differences between the control group (CG) and the DS group were -29% for compressive strength (DS 3.754±0.132 vs. CG 4.835±0.105 g/kgm, $p<.0001$), -34% for bending strength (DS 1.031±0.046 vs. CG 1.379±0.037 g/kgm, $p<.0001$), and -24% for impact strength (DS 0.249±0.008 vs. CG 0.309±0.249 g/kgm, $p<.0001$). The mean of each strength measures was lower in DS subjects than in CG. It was not found any interaction effect between subjects with and without DS and gender.We concluded that mineral density and femoral neck size in DS subjects did not adjust for body size variables with the same rate that was observed in CG. These results suggest that DS subjects may be at higher risk for a lower femoral neck strength.

Disclosures: ***F. Baptista****, None.*

SA091

See Friday Plenary number F091

SA092

Preprocessing and Fractal Analysis in Radiographic Texture Analysis (RTA) of the Calcaneus in Evaluating Patients with and without Vertebral Fractures. M. L. Giger*[1], V. Jaros*[1], T. Vokes[2], M. Chinander*[1], J. Wilkie*[1]. [1]Radiology, University of Chicago, Chicago, IL, USA, [2]Medicine, University of Chicago, Chicago, IL, USA.

We have developed radiographic texture analysis (RTA) methods for the assessment of bone structure from radiographic images of the spine, hip, and heel. Presently, we have reported that the combined analysis of BMD and RTA on images obtained using a clinical densitometer for the calcaneus shows promise in differentiating patients with and without vertebral fractures. In this study, we present our latest methods for optimizing the preprocessing and texture features involved in RTA.
Our database includes images of the calcaneus from 160 subjects (44 with and 116 without vertebral fractures) acquired with a peripheral densitometer system. In addition, BMD of the lumbar spine, femoral neck, and calcaneus are obtained. Radiographic texture analysis consists of (1) extracting a region of interest (ROI) from the digital radiographic image of the heel, (2) preprocessing the ROI of the trabecular structure, (3) characterizing the fractal nature of the trabecular pattern using a Baysian neural network, and (4) estimating the probability that a fracture exists. ROC analysis was employed to evaluate the performance of BMD and RTA in the task of distinguishing between patients with and without vertebral fractures. A resubstitution (self consistency) method was used to determine an upper bound in the performance and a leave-on-out jackknife method was used to determine the robustness of the method.
Az values (area under the ROC curve) of 0.59, 0.65, 0.54 were obtained for spine, hip, and heel BMD alone, respectively in the task of differentiating cases without and with a vertebral fracture. Compared to BMD values alone, texture analysis using a Bayesian neural network to characterise the fractal nature of the radiographic trabecular pattern after preprocessing (Az = 0.78, resubstitution; Az = 0.70, leave-one-out jackknife) was better than spine, hip, and heel BMDs with p-values less than 0.05 (in resubstitution) and with p-values of 0.16, 0.53, 0.08, for spine, hip, and heel, respectively (in the jackknife evaluation).
In conclusion, RTA has the potential to perform as well as hip BMD (and better than heel BMD) in the task of predicting those at risk for vertebral fracture. Continued improvement is expected as new filters and larger databases are obtained.

Disclosures: ***M.L. Giger****, R2 Technology 1.*

SA093

Variation of Site-Specific Trabecular Bone Architecture in Regions Requiring Tendon-to-Bone Healing. D. J. Adams, S. A. Santangelo*. Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT, USA.

Tunnels are drilled into or cored from trabecular bone routinely for surgical repair of tendons and ligaments torn at their osseous insertion, as well as for reconstructing intra-articular tissues with tendon grafts; e.g., the cruciate ligaments of the knee. The initial integrity of fixation and subsequent bone-tendon healing are dependent on the integrity of bone surrounding the tunnel. With the advent of high-resolution micro-computed tomographic imaging (μCT), it is possible to quantify the three-dimensional architecture of bone cores removed during surgical procedures.

In this study, μCT imaging at 15 μm resolution and bone densitometry via high-resolution QCT were used to measure trabecular morphometry and volumetric bone density (mg/cm^3) of 10 mm diameter cylindrical trabecular cores removed at tibial tunnel sites of 19 subjects undergoing surgical reconstruction of a torn anterior cruciate ligament (14-57 years old, 7 male, 12 female). Morphometric measurements were computed for the entire three-dimensional field and subregions of the cores. To estimate the morphometric integrity of the remaining tunnel wall (the interface with tendon and fixation), an outer annulus of each cylindrical core image was "unrolled" computationally, providing images of the host tunnel walls. From this subset of two-dimensional image data, the architectural integrity of the receiving tunnels also was quantified.

Full-field measurements of trabecular bone volume fraction and density from the spectrum of subjects revealed a 2.5-fold range in magnitude at longitudinally matched sites within the cylindrical cores (26-64 % and 147-366 mg/cm^3, respectively), differences that were associated with variation in both trabecular thickness and number. Density of the trabecular bone tissue itself, excluding marrow space, revealed site-matched variation ranging 44% (e.g., 423 – 608 mg/ cm^3). Measurements at the tunnel wall, the region of host bone that would appose tendon in the initial phase of tendon-to-bone healing, revealed a smaller range in bone area fraction of 26-38%.

Morphometric data obtained in this and future micro-imaging studies of otherwise discarded bone samples can accurately quantify the integrity of surgical site-specific trabecular architecture. The information obtained is particularly helpful toward elucidating the interaction of surgical hardware and tissue grafts with host bone, as well as for choosing animal models of tendon-to-bone healing and validating benchtop models of fixation performance. Interfacial bone surface area available at the tunnel wall may also influence the initial rate of tendon-to-bone integration and subsequent functional outcome.

Disclosures: ***D.J. Adams****, None.*

SA094

Serum Tartrate-resistant Acid Phosphatase 5b as a Marker of Bone Metastases in Prostate Cancer. S. L. Alatalo*[1], M. Ala-Houhala*[2], J. Korpela*[2], J. M. Halleen[1], H. K. Väänänen*[1], A. Koskinen*[3], H. Helenius*[3], E. Salminen*[2]. [1]Institution of Biomedicine, Department of Anatomy, University of Turku, Turku, Finland, [2]Department of Oncology, University Hospital, University of Turku, Turku, Finland, [3]Biostatistics, University of Turku, Turku, Finland.

Tartrate-resistant acid phosphatase 5b (TRACP 5b) is a bone resorption marker secreted by osteoclasts during bone resorption. The purpose of this cross-sectional study was to evaluate the potential value of serum TRACP 5b as a marker of bone metastases in prostate cancer. Serum levels of TRACP 5b, total alkaline phosphatase (AFOS), and prostate specific antigen (PSA) were measured from 131 prostate cancer patients. Based upon scintigraphic and radiological findings and clinical follow-up the patients were divided into two groups: patients with verified single or multiple bone metastases (N = 25) and patients without bone metastases (N = 106). Bone marker levels were correlated with the presence or absence of bone metastases. Serum TRACP 5b activity was measured with a solid-phase immunofixed enzyme activity assay. Comparison between groups was done with the non-parametric Wilcoxon's test. Patients with bone metastases group had higher TRACP 5b level 7.81 ± 5.5 U/l compared with patients without bone metastases 3.39 ± 1.24 U/l ($p<0.0001$). The mean serum levels of AFOS were 1152 ± 1614 U/l and 164 ± 49 U/l, respectively ($p<0.0001$), and PSA 328 ± 161 and 12.7 ± 31.9 ($p<0.0001$). Data showed strong association between serum TRACP 5b and bone metastases, and the correlation between TRACP 5b and AFOS was statistically significant, Spearman coefficient 0.556, $p<0.0001$. The clinical specificity of TRACP 5b (percentage of prostate cancer patients without bone metastases within reference values) was 94.3 % (100/106), and the clinical sensitivity (percentage of prostate cancer patients with bone metastases above reference values) was 60.0 % (15/25). These results suggest that serum TRACP 5b is a powerful marker of bone metastases in prostate cancer patients with radiological skeletal metastases. Its role in prediction of dormant bone metastases needs further prospective studies.

Disclosures: ***S.L. Alatalo****, None.*

SA095

Serum Tartrate-resistant Acid Phosphatase 5b and Osteocalcin in Naturally Occurring Osteopetrotic Rats. S. L. Alatalo*[1], K. K. Ivaska*[1], Z. Peng[1], J. M. Halleen[1], S. C. Marks*[2], H. K. Väänänen*[1]. [1]Institute of Biomedicine, Department of Anatomy, University of Turku, Turku, Finland, [2]Department of Cell Biology, University of Massachusetts Medical Center, Worcester, MA, USA.

Three different naturally occurring osteopetrotic rat strains have been identified; toothless (tl/tl), containing severely reduced number of osteoclasts with normal ruffled borders, osteopetrotic (op/op), containing slightly reduced number of osteoclasts with poorly developed ruffled borders and incisors absent (ia/ia), containing markedly increased number of osteoclasts without ruffled borders. The tl/tl mutation is a defect in colony-stimulating factor (CSF-1 or M-CSF), but the mutations in op/op and ia/ia rats are still unknown. We measured the bone resorption marker tartrate-resistant acid phosphatase 5b (TRACP 5b) and the bone formation marker total osteocalcin from the serum of 2-week-old osteopetrotic rats and their normal littermate controls (nlm). The results are shown in table 1. TRACP 5b was significantly decreased and osteocalcin significantly increased in tl/tl rats, while both markers were unchanged in op/op rats. In ia/ia rats, TRACP 5b was significantly increased and osteocalcin unchanged. In vitro osteoclast cultures were made from long bones of 1- to 4-day-old ia/ia rat pups. Despite of their inability to resorb bone, osteoclasts from ia/ia rats secreted more TRACP 5b into the culture medium than osteoclasts from nlm controls. TRACP 5b activities in the soft tissues (liver, spleen and lungs) of adult ia/ia rats were normal, which suggests that serum TRACP 5b originates form osteoclasts. Osteopetrotic phenotype in ia/ia rats was sustained up to 8 months of age, studied by peripheral quantitative computed tomography (pQCT). These results suggest that in various osteopetrosis rat strains, secreted TRACP 5b might reflect the number of osteoclasts rather than their activity. Osteocalcin measurements suggest that bone formation is increased in tl/tl rats but not affected in op/op or ia/ia rats.

Table 1. Serum TRACP 5b and osteocalcin levels in tl/tl, op/op, and ia/ia rats

Mutation	n	TRACP 5b (U/L) (mean ± SD)	Osteocalcin (ng/ml) (mean ± SD)
tl/tl	10	2.7 ± 0.4***	32.7 ± 2.7**
nlm	10	118.4 ± 29.5	26.0 ± 4.7
op/op	4	100.4 ± 16.2	27.0 ± 3.5
nlm	4	115.25 ± 6.7	29.0 ± 6.9
ia/ia	7	482.6 ± 76.1***	22.8 ± 3.2
nlm	6	122.2 ± 18.1	19.7 ± 3.1

Disclosures: ***S.L. Alatalo****, None.*

SA096

A Novel Precise and Sensitive Method for the Determination of Vitamin K Homologues in Human Plasma Using High-Performance Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS). Y. Suhara*, M. Kamao*, N. Tsugawa*, T. Okano. Department of Hygienic Sciences, Kobe Pharmaceutical University, Kobe, Japan.

Vitamin K is believed to be involved in posttranslational modification of the bone matrix protein osteocalcin and its insufficiency is frequently associated with low bone mineral density (BMD) and higher incidence of hip-fracture. Assessment of vitamin K status can be achieved by measuring plasma levels of either vitamin K or undercarboxylated osteocalcin that is predictive of vitamin K deficiency. There was, however, no direct evidence that undercarboxylated osteocalcin is involved in impaired bone mineralization in vitamin K deficiency. Therefore, measurement of vitamin K is as of now the preferred method for establishing a basis for the treatment and prevention of osteoporosis with vitamin K. Plasma vitamin K levels are usually extremely low and require a highly sensitive method for the assay. High-performance liquid chromatography (HPLC) using either a combination of platinum oxide catalyst reduction with fluorescence detection or electrochemical detection has been employed for this purpose, but the separation of concomitants existed in blood samples from vitamin K homologue, especially menaquinone-4, is sometimes difficult in these assays. We report here the development of a novel precise and sensitive method for the determination of vitamin K homologues including phylloquinone, menaquinone-4 and menaquinone-7 in human plasma using HPLC-tandem mass-mass spectrometry with atmospheric-pressure chemical ionization (LC/MS/MS-APCI). The method includes the use of stable isotope (^{18}O) labeled internal standard compounds that were synthesized in our laboratory and the selection of precursor and product ion with a MS/MS multiple reaction monitoring method. Average intra-assay and inter-assay variations (CV) for PK, MK-4 and MK-7 were <10 %. Average spiked recoveries from authentic compounds added to normal human plasma samples for PK, MK-4 and MK-7 were 98-102 %. Mean plasma concentrations of PK, MK-4 and MK-7 from unselected healthy subjects (n=20) were 1.39±0.86 ng/mL, 3.22±0.57 ng/mL and 6.37±7.45 ng/mL, respectively. We conclude that this novel LC/MS/MS-APCI method should be useful for the evaluation of vitamin K status in postmenopausal women and elderly subjects and provides useful information for the treatment and prevention of osteoporosis with vitamin K.

Disclosures: ***Y. Suhara****, None.*

SA097

See Friday Plenary number F097

SA098

Validation of the ELISA CrossLaps™ Assay for the Quantification of C-Terminal Telopeptides in Dog Urine. J. Mayer*, P. Oldfield, A. Bartlett*, S. Y. Smith. CTBR, Montreal, PQ, Canada.

The purpose of this study was to validate an assay for bone resorption in dogs. The CrossLaps enzyme-linked immunosorbent assay (ELISA) was validated for the determination of C-terminal telopeptides of type-1 collagen (CTx) in dog urine. This test is designed to measure collagen type-1 fragments generated by osteoclast activity. Its use will be important in the preclinical testing of medicinal products to support osteoporosis, arthritis

and toxicology studies in which dogs are frequently used and where bone resorption is a required end-point.
The assay is performed using the CrossLaps ELISA kit (cat. no: ICRL-4000, Nordic Bioscience Diagnostics, Denmark). The wells of the plate are pre-coated with a synthetic CrossLaps to which calibrators and quality controls are added followed by the anti-CrossLaps and allowed to incubate. The wells are washed, followed by the addition of the second antibody conjugated with peroxidase, and after further incubation followed by a washing step, a chromogenic substrate is added. The reaction is subsequently stopped using sulfuric acid and the absorbance at 450 measured.
The lower limit of quantitation (LLOQ) of the assay was 248 µg/L. The method has been shown to be highly specific for the determination of bone-related degradation products from C-terminal telopeptides of type I collagen in dog urine. Accuracy and precision are summarized as follows:

QC Sample	Intra-assay	(3 times n=6)	Inter-assay (n=18)	
(µg/L)	CV (%)	Recovery (%)	CV (%)	Recovery (%)
248	9.4-18.9	93.8-104.8	15.1	100.6
552	3.7-8.2	118.0-120.8	5.6	119.9
1104	5.0-6.1	102.2-124.0	9.6	113.4
2208	7.4-9.6	87.2-109.6	12.5	100.0
5037	3.4-5.9	87.5-108.1	10.1	100.0

CTx was shown to be stable in dog urine after three freeze-thaw cycles, at room temperature for at least 6 hours, and at 4°C for at least 23 hours. Long-term storage stability at -20°C is currently on-going.
The use of the CrossLaps assay has been validated for the determination of CTx of type-1 collagen in dog urine. This assay provides a sensitive tool to monitor bone resorption and the effect of bone active agents on resorption activity.

Disclosures: ***J. Mayer****, None.*

SA099

Urinary Gamma-Glutamyl Transpeptidase (GGT) Activity Measurement Is Useful as a New Biochemical Marker for Bone Resorption. I. Kodama, M. Sanada*, M. Tsuda*, K. Ohama*. Obstetrics and Gynecology, Hiroshima University, Hiroshima City, Japan.

The purpose of this study is to investigate the measurement of urinary gamma-glutamyl transpeptidase (GGT) activity is useful as a biochemical marker for bone resorption in postmenopausal women.
A total of 45 postmenopausal Japanese women, aged 48 to 57 years, were admitted to study. Each subject had experienced natural menopause for at least 1 year but not longer than 5 years. All participants were followed up for 12 months of without any lifestyle modification. A total of 26 women (mean age 52.3±1.3) received conjugated equine estrogen (Premarin), 0.625 mg daily each morning for 12 months. Urinary cross-linked N-telopeptides (NTx), GGT activity and serum GGT activity were measured on all subjects before the start of therapy and for 3, 6 and 12 months after therapy. Lumber spine (L2-L4) bone mineral density (BMD) and femoral neck BMD were measured using a dual X-ray absorptiometer (Lunar DPX). These parameters were measured on all subjects before the start of therapy and for 6 and 12 months after therapy.
Correlation between urinary GGT activity and NTx were significant at before the start of therapy, and those relations were being maintained within the period of therapy.
L2-L4 BMD was increased after the start of the HRT, averaging +3.23% and +5.88%, at the 6 months and 12 months, respectively.
Femoral neck BMD was slightly increased compared with baseline value, averaging +0.98 % and +1.96 %, at the 6 months and 12 months after HRT (statistically not significant). In conclusion, we now report that urinary GGT activity measurement is useful as a new biochemical marker for bone resorption.

Disclosures: ***I. Kodama****, None.*

SA100

See Friday Plenary number F100

SA101

Bone Mineral Density and Bone Biochemical Markers in Lupus and Control Women Stratified by Menopause Status. A. B. Chadha[1], E. Shamiyeh*[1], C. Langman[2], H. Price*[2], S. Spies*[1], R. Ramsey-Goldman[1]. [1]Feinberg School of Medicine, Chicago, IL, USA, [2]Childrens' Memorial Hospital, Chicago, IL, USA.

Over the past 2 decades improved survival of lupus women (LW) lead researchers to focus on minimizing morbidity associated with disease and its treatment. Of the various complications facing women with lupus, osteoporosis remains a continuing challenge for clinicians. The goal of this study was to determine if bone mineral density (BMD) correlated with bone biochemical markers (BBM) in LW and control women (CW) matched for age, race, and menopause status. In this cross-sectional study, data collected included: a lifestyle questionnaire and medication survey, BMD of the hip, spine, and distal forearm, urine for N-telopeptide (NTx), and serum for Osteocalcin (OC), and bone alkaline phosphatase (BAP). 80 pairs were studied, 33 postmenopausal (PM) and 47 still menstruating (M). The mean age of LW and CW was 44.3 years, (SD 11.7) and 44.5 years, (SD 11.3) respectively; the mean disease duration for LW was 9.3 years, (SD 8.3). The mean age of menopause was 41.3 years, (SD 7.8) for LW and 44.4 years, (SD 5.8) for CW (NS). When stratified by menopause status, BMD was lower in M LW vs. CW at the hip (0.927 vs. 0.944 g/cm^2) and spine (1.020 vs. 1.047 g/cm^2), and in PM LW vs. CW BMD was lower at the hip (0.848 vs. 0.852 g/cm^2), spine (0.937 vs. 0.982 g/cm^2) and distal forearm(0.645 vs. 0.673 g/cm^2).
There were no significant differences in median BBM between LW and CW stratified by menopause status (data not shown). In PM LW multiple BBMs correlated with BMD at various sites: higher NTx with lower BMD at the hip (r= -0.550, p= 0.001) and spine (r= -0.419, p= 0.02), higher BAP with lower BMD at the hip (r= -0.436, p=0.012) and distal forearm (r= -0.376, p= 0.04), and higher OC with lower BMD at the hip (r= -0.492, p= 0.004). In PM CW multiple BBMs also correlated with various BMD sites: higher NTx with lower BMD at the hip (r= -0.367, p= 0.04), spine (r= -0.346, p= 0.05), and distal forearm (r= -0.518, p= 0.002), higher BAP with lower BMD at the distal forearm (r= -0.609, p= 0.0002), and higher OC with lower BMD at the distal forearm (r= -0.482, p= 0.005). In M LW, higher NTx (r= -0.295, p= 0.05) and BAP (r= -0.336, p=0.02) correlated with lower BMD at the distal forearm. In contrast, in M CW there were no correlations between BBM and BMD. LW had lower BMD than CW at most sites regardless of menopause status. Higher BBMs were associated with lower BMDs in PM and M LW, and only PM CW suggesting higher bone turnover in these groups. Measuring BBMs in addition to BMD many help identify LW at risk for low BMD, especially young M LW. Future studies include longitudinal analysis of LW at baseline and two years and determining weather baseline BBMs are predictors of future BMD.

Disclosures: ***A.B. Chadha****, NIH/NIAMS 2; Arthritis Foundation Clinical Science Grant and Greater Chicago Chapter 2; Proctor and Gamble Pharmaceuticals 2, 5; Merck 2, 5, 8.*

SA102

A Comparative Study of Volumetric, CTx-beta and Helical Peptide Resorption Assays. S. A. Nesbitt, B. M. Nicholls*, G. T. Charras*, M. A. Horton. Medicine, UCL, London, United Kingdom.

Many clinical diagnostic kits monitor skeletal turnover by analysing collagen degradation products. Herein, using an in vitro model, we have used CTx-beta (CTx) and Helical peptide (HP) ELISA assays to measure resorption and compared them with volumetric changes in resorption pits.
Rabbit osteoclasts were cultured on dentine slices for 1, 3, 7 and 10 days and also for 3 days with resorption regulators at doses that altered resorption while maintaining cell numbers (0.05 mM E-64, a cathepsin K inhibitor; 0.1 mM alendronate, a bisphosphonate; 25 nM kistrin, an integrin antagonist; 50 pM TIMP1 protein, a matrix metalloproteinase inhibitor). Resorption culture media were assessed for CTx (Nordic BioSciences, Denmark) and HP (Quidel, USA) by ELISA. Volumetric analysis of 13,589 stained resorption pits was done with an established in-house assay using 3D immunofluorescence confocal microscopy. Percentage changes were calculated from controls and assessed by Mann-Whitney statistics (n = 9-18).
The volumetric and ELISA assays detected significant changes ($p < 0.05$) for resorption in all experiments, except for HP using TIMP1. However, the resorption percentages varied between the assays. Firstly, resorption measured by time with the ELISAs (rows 2-4) showed greater increases (3-20 fold). These data suggest additional processing of the collagen matrix is occuring after resorption. Secondly, the ELISAs produced variable results for the resorption regulators (rows 5-8). Thus, biochemical changes in the resorbed matrix given by the regulators can produce an underestimate (eg. CTx with alendronate, TIMP1 with CTx and HP) or an overestimate of resorption (eg. kistrin with HP) when compared to the volumetric analysis.
In summary, these data suggest that several markers of bone resorption should be used in clinical diagnosis and treatment to account for the variability of measurements given by different ELISA assays.

Resorption % Changes (* relative to day 1, ** relative to day 3 without compounds)

	% Volume change	% CTx change	% Helical peptide change
Time - Day 3 *	250	211	803
Time - Day 7 *	726	4,655	14,761
Time - Day 10 *	1,257	4,056	15,850
Inhibitor - Kistrin **	-33	-37	-57
Inhibitor - Alendronate **	-68	-49	-68
Inhibitor - E-64 **	-86	-85	-99
Stimulator - TIMP1 **	83	22	Not significant

Disclosures: ***S.A. Nesbitt****, None.*

SA103

Alternate Immunoassay for Tartrate-Resistant Acid Phosphatase 5b Activity. R. M. Simons*[1], A. J. Janckila[2], L. T. Yam*[1]. [1]Medicine, University of Louisville, Louisville, KY, USA, [2]Medicine, VAMC, Louisville, KY, USA.

Objective: Osteoclastic tartrate-resistant acid phosphatase (TRACP) isoform 5b may be useful as a serum marker of bone turnover. Immunoassays (EIA) in use for serum TRACP using p-nitrophenyl phosphate (pNPP) as substrate are not entirely specific for TRACP isoform 5b. Our purpose was to develop an alternate immunoassay for TRACP using naphthol ASBI-phosphate (ASBI-P) as a selective substrate for isoform 5b and heparin as a selective inhibitor of isoform 5a.
Methods: Partially purified serum TRACP 5a and 5b were used to determine optimal reaction conditions for TRACP 5b with ASBI-P and heparin. TRACP immunoassay with ASBI-P was then standardized in terms of linearity, analytical recovery, sensitivity and reproducibility. To measure specificity of bone resorption, TRACP levels were correlated with those of bone alkaline phosphatase (BAP) and N-telopeptides of type-I collagen

(NTx) in both end-stage renal disease (ESRD) and rheumatoid arthritis (RA) sera.
Results: Optimum TRACP 5b activity was achieved at pH 6.3 with 10mM ASBI-P and 25 U/mL heparin. The relative biochemical specificity for isoform 5b was greater than 2-fold higher using ASBI-P compared to pNPP. The specificity increased a further 2-fold when heparin was included into either substrate solution. TRACP Immunoassay using ASBI-P followed a quadratic dose-response curve from 0 to 25 U/L with a least detectable limit of 0.24 U/L. The analytical recovery over the range of the assay was 76% with an average coefficient of variation of 16.6%. ASBI-P-based immunoassays of sera from both RA and ESRD correlated highly with standard pNPP EIA (r=0.94). TRACP EIA using ASBI-P and pNPP correlated significantly and to the same degree with BAP and NTx in both RA and ESRD cohorts.
Conclusions: ASBI-P is biochemically more specific for TRACP isoform 5b than pNPP and has equivalent sensitivity. However, in practice, TRACP 5b activity in sera measured with either substrate correlates with other bone turnover markers approximately equally. Larger studies of different diseases are required to determine if this alternate TRACP assay offers significantly greater diagnostic specificity over the well-established assay for osteoclastic TRACP 5b.

Disclosures: ***R.M. Simons****, None.*

SA104

Comparison of Serum Tartrate-resistant Acid Phosphatase 5b with Other Markers of Bone Turnover in Monitoring Alendronate Therapy. J. M. Halleen[1], A. M. Nenonen*[2], S. L. Alatalo*[3], K. K. Ivaska*[3], S. Cheng[4], H. Schmidt-Gayk*[5], K. Uusi-Rasi*[2], A. Heinonen[2], P. Kannus*[2], H. Sievänen*[2], K. Väänänen*[3]. [1]Pharmatest Services Ltd, Turku, Finland, [2]The UKK Institute for Health Promotion Research, Tampere, Finland, [3]Institute of Biomedicine, University of Turku, Turku, Finland, [4]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [5]Department of Endocrinology and Oncology, Laboratory group, Heidelberg, Germany.

The purpose of this study was to compare serum tartrate-resistant acid phosphatase isoform 5b (TRACP 5b) with other markers of bone turnover in monitoring alendronate therapy. This double-blinded study included 164 healthy postmenopausal women that were randomly assigned into two groups, one receiving 5 mg alendronate daily, and the other receiving placebo. All subjects received calcium and vitamin D daily. The resorption markers serum TRACP 5b, serum C-terminal crosslinked telopeptides of type I collagen (CTX), total urinary deoxypyridinoline (DPD), and the serum formation markers procollagen I N-terminal propeptide (PINP), bone-specific alkaline phosphatase (BAP), and total osteocalcin (OC) were assessed at baseline and at 3 months after start of treatment. Bone mineral density (BMD) of the lumbar spine was measured at baseline and at 12 months. Compared with the placebo-group, BMD increased and the markers decreased significantly more in the alendronate-group ($p<0.001$ for each parameter). Least significant change (LSC) was determined for each marker based on its analytical and biological variability. Those subjects that showed a decrease of more than LSC were defined as responders for the treatment. Clinical specificity was determined as the relative amount of non-responders in the placebo-group, and clinical sensitivity as the relative amount of responders in the alendronate-group. The results are summarized in table 1. The column "combined" shows values obtained by multiplying the specificity of each marker with its sensitivity. The highest combined values were obtained for TRACP 5b (0.74) and PINP (0.72). These results suggest that serum TRACP 5b is a specific and sensitive marker for monitoring alendronate treatment.

Table 1: Bone markers in alendronate therapy

Marker	LSC (%)	Specificity	Sensitivity	Combined
TRACP 5b	29,0	0,90	0,82	0,74
PINP	33,8	0,83	0,87	0,72
CTX	60,8	1,00	0,63	0,63
DPD	30,7	0,92	0,61	0,56
OC	19,4	0,70	0,75	0,53
BAP	25,1	0,93	0,55	0,52

Disclosures: ***J.M. Halleen****, SBA-Sciences Ltd 7.*

SA105

CTX and Osteocalcin in a Simple Model for Improved Assessment of Skeletal Response to Hormone Replacement Therapy. P. Qvist[1], I. Byrjaldsen*[1], C. Christiansen[2]. [1]Nordic Bioscience, Herlev, Denmark, [2]Center for Clinical and Basic Research, Ballerup, Denmark.

Biochemical 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. The objective of the present study was to investigate if a combined model using both a bone resorption and a bone formation marker could improve accuracy and consistency in the prediction of the long-term skeletal effect of hormone replacement therapy in postmenopausal women.
Two hundred seventy-eight healthy women within 1-6 years after menopause were included in a double-blind, three year monocenter study of treatment with various doses of 17β-estradiol + gestodene or placebo (Bjarnasson et al., 2000). One hundred and fifty three women completed the study and 135 of those were available for the present analysis.
With cut offs defined as 1%, 35% and 25% for αBMD, CTX and Osteocalcin, respectively, the combined biochemical model, requiring a response in either the resorption or the formation marker, provided sensitivities and specificities in the range 68-95%, for prediction of BMD response and 91-100% for prediction of compliance. This was similar to CTX alone. However, the analysis demonstrated higher sensitivities of the combined model at the 12 months visit, also reflected in an increase in the consistency of the response status reported by the combined biochemical model between the 6 and 12 months visit.

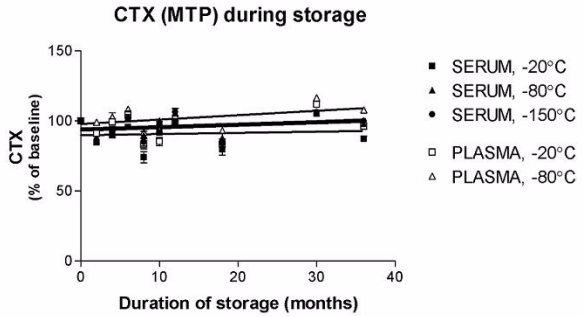
Model	Visit	Prediction of BMD response		Prediction of compliance (HRT vs placebo)		Biochemical Response Consistency	
	(months)	SEN (%)	SPE (%)	SEN (%)	SPE (%)	RESP: (%)	NON-RESP. (%)
Combined Model	6	94.1	76.4	93.9	91.9		
	12	95.2	68.6	96.9	97.3	97.9	90.2
CTX alone	6	94.0	72.5	93.9	97.3		
	12	90.5	76.5	89.8	100.0	91.4	90.2

Disclosures: ***P. Qvist****, Nordic Bioscience 1, 3.*

SA106

Fragments of C-telopeptides of Type I Collagen (CTX) Are Stable in Serum and Plasma Samples during Storage at Low Temperatures for Three Years. P. Qvist[1], N. From*[1], M. Munk*[1], N. Hoyle*[2], C. Christiansen[3]. [1]Nordic Bioscience, Herlev, Denmark, [2]Roche Diagnostics, Penzberg, Germany, [3]Center for Clinical and Basic Research, Ballerup, Denmark.

BACKGROUND: Control of pre-analytical variables is essential for successful application of biological markers, including bone resorption markers, in clinical trials and routine use. The effect of storage temperature on stability of bone resorption markers have not been subject of systematically investigation, and therefore the present study determined the stability of serum CTX in frozen serum and plasma samples stored for three years. METHODS: Serum and EDTA plasma samples were aliquoted and stored at -20°C, -80°C or -150°C until testing for CTX by the microtitre plate or the automated ElecSys formate of the Serum CrossLaps test..RESULTS: No decrease in CTX could be detected in neither serum nor plasma samples after three years of storage at -20°C, -80°C or -150°C, as recoveries were in the range 87-126% compared to baseline. CONCLUSIONS: CTX is stable in frozen serum and plasma samples for at least three years.



Disclosures: ***P. Qvist****, Nordic Bioscience 1, 3.*

SA107

Exclusion of 5 Candidate Genes for Familial Benign Hypercalcaemia, Type 3, Located on Chromosome 19q13. J. J. O. Turner[1], J. M. Stacey*[1], M. P. Whyte[2], R. V. Takker[1]. [1]Nuffield Department of Medicine, Oxford University, Oxford, United Kingdom, [2]Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children, St. Louis, MO, USA.

Familial benign hypercalcaemia (FBH), also referred to as familial hypocalciuric hypercalcaemia (FHH), is a genetically heterogeneous disorder that consists of three types designated, FBH1, FBH2 and FBH3 whose chromosomal locations are 3q21-q24, 19p13.3 and 19q13, respectively. FBH1 is caused by mutations of the calcium sensing receptor (CaSR), but the abnormalities underlying FBH2 and FBH3 are unknown. FBH3, also referred to as the Oklahoma variant (FBHOk), has been mapped to a 10 centimorgan (cM) interval on chromosome 19q13 flanked centromerically by D19S112 and telomerically by D19S317. This interval contains over 100 genes and we have utilised a bioinformatics approach to identify candidate genes based on tissue expression patterns and likely functions of the encoded proteins. This revealed 5 potential candidate genes and DNA sequence analysis of the coding regions and intron/exon boundaries of each of these 5 genes was undertaken using leukocyte DNA from an affected and an unaffected family member. These 5 candidate genes consisted of:1) Histidine Rich Calcium Binding protein (HRC) which has 6 exons and a coding region of 2.1 Kb, and is expressed in muscle and kidney, with a role in intra-cellular calcium binding; 2) rRAS which has 6 exons and a coding region of 0.6 Kb, and is expressed ubiquitously, with a role as a GTPase; 3) G-protein receptor 4 (GPR4) which has 1 exon and a coding region of 1.1 Kb, and is expressed in kidney and lung, with a possible role as a Sphingosylphosphorylcholine receptor; 4) Pleckstrin Homolgy Sec7 Coiled Domain 2 protein (PSCD2) which has 12 exons and a coding region of 1.2 Kb and is expressed ubiquitously, with a role as a cellular adhesion molecule; 5) Tuberoinfundibular peptide of 39 amino acids (TIP39) which has 2 exons and a coding region of 0.1 Kb and is expressed in the CNS and kidney, with a role as a PTH receptor antagonist. DNA sequence analysis did not detect any FBH3-associated mutations involving these 5 candidate genes, but 5 polymorphisms (3 for rRAS and 2 for HRC) with allele frequencies ranging from 24% to 48% were found. Thus, our studies have excluded these 5 candidate genes for FBH3. The identification of the gene causing FBH3 will help to facilitate the characterisation of another CaSR or a mediator of calcium homeostasis.

Disclosures: ***J.J.O. Turner****, None.*

SA108

Functional Phenomics of Bone: High-throughput Determination of Bone Strength. G. H. van Lenthe*[1], T. Kohler*[1], L. R. Donahue[2], R. Müller[1]. [1]Swiss Federal Institute of Technology (ETH) and University of Zurich, Zurich, Switzerland, [2]The Jackson Laboratory, Bar Harbor, ME, USA.

It has been shown that bone strength is under genetic control and that it is a polygenic trait. To elucidate one pathway of skeletal genetic regulation, our long-term goal is to identify genes that regulate bone strength independent of growth hormone (GH) and insulin-like growth factor-1 (IGF-1). We have recently used a spontaneous mutation in the mouse, known as *little*. In *lit/lit* mice (on C57BL/6J background), GH is not detectable, IGF-1 is fixed at low levels, and bone strength is reduced. In a congenic *little* mouse (*little* transferred on C3H/HeJ background; C3.B6-*lit/lit*) we have shown that bone phenotype can vary independently of IGF-1. By crossing these two strains regulatory loci for adult bone strength can be located, provided that both genotype and phenotype can be assessed accurately. Although huge progress has been made for fast acquisition of genomic information, only few approaches have dealt with increasing throughput for phenotypic characterization, let alone for characterization of bone function. Therefore, the aim of this study was to develop and validate analysis tools needed to automatically determine bone phenotype at a functional level.

Using micro-computed tomography, we accurately measured complete femora of males and females of the two parental strains and their heterozygous (*lit/+*) littermates. Microstructural finite element models were created by direct voxel conversion; loading conditions resembled single-legged stance. The elastic behavior of the bone models was correlated to real mechanical tests of femoral neck failure.

The results of our analysis showed that bone stiffness could be determined from mechanical simulations in a fast and fully automated fashion. We found many distinct differences amongst the GH deficient parental strains as well as between the *lit/lit* and *lit/+* littermates. Those differences seemed to be gender specific and were more pronounced on the tissue level than the apparent level.

In conclusion, in this study we have developed a *functional phenomics* approach, a nondestructive technique for high-throughput characterization of bone function. We believe this to be a critical step in determining bone-strength related genes, as direct mechanical testing of these very small femora will be extremely demanding; furthermore, it allows for automated characterization for the large numbers of bones typically required for genetic linkage studies. Additionally, this novel approach will be crucial to interpret results from upcoming small animal *in vivo* imaging systems, where destructive testing cannot be performed but individual bone strength can be assessed over time.

Disclosures: ***G.H. van Lenthe****, None.*

SA109

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SA110

False Positive Rates in Association Studies as a Function of Degree of Admixture. D. L. Koller[1], M. Peacock[2], T. Foroud[1], M. J. Econs[2]. [1]Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Medicine, Indiana University School of Medicine, Indianapolis, IN, USA.

Studies of association between a genetic polymorphism in a candidate gene and bone-related traits such as BMD and fracture are commonly performed in order to identify genes that influence these traits. It is not clear how results from these studies should be interpreted. For example, population stratification or racial admixture can cause false positive results when the population sampled contains two or more subpopulations, usually unobservable, that differ in both polymorphism allele frequency and trait value.

To explore the degree to which stratification can cause spurious positive association results, we used an unrelated sample of 381 Caucasian and 126 African-American females with femoral neck BMD measured by DEXA and 373 microsatellite markers genotyped. Each multi-allelic marker was reduced to a bi-allelic marker for simplicity of interpretation, using the most common allele as one allele and binning all other alleles as the second allele. The mean allele frequency difference between Caucasians and African-Americans for the 373 reduced bi-allelic markers was 0.1. African-Americans have higher mean BMD than Caucasians, and in our sample the African Americans had one-half standard deviation higher mean BMD. Samples were generated with increasing degrees of Caucasian/African-American admixture, with 0%, 1%, 2%, 5%, 10%, 15%, and 20% African-American subjects in the total sample. An ANOVA test of association of BMD with the three marker genotypes (1/1, 1/2, 2/2) was performed in each admixed sample for each of the 373 markers. The rate of positive results for the association test were observed to double as the proportion of admixture increased, with substantial increases even for amounts of admixture as small as 2-5%. These results demonstrate the great importance of controlling for racial admixture where the trait and the allele frequency of the races are different. They also suggest that unobserved admixture may lead to an increase in the nominal false positive rate of an association test.

Disclosures: ***D.L. Koller****, None.*

SA111

An ENU Mutant Mouse Characterized by the Combination of a Dcerease in Bone Size and Increase in Fat Mass with Evidence of Gender Differences. A. K. Srivastava, S. Mohan, J. Wergedal, D. J. Baylink. Musculoskeletal Disease Center, J. L. Pettis VAMC, Loma Linda, CA, USA.

The N-ethyl-N-nitrosourea (ENU) mouse mutagenesis provides a powerful platform for gene function studies. In our ENU mutagenesis screen for dominant musculoskeletal phenotypes using a C57BL/6J (B6) strain of mice, a phenodeviant was discovered which exhibited a highly significant decrease in bone size attended by an increase in fat mass. This mutant phenotype was confirmed in two generations of inheritance-test crosses with wild type B6 females. The affected progeny from inheritance-tests have 8-9% lower body weight. However, three main parameters that assess bone size such as bone area, bone mineral content (BMC), and periosteal circumference were all significantly lower in affected mice even after adjustment for decreased body weight as shown in the table. Interestingly, the total body bone area phenotype was consistently expressed in males (92% affected), whereas only 6% of females exhibited the phenotype (partial). The males with decreased bone size also showed a 15-20% increase ($p<0.01$ both at 10-weeks and 16-weeks age) in fat mass and a 10% decrease in lean mass, as compared to age and sex matched control male mice. The normal WT B6 males have 10-20% higher bone area ($p<0.0001$) and 6% lower fat mass ($p<0.05$) compared to WT B6 females, whereas mutant males were similar to females littermates in total body bone area (8.79 cm^2 in male Vs 8.96 cm^2 in females, $p>0.05$) and a similar (slightly higher) percent fat mass (15.4% in male Vs 14.0% in female, $p<0.05$). These compositional changes in male mutant mice indicate that the mutant gene may regulate gender differences in bone size and fat mass. In conclusion: 1) a germ line mutant mouse with reduced bone size and increased fat content has been identified using genome wide ENU mutagenesis screen for musculoskeletal disorders; 2) gender specific differences in bone size and fat mass were observed in progeny from a mutant mouse; and 3) based on the past findings, we speculate that mutation may involve the gene(s) pathway accounting for male/female differences in bone size and fat mass.

Mean difference in BW adjusted bone area parameters in 16-week old phenodeviant mice compared to WT

	Total Body Bone Area (Excluding skull area)	Total Bone Area (Tibia Midshaft)	BMC (Tibia Midshaft)	Periosteal Circumference (Tibia Midshaft)
Male (n=21)	-10.4%*	-16.9%**	-11.7%**	-8.8%**
Female (n=36)	-2.6%*	-5.1%*	-1.4%#	-2.4*

#p>0.05, *p<0.05, **p<0.001

Disclosures: ***A.K. Srivastava****, None.*

SA112

Evidence for Genetic Regulation of Bone Loss Following Ovariectomy in Mice. K. S. Myers*[1], W. G. Beamer[2], K. L. Shultz[2], L. R. Donahue[2], V. Glatt*[1], C. J. Rosen[2], M. L. Bouxsein[1]. [1]Orthopedic Biomechanics, Beth Israel Deaconess Med Ctr, Boston, MA, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA.

Bone loss following the menopause is variable, with some individuals showing dramatic and rapid bone loss and others appearing relatively resistant to bone loss induced by estrogen deficiency. Whereas it is clear that genetic factors play an important role in determining many skeletal characteristics, including peak bone mass, bone size and bone turnover, less is known about the genetic contributions to age- and menopause-related bone loss [Garnero, JCEM 1996; Li, ASBMR 2002; Beamer, ASBMR 2002]. Thus, we used inbred mouse strains to test the hypothesis that genetic factors play a role in determining bone loss associated with estrogen deficiency. Female mice from five inbred strains (C57Bl/6J (B6), C3H/HeJ (C3H), BALB/cByJ (Balb/c), DBA/2J (DBA) and Castaneus (CAST)) were ovariectomized (OVX) or sham-operated at 4 months of age and euthanized one month later (n=5 to 9 per group). OVX was confirmed by uterine weight at necropsy. Bone loss was assessed by μCT at the mid-femur, 5th lumbar vertebrae and proximal tibia. As shown before, bone density in the sham mice varied with strain ($p<0.0001$ for strain-effect at all trabecular sites). Vertebral trabecular BV/TV (V.BV/TV) was highest in BALB/c (34.2± 1.4%), intermediate in B6 (23.5 ± 0.6%) and DBA (21.7± 1.5%), and lowest in C3H (14.5 ± 1.5%). In comparison, tibia trabecular BV/TV (T.BV/TV) was highest in BalbC and C3H (17.3 and 15.2%, respectively), intermediate in B6 (4.0 ± 0.3%) and DBA (3.6 ± 0.2%), and lowest in CAST (1.5 ± 0.4%). Femoral cortical thickness (CortTh) was highest in C3H (387±6 μm), intermediate in BalbC (278±7 μm) and Cast (302±8 μm), and lowest in B6 (205±6 μm) and DBA (225±4 μm). Vertebral and tibial bone loss following OVX was highly strain dependent (strain x treatment interaction, $p<0.005$ for both sites). DBA and BalbC had the greatest OVX-induced loss in V.BV/TV (-30 and –24% vs sham, respectively, $p<0.001$), whereas C3H had only a 5% decline (ns). Similar results were seen for trabecular number and thickness. In contrast, C3H had 50-100% greater bone loss at the proximal tibia (-39% versus sham, $p<0.005$) than the other strains (-16 to 27% vs sham, $p<0.05$). Femoral CortTh declined only in B6 (-10%, $p<0.01$). These findings confirm that trabecular and cortical bone loss following estrogen-deficiency is genetically regulated, and may be skeletal site-specific. Thus, the search for genes contributing to the regulation of bone loss following estrogen deficiency may ultimately lead to identification of those at greatest risk for rapid bone loss following menopause, and therefore afford the opportunity for early preventative interventions.

Disclosures: ***M.L. Bouxsein****, None.*

SA113

GH/IGF-I Independent Genetic Effects on BMD and Skeletal Morphology are Both Gender Dependent and Independent. L. R. Donahue[1], V. E. Guido*[1], C. J. Rosen[2], L. G. Horton*[1], C. L. Ackert-Bicknell*[1], M. L. Bouxsein[1], K. L. Shultz*[1], W. G. Beamer[1]. [1]The Jackson Laboratory, Bar Harbor, ME, USA, [2]St. Joseph Hospital, Bangor, ME, USA.

The interaction between IGF-I, sex steroids, and bone is comprised of autocrine loops whereby IGF-I levels are regulated by sex steroids via the hypothalamus and by aromatization of androgen to estrogen. Thus, the direct effect of steroids on bone independent of IGF-I is not easily discerned, particularly since steroids can affect the local synthesis of IGF-I in target tissues. The aromatization of androgens to estrogens by osteoblasts provides another autocrine loop for interaction with IGF-I to regulate bone physiology. A genetic model of steroid regulation of bone without the influence of IGF-I could provide insight into gender-specific interventions to impact bone health.

To determine the gender specificity of GH/IGF-I independent skeletal phenotypes, we are utilizing a spontaneous mouse mutation, *little,* with a non-functional GHRHR. A congenic strain has been made by transferring the *little* mutation from the low BMD C57BL/6J (B6) to the high BMD C3H/HeJ (C3) background. In both B6-*lit/lit* and C3.B6-*lit/lit* mice, circulating GH is undetectable, serum IGF-I is low, and femoral BMD (pQCT), areal BMD (PIXImus), femur length, and body mass are reduced compared to *lit/+* mice. Although C3.B6-*lit/lit* mice are of the same body weight and have the same femur length as B6-*lit/lit* mice, C3.B6-*lit/lit* mice have higher areal and femoral BMD. Within B6-*lit/lit* there are no gender differences in these phenotypes, whereas C3.B6-*lit/lit* females have greater femoral BMD than males, while areal BMD and femur length are not different.

A cross between B6-*lit/lit* and C3.B6-*lit/lit* has been made to generate F2s where all mice have low IGF-I but segregating genes from B6 and C3. Preliminary data from 550 F2s indicate that when IGF-I is held constant at low levels (<10% normal), gender-specific and gender independent regulatory loci for the skeleton can be detected. Data are first analyzed with genders together and then separately as shown below.

We would hypothesize that the gender specific loci are sex steroid driven and are independent of interaction with IGF-I. (Linkage Criteria 0.0001; values within table are LOD scores; n=235-253 males; 246-271 females)

QTLs in 550 B6-lit/lit x C3.B6- lit/lit F2 Males and Females

			AREAL BMD		pQCT fem BMD		f length		peri c		
chr	marker	cm	M&F	M	F	M&F	M	F	M&F	M	F
1	14	82				5.6	4.3				
4	170	67	8.3	4.9		6.7			10.7	4.1	6.6
8	292	19					6.4				
13	88	21				6.1					
15	143	21					5.2	4.3			
17	175	18	4.6	4.8					6.1		5
	39	45	4.2	4.7		4.1					

Disclosures: ***L.R. Donahue****, None.*

SA114

Heritability of Hip Structural Phenotypes in the Old Order Amish. E. A. Streeten[1], D. J. McBride[1], J. R. O'Connell*[1], T. I. Pollin*[1], T. J. Beck[2], K. Uusi-Rasi[2], A. R. Shuldiner*[1], A. R. Shuldiner*[1], B. D. Mitchell[1]. [1]Medicine, University of Maryland School of Medicine, Baltimore, MD, USA, [2]Radiology, Johns Hopkins University, Baltimore, MD, USA.

Ambiguities in the dimensions underlying BMD can complicate efforts to identify the genetic contributions to bone mechanical strength and osteoporotic fragility. Structural geometry should provide insights into this issue although the heritability of bone structural dimensions has not been studied. The purpose of this study was to assess heritability of geometric indices of hip structural strength.

Hip Structure Analysis (HSA) was used to analyze DXA images yielding: BMD, bone cross-sectional area (CSA) section modulus (Z), subperiosteal width (W), and buckling ratio (BR). These parameters were measured in the narrow-neck and proximal shaft regions of the hip in 903 men and women, aged 19-91, recruited from large Amish families in Lancaster County, PA. Heritability (h2) was calculated using variance component methods to partition the total phenotypic variation into components attributable to individual-specific covariates, including height, weight, age and sex, and a component due to the additive effects of genes, or heritability.

Adjusted h2 are listed in the table. Independent of age, gender and body size, all of the geometric phenotypes in the femoral neck and shaft regions were highly heritable ($p < 0.0001$). With further adjustment for BMD, heritability estimates were reduced only modestly for most of these parameters, and remained significantly greater than zero.

We conclude that geometric indices of bone strength of the proximal femur derived from DXA data are highly heritable, especially at the proximal shaft. The higher heritability at the shaft may be due to greater measurement precision at that region. The use of hip structural dimensions may help clarify the genetic contributions to bone strength and osteoporotic fragility and should provide additional information over BMD alone.

	Heritability (h2)			
Hip region	CSA	W	Z	BR
Narrow-neck	0.44	0.52	0.34	0.53
Shaft	0.66	0.70	0.84	0.70

Disclosures: ***E.A. Streeten****, None.*

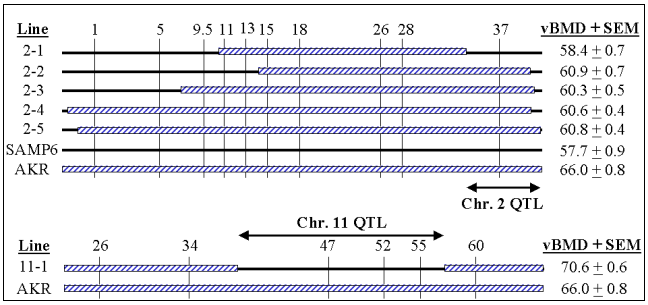
SA115

See Friday Plenary number F115

SA116

Quantitative Trait Loci for Murine Vertebral BMD, Mapped in Interstrain Cross Progeny, Confer Distinct Allelic Effects on Bone Density After Isolation in Congenic Lines. D. Szumska*[1], P. Kang*[2], R. S. Shelton*[3], R. S. Weinstein[3], R. L. Jilka[3], S. C. Manolagas[3], R. J. Shmookler Reis[2]. [1]Geriatrics, Univ. of Arkansas for Medical Sciences and CAVHS, Little Rock, AR, USA, [2]Geriatrics, and Biochem./Molec. Biol., Univ. of Arkansas for Medical Sciences and CAVHS, Little Rock, AR, USA, [3]Center for Osteoporosis and Metabolic Bone Diseases, Univ. of Arkansas for Medical Sciences and CAVHS, Little Rock, AR, USA.

We recently mapped quantitative trait loci (QTLs) for BMD of the mouse spine, measured in mature (4-month) F2 progeny of SAMP6 x AKR and SAMP6 x SAMR1 crosses (Beneš *et al.*, JBMR 15:26–33, 2000). Five significant QTLs were defined, on chromosomes 2, 7, 11, 13 and 16. For major peaks on chromosomes 2 and 11, we have now constructed multiple congenic lines by marker-directed selection of backcross progeny. In each case, a "donor" QTL allele is isolated in the genetic background derived from the other, recipient parental strain. By comparison of vertebral BMD in overlapping recombinants across these QTL regions, the major loci affecting each region have been localized to intervals that can be precisely determined by fine-mapping of introgressed segments. Highly significant allelic effects (each $p<0.01$) were observed on mineral density in the spine. Introduction of an AKR chromosome-2 QTL region in a SAMP6 background increased spine BMD by ~30 μg/mm^2 (Figure, top), most of which is attributable to a QTL near 37 cM, while insertion of a SAMP6 chromosome-11 QTL into an AKR background increased spine BMD by ~46 μg/mm^2 (Figure, bottom). Boundaries between recipient and



donor DNA regions are at present defined only approximately, by genotyping at markers indicated by vertical lines with genetic positions (in cM) above them. Additional markers and recombinant lines will be evaluated to more precisely position the QTLs. Other measures of bone structure and strength, at this and other skeletal sites, are also being assessed for the most informative congenic lines demarcating each implicated QTL region. These measures include bone formation rate, clonogenic assays of osteoblast precursors, self-renewal of mesenchymal stem cells, resistance of femur to three-point bending, and resistance of vertebrae to compression fracture.

Disclosures: ***D. Szumska****, None.*

SA117

Heritability and Familial Correlations of the Bone Synthesis Marker, Serum N-Terminal COL1A1 Propeptide (P1NP). L. J. Miles*[1], A. Blumsohn[2], R. Eastell[2], E. L. Duncan[3], J. A. H. Wass[3], M. A. Brown*[1]. [1]Spondyloarthritis and Bone Disease Research Group, Oxford University Institute of Musculoskeletal Sciences, Oxford, United Kingdom, [2]Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom, [3]Department of Endocrinology and Metabolism, Nuffield Orthopaedic Centre, Oxford, United Kingdom.

Whilst there is clear evidence of the heritability of bone mineral density (BMD), the inheritance of bone turnover is less well established. We measured serum procollagen type I N-terminal propeptide (PINP) in serum by immunoassay (Roche Elecsys), a marker of type 1 collagen synthesis, in 427 individuals belonging to 115 families recruited with probands with low BMD (T-score <-2.5, Z-scores <-2.0) in whom secondary causes of osteoporosis had been excluded. Cases with serum P1NP >3 SD from the mean were excluded (n=2). Multiple linear regression demonstrated correlation between serum P1NP and age+age^2, but not with gender, BMD, height, weight or BMI. Heritability and within family correlations were assessed using the program PAP[1].

Serum P1NP levels were significantly heritable (additive heritability 0.35, p=0.0007). Correlation between siblings was much greater than between parent-offspring pairs (0.35 vs 0.02, p=0.002). Correlations were more positive between same-gender compared with opposite gender pairs. Sister-sister correlation was 0.41, compared with brother-brother correlation of 0.24. Father-son correlation was 0.19 whereas father-daughter correlation was 0.06, and mother-daughter correlation was 0, whereas mother-son correlation was -0.12.

These findings support the hypothesis that gender specific effects influence the inheritance of skeletal phenotypes including BMD and bone turnover. Studies of the COL1A1 Sp1 polymorphism in these families (Miles *et.al*, ASBMR Conference Abstracts 2003) suggest

the presence of parent-of-origin effects on the association of this marker with BMD and serum P1NP levels, consistent with the differences reported here in within-family correlations of P1NP.

[1]Hasstedt, SJ (1993) Gen Epidemiol 10, 145-58.

Reagents for assay of PINP kindly supplied by Roche Diagnostics, Germany.

Disclosures: ***L.J. Miles****, None.*

SA118

See Friday Plenary number F118

SA119

Five Novel QTLs for Bone Mineral Density and Biomechanical Strength Discovered in an Intercross of Red Jungle Fowl and White Leghorn Chicken. H. Brändström*[1], U. Gunnarsson*[1], E. Grundberg*[1], C. Ohlsson*[2], H. Mallmin[3], S. Larsson*[3], L. Andersson*[4], P. Jensen*[5], R. Fredriksson*[4], A. Kindmark[1]. [1]Dept of Medical Sciences, Uppsala University Hospital, Uppsala, Sweden, [2]Division of Endocrinology, Department of Internal Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden, [3]Dept of Orthopedics, Uppsala University Hospital, Uppsala, Sweden, [4]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, [5]Department of biology, IFM, Linköping university, Linköping, Sweden.

Osteoporosis is the cause of skeletal problems in hens. Up to 90% of hens in commercial flocks display broken bones during life. Short generation time and large resource pedigrees make it an attractive model for genetic dissection of multifactorial traits. The genome size (~1200 Mbp) is smaller than in mammals, but has approximately the same gene number.

This Functional Genomics of the Chicken (FunChick) program is based on a unique pedigree, an intercross between 2 divergent populations: the Red Jungle Fowl (RJF, the ancestor of the domestic chicken) and White Leghorn (SLU13, selected for egg production). The pedigree comprises 1,000 animals from 3 generations. The populations differ markedly in growth and body composition.

DNA samples are collected, and genome scans using 120 microsatellite markers have been performed. Bone phenotypic traits: determination of BMD by DXA and volumetric BMD and composition by pQCT have been performed. Also, biomechanical strength is determined by three-point bending and torsion testing.

QTL analyses revealed a large number of chromosomal regions linked to bone phenotypes, with five regions on four chromosomes showing linkage values at the 1% level of significance. The results remain statistically significant after adjustment for body size (weight), and also after including the major QTLs affecting animal growth in the model.LOD scores for these five QTLs range between 3.36-4.10 for BMC, BMD and biomechanical strength parameters. These main bone phenotypic QTLs are located on chicken chromosome 1, 8, 26 and 28.

In the continuation of the project, transcript profiling, positional cloning and functional analysis will be performed to identify the genes controlling these traits. A Unigene array using high-quality cDNA libraries from tissue has been produced using sequences from 30,000 cDNA clones.

The ongoing chicken genome project will provide the full chicken genomic sequence during the coming year. This gives a unique opportunity to find novel candidate genes affecting bone metabolism and biomechanical strength of bone in chicken, whereafter comparative genomics can be used in other model animals and in human genetic studies.

Disclosures: ***A. Kindmark****, AstraZeneca R&D 5.*

SA120

Gender Dependent QTL for Femoral BMD in Mice. K. L. Shultz[1], L. G. Horton*[1], C. L. Ackert-Bicknell[1], L. R. Donahue[1], C. J. Rosen[2], W. G. Beamer[1]. [1]The Jackson Laboratory, Bar Harbor, ME, USA, [2]St. Joseph Hospital, Bangor, ME, USA.

Susceptibility to osteoporosis is apparent for both women and men, however the genetic basis is unknown. In young adult C57BL/6J (B6) inbred mice, males have a significantly greater ($p<0.0001$) femoral BMD (0.510±0.008) than females (0.477±0.005). Conversely, in C3H/HeJ mice, females have a significantly greater ($p<0.03$) femoral BMD (0.721±0.007) than males (0.699±0.008). Based on female genetic analyses, we reported that B6.C3H-1T (1T) congenic mice has two femoral BMD QTLs, *Bmd5* and *Bmd19* (Shultz et al JBMR, 2003). The 1T females have a significantly greater ($p<0.0001$) femoral BMD (0.507± 0.004) than B6 female controls (0.471 ± 0.005).), whereas the 1T males (0.479 ± 0.006) have a lower BMD ($p < 0.003$) than B6 male controls (0.510 ± 0.008). We subdivided 1T into nested congenic sublines to narrow the regions containing the femoral BMD QTLs.

We found that there was a different pattern of BMD for females and males across the sublines (Table below). Among the 7 sublines, only B6.C3H-1-1, B6.C3H-1-5, B6.C3H-1-8, and B6.C3H-1-9 females show a significantly greater femoral BMD than B6 controls. Whereas, in males, only the sublines 1-3, 1-5, and 1-7 show a difference in BMD when compared to B6 controls, and in all case BMD was reduced. Three patterns of BMD changes within the sublines are: a) females increase, males not different; b) females not different, males decrease; and c) females increase, males decrease. The subline, 1-1, is the only subline to contain *Bmd19* and expression appears only in females. Sublines 1-5,1-8 and 1-9 carry a second BMD locus whose expression appears only in females. Sublines 1-3, 1-5, and 1-7 carry two or more BMD QTL that affect male BMD.

These data demonstrate that mapping genes for BMD in one gender will not necessarily generalize to the opposite sex. Haplotype analysis has proven to be a powerful tool for the purpose of characterizing the genotype verses phenotype for each subline by aiding in defining genetic overlap between sublines. As we continue to narrow the regions encompassing each QTL in the hunt for candidate genes, each gender must be considered separately.

Comparison of BMD in B6.C3H sublines for Chr 1 with B6 controls.
Direction of arrow indicates significant increase (↑) or decrease (↓).

Gender	1-1	1-3	1-5	1-7	1-8	1-9	1-10
Female	↑	ns	↑	ns	↑	↑	ns
Male	ns	↓	↓	↓	ns	ns	ns

Disclosures: ***K.L. Shultz****, None.*

SA121

Spinal Osteophytosis and Serum Measures of Bone Turnover in Pedigreed Baboons: Evidence for Pleiotropy and Linkage to Human Chromosome 22q. M. C. Mahaney, L. M. Havill, J. Rogers*. Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, USA.

In pedigreed baboons, we have observed radiographic evidence for osteophytosis of the lumbar spine in association with spinal arthritis and diffuse idiopathic hyperostosis. In earlier statistical genetic analyses we detected a significant additive genetic component to variation in susceptibility to osteophytosis when it was treated as a dichotomous trait. To further characterize the genetic contributions to this trait in a non-human primate model for age-related changes and pathology in bone, we conducted bivariate quantitative genetic analyses of data on osteophytosis (Ost) of lumbar vertebrae 2 through 4, measured on a 4-point ordinal scale (0="absent" - 4="most severe"), in 672 pedigreed baboons for whom we also assayed serum concentrations of 3 measures of bone formation and turnover -- i.e., bone-specific alkaline phosphatase (Bone ALP), osteocalcin (OC), and human cartilage glycoprotein 39 (YKL40), a marker of both skeletal remodeling and cartilage degeneration in osteoarthritis. All 4 traits had significant heritable components (Ost h^2=0.32, Bone ALP h^2=0.18, OC h^2=0.31, YKL40 h^2=0.24) and Ost exhibited significant ($p<0.05$) additive genetic correlations (ρ_G), indicative of pleiotropy, with all three serum measures (Ost-Bone ALP ρ_G=-0.48, Ost-OC ρ_G=-0.59, and Ost-YKL40 ρ_G=0.33). Obtained as ρ_G^2, estimates of the proportion of the additive genetic effect on variance in lumbar vertebral osteophytosis attributable to genes that also affect each of these 3 bone formation/turnover measures range from approximately 10% to 33%. While univariate linkage screens detect only tentative evidence of QTLs influencing Ost (maximum LOD=1.82), bivariate whole genome linkage screens allowing for these correlations yield significant evidence for a QTL with pleiotropic effects on Ost and one of the serum measures -- YKL40 (LOD=2.71, genome-wide P=0.048). The hypothesis of coincident linkage was rejected (p=0.003). This QTL with pleiotropic effects on Ost and YKL40 is localized to a region of the baboon genome between human microsatellite marker loci D22S304 and D22S280, corresponding to an area of human chromosome 22q11.2-q12; an area containing several possible candidate genes, including 2 related to the Wnt signaling pathway (*KREMEN1*, *MAP3K7IP1*), 2 related to cell cycle regulation (*APOBEC36*, *RRP22*), and 1 (*TFIP11*) that may interact with genes involved in odontogenesis and ossification.

Disclosures: ***M.C. Mahaney****, None.*

SA122

See Friday Plenary number F122

SA123

A Large-scale Genome Wide Scan for QTLs Underlying BMD Variation in an Extended Sample. P. Xiao[1], H. Shen*[1], J. R. Long*[2], F. H. Xu*[1], L. J. Zhao*[1], Y. J. Liu*[1], Y. Y. Zhang*[2], D. H. Xiong[1], S. Araujo*[2], Y. Z. Liu*[1], V. Dvornyk*[2], Q. Zhou*[2], K. M. Davies[2], T. Conway*[2], R. R. Recker[2], H. W. Deng[1]. [1]Biomedical Sciences,Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Low bone mineral density (BMD) is a major risk factor for osteoporosis and is under strong genetic control. Previously, we reported a genomewide linkage scan for BMD in 53 pedigrees with 630 Caucasian subjects that contained more than 10,000 informative relative pairs including 1,249 sibling pairs (Deng et al., JCEM. 2002). We observed several regions with suggestive linkage. To confirm the previous results and to identify those genomic regions that may have been undetected in the previous study, we performed another whole genome scan in an extended large sample of 79 pedigrees, in which 1,816 individuals were genotyped using 432 microsatellite markers. This current sample contains more than 70,000 relative pairs informative for linkage analyses including 3,846 sibling pairs, which yields dramatic increase in the statistical power and leads to more reliable results for whole genome linkage scan analyses compared to our earlier whole genome linkage study. The number of informative relative pairs contained in this sample represents one of the largest in the human genetics field in whole-genome linkage scan studies for any complex trait. The raw BMD values at spine (L_{1-4}), hip (combined femoral neck, trochanter, and intertrochanteric region), and wrist (ultra-distal forearm) were adjusted for age, sex, weight, and other significant covariates. Two- and multi-point linkage analyses were performed using a maximum likelihood based variance components method implemented in the computer package SOLAR. Several genomic regions showed suggestive linkage for spine BMD (18q22, 7p15, and 20q13 with maximum LOD score (MLS >1.5) and wrist BMD (7p15, 20p13, and 10p13, MLS > 1.0). Compared with our early genome

scan, linkage to the genomic region 7p15 (44cM) appears to be replicated (MLS=1.5). (Significance for confirmation is different from initial whole genome scan). 1p36 that has been identified in other groups' genome-wide linkage studies (Devoto et al., HMG 2001; Wilson et al., AJHG 2003), also showed suggestive evidence for linkage with wrist BMD in this study (two-point LOD = 1.51 at D1S2660; multi-point MLS = 0.82). This study is one of a few genome scans performed in extended samples with larger statistical power. The results highlight the importance and difficulties of confirming results of genome scans in replicate populations as a prelude to positional gene cloning.

Disclosures: ***P. Xiao****, None.*

SA124

A Large-scale Whole Genome Scan for QTLs Influencing Bone Size Variation in an Extended Sample. D. H. Xiong[1], F. H. Xu*[1], J. R. Long*[2], H. Shen*[1], P. Xiao[1], L. J. Zhao*[1], Y. Y. Zhang*[2], Q. Zhou*[2], S. Araujo*[2], Y. J. Liu*[1], Y. Z. Liu*[1], V. Dvornyk*[2], K. M. Davies*[2], T. Conway*[2], R. R. Recker*[2], H. W. Deng[1]. [1]Biomedical Sciences, Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Bone size, as an important risk factor for osteoporotic fractures, is highly heritable. Previously, we reported a genome wide linkage scan for bone area phenotypes (measured by DXA) in 53 pedigrees with 630 Caucasian subjects that contained more than 10,000 informative relative pairs including 1,249 sibling pairs (Deng et al., AJMG, 2002). A significant linkage was found in genomic region 17q22 for the wrist bone size. We also observed several regions with suggestive linkage. To confirm the previous results and to identify those genomic regions that may have been undetected in the previous study, we performed another whole genome scan in an extended large sample of 79 pedigrees, in which 1,816 individuals were genotyped using 432 microsatellite markers. This current sample contains more than 70,000 relative pairs informative for linkage analyses including 3,846 sibling pairs, which yields dramatic increase in the statistical power and leads to more reliable results for whole genome linkage scan analyses compared to our earlier whole genome linkage study. The number of informative relative pairs contained in this sample represents one of the largest in the human genetics field in whole-genome linkage scan studies for any complex trait. One previously detected region on 17q22 near marker D17S787 was confirmed in this extended sample with a LOD score of 2.27 for two-point analyses and maximum LOD score (MLS) of 1.78 for multi-point analyses. (Significance for confirmation is different from initial genome scan search). This region was also reported for femur head width (one-dimensional measure of bone size) in an earlier whole genome scan study, and it is very close to the candidate gene COL1A1. In addition, we detected suggestive linkage for the following genomic regions that were not detected in our earlier whole genome scan: 14q32 near marker D14S65 (two-point LOD = 1.99, MLS = 2.61) for the spine bone size, 7q36 near marker D7S798 (two-point LOD = 1.98, MLS = 2.20) and 11q12 near marker D11S4191 (two-point LOD = 3.40, MLS = 2.38) for the wrist bone size. However, some genomic regions found earlier with suggestive evidence of linkage did not show any apparent linkage signal in this large-scale extended whole genome linkage study. These findings demonstrate the importance of large sample size and high statistical power in the whole genome scans to search for genes underlying the complex traits such as bone size.

Disclosures: ***D.H. Xiong****, None.*

SA125

4cM Linkage Map of Chromosome 1q and Lumbar Spine BMD. M. J. Econs[1], D. L. Koller[2], S. L. Hui[1], T. M. Fishburn*[1], P. M. Conneally*[2], C. C. Johnston[1], M. Peacock[1], T. Foroud[2]. [1]Medicine, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA.

BMD of the spine is a major risk factor for fracture and has high heritability. We previously performed a genome screen in pre-menopausal Caucasian sister pairs and found significant evidence of linkage of peak BMD at the lumbar spine to markers on chromosome 1q. Since the region of linkage was relatively broad and only a portion of our sample had been genotyped for the markers in this region, we genotyped 16 polymorphic microsatellite markers on chromosome 1q in all available samples. The goal of these analyses was to perform linkage analysis to confirm and refine our previous linkage results using a substantially enlarged sample that had been genotyped for a common set of markers.

BMD, BMC and area for L2-4 was measured using a Lunar DPXL densitometer and was weight and age-adjusted. We used 16 highly polymorphic, microsatellite markers in 938 pre-menopausal, Caucasian sister pairs. Average marker spacing in the critical interval between markers D1S2777 and D1S2823 was 4cM. Multipoint, nonparametric linkage analysis was performed using the maximum likelihood variance components method (Mapmaker/SIBS). Results demonstrated linkage for spine BMD with a maximum LOD score of 4.3 at position 181 cM on the Marshfield map. The multipoint LOD score for the corresponding BMC was 2.8, with the peak in approximately the same position as the peak for BMD. There was no evidence for linkage with bone area, suggesting that the gene(s) affecting BMD on chromosome 1q do not affect bone size.

In summary, our data provide strong evidence that a gene (or genes) on chromosome 1q affects peak BMD of the lumbar spine in pre-menopausal white women.

Disclosures: ***M.J. Econs****, None.*

SA126

See Friday Plenary number F126

SA127

Skeletal Fragility in Laboratory Mice: Affect of Sex and Genotype. M. Shea[1], B. L. Hansen*[1], D. A. Olson*[2], D. Dinulescu*[2], B. Orwoll*[2], J. K. Belknap*[2], E. S. Orwoll[2], R. F. Klein[2]. [1]Orthopaedic Biomechanics Laboratory, Oregon Health & Science University, Portland, OR, USA, [2]Bone and Mineral Unit, Oregon Health & Science University, Portland, OR, USA.

The genetics of skeletal fragility has been pursued with increasing intensity over the past decade. Genetic factors play an important role in determining bone size, shape, and bone mineral density (BMD), all key contributors to bone strength. There is now an ongoing effort using modern genetic analyses to map the chromosomal loci of genes responsible for the heritable differences in BMD. However, much less is known about the specific biomechanical characteristics that more completely describe the integrity of skeletal tissue. In this study we examined sex- and strain-dependent differences in femoral BMD (by pixiMus), geometry (by μCT) and failure properties (by 3-point bending) in 4 mo-old male (M) and female (F) mice from 8 inbred mouse strains: 129S1, Castaneus (Cast), BALB/cByJ (Balb/c), C57Bl/6J (B6), C3H/HeJ, DBA/2J (D2), A/J and AKR/J (n=13-20/gr). Strain and gender differences were determined using two-way ANOVA. Body weight (BW) differed significantly between strains and gender, and there were significant correlations between BW and all parameters. Consequently, within each gender all femoral parameters were corrected for BW. In both M and F datasets, narrow-sense heritability estimates were high for weight-corrected femoral BMD ($h^2 = 0.78$ for both), geometric parameters ($h^2 = 0.66$ to 0.90) and strength ($h^2 = 0.57$ to 0.84). The effect of strain, sex, and strain x sex was significant for all femoral traits ($p < 0.0001$ for all). For example, femoral failure load was highest in C3H/HeJ (31.5 ± 0.7 N), intermediate in A/J, AKR, Balb/c, Cast and DBA (17 to 26 N) and lowest in B6 (16.4 ± 0.6 N) and was higher in M (7/8 strains). Ixx varied 2.5-fold between strains, and similar to failure load was higher in M (7/8 strains). Femoral strength and modulus (intrinsic properties of the bone material) also varied across strains (1.7 – 1.9 fold) but interestingly were both higher in F (8/8 strains). In both M and F mice, femoral failure load correlated strongly with femoral BMD, cortical area and thickness and Ixx (Pearson correlation coefficients 0.66 – 0.86; $p < 0.0001$ for all). These data indicate that the key determinants of bone strength are under significant genetic control. Moreover, the present data highlight the important role of gender in the inheritance of skeletal strength. Since the male phenotype is associated with considerable fracture risk reduction in humans, an improved understanding of the nature of the gender divergence in murine skeletal traits could provide for novel diagnostic, preventative or therapeutic approaches.

Disclosures: ***M. Shea****, None.*

SA128

Heritability of Calcaneal Quantitative Ultrasound Measures in Healthy Adults from The Fels Longitudinal Study. M. Lee, S. A. Czerwinski*, B. Towne*, E. W. Demerath*, W. C. Chumlea*, S. S. Sun*, R. M. Siervogel. Community Health, Wright State University, Kettering, OH, USA.

Quantitative ultrasound (QUS) measurements have been reported to predict osteoporotic fracture risk in postmenopausal women and older men. In addition, QUS measures may reflect important aspects of the internal structure of the calcaneal bone. Many studies have found significant heritabilities of bone mineral density obtained from single or dual-energy X-ray absorptiometry, for example, but few studies have estimated the heritability of calcaneal QUS phenotypes. In the present study, we estimate the heritability of calcaneal QUS measures in healthy adults. The study population includes 200 men and 235 women aged 18 to 60 years (mean ± SD: 40 ± 12 yrs) who belong to 98 extended and nuclear families participating in the Fels Longitudinal Study. Three measures of calcaneal structure were collected from the right and left heel using the Sahara ® bone sonometer (Hologic, Inc., Waltham, MA). These measures include speed of sound (SOS), broadband ultrasound attenuation (BUA), and quantitative ultrasound index (QUI). We used a variance components-based maximum likelihood method (SOLAR) to estimate the heritability of QUS measures while simultaneously adjusting for the effects of age, sex, age*sex, age^2, and age^2*sex.

All QUS measures demonstrated statistically significant heritabilities ($p< 0.0001$). For the right heel, heritability estimates ($h^2 \pm$ SE) were $h^2 = 0.49 \pm 0.13$ for BUA, $h^2 = 0.67 \pm 0.13$ for SOS, $h^2 = 0.64 \pm 0.14$ for QUI. For the left heel, heritabilites were 0.53 ± 0.12 for BUA, 0.59 ± 0.12 for SOS, and 0.57 ± 0.12 for QUI. There was little influence of age and sex on BUA and QUI. For example, sex explained only 4 % of the phenotypic variance for BUA in the right heel ($p< 0.01$). Age had a significant effect on SOS in both the right and left heel ($p < 0.05$). Other covariates, such as age-squared and interactions between age and sex, did not contribute significantly to the variation of QUS measures. This study demonstrates that QUS measures of bone among healthy men and women are highly heritable.

Disclosures: ***M. Lee****, None.*

SA129

Heritability of Peak Bone Mineral Density and Bone Structure in Men. M. Peacock[1], D. Koller[2], S. Hui[1], C. Johnston[1], T. Foroud[2], M. Econs[1]. [1]Medicine, Indiana University, Indianapolis, IN, USA, [2]Medical and Molecular Genetics, Indiana University, Indianapolis, IN, USA.

We have shown that peak bone mineral density (BMD) and bone structure at the hip and spine are highly heritable phenotypes in both white and black women (Endo Rev 2002 23; 303). There are few comparable data in men. Thus the purpose of this study was to establish the heritability of peak BMD and bone structure in men.

BMD was measured on the same DXA machine at lumbar spine (ls) and femoral neck (fn) in 271 pairs of white brothers aged 18-61, median age 33 and 58 pairs of black brothers

aged 20-59, median age 35. . Bone structural variables at the hip including , pelvic axis length (pal), femoral medullary width (fmw), femoral total width (ftw), femoral neck width (fnw) femoral head width (fhw), calcar femorale width (cfw) were measured from radiographs of the hip in 15 degrees internal rotation.
Heritability was calculated using the computer program SOLAR (Am J Hum Genet 1998 62:1198). For BMD, age and weight were used as covariates and for structure, height was used as covariate.
As shown in the table peak BMD at the hip and spine in both white and black brothers was highly heritable and equivalent to the values we have previously reported in women. The structural variables at the hip and spine were also highly heritable and equivalent to the values we have reported in women.
It is concluded that these high heritability estimates in the components of peak bone strength in men warrant not only the identification of QTL underlying BMD and bone structure but also the identification of sex specific QTL by comparing men and women.

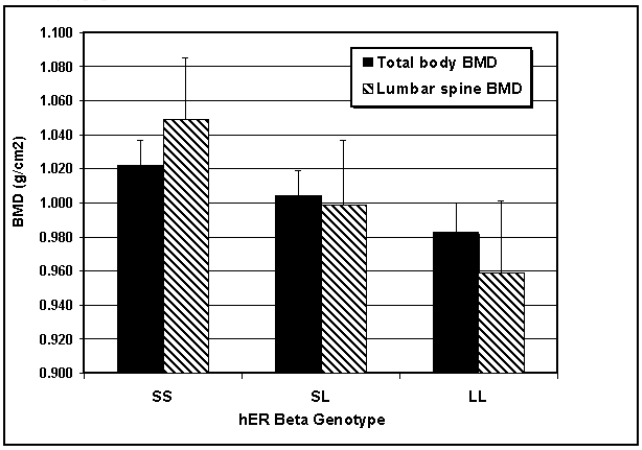
Phenotype	White pairs	Black pairs
ls BMD	0.87	0.61
fn BMD	0.83	0.74
pal	0.72	0.57
fmw	0.76	1.00
ftw	1.00	0.96
fnw	0.86	0.99
fhw	1.00	0.78
cfw	0.39	1.00

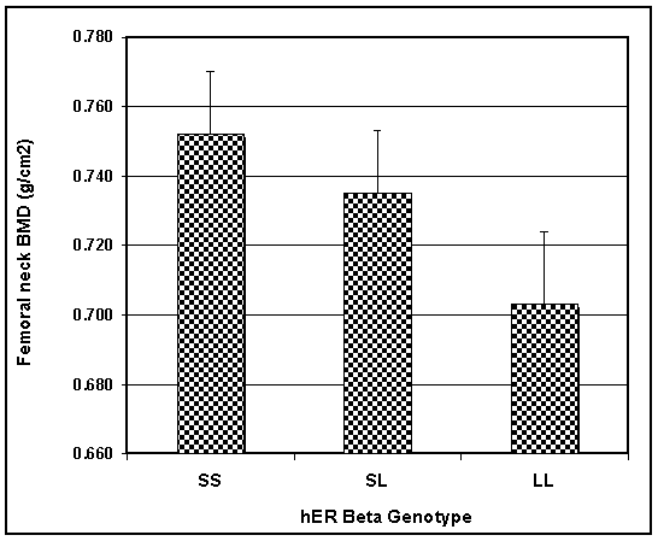
Disclosures: ***M. Peacock****, None.*

SA130

Estrogen Receptor β Dinucleotide (CA) Repeat Polymorphism Is Significantly Associated with Bone Mineral Density in Postmenopausal Women. J. K. Scariano[1], S. G. Simplicio*[2], G. D. Montoya*[1], P. J. Garry*[1], 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.

Modest associations between the length of a highly polymorphic dinucleotide (CA) repeat located near the human estrogen receptor beta gene (hERβ) on chromosome 14, spinal bone mineral density in postmenopausal women and androgen levels in premenopausal women have previously been reported. We measured the size of the hERβ CA repeat in 226 healthy women (age 60-98 yrs). After adjustment for age, body mass index (BMI), hormone replacement (HRT) status and circulating levels of 17-β estradiol (E_2), subjects with shorter CA repeat number (< 25 repeats) had significantly higher bone mineral density measured in the total skeleton (Mean+/–S.E.: 1.022+/-0.015 vs. 0.983+/–0.017 gm/cm^2, p = 0.02), lumbar spine (1.049+/–0.036 vs. 0.959+/–0.042 gm/cm^2, p = 0.03) and femoral neck (0.752+/–0.018 vs. 0.703+/–0.021gm/cm^2, p = 0.02) when compared with women having longer alleles. Women with shorter alleles also had higher circulating estrone and E_2 levels that approached statistical significance as compared with women harboring longer alleles after adjustment for age, BMI, HRT and circulating levels of sex hormone binding globulin. A dosage effect of either a beneficial or detrimental allele was apparent in women having both short and long CA repeats. The hERβ CA repeat size was not associated with the 5-7 year change in BMD we monitored, nor did it correlate with serum levels of biochemical markers of bone turnover such as crosslinked N-telopeptides of type-I collagen (NTx) or bone-specific alkaline phosphatase. Accordingly, hERβ genotype more likely influences the attainment of peak bone mass rather than the rate of decline in BMD after menopause. Our results warrant further investigation of the hERβ CA repeat size in larger populations of women and men with low BMD.





Disclosures: ***J.K. Scariano****, None.*

SA131

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SA132

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SA133

Endogenous Estrogen and Estrogen Receptor Alpha Gene Polymorphisms Predict Renal Calcium and Phosphorus Handling and Intestinal Calcium Absorption in Postmenopausal Women. R. L. Prince[1], I. M. Dick*[1], A. Devine[1], S. S. Dhaliwal*[2]. [1]Medicine, University of Western Australia, Western Australia, Australia, [2]Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Western Australia, Australia.

Postmenopausal endogenous estrogen production is a determinant of bone mass and fracture in elderly women. In addition to effects on bone, estrogen may have direct effects on kidney and bowel. The interaction between circulating estrogen concentrations and estrogen receptor (ER) gene polymorphisms in relation to the renal handling of calcium and phosphorus and intestinal calcium absorption was investigated.
As part of a population based study ER TA repeat genotyping was performed on 483 postmenopausal women. A fasting blood and urine sample was collected in 259 of these subjects. Factors associated with renal calcium and phosphate excretion were measured including estradiol, SHBG and PTH. The free estradiol index (FEI) was calculated. Intestinal calcium absorption (% dose) was estimated in 118 fasting subjects by measuring the appearance of an orally administered ^{45}Ca tracer in the blood. The ^{45}Ca tracer was administered with a 10 mg calcium load.
High plasma estradiol and FEI concentrations were associated with a high total calcium concentration and a high calculated ultrafiltrable calcium concentration. However a low urine calcium excretion was associated with a high FEI after adjustment for ultrafiltrable calcium, PTH and weight (partial r= -0.16, p=0.01). Addition of the TA repeat (> 20/<=20) to the model further improved the prediction of calcium excretion. Mean urinary calcium excretion, after adjustment for weight, PTH, ultrafiltrable calcium and free estradiol concentration, was higher in the group that did not have an ER (TA)n> 20 allele (16.8 µmol/LGF, 95% CI 14.1-19.81) than in the group that had at least one ER (TA)n> 20 allele present (13.4 µmol/LGF, 95% CI 12.0-15.0). After adjustment for PTH and plasma calcium concentrations in a multiple linear regression analysis, a high FEI was associated with a low TmP (partial r = -0.15 p = 0.02), indicating that high FEI was associated with a decreased renal phosphate reabsorption. Intestinal calcium absorption was not associated with the FEI but was higher in the group with an ER TA repeat >20 (41%, 95% CI 37-45) compared to the group that did not have an ER (TA)n> 20 (34%, 95% CI 32-37), after adjustment for the FEI.
An association of the ER receptor TA repeat allele > 20 with increased renal calcium reabsorption and increased intestinal calcium absorption was observed. Thus non-bone effects of estrogen and the estrogen receptor may play a significant role in calcium and phosphorus homeostasis and may be implicated in the pathogenesis of osteoporosis.

Disclosures: ***R.L. Prince****, None.*

SA134

The CYP19 TTTA Repeat Polymorphism Is Associated with Reduced Bone Mineral Density and Fracture. R. L. Prince[1], A. Devine[1], I. M. Dick*[1], S. S. Dhaliwal*[2]. [1]Medicine, University of Western Australia, Western Australia, Australia, [2]Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Western Australia, Australia.

Osteoporosis is a disease that is strongly genetically determined so that polymorphisms present in a range of candidate genes may be involved. The human Aromatase (CYP19) gene, localized on 15q21.2, catalyzes the conversion of testosterone to estradiol, and androstenedione to estrone. A variable number of tandem repeat (VNTR) polymorphism of the CYP19 gene has been associated with reduced bone mineral density (BMD) and increased fracture in early postmenopausal women but the importance of this has not been determined in older women.

We examined the CYP19 TTTA VNTR in a population based study of 1257 Caucasian women over age 70. DXA bone mineral density (BMD) data and calcaneal quantitative ultrasound (QUS) data was obtained. The number of atraumatic prevalent fractures at entry into the study and the number of atraumatic incident fractures during the following three years was assessed. Genotyping of the CYP19 TTTA VNTR was done by PCR amplification.

Genotyping resulted in 10 different PCR fragments ranging in size from 142 to 182 bp, designated A1 to A11. Based on the results of an initial statistical analysis, the data was stratified based on the presence or absence of A4 (158 bp, number of TA repeats=9). A4 was present in 27% of subjects and was associated with higher BMD at all sites of the hip (3.8% total hip, 2.6% femoral neck, 3.8% intertrochanter, 4.5% trochanter) and the lumbar spine (14.7%) compared to the absence of A4. A4 was also associated with higher values for the calcaneal quantitative ultrasound (QUS) parameters BUA (1.8%), SOS (0.5%) and stiffness (4.5%) compared to the absence of A4. A4 was associated with a decreased risk of osteoporosis as defined by WHO criteria (OR 0.56, 95% CI 0.34-0.93). A4 was associated with a decrease in the deoxypryridinoline creatinine ratio (DpdCr) (30.3 ± 10.6 vs 26.9 ± 8.9, p=0.02) and an increase in the Free Estradiol Index (FEI) (0.64 ±0.57 vs 0.71 ± 0.59, p=0.049). Despite the association of A4 with increased BMD and QUS, there was no decrease in prevalent fractures or incident fractures recorded over 3 years observed. However, a significant interaction between BMD and the presence or absence of A4 was observed, such that subjects with low BMD (osteopenia or osteoporosis) and the presence of A4 were protected against incident fracture compared to subjects with a low BMD who did not have an A4 allele (R.R. 0.31, 95% CI 0.1-1.0).

Therefore, a Cyp19 A4 allele was associated with increased FEI, BMD and QUS parameters and reduced bone resorption. Importantly the A4 allele enhanced prediction of incident fracture in women with low BMD.

Disclosures: ***R.L. Prince****, None.*

SA135

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SA136

Promoter Polymorphism of the COLIA1 Gene Is Associated with Spine BMD in Women. F. E. A. McGuigan*[1], T. L. Stewart*[1], D. M. Reid[1], S. C. Main*[1], N. Garcia-Giralt*[2], S. Balcells*[2], X. Nogues*[3], A. Diez-Perez[3], D. Grinberg*[2], S. H. Ralston[1]. [1]Medicine & Therapeutics, University of Aberdeen, Aberdeen, United Kingdom, [2]Genetics, University of Barcelona, Barcelona, Spain, [3]Hospital del Mar, University of Barcelona, Barcelona, Spain.

The COLIA1 gene, which encodes the alpha 1 chain of type I collagen is an important candidate gene for regulation of BMD and bone fragility. A G/T polymorphism affecting an Sp1 binding site within intron 1 of COLIA1 (+1245G/T) has been found to predict fractures independent of BMD, and more recently, two polymorphisms were identified in the COLIA1 promoter (–1997G/T and -1663T7/T8) that have been associated with BMD in Spanish postmenopausal women. In this study, we investigated associations between the promoter and intron 1 polymorphisms of COLIA1 and BMD in a population-based sample of 962 women aged 45-55 years (mean 47.5 +/- 1.42 years) from the UK. There was strong linkage disequilibrium between the +1245G/T and -1663T7/T8 polymorphisms (D' 0.81; p< 10-8) and weaker LD between -1663T7/T8 and –1997G/T (D' 0.17, p< 10-5) and +1245G/T and –1997G/T (D' 0.20, p<10-5). After adjusting for age, weight, height, menopausal status, and HRT use, we found that –1997 T/T homozygotes had significantly higher spine BMD values than –1997 G/T heterozygotes and –1997 G/G homozygotes (Table 1). There was no association between the -1663T7/T8 or +1245G/T polymorphisms and BMD and no association between any polymorphism and hip BMD (not shown). We identified three common haplotypes that together accounted for 89.2% of the alleles. They were (ordered 5' to 3'): G-T8-G (59.2%); T-T7-T (7.2%) and T-T8-G (22.9%). All three haplotypes were significantly associated with BMD (p=0.021-0.024), but the association was primarily driven by variation at the -1997G/T site (not shown). In this study, we found no association between the COLIA1 Sp1 polymorphism and BMD, but found a strong association between –1997 G/T promoter polymorphism and spine BMD. This raises the possibility that these different polymorphisms in COLIA1 may have differential effects on bone mass and bone quality.

Table 1: BMD and COLIA1 Genotype data

	G / G (n=696)	G / T (n=243)	T / T (n=23)	ANOVA p-value
-1997 G / T	1.05 + / - 0.14	1.06 + / -0.16	1.15 + / -0.13	p=0.002
	T8 / T8 (n=657)	T8 / T7 (n=261)	T7 / T7 (n=44)	
-1663T7 / T8	1.05 + / - 0.15	1.05 + / -0.14	1.05 + / - 0.15	p=0.802
	G / G (n=663)	G / T (n=261)	T / T (n=36)	
+1245G / T	1.06 + / - 0.15	1.05 + / - 0.14	1.02 + / - 0.14	p=0.328

Disclosures: ***F.E.A. McGuigan****, None.*

SA137

See Friday Plenary number F137

SA138

The Interleukin 6 G-174C Promoter Polymorphism Is Associated with Hip Bone Loss in Older Men. K. Y. Z. Forrest*[1], J. M. Zmuda[2], J. A. Cauley[2], S. M. Roth*[3], R. E. Ferrell*[2]. [1]Slippery Rock University of Pennsylvania, Slippery Rock, PA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA, [3]University of Maryland, Pittsburgh, PA, USA.

Interleukin 6 (IL6) plays an important role in osteoclast formation and bone resorption. A G to C polymorphism at position -174 in the IL6 promoter region has been associated with IL6 gene transcription, plasma IL6 levels, and with bone metabolism and the rate of bone loss in women. In the present study, we examined the association between the IL6 G-174C polymorphism and the rate of hip bone loss in 296 men aged 50 years and older (mean ± SD, 66 ± 7 years). Total hip bone mineral density (BMD) was measured at baseline and after an average of 7 years, and the annualized percentage (%/yr) and absolute (mg/cm^2/yr) rates of change in BMD calculated. The G-174C polymorphism was genotyped using polymerase chain reaction and fluorescence polarization. Despite similar initial BMD, men with the GG or GC genotypes (n=255) had a significantly greater rate of decline in hip BMD (-0.16 ± 0.67 %/yr) compared to men with the CC genotype (n=41) (0.07 ± 0.51 %/yr). These results remained statistically significant after adjusting for age, baseline BMD, and weight change during follow-up (P≤0.05). We found similar patterns for the annual absolute rate of change in hip BMD by IL6 genotype (P≤0.07). These findings suggest that older men with the IL6 -174G allele may experience an accelerated rate of decline in hip BMD with aging, similar to previous findings reported for older women.

Disclosures: ***K.Y.Z. Forrest****, None.*

SA139

Correlations of the Estrogen Receptor Alpha Coactivator RIZ1 Deletion with Bone Mineral Density in Men and Women. E. Grundberg*[1], H. Brändström*[1], E. L. Ribom*[2], O. Johnell[3], E. Orwoll[4], Ö. Ljunggren[1], H. Mallmin[2], T. Carling*[2], A. Kindmark[1]. [1]Dept of Medical Sciences, Uppsala University, Uppsala, Sweden, [2]Dept of Surgical Sciences, Uppsala University, Uppsala, Sweden, [3]Dept of Orthopedics, Malmo University Hospital, Malmo, Sweden, [4]Oregon Health and Science University, Portland, OR, USA.

ER-alpha is one of the candidate genes that have been most extensively studied in relation to bone mineral density (BMD). ER-alpha requires a number of cofactor for activation of target genes. Recently it has been shown that RIZ1 (Rb-interacting zinc finger) is a coactivator of ER-alpha and strongly enhances ER-alpha function. RIZ1 was initially characterized as a tumor suppressor gene, and maps to chromosome 1p36, which has been reported as a QTL for BMD. Also, in vitro studies have demonstrated that a deleted form of the RIZ1 gene, lacking a proline (P704) at exon 8, responds in a decreased manner to estrogen compared to the wild-type gene. Consequently, we have investigated the correlations of the P704 deletion in the RIZ1 gene with BMD in a population based study of 317 young women and in a subset of 295 men from the Swedish part of the ongoing MrOS study. BMD was measured by dual x-ray absorbtiomtery (DXA) at spine, total body, total hip and heel and by Quantitative Ultrasound (QUS) at the heel. The RIZ1 deletion was amplified by PCR from the individual's leukocyte DNA and analysed for genotype. Individuals homozygous for the deletion were denoted P704- hom, individuals with absence of the deletion on both alleles were denoted P704+ hom and individuals heterozygous for the deletion were denoted Het. The RIZ1 deletion was in Hardy-Weinberg equilibrium in both study populations and the genotype frequencies were P704+ hom 19%, Het 50%, P704- hom 31% in the female cohort and P704+ hom 19%, Het 54%, P704- hom 27% in the male cohort. The results show that after adjustments for height, age, fat and lean mass, BMD at the heel was significantly higher (p=0.02) in women homozygous for the P704+ allele compared to the heterozygous group. Same pattern was shown in men regarding BMD at the heel in relation to RIZ1 genotype, although not statistically significant. A borderline significant (p=0.06) correlation of QUS at the heel with RIZ1 genotype was observed in men after adjustments for BMI, indicating that also men homozygous for the P704+ allele have higher BMD compared to the heterozygous group. No correlations were found between the RIZ1 deletion and total hip either in the female cohort or in the male cohort. Taken together, these findings support the hypothesis that the RIZ1 deletion, lacking a proline at position 704, has an impaired capacity to bind ER-alpha, resulting in a decreased response to estrogen and thus lower BMD of the heel.

Disclosures: ***E. Grundberg****, None.*

SA140

See Friday Plenary number F140

SA141

Analysis of Polymorphisms of Vitamin D Receptor (VDR) and Estrogen Receptor A (ERa) Genes in an Italian Population From Lampedusa Island (Sicily). G. Di Fede*[1], A. Falchetti*[2], L. Masi[2], F. Del Monte*[2], F. Marini*[2], A. Gozzini*[2], C. Berni*[3], V. Ghinoi*[2], N. Napoli*[1], G. B. Rini[1], G. Cusumano*[1], S. Bucchieri*[1], C. Sferrazza*[1], M. C. Pandolfo*[1], M. L. Brandi[2]. [1]Internal Medicine, University of Palermo, Palermo, Italy, [2]Internal Medicine, University of Florence, Florence, Italy, [3]Clinical Physiopathology, University of Florence, Florence, Italy.

Vitamin D receptor (VDR) and Estrogen Receptor a (ERa) gene polymorphisms have been thoroughly reported to account for most of the well established genetic influence BMD, although conflicting results appeared in literature on the possible role that such polymorphisms may play in BMD control. The purpose of this study was to assess the role of VDR and ERa gene polymorphisms in a Lampedusa island postmenopausl women cohort. Total population of Lampedusa consists of 5908 individuals with 2867 women. We analyzed 229 postmenopausal women for VDR and ERa genotypes. Analyses have been performed according to described methods. For FOK1 polymorphism of VDR gene we observed frequencies of F allele equal to 64% and f allele 36%. FF, Ff and ff genotypes were, respectively, 34%, 60% and 6%. PvuII and XbaI polymorphisms of ERa gene exhibit the following frequencies: P 46%, p 54%, X 40% and x 60%. The frequencies of genotypes were: PP 20%, Pp 53%, pp 27%, XX 15%, Xx 50 and xx 35%. In a subgroup of 156 subjects (age range 58.7±9.15 yrs.) BMD was evaluated at lumbar spine (LS) by DXA (Hologic, 2000) and by QUS (Hologic, Achilles) at calcaneal level.
Ancova analysis (including age, age from menopause, weight and height as covariates) was performed to evaluate the association between genotypes and BMD expressed as T-score for DEXA and QUS evaluations. We observed that subjects with ff genotype had a significant lower T-score for BUA, SoS, and LS in comparison with Ff and FF genotypes (BUA: -2.54 SD vs. –1.39 SD and vs. –1.25 SD; p= 0.005; SoS: -1.88 SD, vs. –1.52 SD and vs. –0.81 SD; p= 0.003; LS: -2.82 SD, vs. –1.12 SD and vs. 0.36 SD; p= 0.0008). No statistically significant associations were observed for ERa genotypes both with QUS and DEXA BMD. Finally, when we combined the 3 polymorphisms we found that subjects with association of ff with xx or Xx and with pp genotypes exhibited BMD values significantly lower than single ff genotype (BUA: ffppxx –4.07 SD, ffppXx -3.26 SD; p=0,0005; SoS: ffppxx –2.09 SD, ffppXx –2.24 SD; p=0.0001; LS: ffppxx –3.17 SD, ffppXx –4.99 SD; p=0,002). In conclusion, our preliminary findings strongly suggest that VDR FOK1 polymorphism may play an important role to influence BMD values in postmenopausal women and that interaction between VDR and ERa gene polymorphisms may be important for identification of individuals at higher susceptibility risk for bone loss and fracture.

Disclosures: ***A. Falchetti****, None.*

SA142

Study of Osteonectin Gene Polymorphisms in Men with Idiopathic Osteoporosis. A. M. Delany[1], J. Shubert-Coleman*[2], R. Bahl*[2], T. L. Colvin*[3], J. Powell*[3], E. S. Kurland[3]. [1]St. Francis Hospital & Medical Center, Hartford, CT, USA, [2]Univ. Connecticut Health Center, Farmington, CT, USA, [3]Columbia University College of Physicians & Surgeons, New York, NY, USA.

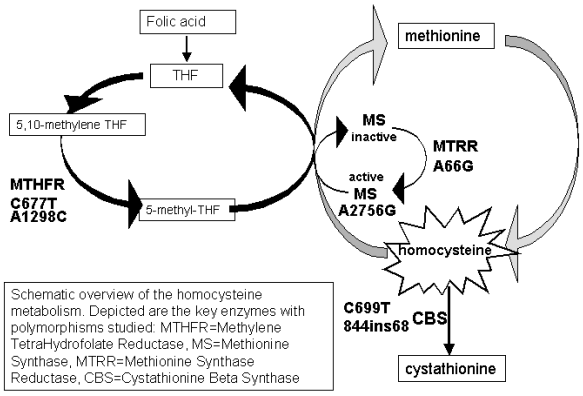
Osteonectin (SPARC, BM-40) is one of the most abundant non-collagen matrix components in bone. Mice bearing a null mutation in the osteonectin gene develop low turnover osteopenia, indicating that osteonectin is important for the maintenance of bone mass. In addition, decreased levels of osteonectin are associated with several animal models of bone fragility and with cultured osteoblasts from patients with osteogenesis imperfecta. For these reasons, we performed a candidate gene study to determine whether polymorphisms in the osteonectin gene are associated with low bone mass in a group of well characterized middle-aged men with idiopathic, low turnover osteoporosis. All patients had Z scores < -2.0 at the lumbar spine, hip or radial shaft, with markers of bone turnover in the low normal range, and reduced indices of bone formation assessed by histomorphometry. Genomic DNA was isolated from patients and from healthy, non-osteoporotic controls (with Z scores > -1.0 at all sites), matched for age, body mass index, and ethnicity (45 and 46 men, respectively). DNA was also isolated from a random sample population reflecting the ethnic mix of the suburban Hartford, CT region (147 individuals). Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis was used to screen the coding exons and splice junctions for variations in osteonectin gene sequence, and polymorphisms were confirmed by sequencing. The human osteonectin gene has 10 exons, 9 of which contain protein coding regions, and we found exons 3 and 10 were polymorphic in our analysis. Exon 3 had 1 single nucleotide polymorphism (SNP) which was silent and 1 SNP in intron C, and their allele frequencies were similar among the osteoporosis patients, matched controls, and random population sample. Exon 10 yielded 1 SNP at base +998 (C to G), in the 3' untranslated region, which is associated with RNA stability. 100% of the random sample population and matched controls were homozygous for the C allele, compared with 91% of the patients. The remaining 9% of the patients had the C/G genotype (different from controls, $p < 0.05$, Fisher's Exact test). It was previously reported that the stability of osteonectin RNA in fibroblasts with the +998 C/C genotype is twice that found in cells with the C/G genotype, providing a mechanism for decreased osteonectin expression in the C/G cells. These data suggest that SNPs in exon 10 of the osteonectin gene may provide a genetic component to the development of idiopathic osteoporosis in a subset of men affected by this heterogeneous disorder.

Disclosures: ***A.M. Delany****, None.*

SA143

Genetic Geterminants of the Homocysteine Metabolism in Relation to Bone Mineral Density and Fracture Risk. J. B. J. van Meurs[1], F. van Rooij*[2], P. Arp*[1], A. Hofman*[2], H. A. P. Pols[1], A. G. Uitterlinden[1]. [1]Internal Medicine, Erasmus MC, Rotterdam, Netherlands, [2]Epidemiology and Biostatistics, Erasmus MC, Rotterdam, Netherlands.

Osteoporosis has a strong genetic component. Previously, we saw that homocysteine levels predict fracture risk. We therefore examined whether genetic determinants of the homocysteine metabolism are associated with BMD and fracture risk. We analysed 6 polymorphisms in four key enzymes of the homocysteine metabolism which were reported previously to affect the homocysteine (hcys) serum level (see figure). We determined these polymorphisms in a population-based sample of 1058 postmenopausal women (aged 55-80 years) and analysed the relation to BMD and incident fractures (n=129, mean follow-up time 7 years). In a subset of 365 women we studied hcys levels in relation to the polymorphisms. All analyses were adjusted for age and BMI.
For the MTRR G66A polymorphism, we observed an allele-dose effect of the A-allele leading to a higher hcys level, but none of the other polymorphisms showed an association with hcys levels. We found a significant association between femoral neck BMD and the MTRR polymorphism (allele-dose=-0.015g/cm^2, p-trend= 0.003). In addition, both the MTHFR polymorphisms were associated with BMD differences. The T-allele of the MTHFR 677 polymorphism was associated with decreased femoral neck BMD (p-trend=0.03) and lumbar spine BMD (p-trend=0.02), while the C-allele of the MTHFR 1298 polymorphism was associated with increased lumbar spine BMD (p-trend=0.05). None of the 6 polymorphisms studied were associated with fracture risk. We conclude that in this study the MTRR G66A polymorphism is associated with hcys levels and BMD. In addition two polymorphisms in another key enzyme of the homocysteine metabolism, MTHFR C677Tand A1298, are associated with BMD differences, but none of the studied polymorphisms were associated with fracture risk. Future analyses of interaction effects will have to be done in larger samples of our population-based study.



Disclosures: ***J.B.J. van Meurs****, None.*

SA144

Normative Values of Soluble RANKL in the Serum of Healthy Adults and Correlation with Various Parameters of Bone Metabolism. S. Kudlacek*[1], P. Pietschmann[2], W. Woloszczuk[3], R. Willvonseder*[1]. [1]Medical Department and LBI of Aging Research, Hospital Barmherzige Brüder Wien, Vienna, Austria, [2]Department of Pathophysiology, University of Vienna, Vienna, Austria, [3]LBI of Endocrinology, University of Vienna, Vienna, Austria.

RANKL (receptor activator of nuclear factor-kappa B ligand) is expressed on stromal and osteoblastic cells and in concert with osteoprotegerin, bone metabolism is regulated. OPG functions as a decoy receptor binding RANKL. This interaction results in inhibition of osteoclast differentiation and finally of bone resorption. Recently those components of bone turnover can be measured in serum samples by commercial available ELISA. Biochemical analysis of skeletal tissue has already been performed but its clinical approach remains further investigation. A sandwich ELISA (Biomedica®, detection limit 0,4 pmol/l) for sRANKL (soluble RANKL) was used, tests were performed in triplicates and samples were incubated overnight. For statistical evaluation we used SAS softare package. We determined soluble serum levels of RANKL and OPG, bone density (lumbar, spine DXA) and basic biochemical parameters of bone metabolism in 1029 normal individuals of a normal Austrian population (1). Overall, serum samples of sRANKL in females (n=635) and males (n=394) were determined. sRANKL levels were 0.37 ± 0.36 pmol/l (10.–90. percentile range 0.04–0.71 pmol/l) for females and 0.46 ± 0.46 pml/l (10.–90.percentile range 0.04–0.85 pmol/l) for males. When we determined sRANKL by decades for females and males seperately and used median we observed a certain decrease in males, whereas sRANKL levels in females kept constant over decades (see Tab.). Correlation with serum parameters and bone density showed a negative correlation of sRANKL with age in females and males ($p<0.05$). sRANKL and OPG were negtively correlated in females whereas positively in males suggesting different mechanisms of osteoporosis between genders. We investigated a certain influence of age on serum levels of bone matrix proteins. Certain levels of RANKL were quantifiable in serum of healthy adults. A wide variation sRANKL levels and their clinical impact has to be investigated.
(1) Kudlacek S., Schneider B., Peterlik M., Leb G., Klaushofer K., Woloszczuk W., Willvonseder R. Serum levels of Osteoprotegerin increase with age in an healthy adult popula-

tion, Bone 2003, in press

sRANKL

gender	n	median±SD	age group
f	229	0,39±0,6	
m	65	0,69±1,2	35
f	220	0,38±0,8	
m	117	0,51±1,1	45
f	150	0,34±0,6	
m	177	0,36±0,8	55
f	31	0,36±1,9	
m	30	0,44±0,7	65
f	5	0,11±0,1	
m	5	0,15±0,1	75

Disclosures: ***S. Kudlacek****, None.*

SA145

Serum Osteoprotegerin as a Novel Marker of Metabolic Bone Disease. F. M. Hannan*[1], A. Fairney[1], P. Kyd*[1], A. Patel*[2]. [1]Endocrinology and Metabolic Medicine, Imperial College, London W2 1NY, United Kingdom, [2]Urology, St. Mary's NHS Trust, London W2 1NY, United Kingdom.

Osteoprotegerin (OPG) is a member of a recently discovered family of cytokines expressed by bone cells and is central to the regulation of bone remodelling. OPG is a soluble protein and is detectable in human serum by enzyme linked immunosorbent assay (ELISA). The relationship between serum OPG and disorders associated with abnormal bone remodelling is unclear.

Using a commercially available OPG ELISA, this study assessed whether serum OPG concentrations are altered in patients with the following metabolic bone disorders: post-menopausal osteoporosis, carcinoma of the prostate (CaP) with skeletal metastases, primary hyperparathyroidism (PHPT) and renal osteodystrophy.

Serum OPG concentrations did not significantly differ in patients with post-menopausal osteoporosis compared with age-matched subjects with no bone disease (median value 3.8pmol/l, range 2.2-7.5 pmol/l vs. median value 3.7pmol/l, range 1.4-5.7pmol/l; p=NS) and OPG levels did not significantly change in osteoporosis patients treated with the anti-resorptive agent alendronate. However, OPG levels were significantly elevated in patients with CaP skeletal metastases compared with patient with locally advanced CaP (median value 7.8pmol/l, range 3.2-17.3pmol/l vs. median value 5.0pmol/l, range 1.8-6.9pmol/l; p=0.024). Serum OPG was also significantly raised in PHPT patients compared with subjects with no bone disease (median value 5.3pmol/l, range 3.4-9.7pmol/l vs. median value 3.4pmol/l, range 1.84-5.21pmol/l; p=0.0008). End stage renal failure (ESRF) patients had markedly elevated OPG concentrations compared with subjects with no renal or bone disease (median 8.1pmol/l, range 2.9-27.4pmol/l vs. median 3.6pmol/l, range 1.3-7.4pmol/l; p<0.0001).

Serum OPG levels appeared to be elevated in disorders with profoundly abnormal remodelling such as skeletal metastatic disease. Whereas post-menopausal osteoporosis, which is associated with mildly abnormal bone turnover did not affect OPG concentrations. It is uncertain whether the markedly raised serum OPG in patients with ESRF represents underlying renal osteodystrophy or is secondary to reduced renal clearance.OPG shows promise as a novel biochemical marker of bone remodelling. However, more work is required to elucidate the metabolism and clearance of this cytokine and larger scale prospective clinical studies are recommended to evaluate the role of serum OPG in the management of metabolic bone disease.

Disclosures: ***F.M. Hannan****, None.*

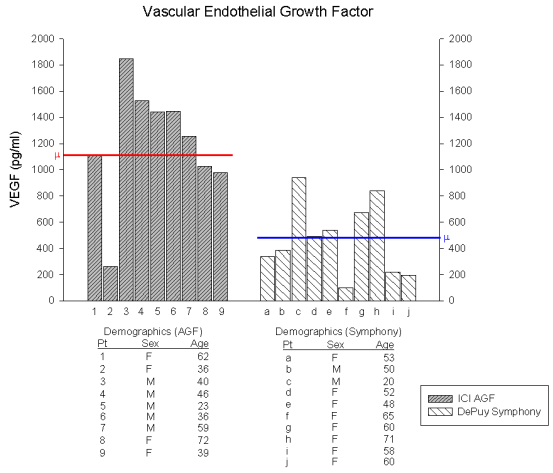
SA146

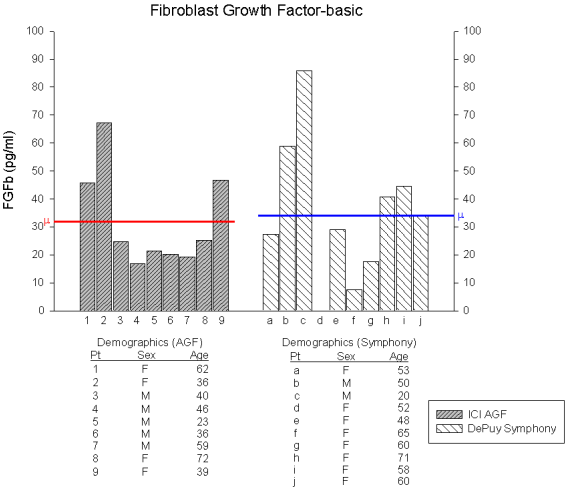
See Friday Plenary number F146

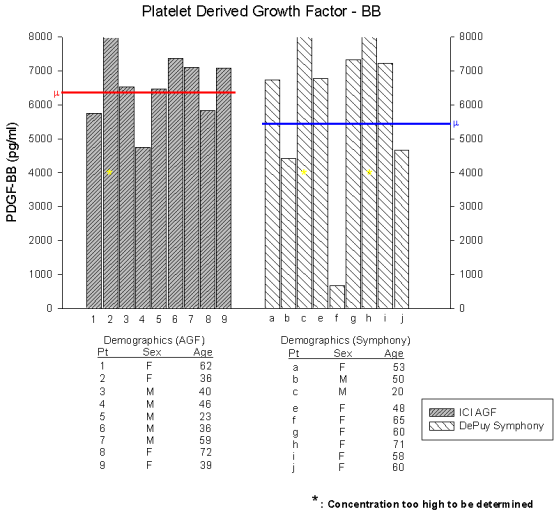
SA147

Cytokine Composition of Commercial Platelet Rich Plasma Concentrates. L. S. Kidder, A. H. Schmidt*, R. F. Kyle*. Midwest Orthropedic & Minneapolis Medical Res Fdns, Minneapolis, MN, USA.

The initial response to a bone injury is the local aggregation of platelets, serving to immediately stem bleeding via clot formation. As the platelets at the site are entrapped within a fibrin meshwork, they begin to degranulate releasing a suite of osteotropic cytokines. In the US, there are currently two commercial methods designed to replicate and enhance this process by extracorporeally concentrating a patient's circulating platelets and placing them at the surgical site. One, (J&J DePuy Biologics; Raynham, MA), employs a centrifuge designed to separate the platelet fraction from an aliquot of whole blood. The other (ICI; Irvine, CA), concentrates the buffy coat fraction pheresised via cell-saver by removing a portion of the water from the plasma by hemoconcentrator filtration. This investigation evaluated the relative ability to of these two processes to concentrate certain osteotropic growth factors. Using quantitative sandwich enzyme immunoassay (ELISA), a survey of 18 orthopaedic patients found that both techniques successfully concentrated Fibroblast Growth Factor-basic (ICI: 258% of baseline; J&J: 275%) and Platelet Derived Growth Factor-BB (126% / 184%) above preconcentration levels. Vascular Endothelial Growth Factor was likewise concentrated by ICI's technique (249%), though the DePuy process failed to do so (97%). By concentrating autologous cytokines, both of these systems may be useful in encouraging bone formation in difficult orthopaedic applications. The relative clinical significance of differences in VEGF concentration remain unknown and a subject for further study.







Disclosures: ***L.S. Kidder****, Interpore Cross Int'l 2.*

SA148

See Friday Plenary number F148

SA149

Osteoprotegerin Levels of Bone Marrow: Associations with Peripheral Blood OPG levels and Bone Metabolism Markers. K. H. Baek*[1], W. Y. Lee*[2], M. I. Kang[1], K. W. Oh[3], J. H. Han[1], K. W. Lee*[1], H. Y. Son*[1], S. K. Kang*[1]. [1]Internal medicine, The Catholic University of Korea, College of Medicine, Seoul, Republic of Korea, [2]Internal medicine, Sungkunkwan University, Seoul, Republic of Korea, [3]Internal medicine, Mizmedi Hospital, Seoul, Republic of Korea.

Osteoprotegerin (OPG) is a potent antiresorptive molecule that binds the final effector for osteoclastogenesis, receptor activator of NF-κB ligand (RANKL). There are some data regarding possible relationships between peripheral blood OPG levels and various markers of bone metabolism. But there has been no study observing OPG levels of bone marrow where cellular interactions are continually occurring as part of bone remodeling process. The present study was carried out to clarify the interrelationships between bone marrow OPG levles and peripheral blood OPG levels and other markers of bone metabolism. In 19 patients (11 men, 8 women; 49.4±12.5 years old; 35 to 75 years old), bone marrow was sampled in order to determine the OPG levels. At the same time, markers of bone metabolism, including serum OPG, osteocalcin, carboxy-terminal cross linked telopeptide of type I collagen (ICTP) and parathyroid hormone, were measured from peripheral blood of each patients. DEXA was performed in all patients to determine the BMD of lumbar spine and femur. The mean bone marrow OPG levels are significantly lower than mean peripheral blood OPG levels (687.4±157.7 pg/ml, 836.5±191.9 pg/ml, $p<0.05$) and the bone marrow OPG correlated well with the peripheral blood OPG ($r=0.61$, $p<0.01$). Both bone marrow and peripheral OPG levels increased with age but more strong correlations were found between marrow OPG levels and age ($r=0.72$, $P<0.01$; $r=0.59$, $P<0.01$). There was significant correlations between the bone marrow OPG and blood PTH levels($r=0.53$, $p<0.05$) but not between the peripheral blood OPG and PTH levels. Bone marrow OPG levels correlated inversely with BMD of femoral neck and ward ($r=-0.48$, $p<0.05$; $r=-0.58$ $p<0.11$), and there was a trend for bone marrow OPG levels to be associated positively with bone resorption marker, ICTP. Peripheral blood OPG levels also correlated inversely with femoral neck BMD, but statistically not significant. Collectively, although bone marrow OPG is mirrored relatively well to peripheral blood OPG, bone marrow OPG may reflect more accurately the bone marrow microenvironment and bone marrow OPG levles better correlate with various markers of bone metabolism than peripheral blood OPG levels.

Disclosures: ***K.H. Baek****, None.*

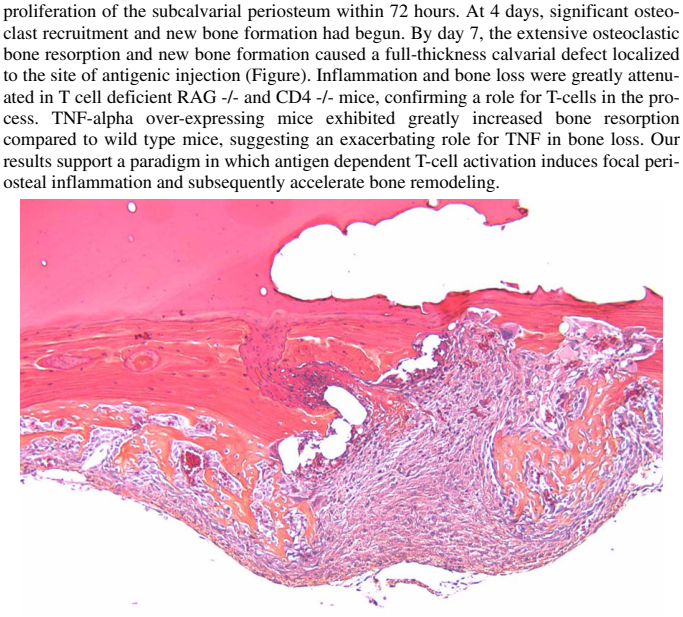
SA150

See Friday Plenary number F150

SA151

An In Vivo Model of T-cell Dependent Accelerated Bone Remodeling. W. Huang*, R. O' Keefe, R. Rosier*, E. Schwarz. Orthopedics, URMC, Rochester, NY, USA.

Several human diseases including ankylosing spondylitis, inflammatory arthritides, and fibrodysplasia ossificans progressiva are characterized by an enigmatic inflammatory T-cell response associated with new bone formation and altered local bone remodeling. To understand this process, we developed a new in vivo model of T-cell dependent accelerated bone remodeling based upon immunization with the classical contact hypersensitivity agent, dinitrofluorobenzene (DNFB). Immunization and challenge with DNFB elicits a strong T-cell dependent (type IV hypersensitivity) inflammatory response. Calvarial injection of minute quantities of DNFB into sensitized mice induced robust inflammation and proliferation of the subcalvarial periosteum within 72 hours. At 4 days, significant osteoclast recruitment and new bone formation had begun. By day 7, the extensive osteoclastic bone resorption and new bone formation caused a full-thickness calvarial defect localized to the site of antigenic injection (Figure). Inflammation and bone loss were greatly attenuated in T cell deficient RAG -/- and CD4 -/- mice, confirming a role for T-cells in the process. TNF-alpha over-expressing mice exhibited greatly increased bone resorption compared to wild type mice, suggesting an exacerbating role for TNF in bone loss. Our results support a paradigm in which antigen dependent T-cell activation induces focal periosteal inflammation and subsequently accelerate bone remodeling.



Disclosures: ***W. Huang****, None.*

SA152

Loss of c-Kit Ligand Function Results in Low Bone Mass and Density in Mice. S. Lotinun, G. L. Evans*, T. E. Hefferan, R. T. Turner. Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

The c-Kit tyrosine kinase receptor ligand, known as stem cell factor (SCF), is expressed in many cells including osteoblasts. However, its action on bone metabolism remains largely unknown. To examine the functional role of SCF/c-Kit signaling in bone growth, we used 6-week-old male mice with a loss of function mutation in the *Steel* (*Sl*) locus that encodes the Kit ligand. *Sl/Sl*^{d} mice grow slower and have shorter bones than wild type (WT) littermates. Bone mineral density (BMD) measured by double energy X-ray absorptiometry using PIXImus small animal densitometer indicates that mice that lack SCF had significantly ($P<0.05$) decreased BMD in tibia (0.047±0.002 vs 0.039±0.001) and femur (0.060±0.002 vs 0.046±0.002), but not lumbar vertebra, largely due to a reduction in bone mineral content. Histomorphometric analysis showed that bone formation rate was suppressed in both cortical and cancellous bone. Cancellous bone volume was 37% lower due to a significant decrease in osteoblast number (Ob.S/BS, 11.8±1.2 vs 7.6±1.6) and activity (MAR, 2.6±0.1 vs 1.8±0.2) and significant increase in osteoclast number (Oc.S/BS, 12.6±1.4 vs 20.0±2.1). To further investigate the cellular mechanism of SCF deficiency-induced osteopenia, we compared functions of osteoblasts derived from humerus of WT and *Sl/Sl*^{d} mice. [^{3}H]Thymidine incorporation studies showed that proliferation of primary osteoblasts derived from mutant mice was significantly suppressed by 46%. Moreover, a decrease in mineralization per DNA content was observed in *Sl/Sl*^{d} osteoblast culture. These results suggest that SCF plays a critical role in bone accretion during growth. Lack of SCF signaling-induced osteopenia is mediated by a combination of suppressed bone formation and increased bone resorption.

Disclosures: ***S. Lotinun****, None.*

SA153

Insulin-Like Growth Factor (IGF)-I is Correlated with the Bone Size, but not with Bone Density. J. Son*[1], B. Kim[1], G. Kim*[1], S. Kim*[2], D. Lee*[2]. [1]Family Practice and Community Health, College of Medicine, Ajou University, Suwon, Republic of Korea, [2]Family medicine, Sungkyunkwan School of Medicine, Seoul, Republic of Korea.

IGF-I is related with bone mass. Bone mass is a function of bone size and volumetric bone mineral density (vBMD). We investigated whether IGF-I is related with bone size or vBMD. 300 men were recruited for this study. Bone mineral density (BMD) was measured at 3rd lumbar vertebra by dual-energy X-ray absorptiometry. Blood was sampled for measuring IGF-I and IGF binding protein (IGFBP)-3. Bone volume were calculated by Carter's method. IGF-I was significantly correlated with age, body mass index (BMI), bone mineral content (BMC), areal BMD (aBMD), height, area, bone volume, and vBMD at the L3 spine($r=-0.404$, 0.135, 0.235, 0.193, 0.329, 0.138, 0.136, 0.144, $P< 0.05$, respectively). After adjusting age and BMI, IGF-I was still correlated with BMC, and bone volume ($r=0.117$, 0.143, $P< 0.05$), but not with aBMD and vBMD ($r=0.056$, 0.096). IGFBP-3 was not correlated with any parameters at the L3 spine($r=0.125$, $P<0.05$), after adjusting age and BMI. We conclude that IGF-I is correlated with BMC, because of bone size, not because of Bone density.

Key words: Insulin growth factor-I, volumetric bone mineral density, bone volume

Disclosures: ***B. Kim****, None.*

SA154

See Friday Plenary number F154

SA155

IGFBP-2 Is PTH Responsive and may Mediate the Anabolic Actions Of IGF-I in Bone. L. G. Horton*[1], K. L. Shultz*[1], C. L. Ackert-Bicknell*[1], W. G. Beamer[1], L. R. Donahue[1], M. L. Adamo[2], X. Ma*[2], D. Clemmons[3], J. Rubin[4], J. Burgess*[5], M. L. Bouxsein[1], C. J. Rosen[5]. [1]The Jackson Laboratory, Bar Harbor, ME, USA, [2]University of Texas Health Science Center, San Antonio, TX, USA, [3]University of North Carolina, Chapel Hill, NC, USA, [4]Emory University Medical School, Decatur, GA, USA, [5]St. Joseph Hospital, Bangor, ME, USA.

Insulin-like growth factor binding protein 2 (IGFBP-2) is a 34 kilodalton binding protein that circulates in relatively high concentrations and is expressed in many tissues, including bone. IGFBP-2 also binds to extracellular matrices, heparin, vitronectin and hydroxyapatite. IGF-II co-administered with IGFBP-2 prevents bone loss in rats, and high levels of IGFBP-2 plus a "big" form of IGF-II in hepatitis C is associated with osteosclerosis. The *Igfbp2* gene is located on Chr 1 in mice at 34.5cM and is a candidate gene for quanitative trait loci (QTL) related to both bone density and serum IGF-I in B6C3HF2 mice. Hence, we postulated that IGFBP-2 is important in mediating the anabolic effects of IGF-I on bone. Since C3H/HeJ (C3H) has higher circulating IGF-I and greater hepatic IGF-I expression than C57BL/6J (B6), we first performed western immunoblots (WIB) for serum IGFBP-2 in females of both strains. Serum IGFBP-2 by WIB was greater in C3H than B6. Additionally, liver IGFBP-2 mRNA by RNAse protection (RPA) was nearly 2-fold higher in female C3H than in female B6 ($p < 0.05$). To define the role of IGFBP-2 and IGF-I in bone *ex vivo,* we studied the effects of PTH and cAMP on calvarial IGF-I and IGFBP-2 from B6 and congenic B6.C3H-6T (6T), a B6 mouse constructed with a portion from Chr 6 of C3H exhibiting low serum IGF-I and reduced BMD [Bouxsein et al, JBMR 2002]. IGF-I secretion at 24 hours in 3-day old B6 and 6T hemi-calvariae cultures was dose dependent and optimal at 25nM PTH and 100μM cAMP. IGF-I in conditioned media

(CM) from hemi-calvariae did not differ by strain, and increased three to four fold over untreated CM after PTH, cAMP, or PTH+cAMP in both B6 and 6T ($p<0.001$). IGFBP-2 secretion did not differ by strain in the untreated CM, but increased nearly two fold ($p<0.05$) in the CM of B6, but not 6T, in response to PTH and cAMP+PTH treatment. Real time PCR from cultured calvariae revealed markedly elevated IGF-I mRNA levels in both strains in response to PTH, cAMP or both, whereas IGFBP-2 mRNA increased only in B6 calvariae (3-fold). OPGL expression increased with all treatments in both strains. We conclude that IGFBP-2 may play a key role in mediating the anabolic activities of IGF-I, both systemically and locally. The absence of an IGFBP-2 response to PTH in 6T calvariae may be important in the pathogenesis of low BMD in this congenic strain.

Disclosures: ***C.J. Rosen****, None.*

SA156

See Friday Plenary number F156

SA157

Parallel PI-3 Kinase and p42/44 MAP Kinase Signaling Pathways Subserve the Mitogenic and Anti-apoptotic Actions of IGF-1 in Osteoblastic Cells. A. Grey, Q. Chen*, X. Xu*, J. Cornish. Medicine, University of Auckland, Auckland, New Zealand.

Insulin-like growth factor 1 (IGF-1) is an endocrine and paracrine regulator of skeletal homeostasis, principally by virtue of its anabolic effects on osteoblastic cells. The molecular mechanisms by which IGF-1 regulates cellular processes vary in a cell-type specific fashion. In the current study we examined the intracellular signaling pathways by which IGF-1 promotes proliferation and survival in SaOS-2 human osteoblastic cells. Inhibition of each of the phosphatidylinositol-3 kinase (PI-3 kinase), p42/44 mitogen-activated protein (MAP) kinase and p70s6 kinase pathways partially inhibited the ability of IGF-1 to stimulate osteoblast proliferation and survival. Since activation of p70s6 kinase is downstream of both PI-3 kinase and p42/44 MAP kinase activation in osteoblasts treated with IGF-1, this ribosomal kinase represents a convergence point for IGF-1 induced PI-3 kinase and p42/44 MAP kinase signaling in osteoblastic cells. In addition, abrogation of PI-3 kinase-dependent Akt signaling, which does not inhibit IGF-1-induced p70s6 kinase phosphorylation, also inhibited the anti-apoptotic effects of IGF-1 in osteoblasts. Finally, interruption of $G_{\beta\gamma}$ signaling partially abrogated the ability of IGF-1 to promote osteoblast survival, without inhibiting signaling through PI-3 kinase/Akt, p42/44 MAP kinases or p70s6 kinase. These data suggest that IGF-1 signals osteoblast mitogenesis and survival through parallel, partly overlapping intracellular pathways involving PI-3 kinase, p42/44 MAP kinases and $G_{\beta\gamma}$ subunits.

Disclosures: ***A. Grey****, None.*

SA158

TGF-b Improves Composite Cortical Bone Grafts Osteointegration in a Rat Model. M. L. E. Faria*[1], S. E. Weisbrode*[2], S. Stevenson*[3]. [1]Comparative Orthopaedic Research Laboratory, University of Wisconsin, Madison, WI, USA, [2]Pathobiology and Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, USA, [3]Orthopaedics, Case Western Reserve University, Cleveland, OH, USA.

TGF-β is a potent multifunctional cytokine capable of immunesuppression and strong osteoinduction. This study was designed to determine the effects of TGF-β1 on histological parameters of cortical bone graft osteointegration. Seventy two Fischer rats were randomly assigned to three treatments groups as recipients of frozen cortical composite femoral bone transplants. Experimental groups received either (I) a single dose of 25ng of TGF-β1, locally administered during bone transplantation; or (II) the same 25ng intraoperatively, followed by continuous local administration of TGF-β 1 during 14 days. Control animals received no TGF-β. All rats were bilaterally implanted with subcutaneous minosmotic pumps delivering either PBS (control and group I rats) or TGF-β1-containing PBS (2ng TGF–1/ µl of PBS), into the center of the femoral grafts. All grafts were composites of frozen cortical bone and fresh syngeneic marrow cells ($1.5x10^8$ cells/ graft). One leg was transplanted with an allogeneic graft (Brown-Norway strain, H-1^n), while the contralateral leg received a syngeneic graft (Fisher strain. H-1^l). Bones were harvested at 1, 2 and 4 months after transplantation. Qualitative and quantitative histology were performed on nondecalcified, toluidine blue stained sections. One, two and three-way ANOVA was used to compare parametric data for main and interactive effects. Host-graft interface healing score were compared by Kruskal-Wallis ANOVA by ranks.
Effects of TGF–1 were most evident at 4 months after surgery. Marrow cell population was decreased by TGF–1 exposure. Allograft bones not exposed to TGF– had higher intracortical porosity than all other grafts and a striking individual variability in this parameter. Total bone area increased in all TGF–1 treated groups. New bone formed mostly on the peiosteum side of the grafts, even in animals receiving TGF–1 intramedullarely. Single administration of TGF–1 was sufficient to induce significant increase in new bone. Continuous administration of TGF–1 did not add to this effect. Grafts exposed to prolonged administration of TGF–1 showed a new bone of a more compact lamellar aspect than groups (where woven appearance predominated). Finally, decreased resorption of the originally transplanted cortex was observed in TGF–1- treated grafts.
Although the mechanisms underlying these results were not identified in here, we concluded that local TGF-β administration enhances bone graft incorporation. We propose possible pathways for these results.

Disclosures: ***M.L.E. Faria****, None.*

SA159

See Friday Plenary number F159

SA160

Expression of Bone Morphogenetic Proteins and their Antagonists during Bone Regeneration. X. Chang*, Y. Shibata*, T. Tsukazaki, A. Yamaguchi. Oral Pathology and Bone Metabolism, Nagasaki University Graduat, Nagasaki, Japan.

Biological activity of bone morphogenetic proteins (BMPs) is regulated by several factors at both extracellular and intracellular levels. Although several lines of evidence demonstrated that BMPs play important roles in bone regeneration, the regulatory mechanism of BMP activity has not been clarified well during bone regeneration. To explore molecular mechanism of BMP action during bone regeneration, we investigated the expressions of mRNAs for BMPs and their possible antagonists that interact with BMPs at extracellular level by GeneChip (affimetrix) and real-time PCR analyses. We made a round hole, 1 mm in a diameter, at diaphysis of femur of 8-week-old male C57BL/6J mice. To verify the reproducibility of the experiments, the bone-healing process was examined daily by histological sections and Northern blot analysis for bone-related mRNAs. These studies revealed that numerous fibroblastic cells appeared on days 3, abundant osteoprogenitors appeared and bone formation occurred on day 5, then abundant bones filled bone defects on day 10. Expression levels of several bone-related mRNAs including osteocalcin increased after day 5. Thus we isolated mRNAs from normal bone (day 0) and regenerating bones 5 and 10 days after injury, and these mRNAs were subjected to GeneChip analysis, which covered 12424 genes. GeneChip analysis revealed up-regulation of BMP-4 and several BMP antagonists including BMP-1, Tolloid-like (Tll), Twisted gastrulation (Tsg), Follistatin (Fst)and Follistatin-like (Fstl) during bone regeneration. This analysis suggested no apparent changes in expression levels of noggin (Nog) and chordin (Chrd). To confirm expression profiles obtained from GeneChip analysis, we investigated the expression levels of these mRNAs by real-time PCR using tissues isolated from days 0, 2, 5 and 10 after injury. Real-time PCR analysis demonstrated that all of the genes above described including Nog and Chrd were up-regulated during bone regeneration, though the expression patterns are slightly different among genes examined. These results demonstrated that not only BMPs but also their antagonists were up-regulated during bone regeneration. The integrated expression profile of genes regulating BMP activity as shown in the present study might conduct proper bone healing.

Disclosures: ***X. Chang****, None.*

SA161

Collagen V(α1) and Nephronectin are Regulated by TGF-β in Osteoblasts. S. Kahai*[1], A. Seth[2]. [1]Laboratory Medicine and Pathobiology and CIHR Group in Matrix Dynamics, University of Toronto, Toronto, ON, Canada, [2]Division of Molecular and Cell Biology Research and Laboratory of Molecular Pathology, Sunnybrook and Women's College Health Sciences Centre, Toronto, ON, Canada.

Production of extracellular organic matrix and its mineralization to form bone are key steps during osteogenesis. Bone matrix contains high concentrations of growth factors, particularly transforming growth factor β (TGF-β), which is known to play important regulatory roles during osteogenesis. Divergent effects of TGF-β1 on bone formation have been reported both in vitro and in vivo depending upon experimental conditions, cells employed, and their stage of maturation. The genome wide expression analysis provided by microarray based methods could reveal the involvement of genes previously not known to be involved in the TGF-β signal transduction network. In this study, cDNA microarray technology has been used to identify and study the expression profile of 8470 genes, some of which could be targets of TGF-β during osteogenesis. Microarray analysis of 8470 genes revealed 97 cDNAs to be differentially expressed in the clonal osteoblastic cell line MC3T3-E1 that had been treated with TGF-β1. From the 97 differentially expressed genes, we selected Collagen V(α1) {Differential expression = + 4.9} and Nephronectin {Differential expression = - 5.2} for further studies. Using Northern Blotting and Real-Time PCR we found that, when MC3T3-E1 cells were treated with TGF-β1, Collagen V(α1) was up-regulated and Nephronectin was down-regulated during the differentiation of preosteoblasts to osteoblasts. By Immunohistochemistry we found significant expression of the Nephronectin protein in the basement membrane of the developing tooth and the developing kidney, and in the developing long bone of E16.5 mouse embryos. Significant Collagen V(α1) mRNA and protein expression were seen by RNA *in-situ* hybridization and Immunohistochemistry respectively, in the developing long bone of E17.5 mouse embryos. In conclusion, by the use of in vivo and in vitro approaches we have discovered that, the Collagen V(α1) and Nephronectin genes are potential targets of TGF-β during osteogenesis.

Disclosures: ***S. Kahai****, None.*

SA162

See Friday Plenary number F162

SA163

TGF-ß regulates chondrocyte proliferation and differentiation via FGF and Smad2 in the perichondrium. R. A. Serra, J. Alvarez*, A. Murkherjee*. Cell Biology, University of Alabama, Birmingham, Birmingham, AL, USA.

TGF-ß inhibits chondrocyte proliferation and hypertrophic differentiation in metatarsal organ cultures by a perichondrium dependent mechanism, suggesting that factors synthesized in the perichondrium are involved. We previously showed that TGF-ß acts through PTHrP to regulate cartilage hypertrophic differentiation. To begin to identify the perichondrial factors that are involved in regulating proliferation by TGF-ß, we studied the effects of IGF-I and FGF on metatarsal organ cultures. As expected, increased proliferation as measured by BrdU incorporation was observed after treatment of organ cultures with IGF-I. A similar effect was seen after the perichondrium was removed from the metatarsals suggesting IGF-I acts directly on the chondrocytes. Treatment with FGF2 resulted in decreased BrdU incorporation into chondrocytes and increased BrdU incorporation in perichondrial cells, similar to what is observed after treatment with TGF-ß. BrdU incorporation was altered after FGF treatment when the perichondrium was removed suggesting FGF acts directly on chondrocytes. To determine if treatment with TGF-ß regulates IGF or FGF signaling, IGF and FGF receptor levels and activation were measured by immunoprecipitation/ immunoblot assays. Treatment with TGF-ß resulted in increased activation as measured by phosphorylation of FGFR3. The overall levels of FGFR3 were not altered by treatment with TGF-ß. In contrast, treatment with TGF-ß resulted in a reduction in the overall levels of IGFR1. The data suggest a model where TGF-ß regulates chondrocyte proliferation by regulating FGF signaling. TGF-ß acts via the Smad family of transcriptional regulators. To determine the mechanism of TGF-ß signaling in the perichondrium, we next tested the role of TGF-ß restricted Smad2 and Smad 3 in chondrocyte proliferation and differentiation in metatarsal cultures. Perichondrium of metatarsals was infected with adenoviruses that express dominant-negative forms of the TGF-ß type II receptor (Ad-DNIIR), Smad2 (Ad-DNS2), and Smad3 (AD-DNS3) as well as a control virus expressing the ß-galacotsidase reporter (Ad-ß-GAL). Infection was detected in about 70% of perichondrial cells in each case. Infection with AD-DNIIR and Ad-DNS2 completely blocked the effects of TGF-ß on chondrocyte proliferation and differentiation. In contrast, infection with Ad-DNS3 only partially blocked signaling by TGF-ß even though high levels of DNS3 were detected by immunoblot. Similar results were observed in metatarsal bones from Smad3-null mice suggesting that Smad3 in the chondrocytes does not contribute to this effect. The data suggest that primarily Smad2 mediates TGF-ß signaling in the perichondrium.

Disclosures: ***R.A. Serra****, None.*

SA164

Mitogenic Mechanical Strain Activates Tyrosine Kinases FAK and PYK2/CAK Beta in Osteoblastic Cells. N. Boutahar*, A. Rattner*, A. Guignandon*, L. Vico, M. Lafage-Proust. Laboratoire de Biologie du Tissu Osseux, INSERM 0366, Saint-Etienne cedex 2, France.

Integrin signaling is one of the mechanisms thought to be involved in the mechanical loading-induced increase in bone formation. However the molecular events involved remain unclear. In this context, we studied the effects of radial cyclic strain (CS)(10 min, 1% @ 0.25Hz) on ROS 17/2.8 and rat primary osteoblastic cells, plated for 72 h on Type I collagen coated silicone membranes, using a FLEXCELL FX-3000 unit. The CS-induced increase in ROS 17/2.8 and primary cell proliferation observed by day 1, was blocked by MAPK inhibitor PD98059 (20µM). Signaling events were then assessed at various time points (0, 30 min, 4 h), after one CS with Western blotting (WB) and co-immunoprecipitation (CI) experiments on cells, serum-starved overnight. CS rapidly and time-dependently promoted phosphorylation of ERK2 at Tyr-187, focal adhesion kinase (FAK) at Tyr-397, the major autophosphorylation site, and at Tyr-925 located in the paxillin binding domain leading to activation of the Ras/Raf/MEK pathway. Cell transfection with FAK mutated at Tyr 397 strongly reduced phosphorylation of ERK2 at Tyr-187. CS also induced Src phosphorylation at Tyr-418 located at the Src catalytic domain. Treatment with the selective Src family kinase inhibitor pyrazolopyrimidine 2 (PP2, 10 µM) markedly reduced the phosphorylation of FAK Tyr-397 in basal conditions. In contrast, PP2 did not prevent FAK phosphorylation at Tyr-397 induced by mechanical strain suggesting a Src-independent activation of FAK. In order to identify other proteins involved in strain-induced signaling, we performed a WB with anti P-Tyr Ab (PY99). We found that, besides the 125 Kd band corresponding to FAK, another 110 Kd protein was highly phosphorylated in both ROS17/2.8 and rat primary cells. We identified this protein as PYK2 , a protein kinase highly homologous to FAK, phosphorylated at Tyr 402. Strain promoted the FAK to PYK association in a time-dependent manner as well as the phosphorylation of effectors of their adhesion-targeting domains, such as the highly homologous proteins paxillin and Hic-5.
In conclusion we found that a mitogenic mechanical stimulus induced phosphorylation of the focal contact proteins FAK and PYK2 that might be at the origin of the activation of the MAPK pathway. Thus, osteoblastic cells may use this PYK2-FAK cooperation to orchestrate cytoskeleton regulation and signaling in response to mechanical strain transmitted in part by extracellular matrix.

Disclosures: ***N. Boutahar****, None.*

SA165

See Friday Plenary number F165

SA166

Gene Expression Profilingafter Mechanical Loading in the Mouse Ulna Model. S. E. Harris[1], M. A. Harris[1], D. Guo*[1], A. G. Robling*[2], L. F. Bonewald[1], C. H. Turner[2]. [1]Oral Biology, UMKC SOD, Kansas City, MO, USA, [2]Orthopedic Surgery, Indiana Univ School of Med, Indianopolis, IN, USA.

Mechanical loading of bone results in various osteogenic stimuli to bone cells, but frequency, amplitude, and intervals between loading bouts can play critical roles in the extent of new bone formation. To begin to understand the genomic regulatory network that is altered after mechanical loading, we have begun an investigation of the gene expression profiles after various times of loading using the mouse ulna loading model. The right ulna was loaded for 1 bout of 60 cycles (2 Hz; peak force of 2.4 N). The left ulna served as an internal control. We developed a method to efficiently extract total RNA, with a yield of 10-15 ug, by combining extract from the diaphyseal region of 5 ulnaes. From 500 ng of total RNA, a full length cDNA population was generated with RACE type primers using a temperature resistant reverse transcriptase at 55°C. From the cDNA population, we determined an optimal PCR strategy to amplify the cDNA to the 1 to 3 ug range using only 19 cycles. From this amplified cDNA, P^{33} probes were prepared for microarray analysis. We have analyzed the gene expression profile at 1 and 2 hrs after a single loading bout using 5K oligonucleotide plastic arrays and for analysis, *Atlas Image Software, Excel, Access,* and the bioinformatics software, *Biomine*. We have been able to analyze approximately 600 genes that are at least 1.5 times above background. For example in the first data set, the osteocyte selective genes, Dentin Matrix Protein1 (Dmp1) and osteoblast/osteocyte factor 45, also known as MEPE are highly expressed. Dmp1 increases approximately 2 fold at 1 hr after loading. CD44 is decreased while several ribosomal protein genes are increased in a coordinate manner. Two annexin genes are upregulated. Most interestingly the cdc42 gene is increased 3 fold, suggesting that after loading the osteocytes and/or periosteal cells are initiating a program for generating cellular extensions such as filapodia or dendritic processes. Cdc42 is also known to increase β-catenin activity. Initial analysis indicates that several muscle-related genes are activated on loading, possibly due to the small muscle contamination in the RNA preparations. Further analysis of these genes and other time points should allow us to begin a systematic search for genes that may be downstream of mechanical stimuli in bone.

Disclosures: ***S.E. Harris****, None.*

SA167

See Friday Plenary number F167

SA168

Mechanotransduction via Glutamate Receptors: Regulation of Osteoclast Activity in Response to Mechanical Stimulation. K. M. Black[1], B. L. Theriault*[2], G. I. Anderson[2]. [1]Pharmacology, Dalhousie University, Halifax, NS, Canada, [2]Pharmacology, Surgery, Biomedical Engineering, Dalhousie University, Halifax, NS, Canada.

The actions of glutamate, an excitatory neurotransmitter involved in pathways of learning and memory, are mediated by a variety of glutamate receptors (gluRs). GluRs expressed in osteoblasts and osteoclasts of the long bones of rat, rabbit, and mouse have are sensitive to mechanical stimulation, the driving force for maintenance of normal bone density. We postulate that gluRs mediate mechanical stimulation to increase bone density in a manner analogous to their role in mediating CNS synaptic plasticity. Marrow-derived murine osteoclasts express glutamate receptors belonging to the NMDA, AMPA, and metabotropic subgroups. Immunocytochemical data suggest that mechanical stimulation decreases the expression of gluRs. In our cultures, gluR antagonists MK801 and NBQX inhibit osteoclast differentiation and to some extent function, implying that these receptors are functional. We wished to further characterize the role and extent of gluR mechanosensitivity in our cultured osteoclasts. Bone marrow cells from femora and tibiae of 7-week old female CD1 mice were seeded onto collagen-I coated silastic membranes, and grown for 6-7 days with mechanical stimulation on days 4, 5, and 6 (1 Hz, 900 cycles). Mature osteoclasts were partially purified from harvested cells using anti-RANK coated immunomagnetic beads and total RNA (day 6) or total protein (day 7) was isolated. Cells were cultured at the same density on glass coverslips for 7 days, immunostained for gluRs and actin or vinculin, and examined using confocal microscopy. Using RT-PCR and Western blots, we confirmed our preliminary immunocytochemical results showing that the expression of osteoclast gluRs is sensitive to mechanical stimulation. Additionally, we were able to show, using RT-PCR analysis of TRAP and calcitonin receptor mRNA, that the bead-purified isolates were highly osteoclast-enriched when compared with unseparated isolates, demonstrating the utility of this strategy to isolate osteoclasts following growth in mixed culture. Using confocal microscopy we found that the gluRs on osteoclasts appear to colocalize with actin and vinculin, which suggests they exist near focal adhesions, further strengthening their hypothesized role as mechanotransducers. Studies are ongoing to elucidate the exact mechanism by which gluRs convert mechanical stimulation into intracellular signals to regulate osteoclast function, and ultimately how they contribute to the maintenance of bone density.

Disclosures: ***G.I. Anderson****, None.*

SA169

See Friday Plenary number F169

SA170

Hypergravity Stimulates the Extracellular Matrix/Integrin-Signaling Axis and Proliferation in Primary Osteoblasts. M. Parra*[1], W. Vercoutere*[1], C. Roden*[1], I. Banerjee*[1], W. Krauser*[2], E. Holton[1], N. Searby*[1], R. Globus[1], E. Almeida*[1]. [1]NASA Ames Research Center, Moffett Field, CA, USA, [2]NASA Astrobiology Institute, Moffett Field, CA, USA.

We set out to determine the molecular mechanisms involved in the proliferative response of primary rat osteoblasts to mechanical stimulation using cell culture centrifugation as a model for hypergravity. We hypothesized that this proliferative response is mediated by specific integrin/Extracellular Matrix (ECM) interactions. To investigate this question we developed a cell culture centrifuge and an automated system that performs cell fixation during hypergravity loading. We generated expression vectors for various focal adhesion and cytoskeletal proteins fused to GFP or dsRed and visualized these structures in transfected (or infected) osteoblasts. The actin cytoskeleton was also visualized using rhodamine-phalloidin staining and Focal Adhesion Kinase (FAK) levels were assessed biochemically. We observed that a 24 hour exposure to 50-g stimulated proliferation compared to the 1-g control when cells were plated on fibronectin, collagen Type I, and collagen Type IV, but not on uncoated tissue culture plastic surfaces. This proliferative response was greatest for osteoblasts grown on fibronectin (2-fold increase over 1-g control) and collagen Type I (1.4 fold increase over 1-g control), suggesting that specific matrices and integrins are involved in the signaling pathways required for proliferation. Exposing osteoblasts grown on different matrices to 10-g or 25-g showed that effects on proliferation depended on both matrix type and loading level. We found that osteoblasts exposed to a short pulse of hypergravity during adhesion spread further and had more GFP-FAK containing focal adhesions compared to their 1-g controls. While overall levels of FAK did not change, more FAK was in the active (phosphorylated) form under hypergravity than in the 1-g controls. Cytoskeletal F-actin organization into filaments was also more prominent after brief exposures to hypergravity during the first five minutes of adhesion. These results suggest that specific integrins sense hypergravity and activate distinct matrix-dependent FAK signaling pathways that can enhance proliferation. Our results also imply that brief exposures to hypergravity accelerate cell adhesion and spreading processes via the focal adhesion-signaling axis. These results support the role of the ECM/integrin-signaling axis in osteoblast response to hypergravity loading.

Disclosures: ***M. Parra****, None.*

SA171

Alterations in Gene Expression as Induced by the Presence/Absence of Mechanical Loads. N. Zhong*[1], M. Squire[1], L. Donahue[2], C. Rubin[1], S. Judex[1], M. Hadjiargyrou[1]. [1]Biomedical Engineering, SUNY, Stony Brook, NY, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA.

Maintenance of normal bone density requires the coordinated activity of osteoblasts and osteoclasts, influenced by functional loading bearing. Attenuation of these loads, which occurs under conditions such as microgravity, can lead to osteopenia, while increases in load, through stimuli such as vibration, can be strongly anabolic. While the bone tissue and cell response to changes in function are well characterized, little is known of the transcriptional activity which regulates these responses. Previously we have shown that 10 minutes per day of whole body vibration at 45 Hz (0.3g) increased trabecular bone formation rates in the tibia of BALB/cByJ by 32%, while hind limb suspension (disuse) decreased bone formation rates by 55%. Here, quantitative real-time RT-PCR was used to explore the underlying molecular basis for these rapid changes in bone cell activity. Sixteen-week old female BALB mice were randomly assigned to control, disuse, and mechanical stimulation groups (n=6 each). Mechanically stimulated mice were placed on a vibrating platform (0.25g, 45 Hz) for 10 min/d, 5 d/wk. After 4 and 21 days, RNA was extracted from the entire left tibiae (including bone marrow and cartilage). For each time point, RNA samples were pooled (2 groups of n=3) and processed for real-time RT-PCR analysis (n=2). All results were normalized to GAPDH. The 15 candidate genes selected for this study were categorized into 3 groups: 1) Bone formation related: BMP-2, collagen type I, osteopontin, osteonectin, cbfa-1, osterix, IGF-1, Nos-2; 2) Bone resorption related: RANKL, cathepsin K, MMP-2, 9, 13; 3) Miscellaneous: Integrin β3 and a novel gene. Following four days of disuse, the largest transcriptional changes were observed with collagen type I (-55%), BMP-2 (-26%), osteonectin (- 44%), osteopontin (+ 28%), MMP-2 (- 35%), and osterix (- 36%). Large changes in gene expression were also observed at 21 days of disuse, especially for Osterix (- 24%) and the novel gene (- 48%). In contrast, 4 days of mechanical stimulation resulted in relatively small increases (<18%) with several genes, while at 21 days of mechanical stimulation - the largest changes in transcriptional activity were seen with MMP-2 (+ 54%), MMP-13 (+ 35%), RANKL (+23%), collagen type I (+20%), BMP-2 (+18%), and Nos-2 (+ 41%). Based on these data, we conclude that a complex, and temporally sensitive, series of transcriptional changes precedes both resorptive and formative adaptations to mechanical challenges. The significance of these molecular changes in defining the anabolic and/or catabolic response remains to be determined.

Disclosures: ***M. Hadjiargyrou****, None.*

SA172

Fresh Femoral Neck Fractures Treated by Osteosynthesis and Fibular Autograft. S. Singh*. Orthopaedics, B.P.K.I.H.S., Dharan, Nepal.

Introduction: Femoral neck fractures in younger adults have a poor prognosis because of high incidence of non-union and aseptic necrosis. Prosthetic replacement of the femoral head is reserved for the older patients. Osteosynthesis is the treatment of choice for displaced fracture neck femur in younger adults. Various types of bone graft supplementation have been advocated to reduce the incidence of nonunion and avascular necrosis. We tried cannulated cancellous screw fixation supplemented by primary fibular autografting in femoral neck fractures to overcome the problems of nonunion and avascular necrosis.
Materials and methods: Thirty-five (M:18, F:17) skeletally mature patients (mean age 49.38 years) of fresh femoral neck fracture were treated by closed reduction and one/two cancellous screw fixation along with ipsilateral fibular autograft between January1999 to September 2002. Weight bearing was allowed only after 3 months, or later if the radiological signs of union was not seen.
Results: Twenty-five patients who had completed a follow-up of two and a half years (mean-32.7 months) were analysed. Twenty-three of the twenty-five patients showed union. In two patients the fracture did not unite. Only two cases showed asymptomatic avascular necrosis. Fibular graft was broken in three cases, screw back out with collapse was seen in two cases. Post-operative radiographs showed penetration of the articular surface by screw and graft in one cases each .
Conclusion: Cancellous screw fixation and primary fibular autografting is a safe, effective and reliable surgical technique for treating fresh femoral neck fractures in younger patients.
Key words: Fractures; Femur neck; Fibular autograft; Cancellous screw

Disclosures: ***S. Singh****, None.*

SA173

See Friday Plenary number F173

SA174

Total and Lean Body Mass Determine Appendicular BMD and BMC in Ad Libitum-Fed and Diet Restricted SENCAR, C57, and DBA Mice. E. J. B. Murray[1], S. S. Murray*[1], M. E. L. Duarte[2]. [1]GRECC/Medicine, VAGLAHS/University of California, Los Angeles, Sepulveda, CA, USA, [2]Histology and Embryology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Dietary restriction (DR) prolongs the lifespan by up to 50% and retards the development of age-related diseases. The effects of DR on bone range from deleterious to beneficial, depending upon the animal model tested, the age at which DR is instituted, and the composition of the diet. In all cases, DR is associated with low body mass, one of the major risk factors for low bone mass and density. The purpose of this research was to determine the effects of aging and DR on axial and appendicular BMD and BMC in three strains of mice that differ in body mass, free radical generation capacity and down-stream oxidative damage (SENCAR > C57 > DBA). Male mice of each strain were killed at 2.5 months of age or randomized to an *ad libitum*-fed or a 30% diet restricted feeding group for up to 1 year. DR mice were fed a diet normalized vs. the *ad libitum*-fed diet with respect to Ca, P, vitamins, and micronutrients. Body composition, BMD, and BMC were determined by high-resolution small animal densitometry with a PixiMUS. The relationships between total body, femoral, humeral, mandibular, and vertebral BMC or BMD and body mass (total, lean, or fat) were determined using the least squares linear regression method with fitting to the polynomial equation $f = y_0 + ax$, where f is the BMD (g/cm^2) or BMD (g) and x is the mass indicated (grams). The major result of the study is the observation that a single regression line describes the relationships between total and lean body mass and total body, femoral, and humeral BMD and BMC in all strains. R values ranged from 0.879 (lean body mass x femoral BMC; $p < 0.0001$) and 0.757 (total body mass x femoral BMC; $p < 0.0001$) to 0.419 (total body mass x total body BMD; $p = 0.0011$). In contrast, fat mass was less significantly correlated with bone mass, with R values ranging from 0.418 (fat mass x vertebral BMD, $p = 0.0011$) to 0.321 (fat mass x femoral BMD, $p = 0.0139$). Thus, our results confirm previous studies indicating that the low BMDs and BMCs observed in DR animals can be accounted for based on body mass and demonstrate that the mechanostat appears to be "set" at a similar level for all strains within a single species. Furthermore, the correlation between fat mass and BMD at a nonweight-bearing site (vertebrae) supports a significant role for adipose cell-derived factors, such as leptin, in regulating bone mass in these three strains of mice.

Disclosures: ***E.J.B. Murray****, None.*

SA175

See Friday Plenary number F175

SA176

Skeletal Responses to Altered Loading of Growing Transgenic Mice that Express a Function-Perturbing Form of Beta-1 Integrin in Osteoblasts. R. K. Globus[1], D. Amblard[1], Y. Nishimura*[1], J. Litzenberger*[1], M. L. Corcoran*[1], E. A. C. Almeida*[1], C. E. Wade*[1], E. Morey-Holton[1], C. D. Damsky*[2], J. B. Kim*[2], T. J. Wronski[3], U. T. Iwaniec*[3], M. C. H. van der Meulen[4]. [1]NASA Ames Research Center, Moffett Field, CA, USA, [2]University of CA., San Francisco, CA, USA, [3]University of Florida, Gainesville, FL, USA, [4]Cornell University, Ithaca, NY, USA.

Cell culture studies demonstrate that integrins mediate critical cell functions, including mechanotransduction, although their roles in responding to mechanical forces in bone are not known. To address this, we analyzed skeletal responses to altered weightbearing in transgenic mice (TG) engineered to express the transmembrane domain and cytoplasmic tail of beta-1 integrin driven by the osteocalcin promoter. When expressed in cultured MLOY4 osteocytes, this fragment was dominant negative for adhesion and migration. To manipulate loading, TG and wildtype mice (WT) were hindlimb unloaded by tail suspension for 1wk (63d old) or 4wk (35d old) with normally–loaded, age-matched controls (CT).

To evaluate recovery from 4wk hindlimb unloading, mice were released to ambulate normally (reloaded). Bones were analyzed for dry mass, ash mass, mineral fraction (ash/dry), and tibial curvature. Comparison of 90d old CT groups revealed TG had a lower body mass (-9.8%), lower tibial ash mass (-14.4%) and lower tibial curvature (-10.1%) compared to WT. In WT, hindlimb unloading for 1wk reduced body mass (-9.0%) and raised serum corticosterone (+121%) compared to CT. Hindlimb unloading for 1wk also caused a significant decrement in mineral fraction (-2.7%) of unloaded tibiae relative to CT, but did not affect loaded humeri. In contrast, hindlimb unloading for 4wk caused lower body mass, bone mass and mineral fraction of both tibiae and humeri compared to CT. TG showed similar, but lesser, effects of hindlimb unloading than WT. Reloading of WT and TG resulted in a partial recovery from the adverse effects of hindlimb unloading. In addition, reloaded mice of both genotypes had lower tibial curvature than CT. We propose skeletal changes due to hindlimb unloading resulted from both reduced mechanical loading and systemic stress. The observed differences due to genotype support the hypothesis that expression of a function-perturbing form of beta-1 integrin in mature osteoblasts reduced sensitivity to mechanical loading.

Disclosures: ***R.K. Globus****, None.*

SA177

See Friday Plenary number F177

SA178

Influence of Physical Activity on Quantitative Ultrasound Variables from 5 Devices: the OPUS-Study. F. E. Thomasius[1], A. Stewart*[2], D. Felsenberg*[1], D. M. Reed*[2], R. Eastell*[3], C. Roux*[4], C. C. Glüer*[5]. [1]Freie Universität, Berlin, Germany, [2]University of Aberdeen, Aberdeen, United Kingdom, [3]University of Sheffield, Sheffield, United Kingdom, [4]René Descartes University, Paris, France, [5]University Hospital Schleswig-Holstein, Kiel, Germany.

Immobility is an important risk factor for bone mineral density loss. We examined the influence of physical activity on quantitative ultrasound (QUS) results in the Osteoporosis & Ultrasound Study (OPUS) which is a European population based study of 2374 postmenopausal women aged 55 to 79 years. Each woman answered a questionnaire including questions on physical activity: 1. hours of walking or bicycling per day (WB) (groups analysed: none (WB-) or > 1 hour (WB+)), 2. physical activity (PA) since leaving school (groups analysed: none (PA-) or > 2 hrs per week (PA+)), 3. Number of months having been confined to bed. QUS scans of the heel (Lunar Achilles+ (Ach), UBIS 5000 (UBIS), OSI/Osteometer DTU-one (DTU), Quidel/Metra QUS-2 (QUS-2)) and of the finger (Igea DBM Sonic Bone Profiler (IGEA)) were performed. Broadband ultrasound attenuation (BUA), speed of sound (SOS), stiffness index (STIFF IND), amplitude dependent speed of sound finger (AD-SoS), ultrasound profile index finger (UBPI), and bone transmission time finger (BTT) were measured. Weak correlations between physical activity and QUS results were shown (correlation coefficients $r < 0.1$, $p<0.05$ except for AD-SoS, BTT and UBPI for number of months confined to bed). Comparison of the mean values of QUS results (groups indicated above) showed significantly higher QUS results for the groups with the higher physical activity.

Table 1: Mean QUS results +/- standard deviation for PA and WB, n= number of cases

QUS results	PA+ (n)	PA- (n)	WB+ (n)	WB- (n)
Ach BUA in dB/MHz	109 +/- 10 * (397)	106 +/- 9 (645)	108 +/- 10 ((968)	105 +/- 11 (50)
DTU BUA in dB/MHz	47 +/- 8 * (454)	46 +/- 8 (925)	47 +/- 8 * (1199)	44 +/- 9 (51)
UBIS BUA in dB/MHz	62 +/- 14 * (430)	59 +/- 14 (823)	61 +/- 14 * (1097)	56 +/- 17 (50)
QUS2 BUA in dB/MHz	78 +/- 17 * (331)	75 +/- 17 (532)	77 +/- 17 * (819)	71 +/- 18 (40)
Ach SOS in m/s	1534 +/- 29 * (397)	1525 +/- 28 (645)	1532 +/- 29 * (968)	1522 +/- 31 (50)
DTU SOS in m/s	1547 +/- 11 * (454)	1544 +/- 10 (925)	1546 +/- 11 * (1199)	1542 +/- 12 (51)
UBIS SOS in m/s	1503 +/- 31* (430)	1498 +/- 29 (823)	1501 +/- 30 * (1097)	1486 +/- 30 (50)
IGEA AD-SoS in m/s	1896 +/- 130* (452)	1877 +/- 130 (918)	1890+/- 130 * (1188)	1855 +/- 113 (51)
IGEA BTT in s	1,37 +/- 0,26 (450)	1,35 +/- 0,25 (912)	1,37 +/- 0,25 (1178)	1,35 +/- 0,26 (51)
IGEA UBPI	0,35 +/- 0,20 * (450)	0, 32 +/- 0,19 (912)	0,33 +/- 0,19 * (1178)	0,28 +/- 0,18 (51)
Ach STIFF IND in %	82 +/-14 * (397)	78 +/- 13 (645)	81 +/- 13 * (968)	76 +/- 16 (50)

* p< 0.05 PA+ vs. PA- and WB + vs. WB-

In conclusion physical activity in the past and present significantly influences QUS parameters in this European population.

Disclosures: ***F.E. Thomasius****, None.*

SA179

See Friday Plenary number F179

SA180

Skeletal Response to Mechanical Loading and Subsequent Deconditioning in Young and Adult Rats. I. Pajamaki*[1], H. Sievanen*[2], T. Vuohelainen*[1], J. Tuukkanen[3], M. Jarvinen*[1], P. Kannus*[1], T. L. N. Järvinen[1]. [1]Department of Surgery, University of Tampere, Tampere, Finland, [2]UKK Institute, Tampere, Finland, [3]University of Oulu, Oulu, Finland.

Fifty young (5-19-week-old) and 50 adult (33-47-week-old) male Sprague-Dawley rats were used to test whether aging modulates the skeletal responsiveness to loading either *quantitatively* or *qualitatively*, and to assess whether the possible mechanical loading-induced changes are better preserved in the young than adult skeleton.

At entry, the rats were randomly assigned into eight groups: Four control groups and four exercise (+deconditioning) groups. Rats in the exercise groups were subjected to 14-week period of progressive running program at the age of 5- or 33- weeks, after which half of the exercised rats (both young and adult) were sacrificed while the remaining rats were allowed to move freely in their cages for a subsequent deconditioning period of 14 weeks. The control rats were kept free in their cages for the entire study period and sacrificed with their respective exercise group (14, 28, 42 and 56 weeks after the entry). At each time point, peripheral quantitative computed tomography and mechanical testing of the femoral neck was performed and total cross-sectional area (tCSA), total bone mineral content (tBMC), total bone mineral density (vBMD), and the breaking load of the femoral neck (Fmax) were determined.

The results are summarized in the Table. No difference was observed in the skeletal responses between the young and adult rats.Similarly, the ability to maintain exercise-induced bone gain did not differ between the age-groups.

In conclusion, this study showed that there was no difference in the skeletal responsiveness to loading between young and adult rats. However, the young skeletons seem to adapt through geometrical changes (increase in bone size) whereas adult skeletons showed increase in bone density. Age did not seem to modulate the ability of the skeleton to maintain the exercise-induced bone benefits, since no difference was observed in the loss of bone in the young and adult rats after cessation of the exercise.

The response (EX vs. CTR, %) of young and adult rats to mechanical loading and deconditioning

	tCSA	tBMC	vBMD	Fmax
Young, exercised	+25***	+28***	+11*	+30**
Adult, exercised	+10	+18***	+23***	+28**
Young, deconditioned	+17*	+18*	+2	+11
Adult, deconditioned	+10	+13*	+2	+6

*p<0.05, **p<0.01, ***p<0.001

Disclosures: ***I. Pajamaki****, None.*

SA181

See Friday Plenary number F181

SA182

Acute Effects of Moderate Intensity Resistance Exercise on Bone Cell Activity. T. J. Whipple*[1], B. Le*[1], L. Demers*[2], M. A. Petit[3], N. Sharkey*[4], N. I. Williams*[4]. [1]Dept. of Orthopaedics, Penn State University, University Park, PA, USA, [2]College of Medicine, Penn State University, Hershey, PA, USA, [3]Dept. of Health Evaluation Sciences, Penn State University, Hershey, PA, USA, [4]Dept. of Kinesiology, Penn State University, University Park, PA, USA.

Resistance exercise is considered to have positive long-term effects on bone mass, but little is known about the mechanisms by which this occurs. The purpose of this study was to determine whether the performance of a single bout of moderate intensity resistance exercise alters biochemical markers of bone cell activity. Indices of bone turnover were measured in nine healthy, untrained young men, before and following a single session of resistance exercise and during a control trial. Blood samples were collected immediately before, immediately after, and at 1, 8, 24, and 48 hours post exercise and analyzed for bone-specific alkaline phosphatase (BAP), type I collagen propeptide (PICP), and type I collagen N-telopeptide (SNTX). Urine from the second morning void was collected over four days (day before, day of, and two days following exercise) and analyzed for type I collagen N-telopeptide (UNTX). **Results:** Exercise resulted in a significant increase ($P < 0.05$) in the ratio of biochemical markers of bone formation to bone resorption eight hours post exercise. **Conclusion:** A single session of moderate intensity resistance exercise performed by untrained young men appears to have stimulated a transient perturbation in biochemical markers of bone cell activity favoring bone formation in the absence of antecedent bone resorption.

Disclosures: ***T.J. Whipple****, None.*

SA183

Protein Kinase A Coordinates Ion Channel Activities to Enhance the Intracellular Calcium Response to PTH or Fluid Shear in Osteoblasts. R. L. Duncan, J. Zhang*, J. A. Bethel*, N. A. Ajubi. Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, USA.

PTH increases the $[Ca^{2+}]_i$ response of osteoblasts to shear that can be mimicked by activation of PKA with forskolin or 8 br-cAMP. While we have previously reported that PTH and cAMP produce increased activation of mechanosensitive channels (MSCC) we postulate that PKA interacts with other ion channels to further enhance the $[Ca^{2+}]_i$ response. We used both patch clamp and $[Ca^{2+}]_i$ imaging of MC3T3-E1 or UMR106.01 osteoblasts to examine the effects of PKA on channel activity and $[Ca^{2+}]_i$. Fluid flow (0.3 ml/min) or PTH treatment (50 nM) increased the activity of a 5-6 pS channel from NP_o values of 0.12 $\pm$ 0.04 to 1.63 $\pm$ 0.41 ($p<0.05$). Addition of 8 br-cAMP (100 μM) or forskolin (10 μM) produced a similar increase. This channel conductance is the same as the increase in single channel conductance we had previously reported for the MSCC in PTH-stimulated UMR106.01 cells. These data suggest that PTH-induced PKA activation is activating this smaller channel, rather than introducing a subconductance state of the MSCC channel. Whole cell patch studies of L-type voltage-sensitive Ca^{2+} channels (VSCC) in the presence of forskolin demonstrated a significant increase in peak currents (263 $\pm$ 37 nA to 407 $\pm$ 59 nA; $p<0.05$) with a slight, but not significant reduction in the depolarization required for activation. We also found that PKA activation was responsible for changes in channel kinetics of an outwardly rectifying K^+ channel. Addition of forskolin or 8 br-cAMP reduced whole cell K^+ currents by 40% within 10 min of treatment. To determine how PKA-induced reduction of the outwardly rectifying K^+ current would influence the $[Ca^{2+}]_i$ response to mechanical stimulation, we hypotonically challenged UMR106.01 cells in the presence of the K^+ channel inhibitors triethylammonium chloride (TEA; 1 mM) or 4-aminopyridine (4-AP; 1mM) and measured the resultant $[Ca^{2+}]_i$ response. K^+ channel inhibition increased the baseline $[Ca^{2+}]_i$, but reduced the peak $[Ca^{2+}]_i$ response to hypotonic challenge. While the hypotonic-induced increase in $[Ca^{2+}]_i$ returned to baseline after 10 min in control cells, inhibition of the outwardly rectifying K^+ channel prolonged the $[Ca^{2+}]_i$ response such that $[Ca^{2+}]_i$ did not return to basal levels after 25 min. These data would suggest that shear activates PKA to increase the activity of a small conductance channel which enhances the shear-induced depolarization. PKA also increases Ca^{2+} entry by altering L-type VSCC kinetics. The Ca^{2+} response is further enhanced by reduced K^+ currents to prevent rapid return of the membrane potential to resting levels.

Disclosures: ***R.L. Duncan****, None.*

SA184

Study of the Effects Produced by 17-Beta-Estradiol and Raloxifene on Fas-Mediated Apoptosis in Primary Human Osteoblast in Culture. C. Garcia-Moreno*[1], M. P. Catalan*[2], A. Ortiz*[2], L. Alvarez*[3], C. de la Piedra[1]. [1]Bone Pathophysiology Lab, Fundacion Jimenez Diaz, Madrid, Spain, [2]Nephrology Department, Fundacion Jimenez Diaz, Madrid, Spain, [3]Orthopaedics Department, Fundacion Jimenez Diaz, Madrid, Spain.

It is known that estrogen deficiency produces an increase in the production of the proinflammatory cytokine TNF-alfa in bone. TNF-alfa stimulates bone resorption and is implicated in bone loss after menopause. TNF-alfa can induce apoptosis in cells of the osteoblast lineage and this mechanism could regulate osteoblast cell number and bone loss. We investigated the effects produced by 17-beta estradiol (E) and raloxifene (R) on Fas-mediated apoptosis in cultured human primary osteoblast-like cells (HOB) primed with TNF-alfa.

Fas-mediated apoptosis induced by agonistic anti-Fas IgM (CH-11) was determined by flow cytometry of permeabilized, propidium iodide stained cells. HOB Fas mRNA levels were measured by semiquantitative rt-PCR, using Fas primers that amplify mRNA from Fas bound to membrane (with death activity). HOB cell membrane Fas receptor expression was analyzed by flow cytometry. HOB were obtained from postmenopausal women (n=3) without bone disease undergoing orthopedic surgery. HOB were cultured in DMEM (Dulbecco´s Modified Eagle Medium) without FBS and pretreated with TNF-alfa (200U/ml) and 10-8M E or R during 48h. Then, we examined Fas-mediated apoptosis induced by CH-11 (1microg/ml) during 24h.

TNF-alfa did not modify spontaneous HOB apoptosis. The combination of CH-11 plus TNF-alfa increased HOB apoptosis 679+/-316% with respect to untreated cells. The addition of 10-8M E or R did not produce any significant effect in the level of apoptosis induced by CH-11 and TNF-alfa. Fas mRNA expression significantly increased in cells pretreated with TNF-alfa at 6, 24, 30 y 48 hours versus untreated cells. Expression of Fas receptor increased significantly (152+/-23%) in cells pretreated with TNF-alfa with respect to HOB (100%). Neither E nor R modulated TNF-alfa induced Fas expression at the mRNA or protein level.

In conclusion, treatment of HOB with TNF-alfa could increase the Fas-mediated apoptosis. Increased Fas mRNA and receptor expression may account for this effect. These events are not affected by E or R, an observation that suggests that these compounds did not impact Fas-mediated apoptosis.

Disclosures: ***C. de la Piedra****, Spanish Institute of Health (FIS) 2; Lilly 2.*

SA185

See Friday Plenary number F185

SA186

Decreased Osteoblast Apoptosis and Increased Bone Formation in Implants of Marrow-Derived Osteoblast Progenitors Overexpressing Bcl-2: *In Vivo* Evidence for a Pivotal Role of Apoptosis in Bone Formation. I. Gubrij, A. A. Ali, T. M. Chambers*, S. B. Berryhill*, X. Liu*, P. Roberson*, C. A. O'Brien, R. S. Weinstein, 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 Medical Sciences, Little Rock, AR, USA.

Increased or decreased osteoblast apoptosis is associated with corresponding changes in osteoblast number caused by glucocorticoid excess, or intermittent administration of PTH, or activation of non-genotropic estrogen-like signaling. Here, we tested directly whether programmed cell death is an important determinant of the rate of bone formation by overexpressing the anti-apoptotic protein Bcl-2 in murine bone marrow-derived osteoblast progenitors, and measuring the amount of bone they form following implantation into mice. Marrow progenitors were transduced with a murine replication-deficient retrovirus expressing human Bcl-2 (hBcl-2), and neomycin phosphotransferase to permit selection of transduced cells. The transduced cells exhibited uniform immunocytochemical staining for hBcl-2, and expression of hBcl-2 was 20-fold higher than the endogenous protein, as measured by Western blotting. The overexpressed hBcl-2 protein was evidently functional as the transduced progenitors were protected from etoposide-induced apoptosis, determined by in situ end labeling (ISEL). The basal level of apoptosis was also decreased from 5% to 2%, whereas proliferation measured by BrdU labeling was unaffected. Bcl-2-transduced progenitors exhibited increased osteoblastogenic capacity following 25 days of culture as evidenced by a 5-fold increase in osteocalcin secretion and calcified bone nodule formation, as compared to progenitors transduced with green fluorescent protein (GFP). To establish the impact of Bcl-2 overexpression on bone formation *in vivo*, hBcl-2-transduced progenitors or GFP controls were embedded in collagen and implanted subcutaneously into immunodeficient mice. Serial undecalcified sections from bone spicules that formed after 4 or 8 weeks were analyzed for the prevalence of osteoblast apoptosis, and bone architecture was measured by image analysis after Von Kossa staining. At both time points, osteoblast apoptosis in spicules from hBcl-2-transduced progenitors was attenuated by 2-fold. Moreover, bone made by Bcl-2-transduced cells exhibited a significant ($P<0.05$) increase in trabecular thickness at 4 weeks (GFP: 16.6 ± 1.7 μm vs. Bcl-2: 20.0 ± 1.8 μm) and at 8 weeks (GFP: 26.1 ± 1.5 μm vs. Bcl-2: 29.7 ± 1.5 μm). We conclude that programmed cell death of osteoblasts, osteoblast progenitors, or both is a major determinant of bone formation.

Disclosures: ***R.L. Jilka****, Anabonix, Inc. 4.*

SA187

Raloxifene Protects Osteoblasts from Apoptosis Induced by Sodium Nitroprusside: Potential Involvement of Ceramide. S. M. P. Olivier*[1], M. Fillet*[2], M. G. Malaise*[1], J. Piette*[2], V. Bours*[2], M. Merville*[2], N. Franchimont[1]. [1]Rheumatology, University of Liège, Liège, Belgium, [2]Center for Cellular and Molecular Therapy, University of Liège, Liège, Belgium.

Raloxifene is a selective estrogen receptor modulator (SERM) possessing estrogen-like agonist effects on bone but its exact cellular mode of action remains unclear. We tested the possibility that raloxifene modifies MC3T3-E1 osteoblastic cell viability induced by two NO-donors, sodium nitroprusside (SNP) and S-nitroso-N-acetyl-penicillamine (SNAP). SNP (1-4mM) and SNAP (0.3-0.5mM) dose-dependently decreased MC3T3-E1 cell viability. Cell viability was maintained when MC3T3-E1 cells were pretreated with raloxifene but not with 17B-estradiol or the bisphosphonate pamidronate. Annexin-V labeling demonstrated that cell death mediated by SNP resulted from apoptosis and raloxifene protected these cells from apoptosis. SNP induced an increase in intracellular ceramide (C22 - C24) that was inhibited by raloxifene. In contrast, in the presence of SNAP, raloxifene had no significant effect on MC3T3-E1 cell viability. However, SNAP induced cell death by late apoptosis or necrosis, enhanced NO production with a different kinetic than SNP and did not modify intracellular ceramide levels. SNP treatment was associated with an increased AP-1 activity that was partially inhibited by both raloxifene and 17B-estradiol indicating that AP-1 modulation was not associated with raloxifene anti-apoptotic effect. Our data demonstrate that raloxifene prevents osteoblast apoptosis induced by SNP, underlining a positive effect of raloxifene on bone quality.

Disclosures: ***N. Franchimont****, Eli Lilly and Co 5.*

SA188

Ghrelin Stimulates Proliferation and Inhibits Apoptosis in MC3T3-E1 Cells. S. W. Kim*, J. A. Kim*, S. J. Heo*, M. J. Jeon*, D. H. Kim*, S. Y. Kim*, C. S. Shin. Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.

Ghrelin is a 28-amino acid peptide that has recently been discovered in human and rat stomach. Ghrelin strongly stimulates the release of growth hormone and is a natural ligand of the growth hormone secretagogue receptor (GHSR), which belongs to a seven transmembrane receptor family. Previous studies have shown that GHSRs are expressed mainly in the brain and pituitary, but are also detected in a variety of tissues including myocardium, adrenal, and gonads. This study was undertaken to investigate the expression and role of ghrelin and GHSR in osteoblasts using mouse calvarial osteoblast cell line MC3T3-E1. We have identified the expression of both ghrelin and GHSR by RT-PCR analysis of MC3T3-E1 cells. Treatment of these cells with ghrelin from 10^{-11} to 10^{-8} M showed dose-dependent stimulation of proliferation as assessed by MTT assay. [^{3}H]-Thymidine uptake was also increased by 41 % after treatment with 10^{-12} M of ghrelin. Moreover, when apop-

tosis was evaluated using fluorescence microscopy and flow cytometry after cell staining with DAPI or Hoechst 33342, ghrelin treatment suppressed serum deprivation- and TNFalpha-induced apoptosis in this cell line.
We examined the mitogen-activated protein kinase (MAPK) pathway as a possible downstream signaling of ghrelin in the regulation of proliferation and apoptosis. Ghrelin (10^{-9}M) elicited a rapid phosphorylation of Erk1/2 (p42/p44 MAPK) in MC3T3-E1 cells, which was abolished by treatment with MEK inhibitor, PD98059 and U0126. Activation of MAPK pathway by ghrelin was abolished by treatment with PKC inhibitor, staurosporin, suggesting that PKC-MEK cascade is used for ghrelin signaling via its receptor in osteoblasts. Ghrelin treatment has no effect on the differentiation of osteoblasts as assessed by alkaline phosphatase activity and expression of osteocalcin. Taken together, ghrelin stimulates cell growth as well as inhibits apoptosis without apparent effect on differentiation. We suggest ghrelin as a direct mitogen and survival factor of osteoblast.

Disclosures: ***C.S. Shin****, None.*

SA189

See Friday Plenary number F189

SA190

Dexamethasone Induces Caspase Activation in Murine Osteoblastic MC3T3-E1 Cells. C. Chua*, B. Chua*, Z. Chen*, C. Landy*, R. C. Hamdy. Osteoporosis Center, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA.

Glucocorticoids are widely used as anti-inflammatory and chemotherapeutic agents. However, prolonged use of glucocorticoids leads to osteoporosis. This study was designed to examine the mechanism of dexamethasone (DEX)-induced apoptosis in murine osteoblastic MC3T3-E1 cells. Total RNA was extracted from MC3T3-E1 cells treated with 10^{-7} M DEX for 6 h. DEX exerted a variety of effects on apoptotic gene expression in osteoblasts. Ribonuclease protection assays revealed that DEX upregulated mRNA levels of caspases-1, -3, -6, -8, -11, -12, and bcl-X_L. Western blot analysis showed enhanced processing of these caspases, with the appearance of their activated enzymes 8 h after DEX treatment. In addition, DEX also induced the activation of caspase-9. DEX elevated the levels of cleaved poly(ADP-ribose) polymerase and lamin A, a caspase-3 and a caspase-6 substrate, respectively. Expression of bcl-X_L protein level was upregulated by DEX. Cytochrome c release was detected in the cytosol of DEX-treated cells. Furthermore, caspase-3 enzyme activity was elevated by 2-fold after DEX treatment for 7 h. Finally, early apoptotic cells were detected in cells treated with DEX for 3 h. Our results demonstrate that DEX-induced apoptosis involves gene activation of a number of caspases and bcl-X_L.

Disclosures: ***C. Chua****, None.*

SA191

Defect of Bone Formation/Remodeling in CDK4 Null Mice. M. C. Rico, J. L. Castaneda*, S. N. Popoff, F. F. Safadi. Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA.

For normal skeletal growth and development the relationship between cell proliferation and differentiation must be tightly regulated. Osteoblasts are committed to enter the cell cycle and proliferate under specific regulatory signals, then differentiate and produce a mineralized matrix. Various cyclins, cyclin dependent kinases (CDKs) and cyclin dependent kinase inhibitors (CDKIs) have been postulated to play a role in osteoblast proliferation and differentiation. Among them is CDK4, a cell cycle protein that plays a major role in the transition of cells from the G_0 phase to the S phase. Previous studies showed that CDK4 null (CDK4-/-) mice develop type I diabetes associated with stunted growth. These animals also exhibited cancellous osteopenia. Western blot analysis was used to confirm the lack of CDK4 protein in bone from CDK4-/- when compared with wild type (WT) animals. In this study, we performed histomorphometric analyses of distal femoral metaphysis, which demonstrated a significant decrease in bone volume and osteoid surface in CDK4-/- compared with WT bones. Densitometry by peripheral quantitative computerized tomography (pQCT) showed a reduction in trabecular bone density in CDK4-/- compared to WT mice. We also examined the expression of osteoblast-related genes, such as alkaline phosphatase, osteocalcin, osteopontin, and type I collagen in bone from CDK4-/- and WT animals. The expression of all these genes were markedly decreased in CDK4-/- compared with WT mice. We also evaluated the expression of Cyclin D, p27 and p21. Cyclin D is a protein involved in the formation of the cyclin D/CDK4 complex, p27 is an inhibitor of cyclin D/CDK4 complex formation and p21 is an inhibitor of the Cyclin D/CDK4/6 complex formation. Western blot analyses demonstrated a decrease of cyclin D, p27 and p21 expression in CDK4-/- compared to WT mice. Cell proliferation was measured using Bromo-deoxyuridine (BrdU) *in vivo*. Cell proliferation was slightly decreased in cells in the periosteum of CDK4-/- compared to WT mice. Expression of ED1 in macrophages/osteoclasts revealed a significant increase in the size and number of the osteoclasts present at the periosteum of CDK4-/- compared with WT mice, suggesting an increase in bone resorption. Our data suggest that CDK4 plays an important role in bone formation/remodeling. Additional studies are being pursued to understand the precise role of CDK4 and its mechanism of action in osteoblasts/osteoclasts.

Disclosures: ***M.C. Rico****, None.*

SA192

See Friday Plenary number F192

SA193

Osteopontin Plays a Role in Regulating Apoptosis and Mineralization under the Control of Inorganic Phosphate. Y. Koyama*[1], K. Kitahara[1], S. R. Ritting[2], K. Tsuji[1], T. Yano*[3], Y. Taketani*[3], A. Nifuji[1], D. T. Denhardt[2], M. Noda[1]. [1]Dpt. of Molecular Phamacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Dept.of Nutritional Sciences, Rutgers University, Piscataway, NJ, USA, [3]University of Tokyo, Tokyo, Japan.

Inorganic phosphate(Pi) is a major component of bone extracellular matrix. Pi has a role in bone remodeling. High levels of Pi induced apoptosis of osteoblasts, and Pi is required for mineralization. Osteopontin is a phosphorylated glycoprotein secreted to the mineralizing extracellular matrix by osteoblasts during bone development. It is reported that OPN may enhance cell survival by inhibiting apoptosis and may have relation with mineralization. Osteopontin RNA and protein in osteoblast are induced in response to elevated extracellular phosphate levels. We investigated whether osteopontin had relation to regulate Pi induced osteoblast apoptosis and mineralization. First, cell survival was estimated by MTT assay. Calvaria out growth cells of wild type (WT) mice and osteopontin knock out (OPN-KO) mice was cultured in the presence of phosphate from 1mM to 10mM. After four days, cell survival was estimated by MTT assay. Cell survival of both WT and OPN-KO was inhibited at high concentration of Pi, but cell survival of OPN-KO was significantly inhibited in moderate concentration of Pi compared to WT. In TUNEL analysis, TUNEL-positive cells were seen after treatment with 3-10 mM/L Pi. As the Pi concentration was raised, there was a profound increase in TUNEL-postive cells. Cell death was identified to cause to apoptosis. Secondly, we investigated mineralization. Calvaria out growth cells of WT and OPN-KO were cultured in the presence of phosphate from 1mM to 10mM. After twelve days, the cells were treated with 1-10 mM Pi. After 48 hours, matrix mineralization was assessed by Alizarin Red staining. High levels of Pi induced in vitro mineralization, but mineralization in OPN-KO cell culture was significantly inhibited in moderate concentration of Pi in compared to WT. This time, cell survival was estimated by MTT. Cell survival was almost not chaged in 1-10 mM Pi. Present study suggests that OPN plays a negative role in Pi induced osteoblast apoptosis, while it promotes mineralization. At the sites of bone remodeling, osteoblasts may be exposed to high concentration of Pi. When Pi concentration was raised, OPN expression may be induced in osteoblasts and OPN could enhance cell survival by inhibiting apoptosis and induce mineralization. Thus, osteopontin plays a role in regulating apoptosis and mineralization under the control of inorganic phosphate.

Disclosures: ***Y. Koyama****, None.*

SA194

Bone-specific Runx2 Levels Are Tightly Regulated during the Cell Cycle to Support a Novel Function in Control of the Switch from Proliferation to Quiescence in Osteoblasts. M. Galindo*, J. L. Stein*, J. B. Lian, G. S. Stein, A. J. van Wijnen. Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, MA, USA.

The Runx2 (CBFA1/AML3/PEBP2αA) transcription factor promotes skeletal cell differentiation by activating bone phenotypic genes in post-proliferative osteoblasts. However, Runx2 is also present in actively dividing osteoprogenitor cells, and recent genetic evidence from our group indicates that the factor performs a dual regulatory role in control of osteoblast growth and differentiation. We addressed here whether this novel cell growth regulatory function of Runx2 is functionally linked to the cell cycle related mechanisms that drive normal osteoblast proliferation.
We show by western and northern blot analyses that serum deprivation of MC3T3 osteoblastic cells, which mediates cell cycle exit into G_0/G_1, upregulates Runx2 protein and mRNA levels. In contrast, Runx2 levels are downregulated within 4 to 8 hr after quiescent cells are induced to proliferate by the reintroduction of serum, and further suppressed prior to entry into S phase. Hence, Runx2 expression is acutely responsive to cessation and stimulation of cell growth. Inhibitor experiments with agents that cause cell cycle arrest in S phase (e.g., hydroxyurea, aphidicolin), G1 phase (e.g., mimosine) or mitosis (e.g., nocodazole) reveal that Runx2 destabilized at the G1/S phase transition. Destabilization of Runx2 is prevented in the presence of the proteasome inhibitor MG132 which is consistent with control of Runx2 levels by specific degradation via the ubiquitin-proteosome pathway prior to S phase. Thus, Runx2 is tightly regulated during the cell cycle. Anti-sense mediated reduction of the low physiological levels of Runx2 in proliferating MC3T3 cells does not accelerate cell cycle progression, but elevation of Runx2 levels by forced expression significantly decreases the rate of cell proliferation. These data establish that Runx2 activity is functionally coupled to osteoblast proliferation and may support exit from the cell cycle to induce a post-proliferative quiescent stage during normal osteoblast differentiation.

Disclosures: ***M. Galindo****, None.*

SA195

Osteoblast Apoptosis and Survival Is Regulated by Integrin-mediated Surface Attachment. V. Grigoriou*[1], C. S. Adams[2], I. M. Shapiro[2], E. A. Cavalcanti-Adam*[3]. [1]Department of Orthodontics, University of Pennsylvania, School of Dental Medicine, Philadelphia, PA, USA, [2]Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA, USA, [3]Institut für Physikalische Chemie, Universität Heidelberg, Heidelberg, Germany.

Although it is recognized that most osteoblasts end their life history by apoptosis, the mechanism by which bone cell apoptosis is regulated remains elusive. Herein we test the hypothesis that cellular attachment chemistry regulates the sensitivity of osteoblasts to the

activity of apoptogens. We use a novel method of grafting RGD molecules to a culture surface to upregulate integrin binding in osteoblasts. Integrin and integrin signaling component expression levels were evaluated in MC3T3E1 osteoblast-like cells cultured on RGD-treated membranes in comparison to RGE-treated membranes for 0.5h, 3h, 24h and 72h. The RGD-modified surface exhibited upregulation of $\alpha_v\beta_3$ integrin and of the β_3 subunit. There was a marked increase in activation of Akt in cells bound to RGD peptides, after 3 days in culture. While there was no change in protein levels of both the proapoptotic protein Bad nor the anti apoptotic protein Bcl-2, there was an increase in pBad, the inactive form of Bad. Apoptotic sensitivity was assessed by MTT assay. Incubation of cells on the RGD-treated membrane completely inhibited apoptosis induced by 0.1 and 0.5 μM staurosporine; 3 mM Pi and 2.4 mM and 2.9 mM Ca^{2+}; and 0.5 mM and 0.1 mM SNP. However, surface modification has no effect on RGD peptide or serum-free induced apoptosis. Furthermore surface modification significantly increased the sensitivity of cells to etoposide. When PI3 kinase was inhibited by LY294002 the surface-stimulated resistance to apoptosis was abrogated. The data presented in this investigation clearly indicate that tethered RGD peptides suppress apoptosis and upregulate osteoblast survival via integrin-mediated attachment.

Disclosures: ***E.A. Cavalcanti-Adam****, None.*

SA196

Inhibition of MEK-ERK-Elk Signaling by Androgens may Influence Osteoblast Apoptosis. K. Wiren, A. Toombs*, X. W. Zhang. Portland VA Medical Center, OHSU, Portland, OR, USA.

While non-aromatizable androgens have significant beneficial effects on skeletal homeostasis independent of conversion to estradiol, the effects of androgens on bone cell metabolism are still poorly understood. The aim of this study is to elucidate the effects of the non-aromatizable androgen 5α-dihydrotestosterone (DHT) on osteoblast viability. We used an osteoblastic model with enhanced androgen responsiveness: MC3T3-E1 cells stably transfected with androgen receptor (AR) under the control of the rat 3.6-kb α1(I)-collagen promoter fragment (colAR-MC3T3). Previously we had demonstrated DHT inhibition of the MEK-ERK-Elk phosphorylation cascade using mitogenic pathway-specific cDNA microarrays, confirmed by Western and reporter analyses. Since the MAPK signaling can modulate both proliferation and apoptosis, we first determined the effect of DHT on osteoblast viability. ColAR-MC3T3 cultures were treated with DHT and the effects on osteoblast viability were measured by MTT assay. A complex response was observed in that continuous short-term DHT treatment (10–^{8}M DHT; 2d) enhanced osteoblast viability ($P<0.05$), but inhibition was observed with longer-term DHT treatment (3 and 5d; $P<0.01$). The inhibition by DHT was abrogated in the presence of the specific androgen receptor antagonist hydroxyflutamide, and was also observed in primary cultures of normal rat calvarial osteoblasts assessed with ^{3}H-thymidine incorporation. A downstream target of Elk-1 is c-*fos*, a component of AP-1. AP-1/luciferase reporter activity also demonstrated complex DHT modulation, with short-term treatment (6 h) activating but extended treatment (24, 48h) inhibiting activity. Apoptosis was assessed after induction by etoposide treatment (50μM; 18h) by measuring either accumulation of cytoplasmic mono- and oligonucelosomes or caspase-3 activity using DEVD cleavage. Continuous treatment with DHT (10–^{8}M DHT; 5 days) enhanced apoptosis over two-fold ($P<0.001$), but as expected similar treatment with 17ß-estradiol inhibited apoptosis. Interestingly, by day 20, continuous treatment with DHT had no significant effect on apoptosis. In conclusion, we have demonstrated that non-aromatizable androgens influence bone cell viability through an effect on MAP kinase signaling. This data further discriminates the effects of androgens (enhances apoptosis) and estrogens (inhibits apoptosis) in proliferating osteoblasts. These observations may help generate hypotheses for investigating the mechanisms of sexual dimorphism in skeletal development and aging.

Disclosures: ***K. Wiren****, None.*

SA197

Cloning and Identification of Novel CREM Isoforms Expressed by Osteoblasts. F. Liu*, Y. Huang, B. E. Kream. Medicine, University of Connecticut Health Center, Farmington, CT, USA.

CREM, the cAMP response element modulator, is a member of the CREB/CREM/ATF family of transcription factors. The CREM gene encodes both antagonists and activators of cAMP-dependent transcription by alternative splicing and the differential use of 4 promoters (P1-P4). The expression of CREM isoforms transcribed from the P1 promoter is cell, tissue and developmental stage specific. We previously showed that osteoblasts express ICER, a dominant negative transcriptional attenuator that is cAMP inducible and transcribed from the P2 promoter of CREM gene. Moreover, preliminary data from our laboratory showed that male CREM knockout mice might have a blunted anabolic response to PTH. Therefore, the goal of the present study was to identify the repertoire of CREM transcripts expressed in osteoblasts. Using RT-PCR and primers that should amplify all P1 promoter initiated full-length transcripts, we cloned and identified 14 CREM isoforms expressed in osteoblastic MC3T3-E1 cells. Interestingly, of the 14 isoforms, 6 were previously unidentified CREM isoforms: CREMτα/γ, CREM-x, CREMτ2α, CREM τ1α, CREM τ1α/γ and CREM τ1γ. CREMτα/γ, and CREM τ1γ are alternative splice variants of CREMτα and CREM τ1, respectively, which contain the γ domain. CREMτ2α is a splice variant of CREM τ2α/γ that excludes the γ domain. CREM τ1α has the similar structure to CREM τ1 except that it contains both of the 2 alternative DNA binding domains of CREM. CREMτ1α/γ is a splice variant of CREM τ1α without the γ domain. Interestingly, CREM-x contains the θ1 exon, which was previously identified only in P3 promoter initiated transcripts, and a previously unidentified exon, termed the L exon, located 100 nucleotides upstream of the θ1 exon and 16.9 k nucleotides downstream of the B exon. Using RT-PCR, we also showed that the expression of CREM P1 promoter initiated transcripts was similar among MC3T3- E1 cells, 6-8 day old mouse calvariae, 2 month old mouse calvariae, primary calvarial osteoblasts and bone marrow stromal cells that had undergone osteogenic differentiation. In conclusion, at least 14 P1 promoter initiated isoforms of the CREM gene were expressed in several murine osteoblast models. Of these, 6 were novel transcripts while 5 resulted from alternative splicing or usage of the γ domain and/or alterative usage of the first DNA binding domain. One new transcript, CREM-x, contains a novel L exon. We speculate that CREM transcription factors may play a regulatory role in PTH-dependent signal transduction in osteoblasts.

Disclosures: ***F. Liu****, None.*

SA198

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SA199

See Friday Plenary number F199

SA200

Fibroblast Growth Factor 2 Directly Inhibits Expression of Osterix mRNA in Osteoblastic Cells. E. Abugunde*, K. Lee, T. Naganawa*, L. Xiao, M. Hurley. Medicine, University of Connecticut Health Center, Farmington, CT, USA.

Osterix is a recently identified novel zinc finger-transcription factor that is essential for osteoblast differentiation and specifically expressed in all developing intramembranous and endochondral bones. Osterix acts downstream of the transcription factor RunX2/Cbfa1 that was previously shown to be required for osteoblast differentiation and bone formation. Currently, there are limited data of regulation of Osterix by growth factors, which influence osteoblast function. Previous studies have shown that continuous treatment with basic fibroblast growth factor (FGF-2) inhibited osteoblast differentiation and bone nodule formation in vitro. We examined whether FGF-2 regulates the expression of Osterix in mouse bone marrow stromal cell cultures. For time course studies, marrow stromal cells harvested from female mice were plated at 10 x106 cells/ well in 6 well dishes in aMEM and 10% FCS and grown to confluence. Cultures were serum deprived and treated with FGF-2 for 30 min to 2 h. Osterix mRNA was determined by RT-PCR analysis using specific primers that were designed according to published sequence. Treatment with FGF-2 (10 nM) decreased the level of Osterix mRNA expression by 60% within 30 min and by 72% at 1 h, and this reduction was maintained at 2 h. Dose response studies showed that treatment with as little as FGF-2 (0.001nM) for 1 h caused maximal reduction in Osterix mRNA. To assess whether new protein synthesis was required for the effect, cultures were pretreated with the protein synthesis inhibitor cycloheximide (CHX 5 μg/ml) prior to addition of FGF-2. CHX did not abrogate the inhibitory effect of FGF-2 on Osterix expression. Since new protein synthesis was not required and a decrease in Osterix mRNA expression was observed within 30 min of FGF-2 treatment, these results suggest that FGF-2 regulated Osterix mRNA at the level of gene transcription. We speculate that inhibition of Osterix expression may play a role in the inhibitory effects of FGF-2 in osteoblast differentiation.

Disclosures: ***E. Abugunde****, None.*

SA201

See Friday Plenary number F201

SA202

See Friday Plenary number F202

SA203

Transcriptional Profiling of Estrogen- and PTH-Regulated Genes During Bone Formation in OVX Mice. D. von Stechow[1], T. Libermann*[2], G. Hattersley[3], J. M. Alexander[4]. [1]Orthopedic Biomechanics Laboratory, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA, [2]Genomics Center, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA, [3]Millennium Pharmaceuticals, Inc., Cambridge, MA, USA, [4]Division of Bone and Mineral Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA.

The aim of this study was to identify the transcriptional mechanisms in bone underlying the anabolic effects of supraphysiologic estradiol (E2) treatment versus intermittent parathyroid hormone[1-34] (PTH) therapy. We utilized an ovariectomized (OVX) mouse model of osteoporosis and transcriptional profiling to identify genes upregulated by either E2 or PTH. Swiss-Webster mice underwent either OVX or Sham operation. Five weeks post-OVX, the mice were administered either E2 and/or PTH, or vehicle for 4 weeks. Femoral bones were analyzed by microCT to confirm the anabolic effect of each treatment. OVX vehicle-treated control mice lost metaphyseal trabecular bone, with significant decrease in trabecular number, thickness, and connectivity. Both E2 and PTH treatments increased trabecular and cortical bone indices above the level of the sham operated controls, fully restoring both bone volume and bone mineral density. Moreover, PTH/E2 combination treatment led to significantly greater increase in cancellous bone and BMD than would be expected from adding together their separate effects. To determine whether PTH and E2 treatments were stimulating similar bone anabolic mechanisms, or were activating

distinct signaling pathways, we compared patterns of gene expression using transcriptional profiling after either E2 or PTH treatment. After 4, 11, and 24 days of treatment, total RNA was collected from both the distal femoral metaphysis and diaphysis. Transcriptional profiling was performed using Affymetrix GeneChip probe arrays, comprised of approximately 36,000 full-length mouse genes and EST clusters from the UniGene database. Several markers of osteoblast activity, including c-fos, RANKL, PHEX, and PTHR1, were consistently upregulated by PTH in both skeletal sites. PTH treatment also increased expression of Cathespin K, confirming the predicted increase in osteoclastic activity. E2 treatment upregulated a largely distinct set of genes, including TGFbeta-3, and BMP1, as well as several genes critical for cell cycle control, including Cyclin D1 and CDK inhibitor 1A. Overall, comparison of transcriptional profiles suggest that anabolic responses in bone to PTH and E2 treatment after OVX-induced osteoporosis involve largely distinct patterns of gene regulation that each result in restoration of bone mass.

Disclosures: ***J.M. Alexander****, Millennium Pharmaceuticals, Inc 2; Merck 2.*

SA204

See Friday Plenary number F204

SA205

See Friday Plenary number F205

SA206

Requirement of the SWI/SNF Chromatin Remodeling Complex to Initiate BMP-2 Induced Skeletal Gene Expression for Osteoblast Differentiation. D. W. Young[1], A. N. Imbalzano*[2], A. J. van Wijnen[1], M. A. Montecino[3], G. S. Stein[1], J. B. Lian[1], J. L. Stein[1]. [1]Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, MA, USA, [2]Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA, [3]Departmento de Biologia Molecular, Universidad de Concepcion, Concepcion, Chile.

Bone Morphogenetic Proteins (BMP) are key regulators of osteoblast differentiation and skeletal-tissue developmental. BMP signals are directed to the nucleus through Smad heterodimers that converge at gene promoters together with tissue specific transcription factors to regulate transcriptional activity. As yet, little is known regarding the mechanisms by which BMP signals are integrated at promoter regulatory regions to induce phenotypic gene activation. In particular, the role of factors that alter chromatin structure has not been addressed in the context of BMP signaling. SWI/SNF chromatin remodeling complexes have emerged as essential machinery to facilitate transcriptional control of cellular differentiation. These multisubunit complexes have been shown to alter nucleosome organization in an ATP dependent manner. Here we addressed the role of SWI/SNF chromatin remodeling factors as effectors in the BMP/SMAD signaling cascade using the alkaline phosphatase (ALP) gene, which is an established model for BMP2 responsive gene regulation and an early marker of the osteoblast phenotype. We used an NIH3T3 derived cell line (B22) that contains a stably integrated tetracycline inducible transgene encoding a Brg1 protein with a mutation in the ATP binding domain. Brg1 is an essential ATPase subunit of the SWI/SNF complex and this mutant has a dominant negative effect on SWI/SNF complex function. We find that treatment of control B22 cells (wild-type SWI/SNF) with recombinant human BMP2 induces alkaline phosphatase activity as detected by histochemical staining and RT-PCR. In sharp contrast, when the mutant Brg1 protein is expressed, causing inhibition of SWI/SNF function, ALP activity and gene expression is not induced by BMP2. Furthermore, even after 14 days of chronic BMP2 treatment ALP activity was undetectable, indicating that this dominant negative effect does not represent a delay in activation. Together these data indicate that SWI/SNF chromatin remodeling complexes are required for induction of alkaline phosphatase gene activation, an early marker of BMP mediated osteogenesis. Our results suggest this activation reflects a requirement for SWI/SNF mediated chromatin remodeling to support the program of gene expression obligatory for osteoblast differentiation.

Disclosures: ***D.W. Young****, None.*

SA207

See Friday Plenary number F207

SA208

Differential Regulation of Gene Expression and Differentiation by Homo- and Heterodimers of the bHLH Protein Twist. Y. Leshem*, V. Andreeva*, C. Muentener*, M. Connerney*, D. Spicer. Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, ME, USA.

Saethre-Chotzen syndrome is the most common autosomal dominant disorder of craniosynostosis, or premature fusion of the cranial sutures. This syndrome is primarily associated with haploinsufficiency of the basic-Helix-Loop-Helix (bHLH) transcription factor TWIST, however the mechanism underlying suture closure due to TWIST haploinsufficiency remains unclear. Twist can form both homodimers (TT) and heterodimers with ubiquitously expressed bHLH E proteins (TE) and we and others have suggested that these dimers may mediate different activities of Twist. Here we propose that the regulation of Twist dimer formation may regulate closure of the cranial sutures and haploinsufficiency of Twist alters the balance of these dimers to promote fusion. Twist is expressed in the suture mesenchyme and osteogenic fronts while the HLH inhibitor Id is only expressed in the osteogenic fronts. Id preferentially dimerizes with E proteins, and thus we hypothesize that within the osteogenic fronts Id dimerizes with the available E proteins forcing Twist to form homodimers, while in the intervening suture mesenchyme Twist forms heterodimers with free E proteins. We have begun to test this hypothesis by creating "forced" homo and heterodimers of Twist and the E protein E12, where the members of the dimers are attached by a linker. Our data with cell lines expressing the different dimers supports this conjecture and indicates that the cellular response to factors such as FGF and BMP differs profoundly dependent on which Twist dimer is expressed. Specifically we find that TE expression causes cell to proliferate and migrate slower, while TT promotes these events. Furthermore, while both Twist dimers inhibit calcification TT promotes early differentiation by inducing Cbfa1 expression and TE inhibits this. We have also identified genes that are differentially regulated by the two dimers and their expression patterns within the sutures are consistent with this regulation and are altered in Twist +/- mice.

Disclosures: ***D. Spicer****, None.*

SA209

See Friday Plenary number F209

SA210

Lentivirus Delivered RNAi Targeting Dlx5 Inhibits Mineralization of Primary Mouse Calvarial Osteoblast Cultures. H. Li*, M. S. Kronenberg, M. Stover*, A. C. Lichtler. Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA.

Previous studies have shown that Dlx5 induces osteoblast differentiation of calvarial osteoblasts and bone marrow stromal cells. Dlx5 knockout mice have defective calvarial development, which is more strongly manifested in Dlx5 and 6 double knockout mice. This phenotype has been attributed to defects in osteoblastic differentiation. This conclusion is not definite, because Dlx5 is expressed at earlier stages of development, and defects in calvaria could be due to improper development of osteoblast precursors or adjacent cell types, rather than a failure of terminal differentiation. The ability to inhibit expression of Dlx5, and other Dlx genes, in primary osteoblast cultures would provide a powerful means for addressing this question. We have chosen to use lentivirus vectors expressing RNAi to achieve this goal. RNAi can efficiently target specific mRNAs for degradation. Pol III promoters are especially suited for delivering small RNAs with hairpin loops containing double stranded RNA regions complementary to specific mRNAs; these are converted into RNAis within the target cell. Lentivirus vectors can deliver stably integrated expression constructs, including Pol III promoters, into non-dividing cells, thus they have the potential for delivering RNAi to essentially all of the cells of a primary osteoblast culture. We obtained the LentiLox RNAi-expressing lentivirus vector from Dr. Luc van Parijis, and cloned two Dlx5 targeted sequences into the vector. This vector also constitutively expresses eGFP. We produced high titer vector preparations from one of those vectors, and transduced primary mouse calvarial osteoblasts during the proliferative phase of culture. This particular vector targets a region of the Dlx5 gene that is conserved in some other Dlx genes, which may produce greater effects on osteoblast differentiation if multiple Dlx gene have the ability to induce this process. Visual assessment of eGFP expression by fluorescence microscopy indicated that transduction efficiency was greater than 80%, which is greater than we have previously achieved with retrovirus vectors. Xylenol Orange staining of a 17 day culture demonstrated very little mineralization, while a parallel culture transduced with a control GFP expressing vector was highly mineralized. Northern blot analysis showed that Dlx5 and Dlx3 mRNA levels were strongly inhibited in the RNAi transduced culture compared to the control. These studies support the importance of Dlx5 in calvarial osteoblast differentiation, and indicate that lentivirus delivered RNAi will be a valuable tool for studying gene function during osteoblast differentiation.

Disclosures: ***H. Li****, None.*

SA211

See Friday Plenary number F211

SA212

See Friday Plenary number F212

SA213

Microarray Analysis of Subpopulations of Cells Within Differentiating Murine Osteoblast Cultures. I. Kalajzic*[1], A. Stahl*[2], W. Yang*[2], S. Wu*[2], S. Johnson*[2], J. Feyen[2], D. Rowe[1]. [1]Genetics and developmental Biology, University of Connecticut Health Center, Farmington, CT, USA, [2]Bristol-Myers Squibb, Princeton, NJ, USA.

The heterogeneity of bone cell cultures complicates the interpretation of microarray studies of osteoblast differentiation. We have utilized mice transgenic for pOBCol3.6GFP and pOBCol2.3GFP to identify preosteoblasts (3.6 low expressing at day 7), early osteoblasts (3.6 high expressing at day 17) and mature osteoblasts (2.3 positive at day 17) in mCOB cultures. Time points were FACS sorted for positive and negative 3.6 and 2.3 cells (at day 7 and 17 respectively) and negative, low positive and strong positive 3.6 cells (day 17). The sorting step increased the representation of GFP positive cells from 30->95% for

3.6 positive cells, 3->98% in 3.6 strongly expressing cells and 8.5->88% in 2.3 expressing cells while the nonfluorescent sorted populations were 99%. Total RNA was extracted from unsorted cells from sister plates of day 7 and 17 cultures. RNA extracted from each population was assessed by Affymetric microarrays using the 74 A, B and C chips. Day 7 and 17 cultures were performed in triplicate and the r^2 correlation between replicate points was greater than 0.98. This study reports the results from the 74A chip which contains 11,916 Ests and approximately 8000 nonredundant unigenes. The arbitrary dynamic range of expression exceeds 3 orders with Col1a1 representing 10^5, integrin ß1 receptor 10^4, cbfa1 10^3, and calcitonin receptor 10^2 (background) and none of these genes show significant variation during differentiation. For genes that are known markers of late osteoblastic differentiation, cell sorting does not greatly increase the sensitivity although it does distinguish which population is responsible for the increase. Genes that are strikingly elevated in late differentiation are DMP, BSP, OC, AP and a number of undefined ESTs. Dkk1, an inhibitor of the Wnt pathway and Dlx3 show a 10-20 fold increase that occurred primarily in 2.3+ cells. No genes of note were induced during the transition of 3.6- to 3.6+ cells. The striking finding was the suppression (10-20 fold) of number of genes in isolated populations as differentiation progressed that was not reflected in the total cell populations. Examples include genes that modify the extracellular matrix, regulate intracellular proteolysis and a variety of growth factor receptors and secreted growth factors. Significant down regulation of Wnt and Igf1 signaling pathways occurred during late differentiation. We anticipate the analysis will be further enriched when the data from Chip B and C are analyzed but the value of using sorted populations of cells for microarray analysis appears to be well founded.

Disclosures: ***I. Kalajzic****, None.*

SA214

Bone Marrow Stem Cells (MSC) and Growth Factors Impregnated in Biodegradable Scaffold for Bone Repair. S. Srouji*[1], M. Peled*[2], I. Blumenfeld*[3], E. Livne[1]. [1]Anatomy and Cell Biology, Technion, Haifa, Israel, [2]Maxillofacial surgery Department, Rambam, Haifa, Israel, [3]Maxillofacial Surgery Department, Rambam, Haifa, Israel.

The need for bone repair is one of the major concerns of reconstructive surgery and in aging and disease. Fracture healing is regulated by osteogenic cells and growth factors. The ability to enhance healing of bone defects and fractures can contribute to prevent the complications of long-term immobilization that are especially fatal in old age. Our aim was to test the ability of biological scaffold containing committed osteogenic stem cells and growth factors to serve as a bone graft substitute for bone repair.
Isolated bone marrow stem cells (MSC) were used for selection of osteogeprogenitor subpopulation, were cultured (1 w) in DMEM medium supplemented with 10% FCS, antibiotics, ascorbic acid, β-glycerophosphate, and dexamethasone. Selected osteogenic subpopulation was identified using Alizarin red, von Kossa staining, osteonectin immunolocalization and mineral deposition. Committed osteoprogenitor cells were cultured (1 w) on hydrogel scaffold, transplanted with growth factors (TGF-β1 10 ng/ml; IGF-I 25 ng/ml) in rat tibia segmental bone defect using an external fixation device for stabilization of the defect site and examined after 2, 4 and 6 weeks by morphology, radiology, 3-D CT and mineral deposition by electron dispersive spectroscopy (EDS). Results indicated biocompatibility of hydrogel scaffold for cultured MSC-derived osteoprogenitor as identified by their proliferation, osteogenic properties and by mineral deposition by EDS in the culture and in the scaffold. Morphology revealed that after 2 weeks calcified material was observed in defects treated with TGF-β. By that time the scaffold was partially degraded and new bone formation was present in the margins of the defect. Biodegradation of the hydrogel was shown to be mediated by matrix metalloproteinases (MMPs).
Radiological images revealed that bone was present in the former site of the defect after 6 weeks of treatment with TGF-β + IGF-I or TGF-β alone. Less pronounced bone induction was observed in IGF-I-treated bone defect, and control specimens revealed partial healing of the bone defect. 3D-CT revealed that the bone restored its shape.
It is concluded that biodegradable scaffold can serve as biocompatible matrix for MSC-derived osteoprogenitor cells and growth factors and that it also provides space for bone regeneration. The biodegradable scaffold containing committed stem cells and growth factors is thus a promising surgical tool for enhancement of bone defect reconstruction and tissue engineering, and will enhance the repair of bone fracture in aging and disease.

Disclosures: ***S. Srouji****, None.*

SA215

See Friday Plenary number F215

SA216

Mechanism of PTHrP Mediated Inhibition of KS483 Osteoblastic Differentiation. G. van der Horst, C. Lowik, M. Karperien. Endocrinology, Leiden University Medical Center, Leiden, Netherlands.

PTH(rP) has been shown earlier to inhibit osteoblastic differentiation *in vitro*. However, the mechanism by which PTH(rP) exerts these effects is not yet known. In this study we investigated the mechanism of PTH(rP) mediated inhibition of osteoblastic differentiation in mouse pre-osteoblastic KS483 cells.
First, we analyzed effects of PTH (1-34), PTH (3-34) and PTHrP (1-34) on osteoblastic differentiation of KS483 cells. PTH(rP) dose dependently inhibited alkaline phosphatase activity (ALP) measured after 72 hrs. In long term differentiation assays, PTH(rP) blocked the formation of ALP positive and mineralized nodule formation dose dependently. No differences were seen when PTH(rP) was administered either continuously or in 1hr pulses at 3 to 4 days intervals. The inhibition required an intact N-terminus of PTH(rP). Furthermore, mRNA expression of Runx2, an essential transcription factor for osteoblastic differentiation, was downregulated by PTH(rP) in KS483 cells treated with PTH(rP) for 72hrs or in long term differentiation assays. Since autocrine BMP signaling has been shown to be crucial for osteoblastic differentiation of KS483 cells, we investigated crosstalk between BMPs and PTH(rP) signaling. Both BMP-4 and BMP-6 induced ALP activity was inhibited by PTH(rP) dose-dependently. In addition, BMPs could reverse inhibition by PTH(rP). Immediate early BMP signaling was not involved since PTH(rP) had no effect on BMP induced phosphorylation of Smads 1,5 and 8. Furthermore, no effect of PTH(rP) was seen on basal or BMP-4 and –6 induced luciferase expression of the BMP reporters Msx2-luc and BRE-luc. The p38 MAPK pathway is also involved in basal and BMP induced osteoblastic differentiation. In KS483 cells, no effects of PTH(rP) were seen on phosphorylation of p38. The p38 MAPK inhibitor SB203580 blocked ALP activity, ALP positive and mineralized nodule formation as well as basal and BMP-induced luciferase expression of Msx2-luc and BRE-luc dose dependently. These data suggest that p38 MAPK is not involved in the inhibitory effect of PTH(rP).
PTHrP could also inhibit differentiation by interfering with another osteogenic pathway, the hedgehog (Hh) signaling pathway. In KS483 cells, recombinant sonic hedgehog (rSHh) induced ALP activity was inhibited by PTH(rP) dose-dependently and rSHh could reverse PTH(rP) mediated inhibition. Furthermore, PTH(rP) decreased mRNA expression of Hh in KS483 cells.
In conclusion, PTH(rP) containing an intact N-terminus, inhibits both basal as well as BMP and Hh stimulated osteoblastic differentiation of KS483 cells. This inhibition is downstream of immediate early BMP signaling and results in a decrease in Runx2 expression.

Disclosures: ***G. van der Horst****, None.*

SA217

See Friday Plenary number F217

SA218

Maintenance of Differentiation Potential of Human Bone Marrow Stromal Cells Immortalized by Human Telomerase Reverse Transcriptase Gene in Spite of Extensive Proliferation. B. M. Abdallah*[1], M. Azadali*[1], F. Jacob*[1], P. Hokland*[2], M. Kassem[1]. [1]Endocrinology, Odense university hospital, Odense, Denmark, [2]Department of Hematology, Aarhus University Hospital, Aarhus, Denmark.

Human bone marrow stromal cells (hMSC) contain a population of stem cells that are capable for differentiation into multiple lineages. However, these cells exhibit senescence-associated growth arrest and phenotypic changes during in vitro culture. We have recently demonstrated that human telomerase reverse transcriptase over-expression (hTERT) in hMSC reconstitutes telomerase activity and extend life span of the cells (Simonsen,J.L. *et al*. Nature Biotech. 20, 592-596, 2002). In the present study we have performed extensive characterization of this cell line at different population doubling levels (PDL) with respect to growth rate, cell morphology, surface markers, ability to differentiate into osteoblasts, adipocytes, chondrocytes, endothelial cells, myocytes and neuronal cells as well as the effect of growth in human serum. Three different cell populations of cells were examined hMSC-TERT2 (PDL 60), hMSC-TERT4(PDL160) and hMSC-TERT20(PDL260). In spite of the presence of differences in the growth rates between these different cell lines, all cells were able to be induced to differentiation into different lineages. In conclusion, hTERT immortalization is a useful method for obtaining enough number of cells with a stable phenotype for mechanistic studies on cell differentiation.

Disclosures: ***M. Kassem****, None.*

SA219

See Friday Plenary number F219

SA220

Potential Paracrine Loop in Bone Involving the Synthesis of ACTH in Osteoclasts and the Presence of Functional ACTH Receptors in Osteoblastic Cells. O. Zhong[1], R. Ling*[2], K. Ding[1], S. Sridhar[1], D. Xie[1], K. Insogna[3], R. Bollag*[1], C. M. Isales[4]. [1]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA, USA, [2]Medical Genomics, Medical College of Georgia, Augusta, GA, USA, [3]Medicine, Yale University School of Medicine, New Haven, CT, USA, [4]Medicine, Medical College of Georgia and the Augusta VA Hospital, Augusta, GA, USA.

Melanocortin receptors belong to the seven transmembrane domain, G-protein coupled family of receptors. Five melanocortin receptors have been described which are widely expressed in the body, including skin, brain, adrenal glands, adipocytes, gut and other peripheral tissues. These receptors are activated by fragments derived from a larger molecule, pro-opiomelanocortin (POMC) and include: ACTH, a b and g-MSH, and b endorphin. Traditional roles for these peptide hormones include regulation of cortisol secretion, skin pigmentation and pain perception. Although the pituitary gland is the traditional site for synthesis, secretion and processing of POMC-derived fragments, it has become clear that other tissues can also synthesize and secrete POMC fragments including keratinocytes, melanocytes, endothelial cells and immune cells among others. Among the latter both lymphocytes and macrophages have been reported to express and secrete POMC fragments. Since osteoclasts in bone are derived from macrophages we investigated whether POMC and the melanocortin receptors were expressed in bone and bone cells. We report that the

five known melanocortin receptors are expressed in normal rat bone as assessed by in situ hybridization. These receptors were variably expressed in different osteoblastic-like cell lines with the MG63 cell line expressing four out of five melanocortin receptors as assessed by Northern blot. 125ACTH, a and b MSH and b endorphin demonstrated high affinity binding to MG63 cells. In attempt to determine the source of ligand for these melanocortin receptors, normal rat osteoclasts were probed for the POMC gene by Northern blot. Osteoclasts did in fact express the POMC gene although at a lower level than normal rat pituitary. Thus, bone cells contain the elements for a novel paracrine hormonal loop where osteoclasts express POMC and osteoblasts express melanocortin receptors.

Disclosures: ***Q. Zhong****, None.*

SA221

Functional Properties of Cultured Normal and Vitamin D Receptor Knockout Mice Calvarial Osteoblasts. T. Okano[1], N. Tsugawa*[1], C. Hirami*[1], S. Kato[2]. [1]Department of Hygienic Sciences, Kobe Pharmaceutical University, Kobe, Japan, [2]Nuclear Information, IMCBM CREST, Tokyo, Japan.

Vitamin D is believed to be essential for bone mineralization and promotes osteogenesis by activation of osteoblast function and stimulation of osteoclast formation. There is, however, little evidence indicating that vitamin D is operative in vivo as a key factor involved in bone metabolism. Inoue et al. reported at the 24th ASBMR annual meeting that ectopically implanted femora of vitamin D receptor knockout (VDRKO) mice in wild-type mice showed a remarkable increase in bone volume compared to those of wild-type mice. Furthermore, in organ culture study, bone volume of fetal VDRKO mice calvaria was already greater than that of wild-type mice before initiation of culture and remarkably increased after 7 days culture. These findings clearly indicate that the role of vitamin D in bone mineralization should be re-evaluated. To clarify the direct effect of $1,25(OH)_2D_3$ on bone metabolism, osteoblastic cells were isolated from calvaria of normal (wild-type) and VDRKO mice by repeated collagenase digestion. Cells were cultured in α-MEM medium with 10 % fetal calf serum. Upon light microscopy, the above two types of primary osteoblastic cells appeared similar, showing rapid proliferation by 5 day, differentiation by 15 day, and mineralization by 20 day of culture. The mRNA levels of bone metabolic markers were measured by real-time RT-PCR at 5, 15, and 20 day of culture. In addition, the degree of mineralization was assessed by von Kossa staining. As the results, the bone formation markers such as alkaline phosphatase, Runx2, Mepe and Phex were strongly expressed in the osteoblastic cells from VDRKO mice compared to those from wild-type mice. On the other hand, the expression of RANKL mRNA was extremely suppressed in the whole stages of proliferation, differentiation and mineralization in the osteoblastic cells from VDRKO mice compared to those from wild-type mice. No significant changes in osteocalcin and osteopontin mRNA gene expression were noted in both wild-type and VDRKO osteoblastic cells. These results suggest that $1,25(OH)_2D_3$ might not be essential for bone mineralization and may work primarily as a negative regulator for bone mineralization by suppression of bone formation and stimulation of bone resorption.

Disclosures: ***T. Okano****, None.*

SA222

See Friday Plenary number F222

SA223

The Role of Splice-Variants of Periostin in Bone Development and Disease. J. Litvin*, A. Selim*, M. O. Montgomery*, M. C. Rico*, K. Lehmann*, F. F. Safadi. Anatomy and Cell Biology, Temple Medical School, Philadelphia, PA, USA.

A splice-variant of Periostin, henceforth referred to as Periostin-Like-Factor (PLF) was identified by using the 'READS' differential display of mRNAs isolated from mouse tissues during various stages of development. Comparison of the predicted amino acid sequence of Periostin in mouse and human tissues with Periostin-Like-Factor in mouse tissues, showed differences in two regions at the carboxy-terminus. These differences are consistent with the two proteins being isoforms resulting from an alternately spliced gene. The alterations in amino acid sequence in these regions are probably functionally significant since the rest of the amino acids are highly conserved across species. During normal mouse embryogenesis and in osteopetrotic bone the temporal and spatial patterns of expression of these isoforms were determined using in situ hybridization and immunohistochemical analyses. We found that they were expressed during embryogenesis in preosteoblast mesenchymal cells and in bone precursor cells in the peri- and endosteum in osteopetrotic bone. Levels of these isoforms were significantly up regulated in osteopetrotic bone compared to normal controls. The expression of these isoforms in preosteoblasts suggests a role in the early stages of osteoblast differentiation. In MC3T3-E1 osteoblast-like cells these proteins were highly expressed at the early stages of differentiation. Western blot analysis of proteins from bone isolated at various stages during development showed two isoforms. We evaluated the effect(s) of second-generation anti-sense PLF and Periostin oligonucleotides on several functional parameters associated with differentiation in MC3T3-E1 cells. Levels of alkaline phosphatase, osteopontin, Cbfa1 and osteocalcin and collagen type I were significantly reduced in anti-sense treated cells compared to controls. It has been shown that as an extracellular matrix product these proteins regulate osteoblast-extracellular matrix interactions. Since they are also located within cells we expect that they are involved in signal transduction via binding to integrin receptors. Collectively, data from our experiments suggest a role for PLF and Periostin in osteoblast differentiation and function.

Disclosures: ***J. Litvin****, None.*

SA224

See Friday Plenary number F224

SA225

Oligomeric RANKL Induces Bone Formation via the ERK Pathway. R. Faccio, J. Lam, K. Aya, K. Homes*, P. Zhou, J. Chappel*, S. L. Cheng, F. P. Ross, S. L. Teitelbaum. Washington University, St Louis, MO, USA.

We have explored the mechanisms underlying the osteogenic ability of RANKL administered to mice as a GST fusion protein. We find that GST-RANKL specifically enhances Col1a1 expression by primary calvarial osteoblasts (pOBs) within 12 hours of exposure, an effect requiring the ERK pathway, since the increase is abrogated following transduction with dominant negative ERK. We next analyzed two signaling molecules downstream of ERK, c-Fos and Fra-1, whose overexpression in vivo leads to osteogenic sarcomas and osteosclerosis, respectively. GST-RANKL, but neither GST nor the soluble extracellular domain of RANKL (sRANKL), induces expression of both c-Fos mRNA and protein, and Fra1 mRNA in pOBs. Downregulation of RANK (and other TNFR) signals involves internalization of the receptor-ligand complex. Our finding that GST-RANKL, but not sRANKL, is an oligomerized molecule suggests that the larger form may be more resistant to endocytosis. This hypothesis was tested by performing confocal microscopy of pOBs, treated with GST-RANKL or sRANKL. Cells were immuno-stained for RANK and co-stained with a lipophilic fluorescent dye to detect the cell membrane. While the GST-RANKL:RANK complex remains on the cell surface for at least one hour, sRANKL:RANK complexes are rapidly internalized. This result suggests that oligomerized RANKL stimulates prolonged intracellular signaling. Whereas sRANKL-induced ERK activation dissipates within 20 min in pOBs, the signal persists for 1-2 hrs when stimulated by GST-RANKL. To establish that the anabolic effect of GST-RANKL does not reflect the GST moiety, we expressed GST-TNFα, another TNF-family GST-fusion protein. We find that GST-TNFα does not impact Col1a1 or Cbfa-1 expression in circumstances in which these moieties are induced by GST-RANKL. Finally, addition of OPG to pOBs in the presence of PTH abrogates the hormone's ability to induce Col1a1 synthesis, establishing that PTH exerts its bone anabolic effect via RANKL. In summary 1) GST-RANKL is a potent inducer of collagen synthesis by pOBs; 2) the bone forming capacity of GST-RANKL reflects its oligomerized state, which leads to prolonged activation of the ERK/c-Fos/Fra-1 signaling pathway; 3) it is the RANKL and not GST component of the fusion protein which specifies its anabolic effect; and 4) PTH exerts its bone anabolic effect via enhancement of RANKL expression. Finally, the recent report that human osteoblasts, like their murine counterparts, express RANK is consistent with the therapeutical potentials of oligomerized RANKL in man. Thus, oligomeric RANKL, a model for the native integral membrane form of the protein, exerts its bone anabolic effect via prolonged activation of the ERK signaling pathway.

Disclosures: ***R. Faccio****, None.*

SA226

Regulated Expression of SFRP-1 by the GeneSwitch System. R. A. Bhat*, B. Stauffer*, H. Ponce-de-Leon*, B. S. Komm*, P. Bodine*. Women's Health Research Institute, Wyeth, Collegeville, PA, USA.

SFRP-1 is a secreted Wnt antagonist. The role of SFRP1 in bone formation has been shown earlier by the targeted disruption of SFRP-1 in mice, which led to decreased osteoblast and osteocyte apoptosis and increased trabacular bone formation. In the present work, we have used the GeneSwitch system to develop methods for the conditional expression of SFRP-1 and the isolation of conditioned media rich in functional SFRP-1 protein.
The GeneSwitch™ system is an inducible expression system that provides exceptionally low uninduced and high-induced expression levels in mammalian cells. The GeneSwitch regulatory protein binds to the Gal4-E1b promoter to activate transcription upon the addition of mifepristone. We have developed an adenovirus recombinant containing the GeneSwitch protein driven by the Gal4-tk promoter, as well as recombinants containing SFRP-1 and the luciferase reporter under the control of the Gal4- E1b promoter. Luciferase activity in A549 cells infected with the GeneSwitch and Luciferase viruses was very low in ethanol treated cells, while the level of luciferase activity increased 200-fold in cells treated with mifepristone. Conditional expression of functional SFRP-1 was demonstrated in A549, human osteoblast (HOB) and CHO cell lines by either co-infection of the cells with the Ad5 SFRP-1 and GeneSwitch viruses or by the infection of GeneSwitch-expressing cell lines with the Adeno recombinant SFRP-1 virus and subsequent treatment with mifepristone. The results are summarized as follows: (a) A 30-fold increase in SFRP-1 transcripts in mifepristone-treated cells compared to minimal or undetectable expression in ethanol-treated cells. (b) Expression of SFRP-1 seen as early as 4 hours post-treatment, with increasing expression reaching the highest level at 20 hours and plateauing thereafter. (c) Demonstration of SFRP-1 protein in conditioned media by Western blotting. (d) Inhibition of Wnt signaling in a paracrine fashion in a co-culture assay. (e) Dose-dependent inhibition of Wnt 3-mediated activation of TCF-Luciferase activity by the conditioned media.
The regulated expression of functional SFRP-1 by the GeneSwitch system and the isolation of SFRP-1 rich conditioned media provide valuable tools for studying SFRP-1 function in osteoblasts and the genes controlled by its expression.

Disclosures: ***R.A. Bhat****, None.*

SA227

See Friday Plenary number F227

SA228

Identification of the Signalling Pathways Required for Interleukin-1 Beta Stimulation of Osteoprotegerin Synthesis in Osteoblastic Cells. C. J. Lambert*[1], C. Ribbens[1], V. Bours*[2], M. Merville*[2], M. G. Malaise*[1], N. Franchimont[1]. [1]Rheumatology, University of Liège, Liège, Belgium, [2]Center of cellular and molecular therapy, University of Liège, Liège, Belgium.

OPG (osteoprotegerin) binds to its natural ligand RANK-L (receptor activator of NF-kB - ligand) and prevents RANK-L from activating its receptor RANK, which is required for osteoclast differentiation, activation and survival. OPG production is finely regulated by osteoresorptive factors. IL-1b (interleukin-1beta), a proinflammatory cytokine, stimulates bone resorption, enhances RANK-L and surpringly OPG expression in osteoblastic cells. The meaning of OPG stimulation by IL-1b is unclear and the mechanisms of OPG gene regulation by proinflammatory cytokines have not been studied extensively. In this study, we confirmed that IL-1b stimulates OPG mRNA expression in MG63 osteoblastic cells, we demonstrated that IL-1b also induces a marked increase in OPG protein levels measured by ELISA and we studied the IL-1b signal transduction pathways required for OPG synthesis. We examined the MAPK (mitogen activated protein kinase) signalling pathway by Western blot analysis and showed that MG63 cells treatment with IL-1b resulted in the phosphorylation of the kinase p38 and ATF-2 (activating transcription factor-2). Phosphorylations of MEK-1 (MAPK extracellular signal-related kinase) and ERK (extracellular signal-related kinase) were also demonstrated but no phosphorylation of JNK (c-Jun N-terminal kinase) was reported after IL-1b treatment. To determine the relevance of the MAPK in OPG stimulation, we studied the effects of SB 203580, PD 98059 and SP 600125, specific inhibitors of the p38, ERK and JNK pathways respectively, on OPG protein levels. We found that ERK signalling pathway is necessary for IL-1b-induced OPG production whereas the p38 signalling pathway is required for both basal and IL-1b-induced OPG production. As expected, JNK inhibition did not decrease OPG protein levels. We next examined the relevance of the nuclear factor NF-kB by using BAY 11-7085 and MG132, two NF-kB inhibitors. In the presence of these inhibitors, OPG protein levels were decreased in IL-1b-treated cultures at concentrations that inhibited NF-kB DNA-binding. In the presence of IL-1b, a concomitant decrease in NF-kB inhibitor IkB-alpha levels was demonstrated by Western blotting. Infecting MG63 cells with an adenovirus expressing a dominant negative IkB-alpha resulted in a marked decrease in OPG protein levels, confirming NF-kB requirement for OPG synthesis.
In conclusion, IL-1b stimulates OPG production by mechanisms dependent on the activation of the kinases p38 and ERK as well as of NF-kB.

Disclosures: ***C.J. Lambert****, None.*

SA229

See Friday Plenary number F229

SA230

Impact of the MAPK pathway on PTHrP Actions in Osteoblasts. C. Chen*[1], A. J. Koh*[2], J. Zhang*[2], E. T. Keller*[3], N. J. D'Silva*[2], L. K. McCauley[1]. [1]Periodontics/Prevention/Geriatrics, University of Michigan, Ann Arbor, MI, USA, [2]University of Michigan, Ann Arbor, MI, USA, [3]Unit for Laboratory Animal Medicin, University of Michigan, Ann Arbor, MI, USA.

Parathyroid hormone-related protein (PTHrP) regulates proliferation and differentiation of osteoblastic cells via binding to the PTH/PTHrP receptor (PTH1R). The PKA pathway governs a majority of the PTHrP effects, but recent evidence also implicates the mitogen-activated protein kinase (MAPK) pathway. The goal of this study was to evaluate the role of MAPK in PTHrP-mediated gene expression and regulation of apoptosis. A specific ERK1/2 phosphorylation inhibitor U0126 was used to block MAPK in differentiated MC3T3-E1 cells. Northern blot analyses indicated that gene expression for osteocalcin (OCN), bone sialoprotein (BSP), and the PTH1R were downregulated by PTHrP, but were not affected by U0126. Gene expression for c-fos, fra-2 and interleukin-6 (IL-6) were all induced by PTHrP. Pre-incubation with U0126 slightly and significantly blocked the upregulation of fra-2 and IL-6 by PTHrP respectively, suggesting that the MAPK pathway is at least in part responsible for these PTHrP actions. Inhibitors of PKA (H-89), PKC (GF109203X), and MAPK (U0126) were used to evaluate PTHrP regulation of IL-6 luciferase promoter constructs. The PKA and MAPK, but not the PKC pathways were found to be responsible for activating IL-6 transcription. Cells transfected with CREB133 (a dominant negative mutant of CREB) or Rap1GAP (an inhibitor of Rap1 activation) demonstrated lower PTHrP-induced IL-6 luciferase activity, but treatment with a cAMP analogue that activates Epac and not PKA did not alter the PTHrP response. Using deletion constructs of the IL-6 promoter, further analysis suggested that both an AP-1 site and a NFkB transcription site were necessary for PTHrP-induced IL-6 gene expression. We have previously shown that PTHrP induces apoptosis in differentiated MC3T3-E1 cells. The cell viability assay indicated that U0126 partially blocked the acute reduction in cell number by PTHrP, whereas western blot analysis indicated that U0126 did not alter the PTHrP-mediated inhibition of AKT (an inhibitor of apoptosis in the phosphorylated state). These data indicate that inactivation of the MAPK pathway by U0126 shows differential regulation of PTHrP-stimulated AP-1 members (affecting fra-2 but not c-fos), and notably reduces PTHrP-stimulated IL-6 transcription. Furthermore, the small GTPase Rap1 and the transcription factors NFkB and CREB also play important roles in the downstream actions of a subset of genes that are regulated by PTHrP.

Disclosures: ***C. Chen****, None.*

SA231

See Friday Plenary number F231

SA232

Activation of Phospholipase A_2 Is Required for P2X7-Mediated Membrane Blebbing in Murine Osteoblasts. N. Panupinthu*[1], H. Z. Ke[2], S. M. Sims*[1], S. J. Dixon[1]. [1]CIHR Group in Skeletal Development and Remodeling, The University of Western Ontario, London, ON, Canada, [2]Global Research and Development, Groton Laboratories, Pfizer Inc., Groton, CT, USA.

Osteoblast activity is tightly controlled by local and systemic factors. In response to inflammatory and mechanical stimuli, nucleotides are released into the extracellular fluid where they can signal through P2 nucleotide receptors on the surface of many cell types, including osteoblasts. There are two classes of P2 nucleotide receptors, P2X (ligand-gated ion channels) and P2Y (G protein-coupled receptors). P2X7 receptor knockout mice exhibit decreased periosteal bone formation. Using molecular and functional approaches, we have identified the expression of P2X7 nucleotide receptors on a subpopulation of murine osteoblasts. The purpose of this study was to investigate the response of osteoblasts to activation of P2X7 receptors. Osteoblast-enriched cultures were obtained by sequential collagenase digestion of calvariae from neonatal wild-type (WT) and P2X7 receptor knockout (KO) mice. In other cell types, activation of P2X7 receptors leads to cytoskeletal rearrangements. Time-lapse video microscopy was used to monitor morphological changes of osteoblasts bathed in divalent cation-free buffer at 37°C. Benzoylbenzoyl-ATP (BzATP, a relatively potent P2X7 agonist) induced dynamic membrane blebbing in a subpopulation of cells from WT, but not KO mice. Blebbing began within 5 min of addition of BzATP and was sustained for at least 30 min in the continued presence of agonist. The effects of BzATP were reversible upon washout, indicating cell viability. In contrast to the effects of BzATP, blebbing was not induced by UTP, which activates P2Y but not P2X7 receptors on osteoblasts. BzATP-induced blebbing was suppressed by the nonspecific phospholipase A_2 (PLA_2) inhibitor, arachidonyl trifluoromethyl ketone ($AACOCF_3$, 100 μM). Blebbing was observed in 16 ± 1% of cells pretreated with $AACOCF_3$ vs. 46 ± 2% of cells pretreated with an inactive analog ($P < 0.05$). Moreover, bromoenol lactone (BEL, 10 μM), a specific inhibitor of Ca^{2+}-independent PLA_2, reduced the percentage of blebbing cells from 43 ± 2% to 11 ± 4% of total ($P < 0.05$). Chelation of extracellular Ca^{2+} using EGTA, or cytosolic Ca^{2+} using BAPTA had little effect on the percentage of cells exhibiting blebbing, consistent with involvement of Ca^{2+}-independent PLA_2. In conclusion, P2X7 receptor activation induces dynamic membrane blebbing in a subpopulation of osteoblasts, a process dependent on PLA_2. Activation of PLA_2 and induction of cytoskeletal changes may contribute to the stimulatory effect of P2X7 receptors on periosteal bone formation.

Disclosures: ***N. Panupinthu****, None.*

SA233

Extracellular Inorganic Phosphate Regulates Glvr-2 Phosphate Transporter/Retrovirus Receptor mRNA Expression in Rat Bone Marrow Stromal Cells. K. Wada*[1], M. Mizuno*[1], T. Komori*[2], M. Tamura[1]. [1]Oral Health Science, Hokkaido University Graduate School of Dental Medicine, Sapporo, Japan, [2]1Division of Oral and Maxillofacial Surgery, Department of Organ Therapeutics, Kobe University Graduate School of Medicine, Kobe, Japan.

In mammalian cells, several observations indicate not only that phosphate transport probably regulates local inorganic phosphate (Pi) concentration, but also that Pi affects normal cellular metabolism, which in turn regulates apoptosis and the process of mineralization. To elucidate how extracellular Pi regulates cellular functions of pre-osteoblastic cells, we investigated the expression of type III sodium-dependent Pi transporters in rat bone marrow stromal cells and ROB-C26 pre-osteoblastic cells. The mRNA expression level of Glvr-2 was increased by the addition of Pi in rat bone marrow stromal cells, but not in ROB-C26 cells or in normal rat kidney (NRK) cells. In contrast, the level of Glvr-1 mRNA was not altered by the addition of extracellular Pi in these cells. The induction of Glvr-2 mRNA by Pi was inhibited in the presence of cycloheximide. Moreover, mitogen-activated protein kinase (MEK) /extracellular-signal-regulated kinase (ERK) pathway inhibitors; U0126 and PD98059 inhibited inducible Glvr-2 mRNA expression, but p38 mitogen-activated protein kinase inhibitor SB203580 did not inhibit the induction of Glvr-2 mRNA expression, suggesting that extracellular Pi regulates *de novo* protein synthesis and MEK/ERK activity in rat bone marrow stromal cells and through these, induction of Glvr-2 mRNA. Although Pi also induced osteopontin mRNA expression in rat bone marrow stromal cells but not in ROB-C26 cells and NRK cells, changes in cell viability with the addition of Pi were similar in both cell types. These data indicate that extracellular Pi regulates Glvr-2 mRNA expression, provide insights into possible mechanisms whereby Pi may regulate protein phosphorylation, and suggest a potential role for the Pi transporter in rat bone marrow stromal cells.

Disclosures: ***K. Wada****, None.*

SA234

Isolation and Characterization of a Bone Marrow Stromal Side Population (SP). S. Uchida, S. Zhang, J. E. Aubin. Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada.

Cells characterized as mesenchymal stem cells (MSCs) reside within bone marrow stroma, however, significant enrichment and rapid isolation of these cells remain elusive. Recently, flow cytometric analysis of freshly isolated hematopoietic and muscle-derived cells has uncovered that efficient Hoechst 33342 dye exclusion via the ABC transporter family defines a side population (SP) enriched in multipotent stem cells leading to the hypothesis that adult stem cells may also reside in SPs of other tissues. We asked whether a SP exists in young adult rat bone marrow stromal cell populations and, if so, is enriched in osteoprogenitors and other precursor populations. RT-PCR analysis of precultured stromal cells revealed the expression of multiple members of the ABC transporter family, including multidrug resistance-associated protein 1 (Mdr1) and breast cancer resistance protein 1 (Bcrp1). Freshly isolated total bone marrow or the stromal fraction precultured for 7 days were therefore stained with Hoechst 33342, analyzed and sorted on a FACStarPlus (Becton Dickinson; BD Bioscience). Cells in the SP, non-SP and total/unfractionated population were re-plated in 96-well plates under osteogenic differentiation conditions. 0.05-0.2% of total marrow or 0.2-0.5% of stromal cells formed a distinct SP. The stromal SP cells gave rise to colony forming units (CFU) of different phenotypes. The percentage of wells with CFU-fibroblast (CFU-F) growth in the SP (63%) was much higher than that in either the non-SP (5%) or the total population (14%). Similarly, the percentage of wells with CFU-alkaline phosphatase (CFU-ALP) growth in the SP (45%) was significantly higher than that in non-SP (2%) or total population (3%). These data demonstrate that rat bone marrow stromal cell populations contain a SP enriched in CFU-F and osteoprogenitors compared to non-SP and total stromal populations. Sorting on the basis of Hoechst 33342 exclusion may be a useful enrichment stratagy for primitive osteoprogenitors and other precursor cell pools including MSCs.

Disclosures: ***S. Uchida****, None.*

SA235

See Friday Plenary number F235

SA236

A Distant Cis-Acting Element Is Responsible for Hormonal Regulation and Cell Type-Specific Expression of the Murine RANKL Gene. Q. Fu, I. P. Foote*, S. C. Manolagas, C. A. O'Brien. Div. Endo/Metab, Center for Osteoporsis & Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. of Arkansas for Med. Sciences, Little Rock, AR, USA.

PTH stimulates osteoclastogenesis and bone resorption in part by stimulating expression of RANKL. Previous studies demonstrate that PTH stimulates RANKL transcription via a protein kinase A (PKA)-CREB pathway in stromal/osteoblastic cells but not other cell types in vitro. Further, basal RANKL expression in vivo is limited primarily to bone and lymphocyte-containing tissues. The mechanisms responsible for this cell type- and tissue-specific expression remain unknown. To determine whether differential PTH receptor expression is responsible for tissue-specificity in vivo, mice were injected with dibutyryl-cAMP (db-cAMP) to broadly activate PKA in many tissues, independent of PTH receptor expression. Db-cAMP stimulated RANKL mRNA by 4-fold in bone tissue, as measured by RNAse protection assay, but did not significantly alter expression in kidney, spleen, liver, or lung. However, db-cAMP elevated IL-6 mRNA in all tissues. These results indicate that cAMP regulation of RANKL is tissue-specific and that mechanisms responsible for this specificity lie downstream of PKA activation. To determine whether cis-acting gene regulatory elements mediate the hormonal responsiveness and cell type-specificity of RANKL expression, we stably transfected murine RANKL promoter reporter constructs into a stromal/osteoblastic cell line that expresses endogenous RANKL (UAMS-32P) and a hepatoma cell line (Hepa), which does not express RANKL. A reporter construct containing 2.1 kb of 5'-flanking region had similar basal activity in UAMS-32P and Hepa cells and was not responsive to PTH or db-cAMP in either cell line. Both cell lines were capable of responding to db-cAMP, as an IL-6 promoter-reporter construct was stimulated by this agent in both lines. UAMS-32P and Hepa cells were then stably transfected with a reporter construct derived from a bacterial artificial chromosome (BAC) containing the entire murine RANKL gene as well as 120 kb of 5'-flanking region. This construct was generated by insertion of an internal ribosome entry site-luciferase cDNA cassette into exon 5 of the RANKL gene by homologous recombination. Expression of this construct was stimulated 5- and 11-fold by PTH and db-cAMP, respectively, in UAMS-32P cells. However, these agents did not stimulate the BAC construct in Hepa cells and the basal activity was also significantly lower in these cells. We conclude that distant elements, residing outside the proximal 2.1 kb 5'-flanking region, mediate both cAMP-responsiveness and cell type-specific expression of the murine RANKL gene.

Disclosures: ***C.A. O'Brien****, None.*

SA237

See Friday Plenary number F237

SA238

Differential Osteogenic Potential in Bone Marrow and Soft Tissue Stromal Cells Mediated by the *Gnas/GNAS1* Gene. R. J. Pignolo[1], S. Blythe*[2], J. Gilmore*[2], F. S. Kaplan[2], E. M. Shore[2]. [1]Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, USA.

The *Gnas* gene, encoding the murine alpha subunit of the stimulatory G-protein of adenylyl cyclase (Gsα), may function as a regulator of osteoblast differentiation in target cells that are, as yet, unidentified. Heterozygous inactivating mutations of the human *GNAS1* gene have been identified in patients with progressive osseous heteroplasia (POH), a disorder in which ectopic osteoblast differentiation and bone formation occurs within soft connective tissues. We have examined osteoblast differentiation in bone marrow and soft tissue stromal cells derived from wild-type mice and mice that are heterozygous for a *Gnas* null allele. Bone marrow derived mesenchymal stem cells (MSCs) were isolated by aspiration of femoral cavities after removal of the metaphyses. Soft tissue stem cells were partially purified from subcutaneous fat tissue. In both cases, cells were placed into osteogenic media and assayed for their ability to form bony nodules. Bone marrow stromal cells from *Gnas* heterozygous mice were found to have a reduced ability to differentiate into osteoblasts. However, soft tissue stromal cells from *Gnas* heterozygous mice appeared to have greater osteogenic potential compared to wild-type cells. These findings suggest that *Gnas* differentially influences cellular differentiation and bone formation based on the type and location of target cells. Thus, inactivating mutations in human *GNAS1* that cause developmental disorders of heterotopic bone formation may depend on the differential response of specific precursor cells to G-protein mediated stimulation as well as their location in soft tissue.

Disclosures: ***R.J. Pignolo****, None.*

SA239

See Friday Plenary number F239

SA240

Bmp-2 Induced Osteogenesis Of Human Marrow Stromal Cells Involves Balancing Smad And Kinase Pathways. A. M. Osyczka*, G. A. Bhargave*, E. B. Golden*, D. L. Diefenderfer, P. S. Leboy. Biochemistry, University of Pennsylvania, Dental Medicine, Philadelphia, PA, USA.

Analyses of marrow stromal cells (MSC) isolated from femurs of over 25 patients undergoing hip arthroplasty have shown that the majority of isolates do not up-regulate alkaline phosphatase (ALP) mRNA and enzyme activity in response to BMP in vitro, although BMP-2 does stimulate transcription of genes for BMP-2, noggin, Id-1 and bone sialoprotein (Diefenderfer, Osyczka, Reilly and Leboy, Conn Tissue Res. 44(S1):305-311; 2003). Human MSC cultured with BMP-2 also show unexpectedly high levels of the transcription factor Msx2 mRNA, but no increase in total Runx2 mRNA.

To examine mechanisms modulating BMP-2 regulation of ALP expression, human MSC were cultured for 4 days in media containing 15% FBS and then were either treated with kinase inhibitors or transferred to serum-free media for an additional 1-3 days. Transfer to serum-free media containing insulin or IGF-1 permits BMP-2 to induce transcription of ALP and to increase expression of osteopontin mRNA. Insulin or IGF-1, alone or in combination with BMP-2, neither stimulates proliferation nor significantly affects ALP expression in the presence of serum. Studies with the p38 inhibitor SB203580 and the PI3K inhibitor Ly294002 indicate that activation of both of these protein kinases is required for BMP-2 stimulation of ALP. In contrast, when human MSC are cultured in serum with the ERK inhibitor PD98059, BMP-2 stimulates ALP to the same extent as dexamethasone, indicating that ERK activity suppresses BMP-2 induction of ALP. These results, along with Western blot analyses imply that the ability of BMP-2 to stimulate ALP in human MSC under serum-free conditions with insulin or IGF-1 is correlated with decreased ERK signaling in the absence of serum. Western blot analyses also indicate that the BMP-regulated Smad pathway is active in human MSC, both with and without ERK inhibition. We suggest that, while several BMP-responsive genes seem to be unaffected by MAP kinase activity, a proper balance between Smad and MAPK pathways is required for BMP-2 to activate ALP transcription in human marrow stromal cell cultures.

Disclosures: ***A.M. Osyczka****, None.*

SA241

The Receptor Activator of Nuclear Factor (NF)kB Ligand (RANKL) Is a New Chemotactic Factor for Human Monocytes. V. Breuil[1], H. Schmid-Antomarchi*[1], A. Schmid-Alliana*[1], L. Euller-Ziegler[2], B. Rossi*[1]. [1]Inserm u 364, IFR50, Nice, France, [2]Rheumatology Department, Hopital l'Archet, Nice, France.

Aim of the study : Due to the role of RANKL in recruitment and fusion of monocyte-derived osteoclasts, we hypothesized that soluble RANKL (sRANKL) could exert chemotactic properties toward monocytic cells.

Methods : Chemotactic responses of MonoMac-6 cells were evaluated using 24-well chemotaxis chambers and polyethylene terephtalate inserts coated with fibronectin. MonoMac-6 cells, incubated for 16 hours with [3H]-methyl thymidine, were placed in the upper well and MCP-1, OPG or sRANKL, at various concentrations, were added to the lower well. After incubation at 37°C, migrated cells were collected at different time points in the lower well, and evaluated by measuring [3H]-methyl thymidine incorporation by scintillation counting. Peripheral blood mononuclear cells (PBMC) were obtained from buffy coats of blood from normal donors, and, for CD14+ experiments, a positive selection was performed using MACS colloidal superparamagnetic microbeads. Cells were seeded in the upper well of a

chemotaxis chambers and, after a 90 mn incubation, filters s were fixed and stained for 30 s with hematoxyline. Five fields were counted on each filter 's lower side.
Results : sRANKL induces the migration of MonoMac-6 monocytic cells as well as human freshly isolated total peripheral blood mononuclear cells (PBMC) and CD14+ purified PBMC. sRANKL induces the migration of MonoMac-6 cells in a dose-dependent manner and with an efficacy similar to MCP-1, the reference chemokine for monocyte. After a 8 hour incubation, sRANKL started to exhibit a chemoattractive effect on MonoMac-6 cells, with an increased effect observed for up to 24 hours. sRANKL elicits an additive chemotactic effect to MCP-1. Furthermore, addition of the sRANKL decoy receptor osteoprotegerin in the lower well or sRANKL in the upper well abrogates the sRANKL-induced migration of MonoMac-6 cells, hallmarking a true specific activity. RNAse protection assay experiments indicate that exposure of MonoMac-6 cells to sRANKL was without any significant effect on the expression of a variety of chemokines, known to attract monocytes.
Conclusion : This study provides the first evidence that RANKL behaves as a chemotactic factor for monocytic cells, emphazing the cross-talk between bone and immune system.

Disclosures: ***V. Breuil****, None.*

SA242

Podosomes and the Internalization of Vacuolar H+-ATPase from the Ruffled Membranes of Osteoclasts. I. R. Hurst*[1], S. Chen*[1], J. Zuo*[1], J. Jiang*[2], B. S. Lee*[3], M. Lu*[4], S. L. Gluck*[4], L. S. Holliday[1]. [1]Orthodontics, University of Florida College of Dentistry, Gainesville, FL, USA, [2]Endodontics, University of Florida College of Dentistry, Gainesville, FL, USA, [3]Physiology and Cell Biology, Ohio State University, Columbus, OH, USA, [4]Medicine/Nephrology, University of Florida College of Medicine, Gainesville, FL, USA.

Inhibition of phosphatidylinositol 3-kinase activity with 100 nM wortmannin triggers internalization of V-ATPase from the ruffled membranes of resorbing osteoclasts into cytosolic vesicles. This process is completed in about 40 min. In resorbing osteoclasts, podosomes are concentrated in the actin ring that surrounds the ruffled membrane and are present at much lower levels in the ruffled membrane area. Podosomes were detected by phalloidin-staining and confocal microscopy of osteoclasts fixed at time points after wortmannin treatment. During the first 10 min podosomes invaded the ruffled membrane area and were found increasingly intercalated within the spaces between membrane ruffles. By 10 minutes intercalation of podosomes and ruffled membranes had progressed to extensive co-localization between microfilaments and V-ATPase. Concurrent with the increasing co-localization between V-ATPase and F-actin increasing amounts of actin was recovered in immunoprecipitations using anti-V-ATPase antibodies. Wortmannin triggered both relocation of podosomes and a 30% reduction in the number of podosomes per osteoclast. Experiments with semi-permeabilized cells suggested wortmannin-treatment did not have profound effects on the dynamics of individual podosomes. Exogenous fluorescent-actin was incorporated into podosomes with or without wortmannin, and treatment with latrunculin A, which sequesters actin monomers, completely disrupted podosomes in the semi-permeabilized cells. Blocking actin turnover in intact wortmannin-treated osteoclasts using latrunculin A or jasplakinolide, which stabilizes filaments, inhibited the formation of cytosolic, V-ATPase containing vesicles. Taken together, these data suggest a role for podosomes, and podosomal dynamics, in the internalization of V-ATPase.

Disclosures: ***L.S. Holliday****, None.*

SA243

See Friday Plenary number F243

SA244

Actin-Related Protein 2/3 Complex Is Required for Osteoclast Differentiation and Actin Ring Formation. I. R. Hurst*[1], J. Zuo*[1], J. Jiang*[2], L. S. Holliday[1]. [1]Orthodontics, University of Florida College of Dentistry, Gainesville, FL, USA, [2]Endodontics, University of Florida College of Dentistry, Gainesville, FL, USA.

Actin-related 2/3 (Arp2/3) complex is associated with most dynamic microfilament-based structures and is found in the podosomes of src-transformed cells. In osteoclasts, podosomes that resemble those from transformed cells form actin ring structures that are required for bone resorption. Two components of the Arp2/3 complex (Arp2 and Arp3) were upregulated 3 fold in Raw 264.7 cells in response to stimulation with osteoprotegerin-ligand. Immunocytochemistry demonstrated that Arp2/3 complex was present in the actin rings of mouse marrow osteoclasts fixed in the process of resorbing bone and in the podosomes of the actin rings of Raw 264.7 cells on coverslips. Arp2/3 complex was found to co-localize in podosomes with F-actin and cortactin, a known regulator of Arp2/3 complex activity, but not with vinculin. Five small interfering RNAs (siRNAs) were designed against Arp2 and one effectively knocked down expression of the target protein. Raw 264.7 cells could be transfected either early (Day 3 after OPGL-stimulation) or late (Day 5) in the differentiation process with efficiencies of 50-80% as judged by a fluorescent oligos. When transfected early with the effective siRNA, the cells were rendered unable to form mature osteoclasts. Thirty hours after late transfection with the effective siRNA, levels of Arp2 were reduced 70% in whole cell lysates when standardized to actin levels and compared with ineffective controls. Many well spread giant cells were evident in cultures late transfected with the effective siRNA after 30 hours of culture; but when F-actin was stained using fluorescent-phalloidin, actin rings were not detected. In control cells transfected by ineffective siRNAs, podosomes and actin rings were normal. In summary, our results suggest that the Arp2/3 complex is vital to osteoclast differentiation and is required for the formation of actin rings.

Disclosures: ***I.R. Hurst****, None.*

SA245

A Specific Inhibitor of Atypical PKCs Blocks Formation of the Sealing Zone in Bone-Resorbing Osteoclasts. H. Zhao[1], S. Ohno*[2], S. L. Teitelbaum[1], F. P. Ross[1]. [1]Department of Pathology, Washington University School of Medicine, St. Louis, MO, USA, [2]Department of Molecular Biology, Yokohama City University School of Medicine, Yokohama, Japan.

Osteoclasts (OCs) are multinucleated bone resorbing cells of hematopoietic origin. Attachment to bone matrix stimulates OC polarization, thus initiating the resorptive process. Bone degradation involves formation of a tight sealing zone and the ruffled border in the plasma membrane juxtaposed to bone, followed by vectorial secretion of protons and proteases into the resorption lacuna. Little is known about the molecular mechanisms underlying these fundamental polarity events. Recent studies have shown that a ternary complex composed of the cell polarity proteins, par-3, par-6 and atypical PKCs (aPKCs), functions as an evolutionarily conserved protein machinery which is essential for establishing cell polarity in a variety of biological contexts. Given these facts, we explored the expression and function of par-3, par-6 and (aPKCs) in OCs and their myeloid precursors. Using the standard culture system of M-CSF dependent bone marrow macrophages cultured with M-CSF and RANKL, total RNA and cell lysates were prepared at different time points representing precursors, pre-OCs and mature cells. RT-PCR and western blot results showed that par-3, par-6 and both aPKC isoforms, PKC-ζ and PKC-λ , are expressed in mature OCs. Importantly, the expression level of par-3 increases during OC differentiation, with maximal expression found in the fully resorptive cells. Immunofluorescence and laser scanning confocal microscopy studies demonstrate that par-3 and aPKCs colocalize with F-actin at the sealing zone of OCs cultured on dentin slices. Together, our results suggest that par-3, par-6 and aPKCs may form a complex and function in the formation and maintenance of tight sealing zone in OCs. To test this hypothesis, we treated mature OCs cultured on dentin slices with specific inhibitors of PKC isoforms. While inhibitors of the classical PKCs, namely PKC-α and -β , and novel PKCs, such as PKC-θ and -ε, fail to alter actin ring formation, an aPKC specific inhibitor decreases this index of OC polarization in a dose- and time-dependent manner. Furthermore, the PI-3K inhibitor, LY294002, which also prevent OC actin ring formation, decreases phosphorylation of aPKCs. In conclusion, 1). Our results have localized components of a cell polarity protein complex containing aPKCs/par-3/par-6 at the OC sealing zone; 2) inhibition of aPKC activity blocks formation of actin rings, suggests a role for the polarity complex in OC polarization. 3). This complex may function as an important downstream effector of PI-3K signaling in regulating osteoclast function.

Disclosures: ***H. Zhao****, None.*

SA246

Actin Ring Formation Stimulated By RANKL Involves a Unique Actin Remodeling Mechanism Distinct from Osteoclast Podosomes and Mediated by CapG. B. D. Bennett[1], P. McAtee*[1], K. Tustison*[1], F. S. Southwick*[2], K. A. Hruska[1]. [1]Pediatrics, Washington University School of Medicine, St. Louis, MO, USA, [2]Department of Medicine, University of Florida College of Medicine, Gainesville, FL, USA.

A quantitative 2Dgel-mass spectrometry based proteome screen of rapidly tyrosine-phosphorylated proteins in osteoclasts detected CapG as the most prominent RANKL target. CapG was shown to cap actin filaments during assembly of the RANKL-stimulated actin ring, a process that was distinct from the gelsolin capped cytoskeleton (podosomes). CapG function was also regulated by ECM, polyphosphoinositides and calcium. CapG mediated organization of actin ring assembly was critical because its deficiency produced a mild osteopetrosis, similar to gelsolin deficiency, in CapG null mice. CapG deficiency was complemented by capping protein (CapZ), preventing the osteopetrosis from being severe. Gelsolin did not associate with the actin ring until disassembly during motility, demonstrating its role in actin severing (CapG does not sever actin filaments). The gelsolin capped cytoskeleton was morphologically separate from actin ring assembly and was focused at the cell periphery, lamellopodia and leading edges of osteoclasts. CapG associated with other actin barbed end regulators such as Scar and Arp2/3 and a specific intracellular calcium pool during assembly of the actin ring. RANKL stimulated calcium sensitization through src, phospholipase C gamma, and PYK2 leading to CapG activation and ring assembly. Calcium stimulated CapG capping activity and gelsolin translocation to the ring leading to its dissassembly. We conclude that CapG is a prominent RANKL effector protein mediating the unique process of osteoclast actin ring assembly during bone resorption. The CapG actin pool is a newly identified actin pool distinct from the podosome, responsible along with CapZ for the unique organization of the actin ring.

Disclosures: ***B.D. Bennett****, None.*

SA247

Activated T Cell Factors Inhibit Bone Formation *In Vitro*. S. Varghese[1], N. Wyzga*[2], F. A. Sylvester[2]. [1]Research, Saint Francis Hospital & Medical Center, Hartford, CT, USA, [2]Pediatric Gastroenterology, Connecticut Children's Medical Center, Hartford, CT, USA.

T cell activation is a hallmark of chronic inflammatory diseases associated with bone loss. We tested the hypothesis that factors produced by activated T cells inhibit osteoblast development from bone marrow stromal cells *in vitro*. We isolated T cells by magnetic cell sorting from spleen of 5-week-old C57BL/6J mice, which yielded T cell fractions that were >95% pure as judged by flow cytometry. The conditioned medium from resting and activated T cell cultures was used as a source of T cell-derived factors. We activated T cells with concanavalin A (Con A), phytohemagglutinin (PHA), *Staphylococcal* enterotoxin A (SEA, a superantigen), or anti-CD3ε and -CD28 (Abs). Bone marrow stromal cells were

harvested from femurs of C57BL/6J mice and grown in culture for 10 to 12 days to allow for stromal cells to settle and form colonies. Stromal cells were then exposed to 0.1 to 10% conditioned medium from resting and activated T cells for 3 weeks. Cultures were stained for calcium nodules with alizarin red and for alkaline phosphatase. Exposure of bone marrow cultures to different doses of conditioned medium from activated T cells reduced bone nodule formation and alkaline phosphatase activity in a dose-dependent manner, especially from T cells treated with either Abs or SEA. Medium from resting T cells had no effect. The decrease in nodule formation in the stromal cell cultures was accompanied by a decrease in cell number, as suggested by hematoxylin staining. We observed that the concentration of IFN-γ was highest in the medium from T cells activated with Abs (279-472 ng/mL) or SEA (2-6 ng/mL) (n=4). We tested if IFN-γ mediated the inhibitory effects of conditioned medium. IFN-γ by itself reduced nodule formation in a dose-dependent manner, with maximum inhibition at 5 ng/mL. However, reduced nodule formation persisted in bone marrow cultures exposed to activated T cell conditioned medium in the presence of an IFN-γ neutralizing antibody. In addition, inhibition of nodule formation occurred in bone marrow cultures from IFN-γ receptor null mice, indicating that other factors produced by activated T cells also contribute to the suppression of bone formation in bone marrow stromal cell cultures. Our studies suggest that soluble factors produced by activated T cells may play a role in inhibiting bone formation. Although IFN-γ present in the medium is inhibitory, it probably works together with other factors to inhibit osteoblast development from bone marrow stromal cells *in vitro*.

Disclosures: ***S. Varghese****, None.*

SA248

See Friday Plenary number F248

SA249

TNFα Induces RANKL and Inhibits OPG Gene Expression in Primary Bone Marrow Stromal Cells. S. Wei, F. P. Ross, S. L. Teitelbaum. Pathology, Washington University School of Medicine, St. Louis, MO, USA.

Enhanced osteoclastogenesis is a hallmark of various forms of bone disease, including the osteopenia involving chronic inflammation of bone or periosseous tissues. These events, which are accompanied by prolonged or excessive release of cytokines, are central to the pathogenesis of inflammatory bone joint destruction. TNFα is among the most potent of the osteoclastogenic cytokines produced in inflammation. While this molecule potently induces macrophages to assume the osteoclast phenotype, it does so only in the context of at least permissive levels of the essential osteoclastogenic molecule, RANK ligand (RANKL), expressed by bone marrow cells of the mesenchymal lineage. The present study was designed to investigate the molecular mechanism(s) by which TNFα regulates RANKL gene expression by marrow stromal cells. We first document, by semi-quantative RT-PCR analysis, that TNFα dose-dependently induces RANKL gene expression in primary murine bone marrow cells purified with an antibody against VCAM-1, a marker constitutively expressed on bone marrow stromal cells and myeloid cells. We next turned to define the molecular mechanism(s) underlying stimulation of RANKL expression by TNFα. We find that TNF enhances expression of IL-1α, another inflammatory cytokine known to induce osteoclast activation. While IL-1α alone does not increase the level of RANKL mRNA, the capacity of TNFα to stimulate this effect is abolished by the addition of excess IL-1 receptor antagonist (IL-1Ra). These data suggest that a synergy may exist between TNFα and IL-1α in the regulation of RANKL gene. This hypothesis is supported by the observation that the combination of a minuscule concentration of TNFα (1 ng/ml) and IL-1α (10 ng/ml) prompts robust upregulation of the RANKL gene. We also find that, within the same mesenchymal cell population, both TNFα and IL-1α inhibit expression of OPG, the decoy receptor for RANKL, thereby further augmenting the osteoclastogenic activity of RANKL. TNFα inhibition of OPG is not attenuated by IL-1Ra, indicating that the mechanism of this effect differs from that which regulates RANKL. Attesting to function, TNF-induced osteoclastogenesis in a coculture of wild-type VCAM-1 positive cells and bone marrow macrophages from TNF receptor double knockout mice, which is about 40% of that induced by 1,25-dihydroxyvitamin D, is inhibited by addition of IL-1Ra by approximate 60%. Thus, excess TNFα associated with states of chronic inflammation acts on the stromal environment to enhance expression of RANKL in synergy with IL-1α, and to inhibit expression of OPG, both leading to accelerated osteoclastogenesis.

Disclosures: ***S. Wei****, None.*

SA250

See Friday Plenary number F250

SA251

The Stage Dependent Effects of Interleukin-6 on Osteoclastogenesis. R. K. Rahman*, S. V. Bukata, J. Gelinas*, W. Huang*, R. J. O'Keefe, J. E. Puzas, R. N. Rosier, E. M. Schwarz. Orthopaedics, University of Rochester School of Medicine, Rochester, NY, USA.

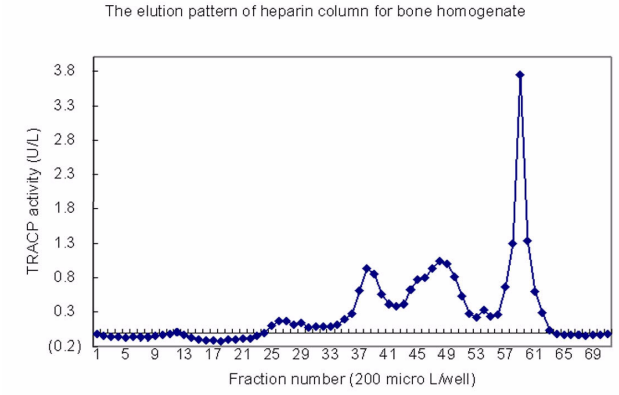
Interleukin-6 (IL-6) is a pleotropic cytokine previously recognized as having both osteoclastogenic and anti-osteoclastogenic properties. A number of studies have proposed IL-6 as a potent stimulator of bone-resorbing cells (osteoclasts), which are formed as a result of monocyte differentiation. As such, they have concluded that anti-IL-6 therapy is advocated for bone loss seen in post-menopausal osteoporosis, erosive arthritis, and wear debris-induced osteolysis. However, others have found that IL-6 inhibits osteoclastogenesis and bone resorption. To resolve this controversy, we evaluated the stage specific effects of IL-6 on the differentiation of osteoclast precursors using cells derived from IL-6 knock out, TNF-transgenic, and wild-type mice. Using murine splenocyte cultures, we found that IL-6 inhibited early precursors, had no effect on mature osteoclast precursors, and stimulated osteoclastogenesis of late precursors into osteoclasts. Flow cytometry for the osteoclast precursor marker CD11b on splenocytes from wild-type and IL-6 k/o mice revealed that IL-6 does not regulate the precursor frequency in vivo. Further studies, employing the murine calvarial model, were undertaken to investigate the effects of IL-6 on bone resorption and in vivo osteoclast formation. IL-6 knock out mice showed increased osteoclast formation and increased bone loss compare to wild-type control. Taken together these results show that the effects of IL-6 are dependent on the stage of differentiation of the osteoclast precursor, and support a model whereby IL-6 inhibits the differentiation of immature pre-osteoclasts, while synergizing with other osteoclastic factors to mediate the differentiation of mature pre-osteoclasts.

Disclosures: ***R.K. Rahman****, None.*

SA252

Tartrate-Resistant Acid Phosphatase (TRACP) in the Rat Bone, Serum and Osteoclast. Y. Igarashi[1], S. Shimizu*[1], K. Manaka*[2], T. Miura*[3], S. Matsuzaki*[1]. [1]Biochemistry, Dokkyo University School of Medicine, Mibu, Japan, [2]Institute for Medical Science, Dokkyo University School of Medicine, Mibu, Japan, [3]Biochemical Laboratry, Nitto Bouseki Co., Ltd., Koriyama, Japan.

Tartrate-resistant acid phosphatase (TRACP) is well-known as a marker of the osteoclast. However, its physiological significance in the osteoclast still remains unclear. In the human serum, Lam (1981) found that there were two types of TRACP 5 i.e. 5a and 5b, separated by native electrophoresis, and that the 5b was derived mostly from the osteoclast. We have shown that these two isoforms were identified by using heparin column chromatography [Igarashi Y. J Chromatogr B Biomed Sci Appl. 2001; 757:269-76]. The objective of our study is to specify the type of TRACP isoforms of rat bone by analyzing various monocytic tissues and cultured osteoclasts. Tibiae were homogenized using a Polytoron homogenizer with 50 mM Tris buffer pH7.5 containing PMS, Triton X-100 and EDTA at 4°C. The osteoclast progenitor cells, purchased from Hokudo (Sapporo, Japan), were cultured with M-CSF (20 ng/ml) and RANKL (20 ng/ml) for 4 days and refreshed with medium every day. Macrophage-rich dish-attached cell fractions were collected from the spleen, alveolus and peripheral blood and incubated with PBS for 1 hour. Each sample was dialyzed against 20 mM Tris-HCl pH 7.2 0.1 M NaCl and absorbed to the heparin column (Amachambiosciences Hitrap heparin HP 1ml). TRACP was eluted with NaCl linear gradient from 0.1 M to 1.335 M. The activities of fractionated TRACPs were photometrically analyzed with 50 mM pNPP, 100 mM citrate buffer pH 5.5. Three TRACP activity peaks were detected in the fractions of the samples from serum, bone, osteoclast and other macrophages. The peripheral blood and spleen macrophages were characterized by higher proportion of the first peak. The serum of 3 week-old rats showed the second peak the highest, but one of the 10 day-old showed the last one the highest. The figure indicated below shows the TRACP pattern of mature bone, which had a prominent third peak and that it was similar to that of alveolar macrophages. Rat osteoclasts expressed three peaks and both the second and the third peak were increased by the stimulation of M-CSF and RANKL. In conclusion, the rat osteoclasts have at least two isoforms of TRACP while human osteoclasts have only one TRACP 5b.



Disclosures: ***Y. Igarashi****, None.*

SA253

See Friday Plenary number F253

SA254

TNFr1 Regulates RANKL Expression and Osteoclastogenesis. Y. Abu-Amer. Orthopedics and Cell Biol. & Physiol., Washington Univ. Sch. of Medicine, St. Louis, MO, USA.

TNF stimulates basal osteoclastogenesis via its type 1 receptor. This is supported by the findings that, 1) TNF accelerates RANKL-primed osteoclastogenesis, and 2) marrow

derived from TNFr1-null mice generates far less osteoclasts compared to wild type (WT). Thus, TNF/TNFr1 might regulate RANKL and possibly RANK expression on osteoclast precursors (OCPs) and stromal cells, respectively. To test this hypothesis, we evaluated osteoclastogenesis using RANKL-primed OCPs, whole marrow cultures and OCP/stromal cell mix and match experiments. We find that RANKL-induced osteoclastogenesis using TNFr1-null OCPs is approximately 64% of the WT. Whole bone marrow cultures from knockout mice generated far less osteoclasts (23%) compared to WT cultures indicating that TNFr1-null OCPs and stromal cells have reduced osteoclastogenic potential.
To examine possible mechanisms for this retarded osteoclastogenesis, we measured mRNA expression of RANK and RANKL in OCP and stromal cells, respectively. RT-PCR revealed that levels of RANKL message in WT stromal cells are markedly increased following vitamin D treatment compared to low levels in TNFr1-null cells. Expression levels of OPG that regulates RANKL were largely unchanged. Thus, the positive ratio of RANKL/OPG, which supports osteoclasts, was overwhelmingly in favor of WT rather than TNFr1-null cultures. Changes in RANK expression on OCPs, that could regulate osteoclastogenesis, were subtle and unlikely to represent the significant changes in osteoclastogenesis. As a result, we reasoned that WT stromal cells might recapitulate retarded osteoclastogenesis by TNFr1-null OCPs. We find that WT stromal cells cocultured with TNFr1-null OCPs induced osteoclastogenesis by knockout OCPs up to 75% compared to WT OCPs. Asserting a direct role for RANKL in this process, OPG was sufficient to block osteoclastogenesis by these cultures. Next, we examined the direct effect of TNFr1 by reintroducing the receptor into TNFr1-null stromal cells. Viral reintroduction of TNFr1 to TNFR1-null stromal cells resumed expression of RANKL mRNA to normal levels which was increased in response to vitamin D. More importantly, osteoclast formation by OCPs resumed in a normal pattern when co-cultured with TNFr1-restored knockout stromal cells. Because exogenous RANKL was not sufficient to induce optimal osteoclastogenesis by TNFr1-null OCPs compared to WT cells, we reintroduced TNFr1 into these cells as well. TNFr1-null OCPs with the newly introduced TNFr1 underwent near optimal osteoclastogenesis (90%) when treated with RANKL or co-cultured with wild type stromal cells (106%). Thus, expression of intact TNFr1 on OCPs and stromal cells is required for optimal induction of basal osteoclastogenesis.

Disclosures: ***Y. Abu-Amer,*** *None.*

SA255

See Friday Plenary number F255

SA256

Development and Characterization of a Novel Human *In Vitro* Bone Resorption Assay Useful for Preclinical Testing of Osteoporosis Drug Candidates. J. P. Rissanen*[1], T. A. Hentunen[2], J. M. Halleen[1]. [1]Pharmatest Services Ltd, Turku, Finland, [2]Institute of Biomedicine, University of Turku, Turku, Finland.

The purpose of this study was to develop a rapid and reliable *in vitro* bone resorption assay that would be a useful tool in preclinical phases of antiresorptive drug development. We cultured commercially available CD34-positive osteoclast precursor cells in the presence of M-CSF, RANKL and TGF-beta1 to allow their differentiation into bone-resorbing osteoclasts. After the differentiation period at day 7, the medium was replaced and the formed mature osteoclasts were allowed to resorb bone for an additional 2-day culture period. Tartrate-resistant acid phosphatase (TRACP) isoform 5b (TRACP 5b) and C-terminal crosslinked telopeptides of type I collagen (CTX) were determined from the culture medium with commercially available methods. Resorption pits were visualized with peroxidase-conjugated Wheat Germ Agglutinin lectin, and the total resorbed area was quantitated. The number of TRACP-positive multinuclear osteoclasts was counted under a microscope. The amount of lactate dehydrogenase released into the culture medium was determined as an index of cell death. After the 9-day culture period, medium CTX showed a highly significant correlation with the total resorbed area ($r > 0.9$, $p < 0.0001$). Medium TRACP 5b correlated with medium CTX ($r > 0.7$, $p < 0.0001$), but not as well as with osteoclast number ($r > 0.9$, $p < 0.0001$), suggesting that TRACP 5b is a marker of osteoclast number rather than their activity. The cathepsin inhibitor E64 proved to be a useful control inhibitor of resorbing activity of mature osteoclasts, osteoprotegerin a useful control inhibitor of osteoclastogenesis, and sodium azide a useful control inducer of cell death. We conclude that with this assay, it is possible to test rapidly the effects of drug candidates on cell death, osteoclastogenesis and resorbing activity of mature osteoclasts from the same osteoclast cultures. Medium CTX values divided by medium TRACP 5b values can be used as a reliable index of the amount of bone resorption per osteoclast. These results show that this novel human *in vitro* bone resorption assay is a fast, convenient and reliable large scale method for screening and optimization of new antiresorptive lead compounds targeted for osteoporosis and related bone disorders.

Disclosures: ***J.P. Rissanen****, None.*

SA257

See Friday Plenary number F257

SA258

Effect of Carboxyl (C) Terminal Fragments of PTH on Osteoclastogenesis. O. Lotz, P. Divieti, H. Jüppner, F. R. Bringhurst. Endocrine Unit, MGH, Boston, MA, USA.

Intact parathyroid hormone, PTH (1-84), is one of the major regulators of cellular differentiation and function in bone metabolism. It acts on cells of the osteoblastic lineage via the G protein-coupled type-1 PTH/PTHrP receptor. Moreover, osteoblasts and osteocytes derived from PTHR-null mice express additional receptors for PTH which specifically recognize the carboxyl-terminal region of the hormone. Unlike the PTHR-1 receptors, these C-terminal PTH receptors (CPTHRs) bind various carboxyl-terminal fragments of PTH (CPTH). These truncating fragments are either generated by the peripheral metabolism of intact PTH or directly secreted, in a calcium-dependent manner, by the parathyroid glands. It has been reported that synthetic PTH C-fragments of PTH (7-84) block the calcemic action of PTH (1-84) and PTH (1-34) in parathyroidectomized rats. We have previously shown that PTH (7-84) reduces the formation of osteoclasts in murine whole bone marrow cultures, treated with M-CSF and RANKL or with $1.25(OH)_2D_3$. To address a direct effect of PTH (7-84) on osteoclastogenesis, we utilized purified marrow macrophages (BMM). As expected, addition of RANKL and M-CSF induced osteoclast formation, as assessed by TRAP staining and TRAP solution assay. BMM cultures exposed additionally to PTH (7-84) (300nM) generated 10-20% fewer osteoclasts. We then performed CPTHR radioligand binding analysis using ^{125}I-(Tyr) hPTH (19-84) as tracer, and observed that BMMs express CPTHRs. The expression in the receptor is reduced by treatment with RANKL. These findings suggest that CPTHR expression might decrease during osteoclast maturation. When BMM cells were cultured for an additional 7 days in medium containing high doses of M-CSF produced by fibroblastic cell line L929, the inhibitory effect of PTH (7-84) was enhanced by 30-40%.
Using this population of BMMs the treatment with (1-84) PTH and PTH (1-34) had no effect on the formation of osteoclasts. Moreover, neither PTH (1-84) nor PTH (1-34) were unable to diminish the inhibition by PTH (7-84). Shorter fragments of CPTH such as 11-84, 19-84, 23-84 and 39-83 had no effect on the formation of osteoclasts. In contrast to these CPTH fragments, we found that a shorter CPTH fragment (53-84) can enhance osteoclastogenesis.
These findings demonstrate that CPTH fragments act on osteoclast precursors cells and that these effects are dependent on the size of the CPTH fragments.

Disclosures: ***O. Lotz****, None.*

SA259

See Friday Plenary number F259

SA260

Two Chloride Channel Genes Required for Human Osteoclasts: CLIC-5 Defects Suppress Early Differentiation and Vacuolar Acidification; ClC-7 Defects Restrict Lacunar Resorption, but Permit Expression of Osteoclast Markers. H. Blair[1], s. Kalla*[1], m. Saito*[2], P. Orchard[3], P. H. Schlesinger[2]. [1]Pathology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Cell Biology and Physiology, Washington University, St. Louis, MO, USA, [3]Medicine, University of Minnesota, Minneapolis, MN, USA.

It is established that an avian intracellular chloride channel $Cl_{or,62}$, the homolog of the human intracellular Cl^--channel CLIC-5, is expressed at high levels at the osteoclasts ruffled border and mediates HCl transport. However, transgenic studies and examination of osteopetrotic families shows that a Cl^--channel homolog, CLC-7 is required for osteoclast formation. We studied the relative roles of these two genes, which are in different intracellular chloride channel families, using CD14 selected human osteoclast precursors differentiated in vitro with RANKL and Csf-1. CLIC-5 expression was modified using an siRNA targeting nt 331-351 of GB AK075163, 30 b upstream of the translation initiation. Results were compared to normal controls and CD14 cells from a natural compound heterozygote with P249R and S744F mutations in each copy of CLC7, which causes severe osteopetrosis. Targeting of the siRNA was confirmed by cy3 labeling of the siRNA within target cells. CLIC-5 suppressed cells showed zero vacuolar acidification using lysotracker labeling. Cell attachment to cell culture substrate or bone was unaffected, but in these cells there was no evidence of osteoclastic maturation including lack of multinucleation and absence of osteoclast markers including MMP9. In contrast, CD14 selected CLC7 double heterozygotes did show vacuolar acidification and cells expressing osteoclast markers including TRAP and cathepsin K were formed, although the amount of lacunar resorption and number of resorbing cells was greatly reduced, typically << 10 % of lacunar resorption in controls, and the lacunae that were formed were atypical, comprising single highly localized deep pits and chains of broad lacunae, common in normal control osteoclasts, were not seen. We conclude that at least two chloride-channel family genes are required for human osteoclastogenesis, CLIC-5, which is required for vacuolar acidification, affects early differentiation and mutants are probably not compatible with life. CLC7 defects are compatible with vacuolar acidification and permit limited survival, but prevent normal lacunar resorption.

Disclosures: ***H. Blair****, None.*

SA261

Osteoclasts from Patients with Autosomal Dominant Osteopetrosis II (ADOII) Have Defective Acidification and Resorption, but Normal Proteolytic Activity In Vitro. K. Henriksen*[1], J. Gram*[2], S. Schaller*[1], J. Delaissé[1], J. Bollerslev*[3], M. A. Karsdal[1]. [1]Nordic Bioscience, Herlev, Denmark, [2]Ribe County Hospital, Ribe, Denmark, [3]Medical Endocrinology, National University Hospital, Oslo, Norway.

Autosomal Dominant Osteopetrosis II (ADOII) is a relatively benign disorder caused by a missense mutation in the *ClCN-7* chloride channel gene that seems to have a dominant-negative effect, with some residual activity.

The osteoclasts from ADOII patients have never been characterized. Here we present an *in vitro* study of the resorptive activity of osteoclasts from the ADOII patients.

Isolated CD14+ monocytes from ADOII patients, harboring a G215R mutation, and from healthy age and sex-matched controls, were cultured in the presence of RANK-L and M-CSF to generate osteoclastic cells and evaluate parameters, such as cell fusion, TRAP activity, ion channel and resorptive activity.

By histological investigations, ADOII osteoclasts have been found to have increased number and size *in vivo*. However, we did not observe any significant changes in the osteoclast formation rate, size or morphology in this study.

When mature ADOII osteoclasts were investigated on fully mineralized bone they formed actin rings, but the resorption level was reduced by 95% when compared to controls. Interestingly, the secretion of TRAP, which is known to depend on acidification, was also significantly reduced in the ADOII osteoclasts. The small residual resorption activity in the ADOII osteoclasts was sensitive to both chloride channel and Cathepsin K inhibitors, showing some acidification, albeit at lower levels than controls.

To prove that the defect is linked to acidification, we tested osteoclasts from ADOII patients and controls for their ability to degrade demineralized bone matrix, and found no significant differences under these conditions. Thus, these observations imply that only processes involved in the dissolution of the inorganic phase, and not the proteolysis of the organic phase, are attenuated by the mutation in the C*lCN-7* gene.

In conclusion, this is the first functional study of human ADOII osteoclasts. We show that the major osteoclastic defect is impaired resorption, likely caused by reduced acidification. We also show that osteoclastogenesis is normal in ADOII osteoclasts, resulting in fully mature actin ring forming osteoclasts. The larger osteoclasts observed *in vivo* , but not *in vitro*, could be a consequence of the absence of signals normally generated during dissolution of the matrix, rather than a consequence of the absence of ClC-7 activity.

Disclosures: ***K. Henriksen****, None.*

SA262

See Friday Plenary number F262

SA263

Targeting Osteoclasts with Zoledronic Acid Prevents Bone Destruction in Collagen-Induced Arthritis. N. A. Sims[1], J. R. Green[2], T. J. Martin[3], M. T. Gillespie[3], E. Romas[1]. [1]Department of Medicine at St. Vincent's Hospital, The University of Melbourne, Melbourne, Australia, [2]Bone Biology Unit, Novartis Pharma, Basel, Switzerland, [3]St. Vincent's Institute of Medical Research, Melbourne, Australia.

Bone destruction is a characteristic feature of rheumatoid arthritis (RA) and constitutes a major cause of progressive disability. Bone loss involves osteoclasts, which are responsible for periarticular joint destruction. Osteoclasts are also evident in animal models of RA such as collagen-induced arthritis (CIA). It has been shown previously that synovial cells in CIA and RA express high levels of receptor activator of NFκB ligand (RANKL), and that treatment of CIA with Fc-OPG blocks osteoclastogenesis thereby preventing focal bone destruction, suggesting the potential of osteoclast inhibitors to prevent structural joint damage in arthritis.

In the present study, we investigated the effects of Zoledronic acid (ZOL), a potent third generation bisphosphonate, in the effector phase of CIA. At the onset of CIA, rats were treated with single doses of ZOL (50 or 100 μg/kg S.C.) and monitored daily for paw swelling. At 2 weeks after onset, joints in the hindpaws were evaluated using histology and radiography. Paraffin-embedded joints were analysed and scored for inflammation, pannus formation, and cartilage and bone destruction using semi-quantitative grading scales.

Histopathologic and radiographic analyses indicated that ZOL prevented structural joint damage, even though it had no impact on inflammatory synovitis. ZOL treatment did not prevent paw swelling, and arthritic scores were mildly increased in rats treated with 100 μg/kg ZOL compared to controls. However, treatment with a single dose of 50 or 100 μg/kg ZOL reduced bone erosion scores (by 65% and 80% respectively) compared to untreated controls. ZOL treatment strikingly diminished radiographic bone erosions in the ankle joints. In contrast, synovial inflammation and cartilage destruction scores were not altered in either untreated or ZOL treated rats.

These findings demonstrate that ZOL has powerful bone protective effects in arthritis that are independent of synovial inflammation. Targeting osteoclasts with potent bisphosphonates like ZOL may be an effective strategy for preventing structural joint damage in RA.

Disclosures: ***N.A. Sims****, None.*

SA264

Inhibition of Osteoclast Bone Resorption by Newly Synthesized NMDA-receptor Modulators. O. Hoffmann[1], T. Pöhler*[2], U. Maurer*[1], M. L. Berger*[3], C. Noe*[2]. [1]Pharmacology and Toxicology, University of Vienna, Vienna, Austria, [2]Pharmaceutical Chemistry, University of Vienna, Vienna, Austria, [3]Brain Research Institute, University of Vienna, Vienna, Austria.

Glutamate is the predominant excitatory transmitter in the central nervous system. It is involved in neuronal functions through the action of two types of membrane receptors, ionotropic receptors and metabotropic receptors. N-Methyl-D-aspartate (NMDA) receptors constitute a family of glutamate receptors that which are crucial for synaptic-like intercellular signaling. Recently, glutamate transporters, receptors, and components of synaptic signaling have been identified in bone. Osteoblasts and osteoclasts (OC) express distinct glutamate receptors and clustering proteins necessary for receptor localization. In vitro incubation of OC with MK-801, a specific NMDA glutamate receptor antagonist inhibits bone resorption. To further elucidate the role of NMDA-receptors in bone, we synthesized novel polyamine inverse agonists based on the structure of aryl-substituted dialkanediamines and tested the capacity of these ligands (IC50 between 120 and 300 nM) to inhibit OC function in vitro. OC derived from rabbit long bones were incubated for 24 hours on bovine bone slices in αMEM + 10% FCS. Contaminating bone cells were then removed and resorption lacunae on bone slices were measured. The known antagonist, MK-801 (1 μM) inhibited bone resorption by 50%. TP266 (1 μM), the most potent synthesized polyamine inverse agonist, completely inhibited bone resorption. The other less potent 8 compounds inhibited OC bone resorption in a concentration dependent manner (100 μM to 10 nM). Inhibition of OC resorption with each compound tested was associated with impaired OC adherence at concentrations higher than 10 μM. To test whether reduced OC resorption was due to cytotoxicity, we measured cell toxicity using a MTS cell toxicity assay. We observed OC toxicity at concentrations of inverse agonists higher than 10 μM, which correlated with impaired OC adherence. DAPI staining at both the low (1 μM) and high (100 μM) compound concentrations did not induce OC apoptosis. Taken together, these data illustrate that low doses of these novel NMDA-receptor blockers inhibit OC resorption 100 times more effectively than the known receptor antagonist, MK-801 and at higher concentrations appear to be cytotoxic. Understanding the role of NMDA receptors in OC is important for further understanding of the mechanisms operating in bone metabolism and may provide new targets for the treatment of osteoporosis. Specific inhibition of NMDA receptor OC resorption may offer a novel therapeutic approach for osteoporosis.

Disclosures: ***O. Hoffmann****, None.*

SA265

Nox and Osteoclasts. S. Yang, Y. Zhang*, W. Ries, L. Key. Pediatrics, Medical University of SC, Charleston, SC, USA.

A new superoxide-generating enzyme (Nox4) is present in osteoclasts and contributes to osteoclastic superoxide production. In this study, we demonstrate that among the Nox family members, only Nox4 has a higher level of expression in osteoclasts than that in precursor cells, suggesting Nox4 is associated with osteoclast differentiation and development. Cotransfection of Nox4/P22 DNA constructs results in 80% increase of osteoclastic superoxide production, indicating that P22 is required for Nox4 to function. Using a real time PCR, we show that expression of cathepsin K and TRAP is increased dramatically in osteoclasts transfected with Nox4/P22, while c-fms remains unchanged. Such increase is correlated with enhanced superoxide generation in osteoclasts transfected with Nox4/p22. Further study reveals that JNK, not NF-κB, is the signaling pathway involved in superoxide-mediated expression of cathepsin K and TRAP in osteoclasts. More interestedly, we found a negative regulation between JNK and NF-κB pathways in osteoclasts, because decreased NF-κB activity correlates with enhanced the JNK activation in osteoclasts.

Superoxide has been shown to directly participate in the process of bone resorption by facilitating matrix protein degradation. In addition to the direct contribution to bone resorption, we show that appropriate amount of superoxide might be needed for osteoclast development and maturation, and superoxide might be involved in triggering the signal transduction pathways necessary for osteoclasts to function.

Disclosures: ***S. Yang****, None.*

SA266

See Friday Plenary number F266

SA267

Annexin II Stimulates RANKL Expression through a MAPK-dependent Pathway in Human Bone Marrow Cells. F. H. Li[1], S. V. Reddy[1], G. D. Roodman[2]. [1]Medicine-Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Medicine-Hematology/Oncology, University of Pittsburgh and Department of Veterans Affairs Medical Center, Pittsburgh, PA, USA.

We previously identified Annexin II (AX-II) as an autocrine/paracrine factor secreted by osteoclasts (OCL), that induces OCL formation and bone resorption *in vitro*. AX-II induced GM-CSF production by bone marrow stromal cells and activated T-cells, which increases OCL precursor proliferation, and further enhanced OCL formation. We have identified a receptor for AX-II on marrow stromal cells that preferentially bound the P11 subunit of the AX-II ($(P_{36})_2P_{(11)})_2$). However, induction of GM-CSF by AX-II did not fully explain its effects on OCL formation. In this study, we tested the capacity of AX-II to induce expression of receptor activator of nuclear factor (NF-κB) ligand (RANKL), and

osteoprotegerin (OPG) as well as the corresponding molecular signaling pathways in human marrow stromal cells (SAKA-T). Western blot assays showed that AX-II significantly increases membrane-bound RANKL (4 folds) compared to control human bone marrow cells. ELISA analysis identified soluble RANKL in the stromal cell conditioned media treated with AXII. Real time PCR analysis further demonstrated a dose dependent decrease in OPG mRNA expression in the human marrow stromal cell line, SAKA-T cells, stimulated with AX-II. Treatment of SAKA-T cells with AX-II activated MAP-kinase (ERKs). PD89059, a specific inhibitor of MEK, abolished the effects of Annexin II on membrane-bound RANKL expression in the SAKA-T cells and human bone marrow cells. These data suggest that the stimulation of OCL formation by AXII is due in part to modulation of the ratio of RANKL and OPG synthesis by marrow stromal cells through a MAPK-dependent pathway. Therefore, AXII is an important physiologic regulator of osteoclastogenesis that can stimulate the proliferation of OCL precursors by GM-CSF, and their differentiation to mature OCL via RANKL.

Disclosures: ***F.H. Li****, None.*

SA268

See Friday Plenary number F268

SA269

Osteoclast Differentiation In the Presence of TGFβ Elevates NFκB Activation and Down Regulates PTEN Expression. A. Gingery[1], M. K. Karst*[2], M. J. Oursler[3]. [1]Biochemistry, University of MN, Duluth, MN, USA, [2]Biology, University of MN, Duluth, MN, USA, [3]Biology and Medical Microbiology and Immunology, University of MN, Duluth, MN, USA.

Understanding the regulation of osteoclast survival is pivotal to understanding osteoclast actions during normal and pathological bone degradation. In our studies we examined the mechanisms of second messenger signaling using osteoclasts generated from murine co-cultures. TGFβ regulates survival of many cells and is released from bone during bone resorption. Thus, there is more active TGFβ present when bone resorption is elevated. Considering these physiological conditions in the normal and pathological bone microenvironment, it is important to discover the impact of differentiation in the presence of TGFβ on osteoclast survival. We investigated the survival signals involved in TGFβ -induced osteoclasts (differentiated in the presence of TGFβ) and compared these with osteoclast that have been differentiated in the absence of TGFβ (naïve osteoclasts). Once mature, stromal cells were removed to study osteoclast survival mechanisms. TGFβ -induced osteoclasts that were further cultured with TGFβ have both increased survival and activation of survival second messengers compared to both naïve osteoclasts and TGFβ -induced osteoclasts withdrawn from TGFβ when further cultured. Since NFκB is known to promote survival we looked at levels of NFκB using EMSA analysis. We found higher levels of NFκB activation in TGFβ -induced osteoclasts compared with naïve osteoclasts. When NFκB activation was inhibited there was reduced osteoclast survival within 90 minutes of further culture. IKK activation results in the phosphorylation of IκB and the subsequent release of NFκB, allowing NFκB to translocate to the nucleus. Activation of IKK was evident within 30 minutes of further culture. TGFβ -induced osteoclasts also have increased levels of active Akt, an upstream activator of NFκB, within in 30 minutes of further culture. Chemical blocking of Akt results in decreased survival within 90 minutes of further culture. Osteoclast survival involves PI3K-mediated activation of Akt/NFκB and MEK/ERK pathways. Chemically blocking PI3K significantly decreases osteoclast survival within 90 minutes of further culture. Since PTEN acts as a repressor of PI3K pathway, we have examined PTEN levels. Interestingly, mature TGFβ -induced osteoclasts have lower levels of PTEN compared to naïve osteoclasts. These data support that signals from PI3K are important components of osteoclast survival and that these survival signals include NFκB activation and may hinge on PTEN regulation. In conclusion we have identified a novel regulator of PI3K survival signaling in osteoclasts, PTEN.

Disclosures: ***A. Gingery****, None.*

SA270

See Friday Plenary number F270

SA271

Genomic Structure and Regulation of Expression of the Microphthalmia Transcription Factor (Mitf) in Osteoclasts. C. L. Hershey, D. E. Fisher. Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.

Microphthalmia transcription factor (Mitf) is a basic/helix-loop-helix/leucine zipper transcription factor key to the development and function of melanocytes and osteoclasts. The murine *mitf*-mutant (*mitf^{mi}/mitf^{mi}*) osteoclasts are predominantly mononuclear, lack a ruffled border and fail to resorb bone matrix resulting in osteopetrosis. Mitf is expressed as multiple isoforms each containing unique first exons, which variably contain open reading frames. At present eight isoforms have been published (Mitf-A, C, Mc, E, H, D, B and M). The expression of the different isoforms is governed by split promoter regulation, with each isoform having its own promoter. To understand how Mitf is regulated within osteoclasts, we wished to obtain a detailed characterization of its genomic structure, including potential transcriptional regulatory elements that may modify Mitf expression *in vivo*. RT-PCR analysis revealed that the A, H and B isoforms are expressed in osteoclasts, whereas the M isoform was not detected. We used genome sequence databases to map the location of the various isoforms and found the human *MITF* locus is ~229kb and the mouse *mitf* locus is ~208kb stretching from the most 5' isoform to the end of the 3'UTR. Previously we have shown that expression of Mitf in melanocytes is responsive to cAMP induction, however, expression in macrophages is not responsive to cAMP, suggesting differential promoter regulation. We used the MatInspector software to analyze the various promoters for potential transcription factor binding sites, including those that are conserved across species. Analysis of ESTs corresponding to the *MITF/mitf* gene has shown that Mitf is transcribed in many tissue types, yet some isoforms are tissue restricted in their expression (e.g. Mitf-M in melanocytes and melanoma). We have also identified a new isoform of Mitf, Mitf-J, from the analysis of ESTs corresponding to the *MITF* gene. By RT-PCR analysis this new isoform is expressed in various cell types including primary osteoclasts and ARPE-19 (retinal pigment epithelium) cells. This study provides a detailed analysis of the human and mouse 200kb Mitf gene. The identification of multiple transcriptional regulatory motifs in Mitf promoters highlights mechanistic links between this functionally important transcription factor and signaling pathways that may regulate its expression in osteoclasts. Efforts are underway to investigate the mechanism of regulation of Mitf expression in osteoclasts, focusing on the isoforms we have shown are expressed in these cells, namely Mitf-A, H, B and J.

Disclosures: ***C.L. Hershey****, None.*

SA272

See Friday Plenary number F272

SA273

Importance of the Calcineurin/NFAT Pathway for Osteoclastogenesis in RAW 264.7 Cells. H. Hirotani*[1], N. A. Tuohy*[1], J. T. Woo[1], P. H. Stern[1], N. A. Clipstone*[2]. [1]Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago, IL, USA, [2]Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

Although best known for its role in T lymphocyte activation, the calcineurin/nuclear factor of activated T cells (NFAT) signaling pathway is also known to be involved in a wide range of other biological responses in a variety of cell types. We investigated the role of the calcineurin/NFAT signaling pathway in the regulation of osteoclast differentiation. RAW 264.7 monocyte-macrophage precursor cells were cultured for 5 days in 96-well plates in alpha-MEM/10% fetal bovine serum/penicillin/streptomycin with or without human soluble RANKL (3-100 ng/ml). Inhibition of calcineurin with either the immunosuppressant drugs cyclosporin A (CsA) and FK506 or the retrovirally-mediated ectopic expression of a specific calcineurin inhibitory peptide, VIVIT, all potently inhibited the RANKL-induced differentiation of the RAW264.7 cells, as indicated by decreases in tartrate-resistant acid phosphatase (TRAP) activity, in the number of TRAP-positive multinucleated cells, and in the ability of the cells to resorb a mineralized matrix substrate. In addition, we found that the NFAT family members NFATc1, NFATc2 and NFATc3 are expressed in RAW264.7 cells and that their expression is upregulated in response to RANKL stimulation. Most importantly, we found ectopic expression of a constitutively active, calcineurin-independent NFATc1 mutant in RAW264.7 cells is sufficient to induce these cells to differentiate into large mature multinucleated osteoclasts capable of resorbing the mineralized substrate. The results demonstrate that calcineurin is an essential downstream effector of the RANKL-induced signal transduction pathway leading towards osteoclast differentiation and furthermore indicate that activation of the NFATc1 transcription factor is sufficient to initiate a genetic program that results in the specification of the mature osteoclast cell fate.

Disclosures: ***H. Hirotani****, None.*

SA274

See Friday Plenary number F274

SA275

The Structurally Unique Osteoclastic Protein-Tyrosine Phosphatase Is Driven by an Intronic Promoter that Is Cell Type-Specific by Virtue of Repressor Elements. K. H. W. Lau, M. Amoui*, J. B. Tillman*, D. J. Baylink. Musculoskeletal Disease Center, Jerry L. Pettis Mem VAMC, Loma Linda, CA, USA.

A structurally unique osteoclastic protein-tyrosine phosphatase (PTP-oc), which is essential for osteoclast activity, shows sequence identity with the intracellular domain of a renal receptor-like transmembrane PTP, termed GLEPP1. Because PTP-oc and GLEPP1 share the same gene, it has been assumed that PTP-oc is a truncated variant of GLEPP1, resulting from alternative splicing. However, the 5'UTR sequence of PTP-oc mRNA contains 217 bp from an intron of GLEPP1. There are no splicing acceptor sites at the PTP-oc transcription site. The intronic sequence flanking the 5'end of the PTP-oc transcription start site also contains potential promoter elements (e.g., TATA and CAT boxes) essential for transcriptional initiation. Accordingly, this study sought to test the hypothesis that the PTP-oc gene has its own intronic, tissue-specific promoter. To test this hypothesis, the basal promoter activity of a 1.3-kb PCR fragment covering the 5'-flanking proximal region of the PTP-oc gene was measured with the luciferase-based assay system. The putative PTP-oc promoter fragment showed relatively strong basal promoter activity in U937 cells, which was approximately 30% of that of SV40 promoter. Mutation of the putative TATA box within the PTP-oc promoter abolished 60% to 90% of its basal promoter activity. The 1.3-kb PTP-oc promoter fragment showed strong promoter activity in cells that express PTP-oc

(i.e., U937 cells and RAW264.7) but not in cells that do not express the enzyme (i.e., skin fibroblasts, TE85 cells, and HEK293 cells). These findings together strongly support the conclusion that the 1.3-kb intronic fragment contains the tissue-specific, PTP-oc proximal promoter. Deletion analysis of the putative PTP-oc promoter revealed that removal of the 5' sequence flanking the putative TATA box enhanced the basal promoter activity by 4- to 5-fold, suggesting that the 5' sequence contains repressor elements. The removal of the putative repressor elements led to the loss of tissue specificity since the truncated promoter showed high promoter activity in all test cell types. In conclusion, this study demonstrates for the first time that 1) there is an intronic promoter within the GLEPP1 gene that drives the expression of the PTP-oc in a cell type-specific manner, 2) the cell type-specificity is conferred by repressive elements, and 3) this GLEPP1/PTP-oc gene system is one of the very few, if not the only system, in which two important tissue-specific enzymes are derived from the same gene through the use of intronic promoters.

Disclosures: ***K.H.W. Lau****, None.*

SA276

See Friday Plenary number F276

SA277

The GTPase Activity of Dynamin in Osteoclasts Is Involved in the Regulation of Bone Resorption. A. Bruzzaniti, L. Neff, A. Sanjay, R. Baron. Yale University, New Haven, CT, USA.

Dynamin is a large GTPase that plays an essential role in receptor-mediated endocytosis. Dynamin is also a substrate of Src, and phosphorylation by Src increases the GTPase activity of dynamin. Recent findings have shown that dynamin also associates with actin and is involved in actin remodeling, a function essential for cell migration. Given that osteoclast (OC) function is dependent both on endocytosis and cell migration, we have explored the possibility that dynamin plays a role in bone resorption. We found that dynamin is expressed in OCs within individual podosomes at early time points following attachment, coincident with the expression of the podosome markers, actin and vinculin. At steady state, i.e. when the actin ring has expanded to the periphery of the cell, dynamin expression was more restricted to the inner edge of the actin ring, suggesting it may have a role in podosome disassembly. The distribution of dynamin to the actin ring was Src-dependent and was disrupted in OCs derived from Src -/- mice, which fail to form an actin ring. Immunoprecipitation studies showed that dynamin constitutively associates with c-Cbl in OCs, independent of its GTPase activity. Interestingly, the dynamin-Cbl association was destabilized by Src kinase activity and conversely, stabilized by inhibition of Src activity or by expression of a Cbl mutant that cannot bind Src, suggesting that binding and subsequent phosphorylation of Cbl by Src were necessary to dissociate dynamin from Cbl. To further examine the functional role of dynamin, we over-expressed dynamin and GTPase-inactive dynamin DynK44A in OCs with adenovirus and characterized their effects on podosome formation, actin remodeling and bone resorption. Osteoclasts over-expressing dynamin exhibited morphological changes including increased membrane ruffling and spreading. In contrast, OCs infected with GTPase-inactive DynK44A were retracted, contained fewer podosomes and exhibited decreased resorption on dentin. Since dynamin-Cbl binding is regulated by Src activity, we then co-expressed dynamin with kinase-inactive SrcK295M in OCs. While SrcK295M-infected cells contained actin patches instead of rings and failed to resorb bone, cells co-infected with dynamin and SrcK295M contained small actin rings. These studies suggest that dynamin, Cbl and Src coordinately participate in signaling events that regulate podosome assembly and disassembly and thereby, osteoclast motility and bone resorption.

Disclosures: ***A. Bruzzaniti****, None.*

SA278

Just How Cellular is Woven Bone? C. J. Hernandez, R. J. Majeska, M. B. Schaffler. Leni and Peter W. May Department of Orthopaedics, Mt. Sinai School of Medicine, New York, NY, USA.

Woven bone tissue forms in cases of rapid bone proliferation. In addition to displaying a more random collagen orientation than other cortical bone tissue, woven bone has been estimated to contain 4 to 8 times more osteocytes per unit volume than cortical tissue. Quantitative data for osteocyte number in woven bone have not been reported, but are of great interest in light of recent studies that have linked osteocyte number to the targeting of bone remodeling and the regulation of bone volume. In this study we evaluate the osteocyte density (Ot.N, cells/mm2 bone tissue) of woven bone and cortical bone of the rat. Adult (400g) male Sprague-Dawley rats (n=5) experienced a controlled femoral fracture using the Bonnarens-Einhorn method in order to induce woven bone; animals were sacrificed 21 days later. Osteocyte density was evaluated on longitudinal sections using light microscopy. Ot.N was measured in woven bone at three sites: 1) in the center of the fracture callus, 2) in buttressing callus on the periosteum adjacent to the fracture and 3) primary spongiosa near the growth plate of the distal femur. These values were compared to osteocyte density measurements in cortical bone far from the fracture. Osteocyte density of woven bone in the center of the fracture callus (1699 ± 291, Mean ± S.D.) and in the primary spongiosa (1403 ± 233) was significantly greater than the osteocyte density in cortical bone (968 ± 120, $p < 0.001$). Osteocyte density in the buttressing callus (1111 ± 300) was not significantly different from that in cortical bone. These findings contradict the widely held notion that woven bone is much more cellular than cortical bone tissue. Rather than the expected 400-800% greater osteocyte density estimated in the literature, woven bone in the rat has, at the most, only 40-80% more osteocytes than cortical tissue. Moreover, osteocyte density was not uniform. Osteocyte density in woven bone of the buttressing callus was similar to that of cortical bone but was less than that near the center of the fracture callus and in the primary spongiosa. A possible explanation is that woven bone in the buttressing callus forms from the periosteum through intramembranous ossification, while woven bone in the center of the callus and at the primary spongiosa are endochondrally derived. Thus, the process through which woven bone tissue is formed determines its cellularity and may influence its bioactive properties.

Disclosures: ***C.J. Hernandez****, None.*

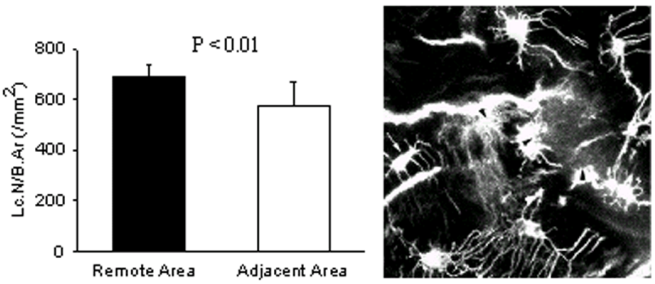
SA279

See Friday Plenary number F279

SA280

Do Osteocyte Lacunae Initiate Microdamage Under Physiological Conditions? S. Qiu[1], D. Rao[1], S. Palnitkar*[1], A. Parfitt[2]. [1]Bone & Mineral Research Laboratory, Henry Ford Hospital, Detroit, MI, USA, [2]Division of Endocrinology and Center for Osteoporosis and Metabolic Bone Disease, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

It has been suggested that the osteocyte lacuna is the stress concentrator in bone which is responsible for the initiation of microdamage. However, this scenario may only occur when the skeleton sustains pathological high loading. There is no evidence that osteocyte lacunae initiate microdamage under physiological conditions.
Rib sections from 9 white men aged 50-59 years were stained with basic fuchsin and examined using bright-field and fluorescent microscopy. We have developed a special method to measure lacunar densities (Lc.N/B.Ar) in the areas adjacent to and remote from linear crack(s) in interstitial bone. In addition, osteocyte lacunae within 50 µm of linear cracks were examined using confocal microscopy.
Lacunar density was 16% lower in the area adjacent to crack(s) than in the area remote from crack(s) ($p < 0.01$)(Fig 1). Damaged osteocyte lacunae were enclosed by clustered tiny cracks or fuzzy stain (arrow heads, Fig 2). The lengths of cracks or the thickness of fuzzy stain were about 10-20 µm, and the cracks around different lacunae rarely connected with each other. Only about 7% of the osteocyte lacunae were encircled by submicroscopic cracks or fuzzy stain ($p < 0.001$).
Confocal microscopy showed that very few of the osteocyte lacunae in normal bone were associated with submicroscopic damage, suggesting that osteocyte lacunae in living bone do not contribute to the initiation of microdamage. This is probably because the maximum strain surrounding the lacunae does not exceed the peak physiological strain. In contrast, microdamage occurs preferentially in bone with impaired osteocyte lacunar-canalicular network.



Disclosures: ***S. Qiu****, None.*

SA281

MLO-Y4 Cells Produce an Unknown Chemoattrantant for Osteoclast Precursors. S. Ko, S. E. Harris, M. Dallas*, L. F. Bonewald. Oral Biology, University of Missouri at Kansas City, Kansas City, MO, USA.

The osteocyte most likely plays a role in bone remodeling by instructing osteoclasts to remove bone at specific sites. This entire process includes recruitment through chemotaxis of osteoclast precursors and proliferation of these precursors followed by fusion and activation of mature osteoclasts. Osteoblasts/stromal cells have been shown to support osteoclast formation and activation upon treatment with bone resorbing cytokines. The two major components necessary for this process are M-CSF and RANKLigand. The osteocyte-like cell line MLO-Y4 supports osteoclast formation and activation in the absence of any resorbing factor through the constitutive production of M-CSF and expression of RANKLigand on their dendritic processes.
To determine if MLO-Y4 cells will also support chemotaxis and proliferation of osteoclast precursors, two cell lines RAW 264.7 and MOCP-5 were utilized and migration was assayed using transwells. These cells form TRAP positive multinucleated cells upon the addition of soluble RANKLigand. Conditioned media alone from MLO-Y4 cells was sufficient to support proliferation and chemotaxis of both osteoclast precursor cell lines, unlike the support of osteoclast formation by bone marrow cells which required cell to cell contact. The MLO-Y4 cells produced much greater amounts of the proliferation activity and chemotactic activity than 2T3 osteoblasts or NIH3T3 fibroblasts. Proliferation was completely inhibited by neutralizing antibody to M-CSF whereas chemotaxis was not. This was surprising as M-CSF is a well-known chemotactic factor. The capacity of recombinant M-CSF to provoke chemotaxis in RAW 264.7 and MOCP-5 cells was determined, but the maximal response did not reach the maximal response of the conditioned media. Gene array analysis was used to identify potential chemoattractants. MLO-Y4 cells express much greater amounts of mRNA for MIP-2α, VEGF, MCP-3 and TGFβ1 compared to 2T3

osteoblast cells. MIP-2α and VEGF did not stimulate transmigration of the osteoclast precursor cells. MCP-3 stimulated RAW 264.7, but not MOCP-5 chemotaxis. The effects of TGFβ1 were modest compared to the conditioned media or to M-CSF. Sufficient neutralizing antibody to TGFβ1 and MCP-3 had no effect. M-CSF does not appear to synergize with either TGFβ or MCP-3. SDF-1, a potent chemoattractant is not made by MLO-Y4 cells. To date, an unknown factor exists that has the potential to synergize with M-CSF to stimulate osteoclast precursor chemotaxis. Identification of this factor should aid in the identification of chemotactic factors for osteoclast precursors made by primary osteocytes.

Disclosures: ***S. Ko****, None.*

SA282

Characterization of a Monoclonal Antibody Specific for Osteocytes by Immunostaining but Not Specific by Western Blot Analysis. K. Zhang*, L. Ye*, Z. Li*, J. Rosser*, L. F. Bonewald. Oral Biology, Dental school, UMKC, Kansas city, MO, USA.

A monoclonal antibody called E11 that reacted specifically with rat osteocytes *in vivo* was generated by Wetterwald and coworkers (Bone 18:125-132, 1996). Previously, we too had generated a mouse osteocyte specific monoclonal antibody called 9C11. These antibodies reacted specifically with osteocytes and not with osteoblasts or other tissues. However, the gene encoding for this antigen was found to be expressed in a number of tissues, but mainly in cell types with cellular projections such as podocytes in kidney, Type 1 alveolar lung cells, and thymic epithelial cells. Keratinocytes stably transfected with this gene develop processes suggesting that the function of the gene product is the generation of cellular extensions. However, the rational for the specificity of E11 and 9C11 antibodies for osteocytes was not clear with such wide-spread gene expression in other tissues. To address this issue, we obtained a hamster monoclonal antibody, 8.1.1, to thymic epithelial cells from Dr. Andrew Farr (J Histochem Cytochem 40:651-664,1992). This antibody recognizes the same gene product as E11 and 9C11. By western blotting, this antibody recognized 40kD bands of similar intensity in MLO-Y4 osteocyte-like cells, MLO-A5 mineralizing cells, 2T3 and MC3T3 osteoblasts, NIH 3T3 fibroblasts and murine calvarial cells. The immunoreactive bands were of slightly different sizes with the MLO-Y4 band the smallest, 40 kD versus 42 kD in the other cells. These same cells express similar amount of mRNA as determined by RT-PCR. However, when immunohistochemical staining was performed on 19-day old mouse long bone sections, only osteocytes were stained by this antibody, not osteoblasts. There was no reactivity with growth plate, bone marrow, nor muscle. These results suggest that the E11 protein is expressed in osteoblasts, but that the epitope in osteoblasts for 8.1.1 antibody is masked or hidden. The data also suggest that the epitope in osteocytes in bone is exposed, perhaps due to post-translational modifications. Comparisons of the antigen from osteoblasts and from osteocytes is underway using 2-dimensional gel electrophoresis and mass spectrometry.

Disclosures: ***K. Zhang****, None.*

SA283

Precision of Height Measurement in Osteoporotic Subjects. K. Siminoski[1], T. Johnson*[2], G. Cline*[2]. [1]Radiology and Diagnostic Imaging, and Internal Medicine, University of Alberta, Edmonton, AB, Canada, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Serial measurement of total body height is a fundamental tool in the assessment of osteoporotic patients, with the primary goal being the detection of vertebral fractures. Given that the average height loss due to vertebral fracture is about 1 cm per fracture, precision error of height measurements has a direct bearing on the use of height monitoring in individual patients.

In this investigation, we have analyzed the long-term precision error of repeated height determinations. Subjects were women from 190 research centers in the placebo arms of the VERT MN/NA risedronate studies (average age 69.0 years; range: 38-85 years) who had data for height and vertebral morphometry. Height was measured with a stadiometer to the closest millimeter. Lateral vertebral radiographs were performed at the same times and were analyzed by digital morphometry from T4 to L4. Vertebral fractures were determined using both quantitative and semiquantitative methods. Only women who did not sustain a new vertebral fracture were studied. Precision errors over one-year intervals were calculated as root-mean-square standard deviations and coefficients of variation. These values were used to derive the amount of change necessary to detect a statistically significant difference on repeat measurement (at a 95% level of confidence). The least significant changes (LSC) for single replicates (LSC-1) and triplicate replicates (LSC-3) were determined.

The numbers of subjects with complete data over the three one-year intervals ranged from 573 to 713. The coefficents of variation ranged from 0.64% to 1.20%. The corresponding LSC-1 (for single measurements) at each time point were 2.8 cm to 5.2 cm, and LSC-3 (for triplicate measurements) ranged from 1.6 cm to 3.0 cm.

These results show that precision variability in height measurement is substantial in comparison to the amount of height loss that occurs due to vertebral fractures, even using a stadiometer. Triplicate measurements at each clinical evaluation reduce the LSC.

Height Measurement Precision

Time Interval (start yr-end yr)	n	CV	LSC-1	LSC-3
0-1	713	0.69	3.0	1.7
1-2	601	0.64	2.8	1.6
2-3	573	1.20	5.2	3.0

CV = coefficient of variation. LSC-1 = least significant change (95% confidence) for single measures at each time. LSC-3 = least significant change for triplicate measures at each time.

Disclosures: ***K. Siminoski****, P&G Pharmaceuticals 2, 5, 8.*

SA284

See Friday Plenary number F284

SA285

Predictive Value of Low Bone Mass for One Year Fracture Outcomes in NORA: Comparison of Results in Women Age 50-64 and Age 65 and Over. E. S. Siris[1], S. K. Brenneman[2], T. W. Weiss*[2], C. Ya-Ting[2]. [1]Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY, USA, [2]Outcomes Research, Merck & Co., Inc., West Point, PA, USA.

Low bone mass and fractures are prevalent in older postmenopausal women. Little information is available about the frequency of low bone mass and its association with fracture in early postmenopausal women. We evaluated the frequency of and association between low bone mass and fracture in women age 50-64 in comparison to their counterparts age 65 and over. NORA enrolled 200,160 postmenopausal women age 50 and older that had no prior diagnosis of osteoporosis. Baseline bone mineral density (BMD) was measured at the heel, forearm, or finger. A one-year follow-up survey requesting incident fractures since baseline was completed by 163,935 women. The association between BMD and fracture was assessed using logistic regression, adjusted for important covariates. In women age 50-64, 31% had low bone mass (T-scores <= -1.0) whereas 62% of women age 65 over had low bone mass. Fracture occurrence and association with BMD is shown in Table below.

	50-64	65 and over
N	87,594	76,341
No. (%) Osteoporotic Fractures	905 (37%)	1535 (63%)
Fracture Rate / 1000 person-years (95% CI)	8.4 (7.9, 9.0)	16.5 (15.6, 17.3)
RR of Fracture / 1 SD decrease in T-score (95% CI)*	1.5 (1.4, 1.6)	1.5 (1.4, 1.5)
RR of fracture per T-score category (95% CI; reference category > -1.0)		
-1.0 to -2.0	1.8 (1.5, 2.0)	1.6 (1.4, 1.8)
≤ -2.0	2.6 (2.1, 3.2)	2.6 (2.2, 2.9)

*RR: Relative Risk based on logistic regression model adjusting for BMD, prior fracture, health status, HRT usage maternal prior fracture, race, cortisone use, and densitometry device.

We conclude that about one third of postmenopausal women in NORA aged 50-64 had low bone mass. Although the absolute fracture rate was lower in these younger women, their relative risk for fracture within one year of the BMD measurement was similar to that in older women in NORA (age 65 and over) for each standard deviation decrease in T-score and by T-score category.

Disclosures: ***E.S. Siris****, Merck & Co., Inc 2, 5, 8; Procter & Gamble/Aventis (Alliance for Better Bone Health) 5, 8; Eli Lilly 2, 5, 8; Wyeth 5, 8.*

SA286

See Friday Plenary number F286

SA287

Comparison of BMD and Fracture Prediction Across Skeletal Sites: Evidence from National Osteoporosis Risk Assessment (NORA). P. D. Miller[1], S. K. Brenneman[2], S. Barlas*[2], T. A. Abbott[2]. [1]Colorado Center for Bone Research, Lakewood, CO, USA, [2]Outcomes Research & Management, Merck & Co., Inc., West Point, PA, USA.

Bone mineral density (BMD) measurements at both peripheral and central skeletal sites have been shown to predict risk of fracture. However, discordance of BMD measurements across sites exists within the same individual. It is not known whether BMD at the central sites is more predictive of fracture than those at the peripheral sites. We compared BMD measurements at 3 skeletal sites, hip, spine, and forearm, to determine the extent to which they concur and their ability to identify women with incident osteoporotic fractures within two years of measurement. Data from the Specialist Arm of NORA were used for this analysis. BMD T-scores were calculated using young adult reference database provided by the manufacturer. Fractures occurring in the subsequent two years were identified by self-report. The current analysis included women who had BMD measured at baseline at all 3 sites: hip (femoral neck), spine (total spine) and forearm (distal radius), and responded to the follow-up surveys. 685 women, mean age 63.7 (SD 8.5) years, reported 45 osteoporosis-related fractures (hip, rib, wrist/forearm and spine). Of the 685 women, 133, 163, and 201 had T-scores ≤ -2.5 at the femoral neck, total spine, and distal radius, respectively. Of the 306 women who were osteoporotic at any site, 51 (17%) were osteoporotic at all 3 sites, 89 (29%) at 2 of 3 sites and 166 (54%) at only one site. Of the 45 osteoporotic fractures, 29 (64%) were identified by T-score ≤ -2.5 at any site. There were no meaningful differences in the proportion of the fractures detected in women with BMD T-scores ≤ -2.5 using single site measures at the femoral neck, total spine and distal radius: 16 (36%), 14 (31%), and 15 (33%) fractures identified, respectively. We conclude that both central and peripheral BMD T-scores of ≤ -2.5 identify women at risk for fracture. However, a single BMD measurement resulting in a T-score > -2.5 does not necessarily rule-out osteoporosis at another site.

Disclosures: ***P.D. Miller****, Merck & Co., Inc. 2, 6, 8; Procter & Gamble Pharmaceuticals 2, 6; Eli Lilly and Company 2, 6; Wyeth 2.*

SA288

See Friday Plenary number F288

SA289

Native Canadian (First Nations) Status is a Marker for High Fracture Risk. W. D. Leslie[1], S. Derksen*[2], C. Metge*[2], L. Lix*[2], E. Salamon*[3], P. Wood Steiman*[4], L. L. Roos*[2]. [1]Dept. of Medicine, St. Boniface General Hospital, Winnipeg, MB, Canada, [2]Manitoba Centre for Health Policy, Winnipeg, MB, Canada, [3]Medicine, St. Boniface General Hospital, Winnipeg, MB, Canada, [4]Island Lake Tribal Council, Island Lake, MB, Canada.

Fracture rates are one index for the prevalence of osteoporosis in a population. Ethnicity is one of many factors that have shown to be determinants of fracture risk and bone density. Native Canadian (First Nations) status in Manitoba defines an ethnic population with unique cultural, socioeconomic and health related factors that could affect fracture rates. We performed a retrospective, population-based matched (1:3) cohort study using the Manitoba administrative data repository from 1987 to 1999. The cohort contained 32,692 First Nations (384,012 person-years follow-up) and 98,076 controls (1,085,778 person-years). Age- and sex-adjusted standardized incidence rate ratios (SIR) are produced for First-Nations and non-First Nations cohorts, and Poisson regression is used to model the incidence fracture data with demographic, socioeconomic, health, and geographic predictors. First Nations had significantly higher rates of any fracture (SIR 2.06, 95 percent CI 2.00 to 2.11). A similar age-related and female-predominant pattern was seen for wrist fractures (SIR 2.96, CI 2.61 to 3.35), spine fractures (SIR 1.93, CI 1.74 to 2.14) and hip fractures (SIR 1.92, CI 1.69 to 2.21). In contrast, craniofacial fractures (SIR 4.96, CI 4.65 to 5.30) were predominant in males and younger adults. Income class (P<.0001), geographic residence area (P<.0001), and diagnosis of diabetes mellitus (P<.0001) were also associated with fracture rates, but adjustment for these factors did not appreciably reduce the marked excess fracture risk seen in First Nations. We conclude that Manitoba First Nations are a previously unidentified group at high risk for fractures.

SIR for fractures: First Nations vs age-matched controls (95% CI).

	Males	Females	Combined
Any site	2.19 (2.12-2.67)	2.26 (2.20-2.36)	2.23 (2.18-2.29
Hip	2.10 (1.68-2.63)	1.70 (1.41-2.05)	1.85 (1.61-2.14)
Wrist	2.78 (2.29-3.39)	3.18 (2.68-3.79)	3.00 (2.63-3.42)
Spine	1.79 (1.54-2.08)	2.18 (1.88-2.51)	2.18 (1.88-2.51)
Craniofacial	4.89 (4.51-5.29)	5.48 (4.88-6.19)	5.07 (4.74-5.42)

Disclosures: ***W.D. Leslie****, None.*

SA290

See Friday Plenary number F290

SA291

Fractures, Bone Mass and Falls in Older Women with Parkinson's Disease. J. L. Schneider*[1], H. A. Fink*[2], K. Yaffe*[1], K. L. Stone[1], S. R. Cummings[3]. [1]University of California, San Francisco, CA, USA, [2]Veterans Administration Medical Center, Minneapolis, MN, USA, [3]University of California and California Pacific Research Institute, San Francisco, CA, USA.

A few studies have suggested that women with Parkinson's Disease (PD) have an increased risk of falling and hip fracture. No studies have examined the relationship between PD and BMD or PD and other types of fractures.

In the Study of Osteoporotic Fractures (SOF), a prospective study of older women, we analyzed the relationship between PD, and BMD, hip and other types of fractures, and falls in 8412 women. Seventy-three women reported a physician diagnosis of PD. Incident falls and fractures were ascertained during a mean 7.3 years of complete follow-up. All fractures were confirmed by radiology reports.

Women with PD had on average 7.2% lower age adjusted total hip BMD (p=0.01) and this was attenuated by adjustment for weight (p=0.22) and 3.7-fold increased risk of multiple falls (2+per year) (Relative Hazard=3.7, 95% CI 1.9-7.1). Women with PD had significantly increased risks of hip and other fractures (table). We estimated that women with PD have 30% risk of hip fracture over 5 years, compared to 5% for women without PD. The increased risk of hip fracture was independent of BMD.

Hazard Ratios for Hip and non-spine, fx associated with PD

	Hip fx HR (95% CI)	Non-spine fx RR (95% CI)
Age adj	2.89 (1.59, 5.25)	1.96 (1.30, 2.95)
Age, weight adj	2.57 (1.27, 5.17)	1.65 (0.99, 2.75)
Age, weight , BMD adj	2.88 (1.36, 6.09)	1.64 (0.95, 2.84)

We conclude that older women with PD have lower BMD, and an increased risk for falls and all types of fractures, including hip fractures. All older women with PD should be evaluated and, perhaps, treated to reduce their high risk of fractures.

Disclosures: ***J.L. Schneider****, None.*

SA292

See Friday Plenary number F292

SA293

Risk Factors for Fractures of Women Aged 48-67 Years. The 10-year Follow-up of OSTPRE Study. R. J. Honkanen. Research Institute of Public Health, University of Kuopio, 70280 Kuopio, Finland.

The Osteoporosis Risk Factor and Prevention (OSTPRE) study cohort was established in 1989 by selecting all women (N=14220) born in 1932-41 and resident in Kuopio Province, Finland. A total of 13100 women responded to baseline postal enquiry in 1989. The study population of the present study consists of those 11074 women who responded also to the 5-year and 10-year enquiries in 1994 and 1999. At baseline, 1865 women reported a fracture since the age of 15 (wrist fracture by 567 women). A total of 1915 women reported a follow-up fracture (ANY) during 1989-99 which could be verified by patient record perusal: 736 women had sustained a distal forearm fracture (DFF) and 381 women an ankle fracture (ANK).

Following variables increased the risk of fracture in multivariate logistic models: fracture history 1.8-fold for ANY fracture (p<0.001), 1.7 for DFF (p<0.001) and 1.4 for ANK (p=0.01); age for ANY (p=0.003) and DFF (p<0.001); use of prescribed drugs by 15 %/drug for ANK (p<0.001) and by 10 %/drug% for ANY (p<0.001); height 4 %/cm for ANY and DFF (p<0.001); smoking by 55 %/10 cigarettes for ANK (p<0.001); weight by 2 %/kg for ANK (p<0.001); physical activity for ANK (p=0.01) and menopause age by 2 % of ANK/year (p=0.02). Following factors were preventive: 100 mg of nutritional calcium prevented 5 % of DFF (p<0.001), 3 % of ANY (p<0.001) and 4 % of ANK (p=0.005); HRT (per one year use) prevented 7 % of DFF (p<0.001), 5 % of ANY (p<0.001) and 4 % of ANK (p=0.04); weight prevented 2 % of DFF/kg (p<0.001) and 1 % of ANY/kg (p=0.001). Following variables were not related to any of these three fracture groups, menarche age, parity, number of self-reported health disorders or work disability. In addition, fracture history predicted tibia, spine, hip and rib fracture.

Fracture history was a potent predictor, while HRT and nutritional calcium had a preventive effect for fractures of these women aged 48-67 years. Smoking and the use of drugs increased the risk of ankle fracture. The effects of putative risk/preventive factors vary by site of fracture.

Disclosures: ***R.J. Honkanen****, None.*

SA294

See Friday Plenary number F294

SA295

See Friday Plenary number F295

SA296

See Friday Plenary number F296

SA297

Heredity for Breast Cancer and Hip Fracture in Relation to Bone Density and Fractures. The Nordos Sudy. E. B. Waern*[1], O. Johnell[2], K. Åkesson*[2], D. Mellström[1]. [1]Dept of Geriatric Medicin, Center for Bone research, Göteborg, Sweden, [2]Dept of Orthopaedics, Malmö University Hospital, Malmö, Sweden.

Heredity for hip fracture is an independent predictor for fractures in women and high bone densiy is a predictor of breast cancer.

The question is if there are differences in bone density and fracture risk among daughters to women with hip fracture and breast cancer respectively. 772 70 year old women from a random sample of the population in Göteborg and Malmö participated in the study. Bone density was measured with Hologic 4500 A. General health was measured in a health questionaire.

91 women, or 12,5 percent, had heredity for hip fracture and 43 women, or 5,9 percent, had heredity for breast cancer. Only 0,6 percent had heredity for both breast cancer and hip fracture.

There were no differences in body weight or body height between women with heredity for breast cancer compared to women with heredity for hip fracture. Women with heredity for breast cancer had higher hip BMD 0.840 (0.140) compared to women with heredity for hip fracture, BMD 0.784 (0.129) p<0.005. The risk for prevalent fractures in women with heredity for breast cancer compared to women with heredity for hip fracture OR 0.221 (0.079-0.615).

A multivariate model showed that BMD hip, OR 1.54 per SD, heredity for hip fracture, OR 1.8 and body height, OR 1.2 per SD, were independant predictors for previous fractures between 50-70 years of age.

We conclude that heredity for hip fracture and breast cancer have important impact on fracture risk. Women with heredity for breast cancer had a higher hip BMD compared to women with heredity for hip fracture.

Disclosures: ***E.B. Waern****, None.*

SA298

See Friday Plenary number F298

SA299

Reduced Mechanosensitivity Is Associated with Fractures in the Old Order Amish. B. D. Mitchell[1], T. J. Beck[2], E. A. Streeten[1], D. J. McBride[1], A. L. Lodge*[1], K. Uusi-Rasi[2], A. R. Shuldiner*[1]. [1]Medicine, University of Maryland School of Medicine, Baltimore, MD, USA, [2]Radiology, Johns Hopkins University, Baltimore, MD, USA.

The sensitivity of bones to mechanical stimuli should be evident in the relationship between bone strength and skeletal load. Reduced mechanosensitivity should cause relatively weaker bones for equivalent skeletal loads and increase susceptibility to traumatic fracture. Underlying geometric differences in weaker bones should also lead to increased osteoporotic fragility in old age. The purpose of this study was to determine whether mechanosensitivity is related to fracture history. As an index of mechanosensitivity we analyzed the ratio of section modulus (index of bending strength) to lean body mass, the latter being a crude measure of muscle load. Total body and hip scans were obtained by DXA in participants of the Amish Family Osteoporosis Study. Section moduli were derived by Hip Structure Analysis (HSA) from hip DXA scans. Mechanosensitivity indexes (MSI) were created using total body lean mass and section moduli measured at three different hip sites: narrow neck (NN), intertrochanter (IT), and shaft (S).

The study population included 682 subjects (263 men, 419 women) ranging in age from 20-88 yrs. A total of 248 subjects (129 men, 119 women) reported having had one or more fractures during their lifetime. This total included 206 subjects reporting fractures resulting from moderate-to-severe trauma ("traumatic" fractures) and 57 reporting fractures resulting from low trauma, such as a fall from a standing height ("fragility" fractures). We compared mean bone mineral density (BMD) and mechanosensitivity between subjects with and without a history of fracture, accounting for correlations among family members. All analyses were adjusted for the effects of age, sex, height, and weight. MSI at the NN and IT were significantly lower in subjects reporting any fracture compared to those with no previous history of fracture ($p = 0.004$ and $p = 0.02$, respectively). In addition, subjects reporting "fragility" fractures had significantly lower MSI at the S ($p = 0.003$), while subjects reporting "traumatic" fractures had significantly lower MSI at the NN ($p = 0.004$). However, BMD was also uniformly lower in those reporting fractures; after simultaneously adjusting for differences in BMD between those with and without a fracture history, MSI at the S remained significantly associated with "fragility" fractures ($p = 0.003$), and MSI at the NN remained borderline associated with "traumatic" fractures ($p = 0.07$). We conclude that differences in mechanosensitivity may alter susceptibility to both "traumatic" and "fragility" fractures.

Disclosures: ***B.D. Mitchell****, None.*

SA300

See Friday Plenary number F300

SA301

Vertebral Deformities Prevalence in Postmenopausal Women: a Population-based National Study. R. Nuti[1], G. Guglielmi[2], G. Capelli*[3], D. de Feo*[4], G. Giuda*[5], U. Senin*[6], S. Adami[7]. [1]Internal Medicine, University of Siena, Siena, Italy, [2]Radiology, Scientific Institute Hospital, Foggia, Italy, [3]Public Health, University of Cassino, Frosinone, Italy, [4]Procter & Gamble, Rome, Italy, [5]Orthopaedics, University of Napoli, Napoli, Italy, [6]Internal Medicine, University of Perugia, Perugia, Italy, [7]Rheumatology, Valeggio Hospital, Verona, Italy.

FEDRO (Fracture Evaluation by Digital Radiography Observational study) is a national study aimed at measuring the prevalence of vertebral fractures (VFs) in a sample of Italian postmenopausal women. Fifty-eight University and Hospital centers in Italy participated in the study. Each center was asked to consecutively enrol ambulatory patients who met inclusion criteria (aged 60+ yrs, 5+ yrs postmenopausal, BMD t-score < -2) over a 6-month period.

A total of 2750 subjects were enrolled in the study. All subjects had undergone an X-ray evaluation of thoracic and lumbar spine. Each film was centrally digitized: T4-L4 vertebral heights ratio were measured by a radiologist using a morphometry software (Spine-X Analyzer; CAM Diagnostics, Milan, Italy). The deformities were defined as: mild, moderate or severe, based on a height ratio decrease of 20%-25%, 25%-35% and more than 35% respectively. Currently data on roughly 1500 women are under revision due to low quality radiographs. The table below shows the prevalence (%) by age and grading of VFs in those 885 subjects with data currently available.

60-64 (n=267)		**65-69 (n=262)**	**70-74 (n=187)**	**75-79 (n=115)**	**80+ (n=54)**	**ALL (n=885)**
1+VFs T4-L4	18.0	22.5	23.0	30.4	35.3	23.1
Mild	9.4	11.8	11.8	13.9	13.0	11.4
Moderate	7.1	9.5	7.5	13.9	16.7	9.4
Severe	1.5	1.2	3.7	2.6	5.6	2.3
2+VFs T4-L4	3.8	9.9	7.5	7.8	20.4	7.9
1+VFs T4-T12	15.7	19.9	19.8	27.0	26.0	19.9
Mild	9.0	12.6	10.7	14.8	13.0	11.4
Moderate	5.6	6.9	5.9	10.5	9.3	6.9
Severe	1.1	0.4	3.2	1.7	3.7	1.6
1+VFs L1-L4	7.1	11.1	7.0	9.6	13.0	9.0
Mild	5.2	6.1	4.3	4.4	3.7	5.1
Moderate	1.5	4.2	2.1	3.5	7.4	3.1
Severe	0.4	0.8	0.6	1.7	1.9	0.8

Preliminary results of this study confirm the high prevalence of undiagnosed VFs in elderly women (at least one VF in 23.1% in population with BMD t-score < -2), showing a strong age-effect trend. Physicians and health authorities should strongly consider the importance of raising awareness at national level on osteoporosis and fractures as a real "social disease".

Disclosures: ***R. Nuti****, None.*

SA302

See Friday Plenary number F302

SA303

See Friday Plenary number F303

SA304

Incident Rates of Hip Fractures in Mexicans Over 50 Years. P. Clark[1], P. Lavielle*[2], J. Salmeron*[3], S. R. Cummings[4]. [1]Clinical Epidemiology Unit, Centro Medico Nacional, IMSS-Faculty of Medicine UNAM, Mexico City, Mexico, [2]Clinical Epidemiology Unit, Centro Medico Nacional, IMSS, Mexico City, Mexico, [3]Epidemiology & Health Systems Unit, IMSS, Morelos, Mexico, [4]SF Coordinating Center, San Francisco, CA, USA.

We have recently shown that the rate of vertebral fractures is similar in Mexico and Whites in the US. The rates of hip fracture in Mexico have never been determined in a population-based study. To describe rates in urban Mexico, cases in individuals over 50 years of age were identified from institutional registers of all hospitals with hip surgery facilities from the two largest Health Systems in Mexico City. Cases were verified against surgical and x rays logs. Age, sex, type of fracture, ICD-10 coding and place of residence were obtain. Age stratified incident rates were developed using the CENSUS 2000 population data.

The rates of hip fracture increase exponentially with age in both genders. Age-standardized rates in Mexico (206 for women and 108 for men) were 2.5 lower than the rates in US non Hispanics for women and 1.6 times lower for men (510 and 174/100 000 for women and men respectably) but similar to rates found in urban Chinese and US Hispanics. This first population based study in Mexicans shows that the risk of hip fracture in Mexican women and men are lower than the US and Europe and similar to several other developing countries.

Age group	Total No. Cases		Population (per thousands)		Annual rate x 100,000 (IC)	
	Men	Women	Men	Women	Men	Women
50-59	29	38	145.99	192.51	20 (13-30)	20(14-28)
60-69	52	105	99.70	138.61	52 (36-73)	76 (58-98)
70-79	95	236	58.62	79.92	162(115-227)	295 (228-385)
80+	143	369	20.77	32.44	688(429-1134)	1,137(803-1711)

Disclosures: ***P. Clark****, None.*

SA305

See Friday Plenary number F305

SA306

Design and Methods for a Community-Based Study of Bone Health in Men: BACH Bone. S. S. Harris[1], A. B. Araujo*[1], M. F. Holick[2], J. B. McKinlay*[1]. [1]New England Research Institutes, Watertown, MA, USA, [2]School of Medicine, Boston University, Boston, MA, USA.

The distribution and determinants of osteoporosis have been far less studied in men and in ethnic and racial minorities than in Caucasian women. The purpose of the BACH Bone Study is to estimate the prevalence of low bone mineral density (BMD) and to examine its medical, hormonal, and behavioral correlates in a diverse, community-based, random sample of adult men. In addition, the study has been designed to facilitate anticipated follow-up measurements.

BACH Bone is a substudy in 900 men from the 6000 men and women now being enrolled in the Boston Area Community Health Survey (BACH). BACH is a survey of uro-gynecologic conditions and related measures in a random sample of Hispanic, African-American, and Caucasian urban adults aged 30-79. Men who complete an in-home BACH visit are invited to participate in BACH Bone by visiting a clinical research center. The BACH visit provides much of the data for BACH Bone, but the clinic visit adds DXA BMD and other bone-related measurements. This approach is efficient because it uses an existing cohort that can only be assembled with costly methods that include extensive field tracing of selected households, and field or telephone eligibility screening. In addition, this approach permits potential participants who might otherwise be wary of clinic-based research to have positive interview experiences in their homes and to receive study stipends before being approached for BACH Bone. As further incentive, the study provides free transportation, bone scan results, and a second stipend.

Data obtained at the BACH visit include demographics, physical measurements, gonadal and pituitary hormone measurements, and medical and lifestyle information. Data obtained at the clinic visit include DXA scans of the hip, spine, whole body, and forearm, and measurements of grip strength, physical and cognitive function, calcium-regulating hormones, and markers of bone turnover. Both studies collect and archive blood samples for future measurements.

Cohort maintenance activities are conducted in anticipation of future follow-up measurements. These include obtaining contact information for a person who will always know how to reach the participant, annual mailings to participants (birthday cards), use of postal service change-of-address notification, and continual updates to our computerized participant information database.

In conclusion, the BACH Bone study combines information from an in-home survey and a clinical site visit in an efficient approach to collecting comprehensive information about skeletal health in a random, multi-ethnic sample of urban men.

Disclosures: ***S.S. Harris****, None.*

SA307

See Friday Plenary number F307

SA308

Men with Idiopathic Vertebral Osteoporosis Have Low Lean (but not Fat) Mass and No Abnormality of COL1A1-Sp1 Genotype. M. W. J. Davie, S. Evans*, H. Davies*, C. Sharp*. Charles Salt Centre, Robt Jones and Agnes Hunt Hospital, Oswestry, United Kingdom.

Osteoporosis in men has been associated with small body size and low serum concentrations of IGF-I. Smaller frame size relates to low bone mass in normal men. We have investigated whether lean body mass and total bone mineral mass (BMC) are low in men with idiopathic vertebral fracture. We have also investigated whether the G>T mutation in the Sp1 binding site of the COLIA1 promoter region is associated with these variables in men.

We have determined COLIA1-Sp1 genotype, body composition, bone density (BMD) and stature in 33 consecutively referred consenting men (mean age 64±10, range 50 –78 yrs) with idiopathic vertebral fracture (IVO) and in 73 healthy (H) volunteers (61±8, 50-78yrs).Indices of body composition were measured using an Hologic QDR4500 densitometer. COLIA1-Sp1 genotypes were determined using a microtitre plate based method with a colorimetric end-point (Axis-Shield, Scotland, UK). Group comparisons were made using the Students t-Test and relationships by simple linear correlation.

Men with IVO were significantly lighter (73±9 vs 82±12 Kg) and shorter (169.5± 8 vs 174.3± 6 cm) than H (both p>0.001). Total body (tb-) BMC (2.1±0.3 vs 2.69±0.4 Kg) and tb-Lean mass (53.6±6 vs 59.3±7 Kg) were also lower in IVO (both p>0.0001). tb-Fat mass was not significantly different. Men with IVO had significantly lower BMD at the lumbar spine (0.880±0.2 vs 1.048±0.2 gcm^2), proximal femur (0.831±0.1 vs 1.017±0.1 gcm^2) and femoral neck (0.682±0.1 vs 0.831±0.1 gcm^2) (all p>0.0001). In both IVO and H men tb-BMC correlated with tb-Lean (r^2=0.3 and r^2=0.49, both p>0.001 respectively) but not with tb-Fat mass.COLIA1-Sp1 alleles were in Hardy-Weinberg equilibrium. Genotype frequencies were GG=72.7 / 68.5; GT=27.3 / 30.1; TT= 0 / 1.4 in IVO and H respectively, and their distributions were not significantly different. Classification of IVO and H groups into GG and GT/TT genotypes revealed no significant differences in any of the measured variables. However, men with the T allele in both IVO and H groups tended to have greater tb-Mass than those with the GG genotype (75±11 vs 72±8 and 83±14 vs 81±11 Kg respectively).

Conclusion: men with IVO are lighter than age-matched healthy men. tb-BMC is low and related to the low tb-Lean mass. Lean body mass, not COLIA1-Sp1, is more closely associated with IVO in men.

Disclosures: ***M.W.J. Davie****, None.*

SA309

See Friday Plenary number F309

SA310

Long-term Physical Activity Increases Bone Bending Strength despite No Change of Areal Bone Mineral Density - the MINOS Study. P. Szulc[1], T. J. Beck[2], P. D. Delmas[1]. [1]INSERM 403 Research Unit, Lyon, France, [2]The Johns Hopkins Outpatient Center, Baltimore, MD, USA.

The long-term effect of physical activity on areal bone mineral density (aBMD) and biomechanical properties of bone is not fully understood. We evaluated the effect of professional physical activity on aBMD as well as on morphological and biomechanical parameters of radius, ulna and femoral neck in 824 men aged 45 to 85 years belonging to the MINOS cohort. The intensity of physical activity was evaluated using a self-reported four level score (low, medium, high, very high) based on the work performed by each participant during the largest part of their professional career. Analyses were adjusted for age, body weight, body height, tobacco smoking, levels of free testosterone and 17β-estradiol, leisure sport activity as well as for lower limb muscular mass for femoral neck and upper limb muscular mass for radius and ulna. aBMD of the three bones did not differ between levels of physical activity. At the radius and ulna, external diameters were 7 and 9 % wider (p<0.0001) and endocortical diameter was 8 and 11 % higher (p<0.0001) in men with the very high physical activity compared with those with the low one. In all three bones, men with the highest physical activity had higher cross-sectional moments of inertia (7.5 to 34 %, p<0.05-0.0001) and higher section moduli (6 to 23 %, p<0.03-0.0001) compared with men with the lowest physical activity. Average buckling ratio was slightly higher at the radius and ulna (7 and 8 %, p<0.05-0.01) but not at the femoral neck.

Our data indicate that the long-lasting high physical activity can modify the morphological and biomechanical parameters of bones in ways that are not apparent in aBMD.

Disclosures: ***P. Szulc****, None.*

SA311

See Friday Plenary number F311

SA312

Prevalent Clinical Fractures and Hip Fractures are Associated with Stiffness Index Measurement in Men: Results from the Population Based ESOPO Study. L. Sinigaglia*[1], M. Varenna*[1], G. Isaia[2], S. Adami[3], S. Giannini[4], S. Maggi*[5], P. Filipponi[6], O. Di Munno*[7], D. de Feo*[8], G. Crepaldi[4]. [1]Rheumatology, Gaetano Pini, Milano, Italy, [2]Internal Medicine, Torino University, Torino, Italy, [3]Rheumatology, Valeggio Hospital, Verona, Italy, [4]Internal Medicine, Padova University, Padova, Italy, [5]CNR Agin Center, Padova, Italy, [6]Internal Medicine, Umbertide Hospital, Perugia, Italy, [7]Rheumatology, University of Pisa, Pisa, Italy, [8]Procter & Gamble, Roma, Italy.

A multicenter population based study was conducted to relate the prevalence of Osteopenia and Osteoporosis with personal fracture history in the Italian general male population. Eighty-three University and Hospital Centers performed the study. Subjects were randomly invited to participate from 1,536 GPs by a specific randomization protocol with 4,981 men aged 60-79 yrs enrolled. Each subject underwent a bone quantitative ultrasound measurement (QUS) on a Lunar Achilles Express. Personal history for clinical fractures (vertebral, hip, rib, wrist and other low-trauma fractures occurred after the age of 50) was recorded together with several life-style and anthropometric variables. In the sample we found 798 men with prevalent fractures after the age of 50. Age, BMI and calcium intake were similar in fractured and non-fractured counterparts whereas patients with fractures had a significantly higher alcohol consumption and a longer history of tabagism (p<0.03). Stiffness Index (SI) was significantly lower in men with prevalent fractures (p=0.0001) after adjustment for the main co-variates such as age, BMI and Calcium intake. Similarly, adjusted means for SI were significantly lower in hip-fractured men as compared to non hip-fractured counterparts (p=0.0001). A logistic model in which the dependent variable was the existence of any prevalent clinical fracture for a total of 4,365 observations showed an OR of 1.2 (95 % CI: 1.1-1.3) for 1 SD decrease of SI. A second model in which a prevalent hip fracture was the dependent variable (3,823 observations) showed an OR of 2.1 (95 % CI:1.5-3.1) for 1 SD decrease in SI.

Logistic procedure. Dependent variable: Prevalent Fractures. N. 4,365 observations

Variable	Unit	Odds Ratio	Lower	Upper
BMI	1unit	1.002	0.991	1.011
Age	1 yrs	1.037*	1.022	1.052
Smoking	Yes / no	1.222*	1.002	1.485
Use of OP drugs	Yes / no	0.869	0.765	2.119
Antihypertensives	Yes / no	0.869	0.738	1.023
Alcohol abuse	Yes / no	1.003*	1.001	1.006
Calcium intake	1 score	1.000*	1. 000	1. 001
Stiffness	- 1 SD	1.272*	1.173	1.381

*p<0.05

This large population-based retrospective study shows that SI ultrasound measurement is strongly related to prevalent clinical fractures and to hip fractures in men older than 60.

Disclosures: ***L. Sinigaglia****, None.*

SA313

See Friday Plenary number F313

SA314

Applying the Osteoporosis Self-Assessment Tool (OST) in Primary Care Practices Uncovers Osteoporosis in Men: Preliminary Report. M. I. Williams*, V. I. Petkov*, S. L. Johnson*, S. Wright*, M. T. Tran*, R. A. Adler. Endocrinology, McGuire Veterans Affairs Medical Center, Richmond, VA, USA.

Despite the availability of reliable diagnostic tests and treatment, osteoporosis (OP) is still under-diagnosed, particularly in men. OST, a simple screening tool for stratifying osteoporosis risk, is calculated by the formula {[weight (kg)-age]*0.2} and correlates well with axial bone mineral density (BMD by DXA) in men and women. We hypothesized that introducing OST as a screening aid in primary care clinics will increase the diagnosis and treatment of OP in men.

Providers in 3 primary care group practices at a single Veterans Affairs Medical Center were randomly assigned to one of the following interventions: 1. Education about OST and an OST pocket nomogram; 2. Education/nomogram plus a computer desktop OST-index calculator; 3. Education/nomogram plus an automatic clinical reminder in the electronic patient record for patients with high and moderate risk of OP according to OST. The 3 practices had similar provider number and patient mix. Outcomes were percent change in DXAs ordered, metabolic bone clinic consults requested, and osteoporosis specific prescriptions issued as compared to a baseline period.

We report preliminary results from a half-year intervention. The distribution of patients according to their OST values was very similar in the 3 group practices during baseline and intervention periods: 4.3% had high risk for OP, 31.8% were at moderate risk and the rest were low risk, similar to other OST studies. At baseline, the practices did not differ significantly in DXAs ordered, bone clinic consults, and OP specific prescriptions ordered. Comparing each practice to its baseline, no significant differences were observed in the practices randomized to education only or to education plus the desktop OST calculator. In contrast, the practice using the electronic BMD reminder ordered many more DXAs (526% increase). During the intervention period 100 DXAs were performed as a result of OST (3% from the practice with education only, 6% from the practice with education plus desktop OST calculator and 91% from the practice with electronic reminder). The prevalence of OP was high: 30% had at least one DXA site (spine, femoral neck, or total hip T-score) $\leq$ -2.5, and 79% had at least one T-score $\leq$ -1. At this preliminary assessment, bone clinic consult and OP medication changes were insignificant because of the time lag between DXA and follow-up.Applying OST as a screening tool for OP, particularly using electronic reminders, can increase the number of DXAs ordered and number of men diagnosed with OP.

Disclosures: ***M.I. Williams****, None.*

SA315

Factors Associated with Low BMD Among Middle-Aged Canadian Chinese Immigrants. A. M. Cheung[1], R. Chaudhry*[1], C. Chan*[1], K. Milstead*[1], N. Diaz-Granados*[1], D. Lui-Yee*[1], O. Gajic-Veljanoski*[1], M. Hamidi*[1], N. Carnide*[1], G. Chan*[2], L. Thompson*[1]. [1]Department of Medicine, University Health Network, Toronto, ON, Canada, [2]Yee Hong Centre, Toronto, ON, Canada.

We conducted a community-based cross-sectional study to explore the relationship between risk factors for osteoporosis and BMD among Chinese-Canadian immigrants aged 45 and older. We recruited through media announcements, health fairs, and community outreach through centres, churches and libraries which predominantly serve the Chinese community in the Greater Toronto Area. Heel BMD was measured using Sahara Quantitative Ultrasound (Hologic, Inc). Data were also collected on height, weight, demographics, diet, physical activity, medication use, co-morbid conditions, fall and fracture history, and health care utilization.

In total, we recruited 1087 participants over a 3 year period (June 2000-March 2003). This analysis includes 650 women and 270 men for whom the data were complete. Participants were primarily immigrants from Hong Kong (64%) & China (18%). The mean duration in Canada was 14.2 years (Range <1 to 53 years). Population characteristics included: mean age 61:64 yrs (Female:Male); height 154:167 cm (F:M); weight 55.5:66.5 kgs (F:M). Overall 39% rated their health as good or very good and 11% as poor or very poor. Mean heel BMD was 0.49:0.51g/cm2 (F:M); mean t-score using a female Caucasian reference population was -0.812:-0.615 (F:M); 3.7:1.5% (F:M) had t-score less than or equal to -2.5 and 41.7:35.2% (F:M) had t-scores between -1 & -2.5. Dietary assessment showed a mean calcium intake of 763 mg/day for women and 722 mg/day for men.

There was a significant inverse relationship between age and BMD. Lower BMD was also significantly associated with smoking and late menarche. BMD was not significantly associated with height, weight, age at menopause, or dietary intake of calcium or vitamin D. Mean BMD for those in the highest tertile for calcium intake was 0.496:0.503 g/cm2 (F:M) as compared to 0.483:0.498 g/cm2 (F:M) in the lowest tertile but the trend was not significant. There was no clear relationship between years since immigration and dietary intake of calcium or vitamin D.

Our preliminary analyses showed that older age, smoking, and late menarche are associated with lower heel BMD among Chinese-Canadian immigrants. These risk factors are similar to those identified in other epidemiologic studies. Calcium intake for our population is similar to that of Asian immigrants in the Ontario Health Survey, which found intake to be lower than that of the general population. Multivariate analysis is being performed to examine the relationship between risk factors, diet and BMD in our study.

Disclosures: ***A.M. Cheung****, Eli Lilly 2.*

SA316

See Friday Plenary number F316

SA317

Race may not Protect Against Low Bone Mineral Density (BMD) in Women with Lupus. A. B. Chadha[1], E. Shamiyeh*[1], S. Manzi*[2], S. Spies*[1], R. Ramsey-Goldman[1]. [1]Feinberg School of Medicine, Chicago, IL, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA.

Women (W) with Lupus are at risk for BMD but it is not clear whether differences in risk exist based on race. The primary aim of this study was to assess risk factors for low BMD at the spine, hip, and distal forearm in both African American (AA) and Caucasian (C) W with lupus. 300 W with lupus completed this cross sectional study (225 C and 75 AA). BMD was measured at the hip, spine, and distal forearm by dual x-ray absorptiometry. All W completed a self-administered risk factor questionnaire and a physician completed the Systemic Lupus International Collaborating Clinics (SLICC, excluding osteoporosis) damage index (DI). Univariate analyses were done on all risk factor variables and BMD scores. The mean age of AA and C W was 39.6 yrs and 42.5 yrs respectively (p= 0.02). These traditional risk factors for low BMD differed significantly between AA and C W: body mass index, BMI (28.7 vs. 26.3 kg/m^2, p= 0.02), daily calcium intake (956 vs. 1285 mg/day, p= 0.0006, mean daily caffeine intake (70 vs. 111 mg/day, p= 0.001), mean daily alcohol intake (0.77 vs. 2.62 g/day, p= < 0.0001), and mean age at menopause (38.5 vs. 42.4 yrs, p= 0.03). These Lupus-related risk factors differed significantly in AA vs. C W: mean age at lupus diagnosis (29.3 vs. 33.7 yrs, p= 0.005), current dose of corticosteroids (8.0 vs. 5.1 mg/day, p= 0.02), duration of corticosteroid use in yrs (8.7 vs. 5.9 yrs, p= 0.02), and the SLICC/DI (1.82 vs. 1.12, p= 0.004). AA W compared to C W had higher BMD raw scores at the hip (0.928 g/cm^2 vs. 0.887 g/cm^2, p= 0.04) and distal forearm (0.689 g/cm2 vs. 0.668 g/cm2, p= 0.04). However, spine BMD raw scores (0.989 g/cm2 vs. 0.981 g/cm2) were similar in AA and C W, but when comparing T scores AA W had significantly lower BMD at the spine than C W (-1.394 vs. -0.599, p= <0.0001) and lower T-scores at the hip (AA vs. C, -0.596 vs. -0.497), but higher T-scores at the distal forearm (0.135 vs. -0.181). Higher BMD raw scores were associated with higher BMI at hip (r= 0.348, p= <0.0001) and spine (r= 0.282, p= <0.0001) only in C W. Lower BMD raw scores at all 3 sites were associated with higher SLICC/DI scores and longer disease duration regardless of race (P<0.05). AA compared to C W with lupus have significantly higher BMD at the hip and distal forearm. However, spine and hip T-scores were lower in AA compared to C W suggesting race may not be protective against low BMD at these sites. In part, this may be due to AA W having lupus at an earlier age, undergoing menopause at an earlier age, having greater disease burden (as measured by SLICC/DI), higher daily use of corticosteroids, and/or longer duration of corticosteroid burden. Measures to prevent osteoporosis need to be implemented early in all lupus W regardless of race.

Disclosures: ***A.B. Chadha****, NIH/NIAMS 2; Arthritis Foundation Clinical Science Grant and Greater Chicago Chapter 2; Proctor and Gamble 2, 5; Merck 2, 5, 8.*

SA318

See Friday Plenary number F318

SA319

A Membranous Bone, Parietal, Shows the Same High MAR and BFR as Endochondral Bone in the C3H (High Bone Density) Mice Compared to B6 (Low Bone Density) Mice. M. H. C. Sheng[1], D. J. Baylink[1], K. H. W. Lau[1], W. G. Beamer[2], J. E. Wergedal[1]. [1]Musculoskeletal Disease Center, J.L. Pettis VAMC, Loma Linda, CA, USA, [2]The Jackson Laboratory, Bar Harbor, MI, USA.

We have previously demonstrated that two inbred mouse strains, the C3H/HeJ (C3H) and C57Bl/6J (B6), displayed a profound difference in femur peak bone density. The higher bone density in C3H mice compared to B6 mice was, in part, due to a greater BFR that was due exclusively to an increase in the mineral apposition rate (MAR), rather than in tetracycline-labeled surface (TLS) per bone surface (BS). To investigate whether membranous bone had a similar phenotype to endochondral bone, 6-week old mice were injected with demeclocycline (25 mg/kg) and tetracycline (30 mg/kg) with a 6-day interval in between and histomorphometric measurements were made on the parietal bone. The results are listed in the table:

Table 1

	B6 (n=6)	C3H (n=5)	% Difference	P
Bone width (μm)	125±2	172±8	38	0.003
TLS/BS (mm/mm)	.963±.049	.956±.013	1	N.S.
MAR (μm/d)	1.42±0.09	1.88±0.11	32	0.01
BFR/BS (mmx10^{-3}/d)	1.02±0.10	1.34±0.06	31	0.03

To further investigate whether differences in MAR and BFR were due to differences in osteoblasts themselves, we isolated osteoblasts from the calvarias of the newborns (n=5, 4 pups per sample) and 6 week old mice (n=6-7, 2 females per sample) by collagenase digestion and established cell cultures. ALP activities were greater by 208% at newborns (P=0.012) and 267% at 6 weeks (P=0.015) in the C3H osteoblasts than those in the B6 osteoblasts. Cell doubling time was similar in the two strains at both ages, consistent with the lack of difference in TLS *in vivo*. In cells from newborn mice, mineralized nodule formation was greater in the C3H osteoblast cultures than that in the B6 osteoblast cultures

(13.4±0.2 % vs. 4.0±0.8 %, P<0.001). In addition, preliminary data indicated that insoluble collagen production was greater by104% in the C3H than B6 osteoblasts. Because the phenotype appears to be present in neonates, the difference between C3H and B6 is not dependent on hormonal differences and is unlikely to be due to a serum factor. We concluded that: 1) the phenotypes of high MAR and BFR in C3H mice were expressed in membranous bone as well as endochondral bone; and 2) greater MAR in the C3H was due to a higher degree of differentiation and greater activity of osteoblasts.

Disclosures: ***M.H.C. Sheng****, None.*

SA320

Increased Release of Substance P and TNFa Underlie Osteoporosis Induced by Mg Restriction in the Rat. R. K. Rude[1], H. E. Gruber[2], H. J. Norton*[2], L. Y. Wei*[1], A. Frausto*[1], B. G. Mills[1]. [1]Medicine, University of Southern California, Los Angeles, CA, USA, [2]Carolinas Medical Center, Charlotte, NC, USA.

Previous studies have demonstrated that severe Mg deficiency results in osteoporosis in rodent models. Here we assess the effects of more moderate dietary Mg restriction (10% of nutrient requirement (NR)) on bone and mineral metabolism over a 6 month experimental period in the rat. At 2, 4, and 6 months, serum Mg, Ca, PTH, and 1,25(OH)2D were measured. Femurs and tibias were collected for mineral content, histomorphometry, and immunocytochemical staining. Data for month 4 and 6 are shown in the Table. Serum and bone (data not shown) Mg were significantly reduced by 2 months and continued so through 6 months. sCa was slightly, but significantly, higher than control at all time points. At 2 months sPTH was elevated in Mg depleted animals but was significantly decreased at 6 months. Serum 1,25-D was significantly reduced at 4 and 6 months of study. Histomorphometry demonstrated decreased bone volume and trabecular thickness, and increased osteoclast (Ocl) numbers over time compared to control. This was confirmed by micro-CT which also showed that trabecular volume, thickness, and number were significantly lowered.

	4 month control	4 month Mg def	6 month control	6 month Mg def
sMg, mg/dl	1.9 +/- 0.2	0.6 +/- 0.1*	1.9 +/- 0.2	0.6 +/- 0.1*
sCa, mg/dl	8.9 +/- 0.5	9.9 +/- 0.7*	8.6 +/- 0.5	9.9 +/- 0.4*
sPTH, pg/ml	152 +/- 93	180 +/- 140	361 +/- 228	141 +/- 56#
1,25-D, pg/ml	46 +/- 31	8 +/- 6*	19 +/- 17	3 +/- 0.5#
BV/TV (%)	24 +/- 3	14 +/- 5	20 +/- 4	9 +/- 4#
TTh (um)	45 +/- 7	35 +/- 5	38+/- 6	32 +/- 3#
NOc/BPm	2.7 +/- 0.4	3.0 +/- 0.5	2.3 +/- 0.8	3.9 +/- 3#

Data are means + SD. *p<.001, #p<.01

TNFa immunocytochemical localization in Ocls was 199% of controls at 2 months, 75% at 4 months and 194% at 6 months. Increased TNFa may be due to increased substance P which was increased to 250% of control at 2 months and 266% at 4 months; however no difference was seen at 6 months. These data demonstrate that Mg intake of 10% NR in the rat causes bone loss which may be secondary to increased release of substance P and TNFa

Disclosures: ***R.K. Rude****, None.*

SA321

Dietary Iron and Calcium Interact to Affect Iron Status and Bone Health in a Response Surface Study of Growing Female Rats. Z. K. Roughead[1], L. K. Johnson*[1], J. L. Wagner*[2]. [1]Grand Forks Human Nutrition Research Center, USDA-ARS, Grand Forks, ND, USA, [2]Department of Physics, University of North Dakota, Grand Forks, ND, USA.

The concerns about inadequate intakes of calcium (Ca) and iron (Fe) have led to extensive fortification of both minerals in the US food supply. The objective of this study was to test the interaction of Ca and Fe over a wide range of intakes using a two-factor central composite response surface design. Weanling female Sprague-Dawley rats (n= 44) were randomly assigned to 9 groups fed AIN-93G basal diets with varying amounts of Fe (3, 5, 17, 60, or 100 ug/g diet), and Ca (1000, 1330, 2646, 5264, or 7000 ug/g diet) for 7 weeks. Semi-partial correlation coefficients (R^2_p) were obtained by stepwise regression analysis and reported at a significant level of p<0.02. Iron status (hemoglobin, hematocrit, red cell distribution, liver nonheme Fe) was primarily determined by dietary Fe (R^2_p = 0.92, 0.93, 0.81, and 0.78, respectively). However, increased Ca intake had an antagonistic effect on Fe status such that, based on the regression model, the predicted maximum hemoglobin of 16.8 g/dL occurred when Fe intake was moderate (43.5 ug/g diet) and Ca intake was minimal (1000 ug/g diet). Although Ca intake was the primary determinant of femur density, accounting for 79% of its variability, Fe intake also affected femur density (R^2_p = 0.10). Femur density was maximal at moderate intakes of Fe and Ca (38 and 4963 ug/g diet, respectively) and decreased slightly with higher intakes of both minerals. Variability in femur breaking strength (N/mm^2) was determined only by Ca intake (R^2_p = 0.70), was maximal with moderate Ca intake of 4120 ug/g diet, and decreased with further increases in Ca intake. While the main constituents of bone matrix (Ca and collagen) were only affected by dietary Ca (R^2_p = 0.42, 0.28, respectively), certain noncollagenous components in bone (osteocalcin, hexosamines) responded to changes in both Ca and Fe intakes. Serum concentrations of insulin-like growth factor-1 (IGF-1) did not respond to either dietary Ca or Fe, but bone IGF-1 concentration was weakly associated with dietary Ca (R^2 = 0.26). In summary, in growing female rats, high Ca intake reduced Fe status. While adequate intakes of both Ca and Fe were essential for maximal bone density, excessive intakes of these minerals reduced bone density and strength. In conclusion, the effects of high Ca intake on Fe status were more pronounced than the effects of Fe nutriture on bone health. These findings indicate that Ca intakes above the adequate amount do not offer additional benefits and may even be detrimental to bone. Additionally, indiscriminate fortification of foods with Ca may compromise the Fe status of vulnerable segments of the population.

Disclosures: ***Z.K. Roughead****, None.*

SA322

See Friday Plenary number F322

SA323

The Effects of Long-Chain Polyunsaturated Fatty Acids on Bone Mass and Bone Metabolism in the Piglet Model. R. C. Mollard*, H. R. Kovacs*, H. A. Weiler*. Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada.

The dietary long chain polyunsaturated fatty acids (LCPUFAs) arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) have been shown to elevate bone mass, however, the optimal amount requires clarification. This study was designed to determine the amount of AA plus DHA that influences bone cell metabolism and mass. Forty male piglets were randomized to receive one of four isocaloric formula: control or LCPUFA supplemented (0.5% AA & 0.1% DHA, 1.0% AA & 0.2% DHA or 2.0% AA & 0.4% DHA wt/wt total fat). After 15 days of study measurements included: bone mineral density (BMD) of whole body, lumbar spine and excised femurs using dual energy x-ray absorptiometry; bone cell metabolism assessed by plasma osteocalcin, urinary N-telopeptide and ex vivo prostaglandin E_2 (PGE_2); and liver fatty acids using gas chromatography. Main effects were identified using a two-factor ANOVA and differences among groups identified using the Bonferroni t-test

. Liver AA and DHA increased in proportion to dietary supplementation. Whole body and lumbar spine BMD was significantly higher (p<0.05) in the group supplemented with 0.5% AA and 0.1% DHA. The group supplemented with 1% AA and 0.2% DHA had significantly lower urinary N-telopeptide (p=0.039), suggesting reduced bone resorption. PGE_2 (p=0.723) and osteocalcin levels (p=0.077) were not significantly different among the groups. Supplementation of AA and DHA at 0.5% and 0.1% wt/wt total fat lead to elevated bone mass, but higher amounts provided no additional benefit.

Disclosures: ***R.C. Mollard****, None.*

SA324

Biochemical Markers of Bone Turnover in Glucocorticoid-Treated Patients Are Altered by Calcium Supplementation: Preliminary Results from ACTIVATE Trial. N. Lane[1], S. Goldring[2], J. Stewart*[3], S. Morris[3]. [1]Department of Medicine, UCSF, San Francisco, CA, USA, [2]Chief of Rheumatology, Beth Israel Deaconess Medical Center, Boston, MA, USA, [3]Medical Research, Aventis, Bridgewater, NJ, USA.

Glucocorticoids (GC) create an uncoupling of bone turnover related to reduced bone formation and increased bone resorption. The ACTIVATE Trial was undertaken to determine whether providing information on bone turnover and vertebral fractures affected compliance with daily risedronate therapy in patients on oral GC. Initial data from this study have provided insights into the effects of oral calcium supplement on biochemical markers of bone turnover in patients on GC. Currently, 139 subjects on GC (≥7.5 mg/d) have been enrolled (40% of total): 106 in chronic treatment category (TREAT; >6 mos GC therapy) and 33 initiating GC treatment (PREV). Baseline serum osteocalcin (OST), osteoprotegerin (OPG), parathyroid hormone (PTH), creatinine and urine N-telopeptide (NTX) were obtained. Mean age of patients in TREAT group was 61.4 years and 67.7 years in PREV. Mean GC doses were 13 mg and 17 mg prednisone in TREAT and PREV, respectively, with a mean duration of usage for 73 months for the TREAT and 0.8 months for the PREV. Oral calcium supplement (defined as at least 500 mg daily) was determined by questionnaire. When biochemical marker values were represented as median and range for each group, calcium supplementation was associated with the following effects (NTXR represents the ratio of the urine NTX/serum creatinine).

Feature	Calcium	TREAT	PRE
NTRX	No	32.3 (10.75-272.6)	42.7(15.9-91.2)
	Yes	29.8(6.3-122.5)	33.6(12.1-86.6)
OST	No	10.7(1.9-31.6)	10.0(4.5-35.0)
	Yes	10.4(3.7-31.5)	8.9(1.1-14.9)
OPG	No	3.98(1.61-7.66)	4.19(2.43-8.84)
	Yes	4.17(1.26-11.14)	4.53(1.10-6.70)
PTH	No	3.30(1.60-10.70)	3.70(1.70-6.90)
	Yes	2.85(1.30-7.30)	2.70(1.60-6.60)

These results indicate that initiation of GC treatment is associated with an increase in bone resorption (elevated NTXR) and that oral calcium supplementation diminishes this effect. In the TREAT group, median NTXRs were lower than in the PREV group. Nevertheless, oral calcium supplement also produced reductions in this index of bone resorption in this group. Although suggestive, more data are required to confirm whether this effect of oral calcium supplement is related to suppression of endogenous PTH and increase in OPG.

Disclosures: ***N. Lane****, Aventis 8; Procter & Gamble 2; Merck 8; Genentech 5.*

SA325

See Friday Plenary number F325

SA326

Bone Loss in Multiple Sclerosis Patients Treated with Corticosteroids. J. J. Stepan[1], E. Havrdová*[2], M. Týblová*[2], D. Horáková*[2], V. Tichá*[2], I. Nováková*[2], V. Zikán*[1]. [1]3rd Department of Internal Medicine, Charles University Faculty of Medicine, Prague, Czech Republic, [2]Department of Neurology, Charles University Faculty of Medicine, Prague, Czech Republic.

Background. Patients with multiple sclerosis (MS) may be at greater risk of fracture because the glucocorticoid (GC) induced bone loss is superimposed on detrimental effects of physical inactivity. The aim of this study was to evaluate the relative contribution of GC treatment, immobilization, and other factors, to decrease in bone mineral density (BMD), in patients with MS. ***Patients and Methods*.** From the out-patient clinic, 515 patents were randomly chosen. The study population involved 404 females (mean ± SD, age, 43 ± 10 yr, weight, 65 ± 13 kg, height, 165 ± 8 cm, duration of MS 14 ± 9 yr) and 111 males (age, 42 ± 11 yr, weight, 76 ± 12 kg, height, 178 ± 7 cm, duration of MS, 14 ± 8 yr). The mean dose of GC was 6.5 ± 2.5 mg/day, 45% of the patients received > 7.5 mg/day. The patients were advised to take the daily recommended dose of calcium and vitamin D. Motor function of the patients was evaluated using the Kurtzke EDSS, a scale useful in measuring the ability to walk that is decisive for normal remodeling of bone. The total amount of used GC was calculated based on documented treatment. All fractures since the start of GC therapy ware counted. BMD was measured using the Delphi A bone densitometer (Hologic, MA) at the lumbar spine, and proximal femur. ***Results*.** BMD > -1 T-score was found in 43% of the patients (age, 42 ± 9 yr, weight, 71 ± 14 kg, total dose of GC, 29 ± 23 g, duration of treatment, 5.9 ± 4.5 yr, KEDDS, 3.9 ± 1.8, fractures in 13% of the patients). Osteopenia (BMD T-score of -1 to -2.5) was found in 40% of the patients (age, 43 ± 11 yr, weight, 66 ± 12 kg, total dose of GC, 36 ± 24 g, duration of treatment, 15 ± 9 yr, KEDDS, 4.6 ± 1.8, fractures in 21% of the patients). Osteoporosis (BMD < 2.5 T-score) was found in 17% of the patients (age, 44 ± 11 yr, weight, 63 ± 15 kg, total dose of GC, 43 ± 25 g, duration of treatment, 8.9 ± 6 yr, fractures in 31% of the patients). In the stepwise regression analysis, the BMD at the lumbar spine, and femoral neck was significantly inversely related to the KEDDS, smoking, and duration of GC treatment, and positively related to weight (Rsqr = 0.21, and 0.33, respectively, $p < 0.001$). The other variables, age, height, sex hormone deficiency, duration of MS, and the cumulative dose of GC did not enter the equation. After controlling for the other factors, prevalence of fractures was determined by the femoral T-score, estrogen deficiency, and duration of GC treatment. ***Conclusion***. In MS treated with low dose GC, immobilization was the main determinant of the low bone mass, which was the main fracture risk factor in these patients.

Disclosures: ***J.J. Stepan****, None.*

SA327

See Friday Plenary number F327

SA328

See Friday Plenary number F328

SA329

Bone Density and Bone Metabolism in Healthy Men Are Unaffected by the Marked DHT Suppression Produced by the Dual 5α-Reductase Inhibitor Dutasteride. R. V. Clark[1], C. S. Huffman*[1], A. M. Matsumoto*[2], R. S. Swerdloff*[3], C. Wang*[3], W. J. Bremner*[2]. [1]GSK Research and Development, Research Triangle Park, NC, USA, [2]Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA, [3]Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA, USA.

The novel dual 5α-reductase inhibitor, dutasteride, has been shown to be effective and well tolerated in patients with benign prostatic hyperplasia (BPH). The objective of this study was to determine whether marked dihydrotestosterone (DHT) suppression observed with dutasteride has an effect on bone mineral density (BMD) and bone metabolism.

In this randomized, double-blind, placebo-controlled study healthy men aged 18–55 were randomized to receive 0.5 mg dutasteride, 5.0 mg finasteride or placebo for 52 weeks, with a further 24 week follow-up after cessation of study medication. Bone density by x-ray absorptiometry was determined at screening, weeks 48–52 and follow-up weeks 20–24. Markers of bone metabolism, serum osteocalcin, serum bone alkaline phosphatase, and urinary n-telopeptide (osteomark) were measured at baseline, weeks 8, 16, 24, and 52, and follow-up weeks 8, 12 and 24.

Ninety-nine subjects were included in the intent-to-treat analysis. The mean reductions in serum DHT concentration in the dutasteride group were >90% at each treatment phase visit compared with 70% for finasteride ($p<0.001$). There were no clinically, nor statistically, significant changes in BMD from baseline, or between groups at weeks 48-52 or follow-up weeks 20-24 (see table).

	Week	Mean % change in BMD		
		Placebo	Dutasteride	Finasteride
Lumbar spine	48-52	0.573	-0.077	0.003
	FU 20-24	0.866	0.094	-0.816
Proximal femur	48-52	-1.823	-0.367	-0.378
	FU 20-24	-0.104	-0.366	0.521

There were no consistent changes or trends seen in any of the bone markers in any treatment group during the treatment period. At follow-up week 24, mean urinary osteomark values were significantly greater in the finasteride group compared with the placebo and dutasteride groups (2-sided p-values=0.017 and 0.003, respectively). However, all parameters remained within normal ranges.

In conclusion, the marked suppression of DHT observed with dutasteride compared with other 5ARIs, had no clinically or statistically significant effect on BMD or bone metabolism.

Disclosures: ***R.V. Clark****, GlaxoSmithKline 3.*

SA330

See Friday Plenary number F330

SA331

See Friday Plenary number F331

SA332

Changes of Nail Ca and Mg Concentrations with Reference to Age, Gender and Bone Mineral Density. Implications for the Detection and Screening of Osteoporosis. S. Ohgitani*[1], Y. Fujii[2], C. Hayashi*[3], H. Nishio*[3], T. Fujita[4]. [1]Division of Clinical Laboratory, Minami-Kyoto National Hospital, Kyoto, Japan, [2]Calcium Research Institute, Kishiwada, Osaka, Japan, [3]Public Health, Kobe University School of Medicine, Hyogo, Japan, [4]Katsuragi Hospital, Kishiwada, Osaka, Japan.

Nails in contact with periosteum are readily sampled and their mineral content may reflect some aspect of mineral metabolism and bone status. Ca and Mg concentrations in the finger and toe nails were therefore measured by using Atomic Absorption Spectrophotometer, Perkin- Elmer Type 408, in 119 males and 175 female between ages of 10 and 90 years without diseases primarily affecting mineral metabolism. In another group of subjects with a mean age of 62 years, lumbar bone mineral density (LBMD) was measured by dual energy X-ray absorptiometry using XR-26 in addition. Fingernail Ca concentration decreased from 927±52 ppm (SD) in the 20s to 529±83 in the 80s with a significant negative correlation with age ($r=-0.288$, $p<0.0001$) and toenail Ca concentration similarly fell with age in males. Decreases in females were less distinct. Fingernail Mg concentrations, on the contrary, increased from 78±14 to 140±6 in corresponding age groups with a significant positive correlation ($r=0.315$, $p<0.0001$) and toenail Mg showed a similar tendency in males, but changes in females were less distinct. In a group with similar age, a significant positive correlation was noted between fingernail Ca concentration and LBMD ($r=0.528$, $p=0.0016$) and a negative correlation between fingernail Mg concentration and LBMD ($r=-0.377$, $p=0.0272$). No significant correlation was noted between toenail Ca or Mg concentration and LBMD. Significant correlation was found between Ca and Mg concentrations at the same site and either Ca or Mg concentrations between finger and toenails. Inverse relationship between Ca and Mg concentrations in males may reflect some kind of antagonism between these two elements, though the mechanism remains to be elucidated. Decrease of Ca concentration and increase of Mg concentration in fingernails in males may be utilized to detect a low LBMD in osteoporosis.

Disclosures: ***S. Ohgitani****, None.*

SA333

See Friday Plenary number F333

SA334

Decreased Vitamin D-Dependent Intestinal Calcium Absorption in Immobilized Rats. T. Sato*[1], K. Morita[2], Y. Kado*[2], H. Yamamoto[2], Y. Taketani[2], Y. Nii*[3], E. Takeda[2]. [1]Clinical Nutrition, University of Tokushima, Tokushima, Japan, [2]Clinical nutrition, University of Tokushima, Tokushima, Japan, [3]Food Technology Division, Tokushima Prefectural Industrial Technology Center, Tokushima, Japan.

Skeletal unloading caused by bed rest or immobilization result in a loss of bone. Previous studies have demonstrated negative calcium balance with increased urinary and fecal excretion during bed rest and space flight. Understanding the molecular mechanism of altered intestinal calcium absorption in the development of disuse bone loss is important for the prevention and treatment. Therefore, various parameters affecting calcium and bone metabolism were investigated in immobilized rats. Each rat of the immobilized group (Wistar male rats), which imitated human bed rest, was kept in Ballman cage III for 2 weeks. Each rat of control group was kept in ordinary cage and fed the food (AIN-93G, 0.6% calcium and 0.6% phosphorus) intake of the immobilized rats. In the second experi-

ment, immobilized rats received a high calcium diet (1.2% calcium and 0.6% phosphorus), a high calcium and high vitamin D diet (20,000IU/100g cholecalciferol, 1.2% calcium and 0.6% phosphorus) or onion diet (7% onion, 0.6% calcium and 0.6% phosphorus) for 2 weeks. In immobilized rats, intestinal net calcium absorption, bone mineral density (BMD) in femur and lumbar vertebrae measured by pQCT, serum level of 1,25(OH)2D, and mRNA levels of renal 1 alpha hydroxylase, duodenal calcium transport protein 1 (CaT1) and calbindin-D9k were decreased and renal 24 hydroxylase mRNA level increased, but colonic CaT1 mRNA level was not decreased. Administration of etidronate (20mg/Kg/day) prevented the reduction of BMD, but did not recover net calcium absorption in immobilized rats. These findings suggest that immobilization stimulated catabolism and depressed production of 1,25(OH)2D and consequently decreased the duodenal 1,25(OH)2D dependent calcium transport activity that is not related with bone resorption. In addition, immobilized rats fed calcium, vitamin D or onion enriched diet interestingly resulted in increased intestinal calcium absorption and BMD without increment of CaT1 or calbindin D9k mRNA levels. Therefore, it might be concluded from present study that those diets are effective to prevent disuse osteoporosis.

Disclosures: ***T. Sato****, None.*

SA335

Oral Daily and Intermittent Ibandronate Significantly Reduce Height Loss in Postmenopausal Osteoporosis. C. H. Chesnut[1], A. Skag*[2], A. Hoiseth*[3], J. Gilbride*[4], R. C. Schimmer[4]. [1]University of Washington, Seattle, WA, USA, [2]Bergen Osteoporosesenter, Bergen, Norway, [3]Sentrum Rontgeninstitutt, Oslo, Norway, [4]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

Ibandronate, a highly potent, nitrogen-containing bisphosphonate, is the subject of an ongoing clinical development program that aims to maximize the potential of simplified, less frequent dosing in postmenopausal osteoporosis (PMO). A recent multicenter, double-blind, randomized study (oral iBandronate Osteoporosis vertebral fracture study in North America and Europe: BONE) investigated the fracture efficacy and safety of ibandronate when administered as an oral daily and intermittent regimen with a between-dose interval of >2 months. A total of 2,946 women (aged 55–80 years, time since menopause ≥5 years) with low bone mineral density (BMD; T-score <–2) in ≥1 vertebra of the lumbar spine (L1–L4) and 1–4 prevalent vertebral fractures (VF) were enrolled into the study. Participants received either oral daily ibandronate (2.5mg; n=982), oral intermittent ibandronate (20mg every other day for 12 doses every 3 months; n=982) or placebo (n=982) for 3 years with daily calcium (500mg) and vitamin D (400IU) supplementation. The primary endpoint was the rate of patients with new incident VF after 3 years. Secondary endpoints included height, BMD, biochemical markers of bone turnover and safety. After 3 years, oral daily and intermittent ibandronate significantly reduced the risk of radiologically confirmed VF (62% [p=0.0001] and 50% [p=0.0006], respectively) relative to placebo. A mean height loss of 5.6mm was observed in the placebo arm. This loss was significantly greater than the loss observed in the oral daily (3.9mm; p=0.0005 vs placebo) and intermittent (4.7mm; p=0.0144 vs placebo) ibandronate arms. The average annual height loss in the oral daily and intermittent ibandronate groups was also significantly less than that observed with placebo (using ANOVA p<0.0001 and p=0.0115, respectively). Significant increases in lumbar spine and hip BMD and sustained decreases in biochemical markers of bone turnover were also observed. Ibandronate was well tolerated, with no significant safety concerns. In summary, oral daily and intermittent ibandronate significantly reduce the risk of VF in women with PMO. This finding is corroborated by the significant and sustained reductions in height loss observed in the active treatment groups compared with placebo. Ongoing studies of ibandronate are evaluating alternative intermittent dosing regimens, such as oral monthly, that aim to provide enhanced treatment outcomes in PMO.

Disclosures: ***C.H. Chesnut****, None.*

SA336

See Friday Plenary number F336

SA337

Relative Contributions of the Early Changes in Bone Resorption and Later Changes in Hip Bone Mineral Density to the Reduction in Vertebral Fracture Risk with Risedronate. A. Blumsohn[1], I. P. Barton*[2], A. Chines[3], R. Eastell[1]. [1]University of Sheffield, Sheffield, United Kingdom, [2]Procter & Gamble Pharmaceuticals, Egham, United Kingdom, [3]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Anti-fracture efficacy of antiresorptive therapies is partially explained by increases in bone mineral density (BMD) and partially by decreases in bone resorption markers. The relative importance of these two factors and whether or not their effects are independent is not known. We examined this by measuring N-telopeptide of type I collagen (NTX) in osteoporotic patients in the risedronate VERT and HIP (Group 1, low BMD) fracture trials. We studied 1245 women (mean age 72 years, SD 6) with either a femoral neck BMD T-score less than -3, or a vertebral fracture who received calcium (and vitamin D if required) and placebo or risedronate 5 mg daily for 3 years. A method based on the Cox-regression model proposed by Li et al. (Statistics in Medicine, 2001) was used to estimate the overall treatment effect in reducing the risk of incident fracture and the effect explained by both NTX and BMD within a single model. The changes in femoral neck BMD at follow-up (mean, -1.1 and +2.0%) and the reductions in urinary NTX (median, 22 and 54%) at 3 to 6 months of placebo or risedronate 5 mg, respectively, were significantly associated (P<0.05) with the reduction in vertebral fracture risk (54% over 3 years). The changes in BMD accounted for 17 and 26% (at one and three years) and those in NTX accounted for 44 and 33% (at one and three years) of risedronate's effect in reducing the risk of vertebral fractures over 3 years, compared with placebo. The estimate of the percent of fracture risk reduction accounted for by NTX was not altered after adjusting for the changes in BMD. In conclusion, the increase in BMD and the decrease in bone resorption in patients taking risedronate each account for some but not all of the reduction in vertebral fracture risk and these effects are independent.

Disclosures: ***A. Blumsohn****, None.*

SA338

See Friday Plenary number F338

SA339

Comparative Efficacy of Hormone Replacement Therapy, Bisphosphonates, Calcitonin, Vitamin D and Vitamin K in Postmenopausal Women with Osteoporosis. Y. Ishida, S. Kawai*. Department of Orthopaedic Surgery, Yamaguchi University School of Medicine, Ube-City, Yamaguchi, Japan.

This randomized controlled trial was conducted at 3 university hospitals to assess the comparative effectiveness of several medications on bone mineral density (BMD), biochemical bone markers, and a new vertebral fracture incidence in postmenopausal women with osteoporosis. A total of 455 postmenopausal women aged 50-75 with osteoporosis were randomly allocated into six groups: 1) hormone replacement therapy (HRT, conjugated estrogen 0.625 mg/day plus medroxyprogesterone 2.5 mg/day); 2) bisphosphonates (alendronate, 5 mg/day or etidronate, 200 mgx14d q3m); 3) eel calcitonin (CT, 20 IU/week); 4) vitamin D3 (alfacalcidol 1 micro g/day); 5) vitamin K2 (45 mg/day); and 6) control (no treatment). Thoracic and lumbar spine radiographs and BMD at distal 1/3 radius were assessed at baseline and at every 3 months, along with markers of bone turnover [serum bone specific alkaline phosphatase, serum osteocalcin, urinary N-telopeptide of type I collagen (NTX), and urinary deoxypyridinoline (DPD)]. Mean changes in BMD relative to baseline after the 2-year treatment in HRT, alendronate, etidronate, CT, vitamin D, vitamin K and control was 2.0%, 2.3%, -1.0%, 1.6%, -3.6%, -1.9% and -3.3%, respectively. In control, the incidence of new vertebral fractures after the 2-year treatment was 25.8%. In HRT, alendronate, etidronate, CT, vitamin D and vitamin K, the fracture incidence was reduced by 63%, 64%, 53%, 58%, 34% and 45%, respectively, compared to control (P=0.02, P=0.01, P=0.01, P=0.04, P=0.27, and P=0.04, respectively, Log-rank test). Logistic regression analysis revealed that changes in BMD relative to baseline at month 3 significantly predicted changes in BMD after 2 years (odds ratio: 4.63 in HRT; 10.54 in alendronate; 8.54 in etidronate; 9.80 in CT; 5.38 in vitamin D; and 2.60 in vitamin K; P<0.01) and the new vertebral fracture risk (odds ratio: 2.17 in HRT; 2.64 in alendronate; 2.54 in etidronate; and 3.53 in CT; P<0.05). Changes in NTX and DPD relative to baseline after 3 months were significant predictors of the incidence of new vertebral fractures (odds ratio: 1.83 and 2.02 in HRT, respectively; 3.04 and 3.87 in alendronate; 2.04 and 1.87 in etidronate; 3.07 and 2.07 in CT; P<0.05). Vitamin K appears to reduce the risk of vertebral fractures without improvement in BMD or reduction of markers of bone resorption, indicating that other mechanisms, not measured by BMD, may also play an important role in vertebral fracture. In conclusion, our results demonstrate the importance of measurements of BMD and markers of bone resorption at month 3 in identifying women for whom drug therapy to prevent vertebral fracture is appropriate.

Disclosures: ***Y. Ishida****, None.*

SA340

See Friday Plenary number F340

SA341

Safety and Tolerability of Risedronate in the Treatment of Osteoporosis in Patients with Renal Insufficiency. M. F. Delaney[1], S. Hurwitz*[2], J. J. Carey[3]. [1]Endocrinology, Diabetes, and Hypertension Division, Brigham and Women's Hospital, Boston, MA, USA, [2]Endocrinology, Diabetes, and Hypertension Division, Brigham and Women' Hospital, Boston, MA, USA, [3]Rheumatology, Cleveland Clinic Foundation, Cleveland, OH, USA.

Osteoporosis is a common disorder effecting more than 10 million Americans. With increasing age, renal insufficiency is more prevalent. The risk of fracture increases with age, so too does the need for safe and effective anti-resorptive therapy for these patients. Risedronate is an oral bisphosphonate approved for the treatment of osteoporosis in patients with a creatinine clearance >30ml/min and is approved to treat steroid-induced bone loss. Often an oral bisphosphonate is the treatment of choice, particularly in patients with a low hip BMD. The safety profile and efficacy of Risedronate is unknown in patients with impaired renal function.

In this retrospective study, we examined patients with mild to moderate renal insufficiency (serum creatinine >1.1 mg/dl) treated with Risedronate for osteoporosis. Patients identified were treated between 1999 and 2003 in Osteoporosis Clinic and were identified from a clinic chart review. All patients had a serum chemistry panel measured prior to starting Risedronate and a follow up at 3-6 months. BMD was performed to establish the diagnosis of osteoporosis using a DEXA QDR 4500A, Hologic, or a Lunar DPX-IQ. Baseline BMD scans were performed in all patients and follow up BMD was available in a subset. All patients were treated with Risedronate, but dosing regimens varied, 5mg daily, and 30mg or 35mg weekly.

We identified 12 patients for this study, 4 men and 8 women, and the mean age is 74 years (range 37-94). Baseline creatinine levels ranged from 1.1 to 1.7 mg/dl (mean 1.4), BUN

ranged from 18 to 45 and calcium ranged from 8.7 to 10 mg/dl. Patients with secondary causes of bone loss were not excluded. Active medical problems included Inflammatory Bowel Disease, Renal Transplant, Hypertension, Diabetes, etc. And concomitant medications included prednisone, hydrochlorthiazide, androderm, prilosec, etc. All received adequate calcium and vitamin D supplementation.
Follow up creatinine levels show a mean value of 1.37mg/dl, that is no significant change since baseline creatinine pre-treatment. No patients were withdrawn from treatment with Risedronate. Baseline BMD T scores ranged from LS –1.2 to –3.5, FN –1 to –4, TR –1.13 to –3.6 and TH –1.07 to –2.9. Follow up BMD scans are not yet available in all patients.
In summary, Risedronate is well tolerated in patients with mild to moderate renal insufficiency. There is no observed nephrotoxicty with FDA-approved doses. Further studies of bisphosphonate therapy in renal insufficiency is recommended.

Disclosures: ***M.F. Delaney****, Procter & Gamble Pharmaceuticals 2, 8; Eli Lilly and Company 2, 8; Roche Pharmaceuticals 2.*

SA342

See Friday Plenary number F342

SA343

Oral Daily and Intermittent Ibandronate have a Similar Safety Profile in Elderly and Younger Patients: Results from the BONE Study. M. P. Ettinger[1], A. Skag*[2], A. Hoiseth*[3], B. Leishman*[4], R. C. Schimmer[4]. [1]Radiant Research and the Regional Osteoporosis Center of South Florida, Stuart, FL, USA, [2]Bergen Osteoporosesenter, Bergen, Norway, [3]Sentrum Rontgeninstitutt, Oslo, Norway, [4]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

Owing to a decline in functional status and the presence of comorbidities, there is a concern that elderly women with osteoporosis may be more vulnerable to bisphosphonate-related adverse events (AEs) than younger women. As such, the safety profile of a bisphosphonate in this patient population is of particular importance. Ibandronate is a highly potent, nitrogen-containing bisphosphonate with proven efficacy when administered with extended between-dose intervals. Most notably, a recent study (oral iBandronate Osteoporosis vertebral fracture trial in North America and Europe: BONE) demonstrated that oral daily (2.5mg) and intermittent (20mg every other day for 12 doses every 3 months) ibandronate regimens significantly reduce the risk of vertebral fracture by 62% (p=0.0001) and 50% (p=0.0006), respectively.[1] Of the 2,946 women enrolled, 1,416 (48%) were aged ≥70 years and 1,530 (52%) were aged <70 years (mean age 74.0 and 63.8 years, respectively). Patients receiving medications likely to irritate the gastrointestinal (GI) tract or with existing upper GI disorders were not excluded. Both ibandronate regimens were well tolerated, with an overall safety profile similar to placebo. The overall incidence of AEs, drug-related AEs and upper GI AEs was comparable among the treatment groups. There was no general effect of age on tolerability: elderly patients (aged ≥70 years) receiving ibandronate therapy did not appear to be at greater risk of experiencing AEs than elderly patients receiving placebo or younger patients receiving ibandronate therapy or placebo. In particular, elderly patients were shown to be at no greater risk of dyspepsia or esophagitis. In summary, oral daily and intermittent ibandronate are well tolerated in postmenopausal osteoporosis, regardless of age. These findings highlight a strong potential for ibandronate therapy in both older and younger patients with PMO.
1. Delmas PD, et al. Osteoporos Int 2002;13(Suppl 1):S15(Abstract O37).

Disclosures: ***M.P. Ettinger****, None.*

SA344

See Friday Plenary number F344

SA345

Early Changes in Serum Osteoprotegerin (OPG) Correlates with Changes in Bone Mineral density following treatment with Risedronate in Post-Menopausal Women with Osteoporosis. S. Sankaralingam*[1], M. Frost[2], I. Fogelman[2], G. Hampson[1]. [1]Chemical Pathology, St Thomas Hospital, London, United Kingdom, [2]Osteoporosis Screening Unit, Guy's Hospital, London, United Kingdom.

Serum osteoprotegerin(OPG) concentration has been shown to increase with age and is higher in post-menopausal women compared to pre-menopausal women. There are few studies of the effects of anti-resorptive treatment on serum OPG in post menopausal osteoporosis. We investigated changes in bone turnover and serum OPG following Risedronate treatment in a group of post menopausal women with osteoporosis. The study group comprised of 18 post menopausal women with newly diagnosed osteoporosis(mean age[SEM]) 67 yrs [1.3]. They were started on 5mg/day of Risedronate for 12 months.Bone mineral density (BMD) was measured at baseline and at 6 and 12 months. Serum levels of bone specific alkaline phosphatase (bsALP), and urinary deoxypyridinoline (DPD) were measured at baseline and at 3, 6 months. Serum OPG and C-terminal telopeptide (CTX) were measured at baseline, 3,6 and 12 months. A significant increase in BMD was observed at the lumbar spine at 6 and 12 months of treatment (mean [SEM]) ***6 months*** 1.74%[0.833]*** p<0.05** and ***12 months*** 4.31% [1.06] * **p<0.005**). There was no significant change in BMD at the femoral neck and total hip. Significant decreases in serum bone specific alkaline phosphatase, serum CTX and urinary DPD were seen at 3 and 6 months (***3 months*** bsALP –28.4% [1.34]* **p<0.0005**, CTX –14.1% [7.28]* **p<0.05**, DPD –22.9% [2.32]* **p<0.005**, ***6 months*** bsALP –22.65%[2.44]*** p< 0.005,** CTX –25.1%[6.08]* **p<0.0005,** DPD –18.1%[3.92]***p<0.005**). Serum OPG concentration did not change significantly at 3 and 6 months. However a significant decrease was seen at the later time point,12 months (–31.6 %[5.8]* **p<0.0005) .** No significant correlations were seen between changes in BMD at 12 month and changes in serum CTX at any time point. However a positive correlation was seen between the changes in BMD at the lumbar spine and % change in serum OPG at 3 month (r = 0.62, p = 0.02). In those patients (38%) where the change in BMD was less than 2% (-0.4 %[1.07]), a significant reduction in serum OPG was observed at 3 month (-26.4%[5.78], p = 0.01) compared to the rest of the study population (BMD : 7.6%[1.15] p =0.0003) where a small increase in serum OPG was seen (8.8%[4.0] p = 0.06).
Early reduction in serum OPG leads to a smaller BMD response at 12 months in patients on Risedronate. Measurement of serum OPG may help in the early assessment of treatment responsitivity.

Disclosures: ***S. Sankaralingam****, None.*

SA346

See Friday Plenary number F346

SA347

The Effect of Risedronate on Bone Mineral Density After Total Hip Arthroplasty. D. F. Scott*, J. N. Woltz*. Orthopaedic Specialty Clinic of Spokane, Spokane, WA, USA.

The purpose of this study is to evaluate the effect of risedronate, an antiresorptive medication, on proximal femoral bone loss after total hip arthroplasty (THA).
Significant proximal femoral remodeling after THA has been observed and characterized by subjective roentgenographic analysis, with regions of relative bone resorption and regions of hypertrophy. Risedronate is an antiresorptive agent effective in increasing bone mineral density (BMD) in postmenopausal women with low bone mass. We hypothesize patients who take risedronate post THA will have less bone loss than patients not taking risedronate.
This prospective, open-label study consists of two statistically similar groups, the control group and the study group. All patients underwent uncemented THA with the same implant and postop protocol. Patients in the study group take 5 mg of risedronate daily beginning 5 to 7 days preoperatively, continuing for 24 months after surgery. Dual energy X-ray absorptiometry (DEXA) scans of the operated proximal femur are performed on all patients preoperatively, 3 to 7 days postop, 6 weeks postop, 6 months postop, 1 year postop, and 2 years postop. Dexa scans are analyzed using Lunar (Lunar Corp., Madison, WI) software and longitudinal changes in BMD compared within and between the two groups.
Using the first postop scan as the baseline, we found the mean decrease in BMD for the control group at latest follow-up to be 7.39 percent and for the study group to be 4.23 percent. Analyzing data of female subjects showed the mean decrease in BMD at latest follow-up to be 11.93 percent in the control group and 4.55 percent in the study group. This was found to be statistically significant (p=.027) using repeated measures analysis.
This short-term data reveals that risedronate significantly reduces bone loss in females after uncemented total hip arthroplasty.

Disclosures: ***D.F. Scott****, Procter & Gamble 2.*

SA348

See Friday Plenary number F348

SA349

Offset of the Action of Clodronate (Bonefos®) After 3 Years of Treatment in Women with Osteoporosis. S. Bal[1], E. McCloskey[1], P. Selby[2], M. Davies[2], J. Robinson*[3], R. M. Francis[4], J. Adams[5], A. Dey*[1], R. Ashford*[1], D. deTakats*[1], M. Beneton*[1], D. Charlesworth*[1], J. Rosnell*[6], J. Kanis[1]. [1]University of Sheffield, Sheffield, United Kingdom, [2]Manchester Royal Infirmary, Manchester, United Kingdom, [3]Crosby Clinical Research Centre, Liverpool, United Kingdom, [4]University of Newcastle, Newcastle, United Kingdom, [5]University of Manchester, Manchester, United Kingdom, [6]Schering Oy, Helsinki, Finland.

In a 3 year double blind placebo controlled study, clodronate 800mg daily (Bonefos®) by mouth increased BMD and reduced the incidence of vertebral fracture in osteoporotic women. During a 2 years blinded extension to the original study, we have examined the reversibility (offset) of the effect of oral clodronate on bone metabolism.
Of 140 women treated with clodronate for 3 years, 72 were randomly assigned to continue therapy while 68 were randomised to an identical placebo. All of the women continued to receive calcium 500mg daily by mouth.
Continuation of clodronate during the 2 years extension was associated with a further 3.3% increase (P<0.0001) in spine BMD (mean increase from study start +7.5%, p<0.0001). Total hip and forearm BMD remained stable over the whole 5 years of therapy (+0.54% and +0.93% respectively from study start, p=NS). In contrast, discontinuation of clodronate after 3 years was associated with a significant decrease in spine and hip BMD by the end of the first year off therapy (-1.19%, p=0.014 and -1.74%, p=0.0038 respectively) but only the changes at the spine were significantly different between the clodronate and placebo groups (p=0.019). A similar pattern was observed at the distal forearm where BMD decreased significantly by the end of the second year off treatment (-2.64 %, p<0.0001). The rates of decrease at the various skeletal sites in the women changing from clodronate to placebo were similar to those observed in women receiving placebo in the original 3

year study. In line with BMD, discontinuation of clodronate resulted in significant increases in all bone turnover markers (fasting urinary calcium/creatinine, hydroxyproline/creatinine, serum CTX and bone specific alkaline phosphatase) within 6-12 months.
We conclude that the offset of effect of clodronate commences within the first post-treatment year after 3 years of treatment. The effect of treatment to decrease bone turnover is rapidly reversible.

Disclosures: ***S. Bal****, None.*

SA350

See Friday Plenary number F350

SA351

Intravenous Pamidronate Increases Bone Density in Postmenopausal Women Previously Treated with Oral Bisphosphonates. A. Rastelli, N. Napoli, R. Civitelli. Div. of Bone and Mineral Diseases, Washington University in St Louis, St Louis, MO, USA.

Pamidronate, an aminobisphosphonate approved in the United States for the treatment of hypercalcemia of malignancy as intravenous (IV) formulation, is also commonly used for osteoporosis treatment in select patients. To evaluate the effectiveness of IV pamidronate on bone density, we retrospectively analyzed 17 patients (mean age 66.8±3.1) who attended our Bone Health clinic and received pamidronate (30 mg IV) at 3-month intervals up to 2 years. All patients had been previously treated with oral bisphosphonates (1 to 4 years) , but developed gastroesophageal discomfort. They were also on 1000-1500 mg of calcium and 400-800 IU of Vitamin D daily. Ten patients had a history of vertebral fractures and five had a hip fracture. All these subjects had one or more DEXA bone mineral density (BMD) measurements of the lumbar spine and proximal femur before starting IV pamidronate, and at least two repeat BMD 6 to 18 months thereafter. A group of 32 postmenopausal women (mean age 61.1±1.9), who were only on calcium and vitamin D supplements, were used as control. Data were expressed as annualized percent changes in BMD calculated by linear regression across all available data points for both groups, starting from the BMD just before initiation of pamidronate therapy (baseline). BMD at the lumbar spine increased during pamidronate therapy (+4.12±1.19 %/yr) compared to the changes that had occurred during the pre-treatment period (+0.10±0.47%/yr). This difference was statistically significant ($p = 0.02$; paired t-test). More to the point, the BMD increase observed during pamidronate therapy was significantly higher than the changes detected in the control group (-0.24 ± 0.44 %/yr; $p< .001$; unpaired t-test). Likewise, BMD of the proximal femur also increased in patients undergoing IV pamidronate (neck: +0.92 ± 0.88 %/yr; total: +5.14 ± 3.7 %/yr) relative to control subjects (neck: -0.04 ± 1.18 %/yr; total: +0.52 ± 0.66 %/yr), although the changes did not reach statistical significance. No adverse side effects or new clinical fractures were reported during IV pamidronate therapy. These data are consistent with a positive effect of pamidronate on BMD in osteoporotic subjects who had already been treated with other bisphosphonates. Larger prospective studies could further evaluate the long-term efficacy and fracture reduction properties of IV pamidronate.

Disclosures: ***A. Rastelli****, None.*

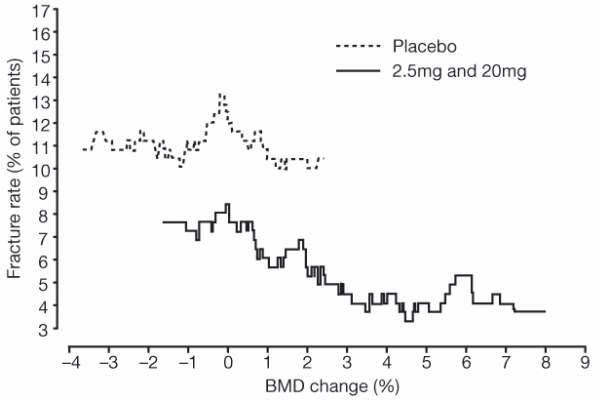
SA352

See Friday Plenary number F352

SA353

Changes in Bone Mineral Density as a Predictor of Vertebral Fracture Efficacy with Ibandronate: Results from a Phase III Fracture Study. R. Wasnich[1], P. D. Miller[2], C. H. Chesnut[3], H. Huss*[4], K. Wilson*[5], R. C. Schimmer[5]. [1]Radiant Research, Honolulu, HI, USA, [2]Colorado Center for Bone Research, Lakewood, CO, USA, [3]University of Washington, Seattle, VA, USA, [4]tbc, Leverkusen, Germany, [5]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

The fracture efficacy of antiresorptive agents is thought to be related to their ability to increase bone mineral density (BMD). A recent study (oral iBandronate Osteoporosis vertebral fracture trial in North America and Europe: BONE) of oral daily and intermittent ibandronate demonstrated significant increases in BMD and substantial reductions in vertebral fracture (VF) risk (62% [p=0.0001] and 50% [p=0.0006], respectively). A moving average procedure was used to explore the relationship between BMD change and VF rate with ibandronate. Increased BMD was associated with reduced VF rate. Most notably, analysis of total hip BMD change at 3 years revealed a decrease in the percentage of patients with VFs from approximately 7% in patients with no increase in BMD to approximately 4% in patients with ≥4% increase in BMD (Figure). Change in total hip BMD was also compared with the number of VFs per 100 patients. VF rate decreased from approximately 12 per 100 patients to 4–5 per 100 patients in patients with >5% increase in BMD. A similar pattern was observed for total hip BMD change at 2 years and lumbar spine (L2–L4) BMD change at 2 and 3 years. To further evaluate the relationship between BMD change and VF rate, a logistic regression model, which included BMD change as a linear term and considered the predictive value of number of prevalent VFs, was employed. Total hip BMD change at year 3 was found to be a significant predictor of VF risk reduction: a 1% increase in total hip BMD was estimated to account for a 7.9% (p=0.0084) reduction in risk. This finding was supported by subsequent analyses without adjustment for the number of prevalent VFs (7.1%; p=0.0165). Similar findings were reported for the change in total hip BMD at 2 years and lumbar spine (L2–L4) BMD at 2 and 3 years, although overall risk reductions were smaller than those at the total hip. Although BMD change does not provide a complete explanation for fracture risk reduction, these findings support the strong relationship between BMD change and VF efficacy with ibandronate.



Relative change in hip BMD vs vertebral fracture rate after 3 years

Disclosures: ***R. Wasnich****, None.*

SA354

See Friday Plenary number F354

SA355

Risedronate Reduces Vertebral Fractures in Men with Osteoporosis. J. D. Ringe*, A. Dorst*, H. Faber*. Department of Internal Medicine IV, Hospital Leverkusen, Teaching Hospital University of Cologne, D-51375 Leverkusen, Germany.

The incidence of vertebral fractures in men is lower than in women, but has recently been approximated to 6/1000 patient years. Causes of male osteoporosis include excess of glucocorticoids, hypogonadism, and other systemic conditions, medications, and lifestyle factors, but often there is no obvious cause. In women with postmenopausal osteoporosis Risedronate has been shown to significantly reduce vertebral and nonvertebral fracture risk as early as 6 months with sustained fracture reduction demonstrated for up to 5 years.
The purpose of this study is to determine the effects of Risedronate on vertebral fractures, spine and hip BMD in men with primary and secondary osteoporosis. In the single center, open label, prospective clinical trial 280 male patients (mean age: 57 years, range 33-77 years) with T-score values of lower than -2.5 SD at lumbar spine (LS) and lower than -2.0 SD at the total hip with or without prevalent vertebral fractures (vert.-fx) were included. The patients were allocated in pair-wise fashion into two treatment groups: Patients in group A (n=140) received Risedronate 5 mg daily plus Calcium 1000 mg and 800 IU Vit. D. In this group 69 patients had prevalent vert.-fx and 71 did not. Of 140 patients in group B, those with a prevalent vert-fx (subgroup B1 n=70) were treated with Alfacalcidol 1 g plus Calcium 500 mg daily, whereas patients without prevalent vert.-fx (subgroup B2, n=70) were treated with Calcium 1000 mg plus 800 IU plain Vitamin D daily. In group A 55 of 140 patients (39%) and in group B 56 of 140 patients (40%) had evidence of secondary osteoporosis. BMD, body height as well as x-rays were obtained at baseline and after 12 months, back pain was recorded on a scale from 0 (no pain) to 3 (severe pain).
After one year of treatment Risedronate increased mean lumbar spine LS-BMD by 4.5% compared to 0.8% in group B ($p<0.0001$). Significant increases of BMD were also observed for total hip and femoral neck BMD. Within one year the risk of new vertebral fractures was significantly reduced by 58% in men on Risedronate (7/140 (Ris) vs. 17/140 (Control), p=0.033, Chi^2-Test). Reduction of back pain after one year was significantly more pronounced in Risedronate versus control patients ($p<0.0001$), and mean height loss in men receiving Risedronate (-0.11cm) was less than in controls (-0.51 cm, $p<0.0001$, Wilcoxon-Test).
In men with primary or secondary osteoporosis, Risedronate reduces the risk of vertebral fractures within one year and increases BMD, consistent with the results from phase III trials in postmenopausal osteoporosis. In addition Risedronate treatment in men reduces height loss and back pain within one year.

Disclosures: ***J.D. Ringe****, None.*

SA356

See Friday Plenary number F356

SA357

Health-Economic Analysis of the Treatment of Osteoporosis with Risedronate. J. G. Brecht*[1], H. Kruse[2], D. Felsenberg*[3], W. Möhrke*[4], A. Oestreich*[4], E. Huppertz*[5]. [1]InForMed GmbH, Ingolstadt, Germany, [2]Abteilung für Nephrologie/Osteologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany, [3]Zentrum für Muskel- und Knochenforschung, Universitätsklinikum Benjamin Franklin, Berlin, Berlin, Germany, [4]Procter & Gamble Pharmaceuticals - Germany, Weiterstadt, Germany, [5]Aventis Pharma Deutschland GmbH, Bad Soden am Taunus, Germany.

Hip fracture is an important and costly problem. The therapy with the bisphosphonate Risedronate effectively prevents hip and other fractures among women with established osteoporosis (McClung, 2001). Risedronate is a first choice therapy option in the German Guidelines of the Dachverband Osteologie for Osteoporosis according to evidence-based medicine criteria for treatment of postmenopausal osteoporosis, osteoporosis of the elderly (women > 75 years) and gluco-corticoid induced osteoporosis (Leitlinie DVO, 2003).

Few economic evaluations of bisphosphonates have been published in Germany. So an assessment of the cost-effectiveness of Risedronate utilizing a Markov model of established post-menopausal osteoporosis based upon randomised clinical trial data was developed. Uncertainty underlying model parameters and outcomes was dealt with using traditional sensitivity analysis and stochastic sensitivity analysis to produce quasi-95%CIs. Baseline model was a cohort of 1000 70-year-old women, who received Risedronate for 3 years and were followed up for an overall observation period of 10 years, modelling transitions through estimated health states and evaluating outcomes.

Over the 3 year period of treatment and 10 year observation Risedronate dominated the current average basic treatment in Germany. In the Risedronate group 33 hip fractures were averted and 32 quality adjusted life years were gained (discounted values).

Treatment with Risedronate is cost saving for the German Social Insurance: The net present value of the associated costs from the perspective of the German Social Insurance is 10.66 million Euro if treated with Risedronate versus 11.00 million Euro in the case of basic treatment, thus net savings of €340 000 for the treatment group per 1000 treated women were calculated.

Furthermore treatment with Risedronate is cost-effective from the perspective of the Statutory Health Insurance with costs per averted hip fracture in the analysed population of €33,856 and cost per QALY gained of €35,690. Both results demonstrate cost effectiveness and are far below the accepted threshold level of €50.000.

Based on this analysis Risedronate is a cost-effective treatment for postmenopausal osteoporosis within the German health care system offering benefits for osteoporotic patients and for budget decision makers.

Disclosures: ***J.G. Brecht****, Procter & Gamble Pharmaceuticals - Germany GmbH 5; Aventis Pharma Deutschland GmbH 5.*

SA358

See Friday Plenary number F358

SA359

Alendronate Effects on Material Properties and Pre- and Post-Yield Behavior of Rat Cortical Bone may not Entirely Depend upon Bone Mass and Mineralization. M. A. Chiappe*[1], G. R. Cointry*[2], R. F. Capozza*[2], G. Iorio*[1], E. Alvarez*[1], J. R. Zanchetta[2], E. J. Roldán[2], J. L. Ferretti[2]. [1]Dept. of Physiology, Faculty of Veterinary Sciences, UBA, Buenos Aires, Argentina, [2]Centro de Estudios de Metabolismo Fosfocálcico, Faculty of Medicine, UNR, Rosario, Argentina.

In order to describe Alendronate (AL) effects on cortical bone, 40 3-month-old rats were ovariectomized (OX) and immediately given sc doses of 0 (OX ctrl, n=13), 5 (OX+5, 13), and 25 ug/kg (OX+25, 14) of AL 2/wk during 6 months, while further 15 remained as sham controls. Their femur diaphyses were studied by DEXA and pQCT and tested in bending.

Despite that no differences in bone mineralization ("areal" or volumetric BMD) and geometry (cortical cross-sectional diameters, area, and bending moment of inertia, CSMI) were observed, the OX reduced all bone tissue stiffness (elastic modulus, E), diaphyseal stiffness, load supported at the yield point, and ultimate load. The post-yield behavior of the bones (difference between ultimate and yield loads) was strikingly improved by OX, presumably as a consequence of the inverse relationship usually observed between effects on crack generation (facilitated in this case) and progression. AL treatment prevented all negative OX effects and improved the bones' ultimate strength over sham values at the highest dose. Despite that the load at the yield point was normal in AL-treated groups (no effects on crack generation), their bones showed the same improvement in the post-yield strength as those from OX rats (resistance to crack progress). The negative correlations observed between bone architecture (CSMI, y) and material quality indicators (E, x) showed significant "anti-anabolic" shifts for the OX group and "anti-catabolic" displacements for the OX+AL groups with respect to controls. This should have reflected negative and positive interactions of OX and AL, respectively, with the feedback control of bone geometry as a function of bone material stiffness according to the mechanical usage of the skeleton (bone "mechanostat" theory). Lack of effects on mineralization and geometry and the effects on post-yield bone behavior suggest that both OX and AL treatment would have affected the microstructural determinants of bone material stiffness / strength which are unrelated to bone mineralization. Data suggest that the improvement induced in the post-yield behavior of bones by both OX and AL treatment should have reflected different effects on those factors. These interesting aspects of bisphosphonate effects, perhaps related to the observed dissociation between bisphosphonate effects on BMD and fracture incidence in clinical studies, deserve further attention.

Disclosures: ***J.L. Ferretti****, None.*

SA360

Femoral Neck Mechanical Quality and MR Microscopic Imaging of a Postmenopausal Osteoporosis Model in Ewes Treated with Calcitonin. Y. Jiang[1], J. J. Zhao[1], J. A. Lynch*[1], X. Ouyang*[1], P. Geusens[2], P. Adriaensens*[2], J. Gelan*[2], M. Azria*[3], H. K. Genant[1]. [1]Osteoporosis and Arthritis Research Group, University of California, San Francisco, CA, USA, [2]Limburgs Universitair Centrum, Diepenbeek, Belgium, [3]Novartis, Basel, Switzerland.

To evaluate an ovariectomy (OVX)-induced osteoporotic model and effects of salmon calcitonin (sCT), an osteclast inhibitor, 28 middle aged (5–8 years old) ewes were randomly and equally allocated into 4 groups: sham (Sham) or OVX injected with vehicle, OVX injected with sCT at 50 U or 100 U. It was a double-blind study with 3 subcutaneous injections a week. They were euthanized 6 months post-OVX. The femoral neck was examined with an MR imager at 9.4 Tesla in axial, coronal, and saggital planes, using a spin echo multislice pulse sequence with TR 1 s, TE 1.8 ms, inplane resolution 78 μ, and slice thickness 1 mm. An internal calibration procedure standardizing image analysis was used to adjust the segmentation threshold. Data from all 3 axial, coronal, and saggital planes were averaged. The operators were blinded to animal codes. The femoral neck BMD was measured by both dual energy QCT and DXA. Compressive testing was performed on trabecular (Tb) cylinder cores of 8 mm in diameter and 10 mm in length aligned with the axis of the neck. Compared with Sham, OVX induced statistically significant changes in the femoral neck micro structure: Tb bone volume fraction –18%, Tb separation +23%, number of free ends +28%, number of nodes –39%, number of Tb branches –23%, and mean length of Tb branches –19%. There was a dose response of sCT effect on the Tb structure. Compared with OVX, treatment of sCT at 100 U statistically significantly improved all the Tb structural parameters to the Sham level, while at 50 U significantly increased the mean length of the Tb branches. OVX significantly decreased Tb biomechanical competence (compressive strain -28%). Treatment with sCT resulted in a significant dose-dependent preservation of compressive stress. Tb bone volume fraction explained 74% of compressive stress. Combination of all structural parameters in a multivariate regression analysis significantly improved the explanation to 84%, and combination of BMD further increased the explanation to 92%. Thus, OVX induces deterioration of the MRI-drived Tb microstructure and of the biomechanical properties in the femoral neck of ewes. sCT treatment prevents OVX induced changes in a dose-dependent manner. The femoral neck Tb microstructure significantly correlates with biomechanical properties, and its combination with BMD further improves the prediction of bone quality. The effects of sCT on the OVX ewes may help explain reduced fracture risk in postmenopausal osteoporotic women treated with sCT.

Disclosures: ***Y. Jiang****, Novartis 2.*

SA361

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SA362

Hip and Spine BMD Increases Following Six Months of Daily Treatment with Fortical® Salmon Calcitonin Nasal Spray. N. Mehta, W. Stern*, A. Sturmer*, A. Malootian*, S. Philip*, S. Mitta*, J. P. Gilligan. Unigene Laboratories Inc, Fairfield, NJ, USA.

A novel nasal spray formulation (Fortical®) has been developed that contains recombinant sCT (rsCT) produced using a direct expression technology in *E. coli.* The pharmacodynamic response of Fortical® was determined in a multi-dose, double blind, parallel design tolerability and pharmacology study using a commercially available nasal spray product as a positive control. One hundred and thirty four osteoporotic women received 6 months of daily dosing of 200 IU per day of Fortical® nasal spray or the positive control, with calcium and vitamin D supplementation. Several markers of bone resorption and bone formation were measured throughout the first 3 months of the study. Spine and hip BMD were measured at baseline and at the end of the six-month dosing period. The key findings from the study were as follows: 1) Fortical® treatment resulted in a modest but statistically significant increase in BMD of 1.3 % at the AP spine and 1.1% at the hip at 6 months, compared to baseline. 2) Plasma levels of the primary end-point β-CTx, were decreased by approximately 40% after the first month, and this decrease persisted through 3 months of Fortical® treatment. 3) Statistically significant decreases in NTx and urinary DPD were also seen throughout the 3 months of measurement. 4) Fortical® significantly decreased the bone formation markers osteocalcin and BSAP at the 3 month time-point compared to baseline. Overall, there was no statistically significant difference in bone markers or BMD between Fortical® and the positive control. Fortical® nasal spray is an alternate sCT nasal spray therapy that achieves equivalent clinical results and has a comparable systemic safety profile to that of a currently marketed sCT nasal spray product, with a formulation that does not contain benzalkonium chloride.

Disclosures: ***N. Mehta****, Unigene Laboratories Inc. 1, 3.*

SA363

Soy Isoflavones: Increased Bone Turnover and Less Bone Loss at the Hip in Postmenopausal Women. L. A. Fitzpatrick[1], A. Vincent[1], A. Oberg*[2]. [1]Endocrinology, Diabetes, Metabolism, Nutrtion & Internal Medicine, Mayo Clinic, Rochester, MN, USA, [2]Biostatistics, Mayo Clinic, Rochester, MN, USA.

Soy phytoestrogens are SERM-like compounds that have lower affinity for binding estrogen receptors alpha and beta compared to estradiol, however, in vivo work has suggested that they have more potent transcriptional activity than 17-β-estradiol. These soy isoflavones exhibit both antiresorptive and anabolic actions on the skeleton in vitro. Genistein, one of the soy isoflavones, stimulates DNA synthesis and markers of differentiation in osteoblast-like cells. Few studies have examined the effect of isolated isoflavones on bone markers and BMD long term to determine the magnitude of their effects and whether isoflavones are antiresorptive or anabolic to bone. We tested the effects of 110 mg of isolated soy isoflavones (1:1 genistein:daidzein) on bone markers and BMD in postmenopausal women over 1 year. Seventy-one healthy postmenopausal women, mean age 58.3 years, were randomly assigned to placebo or 2 isolated isoflavone tablets (ISO) for 6 months; 43 subjects agreed to extend and completed the study for 1 year. The isoflavones were commercially available as "Healthy Woman Soy Menopause" a proprietary, standardized tablet made from highly concentrated soy isoflavones and containing 320 mg of soy extract and 55 mg of isoflavones per tablet. Bone markers (N-telopeptide (NTx), pyridinoline (Pyd), deoxypyridinoline (Dypd), osteocalcin (OC), bone-specific alkaline phosphatase (BSAP)) and DEXA BMD at hip and spine were measured at baseline, 6 and 12 months.

There were significant increases in Dypd and Pyd in both groups, however, the increases were greater in the ISO group at 12 months ($p=0.04$ and $p=0.034$, respectively). BSAP ($p<0.01$) was significantly increased in the ISO group at 12 months compared to baseline levels. Serum NTx decreased in the ISO group ($p=0.045$) and OC increased ($p=0.009$) compared to placebo. However, urine NTx increased in both groups at 12 months ($p<0.02$ for ISO; $p<0.05$ for placebo) but there was no significant difference between groups. Hip BMD fell in the placebo group ($p<0.01$) over 12 months, with no decrease noted in the ISO-treated group. No differences were noted in spine BMD over 12 months in either group.

The changes in the bone markers in the ISO group (with the exception of serum NTx) are suggestive of increased bone turnover that is consistent with the anabolic action of other agents. These minimal changes are reflected in less bone loss at the hip in the ISO-treated group. These data suggest that isolated soy isoflavones may have a small benefit in terms of preventing postmenopausal bone loss, but long-term studies are needed to assess any reduction in fracture risk.

Disclosures: ***L.A. Fitzpatrick****, National Institutes of Health 2, 6; Mayo Foundation 2, 3; Johnson & Johnson 2.*

SA364

See Friday Plenary number F364

SA365

Soy Protein-Based Diet Improves Bone Mineral Density in Osteopenic Female Rats. D. A. Khalil[1], L. Devareddy*[1], L. J. Hammond*[1], D. Y. Soung[1], D. Bellmer*[2], B. H. Arjmandi[1]. [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Food and Agricultural Products Research and Technology, Oklahoma State University, Stillwater, OK, USA.

The objective of this study was to determine if soy protein with graded dose of isoflavones reverses bone loss in an ovariectomized (ovx) osteopenic rat model. Seventy-seven 9 mo old Sprague-Dawley rats were either sham-operated (sham; 1 group) or ovx (5 groups) and fed a semi-purified casein-based diet. After 90 days the occurrence of bone loss was confirmed using dual energy x-ray absorptiometry. Thereafter, rats were assigned to following treatments: sham, ovx (control), ovx + 17β-estradiol (E_2; 10 μg E_2/kg body wt. twice per wk), ovx + soy protein depleted of isoflavones (Soy-; 1.1 mg isoflavones/g protein), ovx + soy protein with normal isoflavone content (Soy; 2.2 mg isoflavones/g protein)), and ovx + soy protein enriched isoflavones (Soy+; 4.4 mg isoflavones/g protein). After 125 days rats were sacrificed and tissues were collected.

	Left tibia		Fourth lumbar	
Group	BMC	BMD	BMC	BMD
Sham	0.3584^{a}	0.1993^{a}	0.1465^{a}	0.2342^{a}
Ovx	0.3103^{c}	0.1825^{c}	0.1189^{b}	0.2055^{b}
Soy-	0.3272^{bc}	0.1882^{bc}	0.1245^{b}	0.2088^{b}
Soy	0.3423^{ab}	0.1908^{b}	0.1273^{b}	0.2124^{b}
Soy+	0.3296^{bc}	0.1899^{b}	0.1243^{b}	0.2097^{b}
Estrogen	0.3273^{bc}	0.1892^{b}	0.1270^{b}	0.2132^{b}

Values in a column not sharing a superscript letter are different from each other ($P<0.05$; $n=12$ per treatment group).

Data analysis indicated that Soy had significantly increased tibial bone mineral content (BMC) but Soy- and Soy+ did not significantly increase tibial BMC. These observations confirm the notion that soy isoflavones may have a biphasic effect on bone. Furthermore, this increase in BMC may be due to the ability of soy in enhancing insulin-like growth factor-I which is known to positively influence BMC. Tibial bone mineral density (BMD) was equally increased by Soy, Soy+, and E_2 but not Soy- indicating the importance of isoflavones in reversal of bone loss. Although 4th lumbar BMC and BMD were slightly improved by soy and E_2 treatments, the values were not significantly different from the ovx control group. Perhaps a longer treatment period would have shown a more pronounced effects on 4th lumbar BMC and BMD.

Disclosures: ***D.A. Khalil****, None.*

SA366

Clinical Outcomes in Osteoporotic Patients with Acute Vertebral Fractures Treated by Percutaneous Vertebroplasty or Conservative Measures: An Interim Analysis. T. H. Diamond, W. A. Clark*. Endocrinology, University of New South Wales, St George Hospital Campus, Sydney, Australia.

Acute osteoporotic vertebral fractures often cause severe back pain, resulting in hospitalisation and an increased morbidity. Percutaneous vertebroplasty (PV) has been a major breakthrough in the management of this syndrome. In an interim analysis, we compare the role of PV to conservative therapy (CT).

126 consecutive osteoporotic patients presenting to St George Hospital with severe back pain due to acute vertebral fractures were enrolled over a 24 month period. 88 patients were treated by PV and 38 by CT alone. All patients received anti-osteoporotic therapies in addition to analgesia. Their osteoporotic risk factors and bone densitometry was measured at baseline, while the patients' pain score and level of function was recorded on presentation, at 24 hours, 6 weeks, 12 and 24 months post therapy. All data was compared using a student t-test or analysis of variance and analysed on an intention to treat analysis.

There were 39 men and 87 women, aged 51-95 years, followed for 362 days (range 32-764). Patients treated by PV did not differ from those treated by CT, with respect to their biochemistry, bone densitometry and radiological evidence of multiple pre-existing vertebral fractures. A significant decrease in the patients' pain score from 20 to 8 (-60%, P=0.0001) and an improvement in their physical functioning from 13 to 18 (+34%, P=0.0001) was noted at 24 hours after PV. Twenty-six patients (30%) were able to cease their analgesia and a further 54 patients (61%) were able to reduce their analgesia by more than 50% after the procedure. By contrast symptomatic improvement in the patients treated by CT was delayed. A decrease in their pain score from 19 to 7 (-63%, P=0.0001) and an improvement in their physical functioning from 14 to 17 (+21%, P=0.01) was only evident at 6 weeks post therapy, with 32 patients (84%) still requiring some form of ongoing analgesia. No significant differences were noted between the two groups at 12 and 24 months of follow up.

Early complications related to PV included a psoas haemorrhage in 1 patient (1.1%) and fractured transverse process in 3 patients (3.4%). There were 11 patients (13%) in the PV and 6 patients (16%) in the CT group who died from non-procedural related diseases. New clinical apparent vertebral fractures occurred in 7 patients (8%) treated by PV and in 2 patients (5%) treated by CT.

In experienced hands, PV is safe and allows for the prompt relief of pain and rapid rehabilitation of patients presenting with acute osteoporotic vertebral fractures. In the interim analysis, PV did not appear to result in an increase in mortality or clinical apparent fractures.

Disclosures: ***T.H. Diamond****, None.*

SA367

See Friday Plenary number F367

SA368

Discrepancy in Follow-Up Absorptiometry Measurements of Lumbar Spine and Proximal Femur. S. Di Gregorio*, L. del-Rio, P. Bassa*, J. Rosales*. Bone densitometry, CETIR.Centre Medic, Barcelona, Spain.

Bone mineral density (BMD) measurement by DXA of at least two skeletal regions (lumbar spine and proximal femur) has shown its utility for assessment of risk fracture as well as a method to evaluate bone mass changes due to metabolic bone diseases or their treatment. Trend in bone changes, however, is not always alike for both regions, irrespective of technical differences and this arises some clinical concern with regard to patient follow-up. In order to investigate the impact of these differences and their origin, a longitudinal study with 3,310 patients who attended our service for evolution of BMD by DXA was undertaken. BMD of lumbar spine and proximal femur were evaluated in every patient with the same DXA device as the previous scan (mean time between scans was 2.9±1.8 years). A "discordant evolution" was established when differences greater and with opposite sign to three times the variation coefficient adjusted for the device used were found between lumbar spine and proximal femur measurements.

In order to avoid confusing factors we furthermore selected a group of patients with the following inclusion criteria: postmenopausal women with no treatment nor diseases that affected BMD including osteoarthritic changes. In this group, changes in BMD, weight, body mass index, calcium intake, physical activity were statistically treated by bivariate correlation, linear regression and multinomial logistic regression.

From the total of patients analyzed, (n= 3310) a discordant evolution was found in 8.5% (n=282). The mean age for the patients with no treatment or diseases (n=107) was 62 years (range 46-83 years). In these patients the most common discordant evolution model (76%) was a BMD increment in lumbar spine and decrease in femoral region (Type I). The loss in spine density and femoral increase (Type II) was only detected in 24% of patients. In both discordant models a consistent statistical positive correlation ($p< 0.0001$) was found between femoral BMD and weight gain and a similar but negative order correlated with the spine density ($p< 0.001$). The relationship was most powerful in the following cases: women aged 65 years or older; early menopausal; calcium intake above 1,000 mg/day, 3 kg or greater weight gain.

The treated group (mostly anti-resorptive) (n= 175; mean age 63 years, range 35-84) showed a similar discordance pattern with a 70% Type I and 30% Type II.
A small but significant discordant trend was found in follow-up BMD measurements in our population. Some factors like changes in anthropometric parameters and especially the body weight could, at least partially, make up for these differences in BMD evolution in the non-treated patient.

Disclosures: ***S. Di Gregorio****, None.*

SA369

Screening Heel Ultrasound from Primary Care Settings Effectively Guides Treatment. D. J. Brown*, R. J. Quinet, W. E. Davis*, L. H. Serebro*, J. M. Zakem*, M. Krousel-Wood*, S. Ishaq*. Rheumatology, Ochsner, New Orleans, LA, USA.

Heel Ultrasound has been found to effectively predict fracture risk. We studied the association between heel ultrasound results and effect on subsequent therapy. **Methods:** 94 female patients who had screening heel ultrasound in the year 2000 at Ochsner Clinic Foundation who were referred from primary care clinics were enrolled. Heel ultrasound was performed using Hologic Sahara heel ultrasound machine. Osteoporosis/osteopenia was defined as T score less than or equal to –1.0. Follow up by the primary care physician and subsequent prescription of estrogen therapy, calcium, raloxifene, bisphosphonates, and calcitonin was observed through chart review. Pearson chi-square test was used for statistical analysis. **Results:** Mean patient age was 66.27 (range 39 – 82 years). Of the 94 patients, 86 were Caucasian, 6 were Hispanic, and 2 were African American. 47.9% of the patients had an abnormal bone density test. Abnormal heel ultrasound was found to be significantly associates with smoking (p=0.016), and weight less than 124 lbs (p=0.022). Prescription of osteoporosis medication was significantly associated with the heel ultrasound result. Patients who had an abnormal result were more likely to have osteoporosis medications prescribed than those who had normal results (X2 = 22.88, p=0.001). Of the individual medications screened for, bisphosphonates were more likely to be prescribed if the heel ultrasound was abnormal (p=0.001). The other medications screened for approached but did not reach statistical significance. **Conclusion:** We concluded that heel ultrasound is an effective instrument in identification of osteoporosis and results guide treatment in primary care clinics.

Disclosures: ***D.J. Brown****, None.*

SA370

See Friday Plenary number F370.

SA371

The Changes of Circulating Osteoprotegerin after Hormone Replacement Therapy in Postmenopausal Women and their Relationship with Estrogen Responsiveness on Bone. Y. Kang*[1], K. Han[2], J. Kim*[1], S. Kim*[1], C. Hwang*[1], I. Moon*[1], C. Yim*[2], H. Chung[2], W. Park*[2], H. Yoon[2], I. Han*[2]. [1]Laboratory of Endocrinology, Samsungcheil Hospital, School of Medicine, Sungkyunkwan University, Seoul, Republic of Korea, [2]Internal Medicine, Samsungcheil Hospital, School of Medicine, Sungkyunkwan University, Seoul, Republic of Korea.

Estrogen replacement reduces the increased rate of bone remodeling after menopause. Osteoprotegerin (OPG) is a negative regulator of osteoclast-mediated bone resorption. The studies in vitro have shown that estrogen stimulates OPG production. We, therefore, analyzed serum for OPG levels and assessed their association with estrogen responsiveness on bone before and after hormone replacement therapy (HRT) in 99 postmenopausal women aged 52.3 ± 4.9 yr. This is the first study conducted under conditions of estrogen replacement in healthy postmenopausal women. The mean circulating level of OPG before HRT was 3.54 ± 1.13 ng/ml. Baseline levels of OPG were correlated neither with bone formation marker, serum osteocalcin (BGP) nor with bone resorption marker, serum carboxyterminal telopeptides (CTx). No significant association of baseline OPG level was found with baseline bone mineral densities (BMDs) measured at lumbar spine and femoral neck. Serum OPG levels measured after 3 months and 1 yr of HRT decreased significantly compared to baseline (p<0.001 in both). The median decrements of circulating OPG were 14.7% (-22.4~ -2.8%, IQR) and 11.8% (-27.0~-2.9%, IQR) at 3 months of HRT and at 1 year of HRT, respectively. The changes in circulating OPG at 3 months of HRT were correlated with the changes in both serum BGP (r=0.226, p=0.029) and serum CTx (r=0.214, p=0.038) at 3 months after HRT. We, however, could not find any association between changes in circulating OPG at 3 months of HRT and changes in BMDs at 1 yr of HRT measured at either lumbar spine or femoral neck. These results suggest that baseline OPG levels do not reflect the bone turnover status and that the serial measurement of serum OPG after HRT is not a useful predictor for the long-term effects of estrogen on bone. The decreased serum concentrations of OPG after HRT seem to be caused by the compensation to suppress the bone resorption by estrogen.

Disclosures: ***Y. Kang****, None.*

SA372

See Friday Plenary number F372

SA373

PSK 3471, a New Designer Estrogen, Prevents Ovariectomy-induced Bone Loss in Mice and Keeps a Safe Profile on Uterus. P. Clément-Lacroix[1], R. Galien[1], F. Marsais*[1], M. Roux*[1], F. Nique*[1], C. Belleville*[1], D. Minet*[1], L. Lepescheux[1], M. Gaillard-Kelly*[2], M. Resche-Rigon[1], R. Baron[1]. [1]ProSkelia Pharmaceuticals, Romainville, France, [2]Oncology, Aventis Pharma, Vitry, France.

Estrogen replacement therapy (ERT) after menopause prevents the development of osteoporosis and reduces the risk of fracture. ERT, however, increases the risk of endometrial and breast cancer. Selective Estrogen Receptor Modulators (SERMs) display antiestrogenic activity in breast and uterus, while triggering estrogenic effects in bone. However increased risk in endometrial cancer is still associated with Tamoxifen (TAM), a first generation SERM. PSK3471 is a new steroidal designer estrogen, which behaves in vitro as an AF2 antagonist like TAM and Raloxifen (RAL). In contrast to TAM, but similarly to RAL, PSK3471 has limited if any, ability to stimulate the AF1 function of ERα. In addition, PSK3471 *per se* does not stimulate proliferation of MCF7 cells and inhibits the E2-induced proliferation of these cells. In the present study, PSK3471 has been evaluated in ovariectomized (OVX) mice. Twelve week-old OVX mice were orally administrated PSK3471 (0.01, 0.03, 0.1, 0.3, 1mg/kg/d), TAM (0.03, 0.1, 0.3mg/kg/d), or RAL (0.1, 0.3, 1, 3mg/kg/d) immediately after surgery, for 4 weeks. MicroCT analysis of proximal tibial metaphysis demonstrated full prevention of OVX-induced trabecular bone loss in mice dosed with PSK3471 (0.1mg/kg/d), a dose equipotent to TAM 0.3mg/kg/d or RAL 3mg/kg/d. Ranking of compounds in this model on the basis of their activity on bone tissue was PSK3471>TAM>RAL. In order to assess possible adverse effects on genital tract, morphometry of uterus and proliferation index (PI) of luminal epithelial cells were evaluated. As expected, ovariectomy induced a dramatic atrophy of the uterus, which was partially prevented in mice treated with either compound. Most importantly, PI of epithelial cells was highly stimulated in mice treated with lower doses of TAM. At higher doses several kystic glands appeared within the endometrium of these mice. In sharp contrast, neither RAL, nor PSK3471 significantly stimulated proliferation of epithelial cells or induced formation of kystic glands at any tested doses. These data indicate that PSK3471 is a promising compound for the prevention and/or treatment of postmenopausal osteoporosis.

Disclosures: ***P. Clément-Lacroix****, None.*

SA374

See Friday Plenary number F374

SA375

Molecular Action of Phytoestrogens in Human Breast Cancer (MCF-7) Cells. W. Chen*[1], R. Y. K. Chan*[1], D. Guo*[2], M. Wong[1]. [1]Central Laboratory of the Institute of Molecular Technology for Drug Discovery and Synthesis, The Hong Kong Polytechnic University, Hong Kong, Hong Kong Special Administrative Region of China, [2]School of Pharmaceutical Sciences, Peking University, Beijing, China.

Estrogen replacement has been an important therapy for the prevention and treatment of osteoporosis in postmenopausal women. However, its undesirable side effects have limited its long-term use. Phytoestrogens are currently receiving considerable attention as potential alternative therapy for reducing the incidence of cancer and for protection against osteoporosis. Elucidating their mechanism of actions can help us to understand the potentially benefit and risk of phytoestrogens consumption. Recent studies demonstrated that cross talk exists between estrogen receptor (ER)-dependent and Insulin-like growth factor I receptor (IGFIR)-dependent pathways in ER-positive breast cancer cells. In the present study, we hypothesize that ginsenoside Rg1 and isoflavone genistein will act like estrogen and mediate their actions via the cross-talk with IGF-I receptor mediated pathway.
MCF-7 cells were routinely cultured in phenol-red free DMEM supplemented with 1% charcoal-stripped fetal bovine serum for 5 days. Then cells were exposed to 1pM Rg1, 1μM genistein or 10nM 17β-estradiol, their effect on cell proliferation were determined by flow cytometry. Protein expression of IGF-IR, IRS-1 and Shc were measured by western blotting analysis and mRNA expression of IGF-IR and IRS-1 were determined by RT-PCR**.** Our results show that Rg1 and genistein significantly increased the proliferative phase (S-phase) and decreased the resting phase (G0/G1 phase) (p<0.05) of MCF-7 cells after 24h treatment. Both Rg1 and genistein increased the expression of IGF-IR and its downstream signaling molecule IRS-1 in a time dependent manner. But no apparent change was found on Shc protein level. And these effects could be blocked by estrogen antagonist ICI 180,782. Cycloheximide, a protein synthesis inhibitor could block the induction of IGF-IR and IRS-1 by both Rg1 and genistein. These data provide the first evidence that the IGF-IR pathway is involved in the proliferative effect of phytoestrogen, Rg1 and genistein, in MCF-7 cells.

Disclosures: ***M. Wong****, None.*

SA376

See Friday Plenary number F376

SA377

No Effect of Estrogen Receptor Gene Polymorphisms on Bone Mineral Density, Bone Loss and Treatment Response to Estrogen. P. B. Rapuri[1], J. C. Gallagher[1], V. Haynatzka*[2]. [1]Bone Metabolism Unit, Creighton University, Omaha, NE, USA, [2]Department of Preventive Medicine, Creighton University, Omaha, NE, USA.

The genetic basis of osteoporosis has been known for several years. Several candidate genes which contribute to bone mineral density (BMD) and bone turnover have been implicated in the pathogenesis of osteoporosis. As estrogen deficiency is a major determinant of bone loss after menopause, association studies have been carried out between estrogen receptor (ER) gene polymorphisms and BMD and the results have been inconsistent. The aim of the present study was to investigate the influence of ER gene polymorphisms on baseline BMD, bone loss rates and response in BMD to estrogen/hormone therapy (ET/HT) in elderly postmenopausal women. The association between ER genotypes and baseline BMD was tested in 489 elderly women (mean age 71± 3 years) recruited for an osteoporosis intervention study. The association between ER genotypes and the rate of bone loss after 3 y was studied in 96 women assigned to the placebo arm and the relation between ER genotypes and response to ET/HT was studied in 79 women receiving the ET/HT for 3 y. The ER gene was genotyped for restriction enzymes PvuII and XBaI. BMD measurements for spine, proximal femur, total body and radius were performed by DEXA and the percent change in BMD over baseline after 3 y of treatment was calculated. The baseline BMD, rate of change in BMD after 3 y in placebo and ET/HT groups were compared between the ER genotypes. The data were analyzed by procedure Mixed from SAS statistical package. Adjustments were made for appropriate covariates like age, weight etc. The observed frequency distribution was PP 21.3%, Pp 51.2% and pp 27.1% for PvuII and XX 14.1%, Xx 48.9% and xx 37.1% for XbaI ER gene polymorphisms. Neither the PuvII nor the XbaI ER gene polymorphisms were associated with baseline BMD. There was no significant association between ER gene polymorphisms and the rate of bone loss after 3 y in women of the placebo group. Also the ER gene polymorphims did not influence the response in BMD to ET/HTafter 3 y. In conclusion, we could not demonstrate any major effect of the ER gene polymorphisms on BMD, rate of bone loss or on the response in BMD to ET/HT in elderly postmenopausal women.

Disclosures: ***P.B. Rapuri****, None.*

SA378

See Friday Plenary number F378

SA379

Association Study of Estrogen Receptor Thymine-Adenine Repeat Polymorphism with Estrogen Responsiveness after HRT on Bone Density and Serum Lipid in Postmenopausal Women. C. Yim*[1], K. Han[1], Y. Kang*[2], J. Kim*[2], S. Kim*[2], C. Hwang*[2], I. Moon*[2], H. Chung[1], W. Park*[1], H. Yoon[1], I. Han*[1]. [1]Internal Medicine, Samsungcheil Hospital, School of Medicine, Sungkyunkwan University, Seoul, Republic of Korea, [2]Laboratory of Endocrinology, Samsungcheil Hospital, School of Medicine, Sungkyunkwan University, Seoul, Republic of Korea.

Several biologically plausible mechanisms have been proposed for estrogen-associated changes in lipid and bone metabolism. These effects are thought to be mediated via estrogen receptor (ER). Several polymorphisms in the gene encoding estrogen receptor alpha may modify the effects of hormone-replacement therapy on lipid and bone density in postmenopausal women. We examined 284 postmenopausal women for thymine-adenine (TA) repeat polymorphism at the ER gene locus and its relationship to bone density and lipid. Their mean age was 52.2 ± 5.0 years. We assessed the association of ER TA repeat polymorphism with either BMD or bone turnover markers. We also analyzed the association between ER TA repeat polymorphism and changes in BMD, bone turnover markers and lipid until 1 year after hormone replacement therapy. The women were stratified by the mean number of TA repeats and assigned to one of two groups : group H, with higher number of repeats (TA>16)(n=110); group L, with lower number of repeats (TA≤16)(n=174). No significant genotypic differences were found in BMD and bone markers. Group L showed significantly greater changes in total and LDL cholesterol levels after 3 month of estrogen replacement therapy than in group H (changes in total cholesterol: -8.4 ± 11.3% vs. -4.2 ± 12.5%, p=0.019), (changes in LDL cholesterol: -18.7 ± 18.4% vs -8.8 ± 30.1%, p=0.006). There was no significant relationship between TA repeat polymorphism and changes in HDL cholesterol, and triglyceride levels after 1 year of estrogen replacement therapy. These data suggest that ER TA repeat polymorphism may predict the response of lipid profile to estrogen replacement therapy, but this ER polymorphism is not associated with changes in BMD or bone turnover markers in Korean women.

Disclosures: ***C. Yim****, None.*

SA380

See Friday Plenary number F380

SA381

Meta-Analysis of the Efficacy of Raloxifene on Reduction of Vertebral Fracture Risk. E. Seeman[1], G. Crans*[2], A. Diez-Perez[2], S. R. Cummings[3]. [1]University of Melbourne, Melbourne, Australia, [2]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA, [3]San Francisco Coordinating Center, San Francisco, CA, USA.

Raloxifene (RLX) has been reported to reduce vertebral fractures (VFx) in the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, but no systematic analysis of the vertebral fracture reduction efficacy has been reported examining the results of several other studies in which vertebral fracture data have been collected. In an attempt to determine whether there is internal consistency of the results, and to more accurately define the point estimate for fracture risk reduction, a meta-analysis was undertaken using all randomized, double-blind, placebo-controlled trials in postmenopausal women in which fracture data was available from prospectively scheduled spinal radiographs. Three prevention studies (GGGF, GGGG, GGGH), two arms of the MORE trial, and two additional treatment studies were included in the analysis. A systematic review of the literature (MedLine, EMBASE) confirmed that all appropriate RLX studies had been identified for this analysis. The effects of raloxifene 60 mg/d (RLX60) and120 mg/d pooled with 150 mg/d (RLX120/150) were analyzed. Intention-to-treat data were used in the analysis, and tests of heterogeneity were performed. If the results of the different studies were not heterogeneous, the weighted relative risk (RR) estimates with 95% CI were calculated. The data from each study are summarized in the table.

Study	Duration	Therapy	VFx#/patients	RR (95% CI)
MORE Non-prevalent VFx	3 years	PL	62/1457	
		RLX60	27/1401	0.45 (0.29, 0.71)
		RLX120	37/1414	0.61 (0.41, 0.92)
MORE Prevalent VFx	3 years	PL	169/835	
		RLX60	121/858	0.70 (0.56, 0.86)
		RLX120	87/863	0.50 (0.39, 0.63)
Prevention studies (GGGF, GGGG, GGGH)	3 years	PL	6/287	
		RLX60	3/281	0.51 (0.13, 2.02)
		RLX150	2/267	0.36 (0.07, 1.76)
Japan Trial	1 year	PL	2/97	
		RLX60	0/90	N/A
		RLX120	1/93	0.52 (0.05, 5.65)
Osteoporosis Trial (GGGN)	1 year	PL	18/45	
		RLX60	21/43	1.22 (0.76, 1.96)
		RLX120	20/45	1.11 (0.68, 1.80)

There was no statistically significant heterogeneity in studies of vertebral fractures for either RLX60 or RLX120/150. Weighted relative risk values (95% CI) were 0.53 (0.39, 0.78) for RLX60 and 0.57 (0.39, 0.80) for RLX120/150. These results show a consistent effect of raloxifene on the reduction of vertebral fracture risk across studies in several populations of postmenopausal women. A significant 47% reduction in vertebral fracture risk has been observed for RLX60. These results confirm that raloxifene substantially reduces the risk of vertebral fracture.

Disclosures: ***E. Seeman****, Eli Lilly and Company 2, 5, 8.*

SA382

See Friday Plenary number F382

SA383

Raloxifene and Estrogen Do Not Affect Microdamage Accumulation or State of Mineralization in Monkey Vertebra. J. Li*[1], D. Burr[1], Z. Fan*[2], J. Y. Rho[2], C. Jerome[3], M. Sato[4], C. Turner[1]. [1]Indiana University School of Medicine, Indianapolis, IN, USA, [2]University of Memphis, Memphis, TN, USA, [3]Skeletech, Inc., Bothell, WA, USA, [4]Eli Lilly & Company, Indianapolis, IN, USA.

Previously, alendronate was shown to increase the degree of mineralization and accumulation of microdamage in animal bones. In an effort to ascertain if long-term suppression of bone turnover with other inhibitors of bone resorption can also affect mineralization and microdamage accumulation, we evaluated bones from cynomolgus macaques treated with raloxifene (R) or conjugated equine estrogens (CEE). Cynomolgus monkeys (Macaca fascicularis) were randomized, ovariectomized (except for Sham controls), and treated orally with vehicle (Sham and Ovx controls), 1 mg/kg raloxifene (R1), 5 mg/kg raloxifene (R5), or 0.04 mg/kg CEE. The functional quality of the mineralized matrix was analyzed post-necropsy by biomechanical testing, histomorphometry, and nanoindentation. A beam of pure cortical bone was machined out of the femoral diaphysis and subjected to materials analysis by biomechanical failure testing. No differences in material strength (ultimate stress) were observed between groups; however the Young's modulus (E) of Sham, CEE and R5 were significantly greater than Ovx. R1 had E that was between Ovx and Sham, but not significantly different from either. Histomorphometry of the lumbar vertebra showed that ovariectomy increased the rate of endocortical bone turnover (BFR/TV) with Ovx BFR/TV 301% of Sham. Relative to Sham, endocortical BFR/BV was 81% for CEE, 129% for R1 and 142% for R5. Trabecular bone turnover (Cn BFR/TV) was 106% for CEE, 138% for R1 and 158% for R5, retative to Sham, but these effects were not significantly different from Ovx. Analysis of microcrack surface density showed a 40% reduction for Ovx relative to Sham. CEE increased microcrack surface density to Sham levels, while the

R5 density was less than Sham. R1 microcrack surface density was not significantly different from Sham and Ovx. No significant differences in crack length were observed between groups. Hardness, which is a measure of the state of mineralization, and Young's modulus were measured for both trabecular bone and the cortex on a micron scale by nanoindentation. No significant differences between groups were observed for either parameter in trabecular or cortical bone. In summary, differences in functional quality of the cortical or trabecular bone were not observed between Sham, Ovx or treated monkeys. CEE increased microcracks from Ovx to Sham levels, whereas raloxifene had no effect on microdamage accumulation. Therefore, neither raloxifene nor CEE adversely affected bone material properties beyond normal levels in nonhuman primates.

Disclosures: ***J. Li****, None.*

SA384

The Release Profiles and Bioactivity of Parathyroid Hormone from Poly(lactic-co-glycolic acid) Microspheres. G. Wei*, G. J. Pettway*, L. K. McCauley, P. X. Ma. The University of Michigan, Ann Arbor, MI, USA.

Human parathyroid hormone (PTH) has recently been approved for systemic administration to treat osteoporosis, and although it has shown promise for applications of local bone regeneration, the methods for local delivery of PTH are not well established. The purpose of this study was to develop and characterize a local delivery system for PTH that provides reliable release of biologically active peptide. Poly(lactic-co-glycolic acid) (PLGA) microspheres containing bovine serum albumin (BSA) or PTH(1-34) were prepared using a double emulsion method with high encapsulation efficiency and controlled particle sizes. The microspheres were characterized with regard to their surface morphology, size, protein loading, degradation and release kinetics, and in vitro and in vivo assessments of biological activity of the released PTH. It was showed that PLGA5050 microspheres degraded rapidly after a 3-week lag time and could be degraded completely within 4 months. In vitro BSA release kinetics from PLGA5050 microspheres was characterized by a burst effect followed by a slow release phase within 1 to 7 weeks and a second burst release at 8 weeks, which was consistent with the degradation study. The PTH incorporated PLGA5050 microspheres released biologically active PTH in the initial 24 hours, and the released PTH stimulated the release of cAMP levels from ROS cells as well as increased serum calcium levels when injected into hypocalcemic mice subcutaneously. Both in vitro and in vivo assays demonstrated that the bioactivity of PTH was maintained largely during the fabrication of PLGA microspheres and upon release. These studies illustrated the feasibility in achieving local delivery of PTH to induce anabolic responses in bone cells by a microsphere encapsulation technique.

Disclosures: ***P.X. Ma****, None.*

SA385

See Friday Plenary number F385

SA386

Skeletal Effects of PTH Are Superior to Alendronate in Senile Intact and Severely Osteopenic Ovariectomized Rats, Especially in the Femoral Neck. Y. L. Ma, A. Schmidt*, R. Cain*, Q. Q. Zeng*, J. Hoover*, H. U. Bryant, M. Sato. Lilly Research Laboratories, Indianapolis, IN, USA.

Skeletal effects of parathyroid hormone (PTH) and alendronate (ABP) were compared in intact and osteopenic ovariectomized (Ovx) rats, during the last 4 months of life. The lifetime of female rats was confirmed to be about 24 months, as half of the intact vehicle controls failed to survive during the last two months (23-24 months of age). Interestingly, all Ovx animals survived until study termination regardless of treatment, as tumor (mammary, uterine, vaginal) incidence was lower post-ovariectomy. Intact or osteopenic, Ovx animals at about 19 months old were treated with human PTH (1-38)10 (Ovx only) or 30ug/kg/d s.c, or ABP (28µg/kg twice weekly s.c) for 4 months. The Ovx animals were ovariectomized at 6 month age and permitted to lose bone for 13 months before treating for the last 4 months. Skeletal efficacy was evaluated by biomechanical, QCT and histomorphometric analyses postnecropsy. Alendronate had no effect on femoral neck strength in either intact or Ovx rats. By comparison, PTH strengthened the femoral neck by 25% at the low dose of Ovx rats and by 15% at the high dose in intact animals. ABP had no effect on the strength of lumbar vertebra in intact animals, but increased strength of Ovx vertebra by 48%. Also in intact animals, ABP increased vertebral BMD by 7%, but had no effect on vertebral BMC, or femoral midshaft BMD. In Ovx rats, ABP also increased vertebral BMD by 16%, vertebral BMC by 16%, compared to Ovx vehicle controls. By comparison, PTH increased vertebral strength by 60%, vertebral BMD by 23%, vertebral BMC by 22% and femoral midshaft BMD by 11% over intact controls. In Ovx rats, PTH dose dependently increased vertebral BMD (37% and 43%, low and high dose, respectively), vertebral BMC (27% and 42%), femoral midshaft BMD (13% and 21%), midshaft BMC (10% and 22%), and midshaft strength (20% and 34%) over controls. Treatment group comparisons showed that PTH is significantly superior to ABP in increasing bone mass, improving the spatial architecture, and enhancing quality of the axial and appendicular skeleton of senile as well as severely osteopenic, ovariectomized rats. Finally, ovariectomy was observed to confer a survival advantage to rats by reducing the incidence of mammary, uterine and vaginal tumors, which was not affected by either PTH or alendronate treatment during the last 4 months of life.

Disclosures: ***Y.L. Ma****, None.*

SA387

See Friday Plenary number F387

SA388

A Single Dose of Sustained-Duration PTH(1-34)-Fc Increases Serum Markers of Bone Formation for 28 Days in Cynomolgus Monkeys. B. J. Stouch*[1], P. Kostenuik[2], H. Gunn*[1], S. Baughman*[1], A. DePaoli*[3], C. R. Dunstan[4], S. W. Martin[1]. [1]Pharmacokinetics and Drug Metabolism, Amgen, Thousand Oaks, CA, USA, [2]Metabolic Disorders, Amgen, Thousand Oaks, CA, USA, [3]Clinical Research, Amgen, Thousand Oaks, CA, USA, [4]ANZAC Research Institute, Concord, Australia.

Daily injection of PTH is a strong anabolic stimulus for bone. Because continuous infusion of PTH is not anabolic, investigators have considered that the short half-life of PTH is essential for this anabolism. We challenged this assumption by engineering a long-acting form of PTH-(1-34), the half-life of which was increased substantially by its fusion to the Fc portion of human IgG1. This PTH-Fc fusion molecule has optimal anabolic effects with less frequent injections relative to PTH-(1-34) in rats. The objective of the current study was to determine the pharmacokinetics and pharmacodynamic response to IV or SC administration of PTH-Fc in female cynomolgus monkeys. Thirty-three monkeys were separated into 11 dosing groups (3/group) and received either a single SC or IV dose of PTH-Fc or a single SC dose of Placebo. Whole blood samples were collected from each animal and analyzed for drug levels, ionized calcium (Ca^{++}) and serum markers of bone formation [osteocalcin (OC) and bone specific alkaline phosphatase (BSALP)]. Urinary N-Telopeptide (NTx) and C-Telopeptide (CTx) (urinary markers of bone resorption) were determined serially from each animal. IV and SC treatments with PTH-Fc led to rapid and transient increases in NTx, CTx and ionized calcium levels during the first 3 days, indicating an increase in bone resorption. Bone formation indices such as BSALP and OC transiently declined during the first week, followed by dose-dependent and sustained increases for up to 28 days. PTH-Fc serum concentration-time profiles (IV 300 µg/kg) declined in a triphasic manner with a terminal half-life of 37.8 hours. At the highest dose (both IV and SC) PTH-Fc serum levels were still greater than 100 pg/mL between 7 and 10 days postdose. PTH-Fc underwent limited extravascular distribution (Vss = 93.3 to 187 mL/kg). Clearance of PTH-Fc decreased 2 to 3 fold as dose increased 30-fold (10 to 300 µg/kg). Following SC administration, maximum concentration occurred 6 to 12 hours post dose and then declined in a similar fashion to that observed following IV administration. The changes in biochemical markers of bone turnover are consistent with the recently reported ability of PTH-Fc to increase BMD in cynomolgus monkeys. A single injection of PTH-Fc resulted in pharmacologic exposure to PTH that persisted for 7-10 days. This finding calls into question the perception that rapid clearance of PTH is essential for bone anabolic effects.

Disclosures: ***B.J. Stouch****, Amgen Inc. 1, 3.*

SA389

Delivery of Osteoprotegerin (OPG)Using an Adeno-Associated Virus (AAV) Gene Therapy Vector Reverse Established Osteopenia in Ovariectomized (OVX) Mice. S. Morony[1], B. Bolon*[2], C. Carter*[3], Z. Geng*[1], M. Daris*[3], P. J. Kostenuik[1], J. Sheng*[3]. [1]Metabolic Disorders Research, Amgen Inc., Thousand Oaks, CA, USA, [2]Pathology, Amgen Inc., Thousand Oaks, CA, USA, [3]Protein Science, Amgen Inc., Thousand Oaks, CA, USA.

We previously demonstrated that recombinant OPG gene therapy using an adenoviral (Ad) vector prevents acute osteopenia in OVX mice when given at the time of OVX (Mol Ther 3: 197, 2001). However, the Ad vector induced mild liver toxicity as indicated by increased liver weight and elevated serum activities of hepatocyte cytosolic enzymes. In the present study, we performed OPG gene therapy in an OVX mouse model of established osteopenia to simultaneously examine (1) the safety and efficacy of an AAV vector and (2) the capacity of AAV-OPG to reverse established osteopenia. Basal bone mineral density (BMD) was assessed by pQCT in 12-week-old CDF1 females (n = 6 – 9/group). Ovaries of all mice were exposed and either touched (sham) or removed (OVX), after which animals were allowed to lose bone mass for 6 weeks. At this time, OVX mice exhibited significant osteopenia by pQCT in the tibia relative to the sham or pre-surgery controls. Animals with established osteopenia were injected once IV with an AAV vector bearing cDNA of human OPG (AAV-OPG) or B-galactosidase (AAV-BGal), after which mice were maintained for 10 weeks. Ten weeks after a single treatment, OVX mice given AAV-OPG had significantly greater tibial BMD compared to age-matched OVX animals given AAV-BGal. Sham-operated mice treated with AAV-OPG also had significantly higher tibial BMD than those that received AAV-BGal. The enhanced BMD in AAV-OPG animals occurred in conjunction with significant increases in bone volume and significant reductions in osteoclast surfaces in the proximal tibial metaphysis. In a separate study, a single IV injection of 4 week old mice with AAV-OPG resulted in systemic exposure to pharmacologic levels of OPG for the lifespan of the mice (>16 months). In contrast to the hepatic lesions induced by Ad, the AAV vector elicited no systemic toxicity as revealed by normal architecture in liver and peripheral lymphoid organs and the absence of AAV-related increases in circulating activities of hepatocellular enzymes. These data demonstrate that a single inoculation with AAV-OPG can reverse established osteopenia in OVX mice without evidence of systemic toxicity. Thus, AAV delivery appears to be a safe and effective method to produce sustained systemic exposure to OPG.

Disclosures: ***S. Morony****, None.*

SA390

Bioactive Compounds of Denshen Prevent Bone Loss in Rat Models of Osteoporosis. L. Cui[1], T. Wu*[1], L. Y. Zou*[1], Y. Y. Liu*[1], Y. F. Deng*[2]. [1]Department of Pharmacology, Guangdong Medical College, Zhanjiang, China, [2]Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical College, Zhanjiang, China.

Danshen (*Salvia miltiorrhiza* Bunge), a traditional herbal medicine, has been widely used in clinical practice in China for the prevention of cardiac diseases, arthritis and other inflammation-related diseases. Tens of individual compounds have been extracted from danshen and their chemical structures have been demonstrated. The purpose of current studies was to determine whether the ethanol extract (EEX) and water extract (WEX) of danshen could prevent bone loss in rat models of osteopenia. The known bioactive individual compounds in EEX are tanshinone IIA and cryptotanshione while known bioactive individual compound in WEX is danshensu. In the first study, EEX at 100 mg/kg (equals to 17.2 mg/kg of tanshinone IIA, 8 mg/kg of cryptotanshione) or vehicle were given by daily oral gavage to 4-month-old ovariectomized (OVX) rats starting one day post-surgery for 10 weeks. Trabecular bone histomorphometric analysis of proximal tibial metaphysis and fourth lumbar vertebral body showed that a significant decrease in trabecular bone volume (TBV) and trabecular number (TBN) and a significant increase in osteoclast surface (OCS/BS) and mineralizing surface (MS/BS) were found in OVX controls compared with sham controls. EEX significantly prevented the decreases in TBV and TBN and the increase in OCS/BS. However, EEX had no effect on MS/BS and uterine weight compared with vehicle-treated OVX controls. In the second study, 4-month-old Spragus-Dawley male rats were received either 2.7 mg/kg/day of prednisone oral gavage or prednisone plus WEX of danshen at dose of 128 mg/kg/day (equals to 25mg/kg/day of Danshensu) for 10 weeks. Treatment with prednisone significantly decreased body weight, bone calcium content, bone hydroxyproline content, and significantly increased serum calcium compared with vehicle controls. Further, decreased TBV and TBN and increased OCS/BS were found in proximal tibial metaphysis in rats treated with prednisone compared with vehicle controls. Treatment with WEX completely prevented the above changes induced by prednisone. Our data illustrate that ethanol extract (EEX) of danshen prevents OVX-induced bone loss and water extract (WEX) of danshen prevents glucocorticoid-induced bone loss in rats. Inhibition of elevated bone resorption is likely the mechanism for their bone protective effects of these extracts. These results demonstrate that the ethanol extract (EEX) and the water extract (WEX) of danshen may have potentials for prevention and treatment of skeletal disorders such as osteoporosis in human.

Disclosures: ***Y.F. Deng****, None.*

SA391

2-Methoxyestradiol Induces Cell Death in Osteoclasts. A. Maran[1], M. J. Oursler[2], G. Gorny*[2], M. Zhang*[1], R. T. Turner[1]. [1]Orthopedic Research, Mayo Clinic, Rochester, MN, USA, [2]Dept. of Biology, University of Minnesota, Duluth, Duluth, MN, USA.

The effects of 2-Methoxyestradiol (2-ME), a naturally occurring metabolite of 17β-estradiol on cultured osteoclast cells were investigated. 2-ME at 5 μM concentrations decreased cell survival by more than 95% in 3 osteoclast model systems (RAW 264.7 cells cultured with RANKL, mouse marrow ST2 cells co-cultured with stromal support cells and spleen cultured without support cells in media supplemented with RANKL and M-CSF) that were examined. 2-ME treatment resulted in the induction of osteoclast apoptosis in RAW 264.7 cells, mouse marrow co-culture system and in spleen cells by 65%, 39% and 55% respectively. Also, 2-ME treatment resulted in the inhibition of osteoclast differentiation in RAW 264.7 cells and mouse marrow co-culture system. 2-ME was ligand specific, its immediate precursor (2-hydroxyestradiol) and the equivalent metabolite of estrone (2-methoxyestrone) did not reduce cell number or induce osteoclast apoptosis. Co-treatment with ICI 182,780 did not block the 2-ME mediated decrease in cell number, suggesting that the cytotoxic effect of 2-ME is not estrogen receptor mediated. Also the 2-ME mediated induction of cell death in RAW 264.7 cells coincided with an increase in gene expression of the proapoptotic cytokines tumor necrosis factor (TNF)-α, interferon-β and transforming growth factor (TGF)-β. TNF-α mRNA levels showed a time-dependent increase with 2-ME treatment and increased by 3-fold at 12 hrs and by 8-fold at the end of 72 hrs. Interferon-β and TGF–1 mRNA levels increased by 7-fold and 2.5-fold respectively at the end of 72 hrs of treatment. These findings suggest that 2-ME could be useful for treating skeletal diseases, such as postmenopausal osteoporosis in which bone resorption is increased. This conclusion is supported by recent *in vivo* studies demonstrating that 2-ME prevents bone loss by inhibiting bone resorption in ovariectomized rats by a non-estrogen receptor mediated pathway.

Disclosures: ***A. Maran****, None.*

SA392

See Friday Plenary number F392

SA393

AMG 162 Maintains Serum Concentrations for up to 9 Months Following a Single Subcutaneous Dose in Healthy Postmenopausal Women. M. C. Peterson[1], B. J. Stouch*[1], D. Chen*[1], S. Baughman*[1], D. L. Holloway[2], A. Rasmussen*[2], P. Leese*[3], L. Galitz*[3], C. R. Dunstan[4], P. J. Bekker[2], A. DePaoli*[2], S. W. Martin[1]. [1]Pharmacokinetics and Drug Metabolism, Amgen Inc., Thousand Oaks, CA, USA, [2]Clinical Research, Amgen Inc., Thousand Oaks, CA, USA, [3]Quintiles, Lenexa, KS, USA, [4]ANZAC Research Institute, Concord, Australia.

RANKL (Receptor Activator of NF kappa B Ligand) is an essential osteoclastic differentiation and activation factor. The pharmacokinetics of AMG 162, a fully human monoclonal antibody to RANKL, was evaluated in postmenopausal women in this randomized, double-blind, placebo-controlled, single-dose, dose escalation study. Six cohorts of 8 to 9 women randomly received a single subcutaneous (SC) injection of either AMG 162 or placebo (3:1 ratio). AMG 162 doses were 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg. Serum levels of AMG 162 were measured by a validated enzyme-linked immunosorbent assay. The pharmacokinetics of AMG 162 following single dose SC administration to postmenopausal women is summarized in the table below.

Following SC administration, AMG 162 serum levels rose rapidly reaching maximum average concentrations of 50 ng/mL (0.01 mg/kg) to 36,400 ng/mL (3.0 mg/kg) at 5.8 to 21 days post-dose. As the dose increased from 0.1 to 3.0 mg/kg, a plateau in serum levels (>2000 ng/mL) was observed which lasted for 14 days at 0.1 mg/kg to 6-months at 3.0 mg/kg. Thereafter, when concentrations dropped below approximately 1000 ng/mL, a more rapid terminal elimination phase was observed that was dose-dependent, with slower rates of elimination at higher doses.

Subcutaneous Pharmacokinetics Parameter Estimates for AMG 162 in Postmenopausal Women

Parameter	Units	AMG 162 SC Dose (mg/kg)					
		0.01 (n=6)	0.03 (n=6)	0.1 (n=6)	0.3 (n=6)	1.0 (n=6)	3.0 (n=7)
Tmax	days	5.8	8	6.8	21	19.2	13.7
Cmax	ng/mL	50	547	721	2230	8990	36400
Plateau Half-life	days	N/A	N/A	N/A	32.4	32	28
Terminal Half-life	days	5.6	5.0	4.9	7.7	11.3	12.6
MRT	days	11.3	16.1	20.5	33.9	43.6	45.1

N/A = Not able to determine

In conclusion, AMG 162 maintained serum levels for a prolonged period (up to 9 months) following a single SC dose of 3.0 mg/kg and potentially allows for infrequent SC dosing in the treatment of bone disorders, such as osteoporosis and cancer related bone diseases.

Disclosures: ***M.C. Peterson****, Amgen Inc. 1, 3.*

SA394

Rapid, Profound, and Sustained Suppression of N-Telopeptide Following Administration of AMG 162, a Fully Human Monoclonal Antibody Against RANKL, in Cynomolgus Monkey. M. C. Peterson[1], B. J. Stouch*[1], D. Chen*[1], S. Baughman*[1], A. DePaoli*[2], C. R. Dunstan[3], S. W. Martin[1]. [1]Pharmacokinetics and Drug Metabolism, Amgen Inc., Thousand Oaks, CA, USA, [2]Clinical Research, Amgen Inc., Thousand Oaks, CA, USA, [3]ANZAC Research Institute, Concord, Australia.

AMG 162 is a fully human monoclonal antibody that binds with high affinity and specificity to RANKL (Receptor Activator of NF kappa B Ligand). A pharmacokinetic (PK) and pharmacodynamic (PD) study of AMG 162 in cynomolgus monkeys following single dose intravenous (IV) and subcutaneous (SC) administration of 0.005 to 3.0 mg/kg has been performed. Thirty-three monkeys were separated into 11 dosing groups: five SC groups, five IV groups, and 1 SC placebo group (n = 3/dose/route). Serum levels of AMG 162 were measured by a validated enzyme-linked immunosorbent assay. Serum N-Telopeptide (sNTx) was used as the PD marker of bone resorption measured by competitive ELISA (Ostex; Bothel, WA). Within 24 hours of dosing, AMG 162 caused a rapid suppression of sNTx. Maximum suppression of sNTx occurred between 1 and 10 days post dose. Average maximum suppression (SD) increased from 14% (9.8) to 71% (4.4) and from 7.7% (7.2) to 75% (3.4) as IV and SC doses increased, respectively. Duration of sNTx suppression increased with dose to a maximum of 56 days (3.0 mg/kg SC). Both extent and duration of effect were similar following IV and SC administration. Following IV administration, AMG 162 demonstrated non-linear PK with a 16-fold decrease in clearance (4.40 to 0.277 mL/hr/kg) as doses increased. Serum IV profiles were generally tri-phasic, exhibiting a rapid distribution phase (t1/2=3.9 hrs), a slower secondary phase (t1/2=96 hrs), and a rapid terminal phase (t1/2=8.4 to 37 hrs). Volume of distribution ranged from 30 to 50 mL/kg, indicating limited distribution outside the systemic circulation. Following SC administration, AMG 162 peak concentrations were observed 10.7 to 96 hours post-dose. In general, as serum levels of AMG 162 decreased below 2000 ng/mL placebo-normalized sNTx levels started to return towards baseline. A PK/PD model was developed and will be presented, in which AMG 162 inhibited the synthesis of sNTx. In conclusion, AMG 162 was a highly effective bone antiresorptive agent, producing rapid and profound suppression of sNTx, and has demonstrated favorable PD and PK profiles following single dose administration to cynomolgus monkeys. These results suggest that AMG 162 may allow for infrequent SC dosing in the treatment of bone disorders, such as osteoporosis and cancer related bone diseases.

Disclosures: ***M.C. Peterson****, Amgen Inc. 1, 3.*

SA395

See Friday Plenary number F395

SA396

An Orally-Bioavailable Cathepsin K Antagonist with Anti-Resorptive Activity In Vivo. D. B. Kimmel[1], B. Pennypacker[1], S. B. Rodan[1], G. Wesolowski[1], L. T. Duong[1], S. Venkatraman*[2], E. Setti*[2], Z. Q. Tian*[2], J. Palmer*[2], R. M. Oballa*[3], J. Robichaud*[3], P. Prasit*[3], J. P. Falgueyret*[3], D. Riendeau*[3], M. D. Percival*[3], G. A. Rodan*[1]. [1]Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA, USA, [2]Medicinal Chemistry, Celera, South San Francisco, CA, USA, [3]Medicinal Chemistry, Merck-Frosst Canada, Kirkland, PQ, Canada.

Cathepsin K (CatK), a cysteine protease, cleaves Type I collagen at acidic and neutral pH and is highly and selectively expressed in osteoclasts. The object was to identify orally bioavailable Cat K inhibitors as potential anti-resorptive agents.
Compound A (*N*-(1-{[(cyanomethyl)amino]carbonyl} cyclo-hexyl)-4-[2-(4-methylpiperazin-1-yl)-1,3-thiazol-4-yl] benzamide) is a fully reversible inhibitor of human, rabbit, and rat CatK (Ki's of 0.2, 0.5, and 5nM, respectively). Compound A inhibits human Cathepsins B, L, and S with Ki's of 1, 6, and 47µM, respectively. Compound A is a potent inhibitor of bone resorption *in vitro* (rabbit osteoclasts on bovine bone assay) with an IC_{50} of 5nM.
When given orally once daily for 27 weeks to adult (7mo, 3.9kg) newly-ovariectomized (OVX) rabbits, Compound A partially (2mg/kg/d, P<.05) or fully (10mg/kg/d, P<.001) prevented bone loss and preserved bone strength in the second lumbar vertebra.
When given orally once daily for 8-11 days to 15-year-old OVX rhesus monkeys at four years post-OVX (0.6, 3, or 15mg/kg/d), A decreased urinary NTx/Cre (n-telopeptides) by 31% (P<.01), 68% (P<.0001), or 76% (P<.0001), respectively. UNTx/Cre returned to control (vehicle monkeys) levels ten days post-treatment cessation.
We conclude that an orally-bioavailable small molecule that selectively (vs. CatB, CatL, and CatS) inhibits rabbit and human CatK with sub nanomolar potency: 1) blocks bone resorption in vitro; 2) fully blocks estrogen deficiency bone loss in adult rabbits; and 3) suppresses bone resorption in estrogen deficient non-human primates in a reversible fashion. Small molecule, selective CatK inhibitors have the potential to prevent and/or treat post-menopausal osteoporosis.

Disclosures: ***D.B. Kimmel****, Merck Research Laboratories 1, 3.*

SA397

HIV Protease Inhibitor Ritonavir Disrupts Osteoclast Cytoskeleton through Preventing RANKL Induced Membrane Recruitment of TRAF6. M. W. Wang, R. Faccio, S. Takeshita, S. L. Teitelbaum, F. P. Ross. Pathology, Washington University School of Medicine, St. Louis, MO, USA.

We previously reported that HIV infected patients treated with protease inhibitors (PIs) have diminished bone mineral density relative to similarly infected individuals not receiving these drugs. Surprising, one of the HIV PIs, ritonavir, was found to be a potentially bone sparing agent based on its ability to abrogate in vitro and in vivo osteoclastogenesis, without affecting osteoblast function. Also, we showed that ritonavir disrupts the osteoclast cytoskeleton with resultant elimination of bone resorption. A specific signaling pathway induced by RANKL that is known to be important in maintaining cytoskeleton integrity, namely PI3 kinase – Akt pathway, was also inhibited by ritonavir.
To elucidate the molecular basis of ritonavir induced osteoclast cytoskeletal disruption, we constructed constitutively active (CA) PI3 kinase (PI3K-CA) and Akt (Akt-CA) containing retroviruses. Osteoclasts transduced with PI3K-CA do not manifest cytoskeletal disruption when treated with ritonavir. In contrast, Akt-CA and control vector transduced cells exhibit cytoskeletal disruption in response to ritonavir. Furthermore, when monitored by phospho-Akt immunoblots, PI3K-CA transduced osteoclasts show enhancement in both the baseline and RANKL stimulated Akt activation, with the enhancement not inhibited by ritonavir. In contrast, the constitutively phosphorylated Akt in Akt-CA transduced osteoclasts is dephosphorylated upon ritonavir exposure. These results indicate that ritonavir inhibition of cytoskeletal disruption acts at or upstream of PI3 kinase activation. The activation of PI3 kinase pathway by RANKL has been shown to require the recruitment of TRAF6-Src-PI3 kinase complex to the membrane surface by ligand activated oligomerization of RANK. Isolation of a lipid raft membrane fraction from pre-osteoclasts demonstrates that ritonavir inhibits RANKL induced recruitment of TRAF6 and Src to the membrane fraction. Establishing specificity, the RANKL induced recruitment of TRAF2 to the lipid raft fraction is enhanced with ritonavir treatment. Thus, the potentially bone sparing HIV PI, ritonavir, inhibits osteoclast function by preventing RANKL induced membrane recruitment of TRAF6 and the subsequent activation of PI3 kinase.

Disclosures: ***M.W. Wang****, None.*

SA398

Potent Inhibition of Cathepsin K Results in Inhibition of Bone Resorption in vitro and in vivo in Non-human Primates. G. B. Stroup[1], M. W. Lark*[1], D. F. Veber*[2], S. M. Blake*[1], L. C. Dare*[1], L. A. Day*[1], K. F. Erhard*[1], S. M. Hwang*[1], I. E. James[1], J. U. Jeong*[1], S. J. Hoffman*[1], R. W. Marquis*[1], M. Mcqueney*[1], H. J. Oh*[1], C. R. Person*[1], J. A. Vasko-Moser*[1], T. Tomaszek*[1], D. S. Yamashita*[1], S. Kumar*[1]. [1]GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, USA, [2]none, None, 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 and bone resorption *in vitro* and *in vivo,* following oral delivery, using estrogen deficient cynomolgus monkeys with elevated bone turnover. Monkeys were used because, unlike other preclinical species, the mature form of the enzyme is identical to human.
Compound A is a potent inhibitor of cathepsin K (Ki = 0.041nM) that is selective versus cathepsins –B (Ki = 15nM), and –S (Ki = 1.6nM). The compound is not selective versus cathepsins -L (Ki = 0.068nM) and –V (Ki = 0.053nM). We have previously shown that cathepsin L inhibition does not prevent human osteoclastic bone resorption and no effect of cathepsin V on bone turnover has been described. Compound A inhibited resorption by human osteoclasts cultured on bone with an IC_{50} of 20nM. In sections of human osteoclastoma tissue this compound also inhibited native cathepsin K activity with an IC_{50} of 45nM. For *in vivo* assessment, cynomolgus macaques were rendered medically ovariectomized (ovx) by depot administration of a gonadotropin releasing hormone agonist (GnRHa) every 28 days. Six weeks after the first dose, estrogen deficiency was verified and bone turnover markers were significantly higher than baseline. Compound A was administered to eight animals at four dose levels (0, 3, 10 and 30 mg/kg) by oral gavage using a Latin Square experimental design. In this design, animals received each treatment over a series of four study days separated by at least one week. Serum and urine markers of bone turnover were evaluated in timed samples collected over 48 hours from the time of dosing. Treatment with compound A resulted in a significant (p<.05) reduction of serum N-telopeptide of type I collagen (NTX) 1.5, 4, 8 and 24 hours after gavage. At 24 h the levels of reduction were 26%, 40% and 43% at 3, 10 and 30 mg/kg respectively. Urinary NTX corrected for creatinine showed significant reductions from 0-24 hours of 20%, 54% and 49%. Results were similar 24- 48 hours after dosing, showing a sustained impact of the compound. These data strongly suggest that inhibition of cathepsin K by compound A is a viable approach to the treatment of osteoporosis and other metabolic bone diseases.

Disclosures: ***G.B. Stroup****, GlaxoSmithKline 3.*

SA399

Bioavailability and Effects of Vitamin D Fortified Cheese on Vitamin D Status in the Elderly. J. L. Johnson*[1], V. V. Mistry*[1], B. L. Specker[2]. [1]Dairy Science, South Dakota State University, Brookings, SD, USA, [2]Martin Endowed Human Nutrition Program, South Dakota State University, Brookings, SD, USA.

Due to changes in the consumption patterns of milk and Process cheese, and to the availability of new methods for the vitamin D fortification of Process cheese, the objective of this study was to determine the effect of 2 months of daily vitamin D3 fortified Process cheese consumption, delivering 600 IU/ vitamin D3 per day, on changes in serum 25-OHD, PTH, and osteocalcin (OC) among the elderly (>60y). In addition, a crossover absorption trial in 4 elderly (>70y) and 4 mid-age adults (18-50y) also was conducted to determine vitamin D2 bioavailability in cheese vs. water. Individuals (N=100) were enrolled in the feeding study and randomly assigned to cheese+vitamin D3, cheese, or no cheese. Cheese (no vit D3, mean vit D intake=529(+/- 69) IU) had an increase in 25-OHD of 1.4 ng/ml (change different from 0, p=0.01), a decrease in PTH of 3.3 pg/ml (p=0.05) and showed no change in OC. The group that consumed the vitamin D3 fortified Process cheese (vit D intake=1034(+/- 54) IU) exhibited a decrease in 25-OHD of –2.4 ng/ml (p=0.0004), with no change in PTH or OC. The control group (mean intake=340(+/-38) IU) showed 25-OHD, PTH and OC concentrations that remained constant. There were differences among the groups in 25-OHD change due to a higher baseline 25-OHD concentration among the cheese+vitamin D3 group at baseline (p<0.001). There were no differences in 25-OHD, PTH, or OC among the groups at study's completion. These results differ from what was hypothesized, so an additional study was conducted to determine whether vitamin D2 was bioavailable from cheese and whether absorption differed by age. The results of this study showed that the elderly individuals absorbed the vitamin D2 from both the water (32750 IU/250ml) dilution and from the Process cheese (5880 IU/2 oz) just as efficiently as the younger individuals. Peak serum vitamin D2 concentration and area under the curve were similar between the elderly and mid-age adults. Vitamin D2 was absorbed more efficiently from Process cheese than from water. Peak serum vitamin D2 concentrations were 14.7 (+/- 1.0) ng/ml per 10,000 IU and 1.7 (+/- 0.4) ng/ml per 10,000 IU (p<0.001) in Process cheese and water respectively, and the area under the curve was 89(+/- 6.6) and 63(+/- 1.5) (p=0.03). In conclusion, we found that vitamin D in fortified cheese is bioavailable and that among elderly 2 months of consuming cheese fortified with 600 IU/vit D was insufficient to maintain serum 25OHD concentrations.

Disclosures: ***J.L. Johnson****, National Dairy Council 2.*

SA400

Oral Vitamin D Supplementation Among 12-14 year Old Black Girls. S. Arunabh*, J. Yeh*, S. Pollack*, J. F. Aloia*. Bone Mineral Research Center, Winthrop University Hospital, Mineola, NY, USA.

Vitamin D insufficiency is a major problem among black women living at higher latitudes and may influence bone mass gain during early years of life. Serum 25-OHD levels >70 nmol/L are now considered optimal. AIM: To assess the relationship between serum 25-OHD, PTH and bone mass in adolescent black girls and determine safety and efficacy of supplementation of 50µg/day (=2000 IU/d) of oral vitamin D3. METHOD:We recruited 21 black girls, 12-14 years of age, during late winter. Height and weight were recorded and diet assessed. Fasting blood sample was drawn for serum 25-OHD and PTH. We performed bone densitometry by DXA (Hologic QDR 4500). 14 girls were randomized to 50 µg/d vitamin D_3 and 7 to a matching placebo in a double-masked study for 12 weeks. All participants were asked to optimize their dietary calcium intake to 1200 mg/d. Serum 25-OHD, PTH, fasting urine calcium and 24 hour urine calcium excretion was assessed at study end. RESULTS: Mean BMI was 22.1Kg/m^2 and pubertal development ranged from Tanner stage 2-5. Mean dietary calcium intake was 573 (±279) mg/d and vitamin D intake of 67IU/d (0-284 IU/d). Mean serum 25-OHD level and PTH levels at baseline were 24.7 (±10.7) nmol/L and 44.0 (±17.9) pg/ml respectively. Serum 25-OHD and PTH levels were inversely related, r= -0.43, p= 0.04.

DXA	serum PTH	
	r	p
Total Body BMC	-0.44	0.04
BMC spine L1-4	-0.39	0.08
BMD Total Femur	-0.60	0.004

The mean serum 25-OHD increased by 27.9 (±14.2) nmol/L in vitamin D3 group as compared to an increase of 11.8 (± 6.7) nmol/L in the placebo ($p<0.04$). All participants, except one, attained a serum 25-OHD level of <70 nmol/L. The PTH levels declined to 39.5 (±15.8) pg/ml in the active group and increased to 54.6 (±28.8) pg/ml in the placebo group. Fasting urinary calcium excretion increased from 6.0±3.1 mg/dl to 10.3±7.7 mg/dl (p=ns). There was no hypercalcemia (>10.6 mg/dl) or hypercalciuria (>5mg/kg/day) seen with vitamin D3 supplementation. CONCLUSION: PTH is inversely related to Total body BMC among the adolescent black girls. This suggests that vitamin D-PTH axis could influence achievement of peak bone mass. In addition, we conclude that daily supplementation with vitamin D3 is an effective strategy to enhance vitamin D nutrition. 50µg per day of vitamin D3 is safe but may not be enough to optimize the serum 25-OHD levels (>70 nmol/L).

Disclosures: ***S. Arunabh****, None.*

SA401

Comparative Effects of Vitamin K and Vitamin D Supplementation on Prevention of Osteopenia in Calcium-Deficient Young Rats. J. Iwamoto[1], J. K. Yeh[2], T. Takeda*[1], S. Ichimura[3]. [1]Department of Sports Medicine, Keio University School of Medicine, Tokyo, Japan, [2]Department of Medicine, Winthrop-University Hospital, Mineola, NY, USA, [3]Department of Orthopaedic Surgery, Kyorin University School of Medicine, Tokyo, Japan.

The aim of this study was to clarify the difference in the effects of vitamin K and vitamin D supplementation on the development of osteopenia in young rats under mild calcium deficiency. Sixty female Sprague-Dawley rats, 6 weeks of age, were randomized by stratified weight method into six groups with 10 rats in each group: baseline control, 0.5% (normal) calcium diet, 0.1% (low) calcium diet, 0.1% calcium diet + vitamin K (30 mg/100 g), 0.1% calcium diet + vitamin D (25 µg/100 g), and 0.1% calcium diet + K + D. After 10 weeks of feeding, serum, urine, and feces were analyzed, and bone histomorphometric analyses were performed on cortical bone of the tibial shaft and cancellous bone of the proximal tibia. Calcium deficiency induced hypocalcemia, increased serum parathyroid hormone (PTH) and 1,25 (OH)2 D3 levels with decreased serum 25 (OH) D3 level, stimulated intestinal calcium absorption and renal calcium reabsorption, and reduced maturation-related cortical bone gain as a result of decreased periosteal bone gain and enlarged marrow cavity, but did not significantly influence maturation-related cancellous bone gain. Vitamin K supplementation in calcium-deficient rats stimulated renal calcium reabsorption, retarded the abnormal elevation of serum PTH level, increased maturation-related cancellous bone gain, and retarded the reduction in maturation-related cortical bone gain. On the other hand, vitamin D supplementation in calcium-deficient rats stimulated intestinal calcium absorption via increased serum 1,25 (OH)2 D3 level with prevention of the abnormal elevation of serum PTH level, prevented hypocalcemia, reduced the maturation-related cancellous bone gain, and prevented the reduction in periosteal bone gain and enhanced enlargement of the marrow cavity with no significant effect on the reduction in maturation-related cortical bone gain. However, no synergistic effect of vitamin K and vitamin D on intestinal calcium absorption, renal calcium reabsorption, and cancellous and cortical bone mass was found. This study shows that vitamin K supplementation stimulates renal calcium reabsorption, increases maturation-related cancellous bone gain, and retards the reduction in maturation-related cortical bone gain, while vitamin D supplementation stimulates intestinal calcium absorption, and prevents the reduction in maturation-related periosteal bone gain by inducing accumulation of calcium from cancellous and endocortical bone.

Disclosures: ***J. Iwamoto****, None.*

SA402

See Friday Plenary number F402

SA403

Effect of Calcitriol on Serum and Urine Calcium. J. C. Gallagher, R. Satpathy, P. B. Rapuri. Bone Metabolism, Creighton University Medical Center, Omaha, NE, USA.

Hypercalcaemia and hypercalciuria are well known side effects associated with the use of larger doses of vitamin D, active metabolites of vitamin D and calcium supplements. Hypercalcaemia due to vitamin D lasts for weeks whereas with Calcitriol it lasts 24-48 hours. The aim of this analysis was to see if there was any relationship between the development of hypercalcaemia and hypercalciuria. We monitored serum and urine calcium in 489 elderly women randomized to treatment with placebo, calcitriol 0.25 mcg bid (C), conjugated equine estrogen 0.625 mg/d or medroxyprogesterone acetate 2.5 mg/d (ET/HT) and a combination group (C+ET/HT). Serum total and ionized calcium, 24 hour urinary calcium (Nova Nucleus) were measured at baseline, 6th week, 12th week and then every 6 months for 3 years. Hypercalcaemia was defined as serum calcium> 10.0 mg/dl for total calcium and >5.28 mg/dl for ionized calcium, hypercalciuria was 24-hour urine calcium > 400 mg. The protocol allowed a continuation of treatment with a repeat test after 1 week in these cases. Over 3 years mean 24-hour urine calcium increased by 19 mg in the placebo group, 7 mg on ET/HT, 72 mg on calcitriol ($p<0.001$) and 79 mg on the combination ($p<0.001$). As a percentage of tests done over 3 years, hypercalciuria > 400 mg/d occurred in 1.2% on placebo, 4% on calcitriol, 0.6% on ET/HT and 2.8% on C+ET/HT. On calcitriol there were 58 cases of hypercalciuria, 76% returned to normal on the second test and 21% on the third test. On C+ET/HT there were 25 cases of hypercalciuria, 80% returned to normal on the second test and 16% returned to normal on the third test. If 300 mg/d was used as the cut-off, the incidence of hypercalciuria was 7% on placebo, 29% on calcitriol, 6% on ET/HT and 18% on the C+ET/HT. Over 3 years mean serum ionized calcium showed no significant change on placebo, ET/HT or C+ET/HT, but increased 0.04 mg/dl on calcitriol ($p<0.001$). Hypercalcaemia occurred in 6% of patients on placebo, 12% on calcitriol, 5% on ET/HT and 5% on C+ET/HT. In all cases, it was mild and ionized calcium rarely exceeded 5.48 mg/dl. Serum calcium returned to normal in 66% of the cases after 1 week and in 98% after 2 weeks, indicating that the episodes were transient. There was no correlation between serum calcium and urine calcium values during 3 years. Only about a quarter of the hypercalcaemic cases were found to have hypercalciuria. In conclusion, hypercalcaemia and hypercalciuria appear to be independent events during treatment with calcitriol and the episodes are less when calcitriol is combined with estrogen.

Disclosures: ***R. Satpathy****, None.*

SA404

Effects of a Long-term Alfacalcidol or Calcitnin Administration on Body Sway in Japanese Elderly Women. T. Koike[1], T. Okawa*[2], M. Wada*[1], T. Kita*[2], K. Takaoka[1]. [1]Orthopaedic Surgery, Osaka City University Medical School, Osaka, Japan, [2]Orthopaedic Surgery, Osaka City Municipal Kosaiin Hospital, Osaka, Japan.

Long-term vitamin D and calcium supplementation are effective in reducing non-vertebral fractures in elderly people. In Japan, alfacalcidol (1 alpha-hydroxycholecalciferol) is very popular as a therapeutic agent for senile osteoporosis. Few studies, however, have demonstrated the increase in bone mineral density (BMD) in the patients who had received alfacalcidol. An alternative explanation for the discrepancy between small increase in BMD and significant reduction in the incidence of non-vertebral fractures might be that alfacalcidol affects factors directly related to fall risk. In this cross-sectional study we investigated the effect of long-term administration with alfacalcidol or elcatonin on BMD, anthropometric measurement and body sway in Japanese healthy old women.
One hundred and forty three women in a nursing home were divided into three groups: group D (n=37) who had received alfacalcidol for over 10 years, group E (n=22) who had received elcatonin for over 10 years and 81 controls (group C). There was no significant differences in age (D: 80.2±7.9, E: 82.4±7.1, C: 79.9±6.7 yrs), body height (D: 144.4±5.9, E: 143.5±6.2, C: 143.2±6.9 cm), body weight (D: 44.0±4.2, E: 46.4±10.0, C: 47.3±9.7 Kg) and lean body mass (D: 32.4±2.4, E: 34.1±5.3, C: 33.9±4.7 Kg) among groups. Lumbar BMD (QDR-2000, Hologic) also did not differ among groups (D: 0.751±0.134, E: 0.788±0.178, C: 0.785±0.175 g/cm2). Body sway was assessed with gravicorder GS-10 (Anima, Japan). Group D showed the significant ($p<0.05$) lower recording area (3.4±2.1 cm2) compared to group E (5.0±3.6 cm2). However, there was no significant difference between group D and controls (Rec. Area 3.9±2.1 cm2). As a performance, those who had received alfacalcidol could stand stably than other groups (mean one-legged standing time; D: 8.7, E: 5.4, C: 4.6 sec, not significant). Although some reports suggested the direct effect of vitamin D on muscle strength, we could not find any differences in grip strength (D: 10.8±4.2, E: 11.0±4.8, C: 11.2±5.3 Kg) or the strength of other skeletal muscles. Our results suggest that long-term administration of alfacalcidol improves body sway and the agent affects coordinative muscle function more than strength. Alfacalcidol therapy should be considered as an approach for fall prevention as well as exercise.

Disclosures: ***T. Koike****, None.*

SA405

Vitamin D Deficiency and Functional Parameters in Community Dwelling Subjects with Hip Fractures. M. S. LeBoff[1], W. Hawkes*[2], S. Hurwitz*[3], J. Yu Yahiro*[4], J. Magaziner[2]. [1]Endocrine, Diabetes,and Hypertension, Brigham and Women's Hospital, Boston, MA, USA, [2]Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD, USA, [3]Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, Boston, MA, USA, [4]Union Memorial Hospital, Baltimore, MD, USA.

Hip fractures rise exponentially with age and are associated with a high morbidity and progressive loss of muscle mass and function. Most hip fracture subjects lose the capacity to function independently. Available data show that, ≤26 % of patients with hip fractures are treated with calcium and vitamin D (J T Harrington et al. Arth. and Rheum 2002). To evaluate the prevalence of vitamin D and the association of baseline vitamin D levels with functional capacity after a hip fracture, we measured 25-hydroxyvitamin D levels and longitudinal performance measures after a hip fracture in 115 community-dwelling subjects with hip fractures from Boston, MA (n=30) and the Baltimore Hip studies, Baltimore MD (n= 85). 25-hydroxyvitamin D (25OHD) levels were measured by RIA. At 2, 6, and 12 months post fracture, grip strength, leg strength, chair rise, and walking were measured in a subset of 44 to 55 subjects from Baltimore cohort. The age (mean ±SD) for the 2 cohorts were 77.9±9.2 and 79.7 ±8 years, respectively. The median 25-OHD levels were 12.96 and 10.20 ng/ml, for patients enrolled from Boston and Baltimore, respectively. Vitamin D deficiency was defined as a 25-hydroxyvitamin D level of <15 ng/ml.

Vitamin D Deficiency in subjects with Hip fracture (N=115)

	25OHD<5 ng/ml		25OHD<15ng/ml	
	N	(%)	**N**	(%)
Combined Groups	14	12%	78	68%
Boston, MA	4	13%	17	57%
Baltimore, MD	10	12%	61	72.9%

A number of performance measures of functioning were evaluated longitudinally post-fracture, but were not significantly associated ($P<0.05$) with baseline vitamin D levels. In sum-

mary, vitamin D deficiency is very prevalent in community-dwelling American women with hip fractures. There were no significant differences in the change in functional parameters in those subjects with or without vitamin D deficiency at baseline, although, the small number of subjects and those with normal vitamin D levels at the time of hip fractures limited the analyses. Vitamin D deficiency should be identified and treated with pharmacological doses of vitamin D in subjects with hip fractures. Vitamin D deficiency can be prevented and correction of vitamin D deficiency may improve hip fracture repair and reduce future fracture risk.

Disclosures: ***M.S. LeBoff****, None.*

SA406

New Bone Formation Is Impaired in a Model of Type 1 Diabetes Mellitus. K. M. Thrailkill, L. Liu, E. C. Wahl*, Z. Liu*, R. C. Bunn*, W. Hogue*, L. J. Suva, D. S. Perrien, J. L. Fowlkes, J. Aronson*, C. K. Lumpkin. Pediatrics, Orthopedics and Physiology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

In humans, the presence of type 1 diabetes mellitus (T1DM) imparts several deleterious consequences for skeletal health, including diminished peak height velocity during the pubertal growth spurt, decreased adult bone density, an increased risk for osteoporosis and fracture, poor bone healing and diabetes-induced skeletal embryopathy, processes which rely on *de novo* bone formation. Mechanisms by which T1DM may impede new bone formation (NBF) are unclear, but may involve metabolic derangements and/or autoimmune-mediated events characteristic of the pathogenesis of this disease. To study the effect of T1DM on NBF, we utilized a model of tibial distraction osteogenesis, uniquely modified for use in the non-obese diabetic (NOD) mouse, a model of T-cell mediated autoimmune DM. Nine week old female NOD mice were monitored weekly for glucosuria as confirmation of diabetic conversion. Seven diabetic (urine glucose 1000-2000 mg/dl) and 11 comparably aged non-diabetic mice (negative urine glucose) were studied. Anesthetized animals underwent application of a two-ring external fixator to the left tibia, using four transosseous pins and a mid-diaphyseal, low-energy osteotomy (day 1). Daily manual distraction was initiated on day 4, at 0.075 mm BID, for 14 days. At sacrifice, the lengthened and the contralateral tibiae were surgically removed and plasma was obtained. Distracted tibiae were analyzed radiographically and histologically. Contralateral tibiae were analyzed using pQCT. Plasma glucoses in diabetic vs. control animals were 457.6 ± 70.4 (mean ± SEM) vs. 176.3 ± 9.9 mg/dl ($p \leq 0.001$). A significant reduction in radiographic total NBF in the distraction gap in diabetic animals compared with controls (27.1 ± 9.9 % vs. 61.0 ± 6.5 %, $p \leq 0.01$) was observed. Impairment in NBF was confirmed using μCT on a representative pair of distraction gaps. At sacrifice, 3 of 7 diabetic animals were ketotic; a further disparity was noted in NBF between ketotic and non-ketotic mice (7.4 ± 5.3% vs. 41.9 ± 12.5%; $p = 0.7$). Histological analysis is in progress. Analysis of contralateral tibias by pQCT, showed no significant differences between diabetic and control groups in trabecular, cortical or total bone density; strength strain indices, reflective of 3-point bending strength, were also equivalent. This study demonstrated marked inhibition of rapid intramembraneous NBF in a model of relatively short term T1DM (max. duration of 4 wks), despite an insufficient duration of disease to effect overall bone remodeling.

Disclosures: ***K.M. Thrailkill****, None.*

SA407

See Friday Plenary number F407

SA408

Increased Length of Long Bones but Decreased Trabecular and Cortical Bone Mineral Density in SOCS2 Deficient Mice. M. Lorentzon[1], C. Greenhalgh*[2], W. S. Alexander*[2], A. L. Eriksson[1], C. Ohlsson[1]. [1]Centre for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]Division of Cancer and Haematology, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Melbourne, Australia.

Growth hormone (GH) is the most important regulator of postnatal body growth. A large part of the growth promoting effect is mediated by GH-stimulated expression of insulin-like growth factor (IGF) 1 in the liver, but also in peripheral tissues. Suppressor of cytokine signaling-2 (SOCS2) is a member of the suppressor of cytokine signalling family. These related proteins have been shown to inhibit cytokine action by interfering with the Janus kinase (JAK) signal transducers and activators of transcription (STAT) signalling pathway. It has been reported that SOCS2 inhibits signalling by GH and that mice lacking SOCS2 (SOCS2 -/-) have increased length of long bones but unchanged levels of IGF-I in serum and in bone (Metcalf, D. 2000, *Nature*: 405: pp1069-1073). In the present study, we show that the longitudinal growth of tibia and femur is unchanged during the early postnatal growth period while it is increased during the later GH-dependent growth period in SOCS2 (-/-) mice, compared to wt mice. Using peripheral quantitative computerized tomography, trabecular bone mineral density (BMD) in the distal metaphyseal area of the femur was found to be decreased by 10 % at 4 weeks of age and by 32 % at 15 weeks of age in SOCS2 -/- mice, compared to wt mice. Cortical BMD, cross sectional bone area, and bone mineral content (BMC), of the femur, was also reduced in 4-week-old SOCS2 -/- mice, but only cortical BMD remained lower in 15-week-old SOCS2 -/-, compared to wt. In conclusion, SOCS2 inactivation results in increased GH-dependent longitudinal bone growth but decreased trabecular and cortical BMD. The effect of SOCS2 inactivation on longitudinal bone growth could be explained by a loss of inhibition of GH-signalling while the mechanism behind the decreased cortical and trabecular BMD remains to be elucidated.

Disclosures: ***M. Lorentzon****, None.*

SA409

See Friday Plenary number F409

SA410

Nail Patella Syndrome: A Potential Model for Altered Architecture with a High Prevalence of Fractures and Mildly Decreased Bone Mineral Density. C. A. Clay, A. Towers*, P. S. Coates, S. M. Sereika*, I. McIntosh*, K. Desai*, S. L. Greenspan. Univ. of Pittsburgh, Pittsburgh, PA, USA.

Nail Patella Syndrome (NPS) is a rare autosomal dominant disorder characterized by hypoplastic or absent patella, dystrophic fingernails and toenails, dysplasia of elbows and iliac horns, and frequently glaucoma and progressive nephropathy. The underlying defect in NPS is caused by loss of function mutations in the transcription factor LMX1B on chromosome 9, which results in an abnormality of collagen type IV. We hypothesized that these patients would have a reduced bone mineral density (BMD). To examine differences in BMD, fracture risk, and scoliosis in patients with NPS, we performed dual x-ray absorptiometry (DXA) in NPS patients [NPS(+), confirmed by genotyping] and their unaffected relatives [NPS(-)]. All participants completed a questionnaire on history of fractures and scoliosis. Results expressed as mean +/- SD.

BMD Z-scores and Percent Fractures

	NPS(+) All (n=43)	NPS(+) Adults (n=34)	NPS(+) Children (n=9)	NPS(-) Controls (n=12)
Age	35±17	41±15	11±2	39±11
Spine Z-score	-0.35±1.30	-0.27±1.36	-0.63±1.07	0.12±0.90
Femoral neck Z-score	-0.55±0.90*	-0.46±0.91*	-0.88±0.79*	0.18±1.08
Trochanter Z-score	-0.84±0.84*	-0.87±0.85*	-0.71±0.83*	0.29±1.01
Intertrochanter Z-score	-0.75±0.91*	-0.90±0.84*	-0.17±0.96	-0.06±0.94
Total hip Z-score	-0.86±0.85*	-0.96±0.82*	-0.47±0.93	0.03±1.02
% Fracture	48.8	52.9	33.3	16.7

Compared to age-matched controls from the Hologic database, NPS(+) patients (6 men, 28 women, 9 children) had BMD at all hip sites that was significantly lower than expected (Z-score=0, *p<0.05) with a similar trend for the spine (p=0.080). Nearly half (48.8%) of the NPS(+) subjects had experienced a fracture, compared to 16.7% of NPS(-) subjects (p=0.055). The odds of fracture for NPS(+) individuals was 4.7 times greater than that of controls (95% CI 0.93 to 24.4). Moreover, the number of fractures per patient was significantly higher than controls, where NPS(+) patients had a mean 1.07 fractures (range 0-5) compared to a mean 0.17 fractures for controls (range 0-1, p=0.035). The number of patients with scoliosis was not significantly different than that observed in the control group (p=0.150). We conclude that NPS(+) patients may be at an increased risk for fracture despite a minimal, yet significant decrease in BMD. Further studies are needed to examine the structural composition, bone architecture, and bone turnover that may account for an increased risk of fractures in NPS(+) patients.

Disclosures: ***C.A. Clay****, None.*

SA411

See Friday Plenary number F411

SA412

Vitamin D Supplementation in Coeliac Disease Patients on a Gluten-free Diet - a Randomised Controlled Trial. S. Venkatachalam*[1], W. Fickling*[2], D. A. Robertson*[2], A. K. Bhalla[1]. [1]Rheumatology, Royal National Hospital for Rheumatic Diseases, Bath, United Kingdom, [2]Gastroenterology, Royal United Hospital, Bath, United Kingdom.

Osteopaenia which is seen in over 50% coeliac disease patients, improves on a gluten-free diet alone. Our objective was to find if Vitamin D supplementation accelerates the bone density change.

Fifty newly diagnosed coeliac disease patients on a strict gluten-free diet were randomized to either 300,000 IU parenteral Vitamin D or normal saline at baseline and 12 months. Duodenal biopsy confirmed the initial diagnosis and was repeated after 3 months to assess response. Patients with osteomalacia and renal disease and those on steroids and bone active medication were excluded. The recruitment was between July 1996 and March 2000. Bone mineral density (BMD) assessment, bone profile and biochemical markers of bone turnover were done at baseline, 6, 12 and 24 months.

Sixteen men and 34 women were recruited and had a mean age of 53.8 years. Twenty- two patients received Vitamin D and 28, normal saline. Mean age was 49.2 years in the active group and 58.4 years in the placebo. Two patients did not complete the 2 years while another died due to an unrelated cause. Spine BMD g /sq cm increased from 0.917 (0.16-Standard Deviation) to 0.929 (0.16) in the active group and from 0.901 (0.18) to 0.935 (0.2) in the placebo group after 2 years. Total hip BMD increased from 0.863(0.2) to 0.888(0.19) in the active and from 0.829 (0.15) to 0.854 (0.16) in the placebo group.

The active group was younger than the placebo group by 9.2 years on average, which could partly explain the baseline BMD differences. Spine BMD could increase spuriously due to osteoarthritis in older age.

Vitamin D supplementation did not significantly influence bone density increase in coeliac disease patients on a gluten-free diet.

Disclosures: ***S. Venkatachalam****, None.*

SA413

Early Lethality in *Hyp* Mice with Targeted Deletion of the *Pth* Gene. X. Y. Bai*[1], D. S. Miao[2], J. R. Li*[2], D. Goltzman[2], A. C. Karaplis[1]. [1]Medicine, SMBD-Jewish General Hospital, McGill University, Montreal, PQ, Canada, [2]Medicine, MUHC, McGill University, Montreal, PQ, Canada.

Inactivating mutations and/or deletions of *PHEX/Phex* are responsible for *XLH* in humans and for the murine homologue, *Hyp*. Although increased circulating levels of PTH with normocalcemia has been reported in *Hyp* mice, hyperparathyroidism in *XLH* is postulated to arise from the standard use of phosphate salts, which induce chronic stimulation of PTH secretion. In this study, we sought to examine the putative role of PTH in the metabolic derangements associated with *XLH*/*Hyp* by generating hemizygous hypophosphatemic (*Hyp/Y*) mice homozygous for the *Pth*-null allele.

Matings between female *Hyp* and male *Pth*-null mice were conducted to initially obtain *Hyp/Pth*$^{+/-}$ progeny and subsequent intercrossings were used to derive *Hyp/Y-Pth*$^{-/-}$ animals. This genotype was not observed in any of the 639 mice examined following weaning. However, immediately after birth, *Hyp/Y-/Pth*$^{-/-}$ mice were present but only very few of them were alive longer than 72 hr after birth.

To investigate the early lethality of the *Hyp/Y-Pth*$^{-/-}$ mice, analysis of serum biochemistry was performed in the four genotypes (wild-type, *Hyp*, *Pth*$^{-/-}$, *Hyp/Y-Pth*$^{-/-}$) at 0, 6, 12, 24, 36, and 48 hrs after birth. In the *Hyp/Y-Pth*$^{-/-}$ mice, serum phosphate levels increased within the first six hours to levels comparable to those in the *Pth*$^{-/-}$ mice, while in *Hyp* mice, the serum concentration had dropped significantly during the first 48 hr after birth. Serum calcium levels started low after birth and remained reduced in both *Pth*$^{-/-}$ and *Hyp/Y-Pth*$^{-/-}$ mice, although more profoundly so in the latter group (5.5 mg/dL vs. 3.8 mg/dL), while in *Hyp* mice, the levels were initially lower than but reached wild-type levels by 24 hr. Circulating PTH levels in *Hyp* mice were significantly higher than wild-type levels throughout the first 48 hr and continued to be so well into adulthood (2.5-fold). Histological analysis of tissues from the compound mutants failed to identify any cause for their early lethality, although minor alterations were evident in the long bones of these animals.

The present findings indicate that the likely cause of death in the *Hyp/Y-Pth*$^{-/-}$ mice is severe hypocalcemia. A potential role for FGF23 in promoting secondary hyperparathyroidism by suppressing renal 1-α hydroxylase activity is proposed. Hence, the combined effect of low 1,25-dihydroxyvitamin D_3 levels and absence of circulating PTH would then lead to severe hypocalcemia and early lethality of the *Hyp/Y-Pth*$^{-/-}$ mice. Hyperparathyroidism, therefore, is an integral component of the pathophysiology of *Hyp/XLH* that may also contribute to the renal phosphate transport defect in this disorder.

Disclosures: ***A.C. Karaplis****, None.*

SA414

See Friday Plenary number F414

SA415

Subcellular Localization and PTH-Dependent Translocation of NaPi-IIa in Renal Proximal Tubular Cells. Y. Taketani, K. Nashiki*, N. Sawada*, H. Yamamoto, M. Ichikawa*, K. Morita, H. Arai, E. Takeda. Department of Clinical Nutrition, University of Tokushima, Tokushima, Japan.

Parathroid hormone (PTH) is most potent and important regulator for the type IIa sodium-dependent phosphate transporter (NaPi-IIa) that plays a key role in the renal phosphate reabsorption to determine Pi homeostasis. PTH inhibits NaPi-IIa activity by leading translocation from apical plasma membrane to intracellular organelle. In this study, we investigated the mechanism of translocation of NaPi-IIa from apical plasma membrane. Our results demonstrated that NaPi-IIa was mostly localized in caveolae of the plasma membrane in OK-N2 cells that stably express human NaPi-IIa or rat kidney by means of both cell fractionation analysis and immunofluorescence analysis. Caveola is a flask-shaped membrane microstructure and a lipid-ordered microdomain enriched in cholesterol and sphingolipids. Caveolae can associate with regulation of many signal transducing molecules as well as endocytosis or potocytosis. We examined whether caveolae can be related to the translocation of NaPi-IIa by PTH. PTH decreased immunoreactive NaPi-IIa in caveolar membrane and Pi transport activity in OK-N2 cells. Methyl-beta-cyclodextrin, which is an inhibitor of caveolae-dependent endocytosis, clearly inhibited the PTH-dependent NaPi-IIa disapperance from caveolae and down-regulation of NaPi-IIa activity. While chrolpromazine that is an inhibitor of clathrin-dependent endocytosis slightly inhibited the PTH action. These data suggest that caveolae would play a key role in the translocation of NaPi-IIa by PTH.

Disclosures: ***Y. Taketani****, None.*

SA416

See Friday Plenary number F416

SA417

The Response of MEPE to Long-Term and Short-Term Phosphate Supplementation in Healthy Volunteers. H. C. Allen[1], N. S. Fedarko*[2], A. Jain*[2], A. Whybro*[1], M. E. Barker*[1], R. Eastell[1], A. Blumsohn[1]. [1]Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom, [2]Department of Medicine, Johns Hopkins University, Baltimore, MD, USA.

Matrix extracellular phosphoglycoprotein (MEPE) is one of several putative humoral mediators of phosphaturia in oncogenic osteomalacia and X-linked hypophosphatemia. The hypophosphatemia and hyperphosphaturia associated with these diseases may be partly regulated by MEPE, suggesting that MEPE may also have a role as a physiological 'phosphatonin'. It is uncertain whether serum MEPE responds to altered phosphate intake in healthy humans and whether it has a physiological role in phosphate metabolism

The aim of this study was to examine the effect of short-term and long-term phosphate supplementation on serum MEPE in healthy adults. To examine long-term phosphate supplementation, samples were collected from twelve healthy men (ages 19 – 38 years). Individuals were studied on their habitual diet for one week and then placed on a standardized diet containing 1000mg/d phosphate and 1000mg/d calcium for another four weeks. An escalating dose of supplemental phosphate was provided on consecutive weeks (phosphate intake 1000mg/d, 2000mg/d, 2500mg/d, 3000mg/d on successive weeks). Blood and urine samples were collected before breakfast at the end of each week. To assess the effect of short-term phosphate supplementation, samples were collected from 10 healthy men and women (ages 22-46, mean 30) after either oral placebo (1400mg sodium bicarbonate and 1260mg potassium bicarbonate) or an oral phosphate supplement (2000mg). Supplement or placebo was given in a single dose at 09:30 after an overnight fast in a randomised crossover design. Blood and urine samples were collected over the next four hours. Serum MEPE immunoreactivity was determined using a competitive ELISA which is not specific for full-length MEPE.

Fasting MEPE decreased significantly by 41%$\pm$11 in response to long-term phosphate supplementation. These findings are consistent with anecdodal evidence following phosphate supplementation in patients using this assay. Serum MEPE increased by to 474.86%$\pm$130.9 ($P<0.01$) following acute phosphate supplementation, but a similar increase was observed following placebo supplementation. It is possible that MEPE may show a circadian rhythm, and that this may contribute to the well described rhythm of renal phosphate handling. MEPE is a likely humoral contributor to pathological phosphaturia in XLH and OM. However, the direction of the MEPE response to chronic phosphate supplementation makes MEPE an unlikely candidate as a physiological phosphotonin.

Disclosures: ***A. Blumsohn****, None.*

SA418

See Friday Plenary number F418

SA419

In Vivo **Studies of Cyclooxygenase-2 Knockout Mice.** M. Xu*[1], D. Goltzman[2], G. A. Gronowicz[1], L. G. Raisz[1], C. C. Pilbeam[1]. [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]McGill Univercity, Montreal, PQ, Canada.

Cyclooxygenase (COX)-2 expression markedly enhances both osteoclast and osteoblast differentiation *in vitro* but the role of COX-2 in bone turnover *in vivo* is unclear. To study COX-2 *in vivo*, we used COX-2 knockout (-/-) mice and their wild type (+/+) littermates bred in a C57Bl/6 x 129 background. Mice were sacrificed at 2, 4 and 10 mo of age. Body weights of COX-2-/- males were the same as COX-2+/+ males at all ages, but weights of 10 mo old COX-2-/- females (23.1 ± 0.8 g, n = 13) were significantly lower than 10 mo old COX-2+/+ females (26.2 ± 0.7 g, n = 19). Previous studies reported that COX-2-/- mice in this background were born with renal abnormalities that resulted in renal dysfunction and early death. As expected, serum creatinine (Cr) levels were significantly elevated in 2 mo old COX-2-/- mice (0.66 ± 0.04 mg/dl, n = 14) relative to 2 mo old COX-2+/+ mice (0.48 ± 0.05 mg/dl, n = 12). At 4 mo of age, Cr levels also tended to be higher in COX-2-/- mice (0.56 ± 0.06 mg/dl, n = 25) than in COX-2+/+ mice (0.45 ± 0.05 mg/dl, n = 31). However, at 10 mo of age, Cr levels in COX-2-/- mice (0.53 ± 0.03 mg/dl, n = 24) were no different from those in COX-2+/+ mice (0.51 ± 0.02 mg/dl, n = 37). Serum calcium (Ca^{++}) levels were significantly elevated in COX-2-/- mice (10.6 ± 0.4 mg/dl, n = 24) relative to COX-2+/+ mice (9.8 ± 0.2 mg/dl, n = 37) at 10 mo of age. Serum PTH levels measured by ELISA tended to be higher in COX-2-/- mice (93 ± 12 ng/dl, n = 23) compared to COX-2+/+ mice (72 ± 7 mg/dl, n = 37) at 10 mo age. $1,25(OH)_2$ vitamin D_3 levels measured by RIA were significantly elevated in COX-2-/- mice (179 ± 15 pg/dl, n = 12) relative to COX-2+/+ mice (123 ± 13 pg/dl, n = 15) at 10 mo age. The combination of elevated serum Ca^{++} and $1,25(OH)_2$ vitamin D_3, without PTH suppression, suggests mild primary hyperparathyroidism in the 10 mo COX-2-/- mice. To avoid the potential effects of renal dysfunction on bone metabolism that may be present in 2 - 4 mo old mice, we are using the10 mo old mice with normal serum Cr to study effects of COX-2 on bone density. Preliminary studies showed significantly lower femoral bone mineral density (BMD) by PIXImus in COX-2-/- females (0.066 ± 0.002 g/cm^2, n = 11) compared to COX-2+/+ females (0.071 ± 0.002 g/cm^2, n = 14). On calvariae histology in 10 mo old males, there was significantly decreased total bone area / total tissue area (TA/TTA) in COX-2-/- males (89.1% ± 2.5%, n = 9) compared to +/+ males (94.7% ± 1.2%, n = 12), with no difference in calvarial width. Our studies suggest that COX-2-/- mice at 10 mo of age have normal renal function, mild primary hyperparathyroidism, and small (6 - 8%) decreases in bone density relative to COX-2+/+ mice.

Disclosures: ***M. Xu****, None.*

SA420

The Effects of Cinacalcet HCl (AMG 073) on Serum Calcium Levels in Patients with Parathyroid Carcinoma or Recurrent Primary HPT after PTX. S. J. Silverberg[1], C. Faiman*[2], J. P. Bilezikian*[1], D. M. Shoback[3], M. R. Rubin[1], L. C. McCary*[4], K. A. Olson*[4], S. A. Turner*[4], M. Peacock[5]. [1]Columbia Presbyterian Med Ctr, New York, NY, USA, [2]Cleveland Clinic Found, Cleveland, OH, USA, [3]Univ of California, San Francisco, Dept of Veterans Affairs Med Ctr, San Francisco, CA, USA, [4]Amgen Inc., Thousand Oaks, CA, USA, [5]Indiana Univ Sch of Med, Indianapolis, IN, USA.

Parathyroid carcinoma and recurrent primary hyperparathyroidism (HPT) post-parathyroidectomy (PTX) with severe hypercalcemia are rare, but patients are usually symptomatic with nephrolithiasis, nephrocalcinosis, renal insufficiency, bone pain, and osteopenia common. Medical management is challenging given the difficulties associated with PTX and the resistance of parathyroid carcinoma to anti-cancer therapy. The novel therapy, cinacalcet HCl, activates the calcium sensing receptor, resulting in decreased secretion of parathyroid hormone (PTH) and reduction in serum calcium. This open-label, dose-titration trial evaluated cinacalcet HCl in 8 patients with parathyroid carcinoma (n = 6) or recurrent primary HPT post PTX (n = 2) with serum calcium > 12.5 mg/dL. Cinacalcet HCl dose was adjusted sequentially (dose range 30 mg bid to 90 mg qid) throughout a titration phase to achieve a clinically relevant reduction in serum calcium of ≥ 1 mg/dL. The end of the titration phase occurred after 16 weeks or when serum calcium was ≤ 10.3 mg/dL.

Mean (range) serum calcium at baseline was 14.8 (11.8 to 17.9) mg/dL and was 11.2 (8.5 to 13.4) mg/dL at the end of titration phase. The proportion of patients who achieved a target reduction in serum calcium of ≥ 1 mg/dL was 75% (6/8). The baseline mean (range) intact PTH (iPTH) was 705 (137 to 1232) pg/mL. Pre-dose iPTH levels were not reduced, although modest reductions in iPTH were seen at 2 and 4 hours post-dose. Serum calcium and iPTH values at baseline and the end of the titration phase are presented below. Cinacalcet HCl was generally well-tolerated, with gastrointestinal side effects attributed to study drug in some patients.

	Normal	Baseline		End of Titration Phase	
	Range	n	Mean	Mean	Mean Change (SE)
Serum calcium (mg/dL)	8.4 - 10.3	8	14.8	11.2	-3.6 (0.97)
				End of Titration Phase	
				Pre-dose Mean	Hour 2 Mean	Hour 4 Mean
iPTH (pg/mL)	10 - 65	7	705	847	624	647

n = Number of subjects with non-missing baseline and end of titration phase lab values

In these patients, cinacalcet HCl was effective in reducing serum calcium levels. The study continues with serum calcium reductions maintained up to 18 months. Cinacalcet HCl is potentially an effective therapy for the treatment of hypercalcemia in patients with parathyroid carcinoma or recurrent primary HPT after PTX.

Disclosures: ***S.J. Silverberg****, None.*

SA421

See Friday Plenary number F421

SA422

Cloning and Expression of Fourteen N-terminally Truncated PTH Fragments and their use for the Validation of Assay Specificities. P. Gao, S. Scheibel*, K. Hakim*, T. L. Cantor. Assay Development and Kit Manufacturing, Scantibodies Laboratory, Inc., Santee, CA, USA.

It was demonstrated that the third generation PTH assay measured human PTH(1-84) without any cross-reaction to the large non-(1-84)PTH fragment (P Gao, et al. J Bone Miner Res 1999;14:S446), which was believed to be N-terminally truncated peptide from the wild type hormone and directly secreted by the parathyroid glands (E Slatopolsky, et al. Kidney International 2000;58:753-761). However, the molecular form or forms of these N-truncated PTH fragments have not been elucidated. To subsequently elucidate which of these candidate N-truncated PTH fragment forms is secreted by the parathyroid gland and to presently demonstrate the specificities of PTH assays, we cloned wild type human PTH(1-84) into IPTG inducible vector pDZ-1; and further mutated and cloned PTH(2-84), (3-84), (4-84), (5-84), (6-84), (7-84), (8-84), (9-84), (10-84), (11-84), (12-84), (13-84), (14-84) and (15-84) into the same expression vector. cDNA sequence analysis revealed correct reading frame of each clone within the vector and also verified the fidelity of each PTH clone sequence. By utilizing appropriate codon optimization strategies in combination with a very strong promotor based pDZ-1 Escherichia coli expression system, we generated recombinant PTH peptides. Using all these recombinant PTH peptides we validated the detection specificities of two routinely used commercial PTH immunoradiometric assays (IRMA), a Whole PTH IRMA or CAP assay (P Gao, et al. J Bone Miner Res 2001;16:605-614) and a Total Intact PTH IRMA. Briefly, wild type recombinant PTH(1-84) as well as the above fourteen N-truncated PTH fragments were spiked and diluted into a PTH zero human plasma at increasing concentrations. These samples were, then, measured using the two PTH assays. The results showed that the Whole PTH IRMA measured the recombinant PTH(1-84) exclusively and did not detect any amount of any of the above N-truncated PTH fragments up to the very high level of 100,000 pg/ml. However, the Total Intact PTH IRMA detected not only the wild type PTH(-84) but also all of the above N-truncated PTH fragments.

In conclusion, we have successfully cloned fourteen N-terminally truncated human PTH fragments with amino acid sequences from 2-84, 3-84, up to 15-84 using a pDZ-1 vector E. coli expression system. It is also demonstrated that the third generation Whole PTH IRMA detects exclusively the wild type human PTH(1-84) without any cross-reaction to any of the N-terminally truncated PTH fragments from PTH(2-84) to PTH(15-84).

Disclosures: ***P. Gao****, Scantibodies Laboratory, Inc. 3.*

SA423

See Friday Plenary number F423

SA424

Correlation of ABC Transporter Gene Expression and Technetium-99-Sestamibi (99-MIBI) Uptake by Parathyroid Tissue. N. C. Greep, F. R. Singer, A. E. Giuliano*, N. M. Hansen*, H. Wang*, D. S. B. Hoon*, H. Takeuchi*. John Wayne Cancer Institute at Saint John's Health Center, Santa Monica, CA, USA.

Previous studies have suggested that p-glycoprotein (P-gp), a membrane pump belonging to the ATP-binding cassette (ABC) family of membrane transporters, is present in parathyroid tissue and capable of transporting 99-MIBI out of a parathyroid cell. We conducted a pilot study to investigate whether the amount of P-gp (also known as multiple drug resistance or MDR1 protein) and another ABC transporter, MDR-associated protein (MRP), correlates with imaging of parathyroid glands in patients with primary hyperparathyroidism. Paraffin-embedded tissues of normal (n=9) and adenomatous parathyroid tissue (n=14) were obtained from patients who previously had undergone parathyroidectomy for primary hyperparathyroidism at Saint John's Health Center. No normal parathyroid glands were detected by 99-MIBI imaging and 11/14 adenomas were detected. Messenger RNA (MDR1, MRP) was assessed using quantitative Real Time reverse transcriptase polymerase chain reaction (RT-PCR) technique. Glyceraldehyde 3 phosphate dehydrogenase mRNA expression was assessed as a house-keeping gene in all specimens to verify the integrity of the mRNA extracted. Protein expression of the transporters was assessed by IHC using polyclonal goat anti-human MDR1 and anti-human MRP antibodies. We found evidence for both MDR1 and MRP in parathyroid tissue using both RT-PCR and IHC. As assessed quantitatively by RT-PCR, levels of the two transporters were significantly correlated (Spearman correlation coefficient = 0.79, $p < 0.0001$). The expression of both genes was greater in normal parathyroid glands compared to adenomatous glands: median MDR1 was 71 vs. 22 ($p= 0.0018$), and median MRP was 877 vs. 248 ($p= 0.0012$). Although our sample size was small, the activity of the two transporter genes was equally low in eleven 99-MIBI positive adenomas and three 99-MIBI negative adenomas. However, the median weight of the excised abnormal glands which had been 99-MIBI negative was much smaller than those which had been 99-MIBI positive (182 mg vs. 489 mg, $p= 0.027$). We conclude that retention of 99-MIBI by parathyroid adenomas is associated with decreased expression of both the MDR1 and MRP membrane transporter proteins. In addition, failure to visualize a parathyroid adenoma by 99-MIBI imaging may reflect the small size of an adenoma. In such cases the resolution of the gamma camera detector is probably inadequate to localize the tumor, despite reduced ABC transporter activity. This study suggests that downregulation of MDR1 and MRP gene expression occurs in parathyroid adenomas and significantly influences 99-MIBI imaging.

Disclosures: ***N.C. Greep****, None.*

SA425

Significance of Metaphyseal Radiolucent Changes Following Ischemic Necrosis of Capital Femoral Epiphysis. H. K. W. Kim, D. Skelton*, E. Quigley*. Shriners Hospitals for Children, Tampa, FL, USA.

Although metaphyseal radiolucent changes are often seen in children with Legg-Calve-Perthes disease, the pathogenesis and the clinical significance of these changes remain controversial. The purpose of this study was to determine the occurrence of metaphyseal radiolucent changes in a piglet model of ischemic necrosis of immature femoral head and to determine the histopathological nature of the metaphyseal changes. The study also investigated whether these lesions are associated with growth disturbance of the proximal femoral physis. The study was approved by the local institutional animal care and oversight committee. Ischemic necrosis was induced in 50 piglets by surgically placing a ligature tightly around the femoral neck. Radiographs and histologic sections of the femoral heads were assessed at 2 to 8 weeks following the induction of ischemia. The radiographs were used to measure the femoral neck lengths to assess for proximal femoral growth disturbance. Thirteen out of 50 femoral heads were found to have metaphyseal radiolucent changes. The metaphyseal radiolucent changes ranged from a focal “cystic” lesion to a diffuse area of radiolucency around the proximal femoral physis. Three distinct types of histological changes were observed. In type I change, a focal thickening of the growth plate was observed extending down to the metaphysis. Some lesions showed cystic degeneration of the thickened cartilage. In type II change, a central disruption of the growth plate and resorption of the metaphyseal bone around the region were observed. Type III change showed diffuse resorption of the growth plate and resorption of the adjacent metaphyseal and epiphyseal bone. The femoral heads with metaphyseal radiolucent changes had shorter femoral necks in comparison to those femoral heads without the metaphyseal radiolucent changes ($p=0.02$), indicating a greater degree of proximal femoral physeal growth disturbance. In conclusion, the metaphyseal radiolucent changes were due to histopathological lesions that involved the growth plate. The study validates the clinical observation that a presence of diffuse metaphyseal radiolucent changes may herald significant growth disturbance of the proximal femur in the patients with Perthes disease. The study provides histopathological basis for the proximal femoral physeal growth disturbance that has not been demonstrated in the past. Our findings may also provide histopathological basis for MRI findings of “metaphyseal cysts” in the patients with Perthes disease.

Disclosures: ***H.K.W. Kim****, None.*

SA426

See Friday Plenary number F426

SA427

Bone Mineral Density (BMD) in Childhood Survivors of Acute Lymphoblastic Leukaemia (ALL) Treated without Cranial Irradiation (XRT). M. Z. Mughal, B. M. D. Brennan*, C. Beane*, A. Auld*, K. A. Ward, R. Ashby*, S. M. Shalet*, O. B. Eden*, R. F. Stevens*, A. Will*, S. A. Roberts*, J. E. Adams. Manchester Children's Hospitals, Christie Hospital & the University of Manchester, Manchester, United Kingdom.

We have previously shown that adult survivors of childhood ALL treated with multi-agent chemotherapy, steroids and cranial XRT had a significant reduction in volumetric spinal BMD. In this cross sectional study, we examined the BMD at distal radius, bone geometry at mid-radius and the lumbar spine BMD in 54, [23 males] childhood survivors of ALL who had completed their treatment without XRT at least one year previously, and in age & gender matched controls. Bone mineral content (BMC) and bone area (BA) of L1 to L4 vertebrae (LS) were measured using the Hologic QDR-4500 dual energy x-ray absorptiometer. The bone mineral apparent density (BMAD) of LS was calculated by dividing LS BMC by LS $BA^{1.5}$.The Stratec XCT 2000 peripheral quantitative computer tomography (pQCT) scanner was used to measure the volumetric total (cortical & trabecular) and trabecular BMD at the distal radius (4% site). The endosteal circumference, periosteal circumference, cortical thickness and the axial moment of inertia (related to bending strength) at the mid radius (50% site) were also measured using the pQCT. Data are presented as median (IQR) values. The growth and bone parameters in each ALL subject were compared with that of gender & age matched control using the Wilcoxcon test.

	Childhood Survivors of ALL	Age & Gender Matched Controls	P
Age (yrs)	11.0 (9.0 to13.7)	11.0 (9.0 to 13.8)	0.91
Height (cm)	146 (137 to 158)	147 (137 to 159)	0.81
Weight (kg)	44 (34 to 54)	40 (33 to 55)	0.44
LS BMAD (g/cm^3)	0.21 (0.19 to 0.23)	0.21 (0.20 to 0.23)	0.15
Distal radial total BMD (mg/cm^3)	302 (279 to 328)	294 (276 to 332)	0.66
Distal radial trab. BMD (mg/cm^3)	172 (157 to 188)	180 (162 to 214)	0.0035
Endosteal circumference (mm)	20.1 (18.0 to 22.4)	18.5 (16.4 to 20.6)	0.019
Periosteal circumference (mm)	33.8 (31.4 to 36.5)	32.5 (29.5 to 36.2)	0.36
Cortical thickness (mm)	2.0 (1.8 to 2.3)	2.2 1.9 to 2.5	0.022
Axial Moment of inertia (mm*4)	574 (419 to 749)	501 (348 to 779)	0.77

The distal radial trabecular but not total BMD was reduced in the ALL subjects. At the mid-radial site, we speculate that ALL or its treatment resulted in endosteal bone loss, cortical bone thinning but the axial moment of inertia was not different from controls, probably due to gain in bone at the periosteal surface.

Disclosures: ***M.Z. Mughal****, None.*

SA428

See Friday Plenary number F428

SA429

Accelerated Skeletal Growth and Mineral Deposition During Maintenance Chemotherapy in Children with Standard Risk Acute Lymphoblastic Leukemia (ALL). L. J. Moyer-Mileur, J. L. Quick*, R. VanOrden*, C. Bruggers*. Pediatrics, University of Utah, Salt Lake City, UT, USA.

Background: Survival of children with acute lymphoblastic leukemia (ALL) has improved dramatically over the past two decades. Diminished bone mineral density (BMD) has been reported as long-term complication in ALL survivors. The investigation of skeletal growth and mineral acquisition during the early stages of cancer therapy, however, are limited. Objective: To track growth and changes in bone characteristics during maintenance chemotherapy in children with standard risk ALL. Design/Methods: A 12-month longitudinal study of 5 children ages 4-9 y (4F/1M) with standard risk ALL was performed. Subjects were recruited form the Hematology/Oncology Clinical, Primary Children's Medical Center, Salt Lake City, UT. Measurements were obtained at the initiation of maintenance chemotherapy (baseline) and repeated at 6 and 12 months. Cross-section measurements of the tibia by pQCT (XCT2000, Orthometrix) to assess cortical and trabecular bone compartments, bone size and strength; whole body, hip, and spine by dual energy x-ray (DXA, Hologic QDR4500A) for body composition and bone density were obtained. A regional reference (n=68, 38M/30F) was used for comparison. Anthropometrics and diet and physical activity histories were also collected. Average height and weight gains during the intensive and maintenance phases of therapy were calculated. ANCOVA controlling for age, gender, and body size was used for comparison of ALL to reference values. Wilcoxon-signed rank tests were used to examine changes in height, weight, and bone characteristics. Results: At baseline, tibia trabecular BMC (mg) and vBMD (mg/cm3) and cortical thickness (mm), total hip areal BMD (g/cm2) and BMAD (g/cm3/2), and whole body BMC adjusted for fat-free mass were significantly lower in ALL children ($p<0.01$). From baseline to 6 months, height, tibia cortical bone, total hip, spine, and whole body bone characteristic gains were accelerated ($p<0.04$) when compared to gains from 6 to 12months. Tibia trabecular BMC and vBMD values did not change. Dietary intake and physical activity met or exceeded advised levels. Conclusions: This preliminary data suggests that children with standard risk ALL experience accelerated linear growth and rapid recovery of compact bone mass once the intensive chemotherapy is stopped and the maintenance phase of chemotherapy is initiated. The chronic, diminished trabecular bone mass may reflect continued, albeit decreased exposure to chemotherapy agents.

Disclosures: ***L.J. Moyer-Mileur****, None.*

SA430

Increased Vertebral Fracture Prevalence is Associated with Time on Dialysis. G. J. Elder, E. Edström-Elder*. Bone and Mineral Program, Garvan Institute of Medical Research, Sydney, Australia.

Patients with end stage renal disease (ESRD) are reported to be at increased risk of fracture, which has been associated with levels of iPTH and bone mineral density (BMD). However both gain and loss of BMD has been reported following commencement of dialysis. In this study prevalence of vertebral fracture was examined in relation to time on dialysis, BMD and biochemical parameters in 63 consecutive patients with ESRD about to undergo a renal or renal-pancreas transplant.

Patients (36 male and 27 female) with median age 42 years (range 22-65) were admitted for transplantation. Haemodialysis or peritoneal dialysis was used in 56 of these, with median time on dialysis 30 months (range 5-120). Insulin dependent diabetes mellitus (IDDM) was the cause of ESRD in 33% of patients. Biochemical parameters were assessed within the 24 hours prior to transplant. Spinal X-rays and BMD by DXA were assessed within 2 weeks of transplantation.

Time on dialysis was positively correlated with lumbar spine and femoral neck Z scores ($p<0.02$; $r^2=0.10$ and $p<0.001$; $r^2=0.19$ respectively). Mean lumbar spine and femoral neck Z scores were normal and as expected were higher at the lumbar spine than the femoral neck (0.12±1.25 vs -0.37±1.26; $p<0.001$). Femoral neck Z scores were lower for patients with IDDM (-1.14±0.85 vs 0.00±1.27; $p<0.001$; ANOVA). Intact PTH values were 4.4 times the assay upper range (median; range 0.4-35 times) and were not associated with time from commencement or mode of dialysis.

Vertebral fracture was detected in 30% of patients and these patients had been on dialysis for longer than those without fracture (46±30 vs 25±20 months; $p<0.003$). However fracture was not associated with BMD, age, gender, IDDM or iPTH levels. Time on dialysis was longer for males than females (38±28 vs 24±18 months; $p<0.05$) and time on dialysis was associated with fracture ($p<0.002$) in logistic regression analysis.

These relatively fit patients had a high prevalence of vertebral fracture associated with time from commencement of dialysis. This may reflect progressive architectural change due to renal osteodystrophy which may mask the influence of BMD in patients with ESRD.

Disclosures: ***G.J. Elder****, None.*

SA431

See Friday Plenary number F431

SA432

Hyperphosphatemia Is Associated with Renal Osteodystrophy and Vascular Calcification in a Murine Model of Chronic Kidney Disease. R. J. Lund*, M. R. Davies*, K. A. Hruska. Renal Division, Washington University School of Medicine, St Louis, MO, USA.

Hyperphosphatemia and a high CaxP are associated with increased mortality and morbidity in ESRD patients. Increased mortality is primarily cardiovascular in nature and associated with increased vascular calcification (VC), even at a young age. We hypothesize that the increase in VC in Chronic Kidney Disease (CKD) may be in part due to altered bone remodeling and disturbances in serum Pi, and can be treated with BMP-7 by reversing the underlying Renal Osteodystrophy.

1)12 week week old C57Bl6 mice were subjected to electrocautery of the right kidney followed in two weeks by left nephrectomy to create CKD, then randomized into 3 Groups: Sham operated mice fed low Pi chow (0.2% Pi, 0.5% Ca) and calcitriol (20 ng/kg tiw sq); CKD mice fed low Pi chow and treated with calcitriol; CKD mice fed low Pi chow and treated with calcitriol and BMP-7 (10 mcg/kg q week). 2) 10-week old LDLR-/- mice (C57Bl6 background) were given CKD by the same procedure. These mice were fed a high cholesterol (15%) diet and developed hyperglycemia, hypercholesterolemia, intimal and medial aortic calcification. Three groups, sham/high fat, CKD/high fat and CKD/high fat + BMP-7 (10 mcg/kg q week) were evaluated. All groups were maintained on their regimens for 12 wks prior to calcein labeling of mineralization fronts, sacrifice and histomorphometry of distal femur metaphyses.

BUN levels were elevated equally in all of the CKD groups; iPTH levels were elevated only in the CKD high fat animals. In study 1 the iPTH suppression by the calcitriol and low Pi diet resulted in an Adynamic Bone Disorder (osteoid volume, osteoblast perimeters, mineralizing surfaces, bone formation rates, and activation frequency were all decreased). The changes were all reversed to normal or greater levels in the BMP-7 group ($p<.05$). Pi levels were higher in the CKD mice 6.1±0.6mg/dl vs 4.5±1.5mg/dl ($p<0.05$) for the mice treated with BMP-7. In study 2, despite high iPTH levels 176.3±186.6pg/ml in CKD vs 33±26.7pg/ml in sham ($p<0.05$), the underlying osteodystrophy in the LDLR-/- mice was consistent with an ABD (decreased OV/TV, ObN, MS/BS, BFR/TV, and Acf). BMP-7 treatment normalized the osteodystrophy, and there was a reduction in VC by histology staining. Pi levels were reduced from 16.4±0.4mg/dl to 10.1±0.4mg/dl with BMP-7 treatment ($p<0.01$) (Sham Pi 9.9±0.6). Serum Ca levels did not differ among the study groups, and the CaxP mirrored the serum Pi results. This study suggests hyperphosphatemia is a link between Renal Osteodystrophy and VC in a CKD diabetic, hypercholesterolemic murine model and can be successfully reversed by treating the Osteodystrophy and altered bone remodeling with BMP-7 therapy.

Disclosures: ***R.J. Lund****, None.*

SA433

Mice Lacking the αvβ3 Integrin Develop Severe Inflammatory Osteolysis. K. Aya[1], C. Pham*[2], S. Wei[1], K. Roth*[3], F. P. Ross[1], S. L. Teitelbaum[1]. [1]Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA, [2]Medicine, Washington University School of Medicine, St. Louis, MO, USA, [3]Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Neo-angiogenesis and osteoclastogenesis are believed central to the pathogenesis of the periarticular bone loss attending inflammatory arthritis. Since the αvβ3 integrin is expressed on osteoclasts and inflamed endothelial cells, and is essential for optimal bone degradation, the heterodimer presents itself as a potential therapeutic target in inflammatory osteolysis. To test this hypothesis we assessed the impact of αvβ3 deletion on the natural history of experimental inflammatory arthritis. To this end we compared the severity of experimental arthritis and attendant bone loss in β3 integrin null (β3-/-) mice and their heterozygous litter mates (β3+/-), the latter being indistinguishable from wild type. These animals were injected with 250μL serum from spontaneously arthritic KRN/NOD mice. The procedure was repeated two days later and at this time was accompanied by administration of 50 μg LPS. The animals were sacrificed on day 14 at which time 100% of each genotype had developed clinically apparent arthritis. Paw swelling, bone erosions and histological scoring of inflammation were slightly but significantly ($p<.05$) reduced in β3-/- as compared to β3+/- arthritic mice but in each circumstance the magnitude of disease was substantial. In keeping with this conclusion,
angiogenesis was indistinguishable between the two strains as was the number of osteoclasts. (β3+/-24.9+/-9.7 vs. β3-/- 24.4+/-14.6/mm2). Furthermore, while increased M-CSF expression compensates for αvβ3 deletion in the osteoclastogenic process in the basal state, we find marrow M-CSF increased to similar levels in both strains of arthritic mice. These data suggest that while αvβ3 blockade holds promise as an anti-osteoporosis strategy, it will be ineffective in preventing the bone loss of inflammatory arthritis.

Disclosures: ***K. Aya****, None.*

SA434

See Friday Plenary number F434

SA435

Bone Loss Due to Glucocorticoids; Update of a Systematic Review of Prospective Studies in Rheumatoid Arthritis and Other Diseases. M. C. Lodder*[1], W. F. Lems[1], P. J. Kostense*[2], A. C. Verhoeven*[2], B. A. C. Dijkmans[1], M. Boers*[2]. [1]Rheumatology, 4 A 42, VU University Medical Center, Amsterdam, Netherlands, [2]Clinical Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, Netherlands.

Objective: To quantify glucocorticoid (GC)-induced bone loss through a meta-analysis of prospective cohorts.
Methods: A previous systematic review up to 1996 (ref) was extended by an exhaustive search of published studies in MEDLINE (1995 to 2002), web of science (2000 and 2001), Cochrane databases (1995 to 2002), and bibliographic references. Prospective studies of patients receiving systemic GC who underwent serial bone mineral density (BMD) measurements by dual energy X- ray absorptiometry were included. Only suppletion with calcium or vitamin D was allowed. The primary outcome was the change in lumbar spine BMD in one year. The overall estimates are weighted means of the individual study arm results (weight=inverse variance).
Results: Of 1727 abstracts, 34 studies (39 study arms) remained. Two transplantation studies with very high GC doses caused heterogeneity and were excluded from further analyses. In the remaining study arms, 20 studied GC starters (602 patients) and 16 patients on chronic GC (405 patients). One study investigated both GC starters and chronic users (142 patients). Mean GC dosis was 8.9 mg (range 3.3 to 16.4 mg) prednisone equivalents. Weighted BMD decrease was –1.61% (95% confidence interval –1.84 to –1.37) per year (n=1149) in the lumbar spine and –1.45% (–1.76 to –1.14) at the hip (n=991). In exploratory regression analyses, the start of GC use, higher mean daily dose of GC, and either no suppletion or suppletion with calcium alone, were independent predictors of high bone loss at the lumbar spine. At the hip, only the start of GC use was such a predictor. The best regression models yield an explained variance of 73% at the spine and 51% at the hip, respectively.
Conclusion: In GC doses up to 16.4 mg prednisone equivalents/day, this review yields strong evidence for a yearly loss of 1.5% BMD in both spine and hip, and reinforces the need for adequate preventive measures, especially in patients starting therapy.
(ref) Verhoeven and Boers: J Rheumatol 1997;24:1495-503.

Disclosures: ***M.C. Lodder****, None.*

SA436

See Friday Plenary number F436

SA437

Autocrine and Paracrine TNF-mediated Apoptosis in Human Osteoblasts. C. W. Borysenko*, Y. Li, R. Bu, L. J. Robinson*, H. C. Blair. Pathology and Physiology & Cell Biology, University of Pittsburgh and Pittsburgh VAMC, Pittsburgh, PA, USA.

TNF is critical to bone destruction and inflammation in hyper-resorptive pathologies, such as rheumatoid arthritis; however, the mechanisms linking TNF to such periarticular bone destruction are controversial. Net bone catabolism can be initiated either by osteoclast formation or by osteoblast apoptosis. There is evidence for TNF stimulation of osteoclast formation via indirect mechanisms. There is also evidence of direct TNF-mediated apoptosis in non-human osteoblasts, but in human osteoblasts TNF-mediated apoptosis is controversial. We studied the occurrence and functional activity of TNF-family ligands and receptors in nontransformed human osteoblasts and osteosarcoma cells. Findings included that TNFRI mRNA is consistently expressed. Typically, MG63 osteosarcoma cells express the cell-surface receptor at low density (~500 copies per cell), consistent with reports that human osteoblasts are insensitive to TNF-induced apoptosis. For comparison, we determined that the TNF-sensitive cell line U937 has an ~ 7-fold higher TNF-binding capacity. However, we found that osteoblast cell density and growth conditions can alter the expression levels of TNF-family members. In media containing fetal bovine serum, osteoblasts and osteosarcoma cells express not only TNFRI but also TNF, albeit with TNF and TNFRI expressed at highly variable levels. Flow cytometry using anti-TNF suggested that this is due to variable growth phases, where in MG63 cultures a 5-10% subpopulation of cells bound anti-TNF at levels consistent with ~ 10-fold higher receptor expression, similar to that of U937 cells. Further, the sub-population with enhanced TNF-binding was highly sensitive to apoptosis, as demonstrated by annexin V binding. Sensitive cells shared morphologic features with the population of nonsensitive cells in most respects but over half of the sensitive cells are less granular and have a smaller diameter, suggesting nonsecretory or inactive osteoblast phenotype. We found that the proportion of sensitive MG63 cells was increased by cytokine withdrawal, suggesting that the local environment of osteoblasts, including growth factors and probably also cell cycle and differentiation phase, regulate the sensitivity of osteoblasts to TNF-mediated apoptosis. Thus, in environments predominated by inflammatory cytokines, such as in rheumatoid arthritis, the proportion of osteoblasts possessing high TNF-binding capacity would be expected to be sensitive to apoptosis. Taken together, these results are consistent with a functional TNF-mediated apoptotic pathway in human osteoblasts, which may be regulated by autocrine, paracrine, or exocrine stimuli.

Disclosures: ***C.W. Borysenko****, None.*

SA438

Combination Therapy with Rapamycin and Cyclosporine A or Prednisone Affects Chondrocyte Proliferation and Differentiation. C. P. Sanchez, Y. He*. Pediatrics, UW Medical School, Madison, WI, USA.

Rapamycin, an immunosuppressant, when administered alone considerably affects endochondral bone formation in young rats. It is unknown, however, if combination therapy with other immunosuppressive agents that are used to maintain the renal allograft has the same effects on growth and the growth plate. Thus, 52 male weanling rats, 48± 5 grams, were randomly divided into 4 groups: Control (N=13) received saline, Pred + Rapa (N=13) received Prednisone and Rapamycin, Rapa + CsA (N=13) received Rapamycin and Cyclosporine A, and Pred + CsA (N=13) received Prednisone and Cyclosporine A. Prednisone was given at 3 mg/kg/day, Rapamycin was given at 2.5 mg/kg/day, and Cyclosporine A was given at 7.5 mg/kg/dose by gavage route. The animals were sacrificed after 14 days (N=24) and after 28 days of treatment (N=28). Body weight and body length were obtained weekly. Blood was collected for serum calcium, phosphorus, intact PTH, and IGF-I. The proximal tibia was collected, decalcified and sections were obtained for growth plate morphometry, in-situ hybridization and immunohistochemistry experiments.
Gain in body weight was less in animals that received Rapa + CsA and Rapa + Pred, 52±10 grams and 50±14 grams, compared to Control, 73±8 grams after 14 days of therapy, $p<0.001$. Gain in body length was shorter in the Rapa+CsA and Rapa+Pred groups, 7±0.8 cm and 7±0.8 cm, compared to Control, 9±0.5 cm, $p<0.001$. Serum calcium, phosphorus and IGF-I levels were lower in the Rapa+Pred animals, 8.8±0.6 mg/dl, 10±0.4 mg/dl, 766±159 pg/ml, compared to Control 10±0.4 mg/dl, 11±0.4 mg/dl and 912±195 pg/ml. Serum PTH levels were lower and tibial length measurements were shorter in all treated animals compared to the Control group. The width of the growth plate and the area occupied by the hypertrophic zone were wider, whereas the area of the proliferative zone was less in the Rapa+CsA animals compared to the other groups. The expression of type II and type X collagen is considerably lower in the Rapa+CsA animals. Gelatinase B staining was much lower in all treated groups. Tartrate staining for acid phosphatase was increased in the Pred+CsA animals and expression for Indian hedgehog protein was less in Pred+CsA and Rapa+Pred animals. There was no difference in the expression of IGF-I protein, IGF-I receptor and PTH/PTHrP receptor in all groups. Thus, rapamycin either alone or in combination with Cyclosporine A or Prednisone affects growth, chondrocyte proliferation and chondrocyte differentiation in young rats.

Disclosures: ***C.P. Sanchez****, Shire Pharmaceuticals 5C.*

SA439

Osteoporosis in Adult Survivors of Pediatric Cardiac Transplantation May Be Related to Hyperparathyroidism, Mild Renal Insufficiency and Increased Bone Turnover. A. Cohen*[1], L. J. Addonizio*[2], J. M. Lamour*[2], P. Gao*[3], E. Shane[1]. [1]Medicine, Columbia University College of Physicians and Surgeons, New York, NY, USA, [2]Pediatrics, Columbia University College of Physicians and Surgeons, New York, NY, USA, [3]Scantibodies Lab, Inc., Santee, CA, USA.

Adults who undergo cardiac transplantation (CTX) commonly develop bone loss and fractures. Hypothesizing that adults who have had a CTX during childhood would also develop osteoporosis, we studied adult survivors of pediatric CTX in a case-control, cross-sectional evaluation of bone mineral density (BMD), indices of mineral metabolism and bone turnover markers. Nine patients (2 women), who were transplanted between ages 12-16, were evaluated (ages 21-32) and compared to age, gender and race matched controls (C). Patients (P) had CTX at a mean of 12±4 years prior to BMD evaluation and were treated with prednisone (mean dose at evaluation: 6±3 mg/day), azathioprine, and cyclosporine or FK506. Mean BMD Z scores in P were -2.3±0.9 at the spine (LS), -1.4±0.6 at the total hip (TH), -1.6±0.7 at the femoral neck (FN), and -3.2±0.7 at the distal radius (DR). BMD Z scores were significantly higher in C (LS: -0.47±1.2, TH: 0.37±0.8, FN: 0.18±1.0, DR: 0.06±0.9; p<0.001 vs. P at all sites by paired t test). Osteoporosis (Z≤-2) was present at the LS, FN and DR in 56%, 33%, and 100% of P, respectively. Although body mass index was similar between P and C, current height compared to midparental height was significantly lower in P (-8.2±4 vs. 0.5±8 cm; p=0.03). Bone mineral apparent density (g/cm^3), which corrects for the effect of smaller bone size of P on BMD, remained significantly lower in P than C at the LS (p=0.007), FN (p=0.001) and DR (p=0.003). By paired t test, parathyroid hormone (PTH) was 3-fold higher in P when measured by the intact assay (P: 75±19, C: 25±7 pg/mL; p<0.001) and 2-fold higher by the "whole" (1-84) assay (P: 32±8, C: 15±4 pg/mL; p=0.001). Serum calcium, phosphorus, 25-OH vitamin D and 1,25 $(OH)_2$ vitamin D were not different between P and C. Bone specific alkaline phosphatase (P: 35±13, C: 26±10 U/L; p=0.007), osteocalcin (P: 12±7, C: 7±2 ng/mL; p=0.06) and serum N-telopeptide (P: 30±14, C: 16±2 nM BCE; p=0.03) were higher in P than C. BUN was higher in P (P: 30±12, C: 17±5 mg/dL; p=0.04) and creatinine clearance tended to be lower (P: 83±42, C: 114±26 ml/min; p=0.07). Testosterone did not differ between male P and C. In summary, BMD is significantly lower in adult survivors of pediatric CTX, particularly at the DR, a site sensitive to excessive PTH secretion. Low BMD in this population may be related to mild renal insufficiency, hyperparathyroidism and increased bone turnover. We conclude that adult survivors of pediatric CTX should be evaluated for osteoporosis.

Disclosures: ***A. Cohen****, None.*

SA440

Bone Density Is Stable After Discontinuing Antiresorptive Therapy During the Second Year after Cardiac Transplantation. V. Addesso[1], A. Cohen*[1], D. J. McMahon*[1], P. Namerow*[2], S. Maybaum*[1], D. Mancini*[1], E. Shane[1]. [1]Medicine, Columbia University, College of Physicians & Surgeons, New York, NY, USA, [2]Population & Family Health, Columbia University, Mailman School of Public Health, New York, NY, USA.

The first year after cardiac transplantation (CTX) is characterized by rapid bone loss. We reported, in a randomized clinical trial (RCT), that alendronate (ALN; 10 mg QD) and calcitriol (1,25D; .25 mcg BID) were comparably effective in preventing bone loss at the spine (LS), femoral neck (FN) and total hip (TH) in this setting. Although we had previously noted that rapid bone loss was confined to the first year (Y) after CTX, others have noted significant bone loss in Y2 after discontinuing 1,25D. To determine if ALN or 1,25D can be safely discontinued one year after CTX, we measured bone density (BMD), mineral metabolism and bone turnover at 12, 18 and 24 months (M) after CTX in subjects who completed the RCT on ALN or 1,25D and in control subjects (CON; n=16) who received no preventive therapy in Y1. Blinding of subjects and investigators was maintained in Y2. Because of the differing pharmacology of ALN and 1,25D, we hypothesized that the 1,25D (n= 25) group would sustain bone loss and the ALN (n=34) group would not.

At 12M after transplantation, before stopping ALN and calcitriol, the 1,25D group had higher serum calcium (SCa) (CON: 9.3+.1; ALN: 9.2+.1; 1,25D: 9.6+.1 mg/dl; p=.02); urine Ca (UCa) also tended to be higher in the 1,25D group (CON: 112+23; ALN: 140+16; 1,25D: 180+19 mg/24h; p=.06) and PTH tended to be lower (CON: 39+7; ALN: 41+5; 1,25D: 27+6 pg/ml; p=.16). CON had higher bone alkaline phosphatase (BAP) (CON: 24.3+2.3; ALN: 18.3+1.6; 1,25D: 18.1+1.8 U/l; p<.04) and serum NTX (CON: 21.4+1.9; ALN: 16.7+1.3; 1,25D: 15.9+1.5 nmol/l; p<.05).

Over the second year, UCa fell (180+19 to 119+19 mg/24h; p=.003) in the 1,25D group, as did serum Ca (9.6+ .1 to 9.3+ .1 mg/dl; p=.03); both remained stable in ALN and CON. Also in 1,25D, BAP increased by 57% (18.1+1.8 to 28.4+2.0 U/l; p=.0001) and serum NTX increased by 30% (15.9+1.5 to 20.5+1.6 nmol/l; p=.0001). Neither changed in ALN. During Y2, LS BMD (g/cm2) increased by 3.2% in CON (1.049+.04 to 1.083+.04; p =.04) and was stable in ALN (1.052+.03 to 1.063+.03) and 1,25D (1.059+.03 to 1.066+.03; p=ns). FN and TH BMD remained stable in all groups.

In summary, after discontinuing ALN and 1,25D, BMD remained stable during the second year after CTX, despite marked increases in bone turnover in the 1,25D subjects. The stability of BMD despite discontinuing these therapies at 12M suggests that patients with normal BMD by one year after CTX may safely be observed without risk of excessive bone loss.

Disclosures: ***V. Addesso****, None.*

SA441

Factors Determining the Prevalence of Osteoporosis and Fractures a Year after Successful Kidney Transplantation. N. A. T. Hamdy[1], M. J. K. Mallat*[2], N. Bravenboer[3], P. Lips[3], J. W. de Fijter*[2]. [1]Endocrinology & Metabolic Diseases, Leiden University Medical Center, Leiden, Netherlands, [2]Nephrology & Transplantation, Leiden University Medical Center, Leiden, Netherlands, [3]Endocrinology, Free University Medical Center, Amsterdam, Netherlands.

Successful renal transplantation (Tx) corrects many of the metabolic abnormalities leading to renal osteodystrophy. Bone disease is however prevalent post-Tx and persistent hyperparathyroidism and use of immunosuppressive agents are the main contributory factors. Data interpretation has been so far confounded by variables such as time elapsed, spanning months to years post-Tx, and variable graft function. We have examined the prevalence of osteoporosis, fractures and histologic bone disease in 33 kidney Tx recipients analysed between 12 and 18 months post-Tx. All patients were asymptomatic, had stable graft function (creatinine clearance > 50 ml/min) and agreed to have a transiliac bone biopsy. There were 19 men and 14 women, 9 of whom were post-menopausal not on HRT. Median age was 50, range 22-67 years. All patients had been on dialysis (CAPD, n= 20; HD, n=13) for a median of 3 years (range 3 month-14 years). Immunosuppression consisted of prednisone and cyclosporin or mycophenolate mofetil. Mean serum creatinine was 121± 5 µ mol/l. Serum calcium was mildly elevated in 8 patients (mean 2.47± 0.03mmol/l), although intact PTH concentration was increased in the majority (26/33 patients: mean 11.69± 0.94 pmol/l). Bone alkaline phosphatase activity was increased in 16 patients (mean: 21.62± 2.71µ g/l). Four patients had low serum 25-hydroxyvitamin D levels. Lumbar spine (LS) and femoral neck (FN) BMD were respectively 0.95± 0.02 and 0.76± 0.02. Ten patients had osteoporosis as defined by a T-score <-2.5 at LS and/or FN. 25 patients had osteopenia at the FN. There was no significant correlation, between age, sex, mode and years on dialysis or any biochemical parameter measured and the presence or absence of osteoporosis. Six osteoporotic patients, one postmenopausal woman and 5 men, had prevalent vertebral fractures. High bone turnover was the predominant histologic feature, and only one (vitamin D replete) patient had evidence for a mineralisation defect. Osteoporosis is prevalent in a 1/3 of patients a year after successful renal Tx and is associated with asymptomatic vertebral fractures in 2/3 of these. Bone turnover is not suppressed despite the use of glucocorticoids. Hyperparathyroidism which is prevalent does not predict the presence of osteoporosis, neither do other biochemical parameters of bone turnover.

Disclosures: ***N.A.T. Hamdy****, None.*

SA442

PTH Anabolic Induced Gene in Bone (PAIGB), a Novel Gene which may Play an Important Role in Bone Formation. J. A. Robinson, V. Susulic*, W. Zhao, Y. Kharode, V. Gironda*, Y. B. Liu*, M. Wasko*, R. Murrills, F. Bex. Women's Health Research Institute, Wyeth Research, Collegeville, PA, USA.

While the role of Parathyroid hormone (PTH) in calcium regulation and bone metabolism is well known, its biological activity on bone is quite complex as demonstrated by its catabolic and anabolic activities. These conflicting activities may in part be attributed to the fact that PTH can signal through at least two different pathways. These pathways include the cAMP dependent protein kinase A (PKA) pathway and the phospholipase C dependent protein kinase C (PKC) pathway. We were particularly interested in the anabolic action of PTH in order to identify new bone anabolic targets. Therefore, studies utilizing rapid amplification of differentially expressed genes (RADE) were performed on tibia RNA samples obtained from PTH treated Sprague Dawley rats . Here we describe the identification of a novel gene designated PAIGB whose expression was induced selectively by a PTH anabolic treatment (40 ug/kg/day s.c. for 8 days) but not by a catabolic treatment with continuous infusion of the same daily dose. PAIGB is a 145 amino acid cytoplasmic protein which is highly conserved between human, mouse and rat species. Analysis of other tissues demonstrated PAIGB expression in the brain, heart and kidney, however, PAIGB expression was not regulated by PTH in these tissues. To localize the expression of PAIGB in bone, PTH 1-34 was administered subcutaneously to mouse calvariae in a regimen previously shown to be anabolic (20 ug/kg/day s.c. for 18 days). Subsequent immunohistochemical analysis of vehicle treated calvariae demonstrated minimal endogenous PAIGB protein expression whereas the PTH treated calvariae demonstrated selective expression in the osteoblasts adjacent to the bone surface within the periosteum and endosteum. In vitro studies demonstrated that PAIGB mRNA expression was maximally induced after 4hr of PTH treatment in rat and human osteoblastic cells. Although we found that other bone forming factors including PGE2, dexamethasone and Vit D3 can induce PAIGB expression modestly, PTH was by far the most potent inducer. Experiments with phosphodiesterase inhibitors (Rolipram), PKC inhibitors (19-27 peptide) and adenylate cyclase activators and inhibitors (Forskolin and SQ22536 respectively) suggest that PAIGB is regulated by PTH through the cAMP/PKA and not the PKC pathway. This conclusion was supported by the fact that PTH 3-34, which has a minimal effect on adenylate cyclase activation, was unable to induce PAIGB expression.

In summary, we describe a novel protein which is rapidly regulated by PTH in osteoblasts through the PKA pathway and which is selectively induced in bone by an anabolic treatment of PTH.

Disclosures: ***J.A. Robinson****, Wyeth Research 3.*

SA443

See Friday Plenary number F443

SA444

In Vitro and In Vivo Characterization Of PTH-Fc Constructs: C-Terminal Truncation of PTH-(1-34) Reduces Receptor Affinity and Pharmacologic Responses. V. Shalhoub[1], H. Tan*[1], M. Grisanti*[1], S. Morony[1], K. S. Warmington*[1], G. Biddlecome*[2], S. Simonet*[1], S. Adamu*[1], T. Boone*[3], D. L. Lacey[1], P. J. Kostenuik[1]. [1]Metabolic Disorders Research, Amgen Inc., Thousand Oaks, CA, USA, [2]HTS Molecular Pharmacology, Amgen Inc., Thousand Oaks, CA, USA, [3]Protein Science, Amgen Inc., Thousand Oaks, CA, USA.

Infrequent injections of a sustained duration PTH construct [PTH-(1-34)-Fc]increase BMD in mice, rats and monkeys. Despite their overall anabolic effect, PTH constructs including PTH-(1-34)-Fc cause increased bone resorption that can lead to hypercalcemia. We tested whether C-terminal truncations of PTH-(1-34) would reduce the calcemic effects of PTH-(1-34)-Fc while maintaining anabolic activity. Recombinant PTH-(1-34)-Fc includes human PTH-(1-34) fused to the Fc fragment of human IgG1. Stepwise C-terminal truncations of PTH-(1-34)-Fc were made down to PTH-(1-20)-Fc. A single SC injection of PTH-(1-34)-Fc caused transient hypercalcemia in young mice. C-terminal truncations had progressively diminished calcemic effects when injected at equimolar doses. Anabolic activity was assessed with semi-quantitative X-ray analysis of young mice treated SC with different PTH-Fcs. PTH-(1-34)-Fc and PTH-(1-31)-Fc were the most potent constructs in this assay, and C-terminal truncations were progressively less potent. In vitro studies revealed that PTH-(1-34) and PTH-(1-34)-Fc had similar binding affinities for the PTH1 receptor, while truncated fragments of PTH-(1-34)-Fc had progressively diminished affinity. Anabolic PTH fragments are typically active in cAMP and PKA assays, so we examined these responses in MC3T3 osteoblasts treated with PTH-Fc constructs. C-terminal truncation of PTH-(1-34)-Fc resulted in progressive decreases in potency in both of these assays. Treatment of MC3T3 cells with PTH-(1-34)-Fc dose-dependently increased IGF-I mRNA (a putative mediator of osteoblast stimulation by PTH), and this induction was significantly diminished with C-terminal truncations. PTH activates osteoclasts in part by decreasing osteoblast production of OPG, a natural inhibitor of bone resorption. PTH-(1-34)-Fc dose-dependently suppressed OPG mRNA in MC3T3 cells, and the truncated PTH-Fcs had progressively diminished potency in this assay. In summary, the stepwise truncation of PTH-(1-34)-Fc from the C-terminus resulted in fragments that have progressively diminished PTH1R binding affinity and decreased potency in assays of PTH1R activation, osteoblastic gene expression, hypercalcemia, and anabolic activity. These data suggest that the catabolic and anabolic effects of PTH constructs are tightly coupled and closely related to their binding affinity for PTH1R.

Disclosures: ***V. Shalhoub****, None.*

SA445

See Friday Plenary number F445

SA446

Hypocalcemia and Parathyroid Hormone Increase a Novel Secreted Phosphoprotein 24 kDa (Spp24) in Kidney and Bone Cells. M. J. Beckman[1], A. Bajwa*[1], R. L. Horst[2], R. Dalgleish*[3]. [1]Biochemistry, Virginia Commonwealth University, Richmond, VA, USA, [2]Natl. Animal Disease Center, Ames, IA, USA, [3]Genetics, University of Leicester, University Road, United Kingdom.

Osteodystrophy is a multifactorial disease of unknown etiology. In this disease, cortical kidney dystrophy leads to impaired renal handling of mineral homeostasis and increased blood levels of parathyroid hormone (PTH). Similar conditions can be generated by dietary calcium (Ca) restriction leading to secondary hyperparathyroidism. Using a dietary model of secondary hyperparathyroidism and a hypercalcemic model induced by $1,25(OH)_2D_3$, cortical kidney tissues from the two models were compared for differential gene expression by differential display RT-PCR (DD) and by oligonucleotide microarray (MA). In both techniques one common gene was preferentially regulated by the conditions of low serum calcium and high PTH, and its expression was suppressed by hypercalcemia. The transcript of this gene was isolated in DD. Sequencing identified the product as the 24 kDa secreted phosphoprotein (Spp24). The regulation pattern of Spp24 in kidney was opposite that of another secreted phosphoprotein, namely Spp1 or osteopontin. The tissue distribution of Spp24 was analyzed by Real-Time RT-PCR and found to have strong expression in liver, kidney and bone and weak expression in muscle, lung and parathyroid gland. Quantitative Real-Time RT-PCR in cortical kidney also confirmed a 3.5-fold increase in Spp24 transcript in hypocalcemic compared to control rat kidney, which was similar in magnitude to the differential expression of Spp24 observed between low and high blood Ca conditions that were observed in MA. This indicates that the increase in Spp24 under low Ca conditions is influenced by PTH. Treatment of human proximal kidney (HK-2) and fetal osteoblast (FOB) cells with PTH 1-34 increased Spp24 transcript 2-fold, whereas, PTH-1-34 treatment to these cell types in the absence of Ca in the medium increased Spp24 transcript levels by an additional 2-fold over the control. The few studies on Spp24 in the literature indicate that this molecule is secreted into the extracellular space and has some ability to combine with the futin-mineral complex that accumulates in serum and bone. In conclusion, Spp24 is a novel phosphoprotein produced in several calciotropic organs in response to changes in serum Ca and additionally in response to PTH. The exact role of Spp24 in Ca metabolism is now under study.

Disclosures: ***M.J. Beckman****, None.*

SA447

See Friday Plenary number F447

SA448

Vitamin D Status as a Major Factor Determining Circulating Levels of Parathyroid Hormone. J. Pepe*[1], E. Romagnoli*[1], I. Nofroni*[2], S. De Geronimo*[1], E. D'Erasmo*[1], S. Minisola[1]. [1]Department of Clinical Sciences, University of Rome, Rome, Italy, [2]Department of Experimental Medicine and Pathology, University of Rome, Rome, Italy.

The aim of the study was to investigate the relative contribution of the major factors regulating calcium homeostasis in determining the circulating levels of PTH. We studied a large group of healthy volunteers which included 137 males (mean age 48.0 ± 18.8 yr; range 17 - 90 yr) and 125 females (50.5 ± 19.9 yr; range 19 - 92 yr). The women's group was subdivided according to gonadal status in 55 fertile and 70 postmenopausal subjects.
Metabolic study included a fasting blood morning sample for the measurement of serum total Ca, Mg, P, albumin, creatinine, total ALP and 25(OH)D levels. Circulating PTH levels have been determined by 3 different IRMA. The first one (PTH S) measures serum hormone levels using two affinity-purified polyclonal antibodies, one specific for the 1-34 portion of PTH molecule and the second specific for the 39-84 sequence of the hormone (N-tact PTHSP, DiaSorin Inc., Stillwater, MN, USA). PTH plasma levels were also measured by two other IRMA (Duo PTH Scantibodies Lab. Inc.; Santee, CA, USA) sharing polyclonal anti-PTH (39-84) coated beds as universal solid phase. The first assay (PTH W) utilizes a second polyclonal antibody, directed against the N-terminal (1-4) aminoacid sequence. The second assay (PTH T) utilizes a second antibody specific for the 7-34 region. Concentrations of fragments lacking 1-6 aminoacid sequence (PTH N-truncated, PTH N-t) were determined by the difference of values between PTH T and PTH W. For each kind of PTH assay, multiple linear regression models of the numerous independent variables examined were constructed.
Vitamin D was the main explicative variable almost in every model both considering the group as a whole (PTH S: $R2 = 0.238$, $p < 0.0001$; PTH W = R2: 0.08, $p < 0.001$; PTH T R2: 0.145, $p < 0.0001$; PTH N-t: R2: 0.081, $p < 0.009$) and separately men and women, pre- and postmenopausal subjects.
In subjects with vitamin D insufficiency ($n = 53$) (serum 25(OH)D < 30 nmol/L), mean serum levels of PTH were significantly higher ($p < 0.001$) than those in subjects of similar age with normal vitamin status ($n = 209$) with all the assays employed (PTH S: 36.5 ± 12.8 ng/L vs 29.6 ± 11.8; PTH W: 23.9 ± 10.5 vs 18.4 ± 9.4; PTH T 39.3 ± 13.9 vs 31.4 ± 13.4) the only exception being represented by the N-t fragments.
This study provides information as regards the factors regulating PTH secretion in physiological conditions, that for the first time have been simultaneously considered; it demonstrates the central role of 25(OH)D. Vitamin D status represents a parameter to be taken into consideration in evaluating subjects with parathyroid hormone values above the normal range.

Disclosures: ***J. Pepe****, None.*

SA449

See Friday Plenary number F449

SA450

Sustained PTH-stimulated cAMP Signaling in Primary Osteoblasts from ßarrestin2 KO Mice Leads to Altered Skeletal Response to Intermittent PTH. D. D. Pierroz[1], V. Glatt[2], R. Rizzoli[1], S. L. Ferrari[1], M. L. Bouxsein[2]. [1]Division of Bone Diseases, University Hospital, Geneva, Switzerland, [2]Orthopedic Biomechanics, Beth Israel Deaconess Medical Center, Boston, MA, USA.

Cytoplasmic ß-arrestin2 (ßarr2) plays an important role in desensitization of PTH-stimulated cAMP signaling. We hypothesized that absence of ßarr2 would lead to sustained cAMP signaling in primary osteoblasts in vitro and altered skeletal response to PTH in vivo.
Thus, we investigated PTH-stimulated cAMP signaling in primary osteoblastic cells from neonatal calvariae of ßarr2 KO and WT mice and the skeletal response to intermittent PTH (20, 40, 80 µg/kg/d) or vehicle (VEH) for 4 wks in adult WT and KO females (n=6-11/group) using pDXA and µCT.
In presence of a phosphodiesterase (PDE) inhibitor, IBMX (1µM), PTH dose-dependent stimulation of cAMP was 50-70% higher in KO compared to WT cells ($p<0.0001$). Moreover, in cells pre-exposed to PTH, cAMP response to rechallenge with PTH (100nM) was significantly desensitized in WT (-34.5%, $p=0.0001$) but not in KO cells. In absence of IBMX, differences in PTH-stimulated cAMP levels between KO and WT cells were even more dramatic (54±1 vs 19±7 fold increase over baseline, respectively, $p<0.001$), and peak cAMP levels were maintained for up to 30 min. in KO cells, whereas at that time they had declined by 33% in WT cells ($p<0.0013$). These results are consistent with ß-arrestin-mediated inhibition of cAMP signaling through both receptor uncoupling from G protein and recruitment of PDE.
Compared to VEH, PTH significantly increased total body (TB) BMC, whole femur density (F. BV/TV), mid-femoral cortical thickness (C.Th), and vertebral trabecular density (V. BV/TV) ($p=0.0011$-0.0001). Significant interaction with genotype was found for C.Th ($p=0.02$) and F. BV/TV ($p=0.07$), indicating increased cortical bone response to PTH in KO, particularly at the lowest PTH dose (C.Th, PTH20 vs VEH, +10%, $p=0.0019$ and +0%, ns, in KO and WT, respectively). In contrast, V. BV/TV and the percent increase in TB BMC were, on average, 10% ($p=0.011$) and 25% ($p=0.06$) lower, respectively, in PTH-

treated KO compared to WT.
These data indicate that absence of ß-arr2 leads to sustained and prolonged cAMP signaling in primary osteoblasts, which is less desensitized in response to PTH. This increased PTH activity in vitro translates in vivo into increased net anabolic effects on cortical but not cancellous bone in KO mice. Consistent with our previous observations in male ßarr2 KO mice, these results suggest that, in absence of ßarr2, some downstream molecular mechanisms for PTH activity in cancellous bone/bone marrow may be dysregulated.

Disclosures: ***D.D. Pierroz****, None.*

SA451

See Friday Plenary number F451

SA452

A High Calcium Intake Promotes Positive Bone Balance via a PTH-Dependent Mechanism. Y. Xue*[1], J. Li*[1], H. Su*[2], A. C. Karaplis[2], D. Goltzman[1], D. Miao[1]. [1]Department of Medicine, Calcium Research Lab, McGill University, Montreal, PQ, Canada, [2]Medicine, SMBD-Jewish General Hospital, McGill University, Montreal, PQ, Canada.

Increased dietary calcium intake reduces bone loss in aging individuals and may increase bone mass and reduce fracture risk. Although this has been ascribed in part to reduction of secondary hyperparathyroidism, the mechanism of calcium action on the skeleton is unclear. To investigate this issue we examined the effect of a high calcium diet on the skeleton of mice with targeted disruption of the PTH gene ($PTH^{-/-}$). $PTH^{-/-}$ mice and wild-type (WT) littermates were fed either a normal (1.0%) or high (2.0%) calcium diet. On a normal calcium diet, serum $1,25(OH)_2D_3$ levels were 113 ± 6 pg/ml in WT mice and 55 ± 9 pg/ml in $PTH^{-/-}$ mice but on the high calcium diet were reduced to 4.5 ± 0.5 pg/ml and 5.0 ± 0.6 pg/ml in WT and $PTH^{-/-}$ mice respectively. On the normal calcium diet serum calcium was 2.5 ± 0.1 mmol/L and 1.6 ± 0.1 mmol/L in WT and $PTH^{-/-}$ mice respectively but on the high calcium diet calcium levels in both groups were within the normal range (2.5 ± 0.1 mmol/L in WT and 2.3 ± 0.1 mmol/L in $PTH^{-/-}$). In the WT mice serum PTH levels fell from 44 ± 8 pg/ml on a normal calcium intake to 18 ± 4 pg/ml on the high calcium intake; PTH levels were undetectable in $PTH^{-/-}$ mice. Bone formation rates (assessed by double calcein-labeling) and alkaline phosphatase positive osteoblast numbers were increased in both mouse models on the high compared to the normal calcium intakes but both increases were greater in WT than in $PTH^{-/-}$ mice. On the high calcium intake TRAP-positive osteoclast size and numbers decreased and the ratio of OPG/RANKL bone mRNA increased in WT but not in $PTH^{-/-}$ mice. Overall, on the high calcium intake, trabecular bone volume rose in WT animals by 61-68% but fell by 7-21% in $PTH^{-/-}$ mice. The results indicate that (1) calcium may not directly alter osteoclastogenesis but may reduce PTH-dependent osteoclast production (2) calcium may enhance bone formation in the absence of PTH but this response in more robust in the presence of PTH. Increases in dietary calcium may therefore increase trabecular volume by enhancing the anabolic action of endogenous PTH.

Disclosures: ***Y. Xue****, None.*

SA453

See Friday Plenary number F453

SA454

Transcriptional Profiling of Embryonic Tibia after PTH Treatment in vivo Reveals Numerous Genes that Are Affected by PTHR Activation. J. Guo[1], G. Short*[2], A. Szczepanik*[1], N. Elmessadi*[3], F. R. Bringhurst[1], H. M. Kronenberg[1]. [1]Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [2]Microaaray Facility, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA, [3]Microarray Facility, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

The PTH/PTHrP receptor (PTHR) delays chondrocyte hypertrophy, and this process is accompanied by global changes in gene expression involving molecular events such as signal transduction and regulation of transcription. To identify genes involved in PTHR-modulated chondrocyte differentiation, we used cDNA oligonucleotide microarray analysis (13,000 murine genes –Operon set) to compare gene expression profiles of E14.5 embryonic tibial explants 6 hr after ex vivo treatment with rPTH(1-34) (100 nM) vs. vehicle alone. Thirty eight genes were significantly up-regulated (ratio > 2.0) and 61 genes were down-regulated (ratio < 0.5) in PTH-treated samples compared to control samples on four separate chips, using 4 independent RNA pools. To validate the potential downstream target genes identified in the arrays, we performed real-time PCR (RT-PCR) and in situ hybridization analysis on a subset of the genes identified as significantly regulated on DNA chips. Results from 14 out of the18 genes tested by RT-PCR confirmed the DNA chip data. Among up-regulated genes confirmed by RT- PCR were Wnt-7B, Wnt inhibitory factor 1 and secreted frizzled-related sequence protein 4. Interestingly, osteopontin, a marker for hypertrophic differentiation, was dramatically up-regulated by PTH and this increased expression of osteopontin in hypertrophic chondrocytes was further verified by in situ hybridization. Decreased expression of Hedgehog-interacting protein, bone sialoprotein and PTHR was confirmed by real-time PCR. In summary, these data could provide a new insight into the understanding of molecular events involved in PTHR-directed chondrocyte differentiation.

Disclosures: ***J. Guo****, None.*

SA455

Effect of α-N-Methylation of Residues in the Receptor-Binding Region of hPTH(1-31)NH_2 on Structure and Adenylyl Cyclase Activation. G. E. Willick*, J. Barbier*, S. MacLean*, J. F. Whitfield. Biological Sciences, National Research Council, Ottawa, ON, Canada.

The functional recognition of a peptide hormone such as the potently anabolic hPTH-(1-31)NH_2 (Ostabolin™) for its receptor depends on both the side-chains of the amino acids and the carbonyl and amide groups of the peptide backbone. Although the effect of amino acid residue mutations is frequently studied, relatively little attention has been directed towards the CO and NH groups of the backbone. These groups frequently are important determinants of the specificity of the hormone-receptor interaction. For example, N-methylating the backbone NH functions of residues modulates the specificity of somatostatin for its receptor sub-types (Rajeswaren *et al* 2001 J Med Chem 44**:** 1305). The S17–V31 residues of hPTH-(1-31)NH_2 were selectively N-methylated and their adenylyl cyclase (AC)-stimulating activities measured in HKRK-B7 cells stably expressing the hPTHR1 receptor. In these cells, hPTH(1-31)NH_2 stimulates both the AC and phospholipase-Cβ signaling pathways. The effect of the methylations on the peptide's secondary structure was measured by circular dichroism (CD) spectroscopy. Methylation of the three C-terminal residues (QDV) had little effect on the α-helix content and did not diminish AC activity. Methylating the N-terminal residues (SME) reduced helix content by 25% and AC-stimulating activity by about 75%, but methylation of R20 resulted in little AC loss despite a similar 25% loss of α-helix. Methylation of residues E22-L28 (EWLRKKL) resulted in a loss of at least 50% of the helix content and an almost total loss of AC-stimulation with the exception of residues E22 and K26, that retained about 10% and 25%, respectively, of AC stimulation activity. These results point to a loss of some AC-stimulating activity associated with the loss of stability of the C-terminal amphiphilic α-helix, but also to the backbone nitrogens associated with V21, W23, L24, R25, K27, and L28 being critical for correct receptor-activating binding.

Disclosures: ***G.E. Willick****, None.*

SA456

See Friday Plenary number F456

SA457

Development of PTH(1-14)-based Antagonist Analogs that Bind to the Juxtamembrane Portion of the PTH-1 Receptor. N. Shimizu*, J. C. Tsang*, A. Khatri*, T. J. Gardella. Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

For GPCRs in general, ligands that function as antagonist and/or inverse agonists can be useful probes of ligand-binding and receptor activation mechanisms. For the PTH-1 receptor (P1R), most antagonists are N-terminally truncated analogs of PTH(1-34) or PTHrP(1-36), which are thought to bind predominantly to the receptor's amino-terminal extracellular ("N") domain. Here we sought to develop antagonists that bind exclusively to the portion of the P1R that contains the seven transmembrane helices and extracellular loops (the "J" domain). We started with the J domain-specific agonist, [Ac_5c(aminocyclopentane carboxylic acid)[1], Aib(aminoisobutyric acid)[3], Gln^{10}, Har^{11}, Ala^{12}, Trp^{14}]PTH(1-14)NH_2 {[M4]PTH(1-14)}, which functions as a potent agonist in cells (COS-7 or LLC-PK1) transfected with either the intact P1R or a P1R construct deleted for the N domain (EC_{50} for cAMP production ~ 2 nM; E_{max} ~ 50-fold basal; Shimizu et al. 2003, J. B. M. R. Vol 17, abstract # SU246). Based on the known importance of the N-terminal residues of PTH for inducing receptor signaling, we introduced single or multiple amino acid substitutions at positions 1-3 of [M4]PTH(1-14). The Analog [Bpa^2,M4]PTH(1-14), in which Val-2 was replaced by benzoylphenylalanine, exhibited adequate binding affinity {IC_{50} ~ 1 μM, vs. ~30 nM for [M4]PTH(1-14)} and very weak agonism (EC_{50} >> 10 μM; E_{max} ~ 5-fold basal). We then introduced Deg (diethylglycine) at positions 1 and 3, as these paired substitutions confer partial agonism to [M4]PTH(1-14) (*ibid*). The resulting analog [$Deg^{1,3}$,Bpa^2,M4]PTH(1-14) maintained adequate affinity (IC_{50} = ~0.8 μM), but was nearly devoid of agonism (E_{max} < 2-fold basal). On the intact P1R, [$Deg^{1,3}$,Bpa^2,M4]PTH(1-14) (10 μM) inhibited the agonist activity of [M4]PTH(1-14) (1 nM) by ~70% but was ineffective against PTH(1-34) (1 nM); PTHrP(5-36) (10 μM) inhibited [M4]PTH(1-14) by ~60% and PTH(1-34) by ~85%. On a P1R construct having the N domain replaced by PTH residues (1-9) (tethered to TM1) and which thus exhibits high "basal" activity, the basal activity was inhibited ~ 50% by [$Deg^{1,3}$, Bpa^2, M4]PTH(1-14) but only ~ 10% by PTHrP(5-36). Analogs [Bpa^2, M4]PTH(1-14) and [$Deg^{1,3}$, Bpa^2, M4]PTH(1-14) were selective inverse agonists, as they depressed the constitutive activity of P1R-H223R but not that of P1R-T410P or P1R-I458R. The results show that PTH analogs that bind only to J domain of the P1R can function as competitive antagonists and inverse agonists. The study thus opens new pathways for pharmacologically exploring ligand-receptor interactions that occur within the J domain of the P1R, particularly those involving the first three residues of PTH.

Disclosures: ***N. Shimizu****, Chugai Pharmaceutical Co. Japan 3.*

SA458

Vitamin D Represses the Herpes Simplex Virus Thymidine Kinase Promoter: Identification of a Repressor Element and Cell-type Specificity. N. J. Koszewski, A. P. Alimov, H. H. Malluche. Division of Nephrology, Bone & Mineral Metabolism, University of Kentucky Medical Center, Lexington, KY, USA.

The herpes simplex virus thymidine kinase (HSVtk) promoter has been widely used as a heterologous promoter in characterizing a variety of DNA response elements, including vitamin D response elements (VDREs). We noted the capacity of vitamin D to suppress reporter gene activity in opossum kidney (OK) cells from constructs lacking functional VDREs, including the parent HSVtk reporter itself. This was strictly dependent on co-expression of the heterodimer receptors, was not seen with an SV40-driven promoter and did not occur when estrogen receptors were co-expressed with the HSVtk reporter and exposed to estradiol. Mobility shift assays using recombinant heterodimer-containing extracts revealed multiple, specific DNA-binding complexes with an HSVtk promoter fragment (-105 to +57). Binding appeared to be restricted to the 3' portion of this fragment (–44 to +48), and interference footprinting confirmed that interactions were localized over four half-sites from ca.-12 to +25. Use of a series of oligonucleotide probes encompassing various half-site combinations resembling DR+3-like motifs revealed single, specific heterodimer binding complexes with each. Transient transfection analysis in OK cells using a mutant HSVtk promoter that incorporated changes to the 3' pair of half-sites continued to exhibit a repressive response when treated with calcitriol. Mutations made to the 5' pair of half-sites resulted in increased basal activity, but an inability to repress transcription in response to hormone, suggesting that these sites conveyed the repressive response. Finally, modest vitamin D-dependent gene activation could be observed in OK cells with an enhancer VDRE linked to the HSVtk-promoter, while strong suppression was observed from a repressor VDRE construct. In contrast, transfection of the same reporters in COS-7 kidney cells revealed robust activation from the enhancer VDRE, but no suppression of reporter activity from the repressor VDRE construct irrespective of the amounts of co-transfected heterodimer expression vectors. In summary, the vitamin D receptor heterodimer forms strong, multiple DNA-binding complexes with the HSVtk promoter that results in hormone-dependent suppression of gene activity. This repression is cell-type specific, however, suggesting that other factors are playing a role in this response.

Disclosures: ***N.J. Koszewski****, None.*

SA459

See Friday Plenary number F459

SA460

A Mutation in the Ligand Binding Domain of the Vitamin D Receptor that Alters 1,25(OH)2D3 Binding, RXR Heterodimerization and Coactivator Binding thereby Causing Hereditary Vitamin D Resistant Rickets. P. J. Malloy*[1], R. Xu*[1], L. Peng*[1], S. Peleg[2], A. Ashwal*[3], D. Feldman[1]. [1]Department of Medicine, Stanford University, Stanford, CA, USA, [2]Department of Endocrine Neoplasia and Hormonal Disorders, University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA, [3]Department of Pediatrics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.

Hereditary vitamin D resistant rickets (HVDRR) is an autosomal recessive disease caused by mutations in the vitamin D receptor (VDR). The patient in this study, a young Saudi Arabian girl, exhibited the typical clinical features of HVDRR but without alopecia. Analysis of her DNA revealed her to be homozygous for a unique T to C mutation in exon 7 of the VDR gene that changed the codon for isoleucine to threonine at amino acid 268 (I268T). Based on the crystallographic studies of the VDR ligand-binding domain (LBD), I268 directly interacts with $1,25(OH)_2D_3$ and is involved in the hydrophobic stabilization of helix H12. We recreated the I268T mutation in the VDR cDNA and analyzed its effects on VDR function. Several defects in $1,25(OH)_2D_3$ action were demonstrated. In ligand binding assays, the I268T mutant VDR exhibited a ~10-fold lower affinity for $[^3H]1,25(OH)_2D_3$ compared to the WT VDR consistent with I268 interaction with the ligand. However, in transactivation assays, the I268T mutant required ~100-fold higher concentrations of $1,25(OH)_2D_3$ to stimulate gene transcription compared to the WT VDR. Since I268 is involved in stabilizing helix H12 we examined coactivator binding using GST-pull down assays. The I268T mutant required ~100-fold more $1,25(OH)_2D_3$ to promote binding to the coactivators SRC-1 and DRIP205. We also examined RXR binding using the yeast two-hybrid system and GST-pull down. The I268T mutant showed a marked decrease in RXR binding compared to the WT VDR. However, the I268T mutant was able to form a complex on the osteopontin vitamin D response element as shown by gel shift assays. We re-tested the mutant in transactivation assays and showed that the responsiveness of the I268T mutant to $1,25(OH)_2D_3$ could be partially rescued by the addition of RXR. In conclusion, we describe a novel mutation in the VDR LBD, I268T that alters ligand binding, RXR heterodimerization, and coactivator binding. These cumulative defects cause resistance to $1,25(OH)_2D_3$ action and result in the syndrome of HVDRR.

Disclosures: ***P.J. Malloy****, None.*

SA461

See Friday Plenary number F461

SA462

Vitamin D Receptor-Mediated Transcription Is Enhanced by Calmodulin-dependent Kinase IV, Potential Interplays between Vitamin D and Calcium Signaling Pathways. T. I. Ellison*, D. R. Dowd*, P. N. MacDonald. Department of Pharmacology, Case Western Reserve University, Cleveland, OH, USA.

The major physiological role of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] is to maintain normal calcium homeostasis, primarily by ensuring adequate absorption of dietary calcium in the small intestine. The biological actions of 1,25(OH)2D3 are mediated through the vitamin D receptor (VDR), a member of the nuclear receptor superfamily of ligand-activated transcription factors. Despite critical roles for 1,25(OH)2D3 in calcium transport across enterocytes, little information exists on the effects of intracellular calcium on the transcriptional activity of liganded VDR. To explore potential interplays between VDR and intracellular calcium regulated pathways, initial studies focused on the calmodulin (CaM)-dependent kinase termed CaM kinase IV. A vitamin D responsive luciferase reporter gene (VDRE4TATA-luciferase) and a VDR expression vector (SG5-VDR) were transfected into COS7 cells or HeLa cells in the absence or presence of a vector encoding a constitutively active form of CaM kinase IV. A dramatic induction of 1,25(OH)2D3-activated reporter gene expression was observed in cells receiving the CaM kinase IV derivative. The response was dependent on the amount of transfected CaM kinase IV plasmid showing a maximal 12-fold induction in 1,25(OH)2D3 responsiveness compared to controls. No effects were observed on basal or unstimulated expression of the reporter gene. Moreover, 30 micromolar KN93, a relatively selective inhibitor of CaM kinase IV, inhibited vitamin D-activated reporter gene expression by 50 %. No effect of this compound was observed on basal or unstimulated reporter gene expression. Together, these data indicate that CaM kinase IV selectively augments VDR- and 1,25(OH)2D3-activated transcription in both HeLa cells and COS7 cells. We also observed potent activation by CaM kinase IV using a heterologous gal4-VDR(93-427) construct and a gal4-responsive luciferase reporter gene (gal4TATA-luciferase). These data suggest the effect of CaM kinase IV is mediated through the VDR ligand binding domain. A potential mechanism for this enhanced activity of VDR was suggested in western immunoblots which indicated an enhanced level (app 2-fold) of VDR protein in response to CaM kinase IV expression. However, it is likely that this does not account for the full stimulatory effect on VDR-activated transcription. To pursue additional mechanisms we are currently examining whether VDR or VDR associated comodulatory proteins are targets for CaM kinase IV phosphorylation using in vivo labeling and immunoprecipitation strategies.

Disclosures: ***T.I. Ellison****, None.*

SA463

See Friday Plenary number F463

SA464

Analysis of Mice Lacking both Vitamin D Receptor and Calbindin-D28k Reveals a Critical Role of Calbindin-D28k in Calcium Homeostasis. W. Zheng*[1], G. Li[2], Y. Jiang*[3], J. Kong*[1], Y. C. Li[1]. [1]Medicine, University of Chicago, Chicago, IL, USA, [2]Trauma and Orthopaedic Surgery, Queen's University Belfast, Belfast, United Kingdom, [3]Radiology, University of Chicago, Chicago, IL, USA.

The role of Calbindin(CaBP)-D28k, a cytosolic calcium-binding protein mainly expressed in the kidney and brain, in calcium homeostasis remains controversial. It has long been speculated that CaBP-D28k plays an important role in transcellular calcium transport in the kidney, largely based on its calcium-binding property and its abundant expression in distal tubules, which is reduced in vitamin D-deficient state. However, recent studies suggest that CaBP-D28k is not essential for calcium metabolism. In particular, genetic ablation of CaBP-D28k in mice has no effect on calcium homeostasis. Our recent studies show that genetic inactivation of the vitamin D receptor (VDR) results in a 90% reduction in CaBP-D9k, but has little effect on CaBP-D28k expression, in the kidney, which is associated with an increase in calcium excretion and development of hypocalcemia in VDR knockout (KO) mice. It is possible that the function of CaBP-D28k is compensated by CaBP-D9k in CaBP-D28kKO mice, whereas the reverse is not true in VDRKO mice. To further investigate the role of CaBP-D28k in calcium reabsorption, we have generated VDR/CaBP-D28k double KO mice, which express no CaBP-D28k and only 10% of CaBP-D9k in the kidney, and the double KO mice were examined in parallel with wild-type (WT), CaBP-D28kKO and VDRKO mice. As shown previously, CaBP-D28kKO mice appear grossly normal and show no calcemic abnormalities as compared with WT mice. While VDRKO mice have lower body weight than WT mice, the double KO mice are even smaller than VDRKO mice and usually die at about two months of age. The tibiae and femurs of the double KO mice are significantly shorter than those of WT, CaBP-D28kKO as well as VDRKO mice. Peripheral quantitative computer tomography demonstrates that the double KO mice have a much greater reduction in total, trabecular and cortical bone mineral density than VDRKO mice as compared to WT mice. Histological analyses reveal deformity in the growth plate region in both VDRKO and the double KO mice; however, the double KO mice show much more severe distortion and less ossification and bone formation than VDRKO mice. The trabecular bones in the metaphysis regions are predominately osteoid with numerous microvascular invasions, and no secondary ossification center is found. Our results indicate that VDR/CaBP-D28kKO mice show much more severe calcemic and bone abnormalities than VDRKO mice, which provides direct in vivo evidence that CaBP-D28k does play a critical role in maintaining normal bone development by regulating calcium homeostasis.

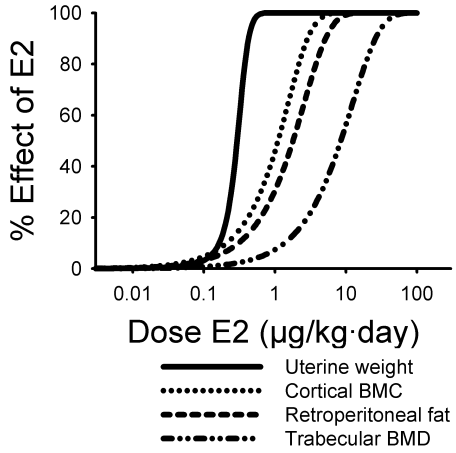
Disclosures: ***W. Zheng****, None.*

SA465

Tissue Specific Differences in Response to Estrogenic Stimulation in Ovariectomized Mice. N. Andersson[1], E. Egecioglu*[2], C. Ohlsson[1]. [1]Centre for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]Department of Physiology, Göteborg University, Göteborg, Sweden.

Estrogen deficiency increases the risk for a wide variety of pathological conditions including osteoporosis, cardiovascular diseases, and neuro-degenerative diseases. Estrogen replacement therapy is associated with side effects such as breast cancer and deep venous thrombosis and agents that can maintain the benefit of estrogen but avoid the risks are therefore needed.

The aim of the present study is to in detail investigate the 17β-estradiol (E2) dose-response pattern in different estrogen sensitive tissues. 8-week-old female C57BL/6 mice were ovariectomized and then treated with E2 for two weeks. Different doses of E2 were administered (0, 0.01, 0.1, 1, 10 and 100 µg/kg·day) using subcutaneously implanted osmotic minipumps. The estrogenic response of peripherally administered E2 was determined in estrogen responsive target organs (E2 increased cortical and trabecular bone mass and uterus weight, while it decreased retroperitoneal fat weight). All organs responded dose-dependently to E2. ED_{50}-values were estimated to be 0.3, 1, 2 and 9 µg/kg·day for uterine weight, cortical bone mineral content (BMC), retroperitoneal fat weight and trabecular bone mineral density (BMD), respectively (see figure).



Regarding the mechanisms of action for estrogen, two major pathways need to be considered: an indirect action through the central nervous system and a direct peripheral action on target tissue. The effect of E2 on uterine hypertrophy is considered to be a direct peripheral effect while its effect on food intake and fat mass is regarded to be central effects. The effect on bone has been suggested to be mediated by peripheral E2 action directly on bone tissue. The present study clearly shows tissue specific differences in response to estrogenic stimulation which may be an indication of the underlying mechanism of action for estrogen. The dose response pattern for E2 in the regulation of cortical and trabecular bone are more similar to the pattern for fat than that for the uterus. Thus, further studies are needed to fully distinguish between the central and the peripheral actions of E2 on bone.

Disclosures: ***N. Andersson****, None.*

SA466

See Friday Plenary number F466

SA467

Differences in Bone Turnover in Subchondral (SC) vs. Epiphyseal/ Metaphyseal Cancellous (EMC) Bone in the Proximal Tibia of Monkeys Are Accentuated by Estrogen Deficiency. K. D. Ham*, C. S. Carlson*. Veterinary Diagnostic Medicine, University of Minnesota, St. Paul, MN, USA.

The purpose of this study was to evaluate the effect of estrogen deficiency on histomorphometry indices in the SC bone versus the EMC bone of the proximal tibia.

Adult female cynomolgus monkeys were ovariectomized (OVX), divided into 2 treatment groups (OVX and OVX estrogen (E2)-treated [Premarin®]) for a 3-year treatment period, and given calcein 14 and 7 days prior to euthanasia. At necropsy a mid coronal section of the right proximal tibia from 20 randomly selected animals/treatment group was embedded in Bioplastic, sectioned at 10µm, and mounted unstained.

Primary static (2X objective) and dynamic (10X objective) histomorphometry measurements were done. SC bone measurements were taken from the medial tibial plateau in a 3.5 mm wide field, beginning 2 mm medial to the central long axis. EMC bone measurements were taken in a 3.5 X 3.5 mm field 3 mm below the lower limit of the SC bone, centered on the central long axis. Derived indices included: MAR; MS/BS; dLS/BS; BFR/BS; BFR/BV; BS/BV; Tb.N; Tb.Sp; Tb.Th and BV/TV. SC bone widths and areas were also calculated. Data were compared using t-tests.

All dynamic indices (except MAR in EMC) in both sites were significantly higher in the OVX group than the OVX E2-treated group. Within each treatment group means for dynamic histomorphometry indices were higher for SC bone than for EMC bone, and many of these differences were significant (see Table). In addition, Tb.N ($p<0.01$) and BS/BV ($p<0.001$) were higher and Tb.Sp ($p<0.01$) and Tb.Th ($p<0.05$) were lower in the OVX E2- treated group than in the OVX group. There were no significant differences in SC bone width or area or in EMC bone volume between the two groups.

In summary, the bone turnover activity is higher in the SC bone than the EMC bone, and this site difference is accentuated by estrogen deficiency. These differences may have implications for the role of SC bone in the development of osteoarthritis in postmenopausal women.

Parameter	ovx SC bone	ovx EMC bone
MAR	1.51 (0.36)	0.91 (0.19)
MS/BS	15.68 (2.97) **	6.45 (1.32)
BFR/BS	120.2 (40.5) *	28.9 (5.94)
dLS/BS	9.25 (2.06) **	3.17 (0.69)
BFR/BV	66.11 (20.3)	69.76 (15.2)
Parameter	ovx-E2 SC bone	ovx-E2 EMC bone
MAR	0.49 (0.2)	0.47 (0.21)
MS/BS	4.96 (1.67)	2.48 (0.93)
BFR/BS	25.38 (12.2)	6.48 (2.83)
dLS/BS	2.38 (0.94) *	0.42 (0.15)
BFR/BV	15.06 (7.24)	21.26 (10.5)

Mean (SE). * $p<0.05$, ** $p<0.01$

Disclosures: ***K.D. Ham****, None.*

SA468

See Friday Plenary number F468

SA469

Testosterone Regulates Skeletal Homeostasis During Puberty Independently of Growth Hormone Receptor Activation in Mice. K. Venken*[1], J. Kopchick*[2], K. Coschigano*[2], S. Boonen[3], R. Bouillon[1], C. Ohlsson[4], D. Vanderschueren[1]. [1]Laboratory for Experimental Medicine and Endocrinology, K.U.Leuven, Leuven, Belgium, [2]Edison Biotechnology Institute, Athens, OH, USA, [3]Center for Metabolic Bone Diseases, K.U.Leuven, Leuven, Belgium, [4]Division of Endocrinology, Department of Internal Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden.

Growth hormone (GH) and androgens are both known to affect skeletal growth during puberty. The aim of the present study was to evaluate a possible interaction between GH and androgen action and, particularly, whether the ability of androgens to stimulate skeletal modeling requires the presence of a functional GH receptor (GHR). To this end, we evaluated the effects of testosterone (T) replacement (1.5 µg T daily via subcutaneous silastic implants) in orchidectomized (orch) male mice with disrupted GHR (GHRKO) and corresponding wild-type mice (WT) during late puberty (6-10 weeks). Results are expressed as % gain or loss compared to orch and analyzed via two-factor ANOVA. Both in GHRKO and WT, T enhanced recruitment of osteoblasts at the outer site of the femur and increased periosteal bone formation rates by 94% and 81% in GHRKO and WT, respectively, as measured by dynamic histomorphometry on femoral cross-sections. This increase of periosteal bone formation was associated with statistically similar increases in body weight (+ 26% and + 14%, resp.) and lean body mass (+ 24% and + 13%, resp.) in GHRKO and WT. At the inner site of the femur however, T reduced bone turnover at the endocortical (bone formation rates -51% and -56% in GHRKO and WT, resp.) and trabecular sites (osteoid perimeter -59% and -39%, resp.). This decrease in bone turnover was reflected by a significant reduction in serum osteocalcin in both models (-28% and -41%, resp.). In line with these findings, T significantly increased trabecular bone volume in GHRKO as well as WT. In these models, T did not affect femoral length and serum IGF-I. The end result of T action on appendicular skeleton was a similar thickening of the cortex and maintenance of trabecular bone in GHRKO and WT. We conclude that, in the context of the mice models studied, T action during puberty on trabecular and cortical bone is independent of GHR activation. These findings support the concept that T action on skeletal modeling does not involve activation of the GH axis.

Disclosures: ***K. Venken****, None.*

SA470

See Friday Plenary number F470

SA471

ERα and ERβ Bind SRC1 with Different Affinities in Normal Human Osteoblast Extracts Using a Modified *in vitro* Assay. D. G. Monroe[1], F. J. Secreto*[1], B. J. Getz*[1], B. L. Riggs[2], S. Khosla[2], T. C. Spelsberg[1]. [1]Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA, [2]Endocrine Unit, Mayo Clinic, Rochester, MN, USA.

Estrogen (i.e. 17β-estradiol; E2) has multiple functions in the maintenance and homeostasis of the skeleton, including important metabolic functions in the bone-forming osteo-

blasts (OB). The functions of E2 are largely mediated through two distinct estrogen receptor isoforms, ERα and ERβ, and both are found coexpressed in normal OBs. Following ligand binding, the ER dimerizes and binds either directly or indirectly to regulatory elements within the control regions of genes. The altered ER protein conformation induced by ligand binding causes recruitment of coactivator complexes that are involved in the activation of transcription. Additionally, it has been shown that binding of alternate ER ligands (i.e. genistein, tamoxifen, raloxifene; SERMs) induces novel, often suboptimal, ER conformations that can potentially have drastic effects on the recruitment of coactivators. The aim of this study is to identify the effect of various SERMs on the ability of ERα and ERβ to recruit SRC1, the founding member of the p160 family of coactivators. We have developed a modified version of the GST-pulldown assay. Glutathione-S transferase (GST) fusion proteins containing the ligand binding domain of either ERα and ERβ were generated and bound with ethanol control, E2, or one of the SERMs (genistein, tamoxifen, or 4-OH tamoxifen). The ligand bound fusions were incubated with hFOB nuclear extracts, a normal human osteoblast cell line developed in our laboratory. Western blot analysis was performed and bound SRC1 protein was detected using a specific antibody. Our analysis indicated that SRC1 was recruited to both ERα and ERβ when either receptor was bound E2 or genistein. However, in both cases, ERα recruited SRC1 more efficiently than ERβ, as indicated through densitometry. Other studies indicated that ER bound with tamoxifen, but not 4-OH tamoxifen, resulted in minor recruitment of SRC1 with ERα . We are currently involved in determining relative affinities for SRC1 binding to the ER isoforms by altering the GST binding and washing conditions. Furthermore, similar GST-pulldown assays to examine the association of other ER coactivators and corepressors (i.e. SRC2, REA, p300) with the ER isoforms are currently underway.

Disclosures: ***D.G. Monroe****, None.*

SA472

See Friday Plenary number F472

SA473

Changes in Gene Expression by Estrogen Receptor Ligands in the Femora of Hindlimb Unloaded Male Rats. M. Sajid*[1], C. Nolan*[1], M. R. Allen*[2], S. A. Bloomfield[2], C. L. Smith[1]. [1]Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA, [2]Health & Kinesiology, Texas A&M University, College Station, TX, USA.

Decreases in mechanical usage that occur during spaceflight, bedrest and hindlimb unloading (HU) induce loss of bone mass in weight bearing bones; this may be accompanied by reductions in serum testosterone. Bone loss in humans and rats with testosterone and/or aromatase deficiencies can be attenuated by 17β-estradiol (E2) treatment, and we have shown that treatment with E2 or the selective estrogen receptor modulator, raloxifene (Ral), attenuates bone loss in mature male rats subjected to 28 d HU. This study's objective was to investigate alterations in gene expression that accompany HU relative to cage control (CC) animals, and to determine the ability of either E2 or Ral to modulate gene expression in unloaded bones in comparison to placebo HU controls. Six-month-old Sprague-Dawley rats were implanted with time-release pellets containing vehicle (Veh), E2 (12 μg/d), or Ral (535 μg/d) and were assigned to one of 4 groups: CC+Veh, HU+Veh, HU+ E2, or HU+Ral (4 rats/group). Femora were obtained 28 d thereafter. Bone mineral densities and areas in the left distal femora were measured *ex vivo* by peripheral quantitative computed tomography scans. E2 and Ral increased total BMD by 13.1% and 7.6%, respectively, in comparison to HU+Veh values. They also attenuated loss of endocortical bone at the metaphysis, preventing the 8.8% increase in marrow area observed in HU+VEH in comparison to CC+Veh rats. Total RNA was isolated from the proximal femora for assessment of various cytokine, and growth and transcription factor mRNAs by quantitative, real-time RT-PCR; target gene expression was normalized to 18S rRNA. Hindlimb unloading resulted in increased transforming growth factor-β (TGFβ), stress activated protein kinase (SAPK) and c-jun mRNA expression; no changes in insulin-like growth factor-1 (IGF-1), interleukin-1α (IL-1α), IL-1β, IL-6 or IL-10 mRNA expression due to 28 days of mechanical unloading were detected. There were no significant effects of E2 or Ral treatment on IGF-1, IL-1α, IL-1β or IL-10 mRNA expression. However, E2 but not Ral repressed IL-6 and c-jun mRNA levels. In addition, both E2 and Ral inhibited SAPK mRNA expression and tended to reduce TGFβ expression in HU animals. These results indicate that E2 and Ral protection from bone loss induced by mechanical unloading is associated with changes in gene expression that may contribute to the mechanisms by which E2 and Ral treatment alleviates disuse-induced osteoporosis.

Disclosures: ***C.L. Smith****, None.*

SA474

Body Fat Is Inversely Associated with 25-Hydroxyvitamin D Levels in Healthy Postmenopausal Women. J. M. Lappe, M. J. Barger-Lux, G. Haynatzki, R. P. Heaney, R. R. Recker, D. Travers-Gustafson. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Optimal vitamin D nutrition is essential for skeletal health. However, low levels of serum 25(OH)D are extremely prevalent. Evidence suggests that percentage of body fat is inversely related to 25(OH)D. Considering the epidemic of obesity in the US, it is important to further explore this relationship. Thus, the purpose of this analysis is to determine the association between serum 25(OH)D levels and percentage body fat in healthy postmenopausal women.

The analysis was conducted on data from 1180 white women randomly selected from the population of healthy postmenopausal women over 55 years of age in a nine-county farming area of Nebraska. Participants were enrolled into an intervention study of calcium and vitamin D. This report includes only data collected at baseline before the intervention was begun.

Each participant underwent a total body scan using DXA (Hologic Inc. Waltham, MA). Percentage body fat (%BF) was calculated as (fat mass [g]/ fat mass + lean mass [g]) X 100. Blood samples were collected after a 3-hour fast. Serum 25(OH)D was measured by RIA. To evaluate seasonal variation, we divided the year into 4 seasons based on serum vitamin D levels in this cohort: Jan-Mar, Apr-June, July-Sept, and Oct- Dec.

Analysis indicated that %BF is inversely associated with 25(OH)D after adjusting for age, season of the year, and vitamin D supplementation ($P<0.0001$). Levels of 25(OH)D decrease in a stepwise manner as quartile of %BF increases. Our findings are congruent with other reports that serum 25-OHD levels vary with adiposity and underscore the importance of considering body fat when evaluating vitamin D requirements.

Baseline Characteristics

Variable	Mean	SD
Age (yrs)	66.7	7.3
Body fat (%)	40.0	5.5
Body mass index (wt/ht x ht)	29.0	5.6
Serum 25(OH)D (nmol/L)	71.8	20.1
Vitamin D supp (IU/d)	318	150
Calcium intake (mg/d)	683	398

Disclosures: ***J.M. Lappe****, None.*

SA475

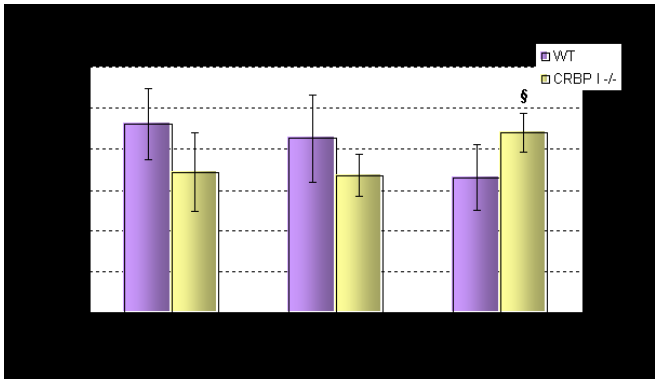
See Friday Plenary number F475

SA476

Bone Status and All-*trans*-Retinoic Acid (atRA) Homeostasis in Mice Lacking Cellular Retinol-Binding Protein I (CRBPI-KO) Before and After Chemical Insult by TCDD. P. Hoegberg*[1], P. M. Lind[1], N. Stern*[1], C. Trossvik*[1], A. Thomassen*[2], R. Blomhoff*[2], T. Jämsä*[3], J. Tukkanen*[3], N. B. Ghyselinck*[4], C. Ohlsson[5], H. Håkansson*[1]. [1]Inst. of Environmental Medicine, Karolinska Institute, Stockholm, Sweden, [2]Inst. for Nutrition Research, Univ. of Oslo, Oslo, Norway, [3]Dep. of Anatomy and Cell Biology, Univ. of Oulu, Oulu, Finland, [4]Igbmc, cnrs-inserm-ulp, Illkirch, France, [5]Dep. of Internal Medicine, Div. of Endocrinology, Sahlgrenska Univ. Hospital, Göteborg, Sweden.

Bone status is known to be affected by vitamin A (retinoids) as well as by organohalogen pollutants. We investigated associations between retinoid homeostasis, organohalogen exposure, and bone status by studying adult male CRBPI-KO mice, known to have an impaired retinoid storage capacity[1]. Wildtype (WT) mice of the same strain background were used as controls. With and without prior exposure to TCDD, we measured the composition of cortical and trabecular bone of tibia and femur by peripheral quantitative computed tomography (pQCT). The strength of tibia and femur was assayed by a three-point-bending test and femoral neck strength was measured by axial loading. Hepatic and serum retinyl ester, retinol, and atRA levels were measured by HPLC, serum 25(OH)D by a commercial RIA, and the osteoclast activity biomarker Trap5b by a serum solid-phase immunofixed enzyme activity assay. We saw no differences in bodyweight between untreated CRBPI-KO and WT mice although mutant tibiae were somewhat shorter. The CRBPI-KO had lower levels of both hepatic retinyl esters, retinol, and atRA levels than the WT at all times and doses. TCDD exposure reduced these levels in both genotypes. Bone mineral density (BMD) and strength were generally lower in CRBPI-KO mice. The effects of TCDD on bone length, composition, and strength 4 weeks after TCDD exposure was highly dependent on whether CRBP I had been knocked out or not; in fact, a disrupted CRBP I gene ameliorated the adverse effects of TCDD on bone. Vitamin D did not differ between the genotypes but were dose-dependently reduced by TCDD, and Trap5b followed approximately the same pattern. This study shows that a binding protein specific for the vitamin A system plays a role not only for proper retinoid storage but also for the homeostasis of the nuclear receptor ligand RA and for bone status.

[1]Ghyselinck *et al* 1999, EMBO J. 18:4903-14.



Disclosures: ***P. Hoegberg****, None.*

SA477

Towards Quantifying the Relationship of Constitutive Skin Color to Daily Skin Dose of Vitamin D3 in Healthy Adults with Ample Summer Sun Exposure. M. Barger-Lux[1], L. Auberry-Adams*[1], J. M. Lappe[1], R. R. Recker[1], R. P. Heaney[2]. [1]Department of Medicine, Creighton University, Omaha, NE, USA, [2]Creighton University, Omaha, NE, USA.

We report here preliminary results of work to quantify the relationship of inherent skin pigmentation and summer increment of vitamin D3 among healthy adults with ample summer sun exposure and limited non-solar sources of vitamin D. The 38 subjects (aged 20 to 45 yr) classified themselves by race as black (n=20), white (n=15), or other (n=3). Data were gathered in late summer (Aug. 29 to Sept. 21). We estimated extent (% body surface area) and duration (hr/wk) of summer sun exposure by interview. We also determined BMI; fasting serum 25(OH)D, vitamin D3, 1,25(OH)2D, PTH, and Ca; fasting urine Ca-to-creatinine ratio; and Ca absorption fraction.
We used a portable colorimeter (SmartProbe, IMS Inc., Milford, CT) that utilizes the CIE L*a*b* color system to measure constitutive skin color of the upper inner arm. There was a strongly positive curvilinear relationship (R2=0.5234) between the "L" readings (a continuous darker-to-lighter scale) and 25(OH)D. The lowest and highest "L" tertiles (i.e., darkest and lightest subgroups) differed significantly in 25(OH)D (47.6 ± 12.5 vs 91.9 ± 17.3 nmol/L); vitamin D3 (0.41 ± 0.31 vs 2.87 ± 2.39 ng/mL; fasting urine Ca-to-creatinine ratio (0.033 ± 0.026 vs 0.121 ± 0.096 g/g); and sun-exposed body surface area (28.1 ± 8.1 vs 40.1 ± 12.6%); They did not differ in 1,25(OH)2D, PTH, serum Ca, or Ca absorption fraction.

Disclosures: ***M. Barger-Lux****, None.*

SA478

ED-71, a Novel Vitamin D Analog, Promotes Bone Formation after Bone Marrow Ablation. N. Okuda*[1], Y. Higuchi*[2], T. Muneta*[1], S. Ito*[3], Y. Asou[3]. [1]Section of Orthopeadic Surgery, Devision of Bio-Matrix, Graduate School Tokyo Medical and Dental University, Tokyo, Japan, [2]Chugai Pharmaceutical Co., LTD, Tokyo, Japan, [3]Division of Molecular Tissue Engineering, Human Genes and Science Center, Tokyo Medical and Dental University, Tokyo, Japan.

1,25-Dihydroxyvitamin D_3 (VitaminD) plays an essential role in bone homeostasis. A novel vitamin D analog 2-β-(3-hydroxypropoxy)-1α,25-dihydroxyvitamin D_3 (ED-71) has high affinity with vitamin D binding protein and has greater activity than vitamin D in preventing bone loss in ovariectomized rat. However, the effect of ED-71 on bone remodeling including bone formation and resorption remains to be elucidated. Bone marrow ablation in the long bone causes vigorous new bone formation within the first week and then subsequent rapid bone resorption in the second week to regenerate bone marrow with normal levels of trabecular bones. It is a highly reproducible in vivo assay to evaluate bone remodeling. The purpose of this study was to evaluate the effect of ED-71 on bone remodeling in vivo by using mice bone ablation model. ICR mice were divided into ED-71 treatment and control groups, and all the femoral bone marrow were ablated by using K-wire . The day after operation, mice of ED-71 treatment group were intraperitoneally administered ED-71 (0.8μg/kg body weight). Mice were sacrificed 3,5,7,14days after surgery. There were no significant differences of body weight between two groups. Femoral bone marrow was observed by micro CT and histological examination. Histological examination showed that 3 and 5 days after surgery, ablated bone marrow in ED-71 treatment group were filled with more abundant matrix compared with control. 7 days after surgery, trabecular bone volume reaches at its maximal level, and more abundant and thicker newly formed trabecular bones were observed in ED-71 treatment group. Histological quantitation and micro CT analysis of the mice sacrificed 7days after surgery revealed that bone volume/tissue volume (BV/TV) were 50% more in ED-71 treatment group. By 2 weeks after surgery, newly formed trabecular bone was resorbed and the amounts of bone were similar between ED-71 treatment and control groups. As for bone resorption, the number of TRAP (tartrate-resistant acid phosphatase)-positive multinuclear cells per trabecular bone surface (N.OC/BS) was not different between ED-71 treated and control mice both 7days and 14days after surgery . These results indicated that ED-71 raised the maximal level of bone volume in the first week of administration, and possibly enhanced the bone formation activity. We concluded that ED-71 promoted bone formation in the region of rapid bone turn over after bone marrow ablation.

Disclosures: ***N. Okuda****, None.*

SA479

See Friday Plenary number F479

SA480

Potent Activity of Gemini Compounds to Suppress Renin Gene Expression. J. Kong*[1], M. Uskokovic*[2], G. Qiao*[1], Y. C. Li[1]. [1]Medicine, University of Chicago, Chicago, IL, USA, [2]BioXell, Inc., Nutley, NJ, USA.

The renin-angiotensin system (RAS) plays a central role in the regulation of blood pressure, electrolyte and extracellular volume homeostasis. Inappropriate activation of the RAS may lead to hypertension, which is one of the major risk factors for stroke, myocardial infarction, congestive heart failure, progressive atherosclerosis and renal failure. Our recent studies have shown that 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] functions as a potent negative endocrine regulator of renin production, and thus plays a critical role in the regulation of the RAS and blood pressure. These studies suggest that vitamin D analogs or derivatives with less calcemic effect and/or more potent renin-suppressing activity than 1,25(OH)2D3 might potentially be used as a new class of anti-hypertensive agents to control renin production and blood pressure. As an initial step to identify such analogs, we have screened 20 vitamin D analog compounds using the As4.1-hVDR cell culture system. The activity of the analogs to suppress renin gene expression was determined by Northern blot analyses and renin promoter/luciferase reporter assays. Interestingly, of the 20 compounds screened, only the Gemini compounds, which have two side-chains at Carbon-20 position, were found to be active in suppressing renin expression, whereas all the non-Gemini compounds were inactive. Of the 13 Gemini compounds, five were inactive or less active than 1,25(OH)2D3, four were as active as 1,25(OH)2D3, and four were 10- to 100-times more active than 1,25(OH)2D3. It is known that some of these Gemini compounds have much higher animal maximal tolerance dose (MTD), thus they may offer an excellent opportunity for further animal testing.

Disclosures: ***J. Kong****, None.*

SA481

Immunoassays for 25 Hydroxyvitamin D Demonstrate Positive Bias Compared with HPLC and Under-Recovery of 25 Hydroxyvitamin D_2 in Hip Fracture Cases. P. Glendenning[1], M. Taranto*[1], J. M. Noble*[2], A. Musk*[1], P. Goldswain*[2], W. Fraser[3], S. Vasikaran[1]. [1]Biochemistry, Royal Perth Hospital, Perth, Australia, [2]Geriatric Medicine, Royal Perth Hospital, Perth, Australia, [3]Chemical Pathology, University of Liverpool, Liverpool, United Kingdom.

Deficiency of vitamin D is commonly associated with fracture. Consequently, 25 hydroxyvitamin D (25 OHD) is frequently measured to define vitamin D nutritional status. The clinical demand for precise and accurate assays to measure 25 OHD has led to the development of a number of immunoassay methods. Lack of standardisation of 25 OHD assay methods has questioned interassay agreement. Recent evidence has challenged the ability of current immunoassays to recognise both 25 OHD_2 and 25 OHD_3 metabolites. We studied 172 patient samples from consecutive hip fracture cases to determine assay agreement when comparing the DiaSorin (DS), IDS radioimmunoassays and the Nichols Advantage automated protein binding assay (CLPBA) to HPLC. 52 of these patients were studied before and 3 months after 1000 IU of daily ergocalciferol (D_2) therapy to assess ability of each immunoassay to recognise 25 OHD_2. Linear regression analysis in pretreatment samples demonstrated a positive Y intercept of between 6 and12 nmol/L for each assay compared to HPLC and a slope that varies from 0.64 (IDS) to 0.97 (DS, CLPBA). Bland Altman analysis demonstrates all 3 immunoassays have a persistent and proportional positive bias relative to HPLC at values from 20 to 50 nmol/L. Consequently, a 25 OHD results of 30 nmol/L by HPLC translates into 42 nmol/L by DS, 33 nmol/L by IDS and 35 nmol/L by CLPBA. A 25 OHD result of 80 nmol/L by HPLC translates into 90 nmo/L by DS, 65 nmol/L by IDS and 83 nmol/L by CLPBA. These results demonstrate, in the absence of standardisation efforts, the need for assay specific decision limits. Pretreatment linear regression analysis was used to apportion 25 OHD_3 value in the post treatment sample by each immunoassay and help approximate D_2 recognition by the different immunoassays. Less than 25 % of 25 OHD_2 measured by HPLC was detected by any immunoassay. It is questionable whether any of the immunoassays are capable of adequate recognition of 25 OHD_2. The above two issues are important when defining 25 OHD decision thresholds for treatment institution and to determine treatment response to D_2 respectively.

Disclosures: ***P. Glendenning****, None.*

SA482

See Friday Plenary number F482

SA483

Fully Automated Method for the Determination of Fat Soluble Vitamins by HPLC. J. M. Quesada Gómez*[1], J. M. Mata Granados*[2], M. D. Luque de Castro*[3]. [1]Mineral Metabolism Unit. University of Córdoba, Hospital Universitario Reina Sofía, Córdoba, Spain, [2]Sanyres, Prasa, Córdoba, Spain, [3]Department of Analytical Chemistry, University of Córdoba, Córdoba, Spain.

Background. There are few methods reported for the simultaneous determination of fat soluble vitamins (namely, 24,25 $(OH)_2$hidroxyvitamin D_3, 25 (OH)hidroxyvitamin D_3,1,25 $(OH)_2$hidroxyvitamin D_3 and vitamins A, E, K_1, K_3, D_3 and D_2).These methods are slow, time consuming and very difficult to automate. The necessity for routine analysis of these vitamins calls for the development of fully automated methods for their simultaneous determination.
Proposed method. A new rapid, automated and sensitive high-performance liquid chromatographic method using 1 mL of serum had been developed for the simultaneous determination of the target analytes, which involves the following steps:
Sample pretreatment by a robotic station: 1 mL of serum was mixed with 0.8 mL ethanol. After vortexing, the mixing was extracted with 4 mL 90:10 hexane-diclormethane mixture. The hexane-diclormethane layer was evaporated to dryness under an N_2 stream. The residue was dissolved in 200 μ L and located in the auto sampler of the chromatograph. Batches of 6 samples were treated with an operational time of 2 h/batch.
Chromatographic separation and detection: the initial mobile phase into which the sample was injected was a 90:10 methanol-water mixture at 1.0 mL min^{-1}, followed by a linear gradient in 4 min to obtain 90:10 methanol-isopropanol, maintained for 8 min. A second gradient was programmed at 1.2 mL min^{-1} to obtain an 1:1 methanol-isopropanol composition in 12 min, maintained until the end of the run. The equilibration time was 5 min. The eluate was monitored with a photodiode-array detector at the the maximum wavelength for each analyte.

Results. The linear range was 1-100 ng mL^{-1} for hydroxymetabolites of vitamin D_3, vitamin A and vitamin E, and was 5-100 ng mL^{-1} for vitamins K_3, K_1, D_3 and D_2. The coefficients of correlation are between 0.998 and 0.995. Limits of detection are between 1.0 and 4.0 ng mL^{-1}. Precision ranges between 5 and 9%.
Acknowledgment: Study supported by Sanyres Grupo PRASA. Córdoba. Spain

Disclosures: ***J.M. Quesada Gómez****, Sanyres (Grupo Prasa. Córdoba. Spain) 2.*

SU001

Relationship of Peak Lean Tissue Accrual to Peak Bone Mineral Accrual in Boys and Girls. R. A. Faulkner, A. D. G. Baxter-Jones*, R. L. Mirwald*, D. A. Bailey. Kinesiology, University of Saskatchewan, Saskatoon, SK, Canada.

It has been postulated that muscle and bone form an operational unit; that is, factors that affect muscle will also influence bone. It also is thought that muscle action is paramount in affecting bone adaptation; if so, then it would be expected that muscle development (or lean tissue as a surrogate of muscle) during the growing years should precede bone mineral accrual. We have shown previously that this is the case for total body mass; however, in theory this relationship should be even stronger at the extremities where muscle action is more isolated. The purpose of this study was to investigate the relationship of the timing of bone-free peak lean mass accrual (PLM) to peak bone mineral mass accrual (PBM) at the arms and legs. Subjects (70 boys and 67 girls) were measured annually (DXA: Hologic 2000 QDR in array mode). Whole year velocity values were calculated for bone-free lean tissue and bone mineral content. The mean age of peak tissue accrual was then calculated as the mean of the peak. Dependent t-tests were done to test for differences between the age of PLM and PBM ($p<.05$). As shown in the following table, PLM occurred prior to PBM at both sites in both boys and girls; however, this difference was not statistically significant at the legs in girls. These preliminary data support the hypotheses that PLM (a surrogate measure for muscle mass) precedes PBM and that muscle and bone development are closely related.

Comparison of age at peak velocity (Mean + S.D.)

	PLM	PBM
Girls (Arms)	12.2 (1.3)*	12.9 (1.3)
Girls (Legs)	12.1 (1.0) NS	12.4 (1.0)
Boys (Arms)	13.7 (1.0)*	14.4 (1.3)
Boys (Legs)	13.6 (1.0)*	14.1 (1.2)

Disclosures: ***R.A. Faulkner****, None.*

SU002

IGF-1 and IGFBP-3 Influence Bone Mass Acquisition in Children via Independent Mechanisms. J. H. Tobias[1], C. D. Steer*[2], P. M. Emmett*[2], A. R. Ness*[2], D. J. Gunnell*[3], J. M. P. Holly*[4]. [1]Rheumatology Unit, University of Bristol, Bristol, United Kingdom, [2]Alspac, University of Bristol, Bristol, United Kingdom, [3]Social Medicine, University of Bristol, Bristol, United Kingdom, [4]Surgery, University of Bristol, Bristol, United Kingdom.

Previous studies suggest that IGFBP-3 augments the stimulation of bone formation by IGF-1, which has been attributed to the ability of IGFBP-3 to prolong IGF-1 half-life. However, since IGFBP-3 is known to exert cellular actions independently of IGF-1, separate effects of IGFBP-3 on bone formation may also be involved. To explore the respective roles of IGF-1 and IGFBP-3 in regulating bone formation, we examined the relationship between serum levels of these proteins and bone mass acquisition in childhood. Serum concentrations of IGF-1 and IGFBP-3 were analysed in samples from 751 children randomly selected from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort at age 7.9, and whole body bone mineral content (BMC) subsequently measured in 652 of these children at age 9.9, using a Lunar Prodigy DXA scanner. Serum levels of IGF-1 and IGFBP-3 were found to be significantly associated with BMC (standardised regression coefficient = 0.299 and 0.297 for IGF-1 and IGFBP-3 respectively, $p < 0.001$). Subsequent analysis suggested that these associations reflected influences on bone growth, since equivalent relationships of IGF-1 and IGFBP-3 were observed to height, weight and DXA-derived skeletal area. Moreover, when BMC was adjusted for DXA-derived skeletal area, a significant association with serum IGF-1 and IGFBP-3 was no longer observed. Multi-variable regression analysis was then carried out to determine whether the relationships of serum IGF-1 and IGFBP-3 to bone growth which we found are inter-dependent. Highly significant associations with parameters related to skeletal growth were still observed after adjusting IGF-1 levels for IGFBP-3, and IGFBP-3 levels for IGF-1 (data shows standardised regression coefficients):-

Parameter	Versus IGF-1 (adjusted for IGFBP-3)	Versus IGFBP-3 (adjusted for IGF-1)
BMC	0.214, $p < 0.001$	0.213, $p < 0.001$
Area	0.229, $p < 0.001$	0.199, $p < 0.001$
Height	0.242, $p < 0.001$	0.201, $p < 0.001$
Weight	0.223, $p < 0.001$	0.180, $p < 0.001$

Moreover, no significant association was observed between these parameters and serum IGF-I/IGFBP-3 ratio. We conclude that serum levels of IGF-1 and IGFBP-3 are independent determinants of bone mass acquisition in childhood, suggesting that these two proteins regulate bone growth in children via distinct mechanisms.

Disclosures: ***J.H. Tobias****, None.*

SU003

Relationship Between Body Composition and Hip Bone Mass in Women of African Ancestry: Tobago Women's Health Study. D. D. Hill[1], J. A. Cauley[1], V. Wheeler*[2], J. M. Zmuda[1], A. Patrick*[2], P. Joseph*[2], C. Baker*[1], G. Beckles*[3], C. Bunker*[1]. [1]U of Pitt, Pittsburgh, PA, USA, [2]Tobago Regional Hospital, Scarborough, Trinidad and Tobago, [3]CDC, Atlanta, GA, USA.

Aging is often accompanied by loss of lean mass and bone mass and an increase in fat mass. Studies examining the interrelationships between body composition and bone mass have primarily on Caucasian women and limited to areal bone measures. To test the hypothesis that lean mass is related to total hip and femoral neck bone mineral density (BMD), independent of fat mass in women of African ancestry, we recruited 355 postmenopausal women, aged 50+ on the Caribbean Island of Tobago.
Body composition and BMD were measured by DXA (Hologic QDR 4500W). We estimated volumetric femoral neck BMD by calculating bone mineral apparent density (BMAD). The mean age, BMI, weight, and height of the women were 63.3 ± 8.2 years, 30.5 ± 5.7 kg/m^2, 81.1 ± 16.1 kg, and 163.1 ± 6.4 cm, respectively. In models considering soft tissue lean mass or fat mass separately, both were related to total hip and femoral neck BMD. We compared mean bone mass across tertiles of lean and fat mass, and tested for an interaction, adjusting for age and height using ANCOVA. Femoral neck BMD and BMAD increased across tertiles of lean mass, but not across fat mass. (Table 1.) We observed similar results for total hip BMD.
Our results suggest that fat mass is not significantly associated with bone mass in this population, after controlling for the effects of covariates. In conclusion, lean mass is independently related to bone mass in postmenopausal Tobagonian women of African ancestry. The stronger association between lean mass and BMD could reflect a multifactorial relationship encompassing bone strength, culture, lifestyle, genetics, hormonal, and growth factors.
Table 1. Age and Height Adjusted Mean Femoral Neck BMD (g/cm^2) and BMAD (g/cm^3) across Tertiles of Lean Mass and Fat Mass

Lean Mass	Fat Mass					
	1		2		3	
	BMD	BMAD	BMD	BMAD	BMD	BMAD
1	0.83	0.18	0.86	0.19	0.79	0.16
2	0.83	0.17	0.89	0.19	0.93	0.20
3	0.99	0.21	0.92	0.19	0.96	0.20
BMD			BMAD			
p (lean mass) < 0.001			p (lean mass) = 0.03			
p (fat mass) = 0.96			p (fat mass) = 0.93			
p (lean x fat mass) = 0.13			p (lean x fat mass) = 0.12			

Disclosures: ***D.D. Hill****, None.*

SU004

Growth "Tracking" of Femoral and Humeral Strength from Childhood to Late Adolescence. C. B. Ruff. Functional Anatomy and Evolution, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

The degree to which skeletal parameters "track", or remain at the same level relative to other individuals during growth and development, has important implications for both early detection and future prediction of increased fracture risk. Several recent studies have indicated an apparently high level of tracking of bone mineral content (BMC), width (BW), area and/or density in children measured over periods ranging from 2 to 6 years. In this study growth tracking of femoral and humeral diaphyseal strength was assessed in a sample of children measured radiographically from infancy to late adolescence. Radiographs were obtained from the archives of the Denver Child Research Study, a longitudinal study carried out in the 1940's-1960's. 20 individuals, 10 males and 10 females, with almost complete radiographic records (average, 34.5 time points at 6 month or yearly intervals) were selected and measured. Periosteal and cortical breadths at midshaft of the femur and 40% of bone length from the distal end of the humerus were measured with sharp-tipped digital calipers, and used to calculate bone section moduli, measures of bending and torsional strength. Comparisons are presented here for a 10 year age interval, from 7 years of age to 17 years of age, representing early school-age to late adolescence. Spearman rank-order correlations of bone strength, within sex, between these two age endpoints are moderate (r=.45-.79), except for the femur in males (r=-.10). Correlations are higher for the humerus (r =.66-.79, $p <.05$) than for the femur (r=-.10-.45, n.s.). In 30% of the comparisons (12/40), individuals cross between bottom and top halves of the strength distributions between 7 and 17 years. These results argue for caution in accepting a high level of growth tracking in mechanically relevant measures of bone strength over extended time periods during pre-adult development. Previous studies of growth tracking that have included different time intervals have found lower correlations for more extended periods and for periods that include early adolescence or childhood in addition to later adolescence. The results presented here are consistent with these findings, and suggest that extrapolations from more limited time intervals to longer periods may be unwarranted. Thus, prediction of bone strength in late adolescence or adulthood from measures taken in earlier in development may be more problematic than previously indicated.

Disclosures: ***C.B. Ruff****, None.*

SU005

Bone Mineral Accrual During Childhood and Adolescence: An Analysis of Size-Correction Techniques. K. S. Davison*[1], R. A. Faulkner[2], D. Drinkwater*[2], D. Bailey[2]. [1]Medicine, McMaster University & University of Laval, Hamilton/Quebec City, ON, Canada, [2]Kinesiology, University of Saskatchewan, Saskatoon, SK, Canada.

The true pattern of bone mineral density changes during childhood and adolescence is unclear owing to a lack of longitudinal investigations, a lack of adequate control for maturational differences, and size-mediated errors in the measurement of areal bone mineral density (aBMD). This investigation incorporated mixed-longitudinal (distance data) and longitudinal (velocity data) designs to describe pediatric changes in bone density at the total body (TB), femoral neck (FN) and lumbar spine (LS). Maturational differences were controlled by aligning participants on their age at peak height velocity (PHV). Changes in areal BMD (aBMD) were compared with densities obtained from two methods of size correction: bone mineral apparent density (BMAD) based on geometric assumptions, and statistically-corrected BMD (sBMD), which utilizes linear regression. Correlations between bone projected area (PA) and density were strongest with aBMD, intermediate with BMAD, and generally insignificant with sBMD, supporting sBMD's size independence. With the distance data aBMD increased over the entire growth period at all sites. Contrastingly, TB BMAD declined initially and then stabilized after PHV, and FN and LS BMAD were generally stable until PHV, increasing afterward. Similarly, TB and LS sBMD decreased until PHV, increasing afterward, and FN sBMD was stable until PHV, increasing thereafter. aBMD velocity was positive at all sites and all ages. In contrast, TB and FN BMAD had a negative velocity until after PHV, and LS BMAD velocity was generally stable until near PHV, with a positive velocity afterwards. sBMD velocity was negative at the TB and LS until PHV and there was a stabilization (males) or decrease in velocity (females) in FN sBMD until PHV. Velocity curves for PA and bone mineral content displayed a consistent dissociation at all sites with bones increasing in area first and later consolidating when rapid growth ceased or slowed. The point of minimal density suggested from the corrected data coincided with PHV and is supported by epidemiological data that reports the highest rates of fracture in adolescents during this time. These results highlight the size-dependence of aBMD and cautions against its use in the pediatric population. Physiologically, sBMD appeared the more appropriate size-correction technique.

Disclosures: ***K.S. Davison****, None.*

SU006

Vitamin D Status and Bone Accretion Profiles in Young Females from Childhood to Young Adulthood. N. E. Badenhop-Stevens*[1], J. D. Landoll*[1], E. Ha*[1], P. Goel*[2], B. Li*[2], B. Hollis*[3], L. Nagode*[4], A. Clairmont*[5], 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 Biosciences, OSU, Columbus, OH, USA, [5]OSU, Columbus, OH, USA.

Effect of vitamin D insufficiency on bone mass accumulation during growth is currently unknown. To evaluate this relationship calcidiol blood levels and bone mineral areal density (BMD) of the proximal forearm (33% site) were measured in a cohort of young females (N=236), participants of a 7-year long randomized study with calcium supplementation from childhood to young adulthood. Blood samples were collected annually while bone measurements were done every 6 months. Serum $25(OH)D_3$ was measured by a radioimmunoassay (RIA) (excluding baseline) with ^{125}I labeled tracer (Hollis et al. Clin Chem 39/3,529, 1993). BMD was measured by DXA, GE-Lunar DPX-L. Bone accretion profiles (linear mixed effect model-LME based on => 7 measuring points) versus years-since-menarche (YSM) were used to assess the effects of vitamin D status (below and above the vitamin D threshold of 31.5 ng/ml) in subjects with cumulative calcium intake above (1494±292) and below (748±161) the median (1006 mg/day). There was no difference in BMD measurements between subjects with cumulative average vitamin D levels below and above the threshold in the high calcium intake group (p=0.88, difference in BMD at YSM 4=-0.70%). However, in the low calcium intake group, the difference between BMD profiles of subjects with cumulative calcidiol levels below and above the threshold was of borderline statistical significance (p=0.08). Furthermore, the subjects above the threshold had much denser bones at YSM 4 (2.7%). Given that the sample size for this subgroups comparison is small, the results of this research indicate that vitamin D status may play an important role with regard to bone mass accumulation in young females accustomed to a relatively low dietary calcium intake.

Disclosures: ***N.E. Badenhop-Stevens****, None.*

SU007

Forearm pQCT Measurements in Young Adult Women Accustomed to Different Calcium Intakes during Adolescence. J. D. Landoll*[1], N. E. Badenhop-Stevens*[1], E. Ha*[1], S. L. Mobley*[1], A. Clairmont*[2], V. Matkovic[1]. [1]Bone and Mineral Metabolism Laboratory, OSU, Columbus, OH, USA, [2]OSU, Columbus, OH, USA.

We previously showed that calcium is an important determinant of bone mineral areal density during growth. In this study, we examined the influence of calcium on volumetric bone mineral density in the same cohort of young women (N=175; average age ~18 y) at the end of a 7-year intervention study with calcium supplementation. Volumetric bone mineral density measurements of the non-dominant radius at the distal (4%) and proximal sites (33%) were performed using a pQCT (Norland-Stratec XCT 2000) densitometer with contour, peel, and separation modes of 2,7,2 (distal site) and 2,2,2 (proximal site), respectively. Calcium-supplemented individuals had higher volumetric density at both the distal radius (394±6 vs 383±6 mg/cm^3), and proximal radius (1002±7 vs 986±6 mg/cm^3), and higher cortical/total area (CA/TA) ratio (0.789±0.005 vs 0.782±0.005) than placebo subjects, however, none of the differences were statistically significant. Dividing the subjects into subgroups according to cumulative calcium intake over time (upper and lower tercile) revealed a significant influence of calcium on volumetric bone mineral density at the distal (404±6 vs 378±8 mg/cm^3, p=0.013) and proximal (1009±7 vs 980 mg/cm^3, p=006) sites, and CA/TA (0.794±0.006 vs 0.774±0.005, p=0.018). There were minimal differences in total cross sectional areas at the distal site (251±4 vs 265±5 mm^2, p=0.03) between the upper and lower tercile subgroups, and no difference at the proximal site (92±1 vs 93±2 mm^2, p=0.5). The above analysis confirmed previous findings, suggesting that calcium exerts its action on bone accretion primarily by influencing volumetric bone mineral density.

Disclosures: ***J.D. Landoll****, None.*

SU008

Bone Mineral Density by Age, Gender and Pubertal Stages in Healthy Lebanese Children and Adolescents. A. Arabi[1], M. Choucair*[2], M. Nabulsi*[3], J. Maalouf*[1], R. Vieth[4], G. El-Hajj Fuleihan[1]. [1]Calcium Metabolism and Osteoporosis Program, American University of Beirut, Beirut, Lebanon, [2]Endocrinology, American University of Beirut, Beirut, Lebanon, [3]Pediatrics, American University of Beirut, Beirut, Lebanon, [4]Mt Sinai Hospital, Toronto University, Toronto, ON, Canada.

Gender, ethnicity and lifestyle factors affect bone mass acquisition during childhood, thus there is a need to have age and sex adjusted Z-scores using ethnic specific data for BMD measurement with DEXA. We have previously demonstrated that peak BMD is lower in healthy Lebanese compared to western standards. This cross-sectional study aims at obtaining normative data of BMD in healthy Lebanese children and adolescents.

363 children aged 10 to 17 years were enrolled in a randomized controlled trial evaluating the impact of vitamin D on musculoskeletal parameters. Data obtained at baseline is used in this study. Each child underwent physical examination including height, weight and Tanner staging of breast and genitalia. BMD and BMC were measured by DEXA using a Hologic 4500 A device. Apparent volumetric BMD (BMAD) of the lumbar spine and the femoral neck were calculated using the following formula: spine BMAD=BMC/A3/2 and femoral neck BMAD= BMC/A2 , where BMC is the bone mineral content and A is the projected area. Low density software was applied to all analyses and subtotal body measurements were used. Children were subdivided into 8 subgroups of one year interval, and mean±SD of BMD, BMC and BMAD were given for each age group and Tanner stage, for boys and girls separately.

The mean age was 13.13±2.04 years. There was a significant effect of age and puberty on all bone parameters by one way ANOVA, except at the femoral neck BMAD in boys. At cortical sites, all bone parameters were higher in boys than in girls, whereas at the lumbar spine girls had higher values, including BMAD. Children who completed their puberty (Tanner V) had mean BMD about 40% higher at the lumbar spine and 20% higher at cortical sites than those who started their pubertal development (Tanner II). At age 17 years, the mean BMD of the lumbar spine were 9.8% lower in boys and 9.5% lower in girls than the peak BMD of the lebanese population. Except at the trochanter, the mean BMD were low compared to western normative values. Z-score ranged between –0.25 and –1.1 (p<10-4) in both sexes.

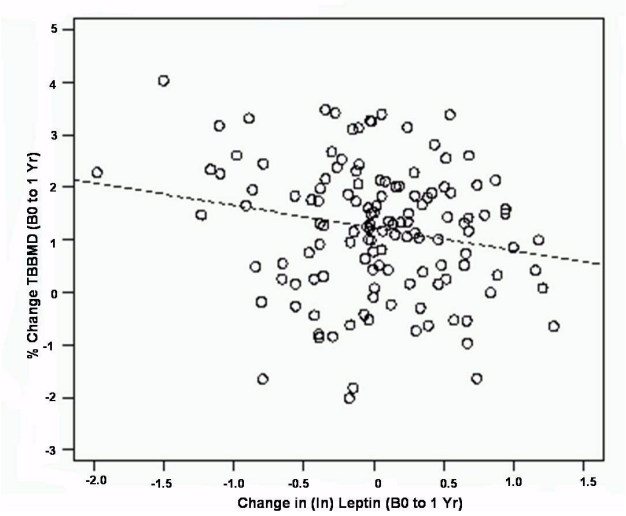
Data obtained from this study confirms previous reports on the powerful effect of puberty and gender on bone acquisition, and provide valuable reference enabling calculation of ethnic, gender, age, and Tanner adjusted Z- scores in our pediatric population

Disclosures: ***A. Arabi****, None.*

SU009

Leptin Negatively Predicts Total Body Bone Mineral Density in Young Healthy Women. C. W. Gunther*[1], R. M. Lyle*[2], G. P. McCabe*[3], D. Teegarden[1]. [1]Foods and Nutrition, Purdue University, West Lafayette, IN, USA, [2]Health and Kinesiology, Purdue University, West Lafayette, IN, USA, [3]Statistics, Purdue University, West Lafayette, IN, USA.

The role of leptin has been widely studied in relationship to body fat and obesity. However, more recently, leptin has been shown to have a significant impact on other biological endpoints, such as bone. For example, leptin has been implicated in the inhibition of bone mass accumulation. In this prospective analysis, the relationship of leptin with total body bone mineral density (TBBMD) was assessed in 132 healthy women, aged 18-30 years old, who were participating in a one year dairy calcium intervention trial. Fasting serum leptin levels (ELISA) and TBBMD (Lunar DXA) were measured at baseline and 12 months. Baseline (B0) means ± SD were: age=20.1±2.4 yrs, weight=62.4±10.3 kg, BMI=22.6±3.3 kg/m^2, fat mass=18.5±7.4 kg, lean mass=39.8±4.4 kg, TBBMD=1.2±0.1 g/cm^2, *ln* leptin=2.4±0.7 (ng/ml). Potential confounders (age, oral contraceptive usage, assignment to the dairy calcium intervention, and B0 and 1 year changes in weight, fat mass, physical activity, dietary calcium, and calories) were assessed. As expected, the 1 year change in fat mass was significantly correlated with the 1 year change in *ln* leptin (r=0.34, p<0.0001). The 1 year change in *ln* leptin negatively predicted the 1 year % change TBBMD in a regression model (R^2=0.04, p=0.03) and remained significant when the 1 year change in fat mass was included in the model. Thus, increased serum leptin levels may contribute to a decrease in the accumulation of bone mass.



Disclosures: ***D. Teegarden****, None.*

SU010

Cheese as a Source of Calcium and its Effects on Body Composition in Pubertal Finnish Girls. A. Lyytikäinen[1], M. Narva*[2], R. Korpela*[2], C. Lamberg-Allardt[3], M. Suuriniemi[4], Q. Wang*[1], S. Cheng[1]. [1]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [2]Valio LTD, Helsinki, Finland, [3]Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki, Finland, [4]Department of Cell Biology, University of Jyväskylä, Jyväskylä, Finland.

The purpose of this study was to compare cheese (16% fat, 100 g/day) and calcium-tablets (calciumcarbonate, 500 mg Ca x 2/d) as a source of supplementation for calcium (1000 mg/d), and their effects on body composition in pre- and peripubertal Finnish girls. Altogether 149 girls aged 10-12 years at Tanner stage I-II were randomized into the tablet (n=75), cheese (n=38), and placebo group (n=36). The total bone mass (TBM), lean tissue mass (LTM), and fat mass (FM) were measured by DXA (Prodigy, GE Lunar). The food consumption and the intake of nutrients were evaluated with a three-day food record. At baseline, the mean intake of energy (1480/1480/1420 kcal/day), calcium (663/694/653 mg/day), protein (14/15/15 E%), and other nutrients, as well as the food consumption were similar between the groups. Neither body composition nor maturation status differed between the groups. No significant differences were found in the compliance between the groups (70/63/72 %). After the two-year intervention, the total calcium intake was 1623 in the tablet, 1335 in the cheese, and 915 mg/day in placebo group. The energy intake increased by 210/260/250 kcal/day in the groups. The tablet group was found to consume more cereals than the cheese and placebo group (p< .05). The energy intake was higher from protein (15/17/15 E%) in cheese group compared to the tablet and placebo group (p< .01), whereas the intake of saturated fatty acids (14/16/15 E%) was higher and intake of carbohydrates (53/49/51 E%) was lower in the cheese group compared to the tablet group (p< .01). There were no significant differences in the percentage increase of the TBM, LTM, and FM between groups. However, when taking into account the speed of maturation (Tanner stage at 24 months) and the compliance, the TBM of the girls at Tanner stage I at the baseline increased significantly in the cheese group compared to the tablet and placebo group (40 vs 36/35 %, p= 0.008). No differences were found in TBM between the girls at Tanner stage II. Our results indicate that adequate calcium intake by high cheese consumption (> 60 g/day) is more beneficial for the total bone mass accrual than the calcium from tablets with similar amount of calcium. This might be due to the high protein content in cheese or increased absorption of calcium induced by caseinphosphopeptides. Our results suggest that components in cheese protein has a vital role in bone mass accrual in growing girls transiting from pre- to peripuberty.

Disclosures: ***A. Lyytikäinen****, None.*

SU011

Comparison of Areal and Volumetric Vertebral Bone Densities Measured by DXA and CT in Pediatric Patients. A. Kovanlikaya*[1], T. A. L. Wren[2], X. D. Liu*[1], P. D. Pitukcheewanont*[3], V. Gilsanz[1]. [1]Radiology, Childrens Hospital Los Angeles, Los Angeles, CA, USA, [2]Orthopaedic Surgery, Childrens Hospital Los Angeles, Los Angeles, CA, USA, [3]Endocrinology and Metabolism, Childrens Hospital Los Angeles, Los Angeles, CA, USA.

Dual-energy x-ray absorptiometry (DXA) values of areal bone mineral density (BMD) in children are influenced by bone size and soft tissue composition, while computed tomography (CT) measures of volumetric bone density (BD) are not. The purpose of this study was to determine the extent to which the assessment of osteoporosis differs when it is performed using DXA versus CT. DXA areal BMD and Z-score (Z_{DXA}) and CT volumetric BD and Z-score (Z_{CT}) were compared in 45 girls and 43 boys ages 3-20 yrs who were referred for assessment of bone density and osteoporosis. Z-score represents the number of standard deviations (SD) the BMD or BD is above or below the mean for sex- and age-matched controls, and patients were considered osteoporotic if they had a Z-score below –2.5 (2.5 SD below normal). Plain radiographs of the spine were blindly and independently reviewed by two experienced pediatric radiologists. Of the 5 patients whose radiographs depicted osteoporosis, 5 had Z_{CT} <=-2.5, and 4 had Z_{DXA} <=-2.5. There was only a moderate correlation between the DXA and CT densities (r=.459, p=.0013 for girls; r=.439, p=.0029 for boys; r=.448, p<.0001 for all subjects) and between the DXA and CT Z-scores (r=.490, p=.0005 for girls; r=.537, p=.0001 for boys; r=.513, p <.0001 for all subjects). Paired t-tests indicated that the DXA Z-scores were systematically lower than the CT Z-scores (p=.0003 for girls; p=.0001 for boys; p<.0001 for all subjects). Nine of the 16 children with Z_{DXA} <=-2.5 had Z_{CT} between +1.0 and –1.0. Consequently, a substantial number of children (6/45 girls and 9/43 boys) who would not be considered osteoporotic based on CT measurements were classified as osteoporotic based on DXA results (see Table). Caution should therefore be exercised in using DXA to assess osteoporosis. Treatment for osteoporosis may not be warranted for many children who have DXA BMD values more than 2.5 SD below normal.

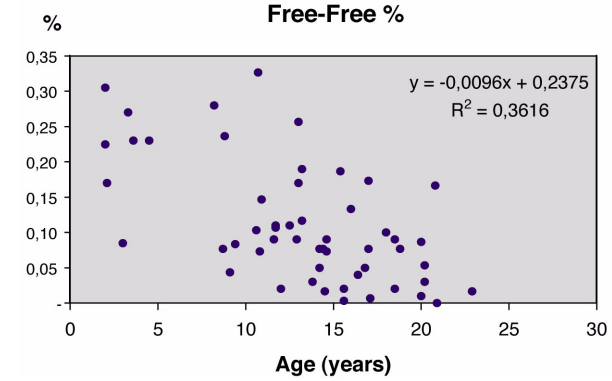
	Not osteoporotic based on DXA	Osteoporotic based on DXA
Not osteoporotic based on CT	37 girls (82%) 31 boys (72%)	6 girls (13%) 9 boys (21%)
Osteoporotic based on CT	1 girl (2%) 0 boys (0%)	1 girl (2%) 3 boys (7%)

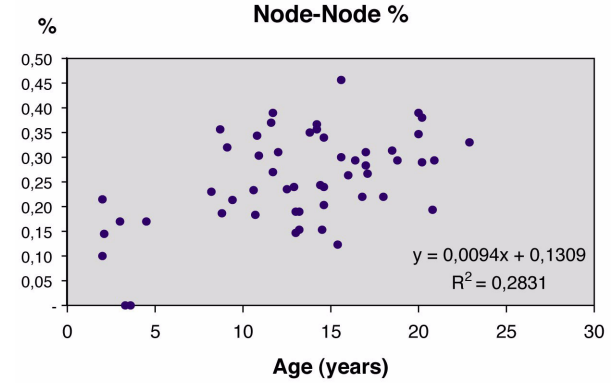
Disclosures: ***A. Kovanlikaya****, None.*

SU012

Micro-Architectural Strut Analysis Study on Paediatric Bone. S. Picard[1], D. Brown*[1], F. Rauch[2], R. Travers[2], F. H. Glorieux[2], J. P. Brown[1]. [1]Groupe de recherche en maladies osseuses, Centre de recherche du CHUQ - Pavillon CHUL, Ste-Foy, PQ, Canada, [2]Shriner's Hospital for Children - Genetics Unit, McGill University, Montreal, PQ, Canada.

Bone quality is defined by a sufficient mineralization of tissue and also by an adequate micro-architecture organization. Quantitative trabecular bone micro-architecture studies from aged skeleton have already been reported but none on pediatric bones. The aim of this study was to perform strut analysis histomorphometric measurements on iliac crest biopsies from children aged 1,5 to 22,9 years. Bone biopsies from 54 healthy Caucasian subjects were obtained during surgery for various orthopaedic conditions. All subjects were ambulatory and no evidence of any metabolic bone disease was found. At least two Masson Goldner's stained slides were selected from each biopsy and JPEG images of the whole section were captured for quantification and measurement. Strut analyses were performed with Nova Prime Bioquant's (R&M biometrics, Nashville) image-analysis software. Trabecular node, free-end and node-to-free ratios have been quantified and calculated for each biopsy. Strut length has also been measured and defined as node-to-node, node-to-free, free-to-free, cortical-to-free, cortical-to-node and node-to-loop struts. The progressive increase in node number per tissue area and node-to-node strut percentage (p = 0.0013) associated with a significant decrease in free-node and free-to-free strut percentages (p = < 0.0001) show an architectural organizational change during skeleton growth. The results may suggest an architecture quality improvement which could contribute in the development of bone quality in growing children.





Disclosures: ***S. Picard****, None.*

SU013

Fecal Calcium Density, Serum PTH, and 24-hr Urinary Calcium as Biological Indicators of Compliance with Calcium and Calcium Efficacy in a 7-year Clinical Trial. E. Ha*[1], J. D. Landoll*[1], L. Nagode*[2], P. Goel*[3], N. E. Badenhop-Stevens*[1], B. Li*[3], S. L. Mobley*[1], A. Clairmont*[4], V. Matkovic[1]. [1]Bone and Mineral Metabolism Laboratory, OSU, Columbus, OH, USA, [2]Veterinary Biosciences, OSU, Columbus, OH, USA, [3]Statistics, OSU, Columbus, OH, USA, [4]OSU, Columbus, OH, USA.

Effectiveness of biological indicators of calcium (Ca) nutritional status in predicting bone accretion at the proximal radius versus years-since-menarche (YSM; linear mixed effect models) was evaluated in a 7-year double blind placebo-controlled clinical trial with Ca supplementation (1000 mg/day) in addition to ~830 mg habitual dietary Ca intake. The data from 236 subjects were analyzed as they had =>7 out of 15 measuring points. Subjects were divided into subgroups based on each cumulative biological indicator above and below the median. The biological indicators of compliance with Ca were: fecal Ca density (FCD, Ca mg/g dry stool weight), serum PTH, and 24 hour urinary Ca excretion. Bone mass measurement by DXA (GE-Lunar DPX-L) were conducted semiannually while fecal (excluding baseline), blood (drawn between 8AM and 5PM), and urine samples were collected annually. FCD was measured according to the method previously described (Heaney RP, 1991). Ca was measured by AAS. Serum PTH was measured by Allegro immunoassay kits (Nichols Institute). The difference in bone accretion profiles between FCD subgroups was highly significant ($p<0.0001$, difference in BMD at YSM 4=2.8%). Average cumulative FCD for the subgroup above the median was 47.3 mg/g while below the median 26.1 mg/g. The difference in bone accretion profiles between PTH subgroups was significant ($p=0.035$, difference in BMD at YSM 4=-1.3%). Average cumulative PTH concentration for the subgroup above the median was 31 pg/ml while below the median 19 pg/ml. Urinary Ca excretion was a poor predictor of Ca efficacy ($p=0.564$; difference in BMD at YSM 4=0.9%). Average urinary Ca excretion in the high urinary calcium subgroup was 147 mg/day and for the low urinary calcium subgroup 65 mg/day. The above research indicate that FCD is a superior marker of Ca nutritional status and compliance with Ca intervention in a clinical trial and serum PTH is a moderately good predictor.

Disclosures: ***E. Ha****, None.*

SU014

Compromised Skeletal Growth and Mineralization following a Period of Dietary Calcium Deficiency Are not Recovered in Prepuberal Pigs. D. K. Schneider*, C. E. Pardo*, K. L. Saddoris*, T. D. Crenshaw. Animal Sciences, University of Wisconsin, Madison, WI, USA.

Dietary Ca deficiency inhibits skeletal mineralization. The potential for recovery of skeletal growth and mineralization following a period of deficiency is not established. Failure to regain skeletal growth and mineralization during early growth stages may compromise peak bone mass. Twenty-four young pigs (6 kg, 3 wk old) were fed diets that differed in Ca (100 vs 70% of Ca requirements) for 4 wk. Both groups were then fed the same diet (108% of Ca requirements) for an additional 5 wk. Pigs were scanned using a Prodigy (GE Lunar) bone densitometer at 0, 2, 4, 6, and 9 wk of the trial. Skeletal mineralization was assessed by bone mineral density (BMD, g/cm^2) and skeletal growth was assessed by bone mineral content (BMC, g). At 4 wk, mineralization (BMD) was reduced ($P<0.001$) by 12% (0.625 vs 0.550 g/cm^2) and skeletal growth (BMC) was reduced ($P<0.001$) by >35% (256 vs 163 g). During recovery no differences were detected ($P>0.50$) in the rate of skeletal growth (BMC, g/d) during wk 4 to 6 (16.9 vs 16.6 g/d) or wk 6 to 9 (21.4 vs 21.2 g/d). The approximately 100 g differences in skeletal growth induced by dietary Ca during the first 4 wk was maintained until wk 9 (BMC, 942 vs 840 g). Likewise, no evidence was detected for recovery of skeletal mineralization. BMD at wk 9 (0.889 vs 0.838 g/cm^2) was lower ($P<0.02$) for pigs previously fed Ca deficient diets. In conclusion, a 35% reduction in BMC of rapidly growing pigs was not compensated during a recovery period that allowed a four- to five-fold increase in total skeletal growth. These results do not support an improvement in efficiency of Ca use following a period of Ca depletion.

Disclosures: ***T.D. Crenshaw****, None.*

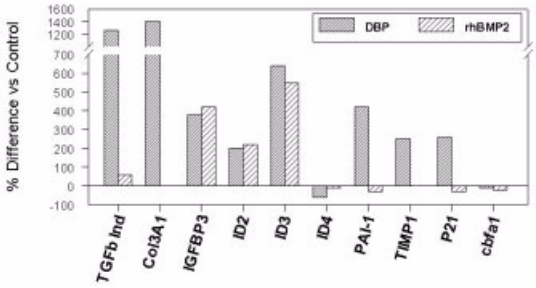
SU015

Comparison of TGF-beta/BMP Pathways Signaled by BMP-2 and Demineralized Bone Powder in Human Dermal Fibroblasts. J. Glowacki, S. Zhou, K. E. Yates. Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA.

Both BMP-2 and Demineralized Bone Powder (DBP) stimulate endochondral osteogenesis in vivo. When human dermal fibroblasts (hDFs) are cultured with DBP in a porous collagen sponge for 7 days, cartilage-specific genes, aggrecan and collagen II, are upregulated and cartilage matrix accumulates around the cells in proximity to the DBP. We reported that this chondroinduction is preceded by shifts in some signaling genes. This study tests the hypothesis that DBP and BMP-2 affect similar signal pathways.

One million hDFs were cultured for 3 days in each porous 3D collagen sponge containing 0 or 3 mg DBP, or 6 μg/4 μL rhBMP-2 or PBS/BSA solution (manufacturer's recommended conc.) The rhBMP was delivered in a 4x4x2 mm coupon of the absorbable collagen and inserted into the porous collagen sponges. The absorbable collagen and rhBMP-2 were generously provided by Wyeth, Cambridge, MA. After 3 days, specimens were prepared for histology, for cDNA macroarrays for BMP/TGFb Signaling Pathways (SuperArray HS-023), RT-PCR, and Northern hybridization.

Histology showed homogeneous cellularity through each of the sponges at day 3. Similarities and differences in gene expression were observed (Figure), confirmed by RT-PCR. First, there were very similar responses of some genes, such as IGFBP3, ID2, and ID3, to DBP and rhBMP-2. This finding suggested that the dosing and timepoint were apt. In contrast, some of the genes that were most dramatically increased by DBP, such as TGFβ-Induced protein (1260%) and Collagen 3A1 (1770%), were barely affected by rhBMP-2. Although there was concordance with ID2 and ID3, ID4 was decreased by DBP by 60% and by rhBMP-2 by only 10%. Both PAI-1 and TIMP1 were greatly increased by DBP, but PAI-1 was decreased by rhBMP-2 (30%) and TIMP was not at all changed. DBP increased cyclin-dependent kinase inhibitor P21/Waf1/Cip1 by 260%, whereas it was decreased (30%) by rhBMP-2. It is possible that DBP's inhibition of proliferation may contribute to its effects to promote differentiation. Cbfa1 was highly expressed in target hDFs but was moderately decreased by both DBP (10%) and rhBMP-2 (20%). Smad 6 and 7 were increased by only rhBMP-2, but smad 2-5 and 9 were low in hDFs and were not modulated by either BMP-2 or DBP. In sum, although BMP was originally isolated as the active factor in DBP, rhBMP-2 alone and DBP do not affect all the same genes.

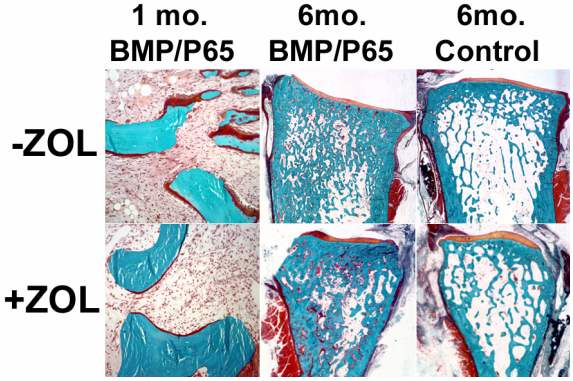


Disclosures: ***J. Glowacki****, None.*

SU016

Intraosseous Delivery of rhBMP-2 in HYAFF-11/P65 Paste Rapidly Increases Trabecular Bone Mass in Nonhuman Primates. E. A. Smith-Adaline, H. J. Seeherman, X. J. Li, H. Kim*, R. Li*, M. L. Bouxsein, J. M. Wozney. Women's Health and Bone Division, Wyeth Research, Cambridge, MA, USA.

Recombinant BMP-2 (rhBMP-2) is a potent osteoinductive factor with the potential to lower the risk of hip and wrist fractures in osteopenic patients by inducing dramatic anabolic bone formation following local injection. However, previous research has shown that local administration of rhBMP-2 can result in transient bone resorption that precedes bone formation (Seeherman HJ, *et al*., ASBMR 2001). This study evaluated the efficacy of intraosseous (IO) delivery of rhBMP-2 to increase bone mass locally when co-administered with bisphosphonates (BP) to block the initial resorption phase. One distal radius of ten adult male cynomolgus monkeys received a single 0.25ml IO injection of 2mg/ml rhBMP-2 delivered in a hyaluronan paste (HYAFF-11/p65, Fidia Advanced Biopolymers) (BMP/p65). The contralateral radius was not treated. Animals were divided into groups receiving 1) no BP (n=4), 2) oral alendronate (2.7mg daily for 4 weeks, n=3), or 3) IV zoledronate (0.067mg/kg weekly for 4 weeks, n=3). Four weeks after BMP/p65 injection, trabecular BMD decreased 28.3±13.2% in animals not receiving BP. While oral alendronate was not effective (ΔBMD = -22.9±8.3%), IV zoledronate eliminated bone resorption at 4 weeks (ΔBMD = +1.6±7.5%) (Fig. 1). Trabecular BMD (pQCT) increased 42.3±15.1% and 45.8±17.8% by 4-6 months respectively in all groups after BMP/p65 treatment compared to baseline values (Fig. 1). In contrast, BP had no effect on BMD of the contralateral untreated radius (6 mo ΔBMD = -0.5±6.4%). Histological evaluation demonstrated both *de novo* and appositional bone formation in response to BMP/p65. Abundant osteoid present on bone surfaces 6 months after treatment indicated an ongoing anabolic effect of BMP at this time. Additional studies in OVX female cynomolgus monkeys demonstrated that a single injection of zoledronate (0.067mg/kg, IV) administered 3 days after IO injection also minimized BMP-induced bone resorption. In summary, local administration of BMP/p65 resulted in a rapid 46% increase in trabecular BMD, and co-administration with zoledronate eliminated the transient resorption phase. This combination treatment represents a promising anabolic therapy for prevention of osteoporosis-related hip, wrist and spine fractures.



Disclosures: ***E.A. Smith-Adaline****, Wyeth 1, 3.*

SU017

Osteoinduction by Genetically Modified Ligamentum Flavum With Human BMP-2 Gene. K. Yang*, S. Moon*, H. Kim*, K. Kim*, U. Kwon*, H. Kim*, J. Jahng, H. Lee*. Department of Orthopaedic Surgery, Yonsei University College of Medicine, Seoul, Republic of Korea.

Ossification of spinal ligament can be found in certain pathologic conditions. Recently spontaneous expression of osteogenic phenotype from degenerated ligamentum flavum (LF) was demonstrated in vitro study. Furthermore in vivo application of recombinant human bone morphogenetic protein-2 (BMP-2) to spinal ligament resulted in ossification of ligament. Biologically modified LF with human BMP-2 gene can be an agent for osteoinduction. Therefore, we implanted genetically modified LF to nude mice to test feasibility of LF as bone graft substitution and a carrier for ex vivo gene therapy.

LF tissue was harvested and cultured. Type 5 adenovirus constructs with lacZ and BMP-2 cDNA (Ad/lacZ, Ad/BMP-2) were prepared. LF cell cultures were exposed to Ad/lacZ and stained with X-gal. Also cultures were exposed to Ad/BMP-2 and stained with alkaline phosphatase, Alizarin Red-S, Von Kossa method. Transgene expression and expression osteocalcin were assessed by immunocytochemistry and Western blot with BMP-2 and osteocalcin antibody. RT-PCRs for mRNA expression of collagen type I, II and osteocalcin were also performed. For in vivo assessment of osteoinductivity by Ad/BMP-2, control group was injected saline and osteoinductive group was injected 1x1011particle to human donor LF tissue, which were implanted to immune-deficient mice. At 1 and 2 month postimplantation, all animals were sacrificed for histological evaluation. Implant tissue were isolated and immunohistochemical staining with osteogenic marker antibody.

Ad/lacZ efficiently transduced LF cells. Culture with Ad/BMP-2 demonstrated transgene expression (BMP-2), expression of osteocalcin in immunocytochemisty and Western blot. Culture with Ad/BMP-2 showed strong reactivity in alkaline phosphatase, Alizarin Red-S, Von Kossa stain. In RT-PCR, cultures with Ad/BMP-2 showed upregulation of mRNA expression for collagen type I and osteocalcin. Newly bone formation was observed in the section from the Ad/BMP-2/hLF tissue implanted side while no bone formation was detected on the control side.

This study demonstrated feasibility of gene transfer to LF and also osteoinductive gene transfer to LF clearly upregulated osteogenic phenotype and induced osteogenesis. In the surgery of spinal stenosis, large amount of LF is removed and destabilized spinal segment is often restabilized with instrumented fusion with autogenous bone graft. The results of this study imply that removed LF can be a carrier for ex-vivo gene therapy and an osteoinductive agent to augment autogenous bone graft through genetic modification.

Disclosures: ***S. Moon****, None.*

SU018

Prostaglandin E EP4 Receptor Agonist Enhances Ectopic Bone Formation Induced by rhBMP-2. H. Terai, R. Sasaoka*, H. Toyoda*, Y. Imai*, R. Sugama*, K. Takaoka. Orthopedic Surgery, Osaka City University Graduate School of Medicine, Osaka, Japan.

For clinical use, it is important to establish methods to maximize the effects of recombinat human bone morphogenetic protein- 2 (rhBMP- 2). In the present study, we investigated the synergic effects of Prostaglandin E EP4 receptor agonist (ONO-4819) in ectopic bone formation induced by rhBMP-2 in mice.

Forty lyophilized disks (6mm diameter) of bovine type I tendon- derived collagen containing 5 μg of rhBMP-2 were implanted respectively on the paravertebral muscle of 4 weeks old ICR mice. These mice were divided into four groups (n=10 per each group) according to the single administrated concentration of Prostaglandin E EP4 receptor agonist; (1) 0 μg/kg, as controls (2) 10 μg/kg (3) 30 μg/kg (4) 100 μg/kg . Prostaglandin E EP4 receptor agonist was injected subcutaneously every 8 hours after implantation until sacrifice. After 3 weeks of implantation, all mice were sacrificed and disks and left tibias were harvested. Soft X- ray photographs were taken for all specimens. All harvested disks and tibias were received histological analysis after measurements of bone mineral density (BMD) and bone mineral content (BMC) by dual energy X-ray absorption (DEXA). In another experimental protocol, serum osteocalcin, phosphate, calcium and ALP were measured under the administration of Prostaglandin E EP4 receptor agonist without rhBMP- 2. Forty- five mice were divided into 3 groups (n=15 per each) according to the administrated concentration and the interval; (I) 0 μg/kg, every 8 hours, as controls (II) 30 μg/kg, every 8 hours (III) 90 μg/kg, every 24 hours. Five mice were sacrificed every week in each group to collect blood samples.

BMD values (mg/cm2) of group 4 (15.7±4.1) and group 5 (16.8±4.5) were significantly higher than in the group 1 (8.1±2.2) ($p< 0.01$). BMC value (mg) of group 4 (7.9±2.8) was significantly higher than in the group 1 (4.4±3.6) and group 2 (3.9±2.1) ($p< 0.01$). No difference was observed in BMD or BMC values of tibiae among any groups. Serum osteocalcin and ALP were significantly higher in group II at 2 weeks ($p< 0.05$).

We concluded that Prostaglandin E EP4 receptor agonist enhanced the ectopic bone formation induced by rhBMP- 2 in mice. The optimal administrative dose was 30 μg/kg when injected subcutaneously every 8 hours, in which dose there was no effect to BMD or BMC of tibiae.

BMC and BMD of implanted collagen disks

Plostaglandin E EP4 agonist	0 μg/kg	10 μg/kg	30 μg/kg	100 μg/kg
BMC (mg)	4.4±3.6	3.9±2.1	7.9±2.8*	7.3±5.0
BMD (mg/cm2)	8.1±2.2	10±2.5	15.7±4.1*	16.8±4.5*

(* : $p< 0.01$)

Disclosures: ***H. Terai****, None.*

SU019

Regulation of BMP Signaling by Smurfs. T. Imamura*, S. Maeda*. Biochemistry, The JFCR Cancer Institute, Tokyo, Japan.

Smurf1, a HECT type E3 ubiquitin ligase, has been reported to interact with receptor-regulated Smads (R-Smads) for bone morphogenetic proteins (BMPs), Smads 1 and 5, and promotes their degradation. Here we show that, in addition to direct binding to BMP-R-Smads, Smurf1 also interacts with inhibitory Smads (I-Smads), Smads 6 and 7, to form E3 ubiquitin ligase complexes to target BMP type I receptors (BMPR-Is) and activated BMP-R-Smads for degradation. Smurf1 interacts with nuclear I-Smads and induces their translocation to the cytoplasm in a nuclear export receptor (CRM1)-dependent fashion. Smurf1 then associates with BMPR-Is, and enhances turnover of the receptors. Furthermore, Smad6-Smurf1 complex binds to activated BMP-R-Smads, and induces their ubiquitination and degradation. Consistence with these data, Smurf1 synergies with Smad6 to inhibit BMP signaling in Xenopus. These results elucidate novel mechanisms whereby I-Smads-Smurf1 complexes inhibit BMP signaling both in vitro and in vivo, by mediating downregulation of activated BMPR-Is and activated BR-Smads.

Disclosures: ***T. Imamura****, None.*

SU020

Use of LMP-1 Fusion Protein to Induce Bone Formation without Risks of Gene Therapy. S. Sangadala*, L. Titus, M. Viggeswarapu, Y. Liu, G. A. Hair*, E. Gibson*, C. Oliver*, S. D. Boden. Orthopaedic Surgery, Emory University, Decatur, GA, USA.

LMP-1, an intracellular osteoinductive protein, can induce multiple BMPs and facilitate spine fusion in vivo. Induction of bone formation by LMP-1 has required gene therapy techniques to deliver its cDNA. The goal of this study was to determine the feasibility of delivering a recombinant LMP-1 fusion protein directly into cells. The fusion protein includes an 11 amino acid cationic transduction domain from the HIV TAT protein that facilitates entry into cells without the need for chemicals or electrical stimulus. Phase I: Athymic rats (N=58) received 3-4 subcutaneous (SQ) implants consisting of a collagen disc loaded with 1-1.5 million buffy coat cells from rabbit, human or non-human primate peripheral blood. The cells were treated with TAT-LMP-1 fusion protein (0.08 to 200 nM) for 30 min before implantation. Phase 2: 46 adult New Zealand white rabbits underwent a posterolateral lumbar spine fusion with a collagen sponge loaded with 6 million autologous buffy coat cells per side after 30 min treatment with TAT-LMP-1 protein (0.25 to 60 nM). Spines and SQ implants were harvested after 4 weeks and assessed by radiographs for bone formation. In rats bone nodules were formed in 2 experiments with the non-lyophilized TAT-LMP-1 protein at doses of 0.6 nM (4/4 implants), 1.2 nM (4/4), or 1.6 nM (2/3). The lyophilized protein appeared more stable and made bone in 3 experiments at 2.5 nM (11/11) when rabbit buffy coat cells were used. In addition human and non-human primate cells transduced with TAT-LMP-1 induced bone formation at 20 nM (6/6, human; 4/6, non-human primate). The challenging rabbit posterolateral spine fusion model also demonstrated the successful use of recombinant TAT-LMP-1 protein. Palpable fusion masses were formed in 2 experiments with the non-lyophylized TAT-LMP-1 protein at 5.0 nM (3/3) or 5.5 nM (2/2). The lyophilized TAT-LMP-1 protein was successful in 3 experiments at 5.0 nM (7/8) and in 2 experiments at 5.5 nM (5/5). In all of the above experiments higher and lower doses of TAT-LMP-1 had a lower rate of success or failed to induce bone formation. This study shows that a short peptide sequence (TAT) added to recombinant LMP-1 protein will facilitate the entry of the protein itself directly into buffy coat cells and initiate ectopic bone formation in rats and spine fusions in adult rabbits. Induction of bone formation by rabbit, human, or non-human primate buffy coat cells transduced with TAT-LMP-1 allows the ectopic rat model to be used to optimize delivery of TAT-LMP-1 prior to spine fusion studies in these species. This approach can avoid the challenges of gene therapy for LMP-1 delivery to induce bone formation.

Disclosures: ***L. Titus****, Medtronic Sofamor Danek 2, 5.*

SU021

Bone Formation Induced by Bone Morphogenetic Protein Is Stimulated by Prostaglandin E EP4 Receptor Agonist in its Early Phase. T. Hiromitsu*, H. Terai, R. Sasaoka*, Y. Imai*, R. Sugama*, K. Takaoka. Orthopedic Surgery, Osaka City Univerity Graduate School of Medicine, Osaka, Japan.

Administration of Prostaglandin E EP4 agonist (EP4, ONO-4819) enhances ectopic bone formation induced by recombinant human bone morphogenetic protein (rhBMP-2) in mice. It is important to investigate the mechanism by which Prostagrandin E EP4 receptor agonist regulates bone formation induced by rhBMP-2. In this study, we examined the effects of Prostaglandin E EP4 agonist *in vivo* and *in vitro* to determine the phase of its action in BMP inducing bone formation.

Mouse MC3T3-E1 cell line and ST-2 cell line were grown in 24- well plates with alpha MEM containing 10% fetal bovine serum. Cells were exposed to various concentration of Prostaglandin E EP4 agonist with or without BMP when they were reached to 60% of confluence. After 3 days of incubation, Alkaline phosphatase (ALP) activity was measured in each well. ALP activity values were analyzed statistically. In another protocol, 15 lyophilized disks (6 mm diameter) of bovine type I tendon- derived collagen containing 5 μg of rhBMP- 2 were implanted respectively on paravertebral muscle of 4 weeks old ICR mice. All mice were received 30 μg/kg of EP4 subcutaneously every 8 hours until sacrifice (3 weeks after implantation). These mice were divided into 3 groups (n=5 in each group) by administrated period of EP4; Group (I) for 3 weeks after implantation, Group (II) for 2 weeks (from 1 week after implantation), Group (III) for 1 week just prior to sacrifice (2weeks after implantation). After 3 weeks of implantation, all disks were harvested and examined bone mineral content (BMC) and bone mineral density (BMD) by dual energy X- ray absorption (DEXA).

Administration of EP4 to ST2 cell lines increased ALP activity levels in dose- dependent manner in the presence and absence of BMP. However, ALP activity levels in 3T3E1 cell lines were not increased in dose- dependent manner in the absence of BMP. In vivo study, the values of BMC and BMD of specimens in Group (I) were significantly higher than in other groups (See table). We concluded that Prostaglandin E EP4 receptor agonist enhanced ectopic bone formation induced by BMP by stimulating osteogenic cells in the early stage of bone formation.

BMC and BMD of implanted collagen disks

Group	(I)	(II)	(III)
BMC (mg)	7.6±2.8*	3.2±1.8	3.1±1.8
BMD (mg/cm2)	15.6±4.2*	8.1±1.6	8.3±2.3

(* : p< 0.01)

Disclosures: ***T. Hiromitsu****, None.*

SU022

Durable Adeno-Associated Virus Delivery of a Systemic Noggin Mutein Prevents BMP Induced Heterotopic Ossification. D. L. Glaser*[1], A. N. Economides*[2], L. Wang*[3], K. V. Sachs*[1], N. Stahl*[2], J. M. Wilson*[3], F. S. Kaplan[1], E. M. Shore[1]. [1]Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, USA, [2]Regeneron Pharmaceuticals, Tarrytown, NY, USA, [3]Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Heterotopic ossification (HO) occurs in a wide variety of diseases, and prevention regimens have had varying success. In patients with fibrodysplasia ossificans progressiva (FOP), a catastrophic genetic disorder of HO with no available treatment, evidence supports an imbalance in the BMP4 pathway with inductive signals overwhelming antagonist signals. We previously demonstrated that a circulating mutein of Noggin (hNOGΔB2), a BMP antagonist, delivered by an adenovirus gene therapy approach was effective in preventing BMP4 induced HO, although transient viral expression with immunogenicity was observed. To develop an effective gene therapy for HO, sustained and controllable expression is required. This study investigated AAV-mediated gene transfer of systemic Noggin mutein for durable expression that is sufficient to prevent induced HO.

A mouse model of BMP-induced HO was used. C57/Bl6 mice (5 groups of 10) were treated with control virus, or one of two serotypes of AAV (2/5 or 2/8TBGhNOGΔB2) at either $1x10^{11}$ or $1x10^{10}$ GC/mouse. Serum Noggins level were monitored by ELISA at 1, 7, 14, 21 and 28 days. On day 28, the abdominal musculature was injected on one side with carrier alone, and on the other with carrier + BMP4. Serum Noggin levels and implants were studied at 7 and 14 days after implant. Histologic stages of bone formation were evaluated by standard methods.

Implants containing BMP4 induced an aggressive, fibroproliferative lesion with cartilage formation at 7d and HO at 14d. In animals treated with AAV 2/8TBGhNOGΔB2 at a viral titer of $1x10^{11}$, implants with BMP4 demonstrated a minimal, mixed inflammatory cell infiltrate at 7d and a thin pseudocapsule several cell layers thick surrounding the unresorbed plug at 14d, indistinguishable from carrier implants with no BMP. HO was evident in low titer AAV 2/8, high and low titer AAV 2/5, and control groups. Serum Noggin levels were detected as early as 7 days after viral injection. By 24d, Noggin levels of <1ug/ml were detectable in the 3 AAV groups that formed HO, while, in the therapeutic group, Noggin levels ranged from 9 ug/ml to 21 ug/ml.

This study demonstrates that delivery of a systemic Noggin mutein through AAV gene therapy provides durable gene delivery at therapeutic levels, suggesting that gene transfer of a circulating BMP4 antagonist may offer a solution to catastrophic disorders of progressive HO when all other modalities have failed.

Disclosures: ***D.L. Glaser****, None.*

SU023

Deficiency of Caf1, a Novel Type Inhibitor of BMP, Promotes Nodule Formation in vitro, Enhances Cancellous Bone Mass and Promotes OrthotopicBbone Formation in Response to BMP Injection in vivo. K. Oikawa[1], T. Nakamura*[2], M. Usui[1], K. Tsuji[1], I. Ishikawa*[3], A. Nifuji[1], T. Noda*[4], T. Yamamoto*[2]. [1]Dept of Molecular Pharmacology, Tokyo Medical and Dental University, Tokyo, Japan, [2]University of Tokyo, Tokyo, Japan, [3]Periodontology, Tokyo Medical and Dental University, Tokyo, Japan, [4]Dept of Cell Biology, Cancer Institute, Tokyo, Japan.

Caf1 (CCR4-associated factor 1), which is a component of CCR4-NOT complex, is involved in gene transcription and mRNA modulation in yeast. In mammal, Caf1 associates with Tob, which belongs to Tob/BTG antiproliferative protein family. Since Tob gene product could modulate BMP signaling, Caf1 may also be involved in the regulation of BMP. This paper examined whether Caf1 deficiency would modulate BMP signaling in vitro and bone mass levels in vivo. Skeletal patterns in Caf1-deficient (-/-) mice and heterozygous littermates (Caf1+/-) were similar to those in wild type mice. Bone mineral density (BMD) of the whole femur and tibia was similar among Caf1-/-, Caf1+/- and wild type mice. However, microCT analysis of the cancellous bone volume revealed enhancement of bone volume per tissue volume (BV/TV) in Caf1-deficient mice compared to heterozygous mice. The bone volume levels in heterozygous mice were similar to those in wild type mice and therefore heterozygous littermate mice were used as control for further analyses. In order to examine the osteoblastic properties of the cells, bone marrow cells were cultured in the presence or absence of beta-glycerophospate and ascorbate. Caf1 deficiency enhancement of the alizarin red positive nodule formation compared to wild type. In contrast, TRAP-positive osteoclast like cell development in the bone marrow cells was similar regardless of the genotypes suggesting that Caf1 deficiency mainly affects the cells in osteoblastic lineage. Further examination indicated that Caf1 deficiency enhanced BMP-stimulated alkaline phosphatase activities in the calvaria-derived osteoblast enriched cells compared to wild type. Caf1 deficiency did not alter the actions of TGF-beta. To test whether BMP signaling could be enhanced by Caf1 deficiency in vivo, BMP was injected onto the calvaria of the mice. BMP injection induced new bone formation on the calvaria in wild type. Caf1 deficiency increased the amount of BMP-induced new bone formation compared to wild type. These observations indicated that Caf1 is a novel type of inhibitor of BMP actions in osteoblasts and suppresses the levels of cancellous bone volume in vivo.

Disclosures: ***K. Oikawa****, None.*

SU024

Necessity of Immune Recognition and Control of a Human Adult Stem Cell, Circulating in Blood as a Monocyte, in order to Avoid some Proliferating Diseases. M. Labat. Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France.

Damage to stem cells has been reported to contribute to neoplasia. Organ stem cells present in blood might represent one single population of pluripotent stem cells in homeostatic equilibrium with the 'reserve' stem cells present in the organs. These circulating stem cells are normally almost quiescent. Under precise circumstances such as wound healing they may proliferate and migrate in order to participate in the regeneration of the damaged tissue. Indeed, such a process has to be tightly controlled. Time-lapse videomicroscopy shows how a subpopulation of CD4+ T lymphocytes, called phagic T lymphocytes, destroy the stem cells when they differentiate in vitro. These stem cells that express constitutively HLA-DR molecules are both the activators and the targets of phagic T lymphocytes that penetrate and circulate inside them until the stem cells 'explode'. It is a beneficial exception to self-tolerance, restricted to normal stem cells, in order to avoid their accumulation out of a repair purpose. In disorders such as fibrosis and chondrosarcoma, these circulating stem cells proliferate, escape destruction by phagic T lymphocytes and accumulate, giving rise in vitro to a 'tissue' evoking the lesion the patient. This process observed in vitro may mimic what happens in vivo at the blood-tissue interface. Hence, failure in the regulation of circulating organ stem cells might be involved in the common early steps leading to fibrosis and some malignancies. On the opposite, excessive activation of phagic T lymphocytes might be involved in autoimmune diseases.

The spontaneous expression by normal circulating stem cells of neural markers, including nestin, and of a specific neural crest marker, suggest a neural crest origin.

Disclosures: ***M. Labat****, None.*

SU025

Role of Twisted Gastrulation (Tsg) in Osteoblast Differentiation and Mineralization. R. Gopalakrishnan[1], N. P. Lowe*[1], A. Petryk*[2]. [1]Oral Sciences, University of Minnesota, Minneapolis, MN, USA, [2]Pediatrics, University of Minnesota, Minneapolis, MN, USA.

Bone morphogenetic proteins (BMPs) play crucial roles in osteoblast differentiation and function. The activity of BMPs in the extracellular matrix of osteoblasts has previously been shown to be regulated by their antagonist, Noggin. Twisted Gastrulation (TSG) is a recently identified protein that also interacts with BMPs, although the nature of the interaction is not completely understood. TSG initially forms a complex with BMP and full-length Chordin to antagonize BMP signaling. However, following cleavage of full-length Chordin by Xolloid, TSG acts as a BMP agonist by dislodging BMP from Chordin and destabilizing the Chordin fragments, thereby facilitating BMP binding to its receptor. The specific role of TSG in osteoblast differentiation and function is not known. In this study, we investigate the role of TSG during osteoblast differentiation and mineralization *in vitro*. Using a strongly mineralizing subclone of MC3T3-E1 cells, we examined the effect of TSG on mineralization. MC3T3-E1 cells were allowed to differentiate (with ascorbic acid) for 10 days in the presence or absence of TSG. TSG treatment during differentiation of osteoblasts completely inhibited mineralization. To determine if the inhibitory effect of TSG on mineralization is related to differentiation, we will examine the mRNA expression of osteoblast differentiation markers such as bone sialoprotein, osteocalcin, alkaline phosphatase, type I collagen and Runx2/Cbfa1 following TSG treatment. Further, we will also examine the effect of TSG treatment on the expression of BMP-2, -4, -7, Noggin, Chordin, Xolloid and TSG in differentiating MC3T3-E1 cells in order to understand the interactions between BMPs, TSG, and known BMP antagonists. To conclude, we have demonstrated that TSG blocks mineralization in MC3T3-E1 cells. Following completion of our proposed experiments, we will begin to understand the role of TSG during osteoblast differentiation and explain the mechanisms involved in its ability to inhibit mineralization.

Disclosures: ***R. Gopalakrishnan****, None.*

SU026

A Conditional Null Allele for the BMP Antagonist Noggin Using a Novel Method for Generating Conditional Knock-Out Mice. E. Gazzerro*[1], E. Canalis[1], X. Wang*[2], R. Raz*[2], W. Auerbach*[2], L. Brunet*[3], M. Boucher*[2], D. Frendeway*[2], A. J. Murphy*[2], T. M. DeChiara*[2], D. M. Valenzuela*[2], R. M. Harland*[3], G. D. Yancopoulos*[2], A. N. Economides*[2]. [1]Department of Research, St. Francis Hospital and Medical Center, Hartford, CT, USA, [2]Regeneron Pharmaceuticals, Inc, Tarrytown, NY, USA, [3]Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA.

Noggin is a secreted protein that acts as an extracellular antagonist of bone morphogenetic proteins (BMPs). Noggin binds to its cognate BMPs and as a result inhibits binding to their receptors. The importance of these interactions is exemplified by the following evidence: (a) osteogenic BMPs induce expression of their antagonists, presumably to maintain

a balance between instructive and inhibitory signals; (b) overexpression or lack of expression of the antagonists lead to profound phenotypic effects: overexpression of noggin causes severe osteopenia whereas noggin null mice display abnormal skeletogenesis and embryonic lethality. Since BMPs and noggin have multiple biological roles pre- and postnatally, it is not surprising that noggin null mice display multiple phenotypic defects and die before birth. Thus it is not possible to dissect the role of BMP inhibition by noggin postnatally. This would be particularly important for understanding the role of BMPs in adult organisms. To this purpose, we generated a Cre-dependent conditional null allele for noggin, using VelocigeneTM, a novel method for rapidly generating genetically modified alleles, ES cells, and mice. LoxP sites were placed within the single exon noggin gene so that noggin expression would not be affected before Cre-induced recombination. After the noggin gene polyadenylation site, but within the floxed region, a PGK/Neo cassette was placed, followed by an open reading frame (ORF) encoding enhanced green fluorescent protein (eGFP) cloned after the 2nd LoxP site. After exposure to Cre recombinase, the ORF of noggin will be deleted and replaced by eGFP, expression of which will indicate deletion of the floxed sequence. The resulting floxed noggin allele was tested in culture. Cos7 cells were transiently transfected with a pcDNA/floxed noggin construct and were shown to secrete noggin protein by Western Immunoblot analysis – they did not express eGFP. Cotransfection of pcDNA/floxed noggin with a Cre vector resulted in expression of eGFP, indicating appropriate recombination and deletion of the gene of interest. Targeted ES cells carrying the floxed allele were generated and after similar testing were used to generate floxed noggin mice. In conclusion, we present a novel methodology to engineer conditional single exon gene knock-out mouse models.

Disclosures: ***E. Gazzerro****, None.*

SU027

BMP-2 Controls Cardiomyocyte Contractility by Activating Phosphatidylinositol 3 Kinase Signaling Pathway. N. Ghosh-Choudhury[1], B. Chandrasekar*[2], S. L. Abboud[1], G. Ghosh Choudhury*[2]. [1]Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [2]Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Cardiac development during vertebrate embryogenesis is critically dependent on the activity of bone morphogenetic protein-2 (BMP-2). We have previously reported that BMP-2 activates phosphatidylinositol 3 kinase (PI 3 K) in cardiomyocyte precursor cell line P19 CL6 (CL6). To determine the role of PI 3 K in BMP-2-induced cardiomyocyte differentiation, we examined the expression of sarcomeric myosin heavy chain (MHC), a marker of mature cardiomyocyte, in CL6 cells. BMP-2 significantly increased expression of MHC, as determined by anti-MHC immuofluorescence, suggesting formation of mature cardiomyocytes. Ly294002, a pharmacological inhibitor of PI 3 K, blocked BMP-2-induced PI 3 K activity, resulting in inhibition of MHC expression. To confirm the involvement of PI 3 K in BMP-2-induced cardiomyocyte differentiation, we used an adenovirus vector containing the constitutively active p110 catalytic subunit of PI 3 K (Ad-Myr-p110). Immunoblot analysis of lysates of CL6 cells infected with Ad-Myr-p110 showed increased expression of constitutively active PI 3 K catalytic subunit, resulting in significant expression of sarcomeric MHC in the absence of BMP-2. These data indicate that PI 3 K positively regulates differentiation of cardiac precursor cells into MHC-expressing mature cardiomyocyte in response to BMP-2. To further understand the functional implication of our finding, we studied the role of BMP-2 in cardiomyocyte contractility, loss of which in many heart diseases results in cardiac dysfunction. BMP-2 significantly increased PI 3 K activity in freshly prepared rat ventricular myocytes with concomitant increase in fractional shortening, a measure of contractility of these cells. Treatment of these primary cells with Ly294002 prior to BMP-2 addition completely inhibited BMP-2-induced PI 3 K activity and contractility. Expression of constitutively active PI 3 K, using adenovirus-mediated gene transfer, resulted in significant increase in cardiomyocyte contractility in the absence of BMP-2. These data for the first time show the role of BMP-2 in cardiomyocyte contractility. Furthermore our data provide the first evidence that PI 3 K regulates cardiomyocyte differentiation and function. Together our data demonstrate a plausible use of BMP-2 in diseases, where cardiomyocyte differentiation and contraction are impaired.

Disclosures: ***N. Ghosh-Choudhury****, None.*

SU028

The Relations between Lumbar Spine, Hip, Distal Femur and Proximal Tibia Bone Mineral Density in Healthy Individuals. K. Beattie*[1], P. Boulos*[2], C. Webber[3], C. Gordon[4], D. Inglis*[1], A. Papaioannou[5], E. Jurriaans*[6], J. D. Adachi[2]. [1]McMaster University, Hamilton, ON, Canada, [2]Dept. of Rheumatology, McMaster University, Hamilton, ON, Canada, [3]Dept. of Nuclear Medicine, McMaster University, Hamilton, ON, Canada, [4]Dept. of Radiology, McMaster University, Hamilton, ON, Canada, [5]Dept. of Geriatrics, McMaster University, Hamilton, ON, Canada, [6]Dept. of Radiology, St. Joseph's Healthcare, Hamilton, ON, Canada.

Objective: To investigate the relation between hip, lumbar spine, distal femur and proximal tibia bone densities in healthy men and women.
Methods: Healthy individuals (no knee pain, history of knee injury or diagnosis of a bone or joint disease) aged 20-69 yrs. were invited to participate. Volunteers underwent a fixed flexion knee X-ray and bone mineral density (BMD) scans of the lumbar spine, hip, distal femur and proximal tibia. Femur and tibia scans were acquired using the lumbar spine protocol. X-ray films were graded according to the Kellgren-Lawrence (K-L) scale. BMDs were evaluated by X-ray technicians and a research assistant.
Results: Of 46 participants, 4 cases were excluded (3 scored ≥2 on K-L scale, 1 had T<-2.5 at lumbar spine). The remaining 42 individuals included 32 females and 10 males, mean age (SD) 41.9 (13.6) years and body mass index (BMI) 25.5 (3.9) kg/m^2. Total proximal tibia and total distal femur BMDs were significantly positively correlated ($r=0.81$, $p<0.01$) as were subchondral femoral and subchondral tibial BMDs ($r=0.642$, $p<0.01$). Linear regression analyses revealed that the femoral neck was a significant predictor of total distal femur BMD (β coeff. 0.745, $p<0.01$) and subchondral femoral BMD (β coeff. 0.617, $p<0.01$). Lumbar spine and total hip BMDs also significantly predicted total distal femur BMD (β coeffs. 0.589 and 0.716, $p<0.01$) and subchondral femoral BMD (β coeffs. 0.321 and 0.522, $p<0.01$). In a multivariable regression analysis with all regions entered as independent variables, femoral neck BMD consistently and significantly predicted total distal femur BMD and subchondral femoral BMD (β coeffs. 0.604 and 0.617, respectively, $p<0.01$). A regression analysis including these same independent variables showed that lumbar spine, femoral neck and trochanter were significant predictors of total proximal tibial BMD at the $p<0.01$ level (β coeffs. 0.251, 0.514 and 0.339 respectively). They also significantly predicted subchondral tibial BMD (β coeffs. 0.321, 0.347 and 0.46, respectively).
Conclusion: Femoral neck and lumbar spine BMD significantly predict both distal femur and proximal tibia BMD in healthy men and women. The trochanter also significantly predicts proximal tibial BMD.

Disclosures: ***A. Papaioannou****, None.*

SU029

Evaluation of Human Trabecular Bone Properties by Scanning Acoustic Microscopy. I. Leguerney*[1], K. Raum*[2], A. Saied[1], H. Follet*[3], G. Boivin[4], P. Laugier[1]. [1]Laboratoire Imagerie Parametrique, CNRS - Paris 6 - UMR 7623, Paris, France, [2]Q-BAM group, Martin Luther University of Halle, Halle, Germany, [3]Laboratoire de Mecanique des Solides, INSA, Lyon, France, [4]Laboratoire Physiopathologie des Osteopathies fragilisantes, INSERM Unity 403, Lyon, France.

The study aims at evaluating local changes of trabecular bone material properties using a 50 MHz - 200 MHz reflection scanning acoustic microscope (SAM). A multi-layer analysis (MLA) method was used to estimate local variations of acoustic impedance of bone that is related to changes in both bone density and elasticity, and to reconstruct acoustic impedance images. This analysis method allows measurements that are independent of the system transfer function, sample topography and inclination.
Measurements were performed on calcaneus samples taken from 13 male and 6 female cadavers between 61 and 91 years of age. After dehydration, embedding in methylmethacrylate and polishing, the bone samples were explored with SAM. For each bone sample, the acoustic impedance (Z) was estimated from the entire sample section of at 50 MHz (30 µm lateral resolution) and from 5 selected regions of interest at 200 MHz (7.5 µm lateral resolution). The mean degree of mineralization of bone (MDMB) was measured over the entire section of bone samples by quantitative microradiography.
No correlation was found between age and acoustic impedance. At 50 MHz, a least square linear fit between Z and MDMB showed a moderate but significant correlation ($R=0.56$, $p<0.05$) between acoustic impedance and mean degree of mineralization for all the samples. No correlation was found between local mean Z-values obtained at 200 MHz and mean degree of mineralization of bone.
These findings indicate that mean degree of mineralization of bone contribute to changes in acoustic impedance. Further analysis are under way and complementary techniques are necessary to establish the influence of bone micromechanical properties on acoustic impedance.
Nevertheless, quantitative acoustic microscopy using MLA method has potential to be a relevant tool for the evaluation of bone properties and follow-up of the therapeutic effects at the tissue level.

Disclosures: ***P. Laugier****, None.*

SU030

Geometrical Changes in Micro-architecture Related to Bone Loss on a Mice Model. F. C. Peyrin*[1], A. Bonnassie*[1], E. Martin-Badosa*[2], D. Amblard*[3], L. Vico*[3]. [1]CREATIS, Villeurbanne, France, [2]Dept. de FAO, Lab. d'Optica, Barcelone, Spain, [3]LBTO INSERM, Saint-Etienne, France.

A tail suspension model of bone loss was investigated to characterise the changes occurring in micro-architecture on C57BL/6J (B6) inbred mice (male, 4-months old). Previous studies using histomorphometry revealed micro-architectural changes, and especially a decrease of trabecular thickness. In B6 mice, two groups were submitted to hind limb unloading through tail suspension (S, n=8) or had the tail suspension device but were not suspended (ANS, n=8). We investigated the distal metaphysis in the left femur using 3D synchrotron radiation microtomography. The voxel size was 6.65 µm, and a ROI encompassing the femoral bone with a total height of 2.35mm was selected in each image. 3D Model independent architecture parameters were computed, in particular bone volume fraction (BV/TV*) and trabecular thickness (Tb.Th*).
We developed a new technique for analysing the geometry's of bone micro-architecture. The 3D original volume is decomposed in three sub-volumes, each of them corresponding to the set of voxels in tube, plate or branching structures respectively. This geometrical approach allows to define new architectural parameters : plate volume to total volume (PV/TV), rod volume to total volume (RV/TV), as well as the direct thickness of each structures respectively denoted as (P.Th*) and (R.Th*). The parameters on the two groups are reported in table 1.

	B6 ANS (n=8)	B6 S (n=8)	P value
Tb.Th* (μm)	34 ± 1	32 ± 2	0.009 †
P.Th* (μm)	33 ± 1	30 ± 2	0.009 †
R.Th* (μm)	34 ± 1	32 ± 2	0.016 †
BV / TV (%)	7.8 ± 0.8	6.9 ± 1.1	0.059
PV / TV (%)	2.53 ± 0.63	2.06 ± 0.62	NS
RV / TV (%)	4.64 ± 0.33	4.19 ± 0.37	0.036 †

Table 1: Geometrical architecture parameters in two groups of mice
(† significantly different (p<0.05). NS, non-significantly different)
The computation of morphological parameters shows a significant thinning of the bone trabeculae with suspension. The geometrical analysis shows that the two groups have a similar dominant rod structure and that rods are significantly thicker than the plates (p=0.012 † in B6 ANS group and p=0.025 † in B6 S group). In addition it shows that suspension induces a statistical significant thinning of both of plate-like and rod-like structures.

Disclosures: ***F.C. Peyrin****, None.*

SU031

Monoclonal Antibodies Production and its Application to Study Possible New Markers in Osteoblastic Cells. L. M. Dos Reis*[1], R. C. Monteiro*[2], M. Benhamou*[2], V. Jorgetti[1]. [1]Nephrology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [2]Nephrologie, INSERM E0225 - Immunopathologie Renale, Recepteurs et Signalisation, Faculté de Médecine Xavier Bichat, France.

Monoclonal antibodies have been used to study the cellular differentiation pathway in osteoblast lineage. However, the majority of the antibodies recognizes antigens present in cells from the mature stage of differentiation and none of them is lineage and cell stage specific. In the present study we obtained monoclonal antibodies against human osteosarcoma cells and osteoblastic like cells by immunizing mice with MG-63. After several steps of tests we sub cloned three monoclonal antibodies: PSP 4-5, PSP 42-22 and PSP 85-9. These antibodies recognizes antigens which size are 100, 20 and 16 kDa, respectively. We evaluated the antigen expression in cryostat sections of undecalcified normal bone, cartilage, adipocyte and cardiac muscle from fetus. Also, in normal osteoblastic like cells and from patients with metabolic bone diseases. Finally, we tested these antibodies in normal fibroblasts like cells from human skin. The three antibodies showed antigen expression in periosteum of undecalcified bone sections. The pattern of expression of PSP 42-22 in this tissue was restricted in some regions. The antibodies were positive also in osteoblasts, osteocytes and in condrocytes. In cardiac muscle the antibody PSP 85-9 showed high antigen expression in some cells. PSP 4-5 detected its antigen in some cells of this tissue and PSP 42-22 in blood vessels. In adipocytes and fibroblasts like cells the antigen expression for PSP 4-5 and 85-9 was also detected. There was a similar pattern of antigen expression with PSP 4-5 e 85-9 in normal osteoblasts like cells and in cells obtained from patients with osteoporosis. In cells obtained from patients with primary and secondary hyperparathyroidism PSP 85-9 antigen expression was higher than PSP 4-5. In contrast, PSP 4-5 antigen expression in osteoblastic like cells from patients with osteomalacia was higher than PSP 85-9. PSP 42-22 was negative in all cells tested by immunocytochemistry. These monoclonal antibodies recognize antigens from undifferentiated to differentiated bone cells and they are not bone specific. We concluded that they may be useful to study the relations between the different cells lineage and in the comprehension of mechanisms involved in metabolic bone diseases.

Disclosures: ***L.M. Dos Reis****, None.*

SU032

Micro-Computed Tomographic Analysis of Endosseous Titanium Implant Integration: A Novel Approach for Assessing the Anchorage of Titanium Implants in Bone. Y. Gabet*[1], D. Kohavi*[2], T. Kohler*[3], R. Müller[3], I. Bab[1]. [1]Bone Laboratory, Hebrew University of Jerusalem, Jerusalem, Israel, [2]Dental Implantology Center, Hebrew University of Jerusalem, Jerusalem, Israel, [3]Institute for Biomedical Engineering, Swiss Federal Institute of Technology (ETH) & University of Zürich, Zürich, Switzerland.

Uncemented endosseous titanium implants constitute the standard of care in restorative dentistry and orthopaedic surgery. However, the mechanisms involved in this unique biological interaction with a foreign substance are poorly understood, mainly due to the absence of robust animal models and tools to analyze the implant-bone system. Here, we report a novel method to quantitatively evaluate endosseous implant anchorage by micro-computed tomography (μCT). A 5 mm long, 1 mm diameter titanium screw was inserted horizontally into the proximal tibial metaphysis of 4 months old male rats. The implantation site was examined 2-8 weeks thereafter by a desktop μCT system at 15 μm resolution. To better penetrate the highly radio-opaque titanium and improve the signal-to-noise ratio the system was operated at 70 KeV and 350 ms integration time, respectively, *vs.* the standard 50 KeV and 100 ms. The titanium and mineralized tissue were individually segmented by a multi-level thresholding procedure using Image Processing Language and topological operators developed with software devised specifically for this purpose. Unlike the traditional assessment of implant anchorage which is limited to the determination of the implant surface fraction in direct contact with bone (percent osseointegration, %OI) in a few histological sections, the present method analyses the %OI as well as the peri-implant trabecular bone (PIB) bone volume (BV/TV) and connectivity (Conn.D) densities directly in three-dimensional μCT images. After the μCT analysis the same specimens were subjected to biomechanical testing by a pullout test. All the μCT parameters showed high correlations with the ultimate force and toughness revealed by the implant pullout test. Surprisingly, the correlation coefficients between the biomechanical and PIB parameters were higher than those with the %OI, approaching the ultimate value (1.00), thus highlighting the critical role of PIB in connecting the implant to the surrounding cortex. The presently developed μCT operational definitions and software for analyzing the implant-bone system comprise an efficient, automatic, non-destructive and highly robust tool proposed as the "gold standard" for the experimental evaluation of endosseous implantation.

Disclosures: ***Y. Gabet****, R&D Pharmaceuticals, Inc. 2, 7.*

SU033

NMR Spectroscopy Shows a Structure Stabilizing Role for Water Found in Bone Mineral. L. W. Beck*[1], E. Wilson*[1], M. D. Morris*[1], D. H. Kohn*[2], M. M. J. Tecklenburg*[3]. [1]Department of Chemistry, University of Michigan, Ann Arbor, MI, USA, [2]Department of Biologic and Materials Science & Department of Biomedial Engineering, University of Michigan, Ann Arbor, MI, USA, [3]Department of Chemistry, University of Central Michigan, Mt. Pleasant, MI, USA.

We propose that water in bone mineral functions to occupy vacancies in the apatitic mineral lattice caused by incorporation of carbonate and other ions in the crystallites. The water content of bone mineral thereby stabilizes the mineral against mechanical weakening caused by these substitutions. Here, proton solid state NMR with magic-angle spinning (MAS) has been used to investigate the water and hydroxide content of deproteinated rat cortical bone tissue and a reference mineral, synthetic carbonated hydroxyapatite (CAP). The reference mineral was obtained by the standard room temperature precipitation resulting in a mineral that is 6-wt% (B-type) carbonate. At least two distinct types of structural water were found in the bone mineral, whereas three distinct types of water were found in the CAP material. The major type of water found in the deproteinated bone mineral sample was observed at 5.6 ppm in the ^{1}H NMR after the sample was heated to 120°C to remove non-structural surface water. This water occupies calcium vacancies in the apatite lattice and is strongly hydrogen-bonded to phosphate or carbonate anions. A second type of structural water was observed in higher abundance in the synthetic carbonated apatite mineral, at 4.3 ppm ^{1}H NMR. This water is assumed to also occupy calcium lattice vacancies. A third type of water, ca. 2-2.5 ppm, is most likely located in the hydrophobic environment of the calcium lined hydroxide channel (apatite lattice). This third type water is present at significantly higher levels in the deproteinated bone mineral as compared to the synthetic CAP material. There is a concomitant decrease in the fraction of hydroxide observed in the bone mineral, which suggests hydroxide is replaced by the structure water (third type).
Table 1. ^{1}H MAS-NMR spectral analysis of deproteinated bone and B-type carbonated hydroxyapatite at 220°C.

Species	Location	NMR shift / ppm	Bone Mineral	CAP
H_2O (I)	Ca vacancy	5.6	54.5%	10.0%
H_2O (II)	Ca vacancy	4.3	not resolved	38.4%
H_2O (III)	in OH^- column	2-2.5	29.0%	trace
$-CH_2-$	fat residue	1-1.6	16.8%	---
OH^-	in column	0.1	15.5%	51.6%

Disclosures: ***L.W. Beck****, None.*

SU034

Visualizing GFP Positive Cells in Frozen Decalcified and Nondecalcified Histological Sections of Bone. X. Jiang*, D. W. Rowe. Genetics & Dev Biology, University of Connecticut Health Center, Farmington, CT, USA.

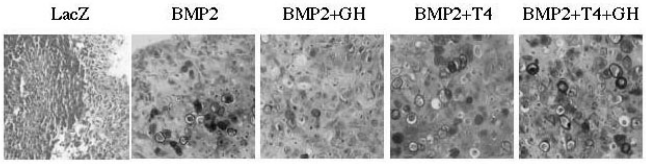
The ability to image specific population of cells within the osteoblast lineage in transgenic mice bearing reporter-GFP constructs can reveal new levels of tissue physiology that cannot be assessed by standard histological techniques. Last year we reported that frozen sections preserved a significantly stronger GFP signal than paraffin sections and allowed GFPcyan, GFPsaph and the widely used eGFP to be visualized. However great skill in preparing the frozen sections was required and the method could not be performed with calcified tissues. The CryoJane adaptation to cryostat sectioning has to potential to overcome these technical problems of tissue sectioning. It uses a piece of adhesive tape to transfer the cut section to a temperature controlled slide thereby preserving tissue integrity. The slide comes precoated with an adhesive that crosslinks the tissue to the slide after a brief flash of UV light. Using this approach we have been able to cut 5-7 μm decalcified sections from mice of any age with a disposable blade and calcified section from mice < 8 months of age with a tungsten blade. The histological sections are imaged with a computer controlled Zeiss Axioplan 200 microscope workstation that records a series of image files that are concatenated into a single file of the majority of the bone section. We will present composite images showing the markedly improved signal of GFP of a frozen versus a paraffin section and the remarkable quality of the frozen section prepared with the CryoJane system. The approach has a very low endogenous fluorescence background and allows weaker GFP signals from other elements within the bone marrow to be appreciated (endothelial cells expressing Tie2eGFP), and to co-localize immunostained and enzymatic stained sections with GFP. Immunostaining intracellular targets that require denaturation of the DNA (BrdU) destroys the GFP signal. Nondecalcified sections can be prepared that show dynamic labeling with calcein and xylenol orange that also preserve the endogenous GFP signal. The step distinguishes those areas expressing GFP that are and are not producing a bone matrix. While we are still learning how to use this approach to histology to a greater advantage, it can reveal information that is not possible with standard histological methods and significantly increases sample throughput for transgene analysis in murine bone. An additional advantage of the tape method is the relative ease such that an inexperienced user can produce their own histological sections.

Disclosures: ***D.W. Rowe****, None.*

SU035

Induction of Mesenchymal Progenitor Cell Differentiation into Chondrocytes by BMP 2 and Promotion of Hypertrophy by Thyroxine. Y. Okubo*[1], K. Bessho*[1], T. Iizuka*[1], H. Reddi[2]. [1]Department of Oral and Maxillofacial Surgery, Graduate School of Medicine, Kyoto university, Kyoto, Japan, [2]Department of Orthopaedic Surgery, School of Medicine, UC Davis, Sacramento, CA, USA.

Mesenchymal stem cells have the potential to differentiate into adipocytes, myoblasts, chondrocytes and osteoblasts. The chondrogenesis of mesenchymal cells is followed by maturation and terminal differentiation and hypertrophy. The faithful mimicry of the sequence *in vitro* will be useful. The induction of chondrogenesis of two mesenchymal progenitor/ stem cell lines, C2C12 and C3H10T1/2 in three-dimensional micromass and pellet cultures was investigated. An adenoviral gene transfer system with bone morphogenetic protein 2 (BMP 2) converted C3H10T1/2 cells to chondrocytes in three-dimensional cultures. However, C2C12 cells were not converted to chondrocytes, but expressed alkaline phosphatase activity, as osteoblast marker. Treatment of C3H10T1/2 with 5-azacytidine prior to BMP2 treatment increased the intensity of Alcian blue staining and alkaline phosphatase (AP) activity on days 14 (Fig) and 21. Next, we examined the effects of thyroxine and growth hormone on the terminal differentiation and hypertrophy of chondrocytes. Thyroxine treatment resulted in an increase in markers of chondrocyte hypertrophy such as AP activity and type X collagen. However, growth hormone alone did not affect the terminal differentiation of chondrocytes. These experiments demonstrated that BMP 2 and thyroxine are sufficient to initiate chondrogenesis and promote hypertrophy of mesenchymal progenitor/ stem cells.



Disclosures: ***Y. Okubo****, None.*

SU036

Study of Growth Plate Homeostasis Markers in Rats with Growth Retardation or Hypophosphatemic Rickets. V. Lascau-Coman*, N. Dion, G. Mailhot*, M. Gascon-Barré, L. G. Ste-Marie. Hôpital Saint-Luc, CHUM Research Centre, Montréal, PQ, Canada.

Normal longitudinal growth depends on a well-defined sequence of chondrocyte maturational stages occurring within the epiphyseal growth plate (GP). During this endochondral ossification, a population of stem cells in the resting zone (RZ) proceeds through proliferation (PZ), differentiation and hypertrophy (HZ) which finally gives way to bone. Several proteins have been involved in chondrocyte proliferation and differentiation such as PTH-related peptide (PTHrP), Indian hedgehog (Ihh) and its receptor Patched (Ptc), fibroblast growth factor 18 (FGF18) and its receptor FGFR3. To ensure normal endochondral ossification and growth, adequate dietary intake of calcium (Ca) and vitamin (D) are essential. It was previously observed that young rats (9 week old) fed a low Ca diet depleted in D (Ca-D-) showed growth retardation whereas young rats fed a D depleted diet but high in Ca (Ca+D-) developed hypophosphatemic rickets. Immunohistochemistry (IHC) performed on rat tibial GP showed that the expression of PTHrP, Ihh, Ptc, FGF18 and FGFR3 was modulated by the diet. When compared to rats fed a normal diet (N), PTHrP and Ihh proteins were decreased in Ca-D- but were abundant in Ca+D-. In Ca+D-, Ptc was present in all GP zones in contrast to Ca-D- and N where Ptc was only localized in PZ.There were no differences among groups in the localization of FGF18 except that its expression was higher in Ca+D- RZ and PZ. IHC of FGFR3 showed a similar expression pattern in all groups but the intensity of staining was lowered in Ca+D- HZ. One of the mechanisms proposed to be part of the fate of hypertrophic chondrocytes is apoptosis. To further characterized growth abnormalities in these rats, we studied chondrocyte apoptosis by performing TUNEL assay and IHC for Caspase 3, the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl-2. Ca-D- were found to have TUNEL and IHC stainings comparable to N. However, there was a marked decrease of apoptosis as indicated by TUNEL and Caspase 3 IHC in Ca+D- HZ compared to N. In addition, Bax IHC staining was lower and conversely, Bcl-2 was higher in Ca+D- than in HZ of N. Taken together, these results suggest that growth retardation observed in Ca-D- is mainly due to a diminution of proliferation and differentiation of GP chondrocytes. In Ca+D- the development of rickets characterized by a widening HZ is partly attributable to the increment of chondrocyte proliferation and differentiation but further amplified by decreased apoptosis. The role of hypophosphatemia and the mechanism by which chondrocyte apoptosis is lowered in this group remain to be elucidated.

Disclosures: ***V. Lascau-Coman****, None.*

SU037

ACTH Enhances Chondrogenesis In Vitro. J. F. Evans*[1], D. Sima*[1], C. Shen*[2], J. F. Aloia[1], J. K. Yeh[1]. [1]Medicine, Winthrop University Hospital, Mineola, NY, USA, [2]Pathology, Texas Tech Health Sciences Center, Lubbock, TX, USA.

Childhood obesity has been linked to increased growth and most forms of obesity are associated with hyperphagia. Recently overfeeding has been linked to increased production of pro-opiomelanocortin (POMC), the melanocortin peptide pre-cursor. Familial Glucocorticoid Deficiency (FGD), a non-obesity syndrome, is also associated with both increased production of melanocortin peptide and increased growth. The association of melanocortin peptide overproduction with enhanced linear growth prompted the current investigation of ACTH's effects on resting zone chondrocytes and bone marrow stromal cells in culture. Resting chondrocytes isolated from the rib of young rats were cultured in differentiation medium plus or minus 10^{-7}M ACTH. Secondary stromal cells derived from long bones of the same rats were grown in basal medium (α-MEM plus 10% charcoal/dextran treated FBS and 50μg/ml ascorbic acid) plus or minus 10^{-7}M ACTH. ^{3}H-thymidine incorporation measurements in Day 3 cultures indicate that ACTH significantly decreases proliferation of chondrocytes (CON 318.06±11.14X10^{3} vs. ACTH 244.14±11.35 x 10^{3} dpm, P=0.001) but significantly increases the proliferation of stromal cells (CON 1563.23±86.20 vs. ACTH 2674.97±136.64 dpm, P<0.001). ACTH significantly increased protein content of both chondrocytes (CON 7.3±0.4 vs. ACTH 8.2±0.4 μg/well, P<0.05) and stromal cells (CON 4.66±0.10 vs. ACTH 7.69±0.46 μg/well, P<0.001). Total collagen was also significantly elevated in ACTH treated chondrocyte cultures throughout the culture period (day 7-28), as measured by ^{3}H-proline incorporation. The increase in protein content and total collagen in the chondrocyte cultures indicates an increased matrix formation while the increase in protein content of the stromal cell cultures may reflect increased cell numbers. Examination of chondrogenic cell and molecular markers in both chondrocyte and stromal cultures indicates an increase in differentiation. Peak AP-activity is significantly greater in ACTH treated chondrocyte (CON 82.36±5.77 vs. ACTH 131.83±11.85 nmol pnp/min/well, P<0.001) and stromal cell cultures (CON 5.59±0.83 vs. ACTH 11.80±0.38 nmol pnp/min/10^{4} cells, P<0.001). mRNA expression of COLL II and AGR is also elevated in ACTH treated chondrocyte and stromal cell cultures. These data show that ACTH increases both chondrocyte differentiation and stromal cell chondrogenesis which implicates ACTH as a regulator of chondrocyte differentiation and a promoter of chondrocyte phenotype development from bone marrow stromal cells. These findings provide a link between enhanced linear growth and the overproduction of melanocortin peptides.

Disclosures: ***J.F. Evans****, None.*

SU038

Hypoxia-Inducible Factor-1α and Effects of Hypoxia on Chondrocytes and Induced Human Chondrocytes. J. Glowacki, C. C. Wykoff*, S. Mizuno. Orthopedic Research, Brigham and Women's Hospital, Boston, MA, USA.

The heterodimeric transcriptional complex hypoxia-inducible factor 1 (HIF-1) is a key mediator of cellular response to hypoxia and directs expression of a large family of hypoxia-inducible genes. HIF-1 comprises an oxygen-sensitive subunit, HIF-1α, and a constitutively expressed subunit, HIF-1β. Hypoxia and hypoxia-mimetics (e.g. deferoxamine, DFO, 150 microM) are capable of inducing the accumulation of HIF-1α protein in normal and cancer cells. Cartilage is an avascular and relatively oxygen-deficient tissue. Hypoxia maintains chondrogenesis in monolayer culture [Dev Bio 19:52, 1969]. HIF-1α was recently identified in mouse fetal cartilage and was required for normal growth plate viability and development [Genes Dev 15:2865, 2001]. Little is known about HIF function in normal adult chondrocytes or neochondrocytes. We tested the hypotheses 1) that HIF-1α regulation in chondrocytes differs from other cell types and 2) that hypoxia enhances chondrogenesis in 3D culture. Freshly isolated bovine articular chondrocytes (bACs), human dermal fibroblasts (hDFs), and control HeLa cells were cultured in normoxia (19%) and in hypoxia (1-5%) in a custom-made incubator by autoregulating air, N_2, and CO_2. The hDFs were also cultured in 3D collagen sponges +/- chondroinductive demineralized bone powder (DBP). Expression of HIF-1α was examined by Western blot. Chondrogenesis was measured by ELISA in bACs, hDFs, and chondroinduced hDFs.

First, hypoxia-inducibility and DFO-inducibility of HIF-1α were confirmed in HeLa and hDFs. Second, HIF-1α was expressed at a relatively high level in the normoxic bACs, followed for 6 days. Third, HIF-1α was not greater in hypoxic bACs; rather, it appeared that HIF-1α was constitutively expressed in normoxia and hypoxia. Fourth, DFO treatment of bACs further increased HIF-1α expression. Fifth, hDFs that were cultured with DBP for 7 days showed histochemical, immunochemical, and molecular evidence of chondroinduction. Those induced neochondrocytes expressed HIF-1α under all conditions. Sixth, hypoxia (2%) enhanced accumulation of cartilage-specific chondroitin 4-sulfate by both bACs (14%, p<0.05) and hDFs with DBP (18%, p<0.05), but not by control hDFs.

In conclusion, we show that 3D chondrogenesis is enhanced in hypoxia and that HIF-1α is expressed in bACs and in induced human chondrocytes. In contrast to HeLa cells and hDFs, bACs demonstrated an impressive normoxic level of HIF-1α expression. Furthermore, the expression of HIF-1α in bACs was constitutive, unresponsive to hypoxia, and increased with exposure to DFO. Enhanced chondrogenesis with hypoxia may be related to viability and other HIF target genes that are unique in chondrocytes.

Disclosures: ***J. Glowacki****, None.*

SU039

Overexpression of $p57^{Kip2}$ Is Not Sufficient for, but Augments, Collagen Type X Induction in Differentiating Chondrocytes. M. Stewart[1], R. M. Kadlcek*[2]. [1]Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Orthopaedics, Case Western Reserve University, Cleveland, OH, USA.

Introduction: The CDK inhibitor $p57^{Kip2}$ has been implicated in regulating collagen type X (Coll X) expression in differentiating chondrocytes (1). This study was carried out to investigate the link between p57 expression and the induction of Coll X in chondrocytes and to determine whether p57 overexpression is sufficient for the induction of hypertrophic differentiation.

Methods: Neonatal rat epiphyseal and growth plate chondrocytes were maintained as non-adherent aggregates in serum-free medium. Hypertrophic differentiation was induced by BMP-2 administration. p57 mRNA and protein levels were assessed by Northern and

Western blot analyses. Associated changes in proliferative activity were monitored by total DNA and ^{3}H thymidine incorporation rates. An adenoviral vector was constructed to assess the phenotypic consequences of p57 overexpression. Vectors expressing LacZ and the related CDK inhibitors $p21^{Cip1}$ and $p27^{Kip1}$ were used as controls and in co-expression studies.
Results: Steady-state levels of p57 mRNA and protein were not affected by BMP-2 treatment, despite transient effects on chondrocyte proliferative activity and the induction of ALP and Coll X. Adenoviral p57 overexpression induced growth arrest in epiphyseal chondrocytes in a dose-dependent manner, as did vectors expressing p21 and p27. p57 overexpression did not induce Coll X, either alone or when co-expressed with p21. Further, pretreatment of epiphyseal chondrocytes with BMP-2 for 4 days to initiate differentiation did not induce "p57-responsiveness" in these cells. Similar results were obtained with more mature tibial growth plate chondrocytes that were actively expressing Coll X at the time of isolation. p57 overexpression did significantly augment Coll X induction by sub maximal BMP-2 doses. This effect was specific to Coll X since ALP was not affected, and was also specific to p57, since parallel experiments using p21 or p27 had no effect on Coll X levels.
Conclusions: These results indicate that growth arrest is not sufficient for expression of the hypertrophic phenotype, but rather occurs in parallel with other aspects of the differentiation pathway, analogous to results derived from keratinocyte and skeletal myoblast differentiation studies(2,3). The study also suggests a specific activity for p57 in controlling Coll X expression in differentiating chondrocytes, independent of its direct effect on cell cycle progression in these cells.
References. 1. Zhang P et al (1997) Nature 387:151-158 2. Harvat BL et al (1998) J Cell Sci 111:1185-1196. 3. Zhang P et al (1999) Genes & Dev 13:213-224

Disclosures: ***M. Stewart****, None.*

SU040

Expression of Mouse Receptor of NF-kappaB Ligand (RANKL) in Growth Plate and Articular Cartilage. K. Kishimoto*, R. Kitazawa, A. Darwanto*, T. Kondo*, S. Maeda*, S. Kitazawa. Division of Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan.

RANKL (receptor activator of NF-kappaB ligand) is a membrane-associated osteoblastic molecule that, along with the macrophage-colony-stimulating factor, is crucial for osteoclast formation. In addition to the osteoblasts that actively support osteoclasts, we and others have reported that RANKL expression is observed in hypertrophic chondrocytes during endochondral ossification in bone tissue. To investigate the molecular mechanism controlling RANKL gene expression in hypertrophic chondrocytes, we examined the expression of RANKL and Runx2/Cbfa1, a key transcription factor of RANKL gene expression, and the methylation status of the RANKL gene promoter. The articular cartilage, a cartilage that does not undergo endochondral ossification, in the joint was used as an internal control for the entire study. Knee joints of male BALB/c mice aged 1-24 weeks were dissected and fixed with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB, pH7.4) at 4 C for 3 days, then decalcified with 20% EDTA in 0.1M PB for 4 days. Tartrate-resistant acid phosphatase (TRAP) staining was used to detect osteoclasts. To characterize hypertrophic chondrocytes, type X-collagen mRNA was detected by *in situ* hybridization (ISH). Immunostaining was carried out using anti-RANKL and Runx2/Cbfa1 antibodies (Santa Cruz). The methylation status of the mouse RANKL gene promoter in both hypertrophic and articular cartilage was analyzed by sodium bisulfite mapping using microdissected mouse bone tissue. By immunostaining and ISH, besides osteoblasts lining the trabecular bone surface, RANKL expression was observed on hypertrophic chondrocytes expressing type-X collagen in the growth plate, but not on chondrocytes in the articular cartilage. A similar localization pattern of Runx2/Cbfa1 was demonstrated by immunostaining. Although the overall methylation in the RANKL promoter region was low, chondrocytes in the articular cartilage showed a higher methylation rate than hypertrophic chondrocytes in the growth plate. These results indicate that RANKL expression in hypertrophic chondrocytes is regulated as in osteoblasts and contributes to the formation of osteo(chondro)clasts during endochondral ossification.

Disclosures: ***K. Kishimoto****, None.*

SU041

Regulation of Chondrogenic Differentiation of ATDC5 Cells: A Role for Estrogens? J. Hoogendam[1], C. Lowik[2], J. Wit*[1], M. Karperien[3]. [1]Pediatrics, LUMC, Leiden, Netherlands, [2]Endocrinology, LUMC, Leiden, Netherlands, [3]Pediatrics and Endocrinology, LUMC, Leiden, Netherlands.

Sex-steroids have profound effects on longitudinal bone growth. They can exert direct effects on growth plate chondrocytes, due to the presence of ERa and ß mRNA and protein. The mechanism of action by which these receptors regulate growth and differentiation of chondrocytes are largely elusive. One possibility is that they influence the activity of the growth restraining PTHrP/Ihh-feedback loop which is operational in the prenatal and postnatal growth plate.
To study the interactions between sex-steroids and the PTHrP/Ihh-feedback loop in more detail, we have started to use the mouse chondrogenic ATDC5 cell line. This cell line differentiates into mineralising hypertrophic chondrocytes in monolayer in the presence of insulin. Hypertrophic differentiation is enhanced by the addition of BMP4 and can be inhibited by PTHrP. RT-PCR analysis identified the presence of ERa, but not ERß mRNA. Hypertrophic differentiation of ATDC5 cells was dramatically accelerated by inducing cell aggregation. Interestingly, under these culture conditions, insulin was not required for terminal chondrocyte differentiation. However, in the presence of insulin aggregates were larger and their integrity was better maintained. Culturing of ATDC5 cells as aggregates in the presence of estrogen reduced terminal chondrocyte differentiation.
In order to study the effect of estrogens on chondrocyte differentiation in more detail, we have generated stable cell lines overexpressing wild type hERa (WT), constitutive active hERa (CA) and dominant negative hERa (DN) using FLP-mediated recombination. For this purpose ATDC5 cells are stable transfected with an FRT-recombination site. One clone, which behaved comparable to the parental cells was selected, and used for generating overexpressing variants of hERa (WT, CA and DN) isogenic cell lines.
In all cell lines overexpression of hERa variants could be detected. Using transient transfection with EREluc, parental and overexpressing cell lines responded differentially to estrogen (E2). The WT was highly sensitive and gave almost a ten fold induction in luciferase activity. The CA overexpressing cell line increased basal activity and did not react to E2. Like the CA, the DN did not react to E2, but had low basal levels.
In conclusion, hypertrophic differentiation of ATDC5 cells can be accelerated by culturing the cells in aggregates. In addition, cell aggregation appears to be more important for hypertrophic differentiation than treatment with insulin. The flp-in stable ATDC5 cell line appears to be a suitable model to study interactions between sex-steroids and the PTHrP/Ihh-feedback loop.

Disclosures: ***J. Hoogendam****, None.*

SU042

Effects of Pyrrolidine Dithiocarbamate on Chicken Chondrocytes. N. C. Rath. USDA, Agricultural Research Service, Fayetteville, AR, USA.

Thiocarbamates are widely used as fungicides, seed fumigants, and accelerators in the vulcanization of rubber. Pyrrolidine dithiocarbamate (PDTC) is used as an antioxidant and a metabolic inhibitor of transcription factor NF-kB. PDTC, and a few other thiocarbamates including, thiram and disulfiram induce tibial dyschondroplasia (TD), a common cartilage defect in meat-type poultry. TD is characterized by the presence of a thickened plug of cartilage in the proximal end of tibial growth plates which impedes bone formation and causes bone distortion, fracture, and lameness. To understand how PDTC affects chondrocyte metabolism to induce TD, we studied its effects on the expression of several chondrocyte-associated genes using avian-leukosis virus transformed chicken epiphyseal growth plate chondrocytes (CEGC) in culture. CEGC were grown at a concentration of 10^5 cells /ml/ well in 24 well plate for14 days before they were treated with 2- and 4µM concentrations of PDTC for 24h. The choice of the concentrations of PDTC was based on studies which showed these to be nontoxic to cells. Total RNA extracted from control and PDTC treated cells, were reverse transcribed and cDNA was amplified using PCR and chicken gene specific primers for glyceraldehyde phosphate dehydrogenase (GAPDH), aggrecan, bone morphogenetic protein (BMP)-4 and 7, collagen types II and X, matrix metalloproteinase-2 (MMP-2), and transforming growth factor-β (TGF-β). The PCR products were analyzed using capillary electrophoresis and laser-induced fluorescence detection which allows both size determination and quantification of amplicons. The expression of different genes was normalized against GAPDH and the results (n=3/ treatment) were analyzed using Duncan's GLM procedure. PDTC at the concentration of both 2 and 4µM down regulated the expression of aggrecan and type II collagen genes in a dose dependent manner but had no effect on other genes. However, at 4µM concentration PDTC also down regulated the expression of BMP-4, collagen X genes without significant effect on MMP-2, or TGF-β. These results suggest that PDTC negatively affects chondrocyte development most likely affecting the maturation of prehypertrophic population of chondrocytes. On the other hand, the hypertrophic population of chondrocytes appear to be less drastically affected since other studies under similar conditions showed that PDTC up to 16µM was not inhibitory to alkaline phosphatase that is responsible for calcification. Extrapolating these *in vitro* observations to an *in vivo* situation of growth plate, it is likely that PDTC prevents maturation of chondrocytes eventually leading to their premature death and accumulation as an unresolved plug of dead cartilage, which characterizes tibial dyschondroplasia.

Disclosures: ***N.C. Rath****, None.*

SU043

Perlecan Domain I Synergizes with BMP-2 to Promote Maturation of Cartilage Condensations. R. R. Gomes*, W. Yang*, M. C. Farach-Carson, D. D. Carson*. Biological Sciences, University of Delaware, Newark, DE, USA.

C3H10T1/2 cells differentiate along a chondrogenic pathway when plated onto the extracellular matrix (ECM) protein perlecan (Pln) or recombinant domain I of Pln (PlnDI). Pln-stimulated condensations undergo chondrogenic maturation in response to exogenous recombinant human bone morphogenic protein 2 (rhBMP-2) treatments. In this investigation, we tested the hypothesis that exogenous rhBMP-2 treatments of PlnDI-stimulated condensations promote chondrogenic maturation in vitro. To test this hypothesis PlnDI was first purified from conditioned media of EBNA-293 cells stably transfected with a vector constitutively expressing PlnDI. Purified PlnDI is decorated with both heparan-sulfate and chondroitin-sulfate side chains, as determined by SDS-PAGE analysis of heparitinase I-III, and chondroitinase ABC treated samples. In addition, dot-blot analysis demonstrated that PlnDI binds basic fibroblast growth factor, and that this binding is heparan sulfate dependent. To test the ability of rhBMP-2 to synergize with PlnDI-stimulated cartilage-like condensations, C3H10T1/2 fibroblasts were seeded in chambered "Permanox" slides uncoated or coated with PlnDI. The cells were maintained in CMRL-1066 media supplemented with ascorbic acid, citrate, and pyruvate. C3H10T1/2 fibroblasts seeded on PlnDI coated wells attach and began to aggregate, forming cartilage-like condensations within minutes of plating. On day 3 post plating, the media was replaced and supplemented with or without rhBMP-2 (100 ng/ml). On day 6 post plating, phase contrast microscopy suggested that cells in rhBMP-2 treated cultures had proliferated relative to untreated cultures. Subsequent observations of cells in rhBMP-2 treated cultures suggested that differentiation also occurs, as changes in cell shape (from fibroblastic to round) and the appearance of increased matrix deposition were noted. By day 12 of culture, indirect immunofluoresence visualized by confocal microscopy revealed that cells within PlnDI-stimulated condensations treated with rhBMP-2 express a late stage marker of chondrogenesis, collagen type X. Morphologically, collagen type X expressing cells appear larger in diameter, hypertrophic, with large nuclei, relative to cells within PlnDI-stimulated condensation not

treated with rhBMP-2. Thus, PlnDI-stimulated cartilage-like condensations respond to rhBMP-2 treatment similar to intact Pln. This in vitro model mimics key events that comprise the early cartilaginous model of bone development.

Disclosures: ***R.R. Gomes****, None.*

SU044

Analysis of Gene Expression Profiles Among Calvarial Bones, Sutures, and Osteogenic Fronts Composing of Cranial Vaults. H. J. Kim[1], W. B. Lee*[1], H. J. Kim*[2], H. M. Ryoo[1]. [1]Biochemistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [2]Pediatric Dentistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea.

Enlargement of the skull vault occurs by appositional growth at the fibrous joints between the bones, termed cranial sutures. However, little is known about patterns of gene expression from cells populating mouse cranial vaults. This study investigated the possible variances of gene expression profile among calvarial bones, sutures, and osteogneic fronts of mouse cranial vaults at embryonic day (E) 15 – E16. Expression was analyzed on a mouse gene array panel of 7,400 mouse cDNAs representing tightly regulated genes with key roles in various biological processes. Of these genes, total 455 were found to have at least 2-fold or higher expression changes in calvarial bones (269 genes), sutures (174 genes), and osteogenic fronts (12 genes). A large number of these genes have never been previously recognized in the context of calvarial development and most genes belong to the following protein families: growth factors and receptors; signal transduction pathway proteins; transcription factors; structural and matrix proteins; immune response-related proteins; transporter, etc. For example, cDNAs for Col1a1, Col3a1, Col5a2, osteopontin, Pkd1, Hoxa5, PDGF receptor beta, Gli, and Ncor1 had higher normalized hybridization intensities in calvarial bones than both in sutures and osteogenic fronts. In suture area, Ftl1, Col11a1, Abl-1, and insulin-like growth factor 2 were higher expressed than other both tissues. Osteogenic fronts had higher expression levels of FGF3, Gfi1, Hoxc4, interleukin 1 alpha, and Sn. The establishment of the expression profiles of these and other genes from each tissue of cranial vault will allow us to distinguish the molecular events for cranial vault development.

Disclosures: ***H.J. Kim****, None.*

SU045

Hypoxia Promotes Chondrocytic Differentiation of C3H10T1/2 Cells induced by Bone Morphogenetic Protein-2 via p38 Kinase Pathway. M. Hirao*, N. Tamai*, A. Myoui, H. Yoshikawa. Department of Orthopaedics, Osaka University Graduate School of Medicine, Suita, Japan.

Cartilage is an avascular tissue that functions at a lower oxygen tension than do most other tissues. On the other hand, ossification is always accompanied by vascularization, suggesting high oxygen requirement. Recently, it was reported that hypoxia-inducible factor 1 (HIF-1), one of the major regulators of the hypoxic response, is essential for chondrocyte growth arrest and survival in *in vivo* experiment. These observations lead us to hypothesize that hypoxia plays a critical role in the chondrocytic differentiation of the cells in mesenchymal lineage. In this study, we investigated the role of oxygen tension on chondrocytic differentiation induced by recombinant human bone morphogenetic protein-2 (rhBMP-2) using pluripotent mesenchymal cell line C3H10T1/2. C3H10T1/2 cells were cultured in normoxia (O2 20%) and hypoxia (O2 5%) conditions in the presence of rhBMP-2 (500ng/ml). Alcian blue staining revealed that more abundant proteoglycan synthesis in the cells cultured in hypoxia than in normoxia. In parallel, type II collagen (*Col2a1*) gene expression by Northern blotting was elevated in hypoxic condition. However, hypoxia did not alter gene expression of sry-Y-box-9 (Sox9), a transcription factor that has been shown to be involved in chondrocyte differentiation. To determine the signaling pathway involved here, activation of Smad 1/5 and p38 mitogen activated protein kinase (MAPK) were evaluated using antibodies specific for phosphorylated form. We found hypoxia upregulated phosphorylation of p38 MAPK but downregulated that of Smad 1/5. Since p38 MAPK has been suggested to be one of the regulators of chondrocyte differentioation, we next determined the effect of p38 MAPK inhibitor (FR167653) on chondrocytic differentiation induced by hypoxia. Interestingly, FR167653 abolished the upregulation of Alcian blue staining and *Col2a1* gene expression induced by hypoxia but did not alter Sox9 expression. Taken together, these results suggest that hypoxia promotes chondrocytic differentiation of C3H10T1/2 cells induced by BMP-2 via p38 MAPK pathway. Oxygen tension may play an important role in regulating BMP's various physiological function.

Disclosures: ***M. Hirao****, None.*

SU046

Heparanase Expression in Developing Mouse Limb and its Relationship with Heparin Binding Growth Factor Delivery System(s). A. J. Brown*, C. Kirn-Safran*, D. D. Carson, M. C. Farach-Carson. Biological Sciences, University of Delaware, Newark, DE, USA.

Heparanase, an endoglucuronidase that cleaves heparan sulfate (HS) chains from various proteoglycans including perlecan (Pln), has been identified in a wide variety of tissues. Pln is a large multi-domain HSPG with a diverse tissue distribution including high expression in the pericellular matrix of cartilage that supports pre-chondrocyte aggregation and early chondrogenic differentiation. We have demonstrated that Pln domain I, when decorated with three glycosaminoglycan (GAG) chains, can trigger aggregate formation, early chondrogenesis, and facilitate heparin binding growth factor (HBGF) delivery. Despite the ability of heparanase to release active HBGFs in various tissues, the importance of heparanase in the chondrogenic pathway has not been determined. To investigate this role, we employed an ATDC5 cell line, a clonal mouse embryonal carcinoma cell line, to study the changes in heparanase expression and function(s) as these cells progress through the chondrogenic pathway. ATDC5 cells serve as good models for endochondral bone formation because under the appropriate conditions they display both early and late stage markers of cartilage differentiation. We propose that heparanase expression during endochondral bone formation is essential for the delivery of HBGFs that regulate cartilage tissue growth and differentiation. Conventional RT PCR was performed to analyze expression of heparanase transcripts in relation to other markers of chondrogenesis. Real time PCR showed that as these cells progress through the chondrogenic pathway, heparanase mRNA expression is transiently increased by 60 fold in maturing chondrocytes. In future studies, Laser Capture Microdissection (LCM) will allow us to obtain homogeneous populations of cells from the long bone of developing mouse limb to visualize heparanase mRNA expression *in vivo*. In addition to investigating the mRNA expression, enzymatic and immunodetection assays will be employed to monitor changes in heparanase protein expression and activity during cartilage development *in vivo* and in the ATDC5 model. Our results support the notion that heparanase is a highly regulated enzyme in the growth plate likely to be involved in HBGF delivery and functions by releasing active HBGFs from HSPGs such as Pln.

Disclosures: ***A.J. Brown****, None.*

SU047

Hepatocyte Growth Factor (HGF) Is Responsible of a High Degree of Bone Remodeling and some Cartilage Matrix Modifications. C. Boileau*[1], J. Martel-Pelletier*[1], M. Guévremont*[1], J. Pelletier*[1], G. Merlino*[2], P. Reboul*[1]. [1]Arthritis Unit, University of Montreal, Montreal, PQ, Canada, [2]Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD, USA.

Our recent in vitro findings suggest that HGF found in human osteoarthritic (OA) cartilage is produced by osteoblasts and could seep from the subchondral bone. Therefore, we investigated whether some modifications of bone and cartilage matrix could be found in knee joints of HGF transgenic mice.

Transgenic mice were compared to an OA model developed in the knee joint of non-transgenic mice of the same strain. OA was induced by an intra-articular injection of mono iodoacetate (MIA, 0.5%) and animals were sacrified 7, 14 and 21 days post-injection. Joints were processed for histological analyses (O-Safranin and toluidine bleu). Using stain intensity, cellularity, structural modifications and remodelling, a histological score was established for bone and cartilage.

Both HGF transgenic mice and MIA induced a loss in both O-Safranin and toluidine bleu staining of cartilage compared to control, indicating a disruption of the proteoglycans. This effect is weaker in HGF tansgenic mice than in MIA model. In MIA model, the proteoglycan loss was maximal at day 14 while a beginning of tissue regeneration was observed at day 21. MIA induced subchondral bone remodeling consisting of formation of large lacunae with osteoclast presence and also irregularities close to the cartilage border. HGF transgenic mouse joints showed almost similar results however with some particularities. Subchondral bone was more remodeled than OA (ie: presented larger lacunae and smaller trabeculae). On the other hand, although some loss of proteoglycans and hypercellularity were found in cartilage, the effect was weaker than in MIA model.

Results obtained with transgenic mice confirmed our in vitro data indicating that HGF source found in cartilage was bone dependent. Moreover, these new data showed that bone was also the main HGF target since high degrees of remodeling were found in HGF transgenic mice. The impact of HGF on the cartilage matrix seemed to be a remodeling effect rather than a complete destruction. Therefore, we may hypothesize that HGF found in human OA cartilage is an attempt to repair the matrix.

Disclosures: ***C. Boileau****, None.*

SU048

Runx1/Cbfa2 Contributes to Proper Endochondral Ossification During Fracture Healing. K. E. McDougall[1], R. M. Belflower*[1], Y. Dong*[1], A. J. van Wijnen[2], J. L. Stein[2], J. B. Lian[2], G. S. Stein[2], E. M. Schwarz[1], R. J. O'Keefe[1], H. Drissi[1]. [1]Department of Orthopaedics, University of Rochester, Rochester, NY, USA, [2]Department of Cell Biology, University of Massachusetts, Worcester, MA, USA.

Runx family members are key regulators of organogenesis and pathogenesis. The leukemia-associated transcription factor Runx1 is required for definitive hematopoiesis, such that mice lacking functional Runx1 protein die during mid-gestation, prior to skeletal formation. We investigated Runx1expression patterns in mouse embryos between E13.5 and E18.5 by in situ hybridization. Runx1 transcript was detected in the sclerotome, concomitant with Sox9 and Collagen type II, and was maintained throughout cartilage development along with Collagen type X in the axial skeleton. Real time RT-PCR revealed that Runx1 mRNA levels are up-regulated between E9.5 and E11.5 and down-regulated thereafter, while Runx2 mRNA continues to be enhanced throughout embryonic development. Since Runx1 is present in these pluripotent mesenchymal condensations, we assessed its expression during chondrocyte differentiation using micromass from mouse limb buds collected at E11.5. While the MASDS isoform of Runx1 is up-regulated throughout chondrocyte hypertrophy, as evidenced by expression levels of cartilage phenotypic genes, the MRIPV isoform is down-regulated between 4 and 10 days of culture. Our results show that Runx1 expression is regulated during embryonic development and chondrocyte differentiation. Both isoforms of Runx1 are expressed and differentially regulated in maturing mesenchymal condensations, suggesting an interplay between Runx1 isoforms in mediating cartilage formation.

We further investigated the function of Runx1 in cartilage, by performing tibial fractures in heterozygous mice (HZ) for the Runx1 gene. Wild-type (WT) littermates were used as controls. X-ray analysis shows that while WT tibia achieved bony union by day 14, radiolu-

cent lines at the fracture site were still evident in the HZ mice. Furthermore, although HZ mice show callus formation as early as day 7, the bone at the fracture site remains uncalcified after 14 days. Histological staining reveals presence of fibrous non-union as well as persistence of uncalcified cartilage in the HZ fractures, while the WT fractures contained bridging bone with little cartilage. Taken together, our results indicate that haplo-insufficiency of Runx1 delays chondrocyte maturation thus suggesting Runx1 may be required for endochondral ossification at the fracture sites.

Disclosures: ***K.E. McDougall****, None.*

SU049

Bonnin Stimulates Osteoblast Differentiation. L. X. Bi, E. G. Mainous, W. L. Buford*, R. R. Throndson*. Departments of Surgery and Orthopaedics, University of Texas Medical Branch, Galveston, TX, USA.

Early studies showed that bone formation is influenced by small electric currents. When an electrode was implanted in bone or bone defects, the new bone formation was observed in the vicinity of the cathode. And our previous studies have also shown that negatively charged resins increase bone formation and accelerate bone defect healing in vivo. In order to investigate potential effects of bonnin, negatively charged peptide, on human preosteoblast, we examined expression of bone morphogenetic protein-7 [BMP-7] and type I collagen, alkaline phosphatase activity (ALP) and mineralization after treatment of cells with bonnin. Human preosteoblast cells were cultured in a-minimum essential medium [a-MEM] and 10% fetal bovine serum with or without bonnin (5ug/ml) for 7, 10, 14 days, respectively. To determine mineralization, the cells were cultured in mineralizing-growth medium. The levels of ALP were assayed using a commercial kit (Sigma Chemical Co., St. Luis, MO). Expression of BMP-7 and type I collagen (polyclonal antibody, Santa Cruz Biotechnology, Inc. CA) was examined using immunoquantitative assay. The mineralization was assessed by Von Kossa technique. ALP activities were significantly elevated (38-47%, P<0.01) in bonnin treated group, compared to control group. BMP-7 expression was increased (21-35% P<0.01) after bonnin treatment compared to control. Expression of type I collagen was increased (23% at day 10 and 38% at day 14, respectively, P<0.001). There was significant increase in the levels of mineralization (2-3 fold, P<0.001) within 14 days of culture. We conclude that bonnin significantly stimulates osteoblast differentiation in vitro. It might be an important regulator of bone formation by accelerating fracture and bone defect healing, and controlling bone diseases.

Disclosures: ***L.X. Bi****, None.*

SU050

Genes that Influence Cell Differentiation Are Altered by Demineralized Bone *In Vitro*. K. E. Yates, S. Zhou, J. Glowacki. Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA.

Demineralized bone powder (DBP) induces endochondral bone formation *in vivo*. An early event in this process (differentiation of chondrocytes) can be modeled *in vitro* with a porous, 3D collagen sponge system [Exp Cell Res 227:89,1996]. We previously showed some of the gene expression shifts on day 3, prior to expression of the chondroblast phenotype [Exp Cell Res 265:203,2001]. In addition, changes in extracellular matrix genes suggested that the DBP-altered environment supports cell differentiation [Conn Tiss Res, in press]. Altogether, those gene expression shifts begin to define a "determined neochondroblast" phenotype at 3 days in the DBP/collagen sponge.

In this study, the hypothesis was that DBP alters expression of differentiation factors (i.e., peptide factors and extracellular matrix components) in determined neochondroblasts. Human dermal fibroblasts were used as targets cells for chondroinduction by DBP. Cells were cultured in DBP/collagen or control collagen sponges for 3 days. RNA was isolated from pooled sponges (n=10 per group) and used for cDNA macroarray and RT-PCR analyses. cDNA synthesis, labeling, hybridization, and data analysis were performed as per the manufacturer's instructions (Human Signal Transduction PathwayFinder and TGFB-BMP Signaling Pathway GE Arrays, Q Series; SuperArray Inc., Bethesda MA).

We found that DBP increased expression of several peptide differentiation factors, receptors, and matrix proteins (Table). Increases in other factors (TGFb1; BMP1; BMP antagonist-1; anti-mullerian hormone) and receptors (activin receptors I, IB, II, and II-like) was detected but could not be quantified due to low levels in control cells. Decreases in some differentiation factor receptors (TGFbR II and III; Patched 1) were detected. It is notable that no changes in expression were found for nodal (a differentiation factor) or BMP receptors IA, IB, or II. Several TGF-b/BMP signaling genes (RUNX1/AML1, RUNX2/cbfa1; Smad 2, 3, 4, 5, and 9) were also not changed by DBP. Of note, many TGFb superfamily proteins (BMP2-11, -15; GDF1-3, 5, 8-11; TGFb2, b3) were below the limits of detection in this system. Overall, the changes reflect coactivation of a unique and specific set of TGFb superfamily signaling and target genes in determined neochondroblasts that may be necessary for initiating post-natal differentiation.

Table. DBP-regulated "differentiation genes."

Functional Class	Gene (GenBank Accession No.)	Fold Change by DBP
Differentiation Factor	TGF-beta induced (NM_000089)	12.6
	IGF-BP3 (M31159)	11.9
	Inhibin alpha (NM_002191)	1.4
Differentiation Factor Receptor	Endoglin (NM_000118)	1.4
Matrix Protein	Collagen type I (NM_000089)	17.7
	Collagen type III (NM_000090)	14.0
	Fibronectin (X02761)	>5.2

Disclosures: ***K.E. Yates****, None.*

SU051

Effects of RGD-Peptide(224) on Bone Metabolism and Mechanical Strength in Ovariectomized Mice. X. Yan*. Department of Biochemistry, BeiJing JI Shui Tan Hospital, Beijing, China.

The purpose of this investigation is to compare the effects of a synthtic RGD peptide (224) with 17β- Estrodiol on bone metabolism and mechanical strength in ovariectomized (OVX) mice. Methods: Bone mineral density (BMD), bone mineral content (BMC), bone strength, bone histomorphometry and biochemistry indicators were measured in all mice. Results: 36 female Kunming mice, weighing an average of 35g, were randomly divided into 4 groups. After 6 weeks treatment RGD peptide(224) at 4.41 ng.kg-1.d-1 and 17β-Estradiol at 30μg.Kg-1d-1, in OVX mice, femal BMD and BMC increased respectively 0.7%, 4% and 5.9%, 5.7%. Maximun load of bone increased respectively 12.9% and 16% and serum AKP decreased 33% and 37% respectively. Conclusion:RGD(Arg-Gly-Asp)-containing sequence into the mutant proinsulin by replaceing C-peptide and four basic amino acid residues with designed CRGDSC hexa-peptide.RGD peptide may maintain BMD,improve BMC and bone mechanical strength and decrease bone turnover in OVX mice.

Disclosures: ***X. Yan****, None.*

SU052

Bisphosphonate Bone Affinity, Differences Predicted By In Vitro Carbonated Apatite Model. Z. J. Henneman*[1], R. Tang*[1], S. Gulde*[1], G. H. Nancollas*[1], R. J. Phipps[2], R. G. G. Russell[3], F. H. Ebetino[2]. [1]University of Buffalo, Buffalo, NY, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [3]University of Oxford, Oxford, United Kingdom.

Bisphosphonates (BPs) are effective inhibitors of bone resorption. Important components in their ability to inhibit bone resorption are their affinity for calcium phosphate surfaces and their ability to target to bone mineral. This can be predicted in vitro through studies of their effects on the dissolution and growth of calcium phosphates. The effects of BPs on the dissolution of a bone-like mineral, hydroxyapatite (HAP), has been previously reported. We now also report data on BP effects on carbonated apatite (CAP), which is thought to be more relevant to human bone. These effects have been studied by a constant composition method at both physiological ionic strength and temperature, 0.15M and 37°C. Adsorption affinity constants, K, were calculated from the kinetics data. For HAP growth at pH=7.4, differences were observed between BPs in the order of zoledronate > alendronate> risedronate. For HAP dissolution at pH = 5.50, the rank order of inhibition was similar but the difference between the BPs was less marked. Adsorption affinity constants for CAP dissolution rank ordered as observed with HAP, but with a greater discrimination between zoledronate, alendronate, and risedronate at pH = 5.50. The degree of inhibition was also markedly dependent on the relative undersaturation, σ, with respect to CAP. When compared at a more physiologically relevant undersaturation, σ = 0.3, K values for risedronate (4.93×10^4 M^{-1}) and alendronate (1.10×10^5 M^{-1}) were only 25% and 50% of that for zoledronate (2.19×10^5 M^{-1}), respectively. The inhibition of CAP dissolution by the BPs was also related to the carbonate content of the crystallites. Lower dissolution rates were obtained with crystallites containing more carbonate. Thus, at σ = 0.3 and pH = 5.50, K values for zoledronate inhibited dissolution of CAP containing 3.1±0.1% and 8.0±0.1% carbonate were 1.47×10^5 and 2.19×10^5 M^{-1}, respectively. These results point to the importance of carbonate in more accurately distinguishing between the ability of BPs to target and affect bone mineral, and further substantiate the significantly lower bone affinity of risedronate compared to alendronate and zoledronate. These differences in bone affinity may contribute to the observed differences in pharmacokinetics among these three BPs in the clinic. Partial support by NIH/NIDCR grant # DE03223.

Disclosures: ***Z.J. Henneman****, None.*

SU053

The Relationship of Antiresorptive Drug Use to Osteoarthritis. L. D. Carbone[1], S. B. Kritchevsky*[1], K. Wildy*[2], K. D. Barrow*[1], M. Visser*[3], F. Harris*[4], T. B. Harris*[5], B. W. E. Wang*[1], M. C. Nevitt*[4]. [1]UTHSC, Memphis, TN, USA, [2]Univ. of Pittsburgh, Pittsburgh, PA, USA, [3]EMGO Institute, Amsterdam, Netherlands, [4]UCSF, San Francisco, CA, USA, [5]NIA, Bethesda, MD, USA.

Subchondral bone changes accompany cartilage degradation in knee OA. Antiresorptive treatments (ARTx) could be beneficial in OA, although there is limited data. We analyzed 1360 women ages 69-81 (44% Black) in the Health ABC study to examine the relationship of current use of ARTx to symptomatic knee OA (Sx) (knee pain plus xray OA in tibiofemoral/patellofemoral area), bone and cartilage changes in the knee by MRI and knee pain severity. Women were interviewed for knee pain and medication use. Those with knee pain, and a random sample without, had PA fixed flexion knee x-rays and bilateral MRI of the knee done on 1.5 T GE Signa scanners (axial/coronal T2 FSE, sagittal T2 FSE with fat saturation). MRIs were read using the Peterfy Whole Organ MRI Scoring (WORMS) method. Logistic and linear regression, with GEE to adjust for inter-knee correlations, were used to examine the association of ARTx use with outcomes. Models were adjusted for age, race, BMI, NSAIDS, thiazides, calcium supplements, smoking, quads strength, whole body BMD and study site. 313 (23%) women had Sx knee OA. In adjusted analyses, there was no association of Sx knee OA with use of any ARTx (OR: 1.11; 95% CI: 0.64-1.90) nor with use of individual ARTx. WORMS data were available for 898 knees. Results were similar for knees with and without pain, so these were combined. Bisphosphonate, but not other ARTx use, was associated with lower bone attrition (BA) and bone marrow edema (BME) scores compared with nonuse (p<0.05). There were no differences in osteophyte (OP), total cartilage (TC) or WOMAC scores by use of any ARTx or

by use of individual ARTx (p>0.11). In women in the Health ABC study, BA and BME were significantly reduced with bisphosphonate use, supporting a potential effect of ARTx in knee OA.

MRI and WOMAC Scores by Use of ARTx (Mean (SD))

OA feature (range)	Nonusers (645 knees)	Estrogen (178 knees)	Bisphosphonate (57 knees)	Raloxifene (18 knees)
BA (0-23)	2.0 (3.0)	1.4 (2.7)	0.6 (1.4)*	3.9 (6.2)
OP (0-85)	13.5 (14.4)	9.4 (13.2)	6.9 (8.8)	23.4 (26.9)
BME (0-20)	2.6 (3.3)	1.5 (2.4)	1.0 (1.7)*	3.8 (5.1)
TC (0-78)	17.5 (16.0)	12.9 (13.7)	14.1 (13.6)	18.6 (18.7)
WOMAC (0-18)	3.3 (4.5)	2.9 (4.4)	2.9 (4.2)	4.5 (4.8)

*p<0.05 Compared to nonusers

Disclosures: ***L.D. Carbone****, Proctor and Gamble 5, 8; Aventis 2, 5, S; Merck 5, 8; Eli Lilly 5, 8.*

SU054

Serotonin Affects Bone Metabolism in Vitro and Leads to Increased Bone Mineral Density (BMD) in Female Rats. B. Gustafsson*, L. Thommesen*, R. Fossmark*, H. L. Waldum*, U. Syversen. Institute of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Faculty of Medicine, Trondheim, Norway.

Serotonin (5-hydroxytryptamine or 5-HT) is a peptide neurotransmitter. Outside the central nervous system it is mainly produced by the enterochromaffin cells of the gut where it participates in the regulation of intestinal motility, fluid secretion and regional blood flow. Cell culture studies have shown that serotonin has proliferative effects on fibroblasts, myofibroblasts, smooth muscle cells and endothelial cells. Serotonin is also known as a regulator of craniofacial morphogenesis. Recent studies have shown the expression of several serotonin receptors in chicken osteocytes and osteoblasts, mouse and rat osteoblasts, as well as the serotonin transporter 5-HTT in rat osteoblasts. In the present study we have studied the effect of serotonin on proliferation of preosteoblasts, the effect on osteoclast differentiation and finally the long-term effects of serotonin on BMD in female rats.

The murine preosteoblast cell line MC3T3-E1 was incubated with serotonin at different concentrations (0.01-50uM), and the proliferation rate was determined by using a ELISA BrdU proliferation kit. Human peripheral blood mononuclear cells were differentiated into osteoclasts by stimulation with RANK-L, MCSF and dexametazone, in the presence or absence of serotonin (0.1-10uM). Tartrate resistant acid phosphatase positive multinucleate giant cells were considered as genuine osteoclasts. Female Sprague-Dawley rats were given daily s.c serotonin injections (5 mg/kg) for 3 months and BMD was thereafter measured by DXA.

Serotonin induced a 10-15 % increase in osteoblast proliferation. This increase was totally abolished in the presence of the PKC inhibitor, GF109203. Serotonin increased the total number of differentiated human osteoclasts by 90-300%, in a dosage-dependent manner. After 3 months total body BMD was significantly higher in the serotonin treated rats, (mean±SEM: 0,1976 ±0,0012 g/cm2) compared to controls, (0,1913 ±0,0015 g/cm2, p=0,004). There was no significant difference in femoral BMD between the 2 groups, which might be explained by the fact that the serotonin treated rats were less active and weighed 5% less than the controls.

Conclusion: Serotonin induces murine osteoblast proliferation and human osteoclast differentiation in vitro. Treatment with serotonin increases total body BMD in female rats. These results indicate that serotonin might have an important regulating role in bone metabolism.

Disclosures: ***U. Syversen****, None.*

SU055

Light-Activated Gene Transduction of Recombinant Adeno-Associated Virus in Human Mesenchymal Stem Cells. H. Ito, J. Goater*, P. Tiyapatanaputi*, P. T. Rubery*, R. J. O'Keefe, E. M. Schwarz. Department of Orthopaedics, University of Rochester Medical Center, Rochester, NY, USA.

Deficiencies in skeletal tissue repair and regeneration lead to conditions like osteoarthritis, osteoporosis and degenerative disc disease. While no cure for these conditions is available, the use of human bone marrow derived-mesenchymal stem cells (HuMSCs) has been shown to have potential for cell-based therapy. Furthermore, recombinant adeno-associated viruses (rAAV) could be used together with HuMSCs for *in vivo* or *ex vivo* gene therapy. Unfortunately, the poor transduction efficiency of these cells remains a significant obstacle. Here, we describe the properties of ultraviolet (UV) light activated gene transduction (LAGT) with rAAV in HuMSCs, an advance toward overcoming this limitation. Using direct fluorescent image analysis and real-time quantitative PCR to evaluate enhanced green fluorescent protein (eGFP) gene expression, we found that the optimal effects of LAGT with limited cytotoxicity occurred at a UV dose of 200J/m^2. Furthermore, this UV irradiation had no effect on either the chondrogenic or osteogenic potential of HuMSCs. Significant effects of LAGT in HuMSCs could be detected as early as 12 hours after exposure and persisted over 21 days, in a time and energy-dependent manner. This LAGT effect was maintained for more than 8 hours after irradiation and required only a 10-minute exposure to rAAV after UV irradiation. Finally, we show that the production of secreted TGFβ1 protein from rAAV-TGFβ1-IRES-eGFP infected HuMSCs is highly inducible by UV irradiation. These results demonstrate that LAGT combined with rAAV is a promising procedure to facilitate gene induction in HuMSCs for human gene therapy.

Disclosures: ***H. Ito****, None.*

SU056

Environmental Effects on Osteogenesis. Z. Valkusz MD*[1], O. Vetró*[2], A. Juhász*[3], A. Petri*[4], M. Gálfi*[2]. [1]Department of Endocrinology, Medical Faculty, University of Szeged, Szeged, Hungary, [2]Environmental Protection Group, University of Szeged, Szeged, Hungary, [3]Department of Psychiatry, Medical Faculty, University of Szeged, Szeged, Hungary, [4]Department of Surgery, Medical Faculty, University of Szeged, Szeged, Hungary.

Bone and cartilage cells differentiate from fibroblasts. These processes are influenced by the extracellular space, and during the development and differentiation of fibrocytes they are determined by the receptors on/in the cells, and genetic factors in nucleus and mitochondria. The modifications of the extracellular enviroment therefore determine the formation and eventually the functionability of cells. The chemical and physical enviromental effects play a role in inducing osteoporosis. We studied the chronic effects of subtoxic doses of chlorobenzene- given to pregnant rats- on embrionic and newborn osteogenesis changes. Model experiments were applied to investigate the causes of observed bone (femur) destruction. During investigation, Wistar rats were treated through gastric tubes with hexachlorobenzene:trichlorobenzene=1:1, and the dosage was 3, 6, 12.5 μg/kg body weight for 30 or 60 days. After treatment, we studied the pregnant, embrionic and newborn animals. The samples were taken from the bone tissues of the treated, untreated control, and solvent-treated control animals. Tissue structure and Ca^{2+} content were examined. Liver function (γGT, SGOT, SGPT) and sturture were also investigated. Monolayer cultures were generated from the fibroblasts of treated and control animals, and membrane fluidity (fluorescent anisotropy), collagen synthesis (protein content in the presence and absence of Collagenase-E) and structure (morphometry) were examined. As a result of the chronic hexa-and trichlorobenzene treatment, femur tissue destruction was observed in all the animals. This general structural damage of the bone tissues is in correlation with a significant change in the Ca^{2+} content of the femur matrices. Although there was found no fundamental change in the amount of procollagen I, destruction was detected in the protein structure.

Disclosures: ***Z. Valkusz MD****, None.*

SU057

From Marrow to Bone and Adipose Tissues: Isolation, Cultivation, and Induction of Differentiation in Adult Rat. C. Shih, H. W. Wu*. Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan Republic of China.

Mesenchymal stem cells(MSCs) are present in a variety of tissues during development, and its potential roles in tissue engineering and cellular and gene therapy are promising in the near future. Bone marrow MSCs contribute to the regeneration of tissues such as bone, cartilage, muscle, ligament, tendon, and adipose tissues. Despite extensive experimentation has defined conditions for their isolation and characterization both in vivo and in vitro, there is still no well-defined protocol for isolation and expansion of these cells. MSCs isoltated primarily by tight adherence to plastic Petri dishes in most experiments shows heterogenerous. More recently, sorting by size differences and surface markers has been developed to obtain more homogenerous population. The purpose of this study was to isolate, characterize, and induce cell differentiation into different lineage cells by density gradient separation method, cell culture, CD marker selection, alkaline phosphatase activity(ALP), type I collagen synthesis, bone colony formation, light and phase contrast inverted microscopy, H&E stain, oil red O stain and flow cytometry. Rat bone marrow cells were harvested from both femur and tibia and MSCs isolated by density gradient separation, cultivation and CD markers (e.g. CD 90 and etc.) selection. Isolated bone marrow MSCs were cultured to semiconfluent and treated with osteogenic differentiation medium(DMEM-LG supplemented with 10% FBS, ascorbic acid, 100 nM dexamethasone, with or without 10 mM beta-GP) or adipogenic differentiation medium (100 nM dexamethasone, indomethacin and insulin). Appearance of cuboid-shaped osteoblastic cells, unmineralized or mineralized colony was noted approximately 5, 10 and 14 days after osteogenic induction. Histological sections showed mineralized matrix with lining osteoblasts and entrapped osteocytes. With adipogenic induction, small sized lipid droplets began to appear within cytoplasm 2-4 days. More and larger lipid droplets accumulation were noted 5-10 days adter induction. We also noted that lipid droplets-containing adipocytes terminally differentiated into round shaped adipocyte and finally became unadherent and floating within the medium. The results of this study indicated that bone marrow MSCs could be isolated, cultured, and induced to become osteogenic and adipogenic cells/tissues.

Disclosures: ***C. Shih****, None.*

SU058

Regeneration of Critical-sized Bone Defect after Transplantation of Rat Marrow Mesenchymal Stem Cells. C. Shih[1], H. W. Wu*[1], J. Shyu[1], P. Chu[2]. [1]Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan Republic of China, [2]Nephrology, Tri-Service General Hospital, Taipei, Taiwan Republic of China.

Bone marrow has been shown to contain a population of mesenchymal stem cells (MSCs) that are capable of forming bone, tendon, cartilage, muscle,and other connective tissues. The repair/regeneration of bone tissue requires 1) MSCs capable of differentiating into osteoblasts, 2) a scaffold to support the attachment and migration of MSCs or osteogenic cells and 3) growth factors that direct these cells to migrate into bone defects, to proliferate, to differentiate into osteoblasts, and form new bone. The purpose of this study was to evaluate the ability of cultured marrow MSCs to elicit repair/regeneration at the site of a critical-sized bone defect in the rat femur. The strategy is based on the hypothesis that such an approach decreases or eliminates the need for chemotaxis of osteoblast progenitor cells

into the defect and their massive proliferation. MSCs has been proved to have the osteogenic potential with addition of differentiation inducing factors in vitro before implantation in this study. Forty 12-month old male Sprague-Dawley male rats were used in this study. A model for the resection of an osteoperiosteal segment and stabilization of the limb was developed, and the defect was left untreated, filled with osteoset that had been loaded with MSCs, MSCs alone, osteoset alone, and isolated adherent light density marrow cells. Bone formation and healing at the site of the defect were evaluated by microradiography, histological, and bone histomorphometric analysis. Microradiographic analysis was performed at 2 week intervals. Eight weeks after surgery, new bone formed throughout the body of the implant by direct conversion of MSCs into osteoblsts, without progression through a cartilaginous intermediate. Microradiographs showed radiopaque area(area of mineralization) began to appear eight weeks (in osteoset with MSCs group) or 10 weeks (in MSCs alone group) after implantation. It is also noted that MSCs were superior to isolated adherent light density marrow cells. The results of this study indicating that osteoset loaded with marrow MSCs effectively repair or regenerate the critical-sized bone defect.

Disclosures: ***C. Shih****, None.*

SU059

Platelet-Derived Growth Factor Stimulates Osteoblastic Migration and Proliferation via Independent Signaling Pathways. M. Mehrotra*, S. Mehrotra*, C. C. Pilbeam. Medicine, University of Connecticut Heath Center, Farmington, CT, USA.

Osteoblastic migration is important for skeletal development as well as bone remodeling and fracture repair. Platelet-derived growth factor (PDGF) is a potent stimulator of both osteoblastic migration and proliferation. We used a wounding model to study migration of osteoblastic MC3T3-E1 cells on a collagen substrate in response to PDGF. Non-tissue-culture-treated Petri dishes were coated with 5 μg/cm^2 of rat-tail collagen type I and cells were plated at 100,000 /cm^2 in DMEM with 10% FCS. 24 h later half the cell layer was removed by gentle scraping and the cultures were treated with PDGF-BB (10 ng/ml) in serum-free DMEM. The average distance of cell migration from the wound line was measured in arbitrary units (pixels) using NIH Image software. PDGF stimulated a 1.8 and 2.4-fold increase in the distance that cells moved out from the wound line over 24 and 48 h, respectively. Treatment with PDGF for 24 h also stimulated a 10-16-fold increase in cell proliferation as measured by ^{3}[H]-thymidine incorporation into replicating DNA ([^{3}H]-TdR) over the last 2 h of culture, normalized to total DNA content. Aphidicolin, 30 μM and 90 μM, inhibited the PDGF stimulated increase in [^{3}H]-TdR by 90% and 98%, respectively, but did not reduce the distance of migration from the wound line. [^{3}H]-TdR results were confirmed by BrdU staining of cells. Previous studies have shown mitogen activated protein (MAP) kinase pathways to be involved in PDGF stimulated fibroblast migration and proliferation. Using flow cytometry, we analyzed the carboxyfluorescein diacetate succinimidyl ester (CFSE) labeled cells to measure the % of cells that were replicating in cultures treated with PDGF, with and without specific MAP kinase inhibitors. An inhibitor of ERK activation, U-0126 (20 μM), reduced the number of replicating cells in both vehicle and PDGF treated cultures by 50%, while a p38 MAP kinase inhibitor, SB-203580 (10 μM), had little effect. Conversely, migration in PDGF treated cultures was reduced 80-95% by the p38 inhibitor and only 25% by the ERK inhibitor. We conclude that PDGF stimulated both proliferation and migration of MC3T3-E1 osteoblasts. Proliferation occured via an ERK pathway. Migration was independent of proliferation and occured via a p38 MAP kinase pathway.

Disclosures: ***M. Mehrotra****, None.*

SU060

Identification of a Biflavone Found in Ginkgo Biloba that Stimulates Bone Formation In Vitro and In Vivo. W. E. Gallwitz, G. Gutierrez, I. R. Garrett, M. Jalomo*, J. Esparza*, A. Escobedo*, B. McCluskey*, P. Rivas*, D. Horn*, G. R. Mundy. OsteoScreen, San Antonio, TX, USA.

There is considerable interest in the effects of various herbal remedies on bone, since many have been proposed to have beneficial consequences. To identify and characterize constituents of herbal preparations that may have stimulatory effects on bone formation, we examined hundreds of natural product compounds frequently found in herbal preparations. We discovered that the biflavone isoginkgetin, one of five biflavones found in Ginkgo biloba leaf extracts, stimulated bone formation in organ cultures of neonatal murine calvaria at concentrations of 5-20 μM. This compound was also tested for its capacity to stimulate bone formation in vivo, using the murine local calvarial injection model. Isoginkgetin at doses from 2-20 mg/kg per day caused a dose-dependent increase up to 40% in local appositional bone growth after 5 days of treatment. To determine the mechanism by which isoginkgetin stimulated bone formation, we examined its capacity to inhibit the 20S proteasome, since we have recently found compounds that are inhibitors of the chymotryptic catalytic activity of the proteasome enhance osteoblast differentiation. We found that isoginkgetin in concentrations of 0.1-3 μM inhibited proteasome activity. Since proteasome inhibitors stimulate osteoblast differentiation in part by their capacity to stimulate BMP-2 expression, we tested the effects of isoginkgetin in concentrations from 0.075-40 μM on BMP-2 promoter activity, using osteoblasts stably transfected with the murine BMP-2 promoter linked to the firefly luciferase gene. We found that isoginkgetin increased BMP-2 gene transcription 3 fold at 5 μM. In conclusion, our results show that the biflavone isoginkgetin, which is a constituent of herbal remedies, stimulates bone formation in vitro and in vivo. Isoginkgetin is a powerful inhibitor of the osteoblast proteasome, and our data is consistent with the notion that isoginkgetin stimulates osteoblast differentiation and bone formation by its effects to enhance BMP-2 transcription.

Disclosures: ***W.E. Gallwitz****, None.*

SU061

Alterations in Bone Formation in a Glucose-Dependent Insulinotropic Peptide Functional Receptor Knockout Mouse on a Low Calcium Diet. D. Xie[1], Q. Zhong[1], K. Ding[1], R. J. Bollag*[1], C. M. Isales[2]. [1]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA, USA, [2]Medicine, Medical College of Georgia and the Augusta VA Hospital, Augusta, GA, USA.

Glucose-dependent insulinotropic peptide (GIP) is a 42 amino acid peptide secreted by endocrine cells in the small intestine. Our laboratory has previously shown that GIP has anabolic effects on osteoblastic-like cells and that daily injection of GIP in rats results in higher bone mass. To further evaluate the role of GIP in bone metabolism we generated a GIP-overexpressing transgenic mouse with a metallothionein inducible promoter. Even at baseline the transgenic mice were found to have a greater than two-fold higher level of GIP than controls (Control: 197.1+/-26.3 vs. MT-GIP: 561+/-130 pM+/-SEM p<0.001), which rose even higher with zinc (25mM): 1,160+/-189 pM/ml+/-SEM. We have previously shown that continuous high levels of GIP lead to GIP receptor downregulation. MT-GIP transgenic mice and controls were started on zinc feeding at one month of age, resulting in receptor downregulation and thus generating a GIP receptor knockout mouse. Twenty mice were then divided into four groups: (1) control (2) control + low calcium diet (0.1% calcium) (3) MT-GIP and (4) MT-GIP on a low calcium diet. Several parameters of bone turnover were measured including osteocalcin and bone mineral density (PIXImus, Lunar Corp.). Osteocalcin (a marker of bone formation) levels were: Group 1: 18.89+/-5.491 and Group 3: 10.89+/-1.74 (ng/ml+SEM). Thus, the MT-GIP have significantly lower osteocalcin levels than control (p<0.01) suggesting that the MT-GIP mice were more prone to lose bone. Similarly, at the end of five months MT-GIP had a lower bone mineral density than controls (0.0503+/-0.0021 vs. 0.0526+/-0.0028 g/cm2+/-SEM). As expected a low calcium diet led to a drop in BMD in both controls and MT-GIP but this was greater in the MT-GIP (0.0400+/-0.003vs 0.0429+/-0033g/cm2+/-SEM). Taken together this data is consistent with an anabolic role for GIP and when the GIP effect is lost (via receptor downregulation) the animals are more prone to lose bone.

Disclosures: ***D. Xie****, None.*

SU062

N-cadherin-mediated Distribution of Stabilized β-catenin Alters the Effect of MAP Kinase and BMP-2 Signaling on Gene Expression during Chondrogenesis. R. Modarresi*[1], J. Roman-Blas*[2], T. Lafond*[1], K. G. Danielson[1], R. S. Tuan[3], M. R. Seghatoleslami*[2]. [1]Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA, USA, [2]Medicine/Rheumatology, Thomas Jefferson University, Philadelphia, PA, USA, [3]Cartilage Biology and Orthopaedics Branch, NIH, NIAMS, Bethesda, MD, USA.

We have examined the effect of calcium dependent adhesion, mediated by N-cadherin, on cell signaling involved in chondrogenesis of multipotential embryonic mouse C3H10T1/2 cells. The activity of chondrogenic genes, type II collagen, aggrecan and Sox 9 was examined in monolayer (nonchondrogenic), or micromass (chondrogenic), cultures of C3H10T1/2, or C3H10T1/2 cell lines expressing a dominant negative form of N-cadherin that is missing the extracellular calcium binding domain (Δ390-10T1/2) and also the MNCD2-10T1/2 subline that expresses N-cadherin at twice the level compared to the endogenous gene. Our findings show that overexpression or inhibition of N-cadherin in C3H10T1/2 cells result in changes in chondrogenic gene expression. In 2.5 day micromass cultures, reduced N-cadherin function in the Δ390-10T1/2 cell line resulted in increased cytoplasmic levels and nuclear localization of ß-catenin which is accompanied by increased ratio of type IIB /type IIA collagen splice forms but no significant effect on aggrecan gene expression. On the other hand, over-expression of normal N-cadherin in the MNCD2-10T1/2 cell line resulted in reduced type II collagen expression as well as total inhibition of aggrecan gene expression. In addition, levels of nuclear and cytoplasmic ß-catenin were markedly reduced but an increase in cell membrane localization of this protein was observed. Therefore, these findings suggest that adherens junctions involving N-cadherin can control the distribution of stabilized ß-catenin (possibly by Wnt signaling), in regulating gene expression. We have further analyzed the SRF activity, a nuclear target of MAP kinase signaling implicated in chondrogenesis. In general, in semi-confluent monolayer cultures (minimum cell-cell contact) of C3H10T1/2, NCD2-10T1/2 or Δ390-10T1/2 cells, there was not a significant change in MAP kinase or BMP-2 regulation of a construct concatamer of SRF element fused with luciferase reporter gene. In micromass cultures, a culture condition required for upregulation of N-cadherin expression and initiation of chondrogenesis, the effect of MAP kinase and BMP-2 on SRF activity was proportional to the nuclear localization of ß-catenin. Specifically, the SRF promoter construct was more responsive to the above signaling in Δ390-10T1/2 micromass cultures compared to C3H10T1/2 or NCD2-10T1/2 cells. Support: NIH grant DE12864.

Disclosures: ***M.R. Seghatoleslami****, None.*

SU063

Developmental Patterning of Fracture Callus Formation: Three-Dimensional Image Analysis and Collagen Gene Expression. L. C. Gerstenfeld, Y. M. Alkhiary, D. M. Cullinane*, F. Nicholls*, D. T. Graves*, T. A. Einhorn. Boston University Medical Center, Boston, MA, USA.

While a standardized set of histological criteria have been developed to assess the metabolic status of bone, identical measurements are not fully applicable to bone repair because of the unique attributes of the endochondral process. Standardized methodologies were developed to assess fracture healing which allowed for the quantitative measure of cartilage and new bone formation, as well as overall fracture callus tissue development and

geometry. Standard mid-diaphyseal transverse closed fractures were generated in the femurs of mature male rats or mice. Fracture calluses from both species were collected at 1, 2, 3, and 5 weeks. Serial transverse sections were taken at 100 micron intervals over a total length of 7500 microns of the callus. The quantities of cartilage, bone and marrow space were determined by differential staining with Safranin-O/Fast Green, and TRAP staining was used to assess osteoclast numbers. Statistically reproducible measurements were obtained from sections taken at 500 micron increments from a minimum of three animals. Using 3D reconstruction software, the developmental patterning of the tissue formation of the fracture calluses were determined. These data showed two morphogenetic fields of endochondral development around the fracture line. The endochondral tissue that forms was not symmetrical. Rather, ~70% of the volume of tissue that forms is on the distal posterior/medial axis, and as it develops proximally the endochondral tissue rotates in an anterior/lateral direction. The confines that defined the volume of original endochondral tissue dictated the pattern of the primary remodeling of the tissue. Serial reconstruction of the pattern of collegen types I and II in situ hybridization were used to establish the developmental patterning of the tissue. Active areas of Type II collagen gene expression were localized on the surface of the callus and centrally around the fracture line. Type I collagen expression was uniformly expressed around the cortical surfaces and was greater proximally and distally away from the fracture line. Both patterns of gene expression and callus formation were identical in both species of bone that were examined. These results demonstrate that traditional sagittal views of fracture callus histology have provided a biased and incomplete description of callus formation. They further suggest that as of yet unidentified aspects of anatomical, biomechanical, and/or developmental principles affect the developmental mechanisms that regulate fracture callus morphogenesis.

Disclosures: ***L.C. Gerstenfeld****, None.*

SU064

The Collagenase, Matrix Metalloproteinase-13 (MMP-13), Is Required for Osteoclast Formation and Function. M. Inada[1], Y. Wang[1], M. H. Byrne*[1], C. Miyaura[2], S. M. Krane[1]. [1]Rheumatology CIID, Mass General Hospital, Boston, MA, USA, [2]Department of Biochemistry, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

To determine potential roles of collagenases in bone remodeling, we targeted a null mutation in mice in the MMP-13 gene splicing out Exon 5 which encodes the critical catalytic, zinc-binding domain. MMP-13 is normally highly expressed in the skeleton during embryonic development and adult bone remodeling. Previously, we presented preliminary results indicating that homozygous mutant mice (MMP-13 -/-) had increased trabecular bone mass (DEXA, microCT), decreased trabecular separation (histomorphometry, microCT) and decreased bone resorption in organ cultures of newborn calvariae in response to parathyroid hormone (PTH) or interleukin-1 alpha, suggesting altered osteoclast formation/function. Here we provide further evidence for altered osteoclast formation/function. In metaphyses of distal femurs from mice 6 weeks of age, osteoclast numbers (TRAP staining) were decreased in MMP-13 -/- vs. MMP-13 +/+ mice by ~ > 40 % (p< 0.01). Bone resorption area was also decreased (p 0.1-0.4 mm2 was strikingly decreased (p< 0.001) and collagen deposition increased (Sirius red staining) in BMC from MMP-13 -/- vs. MMP-13 +/+ mice. This reduction was associated with reduced levels of osteoclast marker mRNAs, measured by Realtime PCR. The functional capacity of the osteoclasts generated in BMC was measured by formation of pits on dentin. The pit area (mm2) /dentin slice area (mm2) formed by osteoclasts generated by PTH was reduced from ~ 8 to ~2 (p<0.01) and that by osteoclasts generated by 1,25(OH)2 vitamin D3 from ~11 to ~2 (p<0.01) in BMC from MMP-13 -/- vs. MMP-13 +/+ mice. Osteoblasts normally support osteoclast formation/function. In wild type BMC, MMP-13 is produced by osteoblasts/stromal cells but not by osteoclasts. We propose therefore that absence of osteoblast MMP-13 and increased uncleaved extracellular collagen are primarily responsible for the decreased osteoclast formation/function we observed in the MMP-13 -/- mice.

Disclosures: ***M. Inada****, None.*

SU065

Differential Effects of Selenium and Iodine on Micro-architecture of Bone in Male and Female Rats. B. J. Stoecker, F. Toure*, E. A. Lucas*, J. B. King*, B. H. Arjmandi. Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA.

Epidemiological studies suggest a relationship between a combined deficiency of selenium (Se) and iodine (I) and impaired bone metabolism. This project investigated the effects of experimental Se and/or I depletion on bone structure in young rats. Dams were fed experimental diets beginning at week 1 of lactation. Pups were weaned at 3 wks of age and a subsample of males and females were fed the experimental diet of their mother for an additional 7 wks. Weight gain was significantly reduced by I deficiency in all rats and by Se deficiency in males. Thyroid weight was increased and serum thyroxine (T4) was reduced by I deficiency. Selenium depletion was confirmed by significantly lower hepatic glutathione peroxidase activity and Se depletion tended (p<0.06) to increase T4. Tri-iodothyronine (T3) was increased in female rats. In male rats selenium depletion reduced T3 levels. Both iodine and selenium depletion decreased bone mineral area and content as measured by DEXA (Hologic QDR4500A Elite with small animal software). Three-dimensional analysis of proximal tibia by micro-computed tomography (μCT) showed a signficantly lower bone volume/total volume (BV/TV) in males than in females. In Se depleted animals, BV/TV was higher when I was adequate. The structure model index (SMI) was lower in females meaning that the structure of the bone was more plate-like. Females also had higher trabecular thickness and lower trabecular separation. In Se-depleted animals, trabecular separation was signficantly higher when I was also depleted. Modes of action of Se and I on bone require further investigation.

Disclosures: ***B.J. Stoecker****, None.*

SU066

Collagen Crosslinking Modifies the Mechanical Properties of Cortical Bone. P. Garnero[1], Y. Proust*[2], O. Borel*[2], E. Gineyts*[2], F. Duboeuf*[2], H. Solberg*[3], P. D. Delmas[2]. [1]Synarc, Inserm Unit 403, Lyon, France, [2]Inserm Unit 403, Lyon, France, [3]Nordic Bioscience, Herlev, Denmark.

Bone mineral density (BMD) is the main determinant of bone strength but the contribution of bone's material properties, particularly the extent of crosslinking (XL), is poorly understood. In vitro studies suggest that enzymatic XL including pyridinoline (PYD) and deoxypyridinoline (DPD) and non-enzymatic glycation end products such as pentosine (PEN) may play a role. We also showed that alterations of the isomerisation of C-telopeptide of type I collagen (CTX) - that can be detected in vivo by the urinary ratio of isomerized (β) to native (α) CTX -are associated with fracture risk independently of BMD and bone turnover. We analyzed the role of increasing XL on mechanical properties of bone specimens of constant size and mineral content.

Calibrated fetal bovine cortical bone specimens (11 different animals, 41 samples in total) characterized by a low degree of postranslational modifications were incubated for 0, 60, 90 and 120 days at 37°C to induce collagen crosslinking in vitro. At each time point, 3 point binding mechanical tests were performed to determine the Young modulus (an index of stiffness) and yield stress (an index of elasticity). We also measured on each sample the concentration of PYD, DPD and PEN by HPLC, α and β CTX by ELISA on trypsin bone digests, and BMD by DXA.

After 60 days of incubation, elasticity was decreased by 40% (p=0.01) -with no significant further change up to 120 days- whereas no significant effect was observed on stiffness and BMD. The decline in elasticity at 60 days was associated with increases of PYD (+98%, p=0.005), DPD (+42%, p=0.013), PEN (+ 55 fold, p=0.005) and β/α CTX ratio (+4.9 fold, p=0.005). In univariate analyses, PYD was significantly associated with stiffness (r= -0.37, p=0.003) and elasticity (r=-0.64, p<0.001). PEN was a significant predictor of both elasticity (r=-0.63, p<0.001) and of its 60 days changes (r=-0.64, p=0.04), higher PEN concentration in bone being associated with lower elasticity. The ratio β/α CTX correlated with elasticity (r= +0.46, p=0.002). In multivariate analyses, the prediction of elasticity was significantly improved by combining BMD data (r^2=0.36) with PYD (r^2 increasing to 0.58, p=0.0003), PEN (r^2 increasing to 0.58, p<0.0001) or β/α CTX (r^2 increasing to 0.49, p=0.003).

These data indicate that the degree of posttranslational modifications of collagen matrix plays an independent role in determining the mechanical competence of cortical bone. Further studies should explore potential alterations of these biochemical parameters of bone matrix in osteoporosis, as they may represent a key component of bone quality.

SU067

Detection by Immunoassay of C-terminal Telopeptides of Bone Collagen Containing Unidentified, Non-fluorescent Cross-links. K. S. Puukka*[1], M. K. Koivula*[1], S. P. Robins[2], K. Savolainen*[3], J. P. Risteli[1]. [1]Clinical Chemistry, University of Oulu, Oulu, Finland, [2]Rowett Reseach Institute, Aberdeen, United Kingdom, [3]Clinical Chemistry, Kuopio University Hospital, Kuopio, Finland.

Two immunoassays, ICTP and CrossLaps®, measure degradation of type I collagen through interaction with carboxyterminal telopeptide structures. ICTP antigen is derived from collagenase or trypsin digestion of bone type I collagen whereas CrossLaps is based on a shorter, synthetic peptide from the same region. The CrossLaps assay is sensitive for physiological bone resorption, but ICTP measures mainly pathological bone destruction. ICTP antigen contains two alfa-1 telopeptides and one helical peptide joined by mature, trivalent cross-links, e.g. hydroxylysylpyridinoline (Pyd) and lysylpyridinoline (Dpd). However, about 60 % of the trivalent cross-links are uncharacterised. To investigate the nature of these unknown cross-links ICTP peptides linking helical and alpha 1 sites were isolated from chymotrypsin digests then treated with cathepsin K. Cross-linked peptide fragments were purified using two successive RP-HPLC fractionations, monitoring both pyridinium fluorescence and CrossLaps reactivity (automatic Elecsys-instrument). The CrossLaps assay used measures only those C-telopeptides where both alfa-1-telopeptides have beta-isomerization of aspartyl residues. Cathepsin K produced five major fragments from the alfa1-alfa1-alfa1-telopeptide, only two of which contained pyridium cross-link fluorescence. The CrossLaps concentration was highest in those fractions where pyridinium cross-links were undetectable. MALTI-TOF analysis revealed differences in the molecular masses of these cross-linked fractions, with CrossLaps detected fragments having m/z values of about 1730 whereas those containing pyridinium cross-links had m/z about 2536. There was also a minor fragment between the main species (m/z about 2180). It is known that ICTP and CrossLaps can be produced from the same type I collagen molecule with different enzymes. However, our results indicate that the ICTP and CrossLaps antigens differ also in the fine structure of collagen molecules. CrossLaps mainly detects only those ICTP antigens where the cross-link is currently uncharacterised. This study provides procedures that will assist in the identification of the unknown cross-links.

Disclosures: ***J.P. Risteli****, None.*

SU068

Bone Acidic Glycoprotein-75 and Bone Sialoprotein Co-Localize in Primary Bone Prior to Mineralization. J. P. Gorski[1], A. Wang*[2], D. Law*[1], K. Powell*[2], R. J. Midura*[2]. [1]Molecular Biology and Biochemistry, Univ. of Missouri-Kansas City, Kansas City, MO, USA, [2]Biomedical Engineering, Cleveland Clinic and Foundation, Cleveland, OH, USA.

Recent studies demonstrate that bone acidic glycoprotein-75 (BAG-75) is a biomarker for biomineralization foci (BMF) in UMR-106-01 osteoblastic cultures, sites of initial bone sialoprotein (BSP) and hydroxyapatite deposition. Our goals were 1) to identify BMF in

primary bone and 2) to define relationships among BAG-75, BSP and mineral during primary bone mineralization in vivo. Primary bone was obtained from the rat tibial marrow ablation model. Our findings can be summarized as follows. First, BMF were identified which are similar in structure to those characterized in UMR cultures, e.g., spherical, punctate, acellular, matrix regions ringed by alkaline phosphatase positive cells. Second, hematoxylin and eosin stained intramedullary tissue five days after ablation is devoid of bone morphology; only cellular mesenchyme and residual fibrin clot were visible. This region contained prominent filaments containing BAG-75 and a network of thinner BAG-75 strands interspersed within the clot. We conclude that BAG-75 is expressed both by condensing mesenchymal cells replacing the fibrin clot, as well as by osteoprogenitor cells derived from pre-existing ossicles of trabecular bone. Third, using confocal microscopy, BAG-75 or BSP immunostaining was compared with nascent mineral detected by Alizarin red-S fluorescence. At the peak of osteogenesis, BSP and BAG-75 co-localized with each other in over 80% of decalcified primary bone at low magnification and this trend persisted at higher magnification yielding 75% co-localization. Both matrix proteins also exhibited a high level of co-localization with the mineral phase in undecalcified sections; there was 3-4 times more detectable protein epitope staining associated with the mineral phase than that identified separately. These values likely underestimate BAG-75 because the mineral phase appears to block antibody access. Fourth, ultrastructural immunoelectron microscopy does not support a direct association with banded fibrils. BAG-75 instead comprises a separate amorphous layer located in close proximity to the fibrillar collagenous network. Within such regions, BAG-75 immunogold particles seem to be preferentially clustered in groups of 2 to 6. Importantly, some of the BSP immunogold signal after double-labeling co-distributes within 50 nm of anti-BAG-75 particles. Our spatial analysis suggests that BAG-75 deposition in primary bone precedes that for BSP, that BSP co-localizes in part with BAG-75 at sub-micron resolution, and that mineral deposition follows BAG-75/BSP co-localization.

Disclosures: ***J.P. Gorski****, None.*

SU069

Evaluation of RANKL and OPG Gene Expression in Human and Murine Fibroblasts in Response to Orthopaedic Particulate Debris. J. T. Ninomiya, B. C. W. Law*, J. A. Struve. Department of Orthopaedic Surgery, Medical College of Wisconsin, Milwaukee, WI, USA.

Implant loosening remains the primary cause of failure in total joint arthroplasty, and this is largely felt to be due to the generation of particulate wear debris, resulting in osteolysis. Histologic examination of osteolytic membranes has revealed a predominance of macrophages and fibroblasts, and these cells are felt to be critical in the process of implant loosening. Although they are capable of producing large amounts of cytokines, prostaglandins, and proteases in response to particulate debris, little is known about their role in the regulation of the proteins RANKL and OPG. Therefore, we chose to assess the effects of orthopaedic particulate debris on the gene expression and secretion of these proteins in both human and murine fibroblasts.

Both human fibroblasts and murine BALB fibroblasts were grown in tissue culture and titanium particles were added to the cells. Controls consisted of cells grown in the absence of particulate debris. For the murine cells, vitamin D-3 was also included in the culture media. Alterations in the gene expression of RANKL and OPG were assessed using RT-PCR, while the effects on protein secretion were determined by ELISA. The functional results of the addition of the particulate debris were evaluated using a murine bone marrow osteoclast maturation assay.

Following the addition of titanium particles to the human fibroblasts, the gene expression of RANKL increased, resulting in a net increase in the RANKL/OPG ratio. This activity peaked between 6 and 12 hours after the particles were added. No detectable changes in RANKL protein levels were observed in conditioned media using ELISA for the secreted form of RANKL.

In contrast, the murine cells increased RANKL gene expression in response to particles in the presence of vitamin D-3, but not to particles or vitamin D-3 alone. Like the human cells, alterations in RANKL gene expression peaked between 6 and 12 hours following addition of the particles.

Taken together, these studies suggest that fibroblasts may alter the generation of osteolysis through modulation of the gene expression of RANKL. Both human and murine fibroblasts produced similar responses to particulate debris, suggesting that either or both of these may be useful models for the evaluation of the role of fibroblasts in the process of implant loosening.

Disclosures: ***J.T. Ninomiya****, None.*

SU070

Mechanisms of Assembly of Latent TGFβ Binding Protein-1 into Bone Extracellular Matrix. Q. Chen*[1], P. Sivakumar*[1], C. Barley*[2], D. M. Peters*[3], S. L. Dallas[1]. [1]Univ. Missouri, Kansas City, MO, USA, [2]Univ. Manchester, Manchester, United Kingdom, [3]Univ. Wisconsin, Madison, WI, USA.

Latent TGFβ binding proteins (LTBPs) are members of the fibrillin superfamily of extracellular matrix (ECM) proteins that bind and regulate transforming growth factor betas (TGFβs). Mutations in this superfamily are associated with heritable disorders that often show skeletal abnormalities. LTBP1 regulates secretion of latent TGFβ as well as its storage and release from the ECM. Given these critical functions, and the implication of TGFβ in fibrotic and other diseases, our goal is to understand the structural arrangement of LTBPs in bone ECM and the molecular mechanisms by which they regulate TGFβ.

We have previously identified fibronectin as a key molecule for assembly of LTBP1 into the ECM of osteoblasts. To further define the relationship between LTBP1 and fibronectin, a series of recombinant LTBP1 fragments were expressed and purified in a mammalian expression system. Transient transfection studies showed that an N-terminal LTBP1 fragment (a.a. 21-487) was sufficient for co-localization with fibronectin in 2T3 osteoblasts. A C-terminal fragment (a.a.1008-1394) also showed a weak association with the ECM. Surprisingly, solid phase binding assays demonstrated no direct interaction of any of the purified LTBP1 fragments with fibronectin or fibronectin fragments. However, when conditioned media containing the recombinant LTBP1 fragments was used, the N-terminal (21-487) and C-terminal (1008-1394) fragments as well as a fragment (526-1014) consisting of a central stretch of 11 tandem EGF-like repeats, bound to immobilized fibronectin, suggesting an indirect association. A heparin binding site was identified in the N-terminal LTBP1 fragment by solid phase binding assays and by heparin sepharose chromatography. Since fibronectin also contains at least two heparin binding sites, we explored the possibility that heparan sulphate containing proteoglycans (HSPGs) may mediate binding between the LTBP1 N-terminus and fibronectin. To mimic the clustered glycosaminoglycan pattern on proteoglycans, heparin coupled to bovine serum albumin was used. Treatment of osteoblast cultures with both heparin and heparin-BSA inhibited incorporation of LTBP1 into the ECM,while chondroitin-6-sulphate had no effect. Solid phase binding assays showed that heparin-BSA could mediate binding of the N-terminal LTBP1 fragment with fibronectin.

These studies suggest that heparin binding sites in fibronectin and LTBP1 may be important for assembly of LTBP1 into the ECM and suggest that HSPGs may mediate binding interactions between these two ECM proteins.

Disclosures: ***Q. Chen****, None.*

SU071

Matrix Metalloproteinase (MMP)-2-deficient Mice Exhibit Hypertelorisms, Cortical Bone Thinnings, and Short Statures, Similar to Human Osteolysis Syndrome. K. Inoue[1], Y. Mikuni-Takagaki[2], O. Kaoru[3], T. Itoh*[4], T. Noguchi*[1], J. S. Park*[1], M. Noda[3], S. Itohara*[1]. [1]Behavioral Genetics, Brain Science Institute, RIKEN, Wako, Japan, [2]Oral Biochemistry, Kanagawa Dental College, Yokosuka, Japan, [3]Molecular Pharmacology, Tokyo Medical & Dental University, Tokyo, Japan, [4]Discovery Research Laboratories, Shionogi & Co. Ltd, Osaka, Japan.

In bone tissues, various extracellular matrix (ECM) proteins are metabolized in concert and the degradation of ECM proteins in bone by matrix metalloproteinases (MMPs) is required for normal turnover. MMP-2 degrades type IV collagen as its major substrate, while it also cleaves type I collagen. However, no functional aspects of MMP-2 have been described in terms of skeletal tissue homeostasis. In the present study, we examined the effects of MMP-2-deficiency on the skeletal systems in mice. MMP-2-deficient mice developed osteoporotic phenotype (reduction in BMD) in long bones after weaning. They showed hypertelorisms, cortical bone thinnings, and short statures, which are similar to those observed in human osteolysis syndrome. It was recently reported that human familial osteolysis syndrome was linked with MMP-2-deficiency. We further analyzed cellular mechanism underlying osteoporotic phenotypes in MMP-2-deficient mice based on histomorphometry. MMP-2-deficiency did not affect bone formation parameters such as mineral apposition rate (MAR) and bone formation rate (BFR) in aged (55 wk) cancellus bones. Similarly, bone resorption parameters such as osteoclast surface and eroded surface were not affected in cancellus bones of aged MMP-2-deficient mice. In contrast, MMP-2-deficiency enhanced endosteal BFR and suppressed periosteal BFR in aged cortical bones. In addition, bone resorption (eroded surface/BS) was enhanced in cortical bones in aged MMP-2-deficient mice. Interestingly, osteoblastic and osteoclastic properties were similar between MMP-2-deficient and wild-type mice when they were examined in bone marrow-derived cell culture experiments. These findings suggest that MMP-2 is an important modulator of bone mass *in vivo*.

Disclosures: ***K. Inoue****, None.*

SU072

The Inhibition of Calcium Phosphate Precipitation by Fetuin is Accompanied by the Formation of a Fetuin-Mineral Complex. P. A. Price, J. E. Lim*. Division of Biology, University of California San Diego, La Jolla, CA, USA.

Previous studies have shown that the fetuin-mineral complex (FMC) is a complex of a calcium phosphate mineral phase and the proteins fetuin and matrix Gla protein that appears in the blood of rats with on-going, vitamin D-induced artery calcification and in the blood of rats treated with doses of etidronate that acutely inhibit bone mineralization [JBC (2002) 277:3926-34]. The present *in vitro* studies were carried out in order to better understand the mechanisms responsible for the generation of the FMC in the rat.

Our working hypothesis is that the FMC is formed *in vivo* under conditions in which fetuin inhibits the precipitation of a calcium phosphate mineral phase, a precipitation that arguably occurs in bone when resorption exceeds formation [JBMR (2002) 17:1171-79]. The first test was carried out in rat serum, since rat serum is known to contain high levels of fetuin (1.5mg/ml), and fetuin is known to be an important serum inhibitor of calcium phosphate precipitation. In this test, the concentration of calcium and phosphate in serum were each increased by 10mM and the modified serum was incubated at 37°C. The serum remained clear with no evidence of calcium phosphate precipitation over 9 days of observation, while precipitation occurred in seconds in the absence of serum under the same ionic conditions. Centrifugation and gel filtration procedures revealed the formation of large amounts of a FMC in the first 3h of this incubation, and the calcium content of the FMC accounted for over 90% of the calcium added to serum. FMC levels remained constant throughout the 9 days of incubation at 37°C.

The second test showed that the addition of purified bovine fetuin to a solution containing 5mM calcium and phosphate at pH 7.4 at 22°C inhibited the precipitation of mineral for over 14 days, while precipitation occurred in minutes without fetuin. There was a biphasic drop in ionic calcium in the fetuin solution, however, from 5 to 3mM in the first hour and from 3 to 0.9mM between 20 and 24h; these changes in ionic calcium proved to be due to

the formation of complexes of calcium, phosphate, and fetuin. The complex found after 24h appears to be identical to the fetuin-mineral complex found in the serum of etidronate-treated rats, while the complex found between 1 and 20h is less stable.
These experiments demonstrate that a fetuin-mineral complex apparently identical to that found in rat blood *in vivo* is indeed generated *in vitro* during the course of the inhibition of calcium phosphate precipitation in serum containing supersaturating amounts of calcium and phosphate, and during the course of inhibition of calcium phosphate precipitation from supersaturated solutions containing purified bovine fetuin.

Disclosures: ***P.A. Price****, None.*

SU073

Bone Repair Is Dramatically Delayed in Dentin Matrix Protein-1 (Dmp1) Deficient Mice. H. Huang[1], L. Ye[1], Y. Xie[1], S. Ko[1], S. E. Harris[1], L. Bonewald[1], Y. Mishina[2], J. Q. Feng[1]. [1]Oral Biology, School of Dentistry, University of Missouri-Kansas City, Kansas City, MO, USA, [2]NIEHS/NIH, Research Triangle Park, NC, USA.

Fracture healing is a complex process that involves both endochondral and intramembranous ossification. Dmp1, an acidic matrix protein, is expressed in both cartilage and bone cells. To address whether Dmp1 is critical for bone repair, rib fractures were generated in wild-type and Dmp1 deficient mice in both sexes and samples collected on days 7, 14 and 21 after fracture. In control animals, Dmp1 expression was highly increased in hypertrophic chondrocytes followed by lower expression in bone cells within the fracture callus, suggesting that Dmp1 is involved in this healing process. In the Dmp1 deficient animals, formation of callus and union was dramatically delayed (see Table). In a search for potential mechanisms for delay of bone healing, we found that Dmp1 null mice display the following: 1) a profound defect in mineralization, not due to a systemic defect in calcium/phosphate metabolism as serum levels of calcium and phosphate were similar to those in the control mice; 2) a severe delay in blood vessel invasion which could be responsible for the reduction in osteoclast numbers in fracture areas as spleen cells from Dmp1 null mice produce similar numbers of osteoclasts as control animals *in vitro*; and 3) delayed expression of Cbfa1, Col I, and Col II suggesting a delay in osteogenesis and chondrogenesis. Based on these observations, defects in vascular invasion in this model could be due to defective matrix produced by chondrocytes and osteoblasts that is incapable of supporting blood vessel invasion.

Table Radiographic Assessments of Fracture Healing

	Day14 after fracture		Day14 after fracture	
	Callus	Union	Callus	Union
Wild-type	5/8	3/8	8/8	6/8
Dmp1-/-	2/8	0/8	3/8	1/8

Disclosures: ***H. Huang****, None.*

SU074

Smad3 Interacts with JunB and Cbfa1/Runx2 for Transforming Growth Factor-beta1-Stimulated Collagenase-3 Expression in Human Breast Cancer Cells. N. Selvamurugan, S. Kwok*, N. C. Partridge. Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA.

The specific characteristics of breast cancer cells and the unique properties of the bone/bone marrow microenvironment are largely responsible for the oberved high frequency of skeletal metastases from breast cancer. A series of recent studies have indicated that collagenase-3 (matrix metalloproteinase-13) is overexpressed in a variety of tumors from diverse sources. Collagenase-3 is characterized by its ability to degrade the extracellular matrix (ECM) and is stimulated by TGF-β1 (enriched in bone matrix) in the human breast cancer cell line, MDA-MB231. Collagenase-3-driven ECM proteolysis may support cancer cell growth by exposing the cells to growth factors and cytokines, resulting in bone degradation (osteolysis). A greater understanding of the regulatory mechanisms that control collagenase-3 expression may provide several new avenues for therapeutic intervention. Hence, our interest is to identify the signaling and molecular mechanisms responsible for TGF-β1-stimulated collagenase-3 expression in human breast cancer cells. We have recently shown that transcriptional activation of collagenase-3 by TGF-β1 is via MAPK and Smad pathways in these cells (N. Selvamurugan, Z. Fung, and N.C. Partridge (2002): FEBS Letters 532, 31-35). To understand the molecular mechanisms responsible for this TGF-β1-response, a functional analysis of the promoter region of the collagenase-3 gene was carried out and we identified the distal RD (runt domain) and proximal RD/AP-1 (activator protein-1) sites as necessary for full TGF-β1-stimulated collagenase-3 promoter activity. Gel shift, real time RT-PCR and Western blot analyses showed increased levels of c-Jun, JunB, and Cbfa1/Runx2 upon TGF-β1 treatment in MDA-MB231 cells. Functional importance of the AP-1 factors and Cbfa1/Runx2 for TGF-β1-stimulated collagenase-3 promoter activity was identified by overexpression of antisense-Fra1 and dominant repressor, Cbfa/ETO expression plasmids, respectively. Further analysis of the proximal RD/AP-1 site by biotinylated DNA precipitation experiments showed no direct binding of Cbfa1/Runx2 to this site. Co-immunoprecipitation *in vitro* studies identified no physical interaction between JunB and Cbfa1/Runx2; whereas Smad3 interacted with both. Taken together, our results suggest that TGF-β1 stimulated JunB and Cbfa1/Runx2 proteins bind to their respective DNA consensus sites and Smad3 stabilizes their interaction to confer functional TGF-β1-stimulation of collagenase-3 expression in MDA-MB231 cells.

Disclosures: ***N. Selvamurugan****, None.*

SU075

Development of an "All-Human" Animal Model of Breast Cancer Metastasis to Bone. C. Kupperwasser*[1], D. Garnet*[2], K. Sperandio*[2], R. A. Weinberg*[1], M. Rosenblatt[2]. [1]Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA, USA, [2]Dept. of Medicine/Division of Bone & Mineral Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA.

A common and serious complication of breast cancer is the development of bone metastasis. Fractures, pain, hypercalcemia, and other disorders often occur as a result of bone metastasis, and the appearance of a skeletal metastasis signals that the disease has entered an incurable phase. We are interested in identifying the mechanisms underlying breast cancer osteotropism, i.e. the molecular basis by which cancer cells adhere to and subsequently establish secondary colonies within bone. In an effort to better understand the biology of cancer osteotropism, we developed a mouse model that replicates the multi-step process by which breast cancer metastasize to bone.
In this model, human bone fragments are implanted subcutaneously in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. These bone grafts are viable and the marrow functional post-engraftment as evidenced by: 1) histologically normal bone cells and architecture; 2) the presence of human immunoglobulin (IgG) in mouse circulation; and 3) the presence of human B-cells in the spleen of mice. In addition, striking neovasculature arises to supply the bone graft. We tested a panel of cell lines for their ability to metastasize to bone after I.V., I.P. or orthotopic (mammary pad) injections. Of the several lines tested, one metastasized to human bone by all three routes of administration, albeit with low frequency. Despite the ectopic extra-skeletal location of the human bone implant, SUM-1315 cells (derived from a metastatic nodule of a patient with breast cancer and bone metastases; generously provided by Dr. S. Ethier) appeared to metastasize preferentially to human bone over the mouse skeleton.
We plan to harvest SUM-1315 metastatic tissue from human bone implants in order to perform gene expression profiling using DNA-microarray analysis. In this manner, we will attempt to identify genes specific for metastases and/or osteotropism. We also plan to refine the model into an assay which can be used to evaluate genes for their role in the osteotropic phenotype or potential skeletal metastasis-specific drugs.

Disclosures: ***M. Rosenblatt****, None.*

SU076

Paracrine Activation of PDGFR Tyrosine Kinase in Osteoblasts Is Critical to Osteosclerotic Bone Metastases in Breast Cancer Producing PDGF-BB. T. Tsutsumimoto*, B. Yi*, P. Williams*, B. Story*, T. Yoneda. Div Endocrinol, Univ TX Hlth Sci Ctr, San Antonio, TX, USA.

Metastatic cancer cell colonization in bone is significantly influenced by the interactions with bone microenvironment. In osteolytic bone metastases, establishment of a vicious cycle between tumor-produced PTH-rP and osteoclastic bone resorption is shown to be critical. On the other hand, tumor-bone partnership in osteosclerotic bone metastases is still poorly understood. Since osteoblasts play a central role and a recent study has suggested the importance of tumor-produced PDGF-BB in osteosclerotic bone metastases, we studied the interactions between tumor-produced PDGF-BB and osteoblasts using a model of the MCF-7 human breast cancer cells transfected with the proto-oncogene Neu (MCF-7/Neu). MCF-7/Neu cells produced hPDGF-BB and caused osteosclerotic bone metastases. MCF-7/Neu cells showed little expression of PDGF receptor-alpha and -beta (PDGFRa and PDGFRb) by RT-PCR, indicating that PDGF-BB is a paracrine rather than an autocrine factor in MCF-7/Neu cells. On the other hand, we found PDGFRa and PDGFRb expression in cells of osteoblast lineage including C3H10T1/2, C2C12, ST2, and MC3T3-E1 cells. PDGFR expressed in these cells were functional, because hPDGF-BB induced tyrosine autophosphorylation of PDGFR and stimulated cell proliferation. Of interest, western analysis showed that hPDGF-BB also increased osteopontin (OPN) production. Furthermore, a selective PDGFR tyrosine kinase (PDGFR TK) inhibitor AG1296 inhibited PDGFR tyrosine autophosphorylation, cell proliferation and OPN production induced by hPDGF-BB but not EGF and FGF-2 in these osteoblastic cells. In contrast, MCF-7/Neu cell growth was not inhibited by AG1296. AG1296 inhibited hPDGF-BB-stimulated bone formation in mouse neonatal calvariae in organ culture, suggesting a critical role of PDGFR TK in bone formation. We then examined the effects of AG1296 on osteosclerotic bone metastases in vivo. AG1296 (1.25mg/mouse, ip, every 4 days for 6 weeks, total 11.25mg/mouse) significantly decreased MCF-7/Neu tumor burden in bone metastases. Furthermore, AG1296 (1mg/mouse, ip, 3 times/week for 3 weeks, total 9 mg/mouse) also significantly decreased bone metastases in the MDA-MB-231 human breast cancer cells transfected with hPDGF-B cDNA. In conclusion, our results suggest that activation of PDGFR TK signaling pathways in osteoblasts by breast cancer-produced PDGF-BB contributes to the promotion of osteosclerotic bone metastases. The results also suggest that PDGFR TK may be a potential molecular target in the treatment of osteosclerotic bone metastases in PDGF-producing breast cancers.

Disclosures: ***T. Tsutsumimoto****, None.*

SU077

Opposite Effects of Clodronate and Ibandronate on the Growth of Estrogen Receptor-Positive Breast Cancer Cells in Steroid-Free Medium. F. Journe*[1], C. Chaboteaux*[1], G. Laurent*[2], F. Place*[1], J. C. Dumon*[1], J. J. Body[1]. [1]Bone Diseases Unit, Institut Bordet, Université Libre de Bruxelles, Brussels, Belgium, [2]Laboratory of Histology, Université de Mons-Hainaut, Mons, Belgium.

The skeleton is the most common site colonized by metastatic breast cancer cells, especially those expressing estrogen receptor (ER). Neoplastic cells stimulate osteoclast-medi-

ated bone resorption and bisphosphonates have emerged as a logical approach for the treatment of bone metastasis. Besides their powerful anti-osteoclast activity, we and others have recently demonstrated that bisphosphonates induce growth inhibition and apoptosis of breast cancer cells in vitro (e.g. Fromigué et al, JBMR, 2000). In the present study, we have investigated the effects of two structurally distinct bisphosphonates on MCF-7 breast cancer cells cultured in steroid free medium, with particular emphasis on ER expression and activity. Tested compounds were clodronate (Clod) and ibandronate (Iban). MCF-7 cells were cultured in RPMI 1640 medium supplemented with charcoal-stripped fetal bovine serum to obtain a steroid-free medium (SFM). Cell growth was estimated by photometry after crystal violet staining. In SFM, Clod stimulated cell growth in dose- and time-dependent manners (mean 46% increase after 3 days of exposure to 10^{-4} M Clod). This mitogenic effect of Clod was not detected when cancer cells were cultured in medium with complete serum. As could be expected, 17β-estradiol (E_2) also produced a growth stimulatory effect in steroid-free conditions (mean 77% increase after 3 days of culture with 10^{-8} M E_2), but combination of E_2 and Clod did not significantly enhance the effect of the latter. Moreover, partial (4-OH-tamoxifen) and pure (RU 58 668) antiestrogens (10^{-7} M) completely suppressed the proliferative activity of Clod, suggesting that it is mediated by an activation of ER. In accordance with this view, Clod induced ER downregulation, weakly increased progesterone receptor expression and stimulated transcription of an estrogen-responsive reporter gene. In contrast, in the same steroid-free conditions, Iban still inhibited cancer cell proliferation (mean 39% decrease after 3 days of incubation with 10^{-4} M Iban in SFM). Furthermore, Iban completely abolished the mitogenic effect of E_2 and reinforced the growth inhibitory action of pure antiestrogen in an additive fashion. In conclusion, we report a previously unknown stimulating effect of Clod on MCF-7 breast cancer cells growth, occurring through direct or indirect ER activation in absence of cognate ligand. In the same conditions, Iban inhibited MCF-7 cell growth, prevented E_2 stimulatory effects and exerted additive effects with pure antiestrogens.

Disclosures: ***F. Journe****, Hoffmann - La Roche 2.*

SU078

Influence of Chemotherapy (AC) on Bone Mineral Density (BMD) and Bone Ultrasonometry (QUS) in Women with Breast Cancer. P. Hadji, C. Maskow*, M. Gottschalk*, V. Ziller*, F. Fischer*. Dept. of Menopause and Osteoporosis, Philipps-University of Marburg, Marburg, Germany.

Introduction: The aim of this prospective, case-control pilot study was to investigate the influence of chemotherapy (Adriamycin/Cyclophosphamid) on BMD and QUS in pre- and postmenopausal women with breast cancer.
Material and Methods: We included 16 patients (12 pre- and 4 postmenopausal), mean age 47,1 ± 8,3 years with an incident diagnose of breast cancer who received a chemotherapy (4 cycles of AC) and 16 age- and BMI-matched controls. Women with metastases, a history of osteoporosis with or without fracture, diseases or treatments known to effect bone metabolism were excluded from the study. BMD was measured by DXA (DPX-L, GE/Lunar) at spine and hip. QUS was performed at the os calcaneus using the Achilles device (GE/Lunar) and at the phalanges using the Bone-Profiler (IGEA). Measurements were performed at baseline (before chemotherapy), after 6 and 12 months and were compared with measurement results of the age- and BMI-matched control group.
Results: DXA results of the spine and hip showed a linear decrease of T- and Z-score in patients with chemotherapy compared controls, but the decrease did not reach statistical significance. In accordance to QUS results, measurement at the os calcaneus showed a similar linear decrease of T- and Z-score without reaching statistical significance. In contrast, QUS results at the phalanges showed a significant decrease of AD-SOS, for T- and Z-Score ($p \leq 0{,}008$), with the largest difference between baseline and 12 months T-score ($p \leq 0{,}001$).
Conclusion: The result of our prospective, case controlled pilot study confirms the deleterious influence of chemotherapy on BMD in women with breast cancer. This effect could be observed by DXA and additionally by QUS for the first time in this regard. Further, large scale longitudinal studies are need to improve our understanding of the mechanism of bone changes during chemotherapy.

Disclosures: ***P. Hadji****, None.*

SU079

Pamidronate Reduces the Severity of Bone Lesions in an Osseous Prostate Cancer Mouse Model by Decreasing Osteoprotegrin and PTHrP. D. W. Burton[1], J. Geller*[2], M. Yang*[2], I. Barken*[2], R. M. Hoffman*[2], L. J. Deftos[1]. [1]Medicine, University of California and SDVAMC, San Diego, CA, USA, [2]AntiCancer, Inc., San Diego, CA, USA.

Prostate cancer is the most prevalent tumor in men and bone metastases are responsible for most of the morbidity associated with this tumor. Bisphosphonates have been shown to attenuate prostate tumor progression in bone, but the mechanism of this action is not clearly understood. This study evaluated the effects of the bisphosphonate, Pamidronate, on the growth of PTHrP-expressing PC-3 prostate cancer cells in a mouse model for prostate cancer in bone. The PC-3 cells were genetically engineered to express green fluorescent protein (GFP) in order to enable convenient and rapid visualization and quantitation of the tumor mass. Immunocompromised mice were injected with PC-3-GFP cells into the bone marrow of the right tibia. The left tibia served as the negative control leg. One month prior to inta-tibial implantation of the prostate cancer cells, the animals received 1.44 mg (low dose) or 7.2 mg (high dose) of Pamidronate i.v./kg body weight. Following the injection of PC-3-GFP cells into the tibias, both groups of mice received monthly doses of Pamidronate i.v. at the same concentrations respectively for 2 months. The control animals received PBS using the same injection protocol. At the end of the study, the mice were imaged for GFP expression, the skeletons analyzed for abnormalities by radiography, and the sera assayed for calcium, PTHrP and selected cytokines and osteokines. The mice that were treated with the high dose of Pamidronate demonstrated a significant reduction in the severity of the bone lesions compared to the control mice as assessed by X-ray and GFP imaging. The low dose group did not demonstrate any significant changes from the control group. The GFP and X-ray score results from the skeletons demonstrated a very high correlation ($r = 0.783$, $P < 0.01$). Once the tumor cells starting growing outside the bone, Pamidronate had little effect on the tumor growth. Serum osteoprotegrin (OPG) and PTHrP levels were significantly decreased in the high dose Pamidronate group compared to the control group (76.5%, $P < 0.001$ and 33.3%, $P < 0.05$, respectively). No significant changes were noted in serum calcium or interleukin-6 or -8 levels between the groups. Our studies demonstrate that GFP imaging can be used to assess the progression of prostate cancer. Pamidronate's effect on decreasing prostate cancer growth in bone was associated with a decrease in serum OPG and PTHrP. Further studies are need to elucidate the signaling cascade of bisphosphonates' effects on the skeletal progression of cancer.

Disclosures: ***D.W. Burton****, None.*

SU080

Regulation of Prostate Cancer Progression in Bone by PTHrP. D. W. Burton[1], I. Barken*[2], J. Geller*[2], R. M. Hoffman*[2], L. J. Deftos[1]. [1]Medicine, University of California and SDVAMC, San Diego, CA, USA, [2]AntiCancer, Inc., San Diego, CA, USA.

We and other investigators have previously reported that PTHrP is widely expressed by human prostate cancer tissue, suggesting that PTHrP might be involved in the progression of this tumor. In order to evaluate the effect of PTHrP on tumor progression in bone, we studied the DU 145 cell line in a mouse model for prostate cancer. The DU 145 cell line was selected because it has a low constitutive PTHrP expression and does not grow well in mouse tumor models. We studied four types of DU 145 cells: 1. Wild type cells, 2. Vector (pCi-neo) transformed cells 3. PTHrP 1-87 transformed cells and 4. PTHrP 1-173 transformed cells. The PC-3 cell line (group 5) was also used as a known prostate cancer cell line that produces extensive bone lesions in immunocompromised mice. The cells were directly injected into the femurs of SCID mice and the mice were evaluated 60 days later for biochemical changes in the sera (see Table 1) and skeletal abnormalities by X-ray (see Figure). Quantitation of the radiographic images of the mouse femurs showed progression of tumor in bone that correlated with PTHrP production by the tumor. The DU 145-PTHrP transformed groups demonstrated increased bone lesions, serum calcium and PTHrP. The PTHrP produced by the DU 145-PTHrP1-173 cells was less than the DU 145-PTHrP1-87, but the femur radiographs nevertheless showed more tumor damage in the DU 145-PTHrP1-173 mice. Our results provide more evidence that PTHrP expression by prostate cancer cells promotes the development of skeletal lesions by the prostate cancer. Furthermore, 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 tumor burden in the bone. 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.

Disclosures: ***D.W. Burton****, None.*

SU081

Apoptotic Effects of Parathyroid Hormone Related Protein in Prostate Cancer Cells. V. V. Maheshwari[1], D. W. Burton[1], R. H. Hastings[2], J. A. Aguilera*[3], F. S. Pardo*[3], L. J. Deftos[1]. [1]Medicine, University of California and SDVAMC, San Diego, CA, USA, [2]Anesthesiology, University of California and SDVAMC, San Diego, CA, USA, [3]Radiology, University of California, San Diego, CA, USA.

Parathyroid hormone-related peptide (PTHrP) is an autocoid regulator cell of growth, acting via autocrine, paracrine and intracrine pathways. Studies by us and our colleagues have demonstrated that PTHrP regulates prostate tumor development, growth, progression and metastasis to bone, but little is known about its role in apoptosis in prostate cancer. We performed apoptosis studies using wild-type DU 145 cells prostate carcinoma cells and DU 145 cells stably transformed to express various PTHrP peptides. We evaluated PTHrP's effect on survival with clonogenic-cell survival assays. Suspended cells were exposed to gamma irradiation (4 Gy) and replated in triplicate into 60-mm dishes. colonies (>50 cells) were counted after 14 days in culture. Multiple clones of DU 145 PTHrP 1-173 expressing cells demonstrated a 2-fold increase in colony formation compared to the vector control transformed cells ($P < 0.01$). Conversely, DU 145 PTHrP 1-87 and 1-141 expressing cells demonstrated a 50% and 30% decrease respectively in colony formation after gamma irradiation compared to the vector control cells. We also evaluated PTHrP's effect on staurosporine-induced apoptosis as measured by nuclear condensation and caspases-3 and -9 activities. The DU 145 PTHrP 1-173 expressing cells showed a reduction in nuclear condensation and caspases-3 and -9 activities ($P < 0.05$, $P < 0.01$ and $P < 0.05$, respectively) compared to the vector-control cells. DU 145 PTHrP 1-87 and 1-141 expressing cells increased caspases-3 and -9 activities slightly compared to vector control cells. The effects of PTHrP peptides on staurosporine-induced apoptosis were studied in wild-type DU 145 cells. PTHrP140-173 peptide treatment decreased caspases-3 and -9 activities and nuclear condensation compared to vehicle treated cells. No significant effects on nuclear condensation and caspases-3 or -9 were observed with treatment with PTHrP 1-34 or scrambled PTHrP 140-173 peptide. Since protective effects on apoptosis were not observed for PTHrP 1-34 peptide or PTHrP 1-87 and 1-141 gene transfer, the human-specific 140-173 region appears responsible for the anti-apoptotic properties of PTHrP in prostate cancer cells through a paracrine mechanism. The pro-apoptotic effects of PTHrP 1-87 and 1-141 may result from an intracrine, nuclear targeting pathway. Further studies are necessary to understand PTHrP's role in prostate cancer apoptosis.

Disclosures: ***D.W. Burton****, None.*

SU082

Osteoblastic Properties of Prostate Cancer Bone Metastases are Dependent on Notch Signaling and ERK Activation. M. Zayzafoon, S. A. Abdulkadir*, J. M. McDonald. Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Prostate cancer bone metastases are characterized by their ability to induce osteoblastic lesions and local bone formation. The pathophysiology of this skeletal response is not currently known. Recently, many reports have indicated that bone metastatic prostate cancer cells are osteomimetic and capable of expressing genes and proteins typically expressed by osteoblasts. The ability of these cells to express osteoblastic genes and to modify their phenotype requires the activation of many pathways, most importantly, the Notch's. Notch1 activation by Dll1 (a Notch ligand) has been recently shown to play a role in inducing osteoblast differentiation. To determine the pattern of Notch expression in skeletal and non-skeletal prostate cancer metastases, we examined gene and protein expression in different metastatic prostate cancer cell lines and in human prostate cancer bone metastases. Our results demonstrate that Notch1 expression is increased 4-5 times in bone derived osteoblastic metastatic cancer cell lines (C4-2B and PCa 2b) compared with lymph node and brain derived cell lines (LNCaP and DU145). Notch1 ligand, Dll1, is expressed only in C4-2B cells. In addition, we demonstrate by immunohistochemistry that Notch1 is present and active in both human prostate cancer bone metastases as well as C4-2B cells. To determine if prostate cancer bone metastases respond to osteogenic induction in a similar way to osteoblasts, C4-2B cells were cultured in osteogenic media that promotes mineralization. C4-2B cells mineralize, like osteoblasts, an effect that is completely inhibited by L-685,458, a Notch activity inhibitor. Interestingly, osteogenic media increases ERK activation independent of Notch signaling. HES-1 expression (a downstream target of Notch activation) is 20-fold increased in C4-2B cells when compared to non-skeletal prostate cancer metastases. This expression is inhibited by 10 uM L-685,458 but not altered by osteogenic media or ERK inhibition by 10 uM U0126. Runt-related transcription factor 2 (Runx2) DNA binding activity is increased in response to osteogenic induction and is inhibited by both L-685,458 and U0126. Supershift analyses indicate that both HES1 and Runx2 are involved in this response. These studies demonstrate that prostate cancer bone metastases acquire osteoblastic properties through independent activation of ERK and of Notch signaling, presumably both pathways being activated in the bone microenvironment.

Disclosures: ***M. Zayzafoon****, None.*

SU083

Pamidronate in the Prevention of Chemotherapy Induced Bone Loss in Premenopausal Women with Breast Cancer. M. Salamoun*[1], A. Shamseddine*[2], A. Chehal*[2], Y. Abou Mourad*[2], Z. Salem*[2], Y. Arslanian*[2], G. El-Hajj Fuleihan[1]. [1]Calcium Metabolism and Osteoporosis Program, American University of Beirut, Beirut, Lebanon, [2]Hematology Oncology Division, American University of Beirut, Beirut, Lebanon.

Overall mortality from breast cancer has decreased worldwide. Improved survival in such patients is partially due to the successful use of adjuvant chemotherapy, a therapy that is not without cost however. Indeed, a common side effect of adjuvant chemotherapy is ovarian failure and ensuing bone loss, bone loss that would be projected to increase these patients' risk of fracture with aging. Limited however is the information on therapies to prevent such anticipated bone loss. This issue is of particular concern in our country, where we observed an increased prevalence of pre-menopausal breast cancer and a lower peak bone mass compared to Western populations.

We describe below the results of a randomized, double-blind controlled trial using pamidronate (APD) 60 mg intravenously versus placebo (PBO) every three months in 38 premenopausal women with non-metastatic breast cancer. Chemotherapy in the APD group included: 16 FAC (5-FU, Adriamycin, Cyclophosphamide) or FAC like therapies, 2 CMF (Cyclophosphamide, Methotrexate, 5-FU); 2 Cisplatinum-vinorelbine followed by FAC; in the PBO 15 FAC/FAC like, 2 CMF and 1 Cisplatinum-vinorelbine followed by FAC. 26 women received tamoxifen: 13 were randomized to APD and 13 to PBO. All subjects were advised to take a calcium/D supplement if their diet was insufficient in these nutrients. Bone mineral density (BMD) was measured using DXA and % change in BMD was compared between the two groups using t-test. Numbers expressed as means (±SD).

The mean age of the patients was 40 (6) yrs, BMI 27 (5) kg/m^2, 21 (60%) patients became amenorrheic within the first year of chemotherapy, 11 (61%) in the APD and 10 (59%) in the PBO group. The mean age at study entry of women who became amenorrheic was 42 (5) years, and was 38(5) years in the women who did not become amenorrheic, p=0.02. At 12 months, BMD increased significantly in the APD group by 2.2 (4)% at the spine and was stable at the total hip 0.6 (5) %, whereas BMD decreased significantly in the PBO group by -2.8(5)% at the spine, and by -2.3 (5)% at the total hip. There was a significant difference in BMD % change at 12 months between the two treatment groups at the lumbar spine only, p=0.002. Treatment was well tolerated, only 1 subject reported a flu-like syndrome with her first injection that did not recur with following ones. Cyclical therapy with APD 60 mg every 3 months is well tolerated, can be conveniently administered at the time of the chemotherapy, and prevents chemotherapy-induced bone loss in pre-menopausal women with breast cancer.

Disclosures: ***G. El-Hajj Fuleihan****, Novartis 2.*

SU084

Breast Cancer Cells Promote Osteoclast Survival by Activating PI3 kinase/ Akt Pathways : Possible Involvement of M-CSF. M. Gallet*, N. Sévennet*, I. El Hajj Dib*, R. Mentaverri*, C. Prouillet*, A. Wattel*, M. Brazier, S. Kamel. Laboratoire de Biologie et Pharmacie Cliniques, UMRO, Amiens, France.

Osteolytic bone metastases are the major complication occurring in breast cancer patients. The osteolytic lesions during bone invasion are believed to be caused by osteoclast-activating factors, which are released by tumor cells in the bone environment. Some of these factors are well known to stimulate osteoclast differentiation and thus bone resorption. Herein, we investigated whether breast cancer cells can directly stimulate osteoclast survival, a mechanism, which contributes to the osteolytic potential of breast cancer cells.

Mature osteoclasts were isolated and purified from long bones of 10-day-old New Zealand rabbits. Conditioned media were prepared from MDA-MB-231 cells. Osteoclast apoptosis was evaluated by Hoechst staining and DNA ladder formation.

MDA-MB-231 conditioned media inhibited significantly and dose-dependently osteoclast apoptosis. When osteoclasts are cultured in 20 % conditioned media harvested from breast cancer cells, apoptosis was decreased by approximately 60%. We then investigated the signaling pathways involved in the anti apoptotic effect of conditioned media. By using PD98059, an ERK1-2 inhibitor and LY294002, a PI3 kinase/Akt inhibitor, we demonstrate that both pathways are involved. LY294002 dramatically inhibited the conditioned media-induced survival suggesting that PI3 kinase/Akt constitutes the major pathway. PI3 kinase/ Akt and ERK pathway are known to be survival pathway triggered by many growth factors and cytokines, such as TNFa, IGFI, IGFII and M-CSF. These factors are all secreted by breast cancer cells and could interact with mature osteoclasts, which express their receptors. In order to identify the possible factors secreted by MDA-MB-231 cancer cells, responsible for the anti apoptotic effect, we used neutralizing antibodies anti-TNFa, anti-IGFI, anti-IGFII and anti-M-CSF. Anti-M-CSF strongly prevented the anti apoptotic effect. Blockade by anti-IGFs was much less pronounced and anti-TNFa was without effect. Finally, using rh-TNFa, rh IGFI, rh IGFII and rh M-CSF ranged from 0.1 to 100 ng/ml, we checked the direct effects of this components on osteoclast apoptosis. We demonstrate that among these factors, only M-CSF increased significantly and dramatically osteoclast survival.

Taken together, these results demonstrate that breast cancer cell-derived factors promote osteoclast survival by activating PI3 kinase/Akt pathway. M-CSF was identified as the major factor responsible for the anti-apoptotic effect suggesting that M-CSF may be a potential therapeutic target in bone metastasis treatment.

Disclosures: ***M. Gallet****, None.*

SU085

Bone Morphogenetic Protein Signaling in Prostate Cancer Cell Lines. K. D. Brubaker, R. L. Vessella*, L. G. Brown*, E. Corey. Urology, University of Washington, Seattle, WA, USA.

Prostate cancer (CaP) cells express bone morphogenetic proteins (BMPs), which stimulate bone formation, implying a role in the development of osteoblastic lesions associated with CaP. Furthermore, CaP cells express BMP receptors (BMPRs) suggesting the possibility of direct effects of BMPs on CaP cells. The objective of this study was to evaluate the effects of BMP-2 and BMP-4 on CaP cells.

BMPs -2 and -4 exhibited growth inhibitory effects on androgen-sensitive LNCaP cells, while eliciting no effect on proliferation of androgen-independent PC-3 cells. Flow cytometric analysis revealed that BMP-2 treatment caused G1 arrest in LNCaP cells. Also, $p21^{CIP1/WAF1}$, a cyclin-dependent kinase inhibitor involved in G1 cell cycle arrest, levels increased in LNCaP cells after BMP-2 treatment. Previously, we reported that both LNCaP and PC-3 cells express BMPR II, but that only LNCaP cells express BMPR IB. It is possible that the inhibitory response of BMPs on CaP cells requires BMPR IB receptor. To determine whether both LNCaP and PC-3 cells posses intact BMP signaling, we evaluated activation of SMAD-1, a signal transducer downstream of BMPRs. Interestingly, the treatment with BMP-2 and BMP-4 increased phosphorylation of SMAD-1 in both LNCaP and PC-3 cells. To further increase our understanding of the effects of BMP treatment on CaP cells, we evaluated the levels of osteoprotegerin (OPG), a factor involved in bone remodeling, in the media of LNCaP and PC-3 cells after BMP treatment. BMP-2 treatment decreased OPG production in LNCaP, but significantly stimulated the levels in PC-3 cells.

In summary, our results demonstrate that BMPs exhibit direct effects on CaP cells, and that these effects are different in androgen-sensitive LNCaP and androgen-insensitive PC-3 cells. Detailed understanding of the effects of BMPs on prostate cancer and its bone metastases will require further studies.

Disclosures: ***E. Corey****, None.*

SU086

Export of TR3 Orphan Nuclear Receptor Mediates Rapid Mitochondrial Effects of Vitamin D Receptor Agonists and Antagonists. J. Barsony, K. Prufer. Laboratory of Cell Biochemistry and Biology, NIH/NIDDK/DHHS, Bethesda, MD, USA.

We have recently discovered that our vitamin D receptor (VDR) antagonist, BCA11, induces growth inhibition in various cancer cells, similarly to VDR agonists such as calcitriol. To understand the mechanisms of this growth inhibition, we explored both the effect of BCA11 on VDR coregulator binding and on TR3 orphan nuclear receptor export. TR3 export has recently been reported to induce mitochondrial permeabilization, cytochrome c release, and growth inhibition. We used GST-pull-down experiments to characterize BCA11 effect on corepressors and coactivators binding to VDR and TR3. To

visualize subcellular trafficking and protein interactions in real-time, we used microscopy on cells expressing fluorescent protein chimeras of VDR, RXR, TR3, and histone deacetylase 3 (HDAC3). GST pull-down assays showed that both VDR agonists and VDR antagonists induce corepressor release, although only the agonists induce coactivator binding. Microscopy showed that TR3 translocates from the nucleus into the cytoplasm within one hour after treatment with calcitriol or BCA11 in MCF7 breast cancer and LNCaP prostate cancer cells. One-hour treatment with calcitriol and BCA11 also induced cytochrome c release from mitochondria as shown by Western blot analysis of MCF7 cell extracts. Experiments in COS7 cells demonstrated VDR-BFP expression was necessary for both calcitriol and BCA11 induced GFP-TR3 export, but VDR was not necessary for TPA induced GFP-TR3 export. Analysis of VDR and TR3 export mechanisms revealed that both calcitriol and BCA11 treatment results in the loss of Crm-1 dependent export of VDR and the gain of Crm-1 mediated export of GFP-TR3. Additional experiments indicated that the swap of corepressor-associated HDAC3 might be responsible for the hormone-induced switch of Crm-1 dependent export from VDR to TR3. Our results reveal a novel mechanism for the rapid hormone-induced TR3 export from the nucleus and suggest that this TR3-mediated mitochondrial pathway plays a role in the growth inhibitory effects of calcitriol agonists and antagonists.

Disclosures: ***J. Barsony****, None.*

SU087

Osteoporosis Screening in Primary Care with Heel Bone Densitometry for Women Aged 65 and Over. J. D. McCrea[1], T. Hewer*[2], D. G. Hayes*[3]. [1]Rheumatology, Cumberland Infirmary, Carlisle, Cumbria, United Kingdom, [2]Bone Densitometry, West Cumberland Hospital, Whitehaven, Cumbria, United Kingdom, [3]Brunswick Medical Group, Carlisle, Cumbria, United Kingdom.

Access to bone mineral densitometry (BMD) in the UK is usually restricted to selective case finding of individuals considered to be at high risk for osteoporotic fracture. This pilot study was designed to examine the feasibility of measuring heel bone BMD in all women over 65 in a primary care setting.

All female patients aged 65 or over (n = 1013) registered with the group were eligible for inclusion in the study. Seventy eight were excluded (previous dxa scan in 49, frailty in 29). Those remaining were invited for a heel BMD scan using a PIXI® (GE Lunar) densitometer. A clinical risk factor questionnaire was also sent to those who agreed to participate. Patients had height and weight measurements performed at the time of scanning. Modified Study of Osteoporotic Fractures risk factor score, Fracture Index score, Osteoporosis Screening Tool score and Femur T score equivalent were calculated for all patients. Manufacturer's T score recommendations were adopted for classification of results: i.e. normal ($T \geq -0.5$), osteopenia ($-1.6 > T \leq -0.6$), osteoporosis ($T \leq -1.6$). Treatment was advised for osteoporotic patients and osteopenic patients on oral steroids or with fractures. Patients with T scores between – 1.3 and – 1.5 without fractures or steroids were referred for central dxa along with patients with normal density and fractures.

One hundred and seventy eight patients declined to participate, 279 failed to reply, 49 did not attend and 4 patients were excluded (previous scan in 3, frailty in 1). Four hundred and thirty two patients entered the study but 3 could not be scanned for technical reasons. Fractures occurring after age 50 were found in 25 of 195 normals (12.8%), in 28 of 106 osteopenics (26.4%) and 34 of 128 osteoporotics (26.6%). A treatment decision was made in 378 patients at the time of scanning (88.1%).

Heel BMD in primary care is a cheap, simple effective means of assessing osteoporosis risk in the elderly female population. Less than 12% of patients needed referral for central dxa scanning. Large numbers of untreated patients with osteoporosis and low trauma fractures were discovered in this study despite the provision of a local central dxa service for 10 years. This study suggests that a selective case finding strategy may fail to identify many individuaIs at high risk for osteoporotic fracture. If, however, the full potential of heel BMD measurement is to be realised in this population, additional efforts may be needed to raise the patient participation rate.

Disclosures: ***J.D. McCrea****, MSD 2, 8.*

SU088

Performance of Computer Assisted Densitometry (CAD): Comparison with Visual Assessment by Experienced Densitometrists. E. N. Schwartz, D. M. Steinberg*. Foundation for Osteoporosis Research and Education, Oakland, CA, USA.

Modern dual-energy x-ray absorptiometry (DXA) systems provide semi-automated analyses of lumbar spine and femur bone mineral density (BMD). However, visual assessment by the user is necessary to a) assure proper acquisition mode and patient positioning, b) assure accurate identification of regions of interest (ROI), and c) identify scans with abnormal conditions that could artificially elevate BMD. Recently, automated software known as Computer Assisted Densitometry (CAD) (GE Medical Systems Lunar) was introduced to assist in identifying scans with common acquisition and analysis irregularities. The aim of this study was to determine how well CAD agreed with the assessment of two experienced DXA users, and to determine if CAD could identify abnormal scans which might be missed by visual assessment.

A total of 71 spine and 70 femur scans were analyzed, with 67% of scans exhibiting some abnormal condition flagged by CAD. Two experienced users (an ISCD-certified densitometrist and an ISCD-certified technologist) together evaluated the scans for abnormalities, unaware of the CAD results. The consensus assessment by the densitometry team was compared to the CAD assessment. In addition, scans where CAD and the visual assessment differed were reviewed to determine if a potential abnormality might have been missed by the densitometry team.

Results showed a strong agreement between CAD classification and visual assessment. Experienced users agreed with the CAD assessment in 76% to 86% of scans prior to knowing the CAD result. Review of scans where disagreement was present, after revealing the CAD recommendations, resulted in an assessment change by the densitometry team in 20% to 40% of cases. Agreement increased to 83% to 92% after CAD assessment was known. Femur results were similar. We conclude that use of CAD can 1) provide valuable information for inexperienced users regarding DXA scan quality and 2) assist experienced densitometrists in identifying potentially abnormal scans which might be missed by visual assessment alone.

Agreement between CAD and Expert Evaluation of Normal and Abnormal Spine Scans

	Blinded to CAD Results		After CAD Results Known	
CAD vs. Experts	**Agree**	**Disagree**	**Agree**	**Disagree**
Spine Centered (%)	86	14	92	8
Spine Straight (%)	79	21	83	17
ROIs Accurate (%)	82	18	86	14
No Unusual High Density (%)	80	20	87	13
No Unusual T-Score Variation (%)	76	24	86	14
No Spine Curvature (%)	83	17	90	10

Disclosures: ***E.N. Schwartz****, GE Medical Systems Lunar 2.*

SU089

Dual Energy X Ray Absorptiometry of Ex Vivo Mouse Long Bones: Site and Side Comparisons. G. E. Lopez-Franco*[1], S. Litscher*[1], T. K. O'Neil*[1], M. Urban-Piette*[1], P. Demant*[2], R. D. Blank[1]. [1]Endocrinology/Medicine, University of Wisconsin Medical School, Madison, WI, USA, [2]Molecular Genetics, Netherlands Cancer Institute, Amsterdam, Netherlands.

Dual energy X ray absorptiometry (DXA) has become a popular analytical technique in mice and other small animals, either as a substitute for more demanding techniques, such as biomechanical testing, histomorphometry, and gravimetry, or as an adjunct to them. As small animal skeletal research continues, more detailed questions regarding differences in the behavior of cortical and trabecular bone and at different anatomical sites are becoming ever more prominent. Such experiments require that site-specific data be generated and interpreted. The existing rodent site-specificity data are therefore insufficient to help guide interpretation of comparative densitometry of left and right bones. Similarly, there are no published data addressing the degree to which contralateral bones are similar in the absence of an experimental intervention. In order to address these gaps in our knowledge, we undertook a comparison of excised mouse femora, humeri, and radii from 383 mice using DXA. At all 3 sites studied, we found that left bones were slightly but significantly ($P < 0.00001$) denser than right bones. Correlation coefficients between left and right sides ranged from 0.53 ius to 0.78 for bone mineral density (BMD), between 0.56 and 0.86 for bone mineral content (BMC), and between 0.45 and 0.66 for bone area. Correlation coefficients between side-averaged femoral and humeral, femoral and radial, and humeral and radial BMD were 0.81, 0.51, and 0.61, respectively. Correlations were lower when limited to ipsilateral or contralateral pairs, but these were equal to each other. Our findings demonstrate the impact of region of interest analyses on measurement precision relative to whole-body analyses and entail the following recommendations for future DXA studies in mice. To the extent that the scientific questions being addressed are amenable to whole body BMD rather than region of interest BMD measurement, the whole body measurement should be preferred. If site-specific differences are being sought, it is desirable to use the largest practical regions of interest and to adjust sample sizes in accordance with the loss of precision entailed by region of interest analysis. In longitudinal studies assessing regions of interest, care should be taken to compare the same side at each measurement. In comparisons of paired bones, it is important either to have a suitable method for randomizing sides for treatment or adequate reporting of assignment of sides to treatment or control.

Disclosures: ***G.E. Lopez-Franco****, None.*

SU090

DXA Bone Mineral Density (BMD) Measurements within the Femoral Neck Are Critically and Clinically-dependent on the Placement of the Region of Interest (ROI). M. T. DiMuzio. The North Shore Osteoporosis Center, Deerfield, IL, USA.

Hip fracture is the most severe clinical outcome related to osteoporosis and its evaluation is critical for establishing the true bone health status of patients. Bone health status, in part, requires the measurement of the BMD of key skeletal sites and is the most sensitive factor in the evaluation of fracture risk. Dual Energy X-ray Absorptiometry (DXA) is the gold standard for determining BMD, however it is sensitive to the specific region of interest (ROI) under evaluation. It has been determined that the most sensitive measure of hip fracture risk is the evaluation of the femoral neck ROI within the proximal femur, since most hip fractures occur at the femoral neck without trauma or are independent of the fall characteristics in patients suffering loss of balance.

In order to evaluate patients at highest risk for hip fracture, it is critical to measure the site where fractures occur within the proximal femur: the femoral neck. The purpose of this study was to determine which ROI along the femoral neck is of most clinical relevance, since a significant disparity exists in the choice of such an ROI between the manufacturers of the most common instruments used for measuring BMD in the world (Hologic and GE-Lunar).

This prospective study uses an Hologic QDR-4500A bone densitometer to determine the BMD along the length of the femoral neck in 250 patients aged 60 to 85 years of age referred for a bone density evaluation. Patients' scans were chosen at random with no pref-

erence for WHO category, sex, weight or body size. A femoral neck ROI (a rectangle of constant width) for each patient was used to measure BMD. Initial measurements were taken at the edge of the greater trochanter and at equal increments along the neck, up to the edge of the head of the femur. The equal increments consisted of a pixel (one cursor stroke). After each movement up along the neck, the BMD was determined, and for each patient, the BMD along the distance between the edge of the greater trochanter and head of the femur was plotted.

Conclusions: In all cases, the BMD increased as one moved up along the length of the femoral neck from the greater trochanter to the head of the femur. The average increase in BMD was 7.8%. Moreover, for all patients, the lowest BMD was measured at the edge of the greater trochanter. Since fracture risk increases with decreasing BMD, it is critical in evaluating a patients hip fracture risk, that BMD measurements of the neck of the femur are made at the edge of the greater trochanter where the ROI has the lowest BMD and highest risk for fracture. Also, in comparing BMD measurements of the proximal femur between manufacturers, close attention must be paid to specific ROIs in order to determine true rates of change.

Disclosures: ***M.T. DiMuzio****, None.*

SU091

Site of Bone Densitometry in Middle-Aged Women: Comparison of Bone Mineral Density of 3 Anatomic Regions. L. W. Turner[1], L. Wallace[2], B. Perry*[3], J. Ballard[4]. [1]Health Science, University of Arkansas, Fayetteville, AR, USA, [2]Medical Sciences, University of Tennessee, Knoxville, TN, USA, [3]Medical Sciences, University of Arkansas, Little Rock, AR, USA, [4]Health and Exercise Science, University of Texas, Tyler, TX, USA.

Because the bone mineral density (BMD) in different anatomic regions is heterogenous the number of women who meet the World Health Organization definition of osteopenia or osteporosis increases with the number of sites examined. The purpose of this study was to compare bone mineral density measures of the left femur, spine (L2-L4) and whole body among middle-aged women. Three hundred and forty two (342) healthy middle-aged women (mean age 49.5 years) were studied using dual-energy x-ray absorptiometry (DXA Lunar, Prodigy, software version 2.5). Subjects lay supine on the scanner table and were wearing no metal when measurements were assessed by a single certified densitometry technologist. Mean t-scores were: -0.267, SD=1.17 for hip; -0.101, SD=1.38 for spine, and 0.269, SD=1.14 for whole body. Correlation coefficients between hip and spine were .73, between hip and total body were .77, and between spine and total body were .78. All correlations were significant ($p<0.0001$). Using WHO definitions, the diagnoses of osteopenia based on a single measurement varied from 28% using the hip region to 22% using the spine area and 13% using the whole body site. Diagnoses of osteoporosis based on a single measurement varied from 2% using the hip area, 4% using the spine site, and 1% using the whole body region. Using a single measurement of the whole body, 3% of osteoporotic patients would have been misclassified as osteopenic and 15% of osteopenic patients would have been misclassified as normal. Six percent more women were diagnosed with osteopenia based on hip measurement compared with spine. Two percent more subjects were diagnosed with osteoporosis based on spine measurement when compared with hip measurement. The choice of anatomic region has considerable impact on diagnoses of osteoporosis and osteopenia. While correlations between anatomic regions are high and statistically significant, considerable variability exists in the classifications of osteoporosis and osteopenia with potential for misdiagnosis, especially when the whole body method is used alone. A combination of densitometry examination using skeletal sites of spine and femur should be used in diagnosing osteopenia and osteoporosis among middle-aged women.

Disclosures: ***L.W. Turner****, None.*

SU092

High Fracture Risk Does Not Increase Bone Mass Measurement in Older Women. J. M. Neuner*[1], A. B. Nattinger*[1], N. C. Binkley[2]. [1]Medicine, Medical College of Wisconsin, Milwaukee, WI, USA, [2]Medicine, University of Wisconsin-Madison, Madison, WI, USA.

BACKGROUND: Use of many preventive strategies decreases with increasing patient age. Little is known about current bone mass measurement in patients at high fracture risk, including older women. Thus, we examined the effect of age on this measurement in female Medicare patients before and after Medicare reimbursement of postmenopausal osteoporosis screening began in 7/98.

METHODS: We studied 43,250 women aged 67 or over from three random population-based 1% samples of those eligible for Medicare parts A/B in several regionally diverse cities and states. After excluding those with prior testing, we determined the percent who underwent bone mass measurement (central DEXA, peripheral DEXA or QUS) in the three independent cohorts each studied over an 18-month period (7/95-12/96, 1/97-6/98, or 7/98-12/99). We examined the effect of age upon testing in the total sample in a logistic regression model with adjustment for time period (cohort), race, history of hip fracture, Medicaid insurance, urban residence, and Charlson-Deyo comorbidity categories (including diagnoses often treated with glucocorticoids). We repeated the analysis with an interaction term between age and time period.

RESULTS: Of subjects in all three cohorts combined, 85% were white and 11% had a prior hip fracture. Bone mass measurement increased over the three time periods for all age groups, but remained low overall even after Medicare payment began (table). Older women underwent less testing during each period. When compared with women aged 85 or older, those 67-74 were 3.2 times (CI 2.7, 3.9) and those 75-84 were 2.4 times (CI 2.0, 2.8) more likely to undergo testing. Hip fracture had no effect upon testing, and the effect of age upon testing did not differ over time (interaction term p >.1).

CONCLUSION: Despite the known increase in fracture risk with age, older female Medicare patients were much less likely than younger women to receive bone mass measurement. Women with prior hip fracture were no more likely to be tested. As the effect of age upon testing did not improve soon after Medicare reimbursement began, efforts to improve adherence with recent osteoporosis screening guidelines should particularly target older women and those with hip fracture.

EFFECT OF AGE ON BONE MASS MEASUREMENT

	Number of pts	BMD 7/98-12/99 (%)	BMD 1/97-6/98 (%)	BMD 6/95-12/96 (%)
Total	43,250	10.1	6.7	3.0
67-74	18,784	13.5	8.4	3.8
75-84	17,718	9.1	6.5	2.9
85 +	6,748	3.7	2.4	1.1

Disclosures: ***J.M. Neuner****, None.*

SU093

Precision and Accuracy of DXA and pQCT For Densitometry of the Rat Femur. J. A. Horton*, G. M. Murray*, J. A. Spadaro, B. S. Margulies*, M. J. Allen*, T. A. Damron*. Orthopedic Surgery, SUNY Upstate Medical University, Syracuse, NY, USA.

Background: Bone mineral density (BMD) and bone mineral content (BMC) are key data in the study of osteoporosis and other pathologic skeletal diseases. Dual-energy X-ray Absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) are used in human and small animal studies of bone density. The purpose of this study was to compare the precision and accuracy of three techniques of measurement of the rat femur.

Method: Validation of relative and absolute accuracy was assessed by scanning the intact and excised femora of 11-week old rats using DXA (Lunar DPX-IQ and PIXImus2) and pQCT (Stratec XCT-2000). The study compared machine measured parameters with standard, non-radiographic measurements using regression analysis and calculated percent difference from standard values to determine the accuracy of each densitometry technique. Machine-specific and subject-specific precision was determined by calculating coefficient of variation of repeated scans, with the subject being repositioned between scans.

Results:

Table 1. Accuracy- Regression Analysis (R^2).

Relative Accuracy by Regression Analysis (R^2)

		DPX-IQ (intact)	DPX-IQ (excised)	PIXImus2	pQCT	Standard Measure
BMC (g)	DPX-IQ (intact)	1	0.818	0.838	0.774	0.851
	DPX-IQ (excised)	x	1	0.969	0.980	0.946
	PIXIMUS	x	x	1	0.924	0.985
	pQCT	x	x	x	1	0.934
aBMD(g/cm^2)	DPX-IQ (intact)	1	0.742	0.680	n.a.	0.721
	DPX-IQ (excised)	x	1	0.946	n.a.	0.892
	PIXIMUS	x	x	1	n.a.	0.954
2-D Projected Area (cm^2)	DPX-IQ (intact)	1	0.390	0.329	n.a.	0.297
	DPX-IQ (excised)	x	1	0.800	n.a.	0.875
	PIXIMUS	x	x	1	n.a.	0.758
Volume (cm^3)	pQCT	n.a.	n.a.	n.a.	x	0.861
vBMD (g/cm^3)	pQCT	n.a.	n.a.	n.a.	x	0.947

Table 2. Accuracy-Mean Percent Difference from Standard Measurement.

Absolute Accuracy of DXA Measurement

BMC (g)	Standard Measure	DPX-IQ (intact)	DPX-IQ (excised)	PIXImus2
Mean ± SD	0.397 ± 0.072	0.439 ± 0.093	0.384 ± 0.071	0.479 ± 0.082
%diff vs Measured	0	10.58%	-3.28%	20.66%
p	x	n.s.	n.s.	<0.001
aBMD (g/cm^2)				
Mean ± SD	0.216 + 0.033	0.256 + 0.043	0.213 + 0.031	0.211 + 0.028
%diff vs Measured	0	18.52%	-1.39%	-2.32%
p	x	<0.0001	n.s.	n.s.
Area (cm^2)				
Mean + SD	1.826 + 0.106	1.674 + 0.257	1.799 + 0.124	2.266 + 0.142
%diff vs Measured	0	-8.35%	-1.48%	-24.10%
p	x	<0.004	n.s.	<0.0001

Absolute Accuracy of pQCT Measurement

Measured vs. pQCT	BMC (g) Measured	BMC (g) pQCT	vBMD (g/cm^3) Measured	vBMD (g/cm^3) pQCT	Volume (cm^3) Measured	Volume (cm^3) pQCT
Mean ± SD	0.397 + 0.072	0.424 + 0.079	0.586 + 0.073	0.770 + 0.065	0.674 + 0.063	0.572 + 0.062
%diff vs Measured	x	6.80%	x	31.40%	x	-15.13%
p	x	n.s.	x	<0.0001	x	<0.0001

Table 3. Results of Machine- and Subject-specific Precision Studies.

Machine-Specific Precision (CV%)

Densitometer	DPX-IQ	PIXImus2	pQCT
BMC	0.57%	1.18%	0.52%
aBMD	0.53%	0.57%	n.a.
2-D Area	0.86%	0.67%	n.a.
vBMD	n.a.	n.a.	0.49%
X-S area	n.a.	n.a.	0.63%

Subject Specific Precision (CV%)

Densitometer	DPX-IQ (intact)	DPX-IQ (excised)	PIXImus2	pQCT
BMC	10.97%	1.81%	0.73%	1.74%
aBMD	13.78%	1.57%	0.99%	na.
2-D Area	4.86%	0.93%	0.96%	na.
vBMD	na.	na.	na.	2.34%
X-S area	na.	na.	na.	1.22%

Discussion: Each of the methods of densitometry examined in this study produced comparable results and was sensitive to small differences. Further, our assessment of the precision and accuracy observed between methods of scanning excised rat femurs supports use of these methods in studies using small animals and may facilitate comparisons of similar data with other laboratories.

Disclosures: ***J.A. Horton****, None.*

SU094

Bony Densitometry and Prediction of Risk of Fracture of the Hip. L. J. A. Lunar*. Endocrinology, Endocrinologycal Foundation, Caracas, Venezuela.

This paper is the result of a multicenter investigation that served as a clinical assay for the validation of a bone densitometer (Degos 7032). The study was based on the comparison of two groups of persons: one of the 50 apparently sound volonteers and the other of 39 patients with hip fracture. To compare both groups it was used for the evaluation the F Fischer's test if the difference between the bone density variances (CMO-width) was significant in the distal extreme of the ratio by using the method of simple photon absorptiometry. Then, another comparison was made by the t of Student test (for unmatched values). The difference between the means was significant. It was concluded that the bone density magnitude is an index that allow to differentiate both groups and that it may be used to monitor the hip fracture risk and to anticipate to the trauma caused by fracture.

Disclosures: ***L.J.A. Lunar*** *, None.*

SU095

Interviews on Osteoporosis in an Area of Health. L. J. A. Lunar*. Endocrinology, Endocrinologycal Foundation, Caracas, Venezuela.

With the aim of knowing the repercussion of osteoporosis among the elderly in a health area, 88 outpatients of both sexes were studied. They were selected by using a simple randomized method, and they stood for 1/6 of all persons 65 years old or over from the health area of the "Endocrinologycal Foundations. This figure represents 91.6 % of the cases that were programmed. It was attained an expectancy of the problem of 50 % with a reliability of 10 %. Every individual underwent conventional radiological examinations of the following segments: dorsal spine, lumbar spine, pelvic bones, thorax, and right hand, in order to analyze the behaviour of senile osteoporosis in an open health area. 100 % of the persons studied showed some radiological sign of osteoporosis. All the skeletal segments were affected, with the exception of the pelvic bones in one patient and the lumbar spine in another. 19 % of these patients had bone fractures. It is concluded that the severity of osteoporosis differs just a little between both sexes and that there is a clear trend to increase with age.

Disclosures: ***L.J.A. Lunar****, Eli Lilly and Company 2.*

SU096

Predictive Value of DXA Measured at Different Sites for Non Vertebral Fracture Risk in a Sample of 586 Elderly Swiss Women Aged > 70 years. K. Lippuner[1], A. W. E. Popp[1], M. Kaufmann*[1], R. Perrelet*[1], M. A. Krieg[2]. [1]Osteoporosis Unit, University Hospital, Berne, Switzerland, [2]CHUV, Lausanne, Switzerland.

Bone density measurements at various skeletal sites are predictive of fracture risk. Whereas prospective studies have been conducted using DXA at the spine, hip, forearm and calcaneus, it is not known whether BMD at the tibia predicts the risk of future fracture. We have previously shown that DXA performed at the tibia allows assessment of cortical (diaphysis) and trabecular (distal epiphysis) bone density in a single weight-bearing bone (J Bone Miner Res 1994 9: 1851-7).

To study the predictive value of tibial BMD for non vertebral fracture risk in comparison to BMD at other skeletal sites, 586 Swiss women from the center of Berne of the SEMOF study (age 76.2 ± 3.0 yrs (mean ± SD) were measured using DXA (QDR 4500, Hologic TM) at the following sites: hip (total hip, femoral neck, trochanter, Wards), forearm (1/3 distal radius, ultradistal radius), and distal tibia (epiphysis, diaphysis). Follow-up lasted 2.5 years and non vertebral fractures were reported by the participants and confirmed by their family physicians or by the hospitals in charge of the patients. To assess the predictive value of BMD at the various sites, COX proportional hazard regression analysis was performed. Results were expressed as relative risks (RR and 95% CI) per decrease in BMD of 1 standard deviation (SD) at each site. Comparison between individual predictive values was performed by comparison of the areas under the ROC curves (AUC).

A total of 60 non vertebral fractures occurred during the follow-up. The respective age-adjusted RRs and AUCs for the various sites are presented in the table.

DXA Site	RR	95% CI	AUC	95% CI
Total hip	1.6	1.3 - 2.1	0.66	0.58 - 0.73
Femoral neck	1.8	1.4 - 2.4	0.67	0.60 - 0.74
Trochanter	1.5	1.2 - 2.0	0.64	0.56 - 0.72
Radius - 1/3	1.9	1.5 - 2.5	0.68	0.61 - 0.75
Radius- UD	1.9	1.4 - 2.5	0.67	0.59 - 0.75
Tibia Epiphysis	2.1	1.6 - 2.7	0.71*	0.64 - 0.77
Tibia Diaphysis	1.7	1.3 - 2.2	0.66	0.59 - 0.73

*p<0.05 compared to tibia diaphysis and trochanter.

We conclude that in our randomly selected cohort of 70 to 80 yrs old women, the predictive value for non vertebral fracture risk was comparable for BMD measured at tibial diaphysis and at standard sites. BMD measured at tibial epiphysis had a significantly better predictive value than BMD measured at the trochanter or at the tibial diaphysis.

Disclosures: ***K. Lippuner****, None.*

SU097

Cost-Saving Classifications for Hip Fracture Prediction. H. Jin*[1], Y. Lu[1], S. T. Harris[1], D. M. Black[2], K. Stone[2], M. C. Hochberg[3], H. K. Genant[1]. [1]Radiology & OARG, University of California, San Francisco, CA, USA, [2]Epidemiology and Biostatistics, University of California, San Francisco, CA, USA, [3]Medicine, University of Maryland, Baltimore, MD, USA.

This paper presents an extension of the Classification And Regression Tree (CART) methods (Brieman, et al, 1984) and its application to cost-saving identification of subjects at high risk of osteoporotic hip fracture within 5 years. Two major modifications were made to the splitting procedures of the standard CART methods: the tree was made more robust, and an algorithm was developed to generate a cost-saving classification rule with classification efficiency not inferior to the optimum rule. We applied the new algorithm to the data from the Study of Osteoporotic Fracture (SOF). Forty-three previously documented predictive variables for osteoporotic hip fracture were examined. The SOF data were randomly separated into two data sets: two thirds were used to generate classification rules (generating data) and the remaining one third was used to compare the rules (validation data). First, using the new algorithm, we generated a robust optimum classification rule for subjects at high risk of 5-year hip fracture without consideration of the cost of the predictive variables. The variables included age, weight, body mass index, height and height loss, bone mineral density (BMD) by dual x-ray absorptiometry (DXA), functional status assessment, visual examination, and prevalent fracture determined by X-ray. This optimum algorithm had a sensitivity and specificity of 76.0% and 74.2% respectively for the data generating the rule, and 65.8% and 73.8% for the validation data. We then generated an alternative cost saving rule with equivalent diagnostic utility, but using only age and BMD by hip DXA scan. This rule had a sensitivity and specificity of 71.4% and 73.6% respectively for the data generating the rule, and 76.3% and 74.6% for the validation data. The two rules were statistically equivalent. We further compared them with the use of a single BMD measurement and results of the standard CART. These comparisons demonstrated the superior performance of the rules generated by our modified algorithm. This paper suggests that our modified CART algorithm can be a useful too to assist clinical decisions and, more importantly, that a DXA hip scan and age can identify subjects at high risk of osteoporotic hip fracture within 5 years as efficiently as more costly and complicated algorithms.

Disclosures: ***Y. Lu****, None.*

SU098

Correcting Phalanx Bone Mass Measurement for Clinical Risk Factors Increases its Validity in the Diagnosis of Osteoporosis. D. Picard, J. P. Brown*, M. Couturier*, L. Rosenthall*, J. Lévesque*, M. Dumont*, L. Ste-Marie*, A. Tenenhouse*, S. Dodin*. Quebec Osteoporosis Study Group, Montreal, PQ, Canada.

Peripheral bone mass measurement by dual-energy X-ray absorptiometry (DXA) is an appealing method for the diagnosis of osteoporosis, especially in distant areas, where central DXA, the gold standard, is not available. On the other hand, clinical risk factors can provide information on bone mineral density (BMD). The aim of this study is to evaluate the ability of phalanx DXA to predict central BMD after being corrected for two important clinical factors that can affect bone mass, age and weight.

We determined BMD of the spine (*s*), femoral neck (*fn*) (1 Hologic, 3 Lunar), as well as phalanx (*p*) with Schick AccuDXA in 831 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-scores were then derived using data from the Canadian Multicentre Osteoporosis Study (Ca*M*os). There was a good correlation between BMD of the different anatomic regions (r = 0.599-0.671, p<0.0001). After applying regression analyses on the gold T-score (lowest between *s* and *fn*) where age, weight and phalanx T-score were retained as the main predictors, we corrected phalanx T-score according to the regression formula obtained . To examine the performance of DXA and corrected phalanx DXA 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 performance was evaluated at optimal cutoff T-scores selected on the basis of highest sensitivity and specificity. Results are reported in the table below. The main improvement of correcting phalanx T-score for age and weight is observed in an increase in sensitivity by 7% translated through a reduction of the false negatives by 32%. Furthermore, among the false negatives, most of the subjects are near the osteoporosis threshold as only 5 present a central T-score < -3. The mean central T-score among the false negatives is -2.91 (+/- 0.39). Hence, this study suggests that correcting phalanx bone mass measurement for age and weight, increases its validity in the diagnosis of osteoporosis as reflected by a greater number of osteoporotic subjects identified without affecting the proportion of correctly identified normal subjects.

	Phalanx	**Corrected Phalanx**
Optimal T-score	-1.48	-1.956
Sensitivity (%)	79	86
Specificity (%)	83	83
False Positives (n)	123	124
False negatives (n)	22	15

Disclosures: ***D. Picard****, None.*

SU099

Equine Third Metacarpal Bone Mineral Density Assessment with a Mobile Dual Energy X-Ray Absorptiometry Device: An In vivo and Ex Vivo study. M. Donabedian*[1], G. Perona*[2], C. Delguste*[3], P. Lebecque*[4], F. Duboeuf*[5], O. M. Lepage[6], W. Martin-Rosset*[7]. [1]Départements NASA & ENA, INRA, Theix, France, [2]Dipartimento di Produzioni Animali, Facoltà di Medicina Veterinaria di Torino, Grugliasco, Italy, [3]Département des Sciences Cliniques, Université de Liège, Liège, Belgium, [4]Département NASA, INRA, Theix, France, [5]Inserm U 403, Hôpital Edouard Herriot, Lyon, France, [6]Département Hippique, Ecole Nationale Vétérinaire de Lyon, Lyon, France, [7]Département ENA, INRA, Theix, France.

Dual energy X-Ray absorptiometry (DXA) is the most widely used osteodensitometric method in man. Until recently, because of anatomical specificities and lack of specific DXA device, only post-mortem or in vivo studies under general anesthesia were possible in horses. The development of a mobile DXA device (PIXI, Lunar Corp) offers new opportunities for in vivo equine bone mineral density (BMD) measurements. The purpose of this study was to analyze in vivo usability, and ex vivo - in vivo precision of this device. Region of interest (ROI) was located 5-cm above the distal end of MCIV. In order to test ex vivo precision on one equine limb, 20 successive BMD measurements of the ROI were achieved without (repeatability) and then with (reproducibility) bone repositioning between each procedure. In vivo, intra and inter operator reproducibility was tested. Horses were not sedated and their left front foot was placed on a wood positioning block in order to keep MCIII vertically and forward. For in vivo intra operator reproducibility testing, 3 different operators' pairs achieved 10 successive BMD measurements on 2 different horses. For inter operator investigations, 4 operators tested 12 combinations of PIXI and foot positioning. Five successive complete sets of 12 combinations were achieved. Coefficient of variation (CV% = standard deviation/mean) was established for each test. Ex vivo repeatability and reproducibility CVs was respectively 1.47 and 1.69 %. In vivo intra operator reproducibility varied between 2.91and 4.06 % depending on horse and operator. In vivo inter operator reproducibility varied between 3.13 and 5.42 % according to the measurement set. Close results for ex vivo repeatability and reproducibility in one hand, and intra and inter operator in vivo reproducibility in the other hand show that positioning process was well standardized and induced few imprecision. With a sensitivity threshold of $2\sqrt{2}$.CV, lowest significant BMD variation would be 8.23 % in an in vivo longitudinal follow up. The present results suggest adequacy of PIXI for metacarpal BMD measurement in the horse in vivo. It would be of interest to test PIXI's accuracy at the high BMD ranges of equine MCIII.

Disclosures: ***M. Donabedian****, None.*

SU100

Discriminant Power of DXA Parameters in Low Trauma Fractures in IJO Subjects. P. Pludowski*[1], M. Lebiedowski*[2], H. Matusik*[1], R. S. Lorenc*[1]. [1]Dep. of Biochemistry and Exp. Medicine, Children's Memorial Health Institute, Warsaw, Poland, [2]Dep. of Rehabilitation, Children's Memorial Health Institute, Warsaw, Poland.

In Idiopathic Juvenile Osteoporosis (IJO) long bone and vertebral fractures are common and etiology is unknown. The aim of study was to test the ability of DXA derived parameters in fracture discrimination. The study population comprised 61 IJO children aged 5-19 yrs (34f, 27m) evaluated during acute and chronic phases of disease as well as 412 healthy children aged 5-19 yrs (187f, 225m). DXA derived total body BMC (TBBMC, an indicator of bone strength), BMD (TBBMD, g/cm2), lean mass (LBM, a surrogate of muscle mass) and body height (BH) were analyzed. BH/LBM and TBBMC/LBM ratios were established (after transformation: X/Y=>ln(X)/ln(Y)) in healthy and diseased children. Logistic regressions (LR) and ROC analyses were performed to evaluate the parameter that most efficiently discriminate healthy and IJO children, with assumption the IJO sustained at least 1 fracture. LR analyses revealed higher chi-square values for TBBMC/LBM ratio (girls 87.25; boys 50.59) than for TBBMD (83.13; 20.62), TBBMC (50.59; 6.99), LBM (11.51; 1.11), BH/LBM (0.87; 4.24) in both genders of IJO children (acute phase). The chi-square values for BH/LBM ratio and for LBM indicated lack or only small relationship between muscle status and probability of fracture condition. Lower significance was shown for the analyses based on results calculated during chronic phase of IJO. In boys chi-square values were very low and not significant (except LBM) indicating no difference with reference data. The ROC showed an analogous decreasing order of significance for the corresponding areas under the curves (AUC). It could be pointed, that during acute phase of IJO, measured TBBMC (AUC girls 80.8%; boys 60.4%), TBBMD (86.7%; 67.6%) and TBBMC/LBM (87.8%; 81.0%) discriminated markedly better healthy from diseased groups than BH/LBM (48.2%; 59.4%) or LBM (67.7%; 47.3%). During chronic phase of IJO the AUC values were markedly lower indicating lack of significant differences between analyzed groups. The ROC assessed "cut off" values were close to those corresponding to the inflexion points of logistic curves. On the basis of AUC values, we found that TBBMC/LBM ratio was the most efficient discriminator in IJO children (irrespective of the phase of disease) in assayed analyses conditions.
Our study pointed on significant advantage of TBBMC/LBM ratio over typically used TBBMD in general assessment of bone mechanical status in IJO cases what postulate on inclusion of this parameter into standard diagnostic procedures in pediatric study of skeletal status.

Disclosures: ***P. Pludowski****, None.*

SU101

Resolution and Magnification Error Using a Cone Beam Bensitometer for Femoral Morphometry. V. Boudousq*, E. Thomas, I. Ruiz*, P. O. Kotzki*. Médecine Nucléaire, Hôpital Carémeau, Nîmes, France.

The bone mineral density (BMD) is the mean determinant of the hip fracture risk. However other factors like the lifestyle, the propensity for falls and the femoral geometry could influence the hip fracture risk. The femoral geometry could be evaluated using pencil beam densitometers. With the fan beam densitometers the spatial resolution was improve but there was an inherent magnification of scanned structures in the direction of the beam inducing a distortion of the image. This distortion of the image did not allow to evaluate easily the femoral geometry.
The purpose of this study is to evaluate in vitro the potentiality of a new cone beam densitometer -the DMS Lexxos- in order to realise femoral morphometry. This densitometer used a 2D digital radiographic detector that did not require scanning for image build up of the lumbar spine or the hip. The system required only two 0.75 s X-rays exposures to complete an examination of these areas. The detector was 20 cm square, provided 512 x 512 pixels, each pixel was 0.4 mm on a side.
The resolution was assessed using a line pair test pattern include in a water container to simulate soft tissue. The resolution was defined as the last bar section in which a clear distinction could be seen between lines and spaces. The resolution was explored in the two principal perpendicular directions.
Magnification and distortion are assessed using a matrix test object placed on the examination table and at different levels from the table between 25 and 295mm. The analysis was on one hand a visual analysis looking for a line distortion and on the other hand some crossing points were identified, the coordonates of the points in pixels were noted and the Euclidean distance was computed.
The resolution results on both the X and Y axis are the same. The resolution was evaluated between 1.4 and 0.5 line pairs /mm for an attenuation varying for 32 to 316 mm of water. The magnification is about 1.17% cm-1 when increasing the distance of the phantom above the examination table, this magnification is isotropic without distortion.

Disclosures: ***V. Boudousq****, None.*

SU102

Precision and Accuracy of the GE Lunar DPX-Bravo Bone Densitometer. R. H. Nord, H. S. Barden, S. Krepanith*, K. G. Faulkner. GE Medical Systems Lunar, Madison, WI, USA.

The DPX Bravo (GE Medical Systems Lunar) is a small footprint, compact DXA system designed to measure the spine, femur and forearm. The spine and both hips can be measured using a single acquisition protocol (OneScan) designed to reduce procedure time by eliminating the need to reposition the subject between scans. The purpose of this study was to evaluate the clinical performance (precision and accuracy) of the DPX Bravo compared to existing DXA systems.
Forty-seven volunteer subjects (18 men and 29 women) ranging in age from 23 to 87 were measured on the DPX Bravo. Each subject was measured at the AP spine (L1-L4), and either at the left forearm or at both femora. A total of 45 spine, 30 femur, and 31 forearm measurements were performed. Precision was evaluated by measuring each site 3 times on the DPX Bravo and computing the root mean square coefficient of variation. Accuracy was evaluated by comparing the DPX Bravo BMD values to measurements on the Prodigy and the DPX Pro, bone densitometers currently available from GE Medical Systems Lunar. Precision error results for the DPX-Bravo are given in the first table. Accuracy is shown as regression results in the second table. Overall comparison results are plotted below.

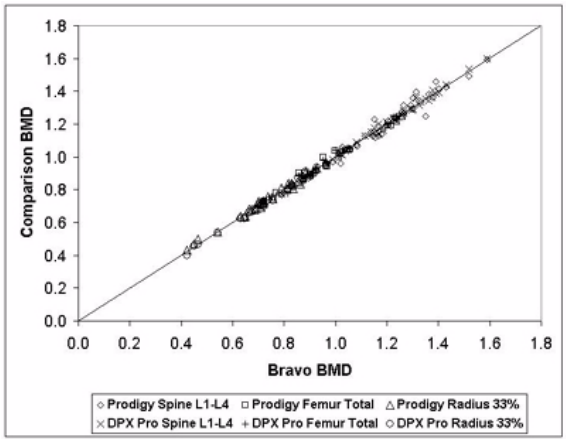
DPX BRAVO PRECISION

	AP Spine L1-L4	Femur Total	Radius 33%
mean BMD	1.199	.955	.716
Std. Dev.	.015	.008	.007
%CV	1.2%	0.8%	1.2%

DPX BRAVO ACCURACY

Prodigy vs. Bravo	AP Spine L1-L4	Femur Total	Radius 33%
Slope	1.003	.997	1.006
R-squared	.966	.982	.983
SEE	.034	.019	.016
DPX-Pro vs. Bravo	**AP Spine L1-L4**	**Femur Total**	**Radius 33%**
Slope	1.002	1.000	.992
R-squared	.993	.989	.990
SEE	.015	.016	.013

We found the clinical precision of the DPX Bravo to be about 1% at the three body sites it is designed to measure. Regression of BMD values with those of currently accepted densitometers gave extremely high correlation coefficients and slopes that did not differ significantly from unity.
We conclude that BMD measurements taken with DPX Bravo will be clinically equivalent, in both precision and accuracy, to those taken with existing GE Lunar densitometers.



Disclosures: ***R.H. Nord****, None.*

SU103

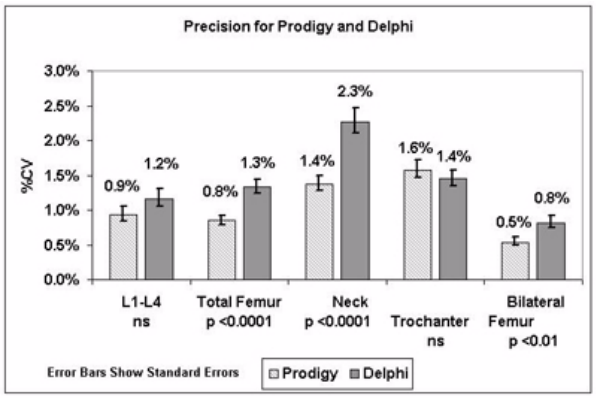
Precision Comparison of Two DXA Densitometers – Prodigy and Delphi. E. M. Lewiecki[1], P. D. Miller[2]. [1]New Mexico Clinical Research & Osteoporosis Center, Albuquerque, NM, USA, [2]Colorado Center for Bone Research, Lakewood, CO, USA.

When measuring bone mineral density (BMD), precision errors can be caused by many factors: differences in patient positioning, variations in scan analysis, and both short- and long-term fluctuations of the densitometry equipment. Minimization of these errors is essential to providing an accurate assessment of BMD change over time. In this study, we compared the short-term precision error of two DXA devices, the Prodigy (GE Medical Systems Lunar) and the Delphi (Hologic). Both are fan-beam DXA devices predominantly used to measure BMD of the spine and proximal femur. In this study, 41 women age 51-80 years (mean age 61) were measured in duplicate on both Prodigy and Delphi systems at either of two centers. The DXA technologists were ISCD certified and used manufacturer recommended scanning and analysis procedures. All scans were performed using the manufacturer recommended 30-second scan modes. BMD precision error was calculated as the root-mean-square standard deviation (RMS SD) and coefficient of variation (RMS-%CV) for the repeated measurements. Data from right and left femora were evaluated individually (single femur precision) and using the combined value (bilateral femur precision). The precision error (variance) of the Prodigy and Delphi measurements at each measurement region was compared using an F-test to determine the significance of any observed differences.

Precision Error for Prodigy and Delphi

	Prodigy %CV (95% CI)	**Delphi % CV** (95% CI)
L1-L4	0.9 (0.80-1.19)	1.2 (0.92-1.48)
Total Femur	0.8 (0.69-1.00)	1.3 (1.04-1.57)
Femur Neck	1.4 (1.10-1.62)	2.3 (1.65-2.68)
Femur Trochanter	1.6 (1.15-1.86)	1.4 (0.99-1.71)
Bilateral Total Femur	0.5 (0.42-0.69)	0.8 (0.61-1.04)

Prodigy precision errors were significantly less than the Delphi at the femoral neck, total femur and bilateral total femur. There was no significant difference in precision error at the spine and trochanter. Using bilateral femur measurements, precision errors were improved for both systems by 40% compared to the single femur results. We conclude that there are skeletal site-specific differences in precision error depending on whether a Prodigy or Delphi is used. In clinical practice, these differences should be considered when determining the time interval for least significant change in BMD to occur.



Disclosures: ***E.M. Lewiecki****, GE Medical Systems Lunar 2.*

SU104

Whole Body and Lateral Vertebral Assessment with a Digital 2D Bone Densitometer. C. Robert-Coutant*[1], M. Julier*[2], L. Gerfault*[1], G. Gonon*[1], L. Gaucher*[2], C. Gagnepain*[2], R. Grando*[2], J. M. Dinten*[1]. [1]Leti, CEA-GRENOBLE, Grenoble Cedex 9, France, [2]Diagnostic Medical Systems, Montpellier, France.

The Lexxos bone densitometer (DMS, France) is the first axial DEXA bone densitometer using a digital 2D radiographic flat panel detector. Previous presentations (SPIE Medical Imaging 2001[1], ASBMR 2001[2]) detailed how Lexxos realizes, without scanning, in two X-Rays flashes, spine, hip and forearm exams and presented its BMD measurement performance. One major Lexxos advantage is a quasi radiological image quality.

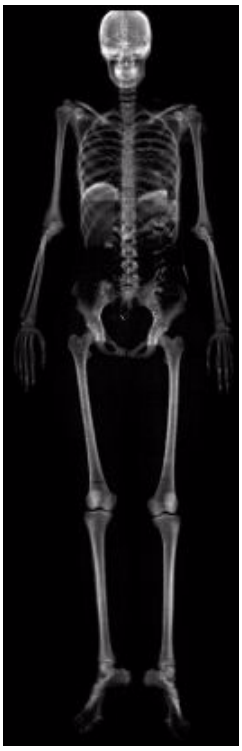
In order to take profit of Lexxos high image quality, its functionalities have been extended to whole body and lateral vertebral examinations. Due to the limited detector size, images are built from a set of acquisitions, composed of overlapping elementary low energy and high energy radiographs. They are combined by a dedicated reconstruction algorithm which includes a breathing artefacts correction process. Reconstructed images are isotropic and free of distortions.

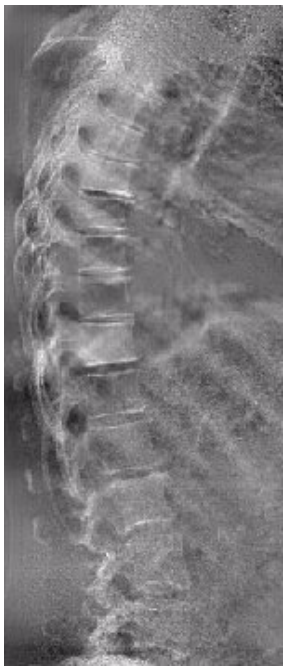
Whole body mode provides with three 1.6mm pixel size images. Whole body bone, fat and lean tissue images are realized in a continuous mode in less than 6 minutes. An automatic ROI detection algorithm provides with BMD, BMC, fat mass, lean tissue mass at the different ROI. Fat and lean tissue spatial distribution can also be analysed on whole body fat and lean tissue images.

Lateral vertebral mode provides with a 0.4mm pixel size vertebral bone image, obtained in a sequential mode in less than 30 seconds. ROI, corresponding to the different lumbar and thoracic vertebra, are automatically detected. A supervised tool enables a semi-quantitative analysis of vertebral deformations on each vertebral region, either on the bone or on the low energy image.

[1] "Dual-Energy X-Rays absorptiometry using a 2D digital radiography detector. Application to bone densitometry", J.M. Dinten et al.; SPIE Medical Imaging 2001

[2] "In vitro short term reproducibility evaluation of a Flash Beam X-Rays bone densitometer"
V. Boudousq et al.; ASBMR 2001





Disclosures: ***J.M. Dinten****, DMS 2.*

SU105

Real Volumetric Compared to Apparent Volumetric and to Areal Bone Mineral Density for the Evaluation of Bone Mass in Turner's Syndrome. A. Lage*[1], I. Verreschi*[1], J. Mendes*[1], M. Huayllas*[2], B. Liberman*[2], B. Mendonça*[3], E. Costa*[3], M. Lazaretti-Castro[1]. [1]Endocrinology, UNIFESP, Sao Paulo, Brazil, [2]Brigadeiro Hospital, Sao Paulo, Brazil, [3]USP, Sao Paulo, Brazil.

Turner Syndrome (TS) is a chromosome abnormality characterized by short stature and hypogonadism. Extremes statures are a known error factor for the bone mineral density (BMD) determination using the conventional dual energy X-ray absorbtiometry (DEXA). In the present cross-sectional study we evaluated lumbar spine BMD in 62 patients with TS aged from 10 to 45 years old obtained by different methods in order to determine the magnitude of the confounding effect of short stature. Using a Hologic 4500 A on postero-anterior (PA) and lateral projections, we obtained the areal (aBMD) and calculated the apparent volumetric (apBMD) and the real volumetric (volBMD) bone mineral densities. One hun-

dred and two normal females (10 to 45 years old) were used as controls, and their BMD values were used as reference. We considered "Normal" Z score for TS patient if >-1. In TS, the mean of Z-score for aBMD was significantly lower than for volBMD (-2.31+1.42 g/cm2 and -0.64+1.55 g/cm3, respectively; P <0.0001). The mean for apBMD was higher than for aBMD (-1.72 + 1.51 g/cm2 and -2.31+1.42 g/cm2, respectively; P <0.0001), even though it was lower than for volBMD (P <0.0001). Most of the patients (83.8%) had abnormal Z-score for aBMD. On the other hand, most of them (58.1%) had normal Z-score for volBMD. For apBMD, 30.6% of the patients had normal Z-score. When we evaluated the patients with normal Z-score for aBMD and volBMD in the age sub-groups, we noticed that in the sub-group of 10 to 12 years old, the percentage of girls that had normal Z-score for aBMD was similar to those with normal volBMD in the proportion of 1:1.3. In the other sub-groups the proportion was much larger culminating in the sub-group of 16 to 21 years old (1:11). When we compared the volumetric and areal Z-score, when patients were normalized for statural-age, we noticed that the mean for areal Z-score for statural-age was higher than the volumetric Z-score (0.02+1.31 and -0.64+1.55, respectively; P = 0.0008). There was no significant difference in the percentage of previous fractures between the 2 groups (TS and control). In conclusion, the aBMD underestimates the bone mass of the lumbar spine in patients with TS. This error is more evident in girls above 12 years old. Volumetric BMD minimized the confounding effect of short stature and approached the bone mass of control patients, while apBMD just partially minimized that effect. The prevalence of fractures in the 2 groups (TS and control) was not different and, therefore, in the analyzed patients, we did not find indications of increased bone fragility.

Disclosures: ***M. Lazaretti-Castro****, None.*

SU106

Increased Awareness of Osteoporosis Improves Radiologists' Reporting of Plain Films and DXA Examinations. L. Lenchik[1], A. J. Laster[2], S. Morgan*[3]. [1]Wake Forest University, Winston-Salem, NC, USA, [2]Duke University, Raleigh-Durham, NC, USA, [3]University of Alabama, Birmingham, AL, USA.

Purpose: Little is known about how physicians' attitude toward a disease influences their practice pattern. The purpose of this study was to determine how increased awareness of osteoporosis influences bone densitometry reporting among radiologists in the United States.
Methods: A one page survey was faxed to a random sample of 11,400 radiologists. Awareness of osteoporosis was determined based on responses indicating agreement (on a scale of 1 to 7) with 15 general statements about epidemiology, diagnosis, and treatment of osteoporosis. The responses were used to calculate the osteoporosis awareness score (scale of 1-14). Six multiple choice questions determined the approach to reporting of plain films and DXA examinations. Reporting practices were compared based on osteoporosis awareness.
Results: 397 (3.5%) complete surveys were returned and used in this analysis. When reporting central DXA examinations, 85% used the T-score < -2.5 for diagnosis of osteoporosis, 77% included the absolute BMD, 57% included fracture risk, and 27% included treatment recommendation. When reporting plain films in elderly patients, 96% routinely reported vertebral fracture, 87% reported osteopenia, and 43% recommended a DXA scan when either osteopenia or fracture (hip or spine) are present. General awareness of osteoporosis was satisfactory (mean score = 8.7/14, SD = 2.7). Radiologists who recommend DXA scans based on plain film findings and those who recommend treatment based on DXA results had higher osteoporosis awareness scores (9.5 vs 8.1 and 9.9 vs 8.3, p<0.0001).
Conclusion: Increased awareness about the epidemiology, diagnosis, and treatment of osteoporosis improves the quality of plain film and DXA reports among radiologists.

Disclosures: ***L. Lenchik****, Procter and Gamble/Aventis 2.*

SU107

Impact of Degenerative Spinal Diseases on Bone Mineral Density of the Lumbar Spine in Elderly Women. S. Muraki*[1], S. Yamamoto[2], H. Ishibashi*[2], T. Horiuchi*[2], T. Hosoi*[2], H. Orimo[2], K. Nakamura[1]. [1]Orthopaedics, Tokyo University School of Medicine, Tokyo, Japan, [2]Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan.

Most of the elderly have degenerative diseases of lumbar spine, which can affect the accuracy of bone mineral density (BMD) of lumbar spine. This study serves as the first to determine the relationship between lumbar spine or femoral neck BMD and the degree of several degenerative diseases at the same. The aim of this study is to determine whether BMD of lumbar spine is related to the degree of degenerative diseases. This study included six hundred and thirty women aged 60 or above (mean age: 73.3 plus/minus 6.9 years) visiting the Osteoporosis Outpatient Clinic in Tokyo Metropolitan Geriatric Medical Center. At entry to this study, subjects were undergone anteroposterior and lateral X-ray of the lumbar spine including L1 to L5. Radiographs were read for the presence and severity of osteophyte (Nathan classification), osteoarthritis (Kellgren method), bone sclerosis, joint space narrowing and spondylolisthesis (Meyerding method) involving L1-2 through L4-5 interspaces. Within one month after taking X-ray, BMD of L2-L4 anteroposterior lumbar spine and femoral neck were measured. The relation between BMD and the score of each item was assessed by regression analysis. Among 630 subjects, 619 (98.3%) had degenerative diseases of lumbar spine. BMD of femoral neck was correlated with age, while, BMD of lumbar spine was not correlated with age. However, among subjects with slight degenerative diseases, lumbar spine BMD was significantly correlated with age. The score of osteophyte, osteoarthritis, bone sclerosis, joint space narrowing and spondylolisthesis were correlated positively with BMD of lumbar spine, however, they had no correlation with BMD of femoral neck. In multiple regression analysis including age, BMI and all items of degenerative diseases, only BMI, osteophyte, bone sclerosis and joint space narrowing were independently correlated with BMD of lumbar spine. According to the multiple regression analysis, adjusted lumbar spine BMD 0.122mg/cm^2 lower than observed BMD. Unlike observed BMD of lumbar spine, adjusted BMD of lumbar spine was correlated with age. This study suggests that degenerative diseases of lumbar spine are important sources of BMD overestimation at this site, thus leading to clinical errors. We conclude that BMD of lumbar spine was related to the degree of degenerative diseases.

Disclosures: ***S. Muraki****, None.*

SU108

Comparison between Osteoprotegerin and Bone Markers in Relation to Age Related Decline in Bone Mass. O. S. Indridason*, L. Franzson*, O. Gunnarsson*, S. L. Gudmundsdottir*, G. Sigurdsson. Department of Medicine, University Hospital, Reykjavik, Iceland.

The purpose of this study was to compare age related differences in osteoprotegerin (OPG) and the bone markers osteocalcin (OC), Collagen crosslinks (CX) and tartrate resistant acid phosphatase (TRAP) in relationship with bone mineral density (BMD).
Data were derived from a cross-sectional study on bone health in a random sample of community dwelling adults aged 30 to 85 years in the Reykjavik area in Iceland. All subjects had whole body, hip and lumbar spine BMD measured (DEXA), gave blood and urine samples and answered a thorough questionnaire on medications and medical history. OPG was measured with an ELISA (Biomedica, Austria), OC and CX with an ECLIA (Roche Diagnostics), TRAP with an ELISA (Suomen Bioanalyttica). We used ANOVA to compare age groups and assessed relationships using partial correlation coefficient. For the current analysis we excluded subjects receiving bisfosfonates, HRT, thiazides or glucocorticoids and those with diseases affecting bone and mineral metabolism. Men and women were analysed separately.
Of 2310 subjects invited over 2 years, 1630 participated. After exclusion, 527 women (age 55.7±16.9) and 496 men (age 58.4±14.9) remained for analysis. In women, BMD at the hip declined steadily after age 50. Women in their 50´s had higher OC (33%), TRAP (30%) and CX (55%) than women in their fourties (P<0.001) but the levels plateaued above age 50. OPG increased steadily with age and this increase was most marked in the oldest age groups rather than around menopause. In men, hip BMD remained stable until after age 70 after which it declined significantly (P<0.001). Significantly higher OC (14%), TRAP (6%) and CX (14%) were seen in these oldest age groups compared to those younger than 70 (P<0.05). OPG increased steadily with age as in women. After controlling for age and weight, neither TRAP nor OPG were significantly related to hip BMD in either sex, but OC and CX were associated with hip BMD, r=-0.16, P<0.001 and r=-0.10, P=0.02 in women, and r=-0.17, P<0.001 and r=-0.18, P<0.001 in men, respectively.
We conclude that age related bone loss starts in women around age 50 but in men at age 70. Serum levels of markers of bone turnover increase when age related bone loss starts and this increase is greater for markers of bone resorption (CX). OPG shows a gradual increase with advancing age independent of bone mineral density. The significance of OPG in maintenance of bone remains to be elucidated.

Disclosures: ***O.S. Indridason****, None.*

SU109

Regional Variations in Cancellous BMD within the Distal Metaphysis of the Rat Femur. H. Lemmon*[1], E. Johnson*[2], D. D. Cody[2], C. G. Ambrose*[3], H. A. Hogan[1]. [1]Mechanical Engineering, Texas A&M University, College Station, TX, USA, [2]M. D. Anderson Cancer Center, University of Texas, Houston, TX, USA, [3]Orthopaedic Surgery, University of Texas Medical School, Houston, TX, USA.

This study was motivated by efforts to measure mechanical properties of cancellous bone tissue in the metaphysis regions of rat femur and tibia specimens. The reduced platen compression (RPC) test utilizes specimens that are 2mm thick and located adjacent to the growth plate, but at a distance intended to avoid primary spongiosa. The specific location of individual specimens is routinely determined using high-resolution contact radiographs (coronal view), which inherently leads to some variability in the locations of the specimens relative to the growth plate region. The purpose of this study was to estimate the effects of this variability on mechanical properties by analyzing variations in bone mineral density (BMD), as a surrogate for mechanical properties. Nine distal femur specimens were selected for analysis from animals from another study, with the goal to encompass a wide range of BMD values. Adult (353 gm avg. initial BW) female Sprague-Dawley rats were sacrificed after 8 weeks. MicroCT scans (model RS-9, GE Medical Systems, London Ontario) were made of the distal third of the right femur (excised) at a resolution of 27 microns. 3D volumes were reconstructed using vendor software. BMD was calculated using a cylindrical region of interest (ROI) that was 2mm long in all cases (to correspond to the size of RPC specimens). The diameter of each ROI was based upon the largest circular region that would inscribe the endocortical perimeter at the proximal extent of the ROI (where the endocortical compartment is smallest) . A reference location for the ROI was defined such that the distal surface of the ROI was aligned with the most proximal part of the growth plate (i.e., junction between epiphysis and metaphysis). BMD was measured for this reference location plus 10 additional locations (5 proximal & 5 distal to reference, in 0.2mm increments). BMD ranged from 1 to 662 gm/cc. To quantify "variability," BMD values were averaged for all specimens at each ROI location and then expressed as a percent of this average. The range (over the 11 locations) of this % for each of the 9 specimens was: 1-30, 7-37, 13-48, 36-100, 43-84, 74-82, 150-186, 175-335, 205-222, resp., which indicates only modest variation as a function of ROI location for most specimens. For ROI locations limited to 0.2mm proximal to 1.0mm distal, the variations become much smaller: 11-30, 11-37, 17-48, 83-100, 74-84, 74-78, 150-179, 175-225, 208-222. These results suggest that specimen locations closer to the growth plate should give more consistent results than those more proximal.

Disclosures: ***H.A. Hogan****, None.*

SU110

Use of Heel Ultrasound to Screen for Osteoporosis: Comparison with Spine and Femur DXA. P. K. Burke. Osteoporosis Diagnostic and Treatment Program, Richmond, VA, USA.

Recently the International Society for Clinical Densitometry (ISCD) recommended the use of peripheral densitometry (including heel ultrasonometry) to identify patients who might have osteoporosis and should therefore undergo bone densitometry at the hip and spine. This recommendation requires the use of a device specific T-score cutpoint on the peripheral device that detects 90% of individuals with osteoporosis (T-score $\leq$ -2.5) at either the spine or hip. In this study, we wished to determine the 90% sensitivity cutpoint that could be used with the Achilles InSight bone ultrasonometer.

The Lunar Achilles InSight (GE Medical Systems) is an imaging ultrasonometer that uses inflatable membranes and an alcohol spray to couple the measurement transducers to the heel. The actual ultrasound measurement takes 8 to 10 seconds; the complete examination (including data entry, patient preparation and positioning) takes 3 to 5 minutes. In this study, 85 female patients (mean age 65 $\pm$ 12 years) referred for bone densitometry were assessed. Each patient had DXA measurements of the spine and both hips using a Lunar Prodigy (GE Medical Systems), as well as heel ultrasound measurement using the Achilles InSight. The lowest T-score at the L1-L4 spine, femoral neck, trochanter and total femur was determined for each patient. Osteoporosis was diagnosed when the lowest T-score was $\leq$ 2.5. Using heel T-scores ranging from 0.0 to –1.6, the sensitivity and specificity for detecting central osteoporosis was calculated. The T-score cutpoint was then determined which correctly identified 90% of the patients with osteoporosis as defined by the spine and hip measurements.

Sensitivity and Specificity of Central Osteoporosis Based on Heel T-Score

Heel T-Score	Sensitivity	Specificity
-0.2	97%	31%
-0.4	97%	33%
-0.6	97%	41%
-0.8	82%	51%
-1.0	79%	59%
-1.2	79%	65%
-1.4	76%	69%

From the DXA results, 34 of the 85 patients were classified as osteoporotic. Sensitivity of the heel T-score ranged from 76% to 97%, reaching 90% at a value between –0.6 and –0.8. At a heel T-score of –0.6, sensitivity was 97% and specificity was 41%. A lower cutpoint of –1.0 provides almost 80% sensitivity but with much better specificity (59%). We conclude that the Achilles InSight can be used as a valid screening tool for osteoporosis according to ISCD recommendations. Using a T-score cutpoint of –0.6 identified more than 90% of patients with osteoporosis at the spine or hip, with a specificity of greater than 40%.

Disclosures: ***P.K. Burke****, GE Medical Systems Lunar 5.*

SU111

Impaired Bone Quality in Bisphosphonate (BISPHOS) Treatment and Renal Transplantation by Ultrasound Critical Angle Reflectometry (UCR). E. Richer*[1], M. Lewis*[1], C. V. Odvina[2], M. A. Vasquez*[3], C. Y. C. Pak[2], P. P. Antich*[1]. [1]Radiology, UT Southwestern Medical Center, Dallas, TX, USA, [2]Center for Mineral Metabolism and Clinical Research, UT Southwestern Medical Center, Dallas, TX, USA, [3]Medicine, UT Southwestern Medical Center, Dallas, TX, USA.

This study was undertaken in order to determine whether intrinsic bone quality is altered following long-term BISPHOS treatment. The UCR technique previously described by us (1) has been incorporated in a new device designed to measure cortical and cancellous bone ultrasound velocities (v) at multiple orientations in the calcaneus *in vivo*. These measurements are consistent with the hypothesis that bone has transverse symmetry, characterized by the relationship $v^2 = a+bx^2+cx^4$, where x = cos (orientation), and v^2 is elasticity normalized to a density of 1 g/cc. By fitting v data to this formula, the minimum (E^*_{min}) and maximum (E^*_{max}) elasticities of trabecular and cortical bone were calculated as a measure of bone material quality.

We obtained E* in 14 normal postmenopausal women (NPO), 9 with postmenopausal osteoporosis on conventional treatment (PO), 25 on BISPHOS treatment, and 11 with renal transplantation on steroids (RT-St). Values were expressed as percentage of values in 14 normal premenopausal women (Table 1). Mild or no decreases (<5%) were observed in NPO. A mild-moderate reduction in E* (~10%) was disclosed in untreated PO. In BISPHOS, trabecular E* declined by ~ 20%, and cortical E* decreased by 26% (mean duration = 3.5 y). Much of the decline in E* occurred during the first 3 y of BISPHOS treatment. In RT-St, the decline in E* was qualitatively similar to that of BISPHOS. In BISPHOS and RT-St, E* was independent of heel bone mineral density (DEXA). In other groups, E* was modestly correlated with bone density.

Table 1. Trabecular and Cortical Elasticities Measured by UCR

Elasticity	Normal Postmenopausal	Osteoporotic Untreated PO	Osteoporotic BISPHOS	RT-St
Trab. E^*_{min}	100.1 ± 3.1	93.1 ± 5.8	83.2 ± 11.5$^+$	85.1 ± 1.8$^+$
Trab. E^*_{max}	98.6 ± 4.3	88.6 ± 6.3$^+$	80.9 ± 9.0$^+$	83.2 ± 1.8$^+$
Cortical E^*_{min}	97.4 ± 5.0	91.5 ± 1.7*	74.0 ± 8.5$^+$	75.0 ± 2.4$^+$
Cortical E^*_{max}	96.8 ± 4.3	87.0 ± 1.7**	74.2 ± 12.6$^+$	71.4 ± 2.6$^+$

* $p < 0.05$; ** $p < 0.01$; $^+$ $p < 0.001$ vs. normal premenopausal women.

In conclusion, quality of cortical and trabecular bone is significantly impaired following long-term BISPHOS treatment, as in a condition associated with low bone turnover and propensity for fractures (RT-St).

1. Antich PP, Anderson JA, Ashman RB, Dowdey JE, et al.: Measurement of mechanical properties of bone material in vitro by ultrasound reflection: methodology and comparison with ultrasound transmission. J Bone Miner Res, 6:417-426, 1991.

Disclosures: ***E. Richer****, None.*

SU112

Bone-Density-Independent Association of Quantitative Ultrasound Measurements and Fracture Risk. T. V. Nguyen, J. R. Center, J. A. Eisman. Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, Australia.

Quantitative ultrasound measurements (QUS) of bone, that may reflect micro-architectural aspects of bone, have been shown to be associated with fracture. However, it is not clear whether this association is independent of bone mineral density (BMD). This study was designed to examine the contributions of cortical QUS and BMD measurements to the prediction of fracture risk in postmenopausal Caucasian women.

Speed of sound (SOS) at the distal radius, tibia, and phalanx (Sunlight Omnisense) and BMD at the lumbar spine and femoral neck (GE Lunar) were measured in 549 women, aged 63.2 ± 12.3 yr (mean ± SD; range: 49 – 88 yr), including low trauma 77 fracture cases. Lower SOS at the distal radius, tibia and phalanx, which were correlated with each other, were associated with increased risk of fracture. Independent predictors of fracture risk (in multivariate analysis) were: distal radius SOS (OR: 1.8 per SD; 95% CI: 1.3 – 2.4), femoral neck BMD (OR per SD: 1.9; 95% CI: 1.4 – 2.4) and age (OR per 5-yr: 1.2; 95% CI: 1.0 – 1.5). Approximately 30% of the women had distal radius SOS T-scores < -2.5; however, only 6.6% of women had both BMD and SOS T-scores < -2.5. Among the 77 fracture cases, only 14 (18.2%) had both BMD and QUS T-scores below -2.5.

In these postmenopausal women, speed of sound at the distal radius was associated with fracture risk, independent of BMD and age. The combination of QUS and BMD measurements may improve the accuracy of identification of women who likely sustain a low trauma fracture.

Disclosures: ***T.V. Nguyen****, None.*

SU113

Calcaneal Quantitative Ultrasound in Japanese Elementary Schoolchildren and Proper Positioning of Heel Using Adapters for CM-100. K. Nakatsuka[1], K. Mimura*[2], T. Yamamoto[3], H. Morii[1], T. Arai*[4]. [1]Osaka City University Medical School, Osaka, Japan, [2]Osaka University of Education, Osaka, Japan, [3]Dept. of Pediatrics, Minoh City Hospital, Osaka, Japan, [4]Furuno Electric corpration, Nishinomiya, Japan.

Assessment of bone health is important in growing children. Although change of bone mass measured by using quantitative ultrasound (QUS) at the calcareous have been previously reported, little is shown on details on how to overcome difficulties in applying QUS devices developed for adults to children. The aims of the present study is to measure calcaneal bone mass by using specially designed adapters which can adjust the position of the heel according to the given foot length of children, and to examine the reliability of the values obtained for these purposes. Subjects consisted of 189 Japanese children living in Osaka Prefecture and belonging to elementary school (6-12 years of age). Body heights, body weights and foot lengths were measured at the same time of QUS measurements. Calcaneal bone mass of right feet were measured using 3 different devices CM-100 (Furuno Electric Co. Japan), AOS-100 (Aloka Co. Japan) and UBIS3000 (DMS Co. France). In case of measurements using CM-100 the heel position of the subjects were adjusted by placing 2 specially designed adapters A and B according to foot lengths. Tomographic imaging of the calcaneal region was also obtained by echo effects of UBIS3000, which makes it easier to adjust manually the heel to the proper position exposed with ultrasonic wave. The lower the SOS values (CM-100) the longer the feet length in the subjects with feet length of 22 or less cm. Although there was no significant differences in SOS between UBIS3000 and CM-100 (Adapter A) in boys, significant differences in SOS were found when CM-100 (Adapter B) and AOS-100 were employed. Similar trends were observed in evaluating differences in SOS among devices in girls. In addition, values of SOS measured by CM-100 (Adapter A) had a greater relationship with that measured by UBIS3000 than those measured by other devices in both boys and girls. In conclusion, proper positioning of heel using an automatic adapter should be recommended for the QUS calcaneal bone mass measurement in growing children.

Disclosures: ***K. Nakatsuka****, None.*

SU114

Quantitative Ultrasound to Evaluate Bone Health in Term and Preterm Infants. H. McDevitt*, C. Tomlinson*, M. White*, S. F. Ahmed. Bone and Endocrine Research Group, Yorkhill Hospital, Glasgow, United Kingdom.

Quantitative ultrasound (QUS) assessment of speed of sound (SOS)has been used extensively in the adult population, and to a lesser extent in paediatrics, as a marker of bone health. It has been used only in limited settings in the neonates. In this study we assessed the ease of use and reliability of the Sunlight Omnisense 7000P in the neonatal population.

Infants were eligible for the study if they were born between 24 and 42 weeks and parents consented to the study. 75 infants (39 male) with gestational age range 25 - 41 weeks and birthweights 790 - 4500g had at least one ultrasound scan performed at the right tibia, 20 of

these 75 infants also had measurements performed at the left tibia and both radii. Overall the Omnisense scanner was easy to use at the tibia in the neonate with low intraobserver error. 23 infants had 3 or more measurements at the right tibia with a mean SOS of 3068 m/s and SD of 45 m/s. Radial measurements were found to be technically much more difficult and time-consuming and the results were found to be similar to tibial measurements.
The 52 term infants tibial SOS varied between 2850 - 3300 m/s with a mean of 3082 m/s and SD 99 m/s. There was no correlation between birthweight or sex and SOS within the term group. 23 preterm infants gestation 24-36 weeks had an average SOS 2925 m/s and SD of 132 m/s. This difference was statistically significant $p<0.0001$ from the term infant group. Within the preterm group there was no relation between sex or birth weight and SOS.
In conclusion, we found that the Omnisense 7000P is able to perform QUS measurements in both term and preterm babies with reproducible results. There is no advantage to measuring multiple sites. SOS measurements are significantly lower in preterm babies. We obtained normative data on tibial SOS for term and preterm babies and this may be of use in future studies. Further studies are needed to establish QUS as a tool for assessing bone mineralisation in term and preterm infants.

Disclosures: ***H. McDevitt****, None.*

SU115

Bone Ultrasonography and Dual X-Ray Absorptiometry in the Assessment of Corticosteroid Induced Osteoporosis. C. Cepollaro*[1], S. Gonnelli[1], A. Montagnani*[1], C. Caffarelli*[1], N. Nikiforakis*[2], M. Martino*[2], P. Rottoli*[2], R. Nuti[1]. [1]Department of Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Siena, Italy, [2]Division of Pulmunary Diseases, University of Siena, Siena, Italy.

Patients treated with glucocorticoids (GCPs) present an increased risk of osteoporosis and fractures. However, in GCPs the reduction in BMD is smaller than that expected given their increased risk of fracture. A possible explanation is that DXA may fail to detect qualitative changes in bone. Quantitative ultrasonography (QUS) has been proposed as a technique that could theoretically provide information on bone structure. The aim of the present study was to investigate the usefulness of QUS, compared to DXA, for detecting bone status in GCPs.
We studied 179 GCPs (56.6±13.9 yrs, 127 women, 52 men) treated with oral prednisone or equivalent at a dose of $\geq$7.5 mg/day for at least 1 year. In all we measured BMD at lumbar spine (BMD-LS) and at femoral subregions (femoral neck: BMD-FN, total hip: BMD-T, trochanter: BMD-TR, intertrochanter: BMD-ITR, Ward's triangle: BMD-W) by DXA (QDR 4500, Hologic), and ultrasound parameters at calcaneus: speed of sound (SOS), broadband ultrasound attenuation (BUA) and Stiffness (S), by Achilles plus (GE.), and at phalanxes: amplitude dependent speed of sound (AD-SoS), and the parameters of the graphic trace by Bone Profiler (Igea). The data were analysed in all the population and after division in tertiles on the basis of cumulative dose (CD).
The T-scores were -2.2 for BMD-LS, -2.1 for BMD-FN, -1.2 for BMD-TR, -1.3 for BMD-ITR, -1.4 for BMD-T, -2.5 for BMD-W, -1.2 for SOS, -2.5 for BUA, -2.2 for S and -2.5 for AD-SoS. A significant correlation was found between CD and femoral subregions, the greatest value was for BMD-FN ($r=-0.33$, $p<0.001$). No significant correlations were found between CD and BMD-LS and QUS parameters. The analysis of variance ANOVA showed that femoral subregions and SOS and S were significantly different on the basis of CD. The discriminatory ability of different parameters to predict a BMD-FN T-score less than –1.5, assessed by ROC curves, resulted better for femoral subregions than for BMD-LS and QUS parameters; among QUS, S and AD-SoS showed the highest values. The analysis of the graphic trace showed a similar pattern with respect to postmenopausal osteoporosis.
We can conclude that: chronic treatment with corticosteroids is able to decrease in DXA and QUS parameters; trabecolar bone appears to be more affected with respect to cortical bone; BMD at proximal femur reflects better the skeletal damage produced by corticosteroids therapy. Among QUS parameters, S and AD-SoS show the greatest reduction in GCPs and the best ability to predict low BMD.

Disclosures: ***C. Cepollaro****, None.*

SU116

Effect of Soft Tissue on Ultrasonic Guided Wave Measurements in Bone. P. Moilanen*[1], P. H. F. Nicholson*[1], Q. Wang*[1], J. Timonen*[2], S. Cheng[1]. [1]Department of Health Sciences, University of Jyvaskyla, Jyvaskyla, Finland, [2]Department of Physics, University of Jyvaskyla, Jyvaskyla, Finland.

Current clinical techniques for ultrasonic assessment of long bones such as the tibia use an axial pulse transmission approach and measure the velocity of the first arriving signal. Such velocities are close to the bulk velocity for cortical bone (3600-4200 m/s). Elsewhere we have described a low frequency axial transmission method capable of measuring an additional "second " wave, consistent with a so-called Lamb A0 guided wave propagating in the cortex with a velocity ranging from 1300-2000 m/s. Guided waves are potentially sensitive to the material and geometric properties of the cortical layer, and may therefore offer an enhanced approach to long bone characterization. However, since the velocity of the second wave in bone overlaps that of soft tissue (1450-1600 m/s), it is possible that signals propagating through soft tissue may interfere with, or be mistaken for, a second wave in bone. In order to clarify this question, measurements were made on water or silicon rubber layers on top of solid substrates (metal, acrylic, animal bone), animal bone with and without skin, and the human tibia in vivo. Ultrasound velocity was measured separately in silicon rubber, and animal and human soft tissue. When the signal was mediated through water, no second wave could be observed. On the other hand, in all cases with soft tissue or tissue-mimics overlying the solid substrates, a second wave was observed. Comparing the second wave velocity to the tissue velocity confirmed, in most cases, that the second wave was consistent with a guided wave in the solid layer rather than a wave in the tissue. Tibial measurements were made in a group of 67 mature female subjects. Difficulties in obtaining a reliable determination of second wave velocity were encountered predominantly in overweight women (BMI>30). In normal women (BMI<30), satisfactory measurements were possible in 82% of subjects. In contrast, approximately 33% of the total group were overweight, and of these 41% could be reliably measured. In conclusion, whilst the in vitro results support the concept of exciting and detecting guided waves in bone through soft tissue, clinical measurements of the second wave using our current methodology are problematic in subjects with thick soft tissue. Options for modifying and improving the measurements with respect to clinical soft tissue effects are being investigated, but it should be noted that overweight women are not at high risk of osteoporosis. The idea of using guided waves for bone characterization remains an attractive potential adjunct to existing measurements.

Disclosures: ***P. Moilanen****, None.*

SU117

Performance Evaluation of the Achilles InSight: Precision, Accuracy, and Comparison to Central DXA. E. Hosszu*[1], S. Meszaros*[2], V. Ferencz*[2], C. Horvath[2]. [1]Department of Pediatrics, Semmelweis University, Budapest, Hungary, [2]Department of Internal Medicine, Semmelweis University, Budapest, Hungary.

Heel ultrasound measurements have been shown to predict fractures in several prospective studies. However, not all heel ultrasonometers have equivalent clinical utility. Differences in technology, coupling, imaging capabilities, measurement time, precision, and relationship to central (spine and hip) measurements exist between systems. In this study, we evaluated a new imaging ultrasonometer, the Achilles InSight.
Fifty-two subjects referred for bone density evaluation were evaluated. Subject age ranged from 23 to 76 years, with an average of 55 $\pm$ 10 years. Each subject had spine and hip DXA measurements using the Prodigy (GE Medical Systems Lunar) as well as heel ultrasound measurements using Achilles InSight (GE Medical Systems Lunar). The InSight provides an ultrasound image of the heel to aid acquisition. In addition, the InSight is capable of using alcohol spray instead of ultrasonic gel for coupling, greatly decreasing measurement time. Precision (RMS standard deviation for the repeat measurements) was determined from a subset of 25 subjects measured twice each with both alcohol and gel. Sensitivity was assessed by comparing the InSight T-score results to the Prodigy values using a paired t -test. Using ISCD recommendations for peripheral screening, the 90% sensitivity cutpoint was determined for detecting osteoporosis (T-score $\leq$ -2.5) at the spine or hip.
Average T-score for the InSight was -1.4 ± 1.1 using alcohol and -1.2 ± 1.3 using gel coupling. This difference, though of little clinical importance, was statistically significant ($p < 0.01$). There was no significant difference in precision error between the alcohol and gel (1.8% vs. 1.7%, $p = 0.44$). Fourteen of the 52 subjects were found to have osteoporosis at the spine or hip. From the ultrasound results, the 90% sensitivity cutpoint was found to be at a T-score of -0.6 for the alcohol and at –0.5 for the gel, with specificity of 32% and 35%, respectively.
We conclude that the Achilles InSight is an accurate and precise imaging ultrasonometer, with precision error less than 2% using either alcohol or gel. Using alcohol coupling, the InSight has significantly reduced preparation, measurement, and clean up time compared to gel-based ultrasonometers. The InSight can be used as an effective screening tool using a T-score cutpoint of –0.6, providing better than 90% sensitivity for detecting subjects with osteoporosis at the spine or hip. In situations where central DXA systems are not readily available, the InSight can be used to identify those who should be considered for spine and hip density measurements.

Disclosures: ***C. Horvath****, None.*

SU118

Performance of a Confocal Acoustic Mapping in Characterization of Trabecular Bone Quality in Human Calcaneus. Y. Xia*[1], W. Lin*[1], E. Mittra*[1], B. Demes*[1], B. Gruber[2], C. Rubin[1], Y. Qin[1]. [1]Biomedical Engineering, SUNY Stony Brook, Stony Brook, NY, USA, [2]Medicine, SUNY Stony Brook, Stony Brook, NY, USA.

Quantitative ultrasound (QUS) measurement of the calcaneus has been increasingly used to assess for osteoporosis. It provides both structural and strength information of bone in a manner which is non-invasive, non-destructive, repeatable, safe and relatively accurate. On this basis, a scanning confocal acoustic imaging system at the region of interest (ROI) has been developed. The objective of this work is to evaluate the performance of confocal ultrasound mapping in characterization of human calcaneus bone. 19 human calcaneus, harvested from cadavers with ages 66~97 have been imaged. Broadband ultrasound attenuation (BUA) and ultrasound velocity (UV) determined from ROI have been performed. To evaluate the capability of high-resolution acoustic images in predicting bone's structural and strength properties, cylindrical samples (10 mm in diameter and 20 mm in medial-lateral length) at ROI are extracted from calcaneus and subjected to micro-CT (~20 micron resolution) scanning and mechanical strength testing. Strong correlations were found between BUA and bone volume fraction (BV/TV) ($R^2 = 0.76$), and between UV and bone's modulus ($R^2 = 0.53$) (Table 1). The correlations are significantly improved ($R^2 > 0.64$) using combined parameters of BUA and UV in linear regression analysis which ultrasound images determined parameters predict structural, e.g., structure morphological index (SMI) ($R^2 = 0.86$), and strength modulus ($R^2 = 0.64$) (Table 1).
These results suggest that high-resolution acoustic mapping is capable of predicting calcaneus bone quantity and quality non-invasively. Structural property parameters of trabeculi, e.g., BMD and BV/TV, is better represented by BUA, while ultrasonic wave velocity has a strong agreement with bone's strength property, e.g., modulus. This imply that overall bone quality is closely associated with both structure and stiffness of bone, in which combined BUA and UV has demonstrated significant performance in predicting mechanical charac-

teristics of trabecular bone. Ultrasonic imaging has shown the great potential to be used as *in vivo* diagnostic modality for assessing skeletal disorder, i.e., osteoporosis.

Table 1. Correlation Coefficients (R^2) between US and Bone Quantity and Quality Parameters

	BUA	UV	Combined BUA and UV
Modulus	0.45	0.53	0.64
Bone Volume Fraction	0.76	0.40	0.80
SMI	0.82	0.39	0.86

Disclosures: ***Y. Xia****, None.*

SU119

Comparison of Quantitative Heel Ultrasound with Osteodensitometry in Postmenopausal Women with Colles' Fracture and Evaluation of Secondary Osteoporosis. G. Tautermann*[1], P. Langer*[1], A. Gohm*[2], R. Zinnecker*[2], K. Benedetto*[2], H. Drexel*[1], G. Hoefle[1]. [1]Endocrinology, Academic Teaching Hospital, Feldkirch, Austria, [2]Traumatology, Academic Teaching Hospital, Feldkirch, Austria.

The aim of this study was to prospectively compare QUS of the calcaneus to BMD of the spine and femur using DXA in a cohort of postmenopausal women with Colles' fracture. The study population consisted of 64 women aged between 48 and 89 years who were referred to an osteoporosis screening programme after a wrist fracture.
Broadband ultrasound attenuation (BUA) and speed of sound (SOS) measurements as well as T score and stiffness evaluation were performed at the calcaneus using a Hologic Sahara ultrasound device.BMD of the lumbar spine, femoral neck and total hip was measured by DXA.Simultaneously bone turnover was assessed by measurement of serum ß- Cross-Laps.The incidence of osteoporosis using the WHO criteria was 27% at the calcaneus, 28 % at the spine and 7.3 % at the femoral neck.The incidence of osteopenia at the spine and femur as evaluated by DXA was 32 % and 40% respectively. Correlation coefficients for ultrasound and DXA T scores were in the range of 0.4.A similar result was obtained when SOS, BUA and stiffness were regressed against lumbar spine BMD and femoral neck BMD.Secondary osteoporosis accounted for 25 % of cases, the majority of which was attributed to alcoholism (7 of 16 individuals or 44%). An endocrinological cause due to primary/secondary hyperparathyroidism or hyperthyroidism was diagnosed in 25 % (4/16) of these patients.Glucocorticoid use was prevalent in 12.5% or 2/16 patients.Fifty nine percent of the study population had a pathologically elevated ß-CrossLap level. We conclude that QUS relates poorly to axial BMD measurements and that ultrasonographic diagnosis of osteoporosis was not reliable as a screening tool in our study population. The high incidence of secondary osteoporosis warrants full lab screening of all osteoporotic patients with Colles' fracture.

Disclosures: ***G. Tautermann****, None.*

SU120

Linkage Disequilibrium Analysis of the PTH/PTHrP Receptor Gene across different Human Populations. L. Fröhlich*, R. C. Gensure, H. Jüppner, M. Bastepe. Endocrine Unit, Massachusetts General Hospital/ Harvard Medical School, Boston, MA, USA.

The use of haplotype blocks in the human genome which contain polymorphisms that are in linkage disequilibrium (LD) could reduce the number of markers required for genome-wide scans of genetic linkage. This approach might provide an important tool in genetic studies to dissect complex multi-factorial diseases. To investigate LD within the human PTH/PTHrP receptor gene, we examined allelic association of four different single nucleotide polymorphisms (SNPs) at this locus. In addition to two previously described polymorphisms (Intron S/E1,BsmI (1): Exon M7, BsrDI(4)), two new SNPs were identified (Intron E1/E2: FokI (2), AvaI (3)) and genotyped for individuals of Caucasian, African-American and Asian origin. Expectation maximization algorithm (EH program) was employed to calculate chi-square values based on haplotype frequencies estimated by the allelic association hypothesis.

LD analysis of the PTH/PTHrP receptor gene.

SNP (Distance)	Overall population	Caucasians n=32	Afr. Americans n=18	Asians n=16
1-4 (24449 bp)	136 (p<0.001)	63 (p<0.001)	36 (p<0.01)	59 (p<0.001)
1,2 (15701 bp)	2.33 (p=1)	9.4 (p<0.1)	1.3 (p=1)	7.5 (p<0.4)
2,3 (154 bp)	24 (p<0.002)	7.3 (p<0.4)	1.3 (p=1)	26 (p<0.002)
3,4 (8594 bp)	32 (p<0.002)	24 (p<0.002)	1.4 (p=1)	26 (p<0.002)

Analysis of the four markers as a group in the overall population showed LD, which also appeared in the subpopulations. However, pairwise analysis of neighboring SNPs indicated no significant association between SNPs 1 and 2 in the overall population. In Caucasians, only SNPs 3 and 4 were in LD, and in African-Americans there was no LD. In Asians, on the other hand, the extent of LD was the same as that of the overall population. Because LD between SNPs 2 and 3 was evident only in the Asian population, we verified this association by direct sequence analysis of genomic DNA from a sample group of double heterozygous individuals. Our results thus demonstrate that LD in the region of the PTH/PTHrP receptor gene varies between subpopulations, even for genetic markers located only 154 bp apart. Based on these results, it might be necessary to consider population-specific differences in the use of haplotype blocks for genome-wide linkage screens.

Disclosures: ***L. Fröhlich****, None.*

SU121

Allele Frequencies at 11 BMD-Affecting Loci Examined Are Different in Africans from Caucasians or Asians. G. Gong*, G. Haynatzki. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

To date, more than 30 candidate genes have been found to be associated with bone mineral density (BMD) in Asian and Caucasian populations. We have recently shown the importance of investigating African populations in the effort to identify susceptibility genes for common diseases such as osteoporosis. We have investigated the allele frequencies of 11 candidate loci in 86 recent immigrants from Africa (Sudanese) shown in the Table (n=86).

Gene	Enzyme	Allele frequency
COL1A1	MscI	0.023
Parathyroid hormone	BstBI	0.843
Osteocalcin	HindIII	0.762
Calcium-sensing receptor	BsaHI	0.981
Calcitonin Receptor	AluI	0.399
Estrogen receptor	PvuII	0.715
Estrogen receptor	XbaI	0.821
Vitamin D receptor	TaqI	0.395
Vitamin D receptor	ApaI	0.378
TGF β1	BstUI	1
a_2HS-Glycoprotein	NIaIII	0.291
Interleukin-6	BsrBI	0.75
Osteoprotegerin	RsaI	0.85

The numbers in the table are the frequencies of alleles with the restriction site. The allele frequencies of these candidate genes in the Sudanese immigrants are significantly different from those of either Asians or Caucasians or both except osteoprotegerin of which no published data are available for comparison. Notably, the allele frequency of COL1A1 is close to 0, similar to that seen in Asian populations. Also there is no polymorphism in TGFβ 1. Their potential association with BMD is under investigation.

Disclosures: ***G. Gong****, None.*

SU122

Association of Molecular Variants, Haplotypes, and Linkage Disequilibrium within the Tumor Necrosis Factor Receptor Associated Factor-Interacting Protein (I-TRAF) Gene with Adult Bone Mineral Density. R. Ishida*[1], Y. Sudo*[1], Y. Ezura[1], H. Yoshida*[2], T. Hosoi[2], S. Inoue[3], M. Shiraki[4], H. Orimo[2], H. Ito*[5], M. Emi*[1]. [1]Molecular Biology, Nippon Medical School, Institute of Gerontology, Kawasaki, Japan, [2]Tokyo Metropolitan Institute of Gerontology and Geriatrics Hospital, Tokyo, Japan, [3]Department of Geriatric Medicine, University of Tokyo, Faculty of Medicine, Tokyo, Japan, [4]Research Institute and Practice for Involutional Diseases, Nagano, Japan, [5]Orthopaedics, Nippon Medical School, Tokyo, Japan.

Osteoporosis is a multi-factorial common disease that results from interplay of multiple environmental and genetic factors. Signaling from tumor necrosis factor (TNF) receptor family is the most potent bone-resorbing system that regulates bone mineral status of individuals. Here in this study, we investigated TNF receptor associated factor-interacting protein (I-TRAF), an essential effecter of this system in genetic studies of 384 Japanese adult women. In the association study for age and body mass index adjusted radial bone mineral density (BMD), we found a significant correlation of the genotypes of a promoter variation -1542T/G with the adjusted BMD ($r = 0.13$, $p = 0.012$). Four promoter variations of I-TRAF gene including -525G/C were in strong linkage disequilibrium (LD) at the level of almost complete LD ($D' = 0.978$, $r^2 = 0.917$, $Chi^2 = 695.2$, $p = 3.4 \times 10^{-153}$). When the adjusted BMD were compared among the three-haplotypic categories focusing on two exclusive haplotypes (-1542T/-525C, frequency 0.74 and -1542G/-525G, frequency 0.24), BMD was lowest among -1542G/-525C homozygotes (mean ± SD = 0.382 ± 0.060 g/cm^2), highest among -1542T/-525G homozygotes (0.405 ± 0.051 g/cm^2), and intermediate among heterozygotes (0.395 ± 0.056 g/cm^2) ($r = 0.11$, $p = 0.030$). The observed trend supported co-dominant effect of the relevant haplotype of I-TRAF gene in determination of radial BMD. Functional promoter assay using luciferase reporter system, significant difference of two constructs harboring the corresponding nucleotides for the two haplotypes -1542G/-525C and -1542T/-525C was detected in osteoblastic MG63 cells. Sequential motif analysis for the transcription factor binding sites revealed that –1542T/G localized within the consensus GATA-2 binding site, which is abolished in the promoter carrying –1542G allele. These results suggested that variation of I-TRAF might be an important determinant for postmenopausal osteoporosis.

Disclosures: ***R. Ishida****, None.*

SU123

Polymorphisms in the RANK Gene Are Associated With Risk of Osteoporotic Fractures and Changes in Bone Mass. B. L. Langdahl[1], L. B. Husted*[1], L. J. Hocking[2], L. Stenkjær*[1], M. Carstens*[1], S. H. Ralston[2]. [1]Endocrinology & Metabolism, Aarhus University Hospital, Aarhus C, Denmark, [2]Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

RANK is the receptor for RANKL and therefore an important factor for osteoclast differentiation and activity. With this key role in the control of resorptive activity the RANK gene has been suggested as a candidate gene for genetic control of bone mass and fracture risk.

We examined the 10 exons with surrounding intron sequences of the RANK gene for polymorphisms in 50 osteoporotic patients. We found 13 polymorphisms, of which some have been reported earlier. Some of the polymorphisms are in complete or very strong linkage disequilibrium. We here report the analyses of the effects of one polymorphism in intron 5: $T^{IVS5-39}$-A, four in intron 6: $G^{IVS6+79}$-A and $A^{IVS6+166}$-G, $A^{IVS6-258}$-C and $A^{IVS6-151}$-G and one polymorphism in exon 9: G^{932}-A on prevalence of osteoporotic fractures, bone mass and bone turnover in 303 osteoporotic patients and 433 normal controls.

The $G^{IVS6+79}$-A polymorphism was significantly associated with risk of osteoporotic fracture. The GG genotype was more common among fracture patients than in normal controls, 33% vs. 25%, $p<0.05$. Lumbar spine BMD (LS-BMD) was 0.883±0.169 g/cm^2 in individuals with the GG genotype compared with 0.902±0.177 g/cm^2 and 0.919±0.175 g/cm^2 in individuals with the GA or AA genotypes, respectively, $p<0.05$. Regression analyses revealed that this polymorphism is significant associated with BMD at the lumbar spine, femoral neck and total hip. Biochemical markers of bone resorption were unaffected by this polymorphism, however, S-BAP was 69±26 U/l in individuals with the GG genotype compared with 60±28 U/l in individuals with the AA genotype, $p<0.05$. This polymorphism is in very strong LD ($D'=1.0$, $p<10^{-6}$) with C^{575}-T in exon 6, which causes a change from Alanine to Valine in position 192.

The A allele of the $A^{IVS6-151}$-G polymorphism in intron 6, present in 40% of normal controls, is associated with increased BMD. LS-BMD was 0.920±0.180 g/cm^2 in individuals carrying the A allele compared with 0.889±0.170 g/cm^2 in individuals with the GG genotype, $p<0.05$. Although the A allele was less frequent in osteoporotic patients, the difference did not reach significance.

None of the other polymorphisms were significantly associated with fracture risk, BMD or biochemical markers of bone turnover.

In conclusion: We have identified 13 polymorphisms in the RANK gene and we have found that one of the polymorphism in intron 6 is associated with increased risk of osteoporotic fractures and changes in BMD and that BMD is influenced by polymorphisms in intron 6.

Disclosures: ***B.L. Langdahl****, None.*

SU124

VDR Polymorphisms and Musculo-Skeletal Parameters in Lebanese Adolescents. J. Maalouf*[1], L. Zahed*[2], R. Vieth[3], M. Nabulsi*[4], M. Choucair*[5], G. El-Hajj Fuleihan[1]. [1]Calcium Metabolism and Osteoporosis Program, American University of Beirut Medical Center, Beirut, Lebanon, [2]Pathology and Laboratory Medicine, American University of Beirut Medical Center, Beirut, Lebanon, [3]Mt. Sinai Hospital, University of Toronto, Toronto, ON, Canada, [4]Department of Pediatrics, American University of Beirut Medical Center, Beirut, Lebanon, [5]Department of Endocrinology, American University of Beirut Medical Center, Beirut, Lebanon.

Peak bone mass is a determinant of fracture risk at older ages. Environmental and genetic factors have been shown to influence it significantly. The list of candidate genes is long, however VDR polymorphisms are particularly relevant in our region in view of the high prevalence of vitamin D deficiency. It remains controversial whether VDR gene polymorphisms are associated with bone mineral density (BMD). Genetic associations may be stronger in younger age groups due to the lesser confounding effect of environmental factors. There are only a few studies investigating the association of VDR polymorphisms and BMD in children or adolescents.

We studied the association between VDR polymorphisms, bone mass and muscle strength using the baseline BMD data from a vitamin D-supplementation trial, enrolling 364 healthy adolescents, 184 boys and 180 girls, ages 10-17years, in a vitamin D supplementation trial. Muscle strength using a squeeze grip ball, and BMD at the lumbar spine, hip, forearm and total body by DEXA using a Hologic 4500A device were measured in all subjects. VDR polymorphism genotypes for two sites were determined by PCR and enzymatic digestion using two enzymes, TaqI and ApaI.

The distribution of VDR alleles for TaqI polymorphism were 51% Tt (+/-), 16% tt (+/+) and 33% TT (-/-) compared to 46% (+/-), 16% (+/+) and 38% (-/-) for ApaI. The Table below shows the results as mean (SD). There was a very small non-significant increase in BMD at all skeletal sites going from the TT, to the Tt to the tt genotype. The pattern was less consistent among the genotypes assessed by ApaI (data not shown). No relationship was found between grip (muscle) strength and VDR genotypes. Subgroup analyses by gender showed a similar pattern.

VDR genotype (TaqI), BMD and Muscle Grip.

	tt (+/+)	Tt (+/-)	TT (-/-)
L1L4 BMD gm/cm^2	0.715 (0.141)	0.711 (0.145)	0.687 (0.138)
1/3 Radius BMD gm/cm^2	0.574 (0.073)	0.573 (0.076)	0.571 (0.076)
Total Hip BMD gm/cm^2	0.814 (0.134)	0.809 (0.150)	0.796 (0.140)
Muscle Grip psi	11.55 (2.81)	12.07 (3.2)	11.96 (3.34)

There was no significant relationship between VDR genotypes and BMD or muscle strength in healthy adolescents. Whether VDR polymorphism affects BMD response to vitamin D supplementation is currently being evaluated.

Disclosures: ***G. El-Hajj Fuleihan****, None.*

SU125

Association of Estrogen and Vitamin D Receptor Gene in Elderly Osteoporotic Korean Women. D. J. Lee[1], I. K. Han[2]. [1]Rheumatology, Samsung Cheil Hospital, Seoul, Republic of Korea, [2]Endocrinology, Samsung Cheil Hospital, Seoul, Republic of Korea.

One hundred women with normal spine BMD and one hundred women with T score below -2.5 were randomly enrolled to examined vitamin D receptor gene and estrogen receptor gene polymorphism.

One hundred and ninety five women, 93 normal BMD and 102 osteoporosis, completed study questionnaire and had drawn venous blood for their vitamin D(Bsm1) and estrogen receptor gene(PvuII) evaluation. Gene results were grouped according to the combinations of two genes.

The distribution of ER PvuII and VDR Bsm1 restriction fragment length polymorphisms was as follows: pp 23.7%,Pp 60.2%,PP 16.1% in normal BMD group, pp 45.1%,Pp 44.1%,PP 10.8%in osteoporosis group, bb 86.0%,Bb14.0%,BB 0% in normal BMD group, bb 87.3% ,Bb 11.8%, BB 1.0% in osteoporosis group respectively. Using two gene polymorphisms participants were subgrouped as, pp-BB, pp-Bb, pp-bb, Pp-BB, Pp-Bb, Pp-bb, PP-BB, PP-Bb, PP-bb and the distribution was as follows: 0%, 2.1%, 32.8%, 0%, 7.2%, 44.6%, 0.5%, 3.6%, 9.2% respectively.

The prevalence of osteoporosis patients in the gene subgroups were 0%, 75%, 67%, 0%, 43%, 46%, 100%, 33%, 35% respectively. Gene polymorphism was independently associated with bone mineral density and thus the presence of any high risk gene polymorphism would result in higher prevalence of osteoporosis patients.

These results indicate that ER and VDR gene polymorphism influence development of osteoporosis in the elderly Korean women.

Disclosures: ***D.J. Lee****, None.*

SU126

Calcium-sensing Receptor Gene Polymorphism is not Associated with Bone Mineral Density in Italian Postmenopausal Women. F. Cetani[1], E. Pardi*[1], S. Borsari*[1], E. Vignali[1], G. Dipollina*[1], A. Picone*[1], V. Braga*[2], S. Adami[2], T. Giacomelli*[1], A. Pinchera*[1], C. Marcocci[1]. [1]Endocrinology, University of Pisa, Pisa, Italy, [2]Reumathology, University of Verona, Verona, Italy.

Calcium-sensing receptor (CaR) is a candidate gene for osteoporosis susceptibility. Several CaR polymorphisms have been identified and an association between the A986S genotype and serum calcium levels has been found in Canadian postmenopausal women.

We investigated whether the presence of 986S allele was associated to BMD and osteoporotic fractures. The study group consisted of 164 Italian postmenopausal women without fragility fracture (Fx^+) and 55 women with fracture (Fx^-). A fragment of exon 7 of CaR gene containing three polymorphisms (A986S, R990G and Q1011E) was amplified by PCR and sequenced. Antropometric characteristic and bone mineral density (BMD) were evaluated. The A986S polymorphism was the most commonly observed (27.9%), whereas the other two CaR polymorphisms, R990G and Q1011E occurred in a minority of cases (8.8% and 5.5%, respectively). There was no significant difference in the frequency distribution of any CaR allele between Fx^- and Fx^+ patients. BMI was found to predict BMD at lumbar spine and femoral neck. The A986S polymorphism and YSM were not independent predictors of BMD at any sites. As far as fracture occurrence, there was no statistically significant difference in the prevalence of fractures between women carrying or not the 986S allele.

In conclusion, our data do not support a role of A986S CaR polymorphism on BMD and on the prevalence of fragility fractures in Italian postmenopausal women.

Disclosures: ***F. Cetani****, None.*

SU127

COL1A1 Sp1 Promoter Polymorphism Influences Serum N-terminal COL1A1 Propeptide (P1NP) Levels. L. J. Miles*[1], J. Colley*[2], A. Blumsohn[3], R. Eastell[3], E. L. Duncan[4], M. Olavesen*[2], J. A. H. Wass[4], M. A. Brown*[1]. [1]Spondyloarthritis and Bone Disease Research Group, Oxford University Institute of Musculoskeletal Sciences, Oxford, United Kingdom, [2]Oxagen Ltd, Abingdon, United Kingdom, [3]Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom, [4]Department of Endocrinology and Metabolism, Nuffield Orthopaedic Centre, Oxford, United Kingdom.

Association between the Sp1 COL1A1 promoter polymorphism, vertebral bone mineral density (BMD) and hip fracture risk has been reported in many studies. The mechanism of this association is uncertain, but it is hypothesised that the polymorphism influences COL1A1 production. To test this hypothesis, we investigated association of the Sp1 polymorphism with a marker of COL1A1 production, serum P1NP.

Serum P1NP was measured by enzyme-linked immunoassay (Roche Elecsys) in 427 individuals belonging to 115 British Caucasian families with extreme low BMD (T-score <-2.5, Z-score <-2.0 at either the lumbar spine or femoral neck (FN)). Secondary causes of osteoporosis were excluded. BMD values were available from a further 168 individuals

from these families. Genotyping of the COL1A1 Sp1 promoter polymorphism was performed using previously described methods[1]. Association was tested between the polymorphism and raw BMD and serum P1NP using the program QTDT[2]. Age, gender, height and weight were used as covariates.
Whilst no overall association was noted between COL1A1 and FN BMD and serum P1NP, association was seen when the parent-of-origin of the COL1A1 allele was taken into account. Considering P1NP levels, when parent-of-origin effects were considered, significant association was seen by total association (p=0.05) and by within-family methods (p=0.008). There was borderline within-family association of FN BMD with the COL1A1 Sp1 polymorphism when parent-of-origin effects were considered (p=0.06). A significant parent-of-origin effect was present (p=0.02), and significant association was seen in the maternally transmitted COL1A1 Sp1 alleles (p=0.01), but not with the paternal alleles. Significant association was noted between height and the COL1A1 Sp1 polymorphism (p=0.04), independent of age and gender.
These studies suggest that the COL1A1 Sp1 polymorphism influences BMD and fracture risk by effects on type 1 collagen production. A parent-of-origin effect was noted, consistent with the pattern of segregation of P1NP levels in these families, (Miles ***et.al***, ASBMR Conference Abstracts 2003). This suggestive finding may indicate the presence of imprinting at this locus.
[1]Grant *et al* (1996) Nat Genet 14, 203-5; [2]Abecasis *et al* (2000) Am J Hum Gen 66, 279-92.
Reagents for assay of PINP kindly supplied by Roche Diagnostics, Germany.

Disclosures: ***L.J. Miles****, None.*

SU128

Relation of Polymorphism in the CYP19 Gene to the Sex Hormone Level in Pre- and Early Pubertal Finnish Girls. A. Mahonen[1], M. Suuriniemi[2], A. Eriksson[3], Q. Wang*[2], A. Lyytikäinen[2], V. Kovanen*[2], M. Alen*[4], C. Ohlsson[3], H. Kröger[1], S. Cheng[2]. [1]University of Kuopio, Kuopio, Finland, [2]University of Jyväskylä, Jyväskylä, Finland, [3]Sahlgrenska University Hospital, Gothenborg, Sweden, [4]Peurunka-Medical Rehabilitation Center, Jyväskylä, Finland.

Estrogens are the main sex steroids involved in skeletal maturation. The conversion of testosterone to estrogen by adipose and muscle tissue -catalyzed by aromatase (CYP19) is the major source of ovary-independent estrogen production. The purpose of this study was to evaluate the associations between a biallelic single nucleotide polymorphism in exon 3 of CYP19 gene and sex hormone levels and different bone properties in pre- and early pubertal Finnish girls. The subjects were healthy 10-12 year-old girls (n=214) with Tanner stage I-III, who enrolled in an intervention study (the CALEX-study). Genotyping of the CYP19 locus at the G/A polymorphic site in exon 3 was performed. Serum 17β-estradiol (E_2), testosterone (T), and sex hormone binding globulin (SHBG) were determined using a time-resolved fluoroimmunoassay (Delfia, Wallac Oy, Turku). The SHBG was used to calculate the free E_2 (fE) and T (fT). Bone properties were measured using different bone assessment modalities (DXA, Prodigy, Lunar; pQCT, XCT 2000, Stratec; QUS-2, Metra Biosystems; Omnisense, Sunlight). Our results showed that girls with the AA genotype had significantly higher T and fT level than girls with the GG genotype (p=0.017 and p=0.027, respectively). They also had higher T / E_2 and fT / fE ratio than the GG girls (p=0.023). Girls with different genotype did not differ in E_2 level, nor in Tanner stage, height, weight, or body mass index. The polymorphism was not significantly associated with bone mineral content, areal bone mineral density (BMD), volumetric BMD, cross-sectional area, or speed of sound at any measured site. However, girls with the AA genotype had significantly lower broadband ultrasound attenuation of the calcaneus than girls with the GG genotype (p=0.011). Our study suggests that the polymorphism in the CYP19 gene may affect the activity or the expression level of the aromatase enzyme.

Disclosures: ***A. Mahonen****, None.*

SU129

Lack of Association between Vitamin D Receptor or Estrogen Receptor Genotypes and Bone Mineral Content or Bone Mineral Density in Young Danish Females. S. Cusack*[1], C. Mølgaard*[2], K. F. Michaelsen*[2], K. D. Cashman[1]. [1]Food and Nutritional Sciences, University College, Cork, Ireland, [2]Research Department of Human Nutrition, Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Peak bone mass (PBM) in early adulthood is considered an important determinant of osteoporosis risk later in life. Acquisition of bone mass during childhood depends on various factors, including genetic factors. Attempts to define genetic factors that influence bone mass have largely focused on adult populations. In contrast little is known about the genetic factors that influence bone density in children and adolescents. Therefore the aim of the present study was to investigate the relationship between polymorphisms in the vitamin D receptor (VDR) and estrogen receptor (ER) genes and bone mineral density (BMD) and bone mineral content (BMC) in a cohort of 222 randomly selected Danish girls, aged 11 – 13 years.
BMD, BMC, and total bone area of the total body and spine, fat content and lean mass were measured by dual energy x-ray absorptiometry (DEXA). The polymorphisms were examined by polymerase chain reaction-restriction fragment length polymorphism analysis (using Fok1 and Taq1, and PvuII and XbaI restriction enzymes) of genomic DNA isolated from peripheral blood leukocytes.
Differences in covariates between genotypes were tested by analysis of variance (ANOVA). Statistically significant different covariates were included in subsequent analyses, testing differences in BMD and BMC between genotypes. Size-adjusted and unadjusted BMD and BMC values were further analysed using 2-factor ANOVA to examine the effects of Fok1 and Taq1 genotype interactions.
The prevalence of FokI and Taq1 VDR gene and PvuII and XbaI ER gene polymorphisms in this cohort were 11.7% ff, 48.7% Ff, and 39.6% FF; 16.7% tt, 50.9% Tt, and 32.4% TT; 24.3% pp, 49.5% Pp, and 26.2% PP; and 29.8% xx, 48% Xx, and 22.2% XX. No significant association (P>0.05) was seen between BMD or BMC (adjusted and unadjusted) and any of the four polymorphic sites examined. Similarly, no significant interaction (P>0.05) between the combined VDR genotypes and BMC or BMD levels were observed. A previous report has indicated that differences of the order of 8.2% and 4.8% in total body BMD exist between FF and ff, and FF and Ff VDR genotype groups in children (Ames et al. 1999). The sample number in this study was sufficient to detect 6.0% and 3.3% differences in total body BMD between FF and ff, and FF and Ff VDR genotypes.
In conclusion, the findings of this study suggest a lack of relationship between polymorphisms of the VDR or ER genes and BMD or BMC levels in a healthy young, Danish girls.

Disclosures: ***S. Cusack****, None.*

SU130

VDR Gene Polymorphism and Bone Ultrasound Parameters in Newborns. D. Merlotti[1], L. Gennari[1], S. Gonnelli[1], A. Montagnani*[1], V. De Paola*[1], A. Calabrò*[1], S. Perrone*[2], G. Martini[1], G. Bonocore*[2], R. Nuti[1]. [1]Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Siena, Italy, [2]Pediatrics, Gynecology and Reproductive Medicine, University of Siena, Siena, Italy.

Bone metabolism is strongly influenced by heredity and environmental factors. Although some studies have reported a relationship between several candidate polymorphic genes and bone mineral density in adults little is known concerning the genetic factors influencing bone mass in children. Moreover the role of genetic polymorphism during intrauterine or early postnatal life is actually unknown. The aim of this study was to evaluate the role of the translation initiation site polymorphism in the vitamin D receptor gene (VDR) in newborns. The VDR genotypes, as detected by Fok I restriction endonuclease, were examined in 83 consecutive healthy full term newborns (43 males and 40 females; gestational age 39.5±1.5 weeks) and in their respective mothers (age range 24-38 years). Quantitative ultrasound (QUS) parameters at distal diaphysis of humerus were assessed within three days of birth by using a Bone Profiler (IGEA, Italy), after an appropriate modification of the calliper and software. Newborn's anthropometric data, such as weight, length, head circumference and APGAR score at 3rd and 5th minute after birth were also collected. Phalangeal QUS measurements were performed in all mothers. All the QUS parameters were slightly but not significantly higher in male than in female newborns. Moreover QUS parameters and anthropometric measurements were weakly correlated with gestational age and maternal calcium intake. A trend approaching statistical significance was observed between Fok I genotype in newborns and QUS parameters or anthropometric measurements at birth. By contrast, maternal Fok I genotype was not significantly correlated with maternal QUS at the phalanxes nor with newborns QUS parameters.
In conclusion, results from this preliminary study do not suggest a major contribution of Fok I polymorphism at the VDR gene and ultrasound bone parameters at birth.

Disclosures: ***D. Merlotti****, None.*

SU131

Lack of Association between the Leptin Polymorphism, Bone Mass or Body Composition in Young and Elderly Women. K. Akesson[1], P. Gerdhem*[1], M. Callreus*[1], V. Lyssenko*[2], O. Johnell[1], K. Obrant[1], L. Groop*[2]. [1]Dept of Orthopedics, Malmö, Sweden, [2]Dept of Endocrinology, Malmö, Sweden.

Background: Bone mineral density BMD is significantly related to total fat mass and body weight. Genes associated with obesity may therefore be explored for potential effects on bone mass. Recent evidence suggests high levels of gene expression of the leptin receptor (LEPR) in bone and that leptin directly stimulates osteoblasts. The LERP Gln223Arg polymorphism has been associated to BMD in men; in women, however, the association is unclear.
Aim: The aim of the present study was to investigate whether a LEPR 3'UTR insertion (I)/deletion(D) polymorphism, which we have shown to be in linkage disequilibrium with Gln223Arg, was associated with bone mass in young women at peak bone mass and elderly women at high risk of fracture.
***Material and methods*:** In this population-based study, 470 young women (age 25±0.1 yrs, BMI 23.0±3.7 kg/cm^2) and 1044 elderly women, (age 75±0.1 yrs, BMI 26.2±4.2 kg/cm^2) were recruited. The primary phenotype was BMD assessed by DXA. The 3'UTR polymorphism was genotyped by PCR and gel electrophoresis. In addition, body composition and calcaneus ultrasound were measured. In the elderly fracture data and bone markers (osteocalcin, CTX) were available.
Results: The LEPR genotype frequencies were I/I 3.0 and 2.6%, I/D 26.4 and 28%, D/D 69.4 and 70.6% in young and elderly, respectively. BMD measured at any site by DXA or calcaneus ultrasound, did not differ significantly between the different genotype carriers, neither between the young nor between the elderly.

Bone Mineral Density according to LEPR Genotype in 25- and 75-year old Women

LERP genotype	I / I (SD)	I / D (SD)	D / D (SD)
Young TB BMD (g / cm)	1,199±0,088	1,177±0,071	1,176±0,074
Young FN BMD (g / cm)	1,116±0,144	1,062±0,114	1,073±0,117
Young LS BMD (g / cm)	1,284±0,160	1,237±0,127	1,244±0,136
Elderly TB BMD (g / cm)	1,017±0,112	1,015±0,099	1,004±0,095
Elderly FN BMD (g / cm)	0,760±0,150	0,759±0,132	0,743±0,125
Elderly LS BMD (g / cm)	1,026±0,219	1,008±0,203	0,985±0,188

The presence of the I-allele was not significantly associated with BMD (TB p=0.67 vs p=0.10, FN p=0.64 vs p=0.08, LS p=0.83 vs p=0.08, young vs elderly). We did not find

any significant association between the LEPR polymorphism and variations in lean or fat mass, body weight or height, or in the elderly to bone turnover. In the elderly 24% of the I/I, 34% I/D and 36% D/D genotype carriers had suffered from an osteoporotic fracture, most commonly of the wrist, between age 50 and 78(N.S).
Conclusions: Our results indicate that the LEPR 3'UTR insertion/deletion polymorphism is not significantly associated to variation in peak bone mass or bone mass and fracture at old age in women. However, there is a trend for an increasing effect of the I-allele with age.

Disclosures: ***K. Akesson****, None.*

SU132

Stimulation of Na-Dependent Phosphate Transport by Platelet-derived Growth Factor in Rat Aortic Smooth Muscle Cells. A. Kakita[1], A. Suzuki[2], Y. Ono*[2], K. Nishiwaki*[3], M. Kotake*[2], Y. Miura*[3], Y. Oiso*[3], M. Itoh*[2]. [1]Department of Internal Medicine, Hekinan Municipal Hospital, Hekinan, Aichi, Japan, [2]Department of Internal Medicine, Fujita Health University, Toyoake, Aichi, Japan, [3]Department of Metabolic Diseases, Nagoya University, Nagoya, Aichi, Japan.

We investigated the effect of platelet-derived growth factor B homodimer (PDGF-BB) on inorganic phosphate (Pi) transport activity, which has been reported to be involved in the mechanism of atherosclerosis, in A-10 rat aortic vascular smooth muscle cells (VSMCs). PDGF-BB time- and dose-dependently stimulated Pi transport in A-10 cells. Using Northern blotting analysis, the PDGF-BB-enhanced Pi transporter (PiT) in A-10 cells was identified as Pit-1 (Glvr-1), a member of the type III Na-dependent PiT. An inhibitor of PDGF receptor tyrosine kinase suppressed PDGF-BB-induced Pi transport. Both a PKC inhibitor calphostin C and PKC down regulation suppressed the stimulatory effect of PDGF-BB on Pi transport. On the other hand, inhibition of mitogen-activated protein (MAP) kinases by selective inhibitors did not affect Pi transport. Ly294002, a phosphatidylinositol (PI) 3-kinase inhibitor, partially attenuated PDGF-BB-induced Pi transport. A selective inhibitor of S6 kinase, rapamycin, reduced this effect of PDGF-BB, while Akt kinase inhibitor did not. In summary, these results indicated that PDGF-BB is a potent and selective stimulator of Pi transport in VSMCs. The mechanism responsible for this effect is not mediated by MAP kinase, but involves activation of PKC, PI 3-kinase and S6 kinase.

Disclosures: ***A. Kakita****, None.*

SU133

Osteoformin Stimulates Osteoblast Differentiation. L. X. Bi, E. Mainous, H. M. Bahrani*, W. L. Buford*. Departments of Surgery and Orthopaedics, University of Texas Medical Branch, Galveston, TX, USA.

Our previous studies have shown that negatively charged resins increase bone formation and accelerate bone defect healing in vivo. In order to investigate potential effects of osteoformin, negatively charged peptide, on human preosteoblast, we examined expression of bone morphogenetic protein-7 [BMP-7] and type I collagen, alkaline phosphatase activity (ALP) and mineralization after treatment of cells with osteoformin. Human preosteoblast cells were cultured in a-minimum essential medium [a-MEM] and 10% fetal bovine serum with or without osteoformin (5ug/ml) for 7, 10, 14 days, respectively. To determine mineralization, the cells were cultured in mineralizing-growth medium. The levels of ALP were assayed using a commercial kit (Sigma Chemical Co., St. Luis, MO). Expression of BMP-7 and type I collagen (polyclonal antibody, Santa Cruz Biotechnology, Inc. CA) was examined using immunoquantitative assay. The mineralization was assessed by Von Kossa technique. ALP activities were significantly elevated (69-109%, P<0.001) in osteoformin treated group, compared to control group. BMP-7 expression was increased (23% at day 10 and 69% at day 14, respectively, P<0.001) after osteofornin treatment compared to control. Expression of type I collagen was increased (45% at day 10 and 52% at day 14, respectively, P<0.001). There was significant increase in the levels of mineralization (2-3 fold, P<0.001) within 14 days of culture. We conclude that osteoformin significantly stimulates osteoblast differentiation in vitro. It might be an important regulator of bone formation by accelerating fracture and bone defect healing, and controlling bone diseases.

Disclosures: ***E. Mainous****, None.*

SU134

Induced Membranes Secrete Growth Factors Including Vascular and Osteoinductive Factors and Could Stimulate Bone Regeneration. P. Pelissier*[1], R. Bareille*[2], A. Masquelet*[3], S. Mathoulin Pelissier*[4], J. Amedee[2]. [1]Service de Chirurgie Plastique, Hôpital Pellegrin-Tondu, Bordeaux, France, [2]INSERM U 577, Bordeaux, France, [3]Service de Chirurgie Orthopédique, Hôpital Avicenne, Bobigny 93009, France, [4]Service de Biostatistiques Institut Bergonié, Bordeaux, France.

One of the used procedures for the treatment of extensive diaphyseal bone defects are the vascularized bone free transfer. A proposed procedure combining induced membranes and cancellous autografts have been developed. The first stage consists in the insertion into the defect of a cement spacer which is responsible for the formation of a pseudosynovial membrane. The second stage is the reconstruction of the defect, by an autologous cancellous bone graft. The aim of this study was to evaluate the histological characteristics of these membranes. This experiment was performed in New Zealand rabbit, four animals were sacrified after 2, 4, 6 and 8 weeks and the membrane surrounding the implants was removed . Four samples of subcutaneous tissue were taken as controls. The first part of the sample was fixed in formalin solution for histological study. The second part was frozen for protein extraction. Histological and immunochemistry studies were carried out after 2, 4, 6 and 8 weeks. Measurement of VEGF, TGFβ1 concentrations was performed using human VEGF EIA kit (Chemicon, USA), human TGFβ1 (Bender Medsystems, Austria), according to the manufacturer's protocol**.** The presence and the amount of BMP-2 into the induced membranes extracts were carried out with an ELISA technique using a monoclonal anti-human BMP-2 antibody (R&D Systems, USA). Rh- BMP-2 used for control (Genetics Institute Cambridge, USA). Finally, influence on human bone marrow stromal cells (HBMSC) differentiation (alkaline phosphatase measurement) and proliferation was studied. Results demonstrate that both VEGF and TGFβ1 identified by immunocytochemistry and quantified by EIA could be in part responsible of the HBMSC cell growth observed in presence of these membranes obtained after 2 or 4 weeks. More interestingly , the induced membrane obtained after 4 weeks exhibits osteoinductive properties due to the maximum secretion of BMP2. In conclusion, these membranes play a role of an *in situ* growth and osteoinductive factors-delivery system. Moreover, cancellous autograft usually included in this membrane could potentially be substituted by a biomaterial loaded with bone marrow stromal cells and then allowing both vascularization of the implant and activation of stromal cells in bone regeneration.

Disclosures: ***J. Amedee****, None.*

SU135

PKR is Regulated by Estrogen in Rat Bone. M. Zhang*, R. T. Turner, A. Maran. Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

PKR is an interferon-inducible, double-stranded RNA-dependent protein kinase that mediates the anti-viral and anti-growth activities of interferon. In addition to its originally known function as a regulator of protein synthesis, PKR has recently been implicated in the regulation of apoptosis, differentiation, growth control, cell cycle progression, stress and transformation. Estrogen is essential for normal growth and remodeling of bone. Although the importance of estrogen in preventing bone loss in adults has been widely studied, the regulatory proteins associated with estrogen actions are incompletely known. The involvement of PKR in estrogen-mediated actions or in the skeletal system has not been previously investigated. In this report, we have investigated the effects of the gonadal hormone 17β-estradiol on PKR gene expression. Western blot analysis of the femoral metaphysis show that proximal tibiae metaphysis from three-month-old ovariectomized (OVX) rats have elevated levels (2.5-fold higher) of PKR protein compared to the intact animals. 17β-Estradiol decreased PKR protein expression in OVX rats at 8 hrs (1.5-fold), 16 hrs (1.2-fold), 24 hrs (3-fold), and 32 hrs (3-fold). These studies demonstrate that PKR levels in bone are increased by OVX and are rapidly decreased following estrogen treatment. These findings suggest that PKR plays a role in mediating the actions of estrogen on bone metabolism.

Disclosures: ***M. Zhang****, None.*

SU136

Monocyte Chemoattractant Protein (MCP)1 Influences Osteoblastic Differentiation. E. A. Kaiser[1], C. Gentzsch*[1], U. Larsen*[1], J. Plutat*[2], R. Sellkau*[2], J. Wodtke*[2], G. Delling*[1]. [1]Bone Pathology, Center of Biomechanics, Hamburg, Germany, [2]Endoklinik, Hamburg, Germany.

Aseptic loosening is a major long term complication arising in 10% of all total hip replacements. The occurring periprosthetic osteolysis is the consequence of net bone loss, which result from an imbalance between bone resorption and new bone formation. Inflammatory mediators produced in the developing synovial membrane-like interfacial tissue (SMILT) play a key role in the osteolysis and implant loosening. The release of the CC chemokine monocyte chemoattractant protein (MCP1) from fibroblasts and osteoblast within the SMILT exposed to titanium and PMMA particles has been described. MCP1 has been demonstrated to be involved in monocytes/macrophage chemotaxis, but as its receptors CCR2 and CCR4 have also been observed on other cell types we were interested in MCP-1 effects on osteoblasts as they can limit osteolysis. Thus in the present study the cell line SAOS2 was differentiated in vitro with dexamethasone, b-glycerol phosphate and ascorbic acid while simultaneously treated with different concentrations of MCP-1 peptide (10, 50, 100 ng/ml). At 4, 7, 11, 14, 18, 21 days of culture proliferation and mineralization of the cell lines were assessed. In SAOS2 cells the differentiation-induced reduction of proliferation was more pronounced and could be perceived at an earlier time point (day 4) as compared to controls (day 7). Further, mineralization also initiated at an earlier time point, on day 14 as compared to day 18. The maximal MCP1 effect, inhibiting proliferation and promoting mineralization, could be observed using 10 ng/ml. Immuncytochemistry revealed a more pronounced CCR2 expression in dexamethasone stimulated cells. Immunohistochemical evaluation of SMILT sections exhibited MCP1 expression in areas where particles accumulated whereas CCR2 expression was distributed in cells surrounding these areas. These results suggest that in addition to the recruitment of monocytes/macrophages, MCP1 could force osteoblast into differentiation, thus potentially exhausting the osteoblast progenitor pool and thereby creating an imbalance between bone resorption and bone formation.

Disclosures: ***E.A. Kaiser****, None.*

SU137

Preptin, Another Peptide Product of the Pancreatic Beta Cell, is Osteogenic *In Vitro* and *In Vivo*. J. Cornish, K. E. Callon*, U. Bava*, M. Watson*, X. Xu*, J. Lin*, V. A. Chan*, A. B. Grey, G. J. S. Cooper*, I. R. Reid. Medicine, University of Auckland, Auckland, New Zealand.

Several hormones that regulate nutritional status also impact on bone metabolism. Preptin, a 34-amino acid peptide hormone that increases glucose-mediated insulin secretion,

has been recently isolated from the same secretory vesicles that contain insulin and amylin from the pancreatic β-cells. Preptin corresponds to Asp^{69} - Leu^{102} of proIGF-II.
We have recently assessed preptin's activities on bone in a number of *in vitro* models. Preptin is anabolic to osteoblasts but, unlike amylin, does not regulate osteoclast activity. Preptin dose-dependently stimulated the proliferation of osteoblasts at periphysiological concentrations (>10^{-11}M). In addition, thymidine incorporation was stimulated in murine neonatal calvarial organ culture, likely reflecting the proliferation of cells from the osteoblast lineage. The mitogenic effect of preptin on osteoblasts depends upon signaling via p42/44 MAP kinases. Preptin also has anti-apoptotic effects in osteoblasts *in vitro* : at 10^{-8}M there is a 22% reduction in the number of TUNEL-positive cells. Preptin not only stimulates osteoblast proliferation but also osteoblast differentiation at 10^{-8}M, significantly increasing the number of mineralized bone nodules in long-term osteoblast cultures. These effects are also seen *in vivo*, when preptin is injected locally over the hemicalvariae of sexually mature male mice. After five daily subcutaneous injections of 16.5 micrograms of preptin, there was a significant increase in bone area, and mineralizing surface.
In conclusion, preptin, a peptide contained within proIGF-II, is anabolic to bone in *in vitro* and *in vivo* models. Since it is secreted from the pancreatic β-cell, it may act in concert with the other β-cell hormones, insulin and amylin, to stimulate bone formation in hyperinsulinemic states, such as obesity. Preptin may also contribute to the osteosclerotic phenotype observed in patients with chronic hepatitis C infection who have increased circulating levels of proIGF-II, which contains the preptin peptide. Thus, the anabolic effects seen in rodent models may be sufficient to influence bone density in humans in some situations.

Disclosures: ***J. Cornish****, None.*

SU138

VEGF Expression Is Altered in the Epiphyseal Cartilage Following Femoral Head Ischemic Injury. H. Bian*[1], T. Randall*[1], A. Garces*[1], L. C. Gerstenfeld[2], T. A. Einhorn[2], H. K. W. Kim[1]. [1]Shriners Hospital for Children, Tampa, FL, USA, [2]School of Medicine, Boston University, Boston, MA, USA.

Ischemic necrosis of the femoral head is a serious condition that can arise following certain injuries or treatments of the pediatric and adult hip. Vascular Endothelial growth factor (VEGF) is an essential mediator of angiogenesis, however, its role in revascularization and repair of the infarcted femoral head has not been clearly defined. The purpose of this investigation was to determine whether a quantitative change in the expression level of VEGF occurs following ischemic necrosis and to correlate these changes with revascularization of the infarcted head. A piglet model of ischemic necrosis was used. Ischemic necrosis was induced surgically by placing a ligature tightly around the femoral neck in 30 piglets. At 2 days to 8 weeks following the induction of ischemia, epiphyseal cartilage was obtained from one half of the femoral head and total RNAs and proteins were isolated and used for western blot analysis and ribonuclease protection assay (RPA). The remaining half of the head was used for histology and immunohistochemistry. Western blot consistently showed increased VEGF expression on the infarcted side compared to the non-operated side at 4 and 8 weeks when revascularization and repair were observed. RPA demonstrated higher level of VEGF mRNA expression as early as 2 days following the induction of ischemia. Immunohistochemical assessment showed high VEGF immunoreactivity (IR) in the hypertrophic zone of the epiphyseal cartilage in the non-operated side. In the infarcted side, however, there was an absence of IR in the hypertrophic zone due to cartilage necrosis. Instead, an increased IR was seen in the proliferative zone of the cartilage. At 8 weeks, there was a persistence of increased IR in the proliferative zone with fibrovascular tissue invasion and resorption of the necrotic cartilage. In the areas where the necrotic cartilage was removed, restoration of endochondral ossification was observed. In conclusion, the level and pattern of VEGF expression was altered following ischemic necrosis. The findings suggest that increased VEGF expression in the proliferative zone induces fibrovascular tissue invasion of the necrotic cartilage that enables restoration of endochondral ossification. By understanding the key factors responsible for revascularization and repair following ischemic necrosis, new treatment strategies to stimulate the repair process can be developed.

Disclosures: ***H. Bian****, None.*

SU139

RANKL AutoVac™ Vaccine: A New Immunotherapeutic Approach for the Treatment of Bone Resorptive Disorders. A. C. Porchia*[1], V. Nardi-Dei*[1], M. Rask*[1], T. Bratt*[1], A. Neisig*[2], J. Backlund*[2], M. Ezban*[1], M. Hertz[2]. [1]Protein Chemistry, Pharmexa, Horsholm, Denmark, [2]Immunology, Pharmexa, Horsholm, Denmark.

The TNF family member Receptor activator of NF-κB Ligand (RANKL) is a type II transmembrane protein found on osteoblasts which functions as a major determinant of osteoclast differentiation and activation. RANKL mediates bone homeostasis through binding to the cognate ligand on osteoclasts, RANK, and a soluble decoy receptor, osteoprotegerin (OPG). An imbalance of this cytokine system with an altered RANKL-to-OPG ratio has been implicated in the pathogenesis of animal models of rheumatoid arthritis, osteolytic tumor metastases, humoral hypercalcemia of malignancy, and various metabolic bone diseases. Furthermore, neutralizing RANKL is effective at reducing bone destruction in models of osteoporosis, RA, bone metastasis, periodontal disease and reducing markers of bone turnover in a phase I clinical trial of postmenopausal women. Since RANKL has emerged as an important therapeutic target, we are developing a RANKL vaccine based on the patented AutoVac™ technology to effectively neutralize RANKL by actively stimulating the endogenous immune system through vaccination. Thus, we have produced RANKL AutoVac™ proteins and verified their biological activity by immunizing rats and by testing the rat sera for their ability to neutralize native RANKL in a competition ELISA, in which sera competes for the binding of RANKL to the OPG receptor. RANKL AutoVac™ proteins were expressed in *E.coli* as insoluble (inclusion bodies) and soluble forms which were purified and characterized for biological evaluation. Our results demonstrate that sera from rats vaccinated with soluble RANKL AutoVac™ can inhibit the interaction between OPG and RANKL having a potential and positive clinical effect in bone loss diseases.

Disclosures: ***M. Hertz****, Pharmexa A/S 1, 3.*

SU140

OPG and TNF-Alpha Antibodies Prevent Inflammation Associated Bone Loss Through Distinct Mechanisms in a Collagen-Induced Arthritis Model. N. Saidenberg-Kermanac'h*[1], A. Corrado*[2], N. Bessis*[1], M. C. de Vernejoul[2], M. C. Boissier*[1], M. E. Cohen-Solal[2]. [1]UPRES EA-3408, Bobigny, France, [2]INSERM U349, Paris cedex 10, France.

Rheumatoid arthritis (RA) is associated with focal and systemic bone loss involving several cytokines such as RANKL and TNF-alpha. Whereas osteoprotegerin (OPG) inhibits bone resorption and osteoporosis, TNF-alpha decreases articular inflammation and bone erosions, but its effect on bone loss remains to be elucidated. The aim of this study is was to evaluate the respective and combined effect of OPG and TNF-alpha on inflammation and on bone remodeling. We used a model of collagen-induced arthritis (CIA). DBA/1 mice immunized with bovine type II collagen. Mice were treated at the onset of arthritis with :1) OPG-Fc (10 mg/kg SC 3 times/week) ; 2) TNF-alpha antibodies (TNF-alpha Ab, 10 mg/kg IP 2 times/week) ; 3) both OPG-Fc + TNF-alpha Ab ; 4) saline (placebo) and 5) one group of mice remained naive. Bone mineral density (BMD) was measured at the total body (Piximus Lunar) at baseline before immunization and at sacrifice allowing to measure bone gain in Bone Mineral Density (BMD). Deoxypyridinolin (D-Pyr) changes were measured in the urines. Histomorphometric parameters were measured at the femur metaphysis.
Clinical arthritic scores significantly improved in TNF-alpha Ab-treated group compared with placebo ($p<0.02$), whereas OPG had no significant effect on this score. OPG and TNF-alpha Ab increased BMD gain compared with placebo (39 ± 2% and 25 ±1% vs 15 ±3%, $p<0.001$ and 0.05 respectively), but OPG was more efficiency to increase BMD gain than TNF-alpha ($p<0.003$). There was no additive effect of both OPG and TNF-alpha on BMD gain (40 ± 3%). In addition, D-Pyr decreased by 65% with OPG compared to 7% with placebo ($p<0.001$) and13% when mice were treated with TNF-alpha Ab (p= NS). Compared with placebo, OPG induced increased Tb bone volume (8.4 ± 1.1 vs 13.4 ± 0.9, $p<0.02$) and decreased Tb spacing (345±91 vs190 ± 13 um, $p<0.02$) as well as decreased BFR (45 ± 8 um2/d vs 0, $p<0.01$). In contrast, TNF-alpha Ab-treated group showed no significant changes in bone volume (11.9 ± 1.5%) and Tb spacing (250 ± 30 um) compared with placebo. However, Tb thickness was higher in TNF-alpha Ab-treated mice than in placebo (30.4 ± 0.8 vs 23.9 ± 1.4um, $p<0.02$) and was close to those of naive mice (32.4 ± 2.3,p:NS), suggesting a protective effect on bone formation. There was no additive effect of OPG and TNF-alpha Ab in any parameters.
In conclusion, systemic administration of OPG and TNF-alpha Ab prevented bone loss in CIA-mice model through distinct mechanisms involving inhibition of bone resorption and formation. Combination of both treatment could be proposed for the prevention bone loss in inflammatory diseases.

Disclosures: ***M.E. Cohen-Solal****, None.*

SU141

Cortikal Osteoporosis and Reduced Bone Strength in theInterleukin-4 and Interleukin-13 Double-KO Mouse Phenotype. C. J. H. Silfversward*[1], C. Ohlsson[2], O. Nilsson*[1], A. R. Frost[1]. [1]Dept. of Orthopedic Surg., Surg sciences, Uppsala, Sweden, [2]Dept. of Internal Medicine, Division of Endocrinology, Gothenburg, Sweden.

Bone loss (osteoporosis or osteolysis) can be caused by inflammation adjacent to bone through the actions of secreted inflammatory mediators. Previous in-vitro studies have revealed that the anti-inflammatory cytokines IL-4 and IL-13 inhibit proliferation of and stimulates IL-6 secretion by human osteoblasts.
In the present in-vivo study, using the IL-13 (-/-) knockout mouse and the IL-4/-13 (--/--) double knockout mouse, we have investigated the physiological role of these cytokines.
The skeletal phenotypes of Balb/cJ knock-out mice were analyzed by DEXA, QCT and biomechanical testing and compared to WT-mice.
When sacrificed, at 20 weeks of age, the double-KO male mice presented with significantly thinner (DEXA and QCT) and weaker (three-point bending) cortical bone compared to the WT controls. A decrease in cortical BMC (tibia -8,2%; $p<0,01$ and femur -8,5%; $p<0,01$) together with cortical area (tibia -7,4%; $p<0,01$ and femur -7,9%; $p<0,01$) and thickness (tibia -3,7%; $p<0,05$ and femur -5,2%; $p<0,01$) was observed. At three-point bending there was a significant reduction of displacement (-11.4%; $p<0,05$), maximal load (-10.6%; $p<0,01$) and total energy (-29.4%; $p<0,002$) to failure.
As these effects were not seen in female IL-4/13 (--/--) mice we are now investigating the hypothetical protective role of estrogen.
In conclusion: IL-4/-13 double inactivated mice have a severe cortical bone loss, resulting in decreased mechanical strength. Thus, the TH2 derived cytokines IL-4/IL-13 are important regulators of cortical bone mass and may be potential targets for the development of new treatment strategies for osteoporosis.

Disclosures: ***C.J.H. Silfversward****, None.*

SU142

IL-12 and IL-18 are Both Required for Normal Bone Remodelling and Normal Trabecular Bone Mass. N. A. Sims[1], D. Mirosavljevic*[2], N. J. Horwood*[2], M. J. Smyth*[3], M. T. Gillespie[2]. [1]Dept of Medicine at St. Vincent's Hospital, The University of Melbourne, Melbourne, Australia, [2]St. Vincent's Institute of Medical Research, Melbourne, Australia, [3]Peter MacCallum Cancer Research Institute, Melbourne, Australia.

Interleukin-18 (IL-18) is a pleiotropic factor that shares structural features with IL-1 and functional activities with IL-12. IL-18 is expressed by osteoblasts and has been reported to inhibit in vitro osteoclast formation by acting upon T cells to promote GM-CSF production. In addition to its ability to enhance GM-CSF production, it also increases IFN-γ, another osteoclast inhibitor. Contrasting with these osteoclast inhibitory actions, IL-18 has also been reported to enhance osteoblast proliferation, an action that appears to be independent of both GM-CSF and IFN-γ. Like IL-18, IL-12 also inhibits osteoclast formation in vitro as a result of its actions on T lymphocytes. When combined, IL-12 and IL-18 are powerful synergistic inhibitors of in vitro osteoclast formation. Since expression of both IL-12 and IL-18 are elevated in inflammatory responses, their postulated actions on osteoblast or osteoclast formation / activity have largely been considered only in the context of inflammatory conditions such as rheumatoid arthritis.

To determine whether IL-12 and IL-18 play are essential for normal bone development, growth and remodeling, we performed histomorphometric analyses of single IL-12$^{-/-}$, IL-18$^{-/-}$ and double IL-12$^{-/-}$IL-18$^{-/-}$ knockout mice. No gross skeletal alterations were evident in any of these mice. However, histomorphometry revealed a significant osteopenia in each of these knockout mice. Furthermore, mice deficient in both IL-12 and IL-18 demonstrated a similar phenotype to the single IL-12 or IL-18 null mice. At 10 week of age, both trabecular bone volume (BV/TV) and trabecular number (TbN) were lower than in wild type strain-matched controls (both were reduced to approximately 83%, 55% and 80% of wild type levels in IL-12$^{-/-}$, IL-18$^{-/-}$ and IL-12$^{-/-}$IL-18$^{-/-}$ mice, respectively). The osteopenia persisted, with the phenotype becoming more severe with age; at 6 months of age, both BV/TV and TbN were less than half that of wild type controls in all three knockouts. This phenotype appeared to result from an increase in osteoclast number (of approximately 46%, 40% and 17% over wild type levels in 10 week old IL-12$^{-/-}$, IL-18$^{-/-}$ and IL-12$^{-/-}$IL-18$^{-/-}$ mice, respectively), as predicted from in vitro experiments.

These results underscore a central role of T lymphocytes in normal skeletal development, in addition to their recognised role in inflammatory conditions such as rheumatoid arthritis and periodontal disease.

Disclosures: ***N.A. Sims****, None.*

SU143

Effects of Oncostatin M on Glucocorticoid Receptors and Cytokine Secretion in Human Osteoblasts. A. Dovio*[1], M. Sartori*[1], B. Ceoloni*[1], L. Saba*[1], S. Racca*[1], A. Fazzari*[2], A. Angeli[1]. [1]Clinical and Biological Sciences, University of Turin, Orbassano, Italy, [2]Clinical Pathophysiology, University of Turin, Turin, Italy.

gp130 family of cytokines includes interleukin (IL)-6, IL-11, leukemia inhibitory factor, oncostatin M (OSM), ciliary neurotrophic factor, cardiotrophin-1 e B-cell stimulating factor-3. Most gp130 cytokines and their receptors are expressed in the bone microenvironment, and are credited with osteoclastogenic activity. As far as osteoblasts are concerned, gp130 cytokines stimulate osteoblast differentiation and inhibit adipocyte differentiation of precursors. Moreover, we have previously demonstrated that IL-6 and IL-11 respectively up- and down-regulate glucocorticoid (GC) receptors (GR) in an autocrine-paracrine way in the human osteosarcoma cell lines Saos-2 and MG-63. GR is a key determinant of tissue sensitivity to GC. The aim of the present study was to extend our investigation to another osteotropic gp130 cytokine, OSM, in both Saos-2 and MG-63 cell lines and primary cultures of human osteoblasts. Cells were incubated with the cytokine (0.05-50 ng/ml) for 20 h; afterwards, supernatants were harvested, GR protein was assessed by whole cell radioligand binding assay followed by Scatchard analysis and western blot, and GR mRNA was measured by RT-PCR. OSM dose-dependently decreased GR number in MG-63 cells without affecting affinity; results were confirmed by western blot analysis. Decreased GR protein was parallelled by decreased GR mRNA. OSM did not exert any effect on GR number in Saos-2 cells, while it decreased GR mRNA in primary cultures of human osteoblasts. Since reciprocal regulation of gp130 cytokines has been previously reported, the effect of OSM on IL-6 and IL-11 release was investigated. OSM significantly increased IL-6 release in all models; on the contrary, IL-11 secretion was increased in Saos-2 cells and decreased in primary cultures, with unconsistent effect in MG-63 cells. No effect upon soluble IL-6 receptor was observed. Our data demonstrate a down-regulatory action of OSM on GR in human osteoblasts. Such action is likely not to be subserved by modulation of IL-6/IL-11 secretion. Lack of effect in Saos-2 cells is possibly due to low number of GR and different pattern of expression of OSM receptors. Further investigation is needed to elucidate the mechanisms of divergent effects on IL-11 release. Our results suggest that gp130 cytokines increase or decrease GC sensitivity of cells of the osteoblastic lineage by modulating GR. Such modulation could be of relevance in the pathogenesis of the biphasic bone loss observed in patients given GC therapy and having high concentrations of inflammatory cytokines in the bone microenvironment.

Disclosures: ***A. Dovio****, None.*

SU144

Bone Histomorphometry and IGF-1, IGFBP-3, and PTH in Young Women Osteoporosis. A. Z. Sawicki*[1], A. Debinski*[2], Z. Polowiec*[3]. [1]Warsaw Osteoporosis Centre, Warsaw, Poland, [2]Mineral Metabolism and Bone Disease, National Food and Nutrition Institute, Warsaw, Poland, [3]Warsaw Osteoporosis Centre "Osteomed", Warsaw, Poland.

The IGF-1 and its binding-protein 3 (IGFBP-3) as well as PTH plays very important role in regulation of bone formation and bone resorption.

The assessment of relationships between bone histomorphometry and IGF-1, IGFBP-3 and PTH in young women osteoporosis were the aim of the study.

Twenty five women aged between 20-39 (X±SD=32.1±6.5) years without known risk factors of osteoporosis underwent transiliac bone biopsy following tetracycline-labelling and bone markers studies. All of them had bone mineral density below -2.5 t-score, measured by DEXA method (Hologic QDR 4500A). None of them had hyperparathyroidism, renal disease, malabsorption and/or long-term corticosteroid treatment or other secondary osteoporosis risk factors. Histomorphometric assessment of their bone including trabecular volume, osteoid volume, osteoclast surface and osteoblast surface according to ASMBR were done. Normal values of static histomorphometric parameters were obtained from 13 women aged 20-39 years without bone disorders who died suddenly in traffic accidents. Serum IGF-1, IGFBP-3 and PTH levels were measured by RIA method. Normal values of IGF-1, IGFBP-3 and PTH levels were obtained from 16 health women aged 20-49 (X±SD=38±5.4) years.

In the study group mean bone volume was significantly decreased (15.80±3.83 vs 23.30±1.07; p<0.001), the mean osteoid volume without mineralization defect and mean osteoclast surface were significantly increased (3.96±2.72 vs. 1.98±0.45; p<0.01: 2.60±1.28 vs. 1.49±0.54; p<0.01 respectively). No significant difference in mean osteoblast surface was found (2.84±2.22 vs 3.50±0.79; NS). In study group mean PTH level was significantly lower then in control group (22.89±9.55 pg/mL vs. 28.14±12.05 pg/mL; p<0.02). No significant differences, between study and control group, in IGF-1 and IGFBP-3 levels was found (307.5±97.8 ng/mL vs.263.4±66.3 ng/ml; NS, 3214.9±637.0 vs. 3481.1±989.2; NS respectively). Significant correlations between IGF-1 and osteoblast surface (R=0.613, p<0.01) as well as between PTH and osteoclast surface (R=0.498, p<0.05) were found. No significant correlations between IGFBP-3 and static histomorphometric parameters were found.

We conclude that high osteoclasts activity in young women osteoporosis is not related to hyperparathyroidism or osteomalacia.

Disclosures: ***A.Z. Sawicki****, State Committe for Scientific Research 2.*

SU145

CSF-1 Regulates Mast Cell Differentiation in Rat Tibia. G. L. Evans*[1], P. R. Odgren[2], C. A. MacKay*[2], R. T. Turner[1]. [1]Orthopedic Research, Mayo Clinic, Rochester, MN, USA, [2]Cell Biology, University of Massachusetts Medical School, Worcester, MA, USA.

In patients, osteoporosis can accompany Systemic Mastocytosis. In animals, the bone loss associated with ovariectomy is accompanied by an increase in mast cell number, and mutations that lead to mast cell deficiency often result in osteopetrosis. These observations suggest that mast cell and osteoclast differentiation are, in part, similarly regulated. Colony stimulating factor-1 (CSF-1) was shown to be essential for osteoclast differentiation and a CSF-1 inactivating mutation was recently identified in the toothless (tl) rat mutation. The purpose of the present study was to determine whether CSF-1 plays an equally important role in mast cell differentiation. Three groups of rats (3-6 w old) were studied; tl/tl (n=9-11), tl/tl treated for 1 w with (10-6 U/d) CSF-1 (n=3) and wild type (n=8-12). Measurements were performed in the proximal tibial metaphysis and osteoclasts were measured in tartrate resistant acid phosphatase stained sections and mast cells in Azure stained sections. As expected, the tl/tl rats had greatly reduced osteoclast number (1/mm2 tl/tl vs. 7/ mm2 wild type, p<.01) and CSF-1 was effective in reversing the defect (20/mm2 treated tl/tl, p<.001). Similarly, the tl/tl rats had greatly reduced mast cell number (1/mm2 tl/tl vs. 25/ mm2 wild type, p<.01). CSF-1 partially restored mast cell number (7/mm2, p<.001). These findings suggest that CSF-1 plays a role in mast cell differentiation. Mast cells produce many cytokines that influence bone resorption. Thus, parallel regulation of mast cell and osteoclast differentiation may be important to local regulation of bone resorption during normal growth, modeling and remodeling, as well as in disease.

Disclosures: ***G.L. Evans****, None.*

SU146

Chondrocytes Respond to the Low Intensity Pulsed Ultrasound by Calcium Influx Pathways. A. Miyauchi[1], T. Sugimoto[2], Y. Takagi[1], K. Jinnai*[1], Y. Yoshimoto[1], K. Chihara*[2], T. Fujita[3], Y. Mikuni-Takagaki[4]. [1]Medicine, National Hyogo-Chuo Hospital, Sanda, Japan, [2]Division of Endocrinology/Metabolism, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan, [3]Calcium Research Institute, Kishiwada, Japan, [4]Oral Biochemistry, Kanagawa Dental College, Yokosuka, Japan.

Mechanical stimuli are essential in maintaining morphology and function of cartilaginous tissues. Low-intensity pulsed ultrasound (LIPUS), which accelerates fracture healing processes in the appendicular skeleton first by cartilaginous induction, provides non-invasive therapeutic treatment for fracture repair and distraction osteogenesis. While we have reported that osteoblasts but not osteocytes are the target cells of the anabolic LIPUS and that their response did not accompany calcium influx (J Bone Miner Res 18:360,2003). Parvizi et al. reported that rat chondrocytes responded to the LIPUS by increasing cytosolic

Ca^{2+} ($[Ca^{2+}]i$) and by proteoglycan synthesis. It suggests that the LIPUS sensing-mechanism of chondrocytes is distinct from that of osteoblastic cells. We, therefore, studied responses of chondrocytes to LIPUS as well as stretch loading. Human articular chondrocytes were preloaded with fura-2, and subcellular $[Ca^{2+}]i$ was monitored using a video image analyzer. Stretch loading by swelling the cells with hypoosmotic solution (182 vs. 363 mOSM) induced a rapid and progressive $[Ca^{2+}]i$ increase (65±6 nM above basal level, n=14). Since more than 90% of the hypotonically-induced $[Ca^{2+}]i$ increase was abolished either by $2x10^{-5}$M Gd^{3+}, a selective inhibitor of stretch-activated cation channels (SA-Cat) , or by Ca^{2+}-free medium, the $[Ca^{2+}]i$ increase is likely caused by an influx of Ca^{2+} through SA-Cat. Meanwhile, LIPUS (90 mW/cm^2) exposure induced a transient $[Ca^{2+}]i$ increase (63±5nM above basal level, n=10) followed by slow $[Ca^{2+}]i$ oscillation(1/150 c/s). A larger $[Ca^{2+}]i$ increase of approximately 90 nM was observed in the submembranous region than in the perinuclear cytoplasm (50 nM). The $[Ca^{2+}]i$ increase was abolished either by Gd^{3+} or Ca^{2+}-free medium. Moreover, preincubation with thapsigargin was not effective and reduced the LIPUS-induced increase by only less than 20%. Taken together, our results indicate that the LIPUS-induced $[Ca^{2+}]i$ increase was primarily caused by an influx of Ca^{2+} through SA-Cat in human chondrocytes. This pathway may be an element in the signal transduction through which LIPUS stimulates chondrocyte maturation and fracture repair.

Disclosures: ***A. Miyauchi****, None.*

SU147

COX-2, but not COX-1, Is Involved in Mechanotransduction in Bone Cells. A. D. Bakker*, E. H. Burger*, C. M. Semeins*, J. Klein-Nulend. Oral Cell Biology, ACTA-VU, Amsterdam, Netherlands.

Cyclooxygenase (COX) is the key enzyme in the production of prostaglandins, which are essential for the anabolic response of bone to mechanical loading. There are two isoforms of COX, a constitutive isoform (COX-1) and an inducible form (COX-2). It is currently unknown which isoform regulates the prostaglandin response to mechanical loading. The aim of this study was therefore to test whether COX-1 or COX-2 determines pulsating fluid flow-induced prostaglandin production by primary bone cells *in vitro*.

Primary mouse and human bone cells were kept under static culture conditions, or were subjected to 1 h of pulsating fluid flow (PFF, 0.6±0.3 Pa at 5 Hz), in the presence or absence of either 10^{-7} M of the specific COX-1 inhibitor SC-560, or 10^{-5} M of the specific COX-2 inhibitor NS-398. After cessation of PFF, cells were post incubated under static conditions for an additional 24 h.

Both mouse and human bone cells reacted to PFF with an increased prostaglandin E_2 production, which continued 24 h after cessation of PFF. NS-398 abolished the stimulating effect of PFF on PGE_2 production both at 1 h and 24 h post-incubation, while SC-560 affected neither the early nor the late response to flow. PFF rapidly stimulated COX-2 mRNA expression at 1 h, but did not affect COX-1 mRNA expression. COX-2 mRNA expression was still significantly enhanced 24 h after cessation of PFF.

We conclude that mechanical loading-induced production of prostaglandins by bone cells is determined by COX-2, but not COX-1. These results explain the observation that COX-2 is essential for the anabolic response of bone to mechanical loading *in vivo*.

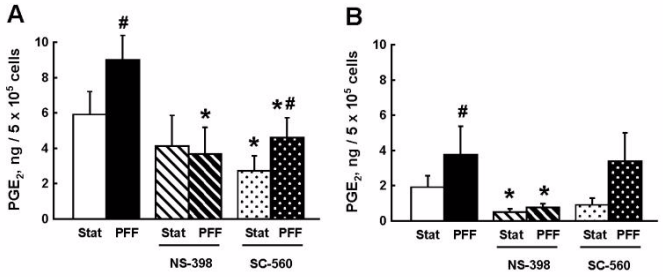


Figure: Effect of PFF, and NS-398 or SC-560, on PGE_2 production by mouse bone cells, after 1 h of treatment (A), and during the 24 h post incubation period (B). Values are mean ± SEM. Stat, stationary culture; PFF, pulsating fluid flow. Stat and PFF, n=9; Stat + NS-398 and PFF + NS-398, n=5; Stat + SC-560 and PFF + SC-560, n=6. # Sign effect of PFF, * Sign effect of NS-398 or SC-560, p<0.05.

Disclosures: ***A.D. Bakker****, None.*

SU148

The Response of Bone Cells from Osteoporotic Donors to Mechanical Loading Is not Altered by Estrogen. A. D. Bakker*[1], P. Lips[2], R. Huiskes[3], E. H. Burger*[1], J. Klein-Nulend[1]. [1]Oral Cell Biology, ACTA-VU, Amsterdam, Netherlands, [2]Endocrinology, Vrije Universiteit Medical Center, Amsterdam, Netherlands, [3]Biomed. eng., Technical University Eindhoven, Amsterdam, Netherlands.

Local bone mass and architecture are determined by mechanical loading, and the subsequent response of osteocytes to the loading-induced fluid flow through the lacuno-canalicular network. Estrogen also has a profound effect on bone mass. It has been suggested that the loss of estrogen during the menopause alters the response of bone cells to mechanical loading, thereby contributing to the rapid loss of bone. The present study aimed to determine whether estrogen modulated the mechanoresponse of bone cells from osteoporotic women *in vitro*.

Bone cell cultures from 11 osteoporotic women between 62 to 90 years of age were subjected to pulsating fluid flow (PFF, 0.6±0.3 Pa at 5 Hz) or static culture for 1 h, in the presence or absence of 10^{-11} M 17β-estradiol (E2).

PFF significantly stimulated PGE_2 production after 5 and 60 min of application. Treatment with E2 enhanced basal PGE_2 production by the bone cells, but did not affect the magnitude of the PGE_2-response to PFF. E2-treatment stimulated endothelial nitric oxide synthase protein expression in the osteoporotic bone cells by 2.5-fold, but did not stimulate basal NO production. 1 h of PFF significantly stimulated NO production by approximately 5-fold. E2-treatment did not affect the magnitude of this NO-response to PFF.

These results suggest that E2 does not alter the response of bone cells from osteoporotic donors to mechanical loading. Thus, the rapid loss of bone during the menopause is unlikely to be caused by a change in mechanosensitivity of the bone cells.

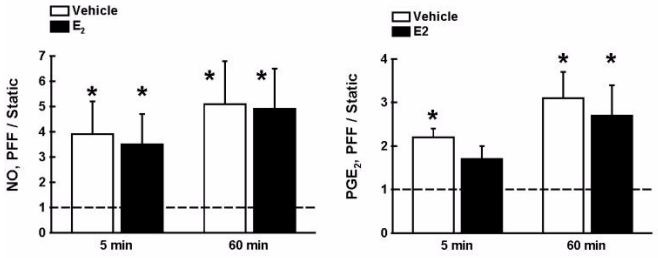


Figure: Effect of E2 on the magnitude of the PGE_2 and NO-response to PFF. Data are expressed as PFF-treated over static culture ratios. Bars are mean ± SEM. There was no difference in magnitude of the PGE_2 or NO-response between E2 and vehicle-treated cells at any time point. * Signficant effect of PFF, p<0.05.

Disclosures: ***A.D. Bakker****, None.*

SU149

Modeled Microgravity Disrupts Integrin Expression and Function during Osteoblastogenesis of Human Mesenchymal Stem Cells. V. E. Meyers*[1], M. Zayzafoon[1], W. E. Gathings*[2], J. M. McDonald[1]. [1]Pathology, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Consortium for Materials Development in Space, University of Alabama in Huntsville, Huntsville, AL, USA.

Spaceflight leads to reduced bone mineral density in weight-bearing bones. This disruption of bone homeostasis is primarily attributed to decreased osteoblast function. Reduced differentiation of human mesenchymal stem cells (hMSC) into osteoblasts likely contributes to this phenomenon. Our lab has previously demonstrated reduced osteoblastic differentiation of hMSC following 7 days culture in modeled microgravity. One possible mechanism contributing to this decline in differentiation is reduced integrin-mediated signaling. When integrins bind extracellular matrix proteins, focal adhesion kinase (FAK) is activated and the transduced signals contribute to the survival and differentiation of hMSC. Here, we begin to characterize the effects of modeled microgravity on integrin expression and function during osteoblastogenesis of hMSC.

To model microgravity, we cultured hMSC for 7 days in a commercially available, NASA-approved rotary cell culture system (RCCS). Cells were seeded onto polystyrene microcarrier beads in either low adhesion 6-well plates, which serve as gravity controls, or 10 mL high aspect ratio vessels (HARVs) used by the RCCS to model microgravity. Cell/bead aggregates were allowed to form for approximately one week in DMEM containing 10% FBS. Osteogenic differentiation was then induced by the addition of β-glycerophosphate, ascorbic acid, and dexamethasone immediately prior to initiation of modeled microgravity. These conditions permit proper osteoblastic differentiation of control cells.

Seven days modeled microgravity was sufficient to cause a 4-fold increase in β_1 integrin subunit protein expression. Increased expression of the α_2 subunit, which dimerizes with β_1 to form the primary functional receptor for type I collagen in osteoblasts, also occurs. However, despite increased integrin protein levels, autophosphorylation of FAK is reduced 4-fold following 7 days modeled microgravity. Protein expression of the α_5 integrin subunit, which dimerizes with β_1 to form a functional receptor for RGD-containing peptides, such as fibronectin, does not appear to be affected by modeled microgravity. In summary, integrin-mediated signaling is disrupted in modeled microgravity despite upregulated or unaltered integrin protein expression. Because integrin signaling is required for hMSC differentiation into osteoblasts, it is likely that this contributes to the reduction in functional osteoblasts and the resulting decline in bone mineral density experienced during spaceflight.

Disclosures: ***V.E. Meyers****, None.*

SU150

Hemichannels Formed by Connexin 43 (Cx43) are Essential for Prostaglandin E_2 (PGE_2) Production by Osteocytes in Response to Mechanical Strain. P. P. Cherian, X. Wang*, E. Sprague*, J. X. Jiang. Biochemistry, University of Texas Health Science Center, San Antonio, TX, USA.

Osteocytes embedded in the matrix of bone express large amounts of Cx43, the component of gap junctions, yet osteocytes are only in contact through the tips of their dendritic processes, an extremely small area compared to the total surface of the cell. These observations suggest that Cx43 may have other functions in addition to forming intercellular chan-

nels. As osteocytes are mechanosensory cells, it was hypothesized that Cx43 may mediate effects of mechanical strain on these cells. Similar to primary osteocytes, Cx43 is highly expressed in the osteocyte-like cell line MLO-Y4 compared to osteoblasts. Previous studies using MLO-Y4 cells have shown that shear stress induced by fluid flow increases functional intercellular coupling, in addition to increases in Cx43 protein expression and PGE_2 production. PGE_2 was demonstrated to be an essential mediator between mechanical strain and gap junction intercellular communication. However, it is not known if Cx43 is essential between mechanical strain, and the production and release of PGE_2. In this study, MLO-Y4 cells were plated at various cell densities ranging from the lowest at which no cells were physically in contact with adjacent cells to the highest with considerable cell-to-cell contact. Plating at low density prevented the formation of intercellular gap junction channels. These cells were subjected to fluid flow treatment at 16 dynes/cm^2 and conditioned media were assayed for PGE_2. The MLO-Y4 cells with no or few intercellular channels produced significantly more PGE_2 per cell than those at higher density. PGE_2 production was exponentially increased at the lowest cell density of $1x10^3$ cells/cm^2, compared to the highest cell density of $1.1x10^5$ cells/cm^2. Both antisense Cx43 oligonucleotides and β-glycyrrhetinic acid, a specific and reversible gap junction channel blocker that can also block hemichannels, significantly reduced PGE_2 production induced by fluid flow at all cell densities tested, especially cells at the lowest density. Moreover, fluid flow shear stress rendered the Cx43 located at the cell surface to be resistant to Triton-X-100 extraction, suggesting the formation of insoluble protein plaques, similar to previously reported gap junctional plaques. Analysis of cell surface biotinylation revealed that fluid flow enhanced the phosphorylation of Cx43 on the cell surface making the molecule more susceptible to labeling by biotin. Our results suggest that hemichannels formed by Cx43, instead of intercellular channels, are likely to play a dominant role in regulating the responses of osteocytes to mechanical stimulation.

Disclosures: ***J.X. Jiang****, None.*

SU151

Zinc Transporter 5 (Znt5) Is an Essential Molecule for Mechanical Stress Signaling in Bone. K. Matsuda*[1], K. Inoue*[2], K. Yamakawa*[1], H. Kawano[1], T. Akune[1], K. Hoshi[1], Y. Nakamura*[2], K. Nakamura[1], H. Kawaguchi[1]. [1]Orthopedic Surgery, Univ. of Tokyo, Tokyo, Japan, [2]Institute of Medical Science, Univ. of Tokyo, Tokyo, Japan.

Mechanical stress is one of the most important factors for maintaining bone mass, although little is known about the molecular mechanism. We recently isolated by a differential display method a novel gene Znt5 whose expression increased in response to expansion load of rabbit aorta. Znt5 has 15 transmembrane domains with a cation efflux domain at its C-terminus, and functions as a zinc transporter. To learn the involvement of this molecule in the mechanical stress signaling in bone, we first examined the expression in bone responding to mechanical stress using several models. In the mouse tail suspension model, Znt5 mRNA level in tibiae and femora was decreased to about one third that of control after 3 weeks of unloading, and was restored to the normal level by reloading. In the culture of mouse newborn calvariae, Znt5 expression was induced by continuous tensile stress by an inserted spring for 9 h. When three kinds of osteoblastic cell lines, MC3T3-E1, MLO-Y4 and MLO-A5, were cultured with stretching stimulation by the Flexor Cell system, about 2-fold increase of Znt5 expression was seen only in MLO-Y4 cells that are known to be the most differentiated osteoblasts. To further investigate the physiological role of Znt5, we generated mice lacking the Znt5 gene ($Znt5^{-/-}$). $Znt5^{-/-}$ mice exhibited slight growth retardation (-10% in length) as compared to wild-type (WT) littermates. Bone densitometry, histomorphometry and 3D-μCT analyses of the $Znt5^{-/-}$ bone showed a marked decrease in the volume of both trabecular and cortex bones (-30% in BMD, -60% in BV/TV) at 8 weeks of age. The bone loss seemed to be due to the impairment of bone formation (-60% in Ob.S/BS, -40% in MAR, -70% in BFR/BS) since bone resorption parameters were normal. Cultured primary osteoblasts derived from $Znt5^{-/-}$ calvariae showed markedly decreased differentiation and mineralization as determined by ALP and Alizarin red stainings, respectively; however, CFU-OB formation from bone marrow cells was normal, suggesting a dysfunction of osteoblasts but not of the precursor cells. When $Znt5^{-/-}$ mice were treated with tail suspension for 3 weeks, the decrease in bone density (-5%) was much less than that in WT (-15%). To explore the downstream signaling of Znt5, we compared the gene expression profile between $Znt5^{-/-}$ and WT, and found that several immediate early genes including Fos, Egr1 and heat shock proteins were decreased in $Zn5^{-/-}$. We conclude that Znt5, whose expression is positively regulated by mechanical stress in mature osteoblasts or osteocytes, plays an essential role in the maintenance of bone volume by mechanical stress.

Disclosures: ***K. Matsuda****, None.*

SU152

Expression of P2Y Purinergic Receptors During Osteoblastic Differentiation. J. You, H. J. Donahue. Dept of Orthopaedics & Rehabilitation, Center for Biomedical Devises & Functional Tissue Engineering, The Pennsylvania State University College of Medicine, Hershey, PA, USA.

Accumulated evidence suggests that one of the ATP receptor subtypes, P2Y (G protein-coupled receptor), may play an important role in cell mechanosensing and differentiation. To date, four mouse P2Y purinergic receptors (P2Y1, P2Y2, P2Y4 and P2Y6) have been cloned. Recently we demonstrated that P2Y receptors (either P2Y2 or P2Y4) are responsible for oscillatory fluid flow-induced intracellular calcium (Ca^{2+}_i) mobilization in mouse MC3T3-E1 osteoblastic cells. The aim of this study was to identify the subtypes of P2Y receptors expressed in MC3T3-E1 cells that are responsible for flow-induced Ca^{2+}_i mobilization. Additionally, we examined whether the expression of P2Y receptors is changed during osteoblastic differentiation. MC3T3-E1 cells were cultured in normal media (MEMα+10%FBS) for 24 hours, then one half of the cultures were placed in differentiation (normal media + β-glycerophosphate + L-ascorbic acid phosphate) media for an additional 48 hours, while the other half remained in normal media. First, using RT-PCR, we examined the expression of all subtypes of P2Y receptors in cells cultured in normal media. Our results demonstrated that P2Y2 was the most abundant P2Y receptor subtype expressed in MC3T3-E1 cells cultured in normal media. We did not detect P2Y1, P2Y4, or P2Y6 receptors in MC3T3-E1 cells in normal media suggesting that these receptors may not be involved in flow-induced Ca^{2+}_i mobilization. In MC3T3-E1 cells cultured in differentiation media we were not only able to detect P2Y2 but also P2Y1, which was not detected in cells cultured in normal media. To examine the functional expression of receptors on MC3T3-E1 cells cultured in differentiation media, we exposed the cells to ADP (100μM), a P2Y1 agonist. ADP significantly induced Ca^{2+}_i mobilization in MC3T3-E1 cells cultured in differentiation media, but not MC3T3-E1 in normal media, suggesting the expression and function of P2Y1 receptors are dependent on cell differentiation. However, the Ca^{2+}_i responses of MC3T3-E1 cells to fluid flow were similar in cells cultured in normal and differentiation media suggesting that P2Y1 may not be involved flow induced Ca^{2+}_i mobilization. Our finding is the first evidence showing that cell differentiation may affect the expression of P2Y receptors in osteoblastic cells and also suggest that P2Y1 receptors are not involved in fluid flow-induced Ca^{2+}_i mobilization.

Disclosures: ***J. You****, None.*

SU153

Gene Expression Profile Comparisons between 2T3 Osteoblasts at Low Density and Confluent Stages with Osteocyte-like MLO-Y4 Cells at High and Low Densities. W. Yang*, M. A. Harris, D. Guo*, A. Krisnaswamy*, L. F. Bonewald, S. E. Harris. Dept. of Oral Biology, U. of Missouri at Kansas City, Kansas city, MO, USA.

The osteocyte is a terminally differentiated cell of osteoblast origin that only remains in physical contact with neighboring cells through the tips of dendritic processes. Markers for osteoblastic differentiation are well-known, but few are recognized for osteocytes. In this study, gene expression profiles for 2T3 cells at low density and confluent stages were compared to those for MLO-Y4 cells. It was postulated that MLO-Y4 cells at low density would have fewer functional gap junctions and therefore less cellular communication compared to cells cultured at higher density. The 2T3 osteoblast-like cells at low density expressed filopodia, reminiscent of early osteoblast precursors. Mouse 5k oligonucleotide microarrays were used to produce gene expression profiles. After triplicate sets of hybridization experiments for each condition, clusters of expression patterns that are characteristic of MLO-Y4 cells and 2T3 osteoblasts at different states were profiled. We then used global intensity and variance to normalize the original data, followed by statistical analysis to validate and exclude genes with high variance. Using percolation clustering and K-median clustering, we have and will continue to derive new biological hypotheses and insight from these data sets. We first selected a set that changed greater than 2-fold between cell lines or states. We then determined a set of genes in which the normalized SD between triplicates was less than 0.5SD. From about 700 genes, we then began our bioinformatics analysis. As might be expected, MLO-Y4 cells have much higher expression of genes that might be involved in the generation and maintenance of dendritic processes such as zyxin and CD44, which are known to interact with E11. MLO-Y4 cells also highly express chemotactic factors for osteoclasts such as Scya7 (MCP-3), VEGF, and TGFb1. Higher gene expression levels for Tob1, a suppressor of growth and BMP/TGFb signaling, Ncor2 (nuclear receptor co-repressor 2), Tgfbr3, and Hoxc10 was found in MLO-Y4 cells compared to 2T3 osteoblasts. We have over 300 genes that are at least 3 fold higher in MLO-Y4 cells than any of the two states of 2T3 cells. Experiments are underway comparing gene expression patterns in MLO-Y4 cells and 2T3 osteoblast that were subjected to 4 dynes/cm^2 of fluid shear stress for 2 hrs followed by analysis at 2 and 24 hrs after stopping flow. Modeling of these gene expression patterns in the MLO-Y4 osteocyte model and 2T3 osteoblast model will be presented, as well as our preliminary studies on the response of these two cell types to mechanical loading.

Disclosures: ***W. Yang****, None.*

SU154

Ultrasound Mimics the Effect of Mechanical Loading *in vivo* on Rat Ulnae and Bone Marrow Cells. L. K. Parry*[1], V. J. Burton*[2], S. Gheduzzi*[2], J. Beresford*[3], M. J. Perry[2], T. M. Skerry[1], V. F. Humphrey*[4]. [1]Veterinary Basic Sciences, Royal Veterinary College, London, United Kingdom, [2]Orthopaedic Surgery, University of Bristol, Bristol, United Kingdom, [3]University of Bath, Bath, United Kingdom, [4]Physics, University of Bath, Bath, United Kingdom.

Mechanical loading influences bone strength, and it has been shown that high frequency low magnitude loads exert positive effects on bone. This experiment was designed to test the hypothesis that ultrasound stimulation exerts positive influences on bone.

Female Wistar rats were anaesthetized, the left ulna loaded cyclically 6 times on alternate days. 40 cycles of load were applied with peak strains of 3,000 microstrain at 0.12 per second, with a 10 second rest period between each cycle. In another group applied transcutaneous ultrasound stimulation consisted of a burst width of 200 microseconds containing 1MHz sine waves, with a repetition rate of 1 kHz and a spatial average-temporal average intensity of 150 mW/cm^2 for the same duration as the loading. In a third group loading and ultrasound stimulation were applied concurrently. Fluorochrome labels were given at the start and 1day before the end of the experiment. The right leg served as a non-loaded control in each animal. At the end of the experiment, left and right ulnae were removed, and marrow cells removed by brief centrifugation before bones were processed for histomorphometry. Cells were cultured for 18 days then fixed and reacted to demonstrate expression of alkaline phosphatase positive colonies. Load induced bone formation on the medial periosteal surface was assessed by measuring interlabel distance.

Loading induced a significant periosteal response, increasing the interlabel distance by

300%, and reduced numbers of AP positive colonies significantly by 25%. The effect of ultrasound was to increase the periosteal bone formation by 50% and inhibit colony formation by the same amount as loading. The effects of loading plus ultrasound were indistinguishable from ultrasound alone.
These data suggest that ultrasound has the ability to induce changes in bone that appear to share common features with mechanical loading. The inhibition of colony formation by loading is part of the integrated structural response of the ulna to loading whereby medial periosteal osteogenesis is stimulated, but endosteal formation (which is part of the growth process in rats of this age) is inhibited to reduce the bone's curvature. The ability of concurrent ultrasound stimulation to suppress the effects of concurrent loading suggests that the interaction between the two stimuli is complex and not simply additive or synergistic. This non-invasive stimulation may induce bone formation safely in those with weak skeletons.

Disclosures: ***L.K. Parry****, None.*

SU155

A Novel Mechanical Stress-induced Signaling Pathway Leading to Smad1/5/8 Activation in Osteoblasts. S. Kido[1], D. Inoue[1], T. Imamura*[2], K. Miyazono*[2], T. Matsumoto[1]. [1]Department of Medicine and Bioregulatory Sciences, University of Tokushima, Tokushima, Japan, [2]Department of Biochemistry, The Cancer Institute of the Japanese Foundation for Cancer Research, Tokyo, Japan.

Mechanical stress to bone plays a critical role in maintaining bone mass and strength. We report here that mechanical loading to osteoblastic cells activates R-Smads 1/5/8, which act downstream of BMP and play an important role in bone formation. Fluid shear stress (FSS) induced endogenous Smad1 phosphorylation and Smad4 translocation to the nucleus within 30 min both in primary osteoblasts and MC3T3-E1 cells. Immunoprecipitation experiments with adenovirally introduced Flag-tagged Smads revealed that FSS also phosphorylated Smad5 and 8 to some extent, but not Smad2 or 3. We found that FSS induced expression of Smad7, an endogenous target of activated R-Smads, at both mRNA and protein levels. FSS also induced transcription from consensus Smad response elements. Moreover, when mice were mechanically loaded in rotating cages, Smad7 expression was induced in hindlimb bones. Therefore, mechanical loading to bone cells resulted in functional Smad activation both in vitro and in vivo. Interestingly, FSS-induced smad1 phosphorylation was inhibited by Smad6 or 7 over-expression, but not by dominant negative ALK3, suggesting that Smad activation by FSS was independent of BMP receptors. Furthermore, Smad activation was inhibited by EGTA or BAPTA, suggesting that it was dependent on extracellular calcium influx. Conversely, increasing extracellular Ca concentrations led to enhanced Smad phosphorylation by FSS. Smad activation by FSS was inhibited by PKC inhibitors or depletion by 24 hr treatment with 50 nM TPA, but not by inhibitors of ERK, p38MAPK or PKA, suggesting a role of PKC. In contrast, Smad activation by an authentic ligand, BMP-2, was not affected by PKC inhibitors. Moreover, we found that FSS activation of Smad was in part inhibited by calmodulin antagonists including W-12, W-13 and compound48/80, but not by a CAMKII inhibitor, KN-62. In vitro binding assay using calmodulin agarose beads revealed a Ca-dependent, direct physical interaction between calmodulin and Smad1/5/8. In summary, we have demonstrated for the first time that Smad1/5/8 lies downstream of mechanical stress-induced osteogenic signaling pathways. Further delineation of this novel signaling pathway may form a molecular basis to understand the mechanism of mechanical stress-induced bone formation and to identify a novel therapeutic target of immobilization osteoporosis.

Disclosures: ***S. Kido****, None.*

SU156

Stretch-induced Parathyroid Hormone-related Peptide (PTHrP) Gene Expression in Bone. X. Chen*[1], C. M. Macica*[1], G. Liang*[1], B. E. Dreyer*[1], K. W. Ng[2], A. E. Broadus[1]. [1]Internal Medicine, Yale University, New Haven, CT, USA, [2]University of Melbourne, Melbourne, Australia.

Mechanical forces play a critical role in regulating skeletal mass and structure. PTHrP is expressed in bone cells under normal physiological conditions and is believed to function locally in an autocrine/paracrine fashion. PTHrP gene expression has been shown to be induced in response to mechanical stretch in several physiological systems. Although the role of PTHrP in bone is unknown, it has been well documented that PTH, which shares a common receptor with PTHrP, can have anabolic effect in bone. PTH has been reported to modulate stretch-activated cation channels (SA-cat) and L-type Ca2+ channels (VOC). However, PTH itself is unlikely to contribute to the local anabolic response induced by mechanical forces. We tested the possibility that PTHrP may act as a local mediator of mechanical stretch using UMR-201-10B osteoblast-like cells in response to variations in tonicity, as a surrogate for mechanical loading.
By RNase protection assay, a three-fold increase of PTHrP mRNA was observed within 30 minutes of a hypotonic challenge (240 mosm vs. 317 mosm isotonic control) in UMR-201-10B osteoblastic cells, and the peak level was reached by 1 hour of treatment. This effect was insensitive to both Gd3+ treatment and to the addition of the VOC inhibitor, nifedipine, eliminating the possible role of both SA-cat and VOC channels in this effect. The increase in PTHrP message was also unaffected by omission of calcium in the incubation medium or depletion of intracellular calcium pool by thapsigargin, suggesting that neither extracellular nor intracellular calcium are involved.
Recent studies have shown that a class of tandem-pore potassium channels belonging to the TREK family are sensitive to stretch and intracellular acidosis but are insensitive to Gd3+. These channels have not been studied in bone cells. Using RT-PCR, we confirmed the presence of the TREK-2 isoform of stretch-activated K channels in UMR-201 cells. We also found PTHrP mRNA was increased in a dose-dependent manner upon intracellular acidification induced by sodium acetate in the medium, while intracellular alkalization by ammonium chloride had no effect.
We propose PTHrP as a candidate participant in the effects of mechanical loading on bone and that TREK-2 channels are involved in mediating this PTHrP response.

Disclosures: ***X. Chen****, None.*

SU157

Early Induction of Cyclooxygenase-2 in Osteoblasts by Fluid Flow Depends on the Protein Kinase C Signaling Pathway. S. Wadhwa, M. Mehrotra*, C. Alander, O. Voznesensky, C. C. Pilbeam. Medicine, University of Connecticut Heath Center, Farmington, CT, USA.

Mechanical loading of bone generates interstitial fluid flow within the mineralized bone matrix that exerts fluid shear stress (FSS) on cells, initiating signaling cascades leading to new gene transcription. One of the genes induced by FSS in osteoblasts is cyclooxygenase-2 (COX-2), which is essential for the anabolic effects of mechanical loading on bone. We previously characterized the maximal (late) COX-2 response to FSS in osteoblastic cells. This response occurred at 4-8 h and was dependent on the protein kinase A (PKA) but not the protein kinase C (PKC) signaling pathway. However, short exposures to FSS may be a better model of the effects of mechanical loading on bone *in vivo* than long exposures. Intuitively, flow duration in a single direction *in vivo* should be restricted by the geometry of the bone. Moreover, long durations of flow (greater than > 1 h) have been shown to cause cell damage. The purpose of this study was to characterize the early COX-2 response of osteoblasts to FSS. We used clonal MC3T3-E1 osteoblastic cells and primary osteoblasts, sequentially digested from calvariae of CD-1 mice transgenic for 371 bp of the COX-2 promoter fused to a luciferase reporter (Pluc mice). Cells were plated on collagen-coated glass slides, grown to confluence, and subjected to 10 dynes/cm^2 of steady laminar flow in a parallel plate flow chamber for 1 h. Controls were maintained in stationary culture. Unlike the late COX-2 mRNA response to FSS, Northern analysis showed that the early COX-2 mRNA response to FSS was independent of new protein synthesis. In both MC3T3-E1 and primary calvarial cells, the early COX-2 mRNA response to FSS was dependent on the PKC signaling pathway. GF109203X (1.25 μg/ml), an inhibitor of the PKC pathway, or 24 h of pretreatment with phorbol myristate acetate (PMA, 1 μM) to down regulate the PKC pathway decreased the FSS induction of COX-2 mRNA by 50-75%. In contrast, PKI, a specific inhibitor of the PKA pathway, had little effect on the early FSS induction of COX-2 mRNA. To see if the PKC-dependent effect was transcriptionally mediated, primary calvarial cells from Pluc mice were subjected to 1 h of FSS and then returned to stationary culture for 2 h before measurement of luciferase activity. FSS stimulated an increase in luciferase activity and this was blocked by GF109203X. Thus, we conclude that the early (primary gene response) and late (peak) induction of COX-2 mRNA expression by FSS occur by different signaling pathways. The activation of different signaling pathways may help to determine the optimal amount and duration of FSS needed to produce anabolic responses in bone.

Disclosures: ***S. Wadhwa****, None.*

SU158

Low Intensity, High Frequency Vibration May Increase Bone Formation in Aged Rats. H. Oxlund, B. S. Oxlund*, T. T. Andreassen. Dept of Connective Tissue Biology, University of Aarhus, Inst of Anatomy, Aarhus, Denmark.

The effect of low intensity, high frequency vibration on bone formation and bone strength was studied in an aged rat model. Twenty-three-month-old male rats were allocated randomly to the following groups: 1) start control, 2) mock vibrated control and 3) vibration at 45 Hz (3.0xg). Vibration was given 30 min/day for 90 days. During vibration the rats were placed in a box on top of the vibration motor. The amplitude of the vibration motor was 1.0 mm. The animals were labelled with calcein at day 79 and with tetracycline at day 86. The tibia mid-diaphysis was studied by mechanical testing and dynamic histomorphometry. The vibration increased (2p=0.03) the periosteal bone formation rate (10^{3} x 1.66 +/- 0.24 cubic micrometer/day, mean +/- SEM) by 2 fold compared with the mock vibrated group (10^{3} x 0.82 +/- 0.27 cubic micrometer/day) at the tibia mid-diaphysis. Furthermore, the percentage labelled circumference of the vibrated group (35.5 +/- 4.3 %) was increased (2p<0.03) by 86 % compared with the mock vibrated group (19.1 +/- 5.4 %). These alterations did not result in significant increases in the breaking strength of the tibia diaphysis of these old rats. In conclusion, the results support the hypothesis of a possible beneficial effect of passive physical loading on the preservation of bone in aged animals.

Disclosures: ***H. Oxlund****, None.*

SU159

Physiological Levels of G-force Induces Cox-2 Gene Expression in MC-3T3-E1 Osteoblasts. M. Hughes-Fulford[1], C. Li*[2]. [1]Medicine, UCSF, Dept of Veterans Affairs and NCIRE, San Francisco, CA, USA, [2]Lab of Cell Growth, NCIRE, San Francisco, CA, USA.

Physiological mechanical loading is required for maintenance of bone integrity and architecture. We have previously reported that as little as 15 minutes of physiological gravity force can induce MAPK and immediate early gene expression. Here we report that one hour after a 15 minute pulse of 120 μstrain of gravity force *cox-2* was significantly induced over five fold. Levels of EP-1, 18s and cpla2 mRNA were not changed during this timeframe. The induction of *cox-2* was inhibited 70% by MEK1/2 inhibitor U0126 (p<0.001) but was not effected by MEK1 or p38 MAPK specific inhibitors. Short-term physiological loading induced ERK 1/2 phosphorylation in a dose dependent manner with maximum phosphorylation saturating at mechanical loading levels of 12.g (p< 0.001) with no effect on total ERK. G-loading did not activate P38 MAPK or JNK. Additionally, a short duration

gravity pulse resulted in the localization of phosphorylated ERK 1/2 to the nucleus while unloaded cells showed no concentration of pERK in the nucleus. The long-term consequence of a single 15 minute gravity pulse was a 64% increase in cell growth ($p<0.001$). U0126 significantly inhibited gravity induced growth by 50% ($p<0.001$). These studies suggest that physiological levels of compressive mechanical stress induce cox-2 message and protein synthesis as well as growth of MC3T3-E1 osteoblasts primarily through an ERK1/2 mediated pathway.

Disclosures: ***M. Hughes-Fulford****, None.*

SU160

T-Type Voltage Sensitive Calcium Channels Modulate the Mechanical Response of Osteoblasts to Fluid Shear. D. Liu[1], E. C. Puente*[2], J. J. Bergh[2], Y. Shao*[2], M. C. Farach-Carson[2], R. L. Duncan[1]. [1]Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Biology, University of Delaware, Newark, DE, USA.

Osteoblasts express several different subtypes of voltage sensitive calcium channels (VSCC) that we postulate play significant roles in osteoblastic function. While we have previously shown that inhibition of L-type VSCC's significantly reduces bone formation in mice subjected to mechanical loading, this inhibition is incomplete. Patch clamp studies in MC3T3-E1 cells demonstrate the presence of a low voltage activated Ca^{2+} channels that exhibit kinetics consistent with those of the T-type VSCC. Immunocytochemistry and RT-PCR demonstrate the presence of both $Ca_V3.1$ (α_{1G}) and $Ca_V3.2$ (α_{1H}) subunits to the T-type VSCC in static MC3T3-E1 cells. To determine if activation of the T-type VSCC is important to the osteoblastic response to fluid shear, MC3T3-E1 cells were grown to confluency on collagen-coated (10 μg/cm^2) glass slides, then subjected to 12 dynes/cm^2 laminar shear using a parallel-plate flow chamber interfaced with a flow loop. One hour prior to shear application, cells were pretreated with either $NiCl_2$ (5 mM) or mibefradil (1 μM) and these inhibitors were maintained in the medium for the duration of the experiment. Cells were exposed to 1 hour of fluid shear, followed by 3 hours post-incubation. After the first 30 minutes of post-incubation, medium samples were removed to assay for PGE_2 release. After the 3 hour post-incubation, COX-2 expression and production were measured. Fluid shear produced a 5-fold increase in COX-2 production compared to static controls ($p<0.001$). $NiCl_2$ significantly reduced this response, while mibefradil only moderately inhibited COX-2 production. Changes of mRNA expression of COX-2 with either $NiCl_2$ or mibefradil inhibition reflected the protein results. PGE_2 release in response to shear corresponded to the increase in COX-2 production compared to static controls ($p<0.05$), while both $NiCl_2$ and mibefradil reduced this release ($p<0.05$). To examine the effect of shear on T-type VSCC expression, mRNA expression of α_{1H} subunit was examined using RT-PCR. After 1 hour of post-incubation, α_{1H} mRNA expression was enhanced 2.5-fold, with a further increase to 4.5-fold observed after 6 hours. By 24 hrs, the increase was 7.5-fold. These data suggest that fluid shear increases the expression and activity of T-type VSCCs ($Ca_V3.1$ and 3.2) and may contribute to the mechanical responses of osteoblasts.

Disclosures: ***D. Liu****, None.*

SU161

Comparison of Dentin Matrix Protein 1 (DMP1) Gene Expression with Experimental and Finite Element Strain Analysis in the Rat Ulnar Fatigue Model. J. Gluhak-Heinrich[1], D. Pavlin[1], S. P. Kotha*[2], L. F. Bonewald[3], M. B. Schaffler[4], S. E. Harris[5]. [1]Orthodontics, U. of Texas Health Science Center, San Antonio, TX, USA, [2]Oral Biology, U. of Missouri at Kansas City, Kansas City, MO, USA, [3]Oral Biology, U. of Missouri at Kansas City, Kansas City, MO, USA, [4]Mount Sinai School of Medicine, New York, NY, USA, [5]Oral Biology, U. of Missoui at Kansas City, Kansas City, MO, USA.

Osteocytes are thought to play critical roles in sensing and conveying messages to other osteocytes and to osteoblasts and osteoclasts. These candidate signals are thought to be required for bone homeostasis under various levels of mechanical and fatigue loading conditions. We have previously shown that the Dentin Matrix Protein I gene is highly and selectively expressed in alveolar osteocytes *in vivo* and is mechanically responsive in the tooth movement model (Gluhak-Heinrich et al, JBMR 2003). DMP1 function in osteocytes is most likely related to its role as a modulator and attenuator of mineralization. DMP1 therefore may play a role in modulating the local microenvironment within the osteocyte lacunae and canalicular walls. These modifications could then alter the dynamics of fluid flow through bone. In order to further explore the DMP1 gene as mechanically responsive in other bone loading systems, we investigated DMP1 expression in a rat ulnar fatigue model. Right ulnae were loaded at 4 Hz with a maximum force of 20N until there was a loss of 30% of the whole bone stiffness. These loading conditions cause considerable microdamage within the diaphyseal cortex. We then assayed for expression of DMP1 mRNA immediately after loading and 2 days later by quantitative *in situ* hybridization using P^{32} labeled RNA probes. In the ulnar midshaft, DMP1 expression was greatly increased on both the tension and compression sides of the diaphyseal cortex. However, a gradient of DMP1 expression in osteocytes near the periosteal surface as compared to osteocytes near the endosteal surface was observed upon quantitation of expression. This pattern of DMP1 expression reflects the known strain gradients in this bending model with higher strains expected near the surface and in the longitudinal axis near the midshaft region. We have now begun a detailed quantitative analysis of DMP1 expression in both the longitudinal axis at various distances from the mid-shaft and in the circumferential axis. By comparison of gene expression with experimental and finite element strain maps, we propose that levels of DMP1 gene expression may represent a sensitive readout of magnitudes of tensile and compressive strains, as well as torsional strains in bone.

Disclosures: ***J. Gluhak-Heinrich****, None.*

SU162

Tibial Cortical Strains Induced by Low-Level Vibrations Are Dependent on Genetic Make-up and Frequency of the Signal. S. Karnik*, E. Choi*, J. Jacobson*, B. Busa*, L. Donahue, C. Rubin, S. Judex. SUNY Stony Brook, Stony Brook, NY, USA.

Despite their low magnitude, vibrational mechanical stimuli induced at high frequencies (>20Hz) can be anabolic to trabecular bone. In genetically distinct strains of mice, we have previously demonstrated that the efficacy of these stimuli is dependent upon the genotype with trabecular bone of C3H/HeJ (C3H) mice being much less responsive than C57BL/6J (B6) mice. While these data may suggest that bone's mechano-sensitivity is regulated by the genome, the dramatic differences in bone mass and morphology between these two strains may alter the magnitude and pattern of the mechanical signals induced in the skeleton. Thus, it is possible that the differential tissue sensitivity is accounted for by interactions between genetic and mechanical factors. Here we collected preliminary data to investigate the different mechanical environments induced by vibrations in these two strains of mice. Single-element miniature strain gauges were implanted (cyanoacrylate) at the antero-medial surface of the proximal tibia of two B6 and two C3H mice. Strain data were recorded while the mice were subjected to a ground-based vertical oscillation at the frequencies of 22.5Hz, 45Hz and 90Hz, at accelerations of 0.2g. In low bone mineral density B6 mice, peak longitudinal normal strains generated in the tibial cortical shell decreased from 11.0 microstrain (μ) at 22.5Hz to 4.5μ at 45Hz to 1.1μ at 90Hz. Cortical normal strains induced in C3H mice at a frequency of 22.5Hz were much lower than in B6 (0.9μ) and were not affected by the frequency of the applied signal; normal strains at 45Hz and 90Hz amounted to 1.2μ. These data indicate a frequency dependency of vibrational mechanical deformations in B6 but not in C3H mice in which the generated strains were consistently low. While these data were collected from the cortical shell and we have only begun to extrapolate matrix strains and stresses to metaphyseal trabecular bone via finite element modeling, the differential mechanical environment induced by vibrations in these two mouse strains raises the possibility of both mechanical and genetic factors regulating trabecular bone adaptation.

Disclosures: ***S. Judex*** *, None.*

SU163

Cortical Bone Augmentation with Mechanical Loading Is Best at a Loading Frequency of 10 Hz. S. J. Warden*, C. H. Turner. Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, USA.

Loading frequency is an important determinant of mechanically-induced adaptation in cortical bone. Researchers have shown an increase in bone formation with increasing loading frequency. This dose-response relationship is limited to loading frequencies below 10 Hz. Studies herein aimed to investigate cortical bone adaptation to loading frequencies of 1, 5, 10, 20 and 30 Hz. Two studies were completed in adult C57BL/6 mice using the ulna axial compression loading model. In the first study, the histomorphometric response of the ulna was studied when loaded for 120 cycles/day for 3 days at one of the five frequencies and one of three strain magnitudes (935, 1750 or 2566 microstrain). In the second study, the change in the ulna mechanical properties was studied following loading for 5 mins/day, 3 days/week for four weeks at one of the five frequencies and one of two strains magnitudes (935 or 1914 microstrain). Results of the first study revealed that loading at each frequency at 2566 microstrain increased all bone formation measurements. The increase in bone formation rate was greatest with loading at 10 Hz than at any other frequency. Results of the second study showed that loading at any frequency and 1914 microstrain increased the energy to failure by between 17.9% and 29.2%, and the ultimate force by between 5.8% and 17.7%. There was no effect of loading frequency on these changes. In both studies, loading at 935 microstrain had no effect irrespective of frequency. The combination of these results shows that cortical bone adaptation to increasing loading frequency does not show a simple dose-response relationship. The bone formation response appears to peak with a loading frequency of 10 Hz, and further increases in frequency do not result in subsequent increases in adaptation. Consequently, for optimal cortical bone building purposes loading should be performed at 10 Hz.

Disclosures: ***S.J. Warden****, None.*

SU164

Is Animal Age a Factor in the Response of Bone to Spaceflight? E. M. Morey-Holton[1], L. P. Garetto*[2], S. B. Doty*[3], B. P. Halloran[4], R. T. Turner[5]. [1]NASA Ames Research Center, Moffett Field, CA, USA, [2]Indiana University Schools of Dentistry and Medicine, Indianapolis, IN, USA, [3]Hospital for Special Surgery, New York City, NY, USA, [4]University of California and Veterans Affairs Medical Center, San Francisco, CA, USA, [5]Mayo Foundation, Rochester, MN, USA.

The rodent bone response to spaceflight may be influenced by a multitude of factors including flight duration, strain (genetic background), and housing. Age may also play a role in the skeletal response. Weanling rats show fewer bone changes than older rats. To determine if the long bones of weanling rats are insensitive to weight-bearing, two spaceflight experiments and a hindlimb unloading experiment were conducted. The hindlimb unloading experiment with individually housed rats was conducted simultaneously with a 9d shuttle flight using 34d old group-housed male rats while 38d old male rats were individually housed during the 14d spaceflight. All animals were injected with fluorochromes before unloading and euthanized at various times (0-14d) following reloading. If no differences in body weight, bone length, or bone formation at the tibiofibular junction were noted at the different time points, data were combined for each group. No significant differences in body weight were found at any time period. The humerus, tibia, and femur

elongated significantly during the flight period with no difference in lengths between groups. The group-housed flight rats showed no change in cortical bone formation rate compared to preflight values, flight controls, or vivarium controls. However, the hindlimb unloading group showed a significant 30% decrease in bone formation rate compared to all other groups. The individually-housed animals flown for 14d showed ~10% suppression of cortical growth. Older singly-housed flight animals appear to show equal or greater bone changes compared to hindlimb unloaded rats. We speculate that the mechanical threshold required for cross-sectional bone growth is reached in group-house weanling rats during spaceflight, perhaps through physical interactions, and that weanling animals are sensitive to loading. However, the threshold is not fully reached in either singly-housed flight or hindlimb unloaded weanling rats. We conclude that age, housing, flight duration, and strain (genetic background) have important roles in rodent skeletal responses to spaceflight.

Disclosures: ***E.M. Morey-Holton****, None.*

SU165

Urinary 25-Hydroxyvitamin D Binding Activity Is Directly Associated with Urinary Sodium of African-American Men during Head-Down Tilt Bed Rest. M. Thierry-Palmer[1], S. Cephas*[1], H. R. Scott*[1], P. Sayavongsa*[1], M. Pasquali*[2], E. Schwartz*[2], R. Lapu-Bula*[3], E. Ofili*[3]. [1]Biochemistry, Morehouse School of Medicine, Atlanta, GA, USA, [2]Pathology, University of Utah, Salt Lake City, UT, USA, [3]Medicine, Morehouse School of Medicine, Atlanta, GA, USA.

The Dahl salt-sensitive rat, a model for salt-induced hypertension, excretes protein-bound 25- hydroxyvitamin D (25-OHD) into urine during low salt intake and excretion is markedly increased during high salt intake (J. Nutr. 133:187-190, 2003). Since the prevalence of salt sensitivity in the African-American population is greater than that in the Caucasian American population, we determined whether healthy normotensive African-American men (29 +/- 2 years old; 122+/- 3 mm Hg, n = 19) involved in a head-down tilt bed rest study excreted 25-OHD binding protein(s) into urine. The subjects were fed a low salt (50 mmol/day, 7 subjects) or high salt (200 mmol/day, 12 subjects) diet during seven days of head-down tilt bed rest. Plasma was collected at day 0 and day 7 and 24 h urine was collected at day 1 and day 7. Binding activity in the urine was determined by binding to radio-labeled 25-OHD3 (20,000 dpm) in the presence and absence of 200-fold unlabeled 25-OHD3. Urinary 25-OHD binding activity varied directly with urinary sodium (r = 0.70, P = 0.0002), as did urinary protein (r = 0.56, P = 0.0004). Baseline values for plasma 25-OHD (26 +/- 4 nmol/L), 24,25-dihydroxyvitamin D (2.4 +/- 0.4 nmol/L), and parathyroid hormone (30 +/- 3 pg/mL) were not significantly affected by seven days head-down tilt bed rest or by high salt intake. Plasma 1,25-dihydroxyvitamin D concentration of subjects on the low salt diet was significantly lower at day 7 than at baseline (55 +/- 17 vs. 120 +/- 10 pmol/L, P = 0.001). Urinary deoxypyridinoline, a marker of bone resorption, was significantly increased from day 1 to day 7 of head-down tilt bed rest (P = 0.01). In conclusion, increased pyridinium cross-links excretion was the major change related to bed rest. Low salt intake, in contrast to high salt intake, resulted in significantly decreased plasma 1,25-dihydroxyvitamin D concentration at the end of bed rest, compared with baseline. Further studies are needed to determine whether there is a contribution of bed rest, as well as salt, to the urinary excretion of 25-OHD binding protein(s).

Disclosures: ***M. Thierry-Palmer****, None.*

SU166

The Effectiveness of Osmotic Mini-Pumps to Deliver Fluorochrome Labels in Rats. L. L. Venton*[1], G. Evans*[2], E. Holton*[3], E. Hill*[4], R. Turner[2], T. Wronski[5], D. Bikle[1], B. P. Halloran[6]. [1]Division of Endocrinology, Veterans Affairs Medical Center, San Francisco, CA, USA, [2]Mayo Foundation, Rochester, MN, USA, [3]NASA Ames Research Center, Moffett Field, CA, USA, [4]Lockheed Martin Space Operations, Moffett Field, CA, USA, [5]Department of Physiological Sciences, University of Florida, Gainsville, FL, USA, [6]Division of Endocrinology, Departments of Medicine and Physiology, Veterans Affairs Medical Center, University of California, San Francisco, CA, USA.

Automated delivery of bone fluorochrome labels could be invaluable in remote locations such as spaceflight. We developed an automated system using polyethylene tubing attached to Alzet osmotic minimpumps to deliver calcein subcutaneously on 5d5 of a 15d study (expt 1) or d12 of an 21d study (expt. 2). Polyethylene-60 tubing was cut to 6.55cm for d5 and 15.75cm for d12. Tubing was sterilized with ethylene oxide, rinsed with fetal bovine serum, and filled with sesame oil (24µl for d5 and 66µl for d12), a small bolus (6µl) of calcein (2.5mg/kg) and then a small bolus (6µl) of sesame oil. The calcein end of the tubing was attached to a saline filled osmotic pump. Male, Fischer 344 rats, 6 weeks or 6 months old, were either sham operated or implanted with minipumps. Three days after surgery, rats were divided into hindlimb unloaded or control groups. Demeclocycline was injected, s.c., on day 0, and calcein was delivered s.c. on day 5 (expt. 1) or 12 (expt. 2). Bone formation rate (BFR) was measured at the tibiofibular junction between the pump administered label (d5 or d12) and the periosteal surface (See Table).

Periosteal Bone Formation Rate (um2/day ± SD)

Age	Delivery Method	Control	Suspended	% Control
Expt. 1 6 week	Inject	31.3±5.5 (n=5)	18.3±0.9 (n=5)	-42
	Pump	32.8±6.5 (n=4)	20.1±4.7 (n=3)	-39
Expt. 1 6 month	Inject	3.2±0.6 (n=5)	1.4±0.8 (n=5)	-56
	Pump	3.0±1.8 (n=5)	ND* (n=4)	ND*
Expt. 2 6 month	Inject	2.3±1.2 (n=5)	0.6±0.9 (n=5)	-74
	Pump	3.1±1.6 (n=5)	0.9±1.1 (n=5)	-71

* ND - no detectable bone formation

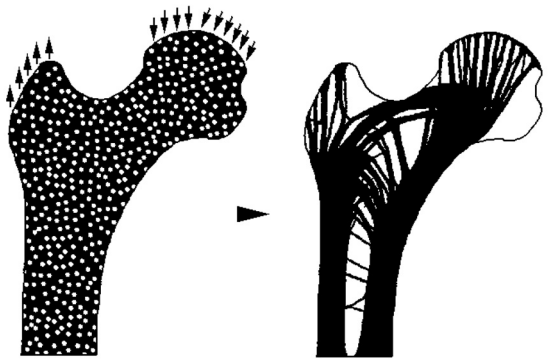
BFRs were not significantly different between the animals receiving calcein by conventional s.c. injection or by minipump. Young animals, as expected, showed less of a decrease in BFR in response to hindlimb unloading than did older animals. These data demonstrate that the minipumps can accurately deliver bone labels at the specified days and that the pump-tubing system could be utilized for many drugs/labels in various remote environments such as space.

Disclosures: ***B.P. Halloran****, None.*

SU167

A Dynamic Bone Architecture Produced by a Simple Reaction Diffusion Model. K. Tezuka[1], Y. Wada*[2], A. Takahashi*[3], M. Kikuchi*[3]. [1]Graduate School of Medicine, Gifu University, Gifu, Japan, [2]Department of Mechanics and Systems Design, Tokyo University of Science, Suwa, Japan, [3]Department of Science and Technology, Tokyo University of Science, Noda, Japan.

Bone is a complex system accompanied with adaptation and repair functions. To understand how bone cells can create a structure adapted to the mechanical environment, we propose a computer model for bone remodeling based on a reaction-diffusion system influenced by mechanical stress. In this model, a hypothetical stimulator of bone formation was produced in response to mechanical stress, and induced another hypothetical molecule that inhibited the activity of the stimulator. These two molecules interacted with each other, diffused independently, and formed a static distribution depending on the local stress value. Bone formation and resorption changed the shape of the model depending on the local concentrations of these two molecules. When an external mechanical stress was applied to a 2-dimensional model of human femoral neck, stimulated bone formation and subsequent activation of bone resorption produced an efficient adaptation of the internal shape of the model bone to a given stress, and demonstrated major structures of trabecular bone similar to those seen in real bone tissue (figure). Parameter analysis suggested that cell to cell communication via diffusion of a hypothetical stimulator of bone formation is important for the efficient adaptation of the entire system. Stepwise change of a parameter affecting the balance between formation and resorption demonstrated deformation of bone structure during osteoporosis. The loss of trabecular bone was much faster than that of cortical bone as observed in osteoporotic patients. Therefore, this simple model will give us an insight how bone cells create a sophisticated architecture adapted to mechanical environment, and serve as a useful tool to understand both physiological and pathological state of bone from structural information.



Disclosures: ***K. Tezuka****, None.*

SU168

Interactive Effects of Parathyroid Hormone and Mechanical Stress on Nitric Oxide and Prostaglandin Production by Mouse Osteoblastic Cells. A. D. Bakker*[1], E. H. Burger*[1], M. Joldersma*[1], C. Netelenbos[2], J. Klein-Nulend[1]. [1]Oral Cell Biology, ACTA-VU, Amsterdam, Netherlands, [2]Endocrinology, Vrije Universiteit Medical Center, Amsterdam, Netherlands.

Local bone remodeling is regulated by mechanical usage, but the overall level of remodeling is determined by systemic hormones such as parathyroid hormone (PTH). Several animal studies suggest that there is an interaction of PTH and strain at the tissue level.

It is, however, still unclear how the effects of mechanical loading and PTH interact.
To investigate whether PTH can modulate mechanotransduction by bone cells, we examined the effect of human PTH-(1-34) on fluid flow-induced prostaglandin E_2 (PGE_2) and nitric oxide (NO) production by mouse osteoblastic cells *in vitro*.
Primary bone cell cultures were obtained as outgrowth from collagenase-stripped fragments of the long bones of adult mice. The bone cells were subjected to mechanical stress in the form of a pulsating fluid flow (PFF; 0.6 ± 0.3 Pa at 5 Hz), in the presence or absence of 10^{-9}M human PTH (1-34). Medium samples were taken at 5 and 30 min after start of fluid flow, and nitric oxide (NO) and prostaglandin E_2 (PGE_2) production were determined as parameter of bone cell responsiveness. NOS activity was determined using a NOSdetect Assay Kit, in bone cell cultures that were subjected to 10^{-9} M PTH for 45 min.
PFF stimulated both NO and PGE_2 production by 2-fold. In the absence of PFF, PTH also caused a 2-fold increase in PGE_2 production, but NO release was not affected. Simultaneous application of PFF and PTH nullified the stimulating effect of PFF on NO production, while PGE_2 production was only stimulated by two-fold. Treatment with PTH alone reduced NO synthase (NOS) enzyme activity to undetectable levels.
We speculate that PTH prevents stress-induced NO production via the inhibition of NOS, which will also inhibit the NO-mediated upregulation of PGE_2 by stress, leaving only the NO-independent PGE_2 upregulation by PTH. These results suggest that mechanical loading and PTH interact at the level of mechanotransduction. As both NO and PGE_2 inhibit osteoclast activity in short term experiments, we now propose that strain-derived NO and PGE_2 serve to locally inhibit osteoclastic bone resorption. The opposite effects of stress and PTH on NO production as found in this *in vitro* study, may thus relate to their opposite effects on osteoclastic bone resorption *in vivo*.
We conclude that PTH and PFF interact in the production of PGE_2 and NO, which may be part of the regulation mechanism of PTH and mechanical loading on bone turnover *in vivo*.

Disclosures: ***A.D. Bakker****, None.*

SU169

The Effect of Selective Agonist for Prostaglandin E Receptor Subtype EP4 (ONO-4819) on the Cortical Bone Response to Mechanical Loading. H. Hagino[1], M. Kuraoka*[2], Y. Kameyama*[2], S. Fukata*[2], T. Okano[2], R. Teshima*[2]. [1]Rehabilitation Division, Tottori University, Yonago, Japan, [2]Department of Orthopedic Surgery, Tottori University, Yonago, Japan.

Purpose: The cortical bone response under selective agonist for prostaglandin E receptor subtype EP4 (ONO-4819) administration was evaluated using a 4-point bending device to clarify the relationship between the effect of ONO-4819 and mechanical loading.
Materials and Methods: Thirty-six 6-month-old female Wistar rats were used. Rats were randomized into 3 groups (N=12/group); EP4-low (6μg/kg BW/day), EP4-high (60 μg/kg BW/day), and EP4-v (vehicle). ONO-4819 was subcutaneously injected in the back twice a day for three weeks. Loads on the right tibia were applied in vivo by 4-point bending. The tibia was loaded at 35 N for 36 cycles at 2Hz three days a week for three weeks and then rats were sacrificed. Bone mineral content (BMC) of the whole body and bone mineral density (BMD) of the total and regional tibia (the region with maximal bending at the central diaphysis) were measured by dual energy X-ray absorptiometry. Histomorphometry was performed at the entire periosteal and endocortical surface of the tibiae.
Results: Whole body BMCs showed a significant difference between EP-v and EP4-high. Regional BMDs of the right tibia (loaded site) were higher in EP4 groups and loaded site, showing significant differences between loaded and non-loaded site and between EP-v and EP4-high. ONO-4819 showed an additive effect on loading at the regional BMD of the tibia. Histomorphometrically labeled surface was higher in EP4 groups than in the vehicle groups, and it was also higher at loaded site than non-loaded site. Bone formation rate was elevated by both EP4 administration and loading.
Conclusion: Selective agonist for PGE receptor EP4 (ONO-4819) has an additive effect on bone response to in vivo external loading by 4-point bending.

Disclosures: ***H. Hagino****, None.*

SU170

Side-to-side Difference in Bone Mineral Density of Tennis Players' Forearms: Determinant Parameters. G. Ducher*[1], C. Jaffré*[1], E. Lespessailles[2], C. L. Benhamou[2], D. Courteix[1]. [1]Laboratoire de la Performance Motrice, UFR STAPS Orleans, Orleans, France, [2]Ipros, CHR Orleans, Orleans, France.

A large side-to-side difference in bone mineral density (BMD) has been reported between both forearms in tennis players. It is assumed that the genetic, hormonal and nutritional influences are similar in the dominant and non-dominant forearms. Such difference is attributed to the mechanical loads encountered by the dominant arm during the tennis stroke. The aim of the study was to identify the training and muscular parameters explaining the greater BMD at this site.
Thirty male tennis players (age: 26.7 ± 6.9 years, total training time: 4337 ± 3801 hours) participated in this study. Grip strength (GS) was measured with a hand-held dynamometer. Lean tissue mass at the forearm and hand (LTM), bone mineral content (BMC), bone area and BMD were determined at the distal radius by DXA. The side-to-side differences were expressed as percentage of the non-dominant values (Δ).
BMD was higher at the dominant radius ($p<0.0001$), with the largest difference obtained at the ultra-distal region (+9.99 ± 5.81%). The BMC difference was greater ($p<0.01$), ranging from 15.14 ± 7.99% at the ultra-distal region to 17.30 ± 9.18% at the mid-distal region ($p<0.0001$). The side-to-side difference in bone area was larger ($p<0.01$) at the mid- and third-distal regions (+11.61 ± 6.65% and +9.67 ± 6.12%, respectively) than at the ultra-distal region (+4.72 ± 4.58%). BMC was still higher at the dominant side after adjusting the value for bone area, except at the mid-distal region. ΔLTM and ΔGS were 17.71 ± 10.33% and 14.34 ± 7.42% respectively ($p<0.0001$). ΔBMC and ΔBMD at the ultra-distal radius correlated with ΔLTM ($r = 0.52$, $p<0.01$ and $r = 0.43$, $p<0.05$, respectively). ΔBMC at the ultra-distal region correlated with ΔGS ($r = 0.39$, $p<0.05$). The starting age of playing was associated with ΔBMC at the ultra-distal radius ($r = -0.42$, $p<0.05$). The total training time correlated with ΔBMC at the third-distal region and ΔBMD at the mid-distal region ($p<0.05$).
The originality of these results was that a correlation between the side-to-side difference in grip strength and in BMC at the ultra-distal radius was observed in tennis players. Tennis playing seems to stimulate higher BMC at the dominant radius through muscular activity and mechanical vibrations. The higher BMC at the ultra-distal site was mainly related to an increase in BMD whereas the mid- and third-distal sites showed a preferential increase in bone area. The side-to-side difference in bone parameters appears to be greater in players who started tennis earlier and in those who have experienced a longer total training time.

Disclosures: ***C.L. Benhamou****, None.*

SU171

Greater Trabecular Bone Connectivity in the Knee of Female Collegiate Gymnasts. C. M. Modlesky*[1], J. M. Slade*[2], S. Majumdar[3], A. Narasimhan*[3], G. A. Dudley*[2]. [1]Department of Health, Nutrition and Exercise Sciences, University of Delaware, Newark, DE, USA, [2]Department of Exercise Science, The University of Georgia, Athens, GA, USA, [3]Magnetic Resonance Science Center, University of California, San Francisco, CA, USA.

Frequent participation in high-load physical activity is associated with increased areal bone mineral density (aBMD); however, its connection to human trabecular bone microarchitecture, an important contributor to bone strength, has not been investigated. The purpose of this study was to determine if the apparent bone volume to total volume (appBV/TV), trabecular number (appTb.N), and trabecular thickness (appTb.Th) are higher and trabecular separation (appTb.Sp) lower in the distal femur and proximal tibia of artistic gymnasts, a group that participates in a substantial amount of high-load physical activity, than controls. Seven female collegiate gymnasts (GYM) and 7 female controls (CON) not different in age, height and weight were tested. Axial images of the distal femur and proximal tibia were collected on a 1.5 T magnetic resonance imager (195x195x1000 microns) using a 3D fast gradient-echo sequence (TE 4.5 ms; TR = 30 ms; 40 degree flip angle) and measures of trabecular bone microarchitecture were assessed using previously described procedures. Results are listed in the table below. Higher appBV/TV (15.6 %, Cohen's d (d) = 1.15) and appTb.N (8 %, d = 1.53) and lower appTb.Sp (13.8 %, d = 1.36) were observed in the proximal tibia of GYM vs CON ($P < 0.05$). Although the differences were less pronounced and not statistically significant ($P > 0.05$), large effect sizes ($d > 0.8$) indicated a similar pattern in the distal femur, with higher appBV/TV (9.4 %, d = 0.87) and appTb.N (5.2 %, d = 0.91) and lower appTb.Sp (9.0%, d =0.93). The findings suggest that the high-load physical activity performed during gymnastics activity is associated with a greater connectivity of trabecular bone in the knee. Future studies assessing the effects of mechanical loading on trabecular bone structure in humans are warranted.

Measures of Trabecular Bone Microarchitecture (*Significant group difference, $P < 0.05$)

	appBV/TV	appTb.N (mm-1)	appTb.Th (mm)	appTb.Sp (mm)
GYM Femur	0.339 ± 0.028	1.425 ± 0.069	0.238 ± 0.017	0.467 ± 0.037
CON Femur	0.310 ± 0.039	1.355 ± 0.085	0.228 ± 0.016	0.513 ± 0.062
GYM Tibia	0.317 ± 0.041*	1.413 ± 0.054*	0.221 ± 0.019	0.483 ± 0.050*
CON Tibia	0.274 ± 0.032	1.308 ± 0.083	0.209 ± 0.013	0.561 ± 0.065

Disclosures: ***C.M. Modlesky****, None.*

SU172

The Effects of Additional Weightbearing during Exercise and Estrogen on Bone Strength in Female Rats. A. M. Tromp*[1], J. Skovhus Thomsen*[2], L. Mosekilde*[2], N. Bravenboer[1], P. Lips[1]. [1]Endocrinology, VU Medical Center, Amsterdam, Netherlands, [2]Cell Biology, Institute of Anatomy, Aarhus, Denmark.

The aim was to investigate the effects of additional weightbearing during exercise and estrogen status on bone strength in female rats.
Sixty female Wistar rats were assigned to sedentary (SED), ovariectomized (SED+OVX), or ovariectomized with estrogen replacement (SED+OVX+E2) groups, or to the exercise groups EX, EX+OVX or EX+OVX+E2. Exercise consisted of running with a backpack (load 20% of bodyweight) for 19 weeks, 5 days/week, 15 min/day. Thereafter, rats were sacrificed and the femur and 4th lumbar vertebra (L4) were prepared for mechanical testing. Testing consisted of 3 point-bending of the femoral diaphysis, compression of the femoral neck and compression of L4. Load-deformation curves and data on maximum load (F_{max}), maximum stress (σ_{max}) and Young's modulus (E) were obtained. After testing, L4 specimens were ashed and apparent ash density (ρ) was determined.
In the 3 point-bending test of the femur, F_{max} did not differ significantly between the groups ($p>0.05$). However, in the femoral neck test, F_{max} was significantly lower in the EX+OVX and SED+OVX groups ($p=0.025$; fig. 1). For L4, F_{max} ($p<0.001$) and σ_{max} ($p<0.001$; fig.2) were significantly lower in the SED and SED+OVX groups but E was not ($p>0.05$). ρ was significantly lower in the EX+OVX and SED+OVX groups ($p<0.001$).
The strength of the femoral neck was decreased in case of estrogen deficiency, while the femoral diaphysis was not. It therefore seems, that the cortical bone of the femoral diaphysis is less sensitive to the effects of exercise and estrogen than the femoral neck which consists of both trabecular and cortical bone. In L4 however, bone strength was increased after exercise and in case of estrogen replacement in the sedentary state. Apparent ash density was decreased after ovariectomy. It therefore seems that, despite a lower ash density of the

lumbar spine, exercise prevented loss of bone strength in the estrogen deficient state.

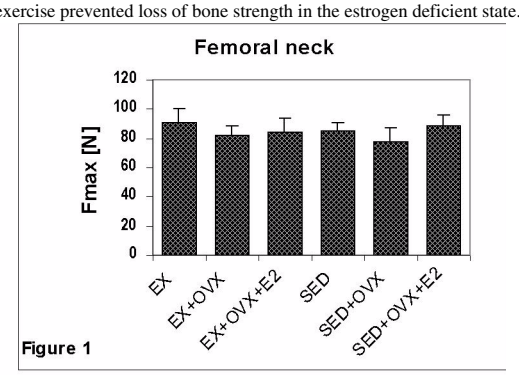


Figure 1

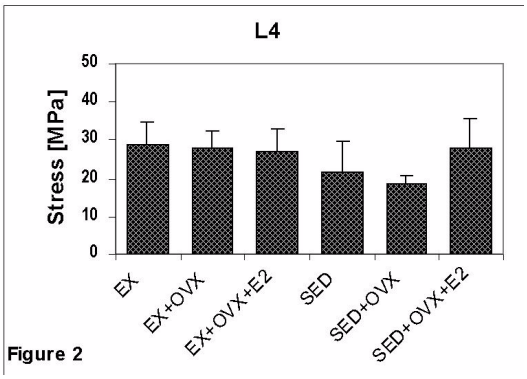


Figure 2

Disclosures: ***A.M. Tromp****, None.*

SU173

Body Weight Supported Treadmill Training in Individuals with Incomplete Spinal Cord Injury: Effects on Bone Mass and Body Composition. L. M. Giangregorio*[1], C. E. Webber[2], S. M. Phillips*[1], A. L. Hicks*[1], N. McCartney*[1]. [1]Kinesiology, McMaster University, Hamilton, ON, Canada, [2]Nuclear Medicine, Hamilton Health Sciences, Hamilton, ON, Canada.

The aim of the present study was to evaluate bone mass changes in men and women with an incomplete spinal cord injury (SCI) after 12 months of body weight supported treadmill training (BWSTT). Thirteen individuals (2 females, 11 males) who had sustained a traumatic, incomplete SCI participated in thrice-weekly BWSTT for a total of 144 sessions (~12 months). The level of lesion ranged from C4 to T12, the average age of the participants was 29 years old, and average time post-injury was 7.70 years (range 1.17 to 24 years). Bone mineral densities of the proximal femur, spine, and whole body were measured using whole body dual-energy x-ray absorptiometry (DXA) scans at baseline and after completion of training. Significance was set at $p < 0.05$. The efficacy of BWSTT for improving walking ability in this group of participants has been published previously (Hicks, McCartney, et al. 2002). Repeated measures multivariate analysis indicated that significant changes occurred at the proximal femur and whole body after 12 months of BWSTT. Post hoc repeated measures analysis of variance revealed a significant decrease in total bone mineral content and area at both the right and left proximal femur, however bone mineral density at these sites was not significantly different after BWSTT. Whole body bone mineral density was significantly reduced after BWSTT, and whole body bone mineral content exhibited a trend towards a decrease ($p<0.08$). Participants did experience significant increases in lean mass, from 45.9kg±2.3 to 47.8kg±2.4 (mean ±SE) before and after training. On average, lean mass in males increased from 48.1kg±2.2 to 50.2kg±2.2, and in females from 33.9kg±3.0 to 34.7kg±1.9. Total body fat mass did not change significantly after BWSTT. Body weight supported treadmill training is an intervention that has been shown to improve walking ability in individuals with spinal cord injury, and may also be a promising intervention for increasing lean mass. However, our data suggests that it does not increase, or prevent the loss of bone in individuals with chronic incomplete spinal cord injury.

Disclosures: ***L.M. Giangregorio****, None.*

SU174

Effect of Impact Loading and Dietary Calcium on the Rat Ulna. J. M. Welch[1], C. M. Weaver[1], C. H. Turner[2]. [1]Foods and Nutrition, Purdue University, West Lafayette, IN, USA, [2]School of Medicine, Indiana University, Indianapolis, IN, USA.

The relationship between dietary calcium and impact exercise on bones is unclear. We investigated what effects impact loading and a diet marginally low in calcium (Ca) have on growing bones. Weanling F–344 female rats were randomized to either a low Ca (CaLow; 0.2%) or a control (CaCon; 0.5%) diet and were further divided into no impact (F0) or impact (F45) loading groups. Impact loaded rats were dropped from a height of 45 cm for a total of 400 impacts over 8 weeks. After completion of the trial, mechanical strength of each ulna was measured by axial compression test. Peripheral quantitative computed tomography (pQCT) was used to measure the cross-sectional area (CSA) and volumetric bone mineral density (vBMD) in whole (tot), cortical (cort), and trabecular (trab) bone at the olecranon process (87% distal) and shaft (45% distal). Mechanical tests indicated that dietary Ca did not significantly affect ulnar shaft strength in either F0 or F45 groups. However, impact loading improved ultimate force by 38.2% in the F45+CaLow group ($p<0.01$) and 32.5% in the F45+CaCon group ($p<0.01$) when compared to the F0+CaLow and F0+CaCon groups respectively. Analysis by pQCT also indicated that the shaft was affected by impact loading but not diet. At this site, vBMDcort was not affected, but CSAcort of the F45+CaLow and F45+CaCon groups was 10.6% and 13.2% greater than that of the F0+CaCon rats (both $p<0.001$). In the olecranon, Ca intake affected only the F0 rats while impact loading influenced both the CaLow and CaCon groups. In the F0 rats, the CaLow diet produced a 5.1% lower vBMDtot ($p<0.05$) with a concomitant 2.4% decrease in vBMDcort ($p<0.01$). Impact loading increased vBMDtot in both dietary groups but the effect was greater in the CaLow (9.1%; $p<0.001$) than the CaCon rats (4.3%; $p<0.05$). This result was accompanied by a 2.6% increase ($p<0.01$) in vBMDcort of the CaLow group with impact loading. Although CSAtot did not differ between any treatments, impact loading increased the CSAcort of proximal ulna in both F0+CaLow (18.3%; $p<0.001$) and F0+CaCon rats (14.4%; $p<0.01$) while decreasing CSAtrab in the F0+CaLow (–45.3%; $p<0.001$) and F0+CaCon (–18.7%; n.s.) groups. Overall, impact loading increased the strength, CSAcort but not vBMDcort in the shaft, and the vBMDtot, vBMDcort, and CSAcort in the proximal metaphysis of the growing ulna in both Ca sufficient and deficient rats. Bones of rats not subject to impact loading were adversely affected by a moderate calcium deficiency, but this effect on the skeleton was averted by impact loading. These results show that impact loading can provide considerable osteogenic stimulation to growing bones in the presence or absence of sufficient dietary calcium.

Disclosures: ***J.M. Welch****, None.*

SU175

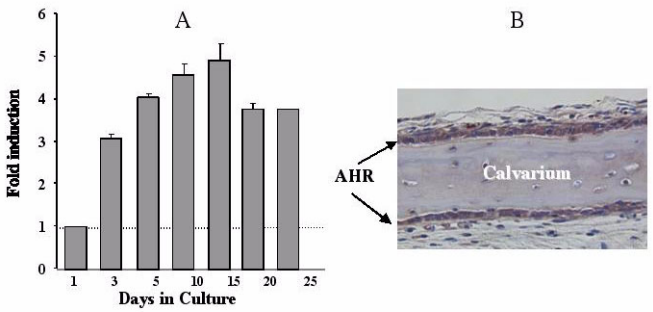
The Aryl Hydrocarbon Receptor: What Is its Role in the Skeleton? J. E. Puzas[1], E. Ryan*[2], H. Drissi[1], R. J. O'Keefe[1], M. J. Zuscik[1], R. N. Rosier[1], E. M. Schwarz[1], T. A. Gasiewicz*[2]. [1]Department of Orthopaedics, University of Rochester School of Medicine, Rochester, NY, USA, [2]Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY, USA.

The aryl hydrocarbon receptor (AHR) was discovered over two decade ago. Its role has been singularly characterized as a mediator of toxic molecules. Its ligands include agents such as dioxin, TCDD and other aromatic hydrocarbons. Moreover, it functions as a polymeric transcription factor for the activation of a number of genes such as the cytochrome p450 oxidases and cyclo-oxygenase 2. We have recently identified the presence of this receptor in developing skeletal elements and osteoblasts. However, its endogenous ligand and physiological role remain undiscovered.

We utilized cultures of osteoblasts derived from neo-natal rat calvaria as well as tissue specimens from both wild type and AHR -/- mice. AHR is expressed in primary cultures of osteoblasts that are induced to differentiate. Both the mRNA and protein levels of the receptor increase through day 15 at which point they begin to decline (Figure 1A). This pattern of expression parallels the induction of a number of early bone-specific genes involved in the maturation of osteoblasts. We also show by immunocytochemistry that the receptor is present in calvarial osteoblasts (Figure 1B). AHR is absent in AHR -/- mice. AHR levels are up-regulated by other toxic agents such as lead (Pb).

Our data also demonstrate that the AHR functions as a transactivating receptor in osteoblasts as evidenced by its ligand-dependent migration to the nucleus and its association with known DNA response elements. Dioxin exposure for 4 hours to osteoblasts in culture induces the expression of Cyp1A1 and Cox II protein levels.

Together these data imply that the AHR may not only mediate the effect of aromatic toxicants on bone cells but, with an as yet unidentified ligand, be involved in the differentiation pathways of the osteoblast.



Disclosures: ***J.E. Puzas****, Lilly Pharmaceuticals 8; Merck Pharmaceuticals 8; Procter & Gamble Pharmaceuticals 8.*

SU176

Functional Elements for Transactivation by PTHrP Are Preserved in Both Human and Mouse RANKL Gene Promoters. R. Kitazawa, S. Kitazawa. Division of Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan.

The role of PTHrP in osteolytic bone metastasis has been linked to the promotion of osteoclastogenesis by the upregulation of RANKL expression on osteoblasts through type I

PTH/PTHrP receptor. We have previously shown that PTHrP treatment of osteoblastic cell increases RANKL mRNA expression by Northern blotting as well as the transcription rate of the gene by nuclear run-on studies; forskolin (FK) shows the inductive effect, whereas H89 abolishes the effect of PTHrP. In this study, to elucidate the molecular mechanism transducing PTHrP signaling, the PKA pathway on human and mouse RANKL gene transcription was analyzed. In both human and mouse RANKL promoters, the basic structure composed of inverted TATA- and CAAT-boxes and VDRE was well preserved. Several CRE-like sequences, although not canonical, were detected in both promoters. By EMSA screening of CRE-like sequences, mouse -940/-933 (TGAGGTCA) and human -1559/-1552 (TAACGTCA) showed specific protein-DNA binding to the nuclear extracts from PTHrP-treated cells; the supershift with an anti-CREB1 antibody was demonstrated. In transfection studies using a series of deletion mutants of mouse (m) and human (h) promoters (0.5-2.3kb insert), PTHrP increased luciferase activity in m-1050-LUC (210%) and h-1974-LUC (180%), but not in m-723-LUC and h-1228-LUC, indicating that m-940/-933 and h-1559/-1552 could be functional CRE. Furthermore, FK enhanced promoter activity in m-1050-LUC and h-1974-LUC, and H89 abolished the inductive effects of PTHrP; inhibitors for p38MAPK (SB203580) and MEK (PD98059) did not suppress the effects of PTHrP. Moreover, reflecting the overlapping of CRE (-940/-933) by VDRE (-937/-922; AGGTCAGCCTGGTTCA) in the mouse RANKL promoter, co-treatment with PTHrP and 1alpha 25(OH)$_2$Vitamin D$_3$ did not exceed the maximum inductive effect of D$_3$ alone. On the other hand, PTHrP and D$_3$ additively increased promoter activity of the human RANKL gene where VDRE (-1584/-1570) and CRE (-1559/-1552) exist separately. These data suggest that 1) the PKA signaling pathway on RANKL gene transactivation is preserved in both mouse and human, 2) CRE and VDRE localize closely and comprise a crucial enhancer element in RANKL gene promoter.

Disclosures: ***R. Kitazawa****, None.*

SU177

Inhibition of Osteoblast Differentiation by TNF-α Is Associated with Suppression of Osterix Expression. L. C. Gilbert, X. He*, A. Kauppi*, J. Rubin, M. S. Nanes. Division of Endocrinology, Department of Medicine, Emory University, VA Medical Center, Decatur, GA, USA.

Tumor necrosis factor-alpha (TNF) plays an important role in the pathophysiology of osteoporosis and inflammatory arthritis by stimulating bone resorption and inhibiting new bone formation. We have previously shown that TNF is a potent inhibitor of osteoblast differentiation in experiments using fetal calvaria cells, marrow stromal cells, or clonal MC3T3E1 preosteoblasts. In addition, we have shown that TNF inhibits the expression of RUNX2 (Cbfa1/AML3/Pebp2alphaA), a critical transcription factor required for differentiation of osteoblasts from their pluripotent progenitors. Here, we test the hypothesis that osterix (Osx), another critical transcription factor acting downstream of RUNX2, is also regulated by TNF. Using the MC3T3E1 pre-osteoblast cell culture model, we used real-time PCR to measure the effect of TNF on steady state levels of Osx mRNA. MC3T3E1 cells were plated on day 0 in MEM + 10% FBS. L-ascorbic acid (50 μg/ml) was added to cells on day 1 and TNF (10 ng/ml) beginning on day 2. Replicate control and TNF-treated cultures were harvested for RNA at 4, 8, and 24 hours after addition of the TNF. TNF reduced the steady state level of Osx mRNA to 55% at 4 hrs, 23% at 8 hrs, and 5% of control cultures at 24 hrs. To determine if the effect of TNF was dose dependent, cells were treated on day 2 of culture with 0.1 to 10 ng/ml. The effect of TNF was dose dependent with an IC_{50} of 0.5-1 ng/ml, a value similar to that observed for inhibition of RUNX2 mRNA and for TNF inhibition of osteoblast differentiation. In order to determine if TNF reduced Osx mRNA by destabilization of the mRNA half-life, cells were treated with TNF and new RNA synthesis was inhibited by actinomycin D. RNA was isolated at time points between 2-14 hrs following addition of the actinomycin D and residual Osx mRNA was measured by real-time PCR. The half-life of Osx mRNA was short. TNF destabilized Osx mRNA, suggesting that this could contribute, in part, toward the reduction in Osx expression. We conclude that TNF inhibits the expression of Osx mRNA. TNF inhibition of transcription factors required for the differentiation of the osteoblast phenotype, including RUNX2 and Osx, may contribute to skeletal loss in the postmenopausal state and in rheumatoid arthritis.

Disclosures: ***L.C. Gilbert****, None.*

SU178

The Extracellular Matrix Protein hCYR61 (CCN1) Modulates Signal Transduction Pathways Associated with Proliferation and Cell Differentiation in Human Osteoblasts. N. Schuetze[1], F. Graedler*[2], M. Kunz*[1], S. Balling*[1], C. Hendrich*[1], J. Eulert*[1], J. Adamski*[2], F. Jakob*[1]. [1]Molecular Orthopaedics, Orthopaedic University Hospital, Wuerzburg, Germany, [2]Institute of Experimental Genetics, GSF National Research Center for Environment and Health, Neuherberg, Germany.

The human cystein rich protein 61 (hCYR61) belongs to the CCN family of proteins. They act in cellular processes such as differentiation, proliferation, tissue regeneration and angiogenesis. Previously, we have shown that the hCYR61 protein expression in human bone correlates with conditions of remodeling and repair. Here we aimed to elucidate the hCYR61 function in human osteoblasts using cDNA microarray analysis. The protein was expressed in the baculovirus system as an IgGFc fusion protein and purified by affinity chromatography with protein G sepharose. Array analysis was performed to compare expression profiles of RNA from hFOB cells treated or not with rhCYR61 for 24 hours. The RNA samples were transcribed and indirectly labelled with the fluorescent dyes Cy3 and Cy5. "Colour flip" was done to aim for the identification of reproducibly regulated gene products only. Raw data were analyzed with the GenePix software and an in-house developed program for data normalization and extraction. RT-PCR was performed with the identical RNA and with RNA from additional time points of rhCYR61 treatment to validate array results and to find further targets. The rhCYR61 protein was detected as a single band in SDS electrophoresis followed by silver staining and western blotting. The chip contained 1920 PCR products spotted in duplicate for genes involved in bone cell development and function. Array analysis showed 28 reproducibly regulated gene products (= candidates; 13 up, 15 down, cut-off 2-fold regulation). Two thirds of the candidates proved to be regulated by rhCYR61 in RT-PCR analysis. Regulated gene products were membrane receptors (including Notch and Hedgehog signal pathway components) as well as intracellular signal molecules and transcription factors, mostly associated with cell proliferation and differentiation. Additional RT-PCR analysis identified downstream target genes of the Notch pathway to be regulated by rhCYR61. Results were coherent with the array analysis and indicate that downstream mediators (Hey and Hes genes) are regulated by rhCYR61. Interestingly, several regulated gene products belonged to the Hedgehog signal transduction pathway as membrane components or as downstream mediators, further strenghtening the validity of expression profiling with cDNA microarrays and RT-PCR. hCYR61 appears to be a control factor for proliferation and differentiation of human osteoblasts.

Disclosures: ***N. Schuetze****, None.*

SU179

Tob-Deficiency Preserves Bone Mass After Ovariectomy in Association with the Enhancement of Ovariectomy-Induced Osteogenic Gene Expression Levels in Bone Marrow. M. Usui[1], Y. Yoshida*[2], K. Tsuji[1], I. Ishikawa*[3], 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, [3]Periodontology, Tokyo Medical and Dental University, Tokyo, Japan.

Tob (transducer of ErbB2) belongs to a novel antiproliferative gene family, which include molecules containing a Tob-homology domain, such as Tob, Tob2, ANA/BTG3, BTG2/PC3/TIS21, PC3B and BTG1. Tob-deficiency (TD) increases bone formation without alteration in bone resorption to yield high levels of bone mass in adult. This enhancement in bone formation is based on the Tob action as an inhibitor against BMP (Yoshida Y. et al., Cell, 2000). We recently observed that TD preserved bone mass even after the bone loss due to ovariectomy (OVX). Bone histomorphometry revealed TD-enhancement in the levels of bone formation parameters but not bone resorption parameters after OVX. However, the alteration in gene expression underlying these TD-induced phenomena has not been known. The aim of this paper was to elucidate the molecular bases, which cause TD effects on OVX-induced bone loss. We carried out RT-PCR analyses on the transcripts of the genes expressed in the femoral bone marrow (BM) and calvaria of mice subjected to OVX. Two weeks after OVX, RNA was obtained from femoral BM and calvaria. In the BM obtained from wild-type (WT) mice, OVX up-regulated mRNA expression levels of bone formation (BF)-related osteogenic genes such as alkaline phosphotase, type I collagen, osteocalcin, Cbfa1 and osterix as well as those of bone resorption (BR)-related genes encoding RANK, RANKL and OPG as reported previously. Similar to BM in WT mice, expression of BR-related genes in the BM of Tob deficient (Tob KO) mice was up-regulated by OVX to the levels comparable to those in WT mice. In contrast, the levels of OVX-induced enhancement of BF-related gene expression in BM were significantly higher in Tob KO mice than those in WT mice. Furthermore, we also examined RNA prepared from calvariae, which mainly consist of cortical bone. Similar to BM, OVX increased mRNA expression of BF-related and BR-related genes in calvaria in WT mice. However, in contrast BM, the levels of OVX-induced enhancement of BF-related gene expression in WT mice and those in Tob KO mice were similar in calvaria. These data indicate that TD preserves bone mass after OVX through the enhancement of the expression of BF-related genes in bone marrow environment.

Disclosures: ***M. Usui****, None.*

SU180

Synergistic Effects of TGFβ and VEGF on Rat Bone Marrow Stromal Cells *In Vitro*. S. Kuroda, D. R. Sumner, A. S. Virdi. Department of Anatomy and Cell Biology, Rush University, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, USA.

Bone regeneration is essential for healing bone defects. Growth factors have been increasingly investigated for their capacity to enhance the regeneration process. Recently, gene therapy has emerged as an effective approach to deliver therapeutic proteins in a more physiological and persistent manner. Transforming Growth Factor beta (TGFβ) has been implicated in osteoblast proliferation and differentiation. Vascular Endothelial Growth Factor (VEGF) plays an important role in angiogenesis and may be intimately related to bone development and healing as both intramembranous and endochondral ossification are associated with capillary development. In this study, we introduced transgenes for mouse TGFβ and/or mouse VEGF into rat marrow stromal cells (rMSC), and investigated their synergistic effects on proliferation, differentiation and osteogenic gene expression. rMSC were seeded at 1x10^5/well in 24-well plate and after 24 hours, transfer of 1 μg plasmid carrying mouse TGFβ and/or mouse VEGF full length cDNA was performed by LipofectAmine (Invitrogen) reagents. The cultures were assessed for alkaline phosphatase (ALP) staining and nodule formation, ALP activity and DNA content, and gene expression analysis by real-time PCR at d1, 2, 4, 7, 14 and 28. All cultures carrying transgenes showed less intensities for ALP staining at d7 and 14, which approached control (no transgene) levels by d28. At this time point more nodule formation appeared than in control. However, less mineralization was seen in each of the transgene carrying cultures when the medium contained dexamethasone (10^{-8}) M. DNA assay showed higher proliferation in all cultures with transgenes by d28. While ALP levels also became higher, its levels per DNA were lower at the same time point due to the higher DNA levels. Most of 15 genes analyzed for expression, including: ALP, BMPs, Cbfa1, IGF1, RANKL and RANK, showed an increase due to each transgene at d2 or d4 with even greater effects in cultures transfected with both

TGFβ and VEGF. These results demonstrated that transgenes for TGFβ andVEGF exhibited mitogenic effects and enhanced osteogenic differentiation of bone marrow stromal cells, and that TGFβ and VEGF act in a synergistic fashion.

Disclosures: ***S. Kuroda****, None.*

SU181

Analysis of Gene Expression During Osteogenic Differentiation of Human Periosteal Cells Using cDNA Array Technology. S. Honsawek*[1], C. J. Osgood*[2], L. Wolfinbarger[3]. [1]Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, [2]Department of Biological Sciences, Old Dominion University, Norfolk, VA, USA, [3]Research and Development, LifeNet, Virginia Beach, VA, USA.

The current study was undertaken to examine gene expression profile of osteogenic differentiation in human periosteal (HPO) cells using cDNA array analysis. HPO cell lines typically contain osteoprogenitor cells which can differentiate into osteoblasts. Numerous studies have demonstrated that demineralized bone matrix (DBM) comprises bone morphogenetic proteins (BMPs) that are essential regulators for inducing bone formation. We reported that DBM-conditioned media (DBM-CM) promoted osteoblast differentiation of periosteal cells in culture. *In vitro* alkaline phosphatase assay showed an increase in alkaline phosphatase activity in the DBM-CM treated cells. Additionally, histochemical detection of alkaline phosphatase revealed osteoblast-like cells in the HPO cells treated with DBM-CM. To attain a profile of gene expression during osteogenic differentiation, total RNA was harvested from HPO cells in the absence or presence of DBM-CM at day 7 and used as template for synthesis of biotin labeled cDNA probes. These cDNA probes were then hybridized to cDNA gene arrays. We demonstrated that several differentially expressed genes such as biglycan, TGF-β, and TGF-βR1 were up-regulated, whereas collagen14A1 was down-regulated. To verify the cDNA array results, RT-PCR analysis of parallel samples was performed on 2 selected genes, one was strongly up-regulated (biglycan) and the other was highly down-regulated (collagen14A1). The results revealed identical expression pattern for selected genes as shown by the cDNA array analysis. To examine the temporal expression of biglycan and collagen14A1 in HPO cells untreated or treated with DBM-CM, poly $(A)^+$ RNA was isolated for RT-PCR analysis at days 0, 4, 7, 10, and 14. The present study revealed that there was an increase in the level of biglycan expression and a decrease in the level of collagen14A1 expression following DBM-CM treatment. Our study demonstrated the potential of cDNA gene expression array technology to identify the gene expression involved in the cellular response to DBM-CM and to provide insight to the biological process of osteogenic differentiation.

Disclosures: ***S. Honsawek****, None.*

SU182

PTH Induces NGFI-B Nuclear Orphan Receptors in vivo. F. Q. Pirih*, O. Bezouglaia*, S. Tetradis. Dentistry, UCLA, Los Angeles, CA, USA.

Parathyroid hormone (PTH), a major regulator of osteoblastic function, induces transcription of several genes, including primary response genes (PRGs). Transcription factors are a subset of PRGs of particular importance since they propagate the activation signal downstream, by regulating secondary response gene expression. Among the transcription factors activated by PTH is the nerve growth factor inducible factor B (NGFI-B) family of orphan nuclear receptors, which is composed of three members: Nurr1, Nur77 and NOR-1. NGFI-B orphan receptors are constitutively expressed in the nervous system and can be induced in multiple tissues including PTH-treated osteoblasts. We have previously reported that PTH induces Nurr1, Nur77 and NOR-1 gene expression primarily through the cAMP protein kinase A pathway *in vitro*. We report here that PTH rapidly and transiently induced Nurr1, Nur77 and NOR-1 mRNA expression in bone and kidney *in vivo*. Unless otherwise specified all experiments were done using 1-month-old CD-1 male mice that received PTH (80μg/kg) i.p. injections. Nurr1, Nur77 and NOR-1 gene expression was assayed by semi-quantitative RT-PCR. PTH maximally induced Nurr1 and Nur77 from .5-1 h and returned to baseline levels at 2 h, while NOR-1 was maximally induced at 1-2 h and returned to baseline levels at 4 h in calvaria, long bone and kidney. Nur77 had the highest level of mRNA expression after PTH injection, followed by Nurr1 and lastly NOR-1. The magnitude and pattern of NGFI-B induction *in vivo* closely mimicked NGFI-B the induction in cultures of primary mouse osteoblast treated with 10nM PTH. To characterize NGFI-B expression at different stages of mouse development, 1-, 3- and 5-month old mice received PTH injections. There were no differences in NGFI-B mRNA expression among different stages of mouse development. To determine if Nurr1, Nur77 and NOR-1 gene expression is altered by intermittent administration of PTH, mice were injected with vehicle or PTH once a day for 1 wk. No difference was seen in the mRNA induction of NGFI-B members after freshly added PTH in calvaria and kidney. To determine which pathway mainly activates NGFI-B gene expression *in vivo*, mice were treated with 10-80 μg/kg of PTH (3-34), which does not activate PKA signaling but activates PKC and calcium pathways. This treatment did not induce NGFI-B mRNA levels, confirming our *in vitro* results with primary mouse osteoblasts. PTH-regulated induction of the NGFI-B family *in vivo* suggests that these genes may play an important role in the early response of osteoblasts to PTH.

Disclosures: ***F.Q. Pirih****, None.*

SU183

Mutations of Runx2 Binding Sites in the Rat Col1a1 Promoter Have Little Effect on Expression of Promoter-Reporter Constructs in Transgenic Mice. H. Chin*[1], M. S. Kronenberg[1], C. Wang*[2], A. C. Lichtler[1]. [1]Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA, [2]Transgenic Core Facility, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan Republic of China.

Runx2/Cbfa1 is a transcription factor that is necessary for formation of bone. It is expressed in osteoblasts, and has been shown to positively regulate expression of the osteocalcin promoter. Since induction of high levels of expression of type I collagen is necessary for synthesis of bone matrix, it would be expected that Runx2 should upregulate the Col1a1 promoter in osteoblastic cells. Despite this expectation, our studies, reported at previous ASBMR meetings, indicate that mutation of Runx2 binding sites in rat Col1a1 promoter-reporter constructs does not inhibit expression in transfected ROS 17/2.8 cells, and furthermore, overexpression of Runx2 inhibits expression of these constructs. To further investigate the role of the Col1a1 promoter's Runx2 binding sites in expression in osteoblasts, we analyzed transgenic mice containing Col1a1 promoter-reporter constructs. The first series of experiments were designed to quantitatively compare expression of a wild type -1719 bp Col1a1 promoter fragment, fused to CAT, to the same promoter with one, three or five Runx2 binding sites mutated. In these experiments the constructs were injected into fertilized mouse eggs, transgenic animals were identified, and calvaria were analyzed for CAT activity. We analyzed at least three to six separate founder animals containing the wild type promoter or mutations in one, three or five of the Runx2 binding sites. Although there was significant variability between founder animals containing the same constructs, there was no apparent difference in expression between the wild type and the mutant constructs, supporting the observations that we have previously made using transfected ROS 17/2.8 cells. In a second series of experiments, the wild type and mutant constructs were linked to a GFP reporter gene to facilitate evaluation of the pattern of expression of the constructs in bone. Our evaluation of GFP expression in whole neonates indicates that there is no apparent difference in the pattern of expression of the wild type and mutant constructs. More detailed histological studies are underway. Thus our experiments in transgenic mice support our previous studies in transfected ROS 17/2.8 cells and fail to support the expectation that Runx2 is a direct inducer of the Col1a1 promoter during osteoblast differentiation.

Disclosures: ***A.C. Lichtler****, None.*

SU184

Interrelationship of Runx2 and BMP2 Signaling in Calvarial Bone and Suture Development. K. Choi[1], S. Lee*[1], M. Park*[1], H. Shin[2], S. Nam*[3], Y. Kim*[3], J. Wozney[4], T. Komori[5], H. Ryoo[1], H. Kim*[3]. [1]Department of Biochemistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [2]Department of Oral Pathology, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [3]Department of Pediatric Dentistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [4]Genetics Institute, Inc., Cambridge, MA, USA, [5]Japan Science and Technology Corporation, Suita, Osaka 565-0871, Japan.

Runx2, a key player of skeletogenesis, has been known as one of the downstream target genes of BMP signaling. However, little has been known their relationships in calvarial bone and suture development. In this study, we employed BMP2 bead and organ culture system using E15 mouse calvaria, and also used Runx2 knockout mice. The observation sites of the calvarial suture organ was osteogenic fronts, calvarial bones and sutural mesenchyme. In our previous study Dlx5, Msx2 and Runx2 have been known as downstream target genes of BMP2 in cell culture system. The BMP2 beads induced Dlx5 and Msx2 gene expression, however, it didn't induced Runx2 gene expression in the calvarial suture organ culture. To explore this discrepancy between ex vivo organ culture and in vitro cell culture system, we determined the expression of BMP2, Dlx5, Msx2 and Runx2 in both endogenous mouse calvaria itself by in situ hybridization. The expression of BMP2 and Dlx5 was observed at osteogenic fronts and calvarial bones and Msx2 was expressed at the sutural mesenchyme in wild-type and Runx2 heterozygous mice. However, neither BMP2 nor the Dlx5 and Msx2, downstream genes of BMP2, were expressed in Runx2 null mice. These results indicate that BMP2 is not an upstream signal of Runx2 gene at least in mouse calvarial bone and suture development. Conclusively, we suggest that Runx2 might be upstream gene of BMP2 signaling in mouse calvarial bone growth.

Disclosures: ***K. Choi****, None.*

SU185

SP1/SP3 and Myeloid Zinc Finger 1 (MZF 1)-like Factors Regulate Human N-cadherin Promoter Activity in Osteoblasts. S. Le Mée*, P. J. Marie. Hopital Lariboisiere, INSERM U349, Paris Cedex 10, France.

Recent studies indicate that N-cadherin plays an important role in cell-cell adhesion and osteoblast differentiation program. In this study, we have determined the molecular mechanisms of transcriptional regulation of N-cadherin expression in human osteoblasts. We isolated 3682 base pairs of 5' flanking region of the previously characterized N-cadherin gene from a human BAC library. Nucleotide sequence analyss revealed 80% and 53% identity, respectively, between human/mouse and human/chicken. To characterize the DNA sequences involved in the activity of the basal N-cadherin promoter, a series of deletion mutants were made in pGL3 basic vector, and constructs were transiently transfected in Immortalized Human Neonatal Calvaria (IHNC) osteoblastic cells. The minimal sequence providing the highest luciferase activity covered 329 nucleotides upstream of the ATG. This region does not contain TATA-box nor CAAT-box but is characterized by a high con-

tent of GC and a GA-rich binding core that may potentially bind a transcription factor of the zinc finger family. Gel retardation analyses showed that two binding sites are involved in the regulation of the N-cadherin promoter. When nuclear extracts from IHNC cells were incubated with -305/-270 wild-type probe containing a putative SP1 binding site, two specific complexes, C1 and C2, were formed. These two complexes were not formed in the presence of a -305/-270 probe mutated in the SP1 binding site. Moreover, a consensus SP1 probe was as efficient as the -305/-270 wild-type probe in competition experiments whereas a -305/-270 mutated probe did not compete at all. Supershift EMSAs were conducted to identify the composition of the two complexes. The addition of anti-SP1 antibody reduced the formation of C1 whereas the addition of anti-SP3 antibody supershifted C2. When nuclear extracts from IHCN cells were incubated with -163/-131 wild-type probe containing the GA-rich binding core, one specific complex was formed. Moreover, the formation of this complex was abrogated in the presence of the -163/-131 probe mutated in the GA repeat. We used several consensus zinc finger probes to identify the nuclear factor involved in the formation of this complex. Only consensus oligonucleotide for Myeloid Zinc Finger 1 (MZF 1), a transcription factor expressed in hematopoietic progenitor cells, exhibited a clear competition for the complex. When SP and MZF 1 binding sites were mutated, the N-cadherin promoter activity decreased by 30% and 50%, respectively. These results identify for the first time critical regulatory regions for the human N-cadherin basal promoter activity in osteoblasts, and reveal a novel role for MZF 1 in the control of N-cadherin expression.

Disclosures: ***S. Le Mée****, None.*

SU186

Chromatin Remodeling of the Proximal Rat Osteocalcin Promoter is Dependent on Vitamin D Receptor Binding at the Distal Promoter Element. S. Gutierrez*[1], A. Javed[2], M. Montecino[1], A. J. van Wijnen[2], G. S. Stein[2], J. B. Lian[2], J. L. Stein*[2]. [1]Departmento de Biologia Molecular, Universidad de Concepcion, Concepcion, Chile, [2]Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, MA, USA.

Gene activation involves modifications in chromatin organization. Initiation of rat OC gene transcription is accompanied by changes in chromatin structure that are detected by nuclease accessibility. Two DNase I hypersensitive sites (DHS) are observed only in the actively transcribed OC promoter: DHS I (-600 to -400 bp) in the distal promoter encompasses the vitamin D response element (VDRE) and two flanking Runx sites; DHS II (-170 to -70 bp) in the proximal promoter spans Runx, C/EBP, OC-Box, and TATA elements. Vitamin D3 (VD) treatment results in transcriptional enhancement and intensification of the DHS. We addressed the role of the VDRE in maintaining chromatin architecture of the rat OC promoter, by introducing point mutations in the two steroid half elements of the VDRE (mVDRE) that do not affect binding of YY-1 or AP-1 to their sites which overlap the VDRE. This mVDRE OC promoter construct was stably integrated into ROS 17/2.8 osteoblastic cells and four monoclonal lines were studied. As expected, mutation of the VDRE results in a complete loss of VD responsiveness of OC activity. Our results show that in basal conditions the two DHS sites were not altered in the mVDRE promoter. However, in response to VD a decrease in DNase I accessibility of the mVDRE promoter was observed at the distal as well as the proximal DHS, reflecting a change in chromatin status. As another index of chromatin structure, we assayed restriction enzyme (RE) accessibility of the OC promoters. VD treatment increased RE accessibility in the distal endogenous (WT) promoter surrounding the VDRE but not in the distal mVDRE promoter. Surprisingly, the WT proximal promoter showed high RE accessibility, with a modest increase in response to vitamin D, and both of these effects were abrogated in the mVDRE promoter. These observations show for the first time that the VDRE influences chromatin structure at the proximal OC promoter. Increased histone H3 acetylation and association of RNA polymerase II were also observed with the mVDRE promoter compared to the WT gene, suggesting higher basal transcription in the absence of VD responsiveness. Thus binding of the VDR to the rat OC promoter appears to be required to complete the chromatin remodeling process across both the distal and proximal DHS domains associated with transcriptional activation of the OC gene.

Disclosures: ***S. Gutierrez****, None.*

SU187

Expression of LRP1 by Human Osteoblast-like Cells: A Mechanism for the Delivery of Dietary Lipids to Bone. A. C. Niemeier*[1], J. Heeren*[2], M. Kassem[3], U. Beisiegel*[2], W. Ruether*[1]. [1]Orthopaedics, University Hospital Hamburg-Eppendorf, Hamburg, Germany, [2]Molecular Cell Biology, University Hospital Hamburg-Eppendorf, Hamburg, Germany, [3]Endocrinology, University Hospital Odense, Odense, Denmark.

Accumulating clinical and experimental data demonstrate the importance of dietary lipids and lipophilic substances, such as vitamin K for bone formation. However, little is known about the mechanisms underlying the delivery of lipids to bone. Thus, we investigated the expression and function of lipoprotein receptors in three different human osteoblast-like cell lines: Osteosarcoma cell lines MG63, SaOS-2 and telomerase-immortalized human bone marrow stromal cells (hMSC-TERT) after 1,25(OH)vitaminD3 induction of osteoblastic differentiation, which was documented by increased osteocalcin expression (immunofluorescence and RT-PCR). In comparison to the human hepatoma cell line HuH7, human osteoblasts exhibited high levels of expression of known chylomicron remnant receptors : LRP1, VLDLR and LDLR proteins as shown by Western blot analysis and immunofluorescence. Uptake of Cy3-fluorescence-labeled chylomicron remnants by osteoblasts resembled the typical pattern of receptor-mediated endocytosis and was stimulated by the addition of exogenous apolipoprotein E and lipoprotein lipase. Quantitative radioactive uptake experiments with 125I-chylomicron remnants displayed that inhibitors of ligand binding to LRP1, such as RAP and lactoferrin, consistently reduced uptake by about 30%, suggesting that among the expressed receptors, LRP plays a predominant role. Immunohistochemistry of normal human bone sections showed strong LRP1 expression by osteoblasts and marrow stromal cells, but not by osteocytes. In conclusion, human osteoblasts possess functional receptors of the LDLR family with a capacity for chylomicron remnant endocytosis , a novel mechanism for the delivery of dietary lipids and lipophilic vitamins to human bone.

Disclosures: ***A.C. Niemeier****, None.*

SU188

LIM Mineralization Protein-1 Stimulates Transcription of Procollagen Type I Alpha 2 Promoter in Human Osteoblasts by an AT-rich Cis-acting Element. T. A. Linkhart[1], A. Delgado*[2], C. Stivers*[2], G. Gutierrez*[2], D. D. Strong[3]. [1]Pediatrics and Biochemistry, Loma Linda University and VAMC, Loma Linda, CA, USA, [2]Loma Linda VAMC, Loma Linda, CA, USA, [3]Medicine and Biochemistry, Loma Linda University and VAMC, Loma Linda, CA, USA.

LIM mineralization protein LMP-1 has been shown to induce osteoblast differentiation in vitro and increase bone formation in vivo. The mechanisms that underlie LMP-1 induction of bone formation, however are not clear. LMP-1 is a member of a diverse LIM domain protein family, and is identical to human Enigma. LMP-1 contains 3 LIM domains, named for homology to homeodomain proteins. We have identified human LMP-1 as a protein that interacted with IGF binding protein-6 in a yeast two-hybrid screen. To identify mechanisms of LMP-1 action on osteoblasts we investigated effects of LMP-1 expression on activity of the human Type 1 alpha 2 (hCol 1a2) and rat Type 1 alpha 1 (rCol 1a1) procollagen promoters. A hCol 1a2 promoter fragment (bp –267 to + 45) from genomic DNA was inserted into the pGL3Basic luciferase reporter gene vector. The rat Col 1a1 promoter was subcloned from ColCAT2.3 (D. Rowe U. Connecticut) into pGL3Basic. In transient transfection assays with TE85 human osteosarcoma cells, basal promoter activities were 40 fold greater than the pGL3 vector alone. To determine effects of LMP-1, TE85 cells were co-transfected with hCol 1a2 or rat 2.3 Col 1a1 promoter vectors and a human LMP-1 pcDNA3 expression vector (G. Gill, U.C. San Diego). LMP-1 expression resulted in reproducible 2 to 3 fold increases in activities of the collagen promoters but had no effect on the empty pGL3Basic vector. These results suggest that a function of LMP-1 in osteoblasts is to increase expression of Type I collagen. To identify promoter sequences involved in LMP-1 induction of the Col 1a2 promoter, we performed site directed mutagenesis of presumptive transcription factor binding sites in the promoter sequence and identified an AT rich site that may mediate transcriptional activation by LMP-1. Mutation of this site did not affect basal promoter activity but prevented activation by LMP-1. Overexpression of truncated LMP-1 (AA1-156) lacking the LIM domains failed to increase Col 1a2 promoter activity, suggesting an important role for the Lim domains. Summary: An AT rich sequence in the proximal region of the LMP-1 promoter sequence may be involved in mediating LMP-1 induction of Col 1a2 gene transcription. This transcriptional activation may require functions of the LIM domains.

Disclosures: ***T.A. Linkhart****, None.*

SU189

Inorganic Phosphate Regulation of a Discrete Set of Genes in MC3T3-E1 Osteoblasts Requires ERK1/2 and PKC. G. R. Beck. Center for Cancer Research, National Cancer Institute, Frederick, MD, USA.

The generation of inorganic phosphate by alkaline phosphatase during osteoblast differentiation represents a potentially important cellular signaling molecule although the consequences are relatively unknown. We have recently described a discrete set of genes both positively and negatively regulated by elevated phosphate in the murine pre-osteoblast cell line MC3T3-E1. Some of the increased genes include osteopontin, HMGA1, HMGA2, Fra-2 and Nrf2 a transcription factor involved in the regulation of many phase II detoxifying enzymes. The down regulated genes encode mostly for extracellular proteins including collagen type-1, periostin and thrombospondin among others. Because of the potential significance of phosphate as a signaling molecule in the process of osteoblast differentiation we were interested in defining possible mechanism for its ability to alter gene expression. Here we report that two families of signaling proteins, ERK1/2 and PKC are required for the regulation of genes responsive to inorganic phosphate. Analysis of the mitogen activated signaling kinases (MAPKs), ERK1/2, p38 and c-jun N-terminal kinase (JNK), reveal that specifically, ERK1/2 is required for the regulation of all phosphate responsive genes analyzed to date. Additionally we demonstrate that phosphate causes a biphasic phosphorylation of ERK1/2, with an initial activation at 15 to 45 minutes followed by a more gradual activation starting at 8 to 12 hours. Evaluation of other protein kinase signaling molecules reveals that PKC is also required in the phosphate-signaling pathway. Analysis of the timing of expression of phosphate responsive genes reveals that they can be separated into at least 2 groups, the first are those that respond early after phosphate treatment and correspond to the first ERK1/2 activation. This group consists mainly of transcription factors and includes Nrf2 and members of the AP-1 family of proteins. The regulation of the second group corresponds to the second ERK1/2 activation and includes osteopontin, HMGA1 and HMGA2 among others. The data presented here identifies ERK1/2 and PKC as important signal transduction molecules in the regulation of phosphate responsive genes in osteoblast cells.

Disclosures: ***G.R. Beck****, None.*

SU190

Pro-Osteogenic and Anti-Adipogenic Effects of Oxysterols in Marrow Stromal Cells are Mediated via COX/PLA2- and MAPK-Dependent Mechanisms. F. Parhami[1], B. Basseri*[1], H. T. Kha*[1], D. Shouhed*[1], J. A. Richardson*[1], S. Tetradis[2], T. J. Hahn[3]. [1]Medicine, UCLA, Los Angeles, CA, USA, [2]Dentistry, UCLA, Los Angeles, CA, USA, [3]Medicine, West L.A. VA Medical Center, Los Angeles, CA, USA.

Identification of anabolic agents that induce osteogenic differentiation of osteoprogenitor cells, inhibit their adipogenic differentiation, and enhance bone formation is of great importance for improved prevention and management of osteoporosis. Oxysterols are naturally occurring products of cholesterol oxidation, have multiple biologic activities, and are made in part by cellular cytochrome P450 enzymes. We previously reported that specific combinations of oxysterols, namely 22(R)- or 22(S)-hydroxycholesterol with 20(S)-hydroxycholesterol have novel pro-osteogenic and anti-adipogenic effects when applied to pluripotent M2-10B4 (M2) mouse marrow stromal cells (MSC) in vitro. Osteogenic effects of these oxysterols were also induced in primary mouse MSC, C3H10T1/2 embryonic fibroblastic cells, MC3T3-E1 preosteoblastic cells, and SAOS-2 human osteosarcoma cells, as assessed by the induction of markers of osteogenic differentiation alkaline phosphatase (ALP) activity, osteocalcin (Osc) gene expression, and mineralization. Further studies demonstrated that oxysterol-induced ALP activity, Osc mRNA expression and mineralization in M2 cells were inhibited by cyclooxygenase 1 and 2 (COX-1 and COX-2) inhibitors, SC-560 (20 μM) and NS-398 (80 μM), respectively. Osteogenic responses of M2 cells to the oxysterols were also inhibited by phospholipase A2 (PLA2) inhibitors, ACA (25 μM) and AACOCF3 (20 μM). Prostaglandin E2 and arachidonic acid rescued the cells from the inhibitory effects of COX and PLA2 inhibitors, respectively. In addition, MAPK kinase (MEK) inhibitor, PD98059, inhibited oxysterol-induced mineralization, but not ALP activity or Osc expression in M2 cells. Ten minutes to 8 hours treatment with oxysterols induced a sustained phosphorylation and activation of Erk1/2. Moreover, the inhibitory effects of the osteogenic oxysterols on adipogenesis of M2 cells were completely inhibited by PD98059, but not by NS-398 or SC-560. These results suggest that the osteogenic and anti-adipogenic effects of oxysterols are mediated via COX/PLA2- and MAPK-dependent mechanisms. The osteogenic oxysterols also acted synergistically with BMP-2 and BMP-7 in inducing osteogenic differentiation of M2 cells. We suggest that oxysterols and their downstream targets may be important regulators of lineage-specific differentiation of MSC. The use of lipid-based rather than protein- or peptide-based molecules introduces a whole new strategy for creating therapeutics for osteoporosis intervention.

Disclosures: ***F. Parhami****, None.*

SU191

Near Infrared Light Irradiation Alters the Gene Expression of RANKL and OPG in Response to PTH. J. T. Ninomiya, S. K. Lee*, J. A. Struve. Department of Orthopaedic Surgery, Medical College of Wisconsin, Milwaukee, WI, USA.

Light irradiation in the near infrared region (NIR)has been shown to promote a variety of biologic effects, including cellular proliferation and wound healing. However, the effects of NIR light on bone are less well characterized. Therefore, we hypothesized that NIR light irradiation would enhance bone formation, and theorized that a potential mechanism of this action was through alteration of the gene expression of the proteins RANKL and OPG in response to stimulus with PTH.

Murine MC3T3-E1 cells were grown in tissue culture, and were irradiated with NIR light at 770, 830, and 880 nm at 4J. Controls consisted of non-irradiated cells.The cells received a daily dose of PTH (1-34), and following incubation the RNA was collected. Alterations in the gene expression of RANKL and OPG were determined by RT-PCR.

Parathyroid hormone increased RANKL gene expression in the osteoblasts. None of the three wavelengths altered RANKL or OPG gene expression in the absence of PTH. However, light of 830 nm produced a significant decrease in the RANKL/OPG ratio following the addition of PTH, and this was due to a decrease in RANKL gene expression.

We conclude that NIR light alters the gene expression of RANKL in osteoblasts that are stimulated with PTH. A decrease in the RANKL/OPG ratio would likely lead to less osteoclast maturation and possible less bone resorption. These findings have important implications for use in fracture healing and the prevention of bone loss during prolonged space flight.

Disclosures: ***J.T. Ninomiya****, None.*

SU192

Stimulation of *in-vitro* Osteoblastic Activity by Flavonoids. M. Gros-van Hest[1], C. Beumer*[1], Z. Dang[2], K. C. T. Knipping*[1], A. van Helvoort*[1], I. Schoenmakers[1]. [1]Biomedical Research, Numico Research, Wageningen, Netherlands, [2]Endocrinology, Leiden University Medical Center, Leiden, Netherlands.

In the prevention of osteoporosis, components reducing bone loss and stimulating bone formation may be beneficial to maintain healthy bone mineral density.

In the search of components stimulating bone formation, several flavonoids were tested on the mouse osteoprogenitor cell line KS483 and on the mouse pre-osteoblastic cell line MC3T3-E1.

KS483 cells were cultured in phenol red free alpha-MEM supplemented with 10% charcoal stripped FCS (M-FCSs) for 17 days. Three hours after seeding, half of the medium was replaced with medium containing 17ß-estradiol (10^{-7}M), flavonoids (10^{-4} to 10^{-8}M) and plant extracts (1-100μg/ml) containing these same flavonoids. Ascorbic acid (50μg/ml) and ß-glycerophosphate (10mM) were added to the culture medium from day 3 and 10, respectively.

MC3T3-E1 cells were pre-cultured in phenol red free alpha-MEM supplemented with 10% non-charcoal stripped FCS (M-FCSns), ascorbic acid and BMP-4 (10ng/ml) for 3 days. Thereafter, M-FCSns was replaced with M-FCSs containing ascorbic acid and ß-glycerophosphate and cells were exposed to 17ß-estradiol, flavonoids and plant extracts containing these same flavonoids for 7 days. All cells were refreshed twice a week.

Alkaline phosphatase activity, calcium deposition and DNA were determined and nodules were visualized with Alizarin staining.

At a concentration of 10^{-5}M, one of the flavonoids increased the number and size of nodules formed by both KS483 and MC3T3-E1 cells. This was reflected in a significantly higher calcium deposition and alkaline phosphatase activity when compared to controls, ß-estradiol and the other flavonoids tested. Also the flavonoid containing plant extracts increased nodule formation, alkaline phosphatase activity and calcium deposition in MC3T3-E1 cells.

Toxicity of the flavonoids and flavonoid containing extracts was first observed at 10^{-4}M and 100μg/ml respectively, as reflected by low DNA content and poor cell morphology.

In vivo studies will have to prove the safety, bioavailability and efficacy of this component and/or its metabolites to protect against post-menopausal bone loss.

Disclosures: ***M. Gros-van Hest****, None.*

SU193

Traumatic Brain Injury Stimulates Systemic Bone Formation: Possible Role for Hypothalamic Leptin Signaling. Y. Tam*[1], E. Regev*[2], N. Casap*[2], A. Shteyer*[2], M. Attar-Namdar*[1], A. Alexandrovich*[3], O. Lahat*[1], E. Shohami*[3], I. A. Bab[1]. [1]Bone Laboratory, Hebrew University of Jerusalem, Jerusalem, Israel, [2]Oral and Maxillofacial Surgery, Hebrew University of Jerusalem, Jerusalem, Israel, [3]Pharmacology, Hebrew University of Jerusalem, Jerusalem, Israel.

The common clinical finding of heterotopic ossification and enhanced fracture healing in traumatic brain injury (TBI) patients has attracted little attention and the underlying mechanisms are poorly understood. The consistency of the TBI-induced peripheral osteogenic effect lead us to initiate a study on its mechanisms in an established mouse model of closed head injury (CHI). Because leptin has been suggested as a master hormone, negatively regulating bone formation via a hypothalamic relay, we investigated the temporal relationship between peripheral osteoblastic activity and hypothalamic leptin signaling during a 16-day post-CHI period. CHI was induced in mice under ether anesthesia by a calibrated weight drop device resulting in a focal injury to the left hemisphere. The severity of injury and rate of recovery were assessed 1 hr – 16 days post-CHI by a Neurological Severity Score, and the mice were sacrificed for *in vivo* assessment of bone formation and hypothalamic gene expression. Dynamic bone histomorphometric analysis showed stimulation of trabecular bone formation rate in the distal femoral metaphysis peaking on days 4 and 12 post-CHI, consequent to increases in both mineral appositional rate (MAR) and mineralizing perimeter (Min.Peri), suggesting a CHI-induced enhancement of both osteoblast activity and number. Real-time RT-PCR demonstrated impaired expression of ObRb, the signal-transducing form of the leptin receptor, 1.5 hrs post-CHI, followed by progressive recovery in spite of a sustained neurological damage. The ObRb expression exhibited a very high negative correlation (r= -0.9) with the MAR, but not not with the Min.Peri. The mRNA levels of other genes involved in leptin signaling was unaffected by CHI. Periosteal bone formation in the mid-femoral diaphysis was enhanced in almost all mice tested on day 4 after CHI. This was followed by a linear decrease in the number of such animals to control level by day 16 and was not related to ObRb expression. Endosteal bone formation was unaffected by CHI. Collectively, these data suggest an association between hypothalamic leptin signaling and TBI-induced enhancement in trabecular osteoblast activity. The involvement of other neuroendocrine systems, which regulate osteoblast number and periosteal bone formation, is also implied. The mouse model of CHI-induced increase in systemic bone formation constitutes a novel tool to study the central regulation of bone remodeling.

Disclosures: ***Y. Tam****, None.*

SU194

Osteoblasts Express Polycystin-1 and Polycystin-2. K. G. Oberman*, J. M. David*, D. C. Usher*, N. J. Karin. Biological Sciences, University of Delaware, Newark, DE, USA.

Autosomal dominant polycystic kidney disease (ADPKD) is a severe and common genetic disorder in humans caused by mutations in one of two genes, PKD1 or PKD2. ADPKD patients exhibit multiple fluid-filled cysts in their kidneys, frequently leading to end-stage renal failure. The physiological function of polycystin-1 (Pc-1) and polycystin-2 (Pc-2), the protein products of the PKD genes, is not clear in any tissue, however Pc-1 has similarities to cell adhesion molecules and Pc-2 is a cation channel with a high permeability to Ca2+. RT-PCR revealed that mRNA encoding polycystins is expressed in mouse MC3T3-E1 pre-osteoblastic cells and in primary rat calvarial osteoblasts. Quantitative RT-PCR measurements indicated elevated expression of both polycystins during in vitro differentiation of MC3T3-E1 cells. Pc-1 and Pc-2 transcripts were found in other mouse cells of mesenchymal origin, pre-adipocytes and C2C12 skeletal myoblasts, but developmentally-regulated expression was not detected. Sequence analysis revealed that MC3T3-E1 pre-osteoblasts also express a novel, alternatively spliced variant of the PKD2 gene product: a 108-base region of the Pc-2 mRNA encoding amino acids 117-152 is deleted in the pre-osteoblast transcript. The deleted region of the protein includes a consensus protein kinase C phosphorylation site. These data suggest a role for polycystins in osteoblast function and may be related to the skeletal deformities seen in PKD1-null mice [Boulter et al., PNAS 98:12174 (2001)].

Disclosures: ***N.J. Karin****, None.*

SU195

Molecular Nature of Functional Complexes of L-type Voltage-Sensitive Calcium Channels (VSCCs) in MC3T3-E1 Osteoblasts. Y. Shao*, J. J. Bergh*, K. Akanbi*, M. C. Farach-Carson. Biological sciences, University of Delaware, Newark, DE, USA.

Plasma membrane VSCCs serve as key regulators of Ca^{2+} permeability. In osteoblasts, the L-type VSCC consists of a pore forming α_1 subunit, a disulfide-linked $\alpha_2\delta$ dimer, an intracellular β subunit, but no γ subunit (Bergh et al, in press). Subunit composition plays an important role in fine-tuning of VSCC expression and function. Ten α_1, four β, and three $\alpha_2\delta$ subunits have been identified, giving 120 potential functional complexes even without a γ subunit. Previous studies in our laboratory have shown that the major functional α_1 subunit present in proliferating osteoblasts is the α_{1c} ($Ca_v1.2$). There are twelve functional complexes that could form with α_{1c} subunit alone. Additionally, a novel 700KDa phosphoprotein AHNAK was recently identified that interacts with β_2 subunits of $Ca_v1.2$ channels and links VSCCs to actin microfilaments via a region at the carboxyl-terminus of AHNAK. It is likely that AHNAK plays an important regulatory role linking VSCCs and the intracellular signaling pathways to a variety of protein kinases and actin-based cytoskeletal changes associated with Ca^{+2} signaling. We are using a combination of approaches to investigate exactly which auxiliary subunits form functional complexes with α_{1c} in MC3T3-E1 cells and in bone sections. RT-PCR with subunit specific primers, multi-photon confocal co-immunolocalization studies using subunit-specific antibodies, and co-immunoprecipitation assays allow us to detect and identify the $Ca_v1.2$ channel complexes. Immunostaining and RT-PCR results reveal that MC3T3-E1 cells express β_1, β_2, β_3, and $\alpha_2\delta_1$ subunits. β_4 subunits are expressed at very low levels. RT-PCR also demonstrated significant amounts of AHNAK mRNA present in MC3T3-E1 cells. Immunostaining with antibodies against the repetitive sequence in the central conserved domain confirmed the subcellular localization of AHNAK mostly along the plasma membrane of MC3T3-E1 cells, consistent with its ability to interact with β_2. Ongoing studies employ co-immunoprecipitation and Western blotting to identify the direct interaction between the α_{1c} subunit of L-type VSCCs and individual auxiliary subunits. Given the abundance and plasma membrane localization of the β_2 subunit in mouse osteoblasts, we are investigating the potential functional interactions between osteoblastic $\alpha_{1c,}$ the β_2 subunit and AHNAK. For the functional study of VSCCs, we have successfully designed the pU1RBα_{1c}6 ribozyme plasmid that specifically knocks down α_{1c} subunit expression and abolishes L-type depolarization. We will utilize the availability of these knockdown cells to determine if loss of α_{1c} disassembles complexes containing β_2, $\alpha_2\delta_1$ and AHNAK.

Disclosures: ***Y. Shao****, None.*

SU196

Uremic Serum Stimulates In Vitro Osteoblast Differentiation. A. Hufkens*, S. C. E. Verberckmoes*, M. E. De Broe, P. C. D'Haese*. Nephrology - Hypertension, University of Antwerp, Wilrijk, Belgium.

As a consequence of decreased phosphate excretion, disturbed parathyroid hormone (PTH) and vitamin D metabolism, end-stage renal failure patients may develop secondary hyperparathyroidism; a particular form of renal osteodystrophy, characterized by an increased bone turnover, resulting from an increased osteoblastic/osteoclastic activity. It is now well recognized that PTH plays a key role in this cellular response. However, to which extent other factors may trigger the increased bone cellular activity also, is not well known yet.

In the present study, an in vitro experiment, using primary osteoblasts, isolated from 20 day old fetal rat calvaria, was set up. At confluence, the cultures were divided into two groups that were further grown up with α-MEM medium supplemented with 50 µg/ml ascorbic acid, 2 mM β-glycerophosphate and either 10% heat inactivated human uremic serum (collected from patients prior to dialysis, N = 25) or 10% heat inactivated human normal serum (collected from age and gender-matched healthy volunteers, N = 9). The culture medium was changed every 2-3 days until 14 days post confluence. Alkaline phosphatase (ALP) activity, used as a marker of osteoblastic activity/differentiation was measured in the conditioned medium at the time points of medium refreshments. Fourteen days post confluence cells were von Kossa stained to identify the presence of calcified nodules. Number and size of nodules were counted by computer aided image analysis. Cellular samples were taken to determine total DNA-content. Data were then expressed per amount of DNA to correct for possible differences in cell number. Total nodular Ca-deposition was determined in a 0.6M HCl extract of the cultures. Uremic and normal sera were analysed for the most relevant biochemical parameters such as PTH, ALP-activity, urea and electrolytes.

Area under the time curve (AUC) of ALP activity and total Ca-deposition were significantly higher in the uremic cell cultures vs. those grown in normal serum (AUC-ALP: 132.5 ± 22.2 vs. 61.3 ± 26.6 U/l; Ca-deposition: 139.3 ± 20.5 vs. 40.2 ± 27.8 µg Ca/well; $p \leq 0.05$). Quantification of the nodules revealed a significantly increased amount of nodules (number and size) in the uremic cultures vs. the normal serum group (number: 19.6 ± 2.3 vs. 12.8 ± 0.6 nodules/cm2; size: 209 . 10^3 ± 19 . 10^3 vs. 130 . 10^3 ± 24 . 10^3; $p \leq 0.05$).

These results allow us to suggest that an uremic environment stimulates the osteoblastic differentiation. Results obtained in the present pilot study are further investigated in detail using advanced techniques such as RT-PCR and proteomics.

Disclosures: ***S.C.E. Verberckmoes****, None.*

SU197

A Threshold Level of Hyaluronic Acid Is Critical for Osteoblast Differentiation: Insights into the Leukemia Inhibitory Factor-Mediated Arrest of Osteoprogenitor Cells Differentiation. D. Falconi, J. E. Aubin. Department of Medical Genetics and Microbiology, University of Toronto, Toronto, ON, Canada.

We have previously shown, using the rat calvaria (RC) cell culture system, that the gp130 family cytokine leukemia inhibitory factor (LIF) blocks osteoblast development in a differentiation stage-specific manner. This effect is also seen *in vivo* when neonatal rats receive sub-cutaneous injections of LIF above the calvaria, leading to dose-dependant widening of the sagittal suture. Using a differential display approach, we identified genes modified by LIF treatment of RC cells and found that hyaluronic acid synthase 2 (HAS2), an enzyme responsible for the synthesis and secretion of high molecular weight hyaluronic acid (HA) molecules, was up-regulated by LIF specifically during the osteoprogenitor inhibition-sensitive time window. Here we report the results of studies assessing the role of the HA/HAS2 system in osteoblast differentiation and the LIF-mediated arrest of osteoblast differentiation. Histochemical analyses with a biotinylated-HA binding protein (HABP) revealed that, in control-treated cells, high HA expression is restricted to the cells at the periphery of developing bone nodules, i.e. osteoblast precursor cells. However, in LIF-treated cultures, HA is expressed highly by all the cells within the few, immature bone nodules that formed, consistent with our previous finding that LIF blocks osteoblast differentiation at the late osteoprogenitor stage. Increasing the concentration of HA in the culture medium, either by adding exogenous HA or by overexpressing HAS2, lead to a dose-dependant decrease in bone nodule numbers and, importantly, the culture time for the HA-associated inhibition of bone nodule formation overlaps with the LIF-associated inhibition. Notably, however, decreasing the concentration of HA through the use of antisense oligonucleotides or hyaluronate lyase did not lead to an increase in bone nodule number or a reversal of the LIF effect. Instead, these treatments produced a decrease in bone nodule numbers in control and LIF-treated cells. Taken together, these results indicate that critical or threshold levels of HA are important for osteoblast differentiation and bone formation and that LIF abrogates the differentiation of osteoprogenitor cells by increasing the levels of HA through up-regulation of HAS2 expression.

Disclosures: ***D. Falconi****, None.*

SU198

Annexin 2 and Lipid Raft Involvement in Osteoblastic Mineralization. J. M. Gillette[1], R. Globus[2], S. M. Nielsen-Preiss[3]. [1]Cellular and Developmental Biology, University of Colorado Health Sciences Center, Denver, CO, USA, [2]Gravitational Research Branch, NASA Ames Research Center, Moffett Field, CA, USA, [3]Orthopaedics, University of Colorado Health Sciences Center, Denver, CO, USA.

The experiments described herein were designed to elucidate the roles of lipid rafts and annexin 2 in the process of mineralization. Lipid rafts function to organize membranes into a series of discrete microdomains to facilitate protein interactions. Recently, our laboratory has established annexin 2, a cytoplasmic and membrane-associated calcium-binding protein, as a facilitator of osteoblastic mineralization, through a potentially novel mechanism. Annexin 2 overexpression in SaOSLM2 cells resulted in a two-fold increase in alkaline phosphatase activity and enhanced mineralization by SaOSLM2 cells. Futhermore, annexin 2 and alkaline phosphatase were localized to osteoblastic membrane microstructures (lipid rafts) which are cholesterol-mediated and triton X-100 insoluble. In separate experiments lipid rafts were isolated at days 3 and 12 during differention from primary fetal rat calvarial cells which were isolated by collagenase digestion. Lipid rafts were then assayed for annexin 2 and alkaline phosphatase activity. Prior to the onset of mineralization (day 3), annexin 2 was not detected in lipid rafts, although annexin 2 expression was present in a total cell lysate throughout the mineralization process. However, following the two weeks of differentiation, annexin 2 and alkaline phosphatase activity were co-localized to lipid rafts. Thus, the translocation of annexin 2 to lipid rafts in primary osteoblasts coincided with an increase in alkaline phosphatase activity during differentiation of primary osteoblasts. Together, our results support the hypothesis that lipid rafts provide a physical entity for the temporal and spatial regulation of proteins required to initiate mineralization.

Disclosures: ***J.M. Gillette****, None.*

SU199

Osteoactivin-Derived Peptides Induce Osteoblast Differentiation in MC3T3-E1 Cells. A. Selim*[1], J. L. Castaneda*[1], T. A. Owen[2], S. N. Popoff[1], F. F. Safadi[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.

We previously identified novel gene called osteoactivin (OA) in bone. OA was identified by differential display using total RNA from wild type compared to osteopetrotic (op) long bone and calvaria. In this study, we examined the role of OA in osteoblast differentiation in vitro using two anti-OA antibodies: anti-OA antibody 27 (Ab-27) and anti-OA 551 (Ab-551). These antibodies were raised against different regions of the molecule; Ab-27 was raised against the N-terminus and Ab-551 was raised against the C-terminus, a sequence that contains an RGD motif. We found that only Ab-551 significantly decreased osteoblast differentiation including, alkaline phosphatase activity, nodule formation and matrix mineralization. In order to test the role of the RGD motif of OA protein in osteoblast differentiation, we designed two peptides that mimic the sequence of the OA peptide used to generate Ab-551. The first peptide (OA-D) has the RGD domain and the second peptide (OA-E) has E (Glutamic acid) in the place of D (Aspartic acid). We examined the

effect of these two peptides on osteoblast proliferation and differentiation in vitro. Although both peptides had no significant effect on osteoblast proliferation and/or viability, they significantly induced alkaline phosphatase activity, nodule formation and calcium deposition. Bioinformatic analysis of these peptides showed the presence of a serine residue that is potentially phosphorylated by casein kinase II (CK-II). Further analysis of other OA protein family members showed that there is conserved serine residue close to C-terminus, which matches the position of serine residue of the OA peptides. CK-II is known to phosphorylates many osteoblast-related proteins that regulate osteoblast development and differentiation such as osteopontin and vitronectin. Collectively, these data show that both OA-D and OA-E peptides significantly induced osteoblast differentiation in vitro and the effect of these peptides is RGD independent. Additional studies are warranted to determine if phosphorylation of the OA peptides by CK-II might be involved in their mechanism of action during osteoblast differentiation.

Disclosures: ***A. Selim****, None.*

SU200

Enhanced Bone Growth in Transgenic Mice Overexpressing Osteocrin, a Novel Secreted Bone Protein. P. Moffatt, F. Lafreniere*, M. Bessette*, K. Sellin*, E. Godin*, M. Gaumond*, C. Lanctot*, G. P. Thomas. Phenogene Therapeutiques, Montreal, PQ, Canada.

Previously we reported the identification of a novel bone protein, PGTI0306. Due to its highly bone specific expression pattern and putative pro-hormone like processing we have now termed the molecule "osteocrin". Osteocrin is a 103aa-secreted protein with two conserved putative dibasic cleavage sites reminiscent of prohormones. Highly expressed in osteoblasts, osteocrin is down regulated in aged bone and *in vitro* is associated with matrix production. Treatment with $1,25(OH)_2D_3$ down regulates osteocrin in a rapid dose-dependent manner further suggesting skeletal functionality.

To assess the role of osteocrin in bone we generated transgenic mice specifically expressing osteocrin in osteoblasts using 3.6Kb of the rat collagen type I promoter. Analysis by Northern blot demonstrated bone-specific transgene expression in three transgenic lines. In all transgenic lines, long bones were on average 7-10% longer than in wild-type littermates ($P<0.001$). The tails of the osteocrin transgenic mice were also significantly longer (10-13%, $p<0.001$) and the mice exhibited a marked kyphosis. DEXA analysis showed no differences in either BMD or BMC and there was no change in body weight. The increased bone length was apparent at 8 weeks of age and was maintained at 8 months of age suggesting a developmental or growth phenomenon.

Bone marrow stromal cultures from osteocrin transgenic mice expressed high levels of osteocrin protein from approximately day 8 in culture which increased when the cultures were differentiated with dexamethasone. Alkaline phosphatase and osteocalcin expression was significantly lower in osteocrin transgenic marrow cultures than in wildtype cultures. This correlates with our previous report of inhibition of alkaline phosphatase, osteocalcin and mineralisation in primary rat calvarial cultures chronically treated with osteocrin-conditioned media.

These data demonstrate osteocrin as an important anabolic factor in osteogenic regulation. Osteocrin may act directly on osteoblasts or indirectly via chondrocytes to stimulate bone growth. Continuing histological and histomorphometric analyses will further elucidate the mechanism underlying the elevated bone growth in these mice.

Disclosures: ***G.P. Thomas****, None.*

SU201

Direct Effect of an Angiogenesis Inhibitor on Osteoblast-like Cells and Bone Marrow Stromal Cells In Vitro. R. J. Majeska, M. B. Schaffler, M. R. Hausman*. Orthopaedics, Mount Sinai School of Medicine, New York, NY, USA.

The angiogenesis inhibitor TNP-470, which inhibits endothelial cell proliferation in vitro, impairs bone and cartilage formation in vivo. The purpose of this study was to test in vitro whether TNP-470 might also act directly on osteoblastic cells or their progenitors. Cultures of human endothelial cells (HUVEC), osteoblast-like cell lines (ROS 17/2.8, MC3T3) and mouse calvarial cells were plated at 0.5 - 1 x104 cells/cm2 , then treated with TNP-470 (25 pM - 0.25 mM). Cell growth and alkaline phosphatase (ALP) activity were measured at selected times between 4-14 days. Primary mouse bone marrow cell cultures were also tested for ALP+ colony formation in the presence of TNP-470 . TNP-470 inhibited HUVEC growth (30 - 50%, $p<0.05$) at concentrations as low as 0.025 nM. TNP-470 also inhibited growth of ROS 17/2.8, MC3T3 and mouse calvaria cells to a similar extent, but at higher concentrations (0.25 - 2.5 nM). TNP-470 inhibition of osteoblastic cell growth was accompanied by inhibition of ALP activity. TNP-470 at 2.5 nM and 25 nM reduced the number of colonies formed by mouse marrow stromal cells by 50%, but had no effect on the % of ALP+ colonies. These findings support the concept that TNP-470 inhibition of cell proliferation is selective for vascular cells; however, proliferation of osteoblastic cells and their progenitors is also inhibited by TNP-470 albeit at higher concentrations. Whether the reduction in osteoblastic cell ALP activity is associated with lower cell growth is not clear; however, colony formation data indicate that TNP-470 does not appear to inhibit the growth of ALP+ and ALP- cells differentially. Thus TNP-470 can act directly to inhibit bone cell growth and function, but these effects are not likely to play a substantial role in the dramatic inhibition of skeletal development and repair produced by TNP-470 in vivo.

Disclosures: ***R.J. Majeska****, None.*

SU202

Connective Tissue Growth Factor (CTGF) Expression During Diabetic Wound Healing in a Tooth Extraction Model. R. A. Aswad*[1], M. C. Rico*[1], R. A. Kanaan*[1], H. Devlin*[2], F. F. Safadi[1], S. N. Popoff[1]. [1]Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA, [2]Restorative Dentistry, Temple University School of Dentistry, Philadelphia, PA, USA.

Delayed healing and infection often complicate healing of the tooth extraction socket in patients with diabetes mellitus. Streptozotocin, the diabetogenic drug of choice, produces in rats an elevated blood basal plasma glucose level and an impaired insulin response to glucose injections. The molar extraction socket of rats treated with this drug exhibits delayed healing with increased bone resorption. In calvarial defect, healing of streptozotocin-treated rats, exuberant formation of primitive bone was observed. It was concluded that uncontrolled diabetes exerts a direct influence on bone healing by inhibiting mineralization and remodeling. CTGF is a secreted, extracellular matrix-associated protein that regulates diverse cellular functions. CTGF mRNA and protein production has been demonstrated in multiple cell types including; fibroblasts, endothelial cells, osteoblasts and mesangial cells. CTGF has also been shown to be up-regulated in diabetic nephropathy. In this study, we examined CTGF expression in socket following tooth extraction in streptozotocin- treated and normal rats. Three and five days following extraction, CTGF was maximally expressed in fibroblasts, endothelial cells and osteoblast progenitors in sockets from diabetic rats when compared to normal. This expression level was decreased by 7 and 10 days in diabetic rats but not in the normal rats. These data suggest that CTGF plays a role in wound healing and is abnormally expressed in diabetic wounds. We next examined whether CTGF expression in osteoblasts is altered in response to glucose treatment. MC3T3-E1 cells were treated with different doses of glucose and examined for their proliferation and differentiation as well as CTGF expression. Glucose-treated cells showed an increase in cell proliferation, and decreased alkaline phosphatase expression and calcium deposition. CTGF expression determined by RT-PCR was increased in response to glucose treatment. These data suggest that glucose modulates osteoblast differentiation and this could be mediated through CTGF. Future studies are warranted to evaluate the mechanism by which glucose alters osteoblast differentiation by CTGF is yet to be determined.

Disclosures: ***R.A. Aswad****, None.*

SU203

Osmoadaptation Impairs Osteoblast Differentiation. L. R. McCabe, S. Botolin*. Physiology, Michigan State University, East Lansing, MI, USA.

Diabetes Mellitus type I is often accompanied with complications including bone loss and increased fracture rate. We hypothesize that osteoblast osmoadaptation to increased blood glucose and its associated increase in blood osmolarity results in impaired osteoblast function, ultimately contributing to the development of osteoporosis. To investigate this hypothesis we have taken *in vitro* and *in vivo* approaches. To examine in vitro effects, MC3T3-E1 osteoblasts were exposed to hyperosmotic media (320 mOsm) for short (1 hour) and long (1-7 days) periods of time. Exposure of osteoblasts to acute hyperosmotic stress was associated with significant changes in gene expression, cell signaling, cell shape and mechanisms necessary for osmoadaptation, such as connexin 43 and HSP27 expression. Here we show that during the process of long term osmoadaptation osteoblast expression of RUNX2 and osteocalcin, markers of osteoblast differentiation, are significantly suppressed. Expression of both markers decreased to 50-30% of control levels. Mannitol treatment, an osmotic control, caused the greatest suppression. To address *in vivo* effects of diabetes on osteoblast osmoadaptation and gene expression we developed a mouse model of long-term diabetes (4-6 weeks). We measured blood glucose and osmolarity and found an increase in both, by greater than 20 mM and 20 mOsm, respectively. Bone histomorphometry on these animals demonstrated significant changes. These *in vivo* and *in vitro* findings support our hypothesis that poorly controlled diabetes accompanied with increased blood osmolarity and glucose levels can contribute to the development of osteoporosis as a consequence of osteoblast dysfunction.

Disclosures: ***S. Botolin****, None.*

SU204

Osteoblast Lineage Differentiation Potential in the OIM Mice. I. Kalajzic*, X. Jiang*, K. Mack*, J. Delaney*, D. Rowe. Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA.

Osteogenesis imperfecta murine (OIM) is a mouse model that reassembles severe type III human OI. Recent studies suggested two pathophysiological mechanisms for the bone disease. The first is a state of high bone turnover secondary to the accumulation of defective bone matrix as a consequence of the underlying molecular mutation within the COL1A1 or Col1A2 genes. Second is an impairment of full osteoblastic differentiation in cells carrying an OI mutation. The combination of the two effects leads to diminished bone mass because bone formation cannot keep pace with bone resorption. We have previously developed mice transgenic for pOBCol3.6GFP and pOBCol2.3GFP and have shown that they can be used as visual markers of preosteoblast, early osteoblast and mature osteoblast differentiation. This study was designed to assess the validity of these two mechanisms using the visual transgenes that were breed into OIM mice. The analysis was performed on MSF cultures derived from the OIM/GFP mice using repetitive imaging for GFP expression of the same culture plate throughout the culture period from which the tempo and magnitude of lineage differentiation can be assessed. Cultures from the 2.3 oim/oim mice show a marked impairment in the number of cells with GFP expression relative to 2.3 +/+ litermates. The 3.6 oim/oim cultures showed a similar number of low intensity GFP+ cells (preosteoblasts) but an impaired number of high expressing GFP cells (early osteoblasts). Conventional markers of osteoblast differentiation confirmed the GFP data. Decreased AP

histochemical activity and decreased expression of bone markers (Col1a1, BSP, OC) and mineralization became apparent in the oim/oim mice after 12 day of culture. Frozen decalcified sections of bone were prepared using the CryoJane tape system and the entire bone area was imaged and reconstructed as an intact bone section. The bone from the 3.6 oim/oim mice showed a dramatic increase in the number and strength of GFP positive cells lining the endosteal, periosteal and trabecular surfaces compared to 3.6 +/+ mice. In contrast GFP expression was similar between 2.3 oim/oim and 2.3 +/+ mice. This data indicates that the lineage is under continuous stimulation that could result in premature senescence. Currently we are testing that possibility by evaluating the capacity of the osteoprogenitor lineage to generate mature osteoblasts with advancinmg age and by mechanical loading of aged GFP oim/+ mice.

Disclosures: ***I. Kalajzic****, None.*

SU205

Effects of Extracellular Matrix Proteins on Bone Differentiation around Titan Implants. C. S. Berntsen*, E. A. Riksen*, H. S. Berner*, J. E. Reseland*, I. Slaby*, D. Dutch*, J. E. E. Ellingsen*, S. P. Lyngstadaas*. Faculty of Dentistry, University of Oslo, Oslo, Norway.

In the enamel matrix amelogenin is the main component, comprising approximately 90% of all proteins secreted by ameloblasts. The others are amelin, enamelin, serum proteins, tuftelin and enzymes. Amelogenin in the form of Emdogain® (EMD) is a formulation of pig amelogenin proteins in polyglycol alginate. Amelogenin and amelin are involved in biomineralization of dental hard tissues, secreted by ameloblasts and odontoblasts during tooth formation. The purposes of these studies were to investigate the effects of extracellular matrix proteins; EMD, rAmelogenin and rAmelin, on bone growth on titanium implants. Bone growth was studied in new zealand white adult female rabbits. Flat coin shaped implants are placed onto calibrated leveled defects made in the cortical bone of tibia. Proteins were applied on the implant site immediately before placement of implants. After eight weeks animals were sacrificed, implants exposed and tibia immediately placed in a pull-out test machine. During the pull out test implants were stressed with an increasing force perpendicular to the test surface, at constant speed, until detaching from the bone. A load versus time plot was recorded. After implant detachment, bone fluid from the implant site was collected for LDH activity analysis. Effect of matrix proteins on differentiation, proliferation and cell death were tested on osteoblasts (MC3T3) and osteosarcomas (SaOs) , cultured on plastic, glass and titanium. The *in vivo* results demonstrated an positive effect of rAmelin, EMD, and to some extend rAmelogenin on bonding forces as compared to control. rAmelogenin had a 20% increased cytotoxicity, measured as LDH activity in bone fluid, whereas EMD and rAmelin were not different from control. We demonstrated that rAmelogenin and rAmelin were actively taken up by cultured osteoblasts**.** No significant effect on proliferation of osteoblasts was observed by rAmelin and rAmelogenin. EMD, and to less extend rAmelogenin, enhanced differentiation as monitored by increased osteocalcin secretion to the media, whereas rAmelin had no significant effect. rAmelin significantly induced secretion of IL-6 to the media, whereas rAmelogenin had no significant effect. IL-6 may upregulate the activity of osteoclasts, and thus affect bone turnover. Pre-treatment of titanium implants with extracellular matrices proteins may be a strategy for promoting bone growth and inducing differentiation of osteoblasts.

Disclosures: ***C.S. Berntsen****, None.*

SU206

A Proton-Sensing G Protein-Coupled Receptor Expressed in Osteoblast-like Cells. M. Ludwig*, M. Vanek*, C. E. Jones*, U. Junker*, H. Hofstetter*, R. M. Wolf*, K. Seuwen*. Bone Metabolism, Novartis Institutes for Biomedical Research, Basel, Switzerland.

G protein-coupled receptors (GPCRs) represent the largest gene family in the human genome, with more than 800 members. These receptors respond to a variety of ligands including small molecule neurotransmitters, peptides, large proteins, lipids, but also calcium and photons. For many GPCRs ligands are still unknown. Working with recombinant cell systems expressing a specific orphan receptor (R412), we observed a strong apparent basal activity of the phosphoinositide signalling system which was not modulated by known GPCR ligands. Further experiments showed that the measured signal was strongly pH-dependent, close to zero at pH 7.8, maximal at pH 6.8, and independent of other assay buffer constituents. Activation at slightly acidic pH was strong, comparable to activation of other GPCRs by their cognate ligands. Inspecting the putative secondary structure of R412 we observed specific histidines at the extracellular surface of the receptor, that were most likely involved in pH sensing. Site-directed mutagenesis confirmed this hypothesis. The skeleton participates in pH homeostasis and osteoblasts were shown to respond to pH changes in the range of pH 6.8 – 7.4. We detected expression of mRNA for R412 in MG63 osteosarcoma cells and in primary human osteoblast precursors isolated from trabecular bone. These cells showed strong pH-dependent inositol phosphate formation matching that observed in fibroblasts expressing ectopic R412. Our data suggest that R412 is a proton-sensing receptor involved in pH homeostasis and bone metabolism.

Disclosures: ***M. ludwig****, None.*

SU207

Role of Alternative Signaling Pathways in the Activation of a Cyclic AMP Response Element (CRE)-Luciferase Reporter System in an Osteoblast-like Cell Line. R. J. Murrills, B. M. Bhat, J. L. Andrews*, R. L. Rupert*, V. E. Coleburn*, F. J. Bex. Women's Health and Bone, Wyeth Research, Collegeville, PA, USA.

Parathyroid hormone (PTH) is known to activate both cAMP and calcium/PLC/PKC signaling pathways following binding to the PTH1 receptor in osteoblasts. In addition, the cAMP pathway can also activate the MAPK pathway. Previous work has shown that a discrepancy can exist between the potency of a truncated PTH peptide in stimulating cAMP and its activity on a CRE-Luciferase reporter, suggesting that other signaling pathways in addition to cAMP may be involved in the activation of the CRE. There is evidence, for example, from other systems that the PKC, calcium and MAPK pathways can each phosphorylate CREB and potentially activate CREs. We have constructed a CRE-Luciferase reporter containing multiple copies of the CRE and stably transfected it into the osteoblast cell line Saos-2. We then tested the ability of modulators of alternative pathways to either activate the CRE or block the PTH-induced activation of the CRE.
Forskolin, an adenylate cyclase activator, and the phosphodiesterase inhibitors IBMX and rolipram each activated the reporter, confirming the role of cAMP in the activation of the CRE. In addition, the protein kinase A (PKA) inhibitor H-89 blocked the effect of PTH in activating the CRE-reporter. Interestingly, a MAPK inhibitor PD-98059, which blocks a pathway outside of the main cAMP/PKA pathway, also partially inhibited the activity of PTH on the CRE reporter. Furthermore, phorbol myristate acetate, an activator of PKC, was capable of inducing a significant increase in CRE-Luciferase reporter activity, implicating the PKC pathway as an additional potential activator of the CRE. A23187, a calcium ionophore, had only a small effect on the reporter, which was not significant, while the calmodulin kinase II inhibitor KN-93 could not significantly block the effect of PTH.
We conclude that, in addition to the cAMP/PKA pathway, the PKC and MAPK pathways may also play a role in activating the CRE in this osteoblast-like system.

Disclosures: ***R.J. Murrills****, None.*

SU208

Stimulation of IL-6 Expression by TNFα in Osteoblast Does Not Depend on p38 Activation. J. C. Dai, X. Chen, E. M. Greenfield. Orthopaedics, Case Western Reserve University, Cleveland, OH, USA.

We have previously shown that TNFα stimulates biphasic expression of IL-6 mRNA and IL-6 protein in MC3T3-E1 osteoblastic cells (JBMR 17:S327, 2002). Thus, both IL-6 mRNA and protein are rapidly induced during the early phase of stimulation by TNFα (0-2 hours) followed by a period of declining IL-6 mRNA levels and little IL-6 protein secretion despite the continuous presence of TNFα. A late phase of increased IL-6 mRNA and protein expression occurs 12-24 hours after stimulation by TNFα, leading to a 5-10 fold increase in IL-6 protein secretion. Other investigators have shown that p38 MAP kinase is activated by TNFα in osteoblasts and that p38 inhibitors partially inhibit stimulation of IL-6 mRNA by TNFα. We therefore examined whether the early or late phases of stimulation by TNFα, or both, depend on activation of p38. Western blotting showed that p38 phosphorylation was rapidly stimulated by TNFα with maximal phosphorylation detected after 5 minutes of exposure. p38 phosphorylation slowly declined to a nadir at 8-12 hours and, then, increased at 20-24 hours. This biphasic pattern of p38 phosphorylation is similar to the biphasic expression of IL-6 induced by TNFα. Since the commonly used inhibitors of p38 have recently been shown to have potent non-specific effects, we used three analogues to determine whether p38 activation is responsible for stimulation of IL-6 expression by TNFα: SB202190, one of the commonly used inhibitors of p38; SB220025, a newly described p38 inhibitor that is more specific; and SB202474, an inactive analogue. As expected, both p38 inhibitors completely blocked the cellular phosphorylation of MAPKAPK-2, a prominent p38 substrate, during both the early and late phases of stimulation by TNFα, while the inactive analogue had no detectable effect. Despite their similar effects on p38 activity, the two p38 inhibitors had divergent effects on TNFα-induced IL-6 expression. ELISA measurements showed that SB202190 strongly inhibited IL-6 secretion by 88% ($p<0.0002$) in the early phase and by 71% ($p<0.001$) in the late phase. In contrast, SB220025 and the inactive analogue had indistinguishable effects ($p>0.5$) in the early phase and SB220025 increased IL-6 secretion by 78% ($p<0.001$) during the late phase. Similar effects were also observed at the mRNA level. Since SB220025 inhibited TNFα-induced p38 activity without inhibiting expression of IL-6 mRNA or IL-6 protein, activation of p38 is not required for stimulation of IL-6 expression in either the early or late phases. Moreover, since SB220025 increased IL-6 expression during the late phase of stimulation by TNFα but not during the early phase, p38 activity may downregulate IL-6 expression specifically during the late phase.

Disclosures: ***J.C. Dai****, None.*

SU209

Parathyroid Hormone (PTH)-Induced Ca^{2+} Signaling in Osteoblasts is Modulated by the Level of PTH1R Expression. B. J. Votta, A. M. Rodriguez-Rojas*, S. M. Hwang*, D. J. Rickard, M. E. Nuttall, S. M. Blake, S. Kumar. MusculoSkeletal Diseases, GlaxoSmithKline, King of Prussia, PA, USA.

In osteoblasts PTH binds to the type 1 PTH receptor (PTH1R) and subsequently activates both the Gs/adenylate cyclase/cAMP/PKA and the Gq/Gi/PLC/IP3/Ca signaling pathways. The relative importance of these different signaling pathways in mediating the net physiological effects of PTH on bone remodeling remains unclear. In HEK293 cells stably expressing high levels of the human PTH1R, we have observed robust signaling via both the cAMP and $[Ca^{2+}]_i$ pathways in response to PTH[1-34]. In contrast, in SaOS-2,

ROS 17/2.8, and primary rat osteoblasts expressing lower levels of the PTH1R, PTH[1-34] consistently elicited robust cAMP responses, but effects on $[Ca^{2+}]_i$ were barely detectable. In order to investigate the effect of the level of PTH1R expression on $[Ca^{2+}]_i$ signaling SaOS-2 cells were transiently transfected with increasing amounts of a human PTH1R-enhanced green fluorescent protein (GFP) expression construct. The relative levels of PTH1R expression following transfection were quantitated using a ^{125}I-PTH[1-34] radioligand binding assay. Gs-mediated signaling was monitored by assessing cAMP production (ELISA) in response to PTH[1-34] in the presence of IBMX. Gq/Gi-mediated signaling was monitored by assessing changes in $[Ca^{2+}]_i$ in Fura-2 loaded cells using a FlexStation. Cells transfected with the GFP-vector alone exhibited a transfection efficiency similar to those transfected with the PTH1R-GFP construct, but showed no increases in PTH receptor number, or PTH-stimulated cAMP or $[Ca^{2+}]_i$ compared to untransfected cells. Forty-eight hours following transfection with the PTH1R-GFP construct, SaOS-2 cells exhibited a 4- to 10-fold increase in receptor number. A similar increase in the maximal cAMP response to PTH[1-34], but not to Forskolin, was also observed. Interestingly, a corresponding increase in the magnitude of the $[Ca^{2+}]_i$ response was also observed indicating that efficient coupling of PTH1R to Gq/Gi, and hence PLC/$[Ca^{2+}]_i$, may require higher levels of receptor expression than does coupling to Gs. These data suggest that in addition to the mechanisms already described the level of PTH1R expression may qualitatively and quantitatively modulate PTH responsiveness in osteoblast-like cells.

Disclosures: ***B.J. Votta****, None.*

SU210

In Osteoblastic Cells, the JNK Pathway Mediates Cell Proliferation by Mitogens and Is a Negative Regulator of Early Differentiation. J. Caverzasio, J. Lemonnier*, C. Ghayor*. Dept of Geriatrics, Division of Bone Diseases, Geneva, Switzerland.

Mitogen-activated protein kinase (MAPK) cascades are central signalling transduction pathways induced by mitogens and stresses. At least four MAPK cascades exist, ERK, p38, JNK and ERK5. Recent studies indicate that various receptor systems can induce activation of the JNK (c-Jun N-terminal Kinase) cascade in mammalian cells including G protein-coupled receptors, BMP receptors as well as the Wnt/LRP receptors. In osteoblastic cells, recent studies suggest that the ERK pathway is implicated in mediating cell proliferation by mitogens whereas the p38 pathway is involved in regulating expression of alkaline phosphatase in response to various growth factors. The role of the JNK pathway in osteoblastic cell regulation is not known and was investigated in the present study. We previously shown that JNK is activated by serum growth factors (SGFs) in MC3T3-E1 cells and recently observed a similar response in calvaria-derived osteoblastic cells. The role of JNK in mediating cell proliferation and differentiation by SGFs was studied in MC3T3-E1 cells using the selective SP600125 JNK inhibitor. We found that incubation of preconfluent cells with this inhibitor dose-dependently (10-25 microM) reduced DNA synthesis and cell number with a complete inhibitory effect at 25 microM. This dose of SP600125 had no effect on activation of ERK induced by SGFs. In early differentiating cells, treatment of cells with SP600125 surprisingly induced a time- and dose-related increase in alkaline phosphatase activity. With 20 microM SP600125, a significant effect was already observed after 48 h incubation with a pronounced and maximal effect after 5 days treatment (4 fold stimulation). This effect of SP600125 was associated with a significant increase in SGFs-induced activation of p38, a MAP kinase pathway that we previously found to be required for expression of ALP in MC3T3-E1 osteoblastic cells.
In conclusion, results presented in this study indicate that JNK plays an essential role in mediating cell proliferation by growth factors in osteoblastic cells. They also suggest that JNK is a negative regulator of alkaline phosphatase expression, an effect probably mediated by a yet unknown negative cross regulation of JNK on the p38 pathway. Taken together these data suggest that the JNK pathway is an essential signalling pathway for controlling the proliferation and early differentiation of osteoblastic cells.

Disclosures: ***J. Caverzasio****, None.*

SU211

Protein Kinase D Is an Essential Signalling Molecule for the Regulation of Osteoblastic Cell Growth and Differentiation. C. Ghayor*, J. Lemonnier*, J. Caverzasio. Dept of Geriatrics, Division of Bone Diseases, Geneva, Switzerland.

The development of osteoblasts is dependent of yet incompletely understood signalling cascades that support proliferation and differentiation. We recently described that BMP-2 induces activation of the MAP kinases p38 and JNK and that both pathways are implicated in BMP-2-induced osteoblastic cell differentiation. More recently, we provided compelling evidences that activation of p38 and JNK by BMP-2 involves protein kinase D (PKD).
In the present study, we investigated the role of this new PKD/JNK-p38 pathway in mediating osteoblastic cell proliferation and differentiation by serum growth factors (SGFs). Exposure of unstimulated MC3T3-E1 cells to 10% FCS induced a rapid and transient activation of PKD with maximal activation/phosphorylation of PKD on Ser744/748 and Ser916 after 1 h incubation. Associated with this effect, there was a corresponding transient and maximal activation of JNK and p38. This latter effect was completely blocked by the selective PKD inhibitor G06976 (10 microM). We found that this new signalling pathway is also activated by SGFs in cultured mouse calvaria-derived osteoblastic cells with similar kinetic and potency of activation compared with that found in MC3T3-E1 cells. The role of PKD in mediating cell growth and differentiation was then analyzed in two clones of MC3T3-E1 cells stably expressing PKD antisense oligonucleotide (AS-PKD) and having a selective and markedly reduced expression of PKD compared with vector transfected cells (V-PKD). Results indicate that the size of AS-PKD growing cells appeared to be increased and cell number significantly reduced by 50 to 60 % compared with V-PKD cells ($p<0.001$). In confluent AS-PKD cells, expression of markers of osteoblastic differentiation such as collagen I, alkaline phosphatase and osteocalcin were significantly ($p<0.001$) and respectively reduced by 40-50%, 60-70% and 70-80% compared with V-PKD cells. Associated with this effect, activation of JNK and p38 was nearly completely blunted in AS-PKD compared with V-PKD cells.
In conclusion, data presented in this study indicate that PKD is activated by serum growth factors in MC3T3-E1 and primary cultured calvaria-derived osteoblastic cells and that this signalling molecule is involved in regulating osteoblastic cell proliferation. They also suggest that PKD mediates activation of JNK and p38 and that this pathway is essential for the differentiation of osteoblastic cells. Together, these observations strongly suggest that PKD is an important signalling molecule for the regulation of the growth and differentiation of osteoblastic cells.

Disclosures: ***J. Caverzasio****, None.*

SU212

Bisphosphonates Affect Signaling Pathways Utilized by Teriparatide [rhPTH(1-34)]. Q. Sun*[1], R. W. Katz[2], S. A. Morris[3], J. P. Bilezikian[1]. [1]Division of Endocrinology, College of Physicians and Surgeons, Columbia University, New York, NY, USA, [2]School of Oral and Dental Surgery, Columbia University, New York, NY, USA, [3]Aventis Pharmaceuticals, Bridgewater, NJ, USA.

There are questions about the antecedant use of bisphosphonates (BP) on the subsequent effects of teriparatide [PTH(1-34)] for the treatment of osteoporosis. This clinical question requires a more basic understanding of how various BP may influence PTH-dpendent signal transduction pathways, but also the reversibility of these effects. In this study, we compared the effect of Alendronate (ALE) or Risedronate (RIS) on the subsequent ability of PTH to stimulate adenylyl cyclase activity and inositol phosphate accumulation.
When a kidney cell line stably transfected with PTH type 1 receptor was exposed to either ALE or RIS (10 pM - 1 mM), maximal PTH dependent inositol phosphate formation was suppressed by ~30-50% at a 0.1 mM concentration. Suppression was noted with 60 minutes of pretreatment; longer incubations did not increase or reduce suppression. Recovery from BP suppression was evaluated by removal of BP medium prior to PTH stimulation. After 24-48 hours of bisphosphonate exposure, the suppression of PTH inositol phosphate accumulation was completely reversible within two hours. Preliminary studies show that ALE caused more suppression than RIS at equivalent concentrations and that the recovery was faster with RIS than with ALE.
SAOS2 human osteosarcoma cells were exposed to graded concentrations of ALE or RIS (10 pM to 1 mM) for one hour and adenylyl cyclase activity (ACA) of the cell homogenate was determined +/- PTH. ALE did not reproducibly suppress PTH-ACA, but RIS at 0.1 micromolar suppressed PTH-ACA by ~30 %. When cells were incubated for a longer period of time at a 1 micromolar concentration (24-48 hours), ALE and RIS appeared to weakly suppress PTH-ACA in an inconsistent fashion.
These findings confirm our hypothesis that bisphosphonates have measurable effects upon key signaling pathways stimulated by PTH, but these effects are subtle. The physiologic significance of the actual effects of these BP on PTH signaling are not known. However, the differences in reversibility of the PLC suppression by ALE and RIS may provide insight into the clinical impact of previous BP treatment followed by teriparatide therapy.

Disclosures: ***Q. Sun****, Proctor and Gamble-Aventis Pharma R.*

SU213

Role of Pten and Akt in the Regulation of Growth and Apoptosis in Human Osteoblastic Cells. S. M. Nielsen-Preiss[1], S. R. Silva*[1], J. M. Gillette[2], S. Stroh*[3]. [1]Orthopaedics, UCHSC, Denver, CO, USA, [2]Cell and Developmental Biology, UCHSC, Denver, CO, USA, [3]University of Colorado, Boulder, CO, USA.

Cancer cells are characterized by either an increased ability to proliferate or a diminished capacity to undergo programmed cell death. PTEN is instrumental in regulating the balance between cell growth and death in several cell types and has been described as a tumor suppressor. In a subset of human osteosarcomas the chromosomal arm on which PTEN is located has been deleted. Therefore, we predicted that the loss of PTEN expression was contributing to increased Akt activation and the subsequent growth and survival of osteosarcoma tumor cells. Analysis of several human osteosarcoma cell lines and normal osteoblasts revealed relatively abundant levels of PTEN expression. Furthermore, stimulation of cell growth or induction of apoptosis in osteosarcoma cells failed to affect PTEN expression or activity. Therefore, routine regulation of osteoblastic cell growth and survival appears to be independent of changes in PTEN expression. Subsequently, the activation of a downstream target of PTEN activity was analyzed.
Akt is a growth factor-responsive kinase that contributes to cellular growth and survival. We predicted that although we were unable to detect changes in PTEN levels, inappropriate activation of Akt could support uncontrolled cell growth and survival and thus still implicate this pathway in the osteosarcoma phenotype. Analysis of Akt expression in several osteosarcoma cell lines and normal osteoblasts also revealed uniformly low levels of activated (phosphorylated) Akt, even following growth stimuli. In addition, osteosarcoma cell growth was only minimally affected by high concentrations of inhibitors of phosphoinositol-3 kinase, an upstream activator of the Akt signaling pathway. These data further suggest that the Akt pathway in not the predominant signaling cascade required for osteoblastic growth.
Incidentally, inhibition of PTEN activity resulted in increased levels of Akt phosphorylation and enhanced cell proliferation, implying that the Akt signaling pathway is intact and functional, but normally suppressed. These data suggest that abundant levels of PTEN normally maintain Akt in an inactive form in osteoblastic cells. Suppression of PTEN, perhaps by chromosomal loss, can provide a mechanism for the activation of Akt and thus may contribute to osteosarcoma growth and survival.

Disclosures: ***S.M. Nielsen-Preiss****, None.*

SU214

Regulation of Phospholipase D in Osteoblastic Cells by $G\alpha_{12}$ and $G\alpha_{13}$, Mevastatin and Alendronate. A. T. Singh[1], T. Voyno-Yasnetskaya*[2], P. H. Stern[1]. [1]Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago, IL, USA, [2]Department of Pharmacology, University of Illinois at Chicago, Chicago, IL, USA.

Phospholipase D (PLD) is an important activator of signaling in many cell types including osteoblasts, and leads to a wide range of biological responses. In UMR-106 osteoblastic cells, parathyroid hormone (PTH) stimulation of PLD activity leads to membrane translocation of PKCα and increased interleukin-6 expression and is dependent on the small GTP binding protein RhoA. Rho A can be activated through heterotrimeric G proteins of the $G\alpha_{12}/G\alpha_{13}$ class, and translocation of Rho A to the plasma membrane involves its modification by geranylgeranylation. The current studies were designed to determine the effects of $G\alpha_{12}$ and $G\alpha_{13}$ on PLD activity in UMR-106 osteoblastic cells, as well as to assess whether pharmacological agents that can decrease geranylgeranylation interfere with PTH stimulation of PLD activity. UMR-106 cells were cutured in DMEM/15% heat-inactivated horse serum/penicillin in 6-well plates. Phospholipase D activity was assessed by the transphosphatidylation of ethanol. Expression of constitutively active $G\alpha_{12}$ and $G\alpha_{13}$ in the UMR-106 cells markedly increased PLD activity. In contrast, activation of the PKA pathway by forskolin (0.04 mM, 3-30 min) did not affect PLD, and 60 min preincubation with the PKA antagonist PKI (0.01 mM) did not influence the PLD response elicited by 5 min treatment with 10 nM PTH. PLD activity was increased by geranylgeranyl pyrophosphate (0.01 mM, 60 min). The PTH-stimulated increase in PLD was inhibited by the HMGCoA reductase inhibitor mevastatin (0.2 – 5 μM) and the bisphosphonate alendronate (10μM), both of which can decrease the production of geranylgeranyl groups. Neither inhibitor affected basal PLD activity. The results indicate the involvement of heterotrimeric G proteins of the $G\alpha_{12}$ and $G\alpha_{13}$, but not the Gs class in the upstream activation of PLD in osteoblastic cells. Further, the findings identify PLD as a osteoblast target for statins and aminobisphosphonates, possibly through effects on Rho signaling.

Disclosures: ***A.T. Singh****, None.*

SU215

TNF-alpha Expression Is Transcriptionally Regulated by RANK Ligand. W. Zou*[1], H. Drissi[2], Z. Bar-Shavit[1]. [1]Experimental Medicine and Cancer Research, Hebrew University, Jerusalem, Israel, [2]Center for Musculoskeletal Research, University of Rochester, Rochester, NY, USA.

Tumor necrosis factor (TNF)-α is known for its osteoclastogenic and resorptive activities. Induction of osteoclastogenesis by receptor activator of NF-κB ligand (RANKL) is accompanied by increased TNF-α expression. In the present study we investigated the mechanisms by which RANKL induces expression of TNF-α in osteoclast precursors using murine bone marrow derived cells (BMMs) and the macrophage-like cell-line, RAW 264.7 (RAW). We found that RANKL is a potent osteoclastogenic stimulator in both models, whereas TNF-α exhibits very low osteoclastogenic activity. Anti-TNF-α antibody significantly inhibits RANKL-induced osteoclastogenesis while RANKL up-regulates TNF-α expression with similar kinetics in these two cell models. We first examined if RANKL-mediated increase in TNF-α expression, involves increased stability of its transcript. BMMs and RAWs were treated with or without RANKL for 20 minutes, and then a transcription inhibitor (DRB) was added. At different time points, TNF-α and L32 mRNA levels were examined. The rate of TNF-α mRNA degradation was not altered by RANKL indicating that this effect is not due to mRNA stability. We therefore measured the transcription rate of TNF-α by run-on assay in RAW cells after treatment with RANKL (50 ng/ml, 25 minutes). RANKL increases TNF-α transcription rate by 2.5-fold in RAW cells. We further characterized this transcriptional induction of TNF-α by RANKL. Gel shift assays using nuclear extracts derived from RANKL-treated RAWs cells show increased specific NF-κB binding activity on the murine TNF-α promoter. We finally used 1260 bp of the murine TNF-α promoter fused to luciferase (1260TNF/Luc), as well as several 5' deletion mutants of this promoter (containing 656, 529, 514 and 210 bp, 656TNF/Luc, 529TNF/Luc, 514TNF/Luc and 210TNF/Luc, respectively) to stably transfect RAW cells. 1260TNF/Luc and 656TNF/Luc promoter activity was increased in response to RANKL, whereas treatment of 529TNF/Luc, 514TNF/Luc and 210TNF/Luc stable cell lines did not elicit significant reporter gene expression. In conclusion, RANKL induces TNF-α expression via a transcriptional mechanism, depending on some, but not all of the NF-κB sites in the TNF promoter.

Disclosures: ***W. Zou****, None.*

SU216

Tumor Necrosis Factor-alpha Inhibits the Formation of Osteoclasts in vitro through the p55 Receptor on Osteoblasts. R. Balga*[1], C. Mueller*[2], W. Hofstetter[1]. [1]Department Clinical Research, University of Bern, Bern, Switzerland, [2]Department for Pathology, University of Bern, Bern, Switzerland.

Tumor necrosis factor-alpha (TNFα) is a pleiotropic cytokine, acting in synergism with other cytokines such as IL-1 or receptor activator of NF-kappaB ligand (RANKL) during the recruitment of osteoclasts. It was suggested that TNFα plays a critical role in the induction of bone loss after estrogen depletion. We have found, however, that in mice deficient for TNFα or for the p55 TNFα receptor, ovariectomy induces a decrease in bone mass. To address this discrepancy, in the present study the effects of TNFα on the recruitment of osteoclasts in vitro were further investigated.
The role of TNFα in osteoclastogenesis was studied in two culture systems. Bone marrow cells (BMC) were cultured in the presence of colony stimulating factor-1 (CSF-1) and RANKL, or BMC were cultured with primary osteoblasts. In both culture systems the cells were grown in the presence or absence of TNFα. After 6 days, the cells were stained for tartrate resistant acid phosphatase (TRAP), and TRAP+ multinucleated osteoclasts (MNC) were counted. In cultures of BMC with CSF-1 and RANKL, the number of MNC was not affected by TNFα (mean ± SE: 106 ± 5 [-TNFα] vs. 100 ± 1 [+TNFα]). In co-cultures of BMC and wt osteoblasts, the formation of osteoclasts was found to be inhibited by TNFα (mean ± SE: 305 ± 28 vs. 1 ± 2). If wt BMC were cultured with osteoblasts lacking the p55 receptor, the inhibitory effect of the cytokine was blocked and the number of TRAP+ MNC formed was even increased (mean ± SE: 108 ± 8 vs. 188 ± 14).
To investigate, whether the effect of TNFα is mediated through the release of soluble factors, conditioned media (CM) from wt or p55-/- osteoblasts treated with the cytokine were added to cultures of wt BMC and osteoblasts from wt or p55-/- mice. CM from wt osteoblasts treated with TNFα inhibited the formation of MNC (mean ± SE: 173 ± 6 vs. 26 ± 8), whereas CM from p55-/- osteoblasts treated with TNFα did not affect osteoclastogenesis (mean ± SE: 133 ± 1 vs. 133 ± 17). The effect of TNFα was dependent on 1,25(OH)2D3, since osteoblasts released the inhibitory activity on osteoclast formation only when cultured in the presence of 1,25(OH)2D3 and TNFα. To exclude toxic effects of the cytokine, the number of osteoblasts and the levels of transcripts encoding RANKL and osteoprotegerin (OPG) were determined. Both of these parameters were not affected by TNFα.
The results of this study show that the role of TNFα in bone is complex and not restricted to the stimulation of bone resorption. TNFα exerts an inhibitory effect on the formation of osteoclasts in vitro, this effect being dependent on the presence of functional p55 TNF receptors on the cells of the osteoblast lineage.

Disclosures: ***R. Balga****, None.*

SU217

Cytokine Imbalance in Celiac Disease and Parallel Direct Stimulation of Osteoclastogenesis and Osteoblast Activity in Vitro. A. Teti[1], A. Taranta*[1], D. Fortunati*[1], M. Longo*[1], N. Rucci[1], S. Migliaccio[1], M. T. Bardella*[2], S. Saraifoger*[2], A. Dubini*[2], M. L. Bianchi[2]. [1]Experimental Medicine, University of L'Aquila, L'Aquila, Italy, [2]Bone Metabolic Unit, Istituto Auxologico Italiano, IRCCS, Milan, Italy.

Celiac disease is an auto-immune disorder characterized by atrophy of the intestine villi triggered by ingestion of gluten in genetically susceptible individuals. The association between celiac disease and low bone mineral density (BMD) has been recognized but the mechanisms of disturbance are poorly understood. We investigated 42 patients, 25 on gluten-free diet (GFD) (age 35.7+/-7.9, 19 females, 5 males), 17 not on GFD (age 41.3+/-10.8, 13 females, 4 males), and 21 normal controls (age 32.4+/-4.6, 17 females, 4 males). Patients presented a significant increase of the bone resorption marker N-terminal telopeptide of procollagen type I, but normal osteocalcin, calcium, PTH and 1,25(OH)2 D3 levels. IL-6, IL-1beta, TNFalpha and TNFbeta were similar to controls. The inhibitory cytokine IL-12 was reduced in all celiac patients, while IL-18 only in those on GFD. The RANKL/OPG ratio was significantly higher (2.5-fold) in the celiac patients not on GFD, whereas it was not different from controls in the patients on GFD. Peripheral blood mononuclear cells from healthy donors cultured in media supplemented with 25 ng/ml M-CSF, 100 nM PTH, sub-optimal concentration of RANKL (0.5 ng/ml) and 5% sera of our patients not on GFD showed a dramatic increase of the number of TRAP-positive multinucleated cells relative to similar cultures supplemented with normal control sera. A lesser increase was instead observed with sera from celiac patients on GFD. The effect was clearly visible after one week of exposure and persisted throughout three weeks. Cultured human osteoblasts from healthy individuals were also subjected to incubation with 5% of our serum pools. No significant modulations of the stimulatory cytokines IL-6 and IL-1beta were observed, and TNFalpha was undetectable. The inhibitory cytokine IL-18, on the other hand, was found to be reduced by exposure to sera from all celiac patients, regardless of the diet regimen, whereas IL-12 was unremarkable. OPG expression was lower upon incubation with the sera from celiac patients not on GFD. RANKL and PTHrP could not be detected in any of our human osteoblast cultures. Proliferation, alkaline phosphatase and nodule mineralization were increased in osteoblast cultures containing sera from celiac patients, either on or not on GFD, but to a remarkably higher extent in the latter. We conclude that in celiac disease bone loss is likely to be due to an imbalance of factors affecting bone-turnover which may directly affect osteoclastogenesis and osteoblast activity.

Disclosures: ***A. Teti****, None.*

SU218

Role of Osteoclast Inhibitory Peptide-1(OIP-1/hSca) in Interleukin-12 Inhibition of Osteoclast Formation. N. Kawanabe[1], M. Koide*[1], G. D. Roodman[2], S. V. Reddy[1]. [1]Medicine-Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Medicine-Hematology/Oncology, University of Pittsburgh and Department of Veterans Affairs Medical Center, Pittsburgh, PA, USA.

Osteoclast formation and activity is regulated by local factors produced in the bone microenvironment. Immune cell products such as IFN-γ and IL-12 are potent inhibitors of osteoclast formation. Furthermore, IL-12 has been shown to stimulate IFN-γ production by T-cells. More recently, we found that IFN-γ stimulated osteoclast inhibitory peptide-1 (OIP-1/hSca) expression in osteoclast precursor cells, and that anti-OIP-1 c-peptide specific antibody significantly neutralized IFN-γs inhibition of osteoclast differentiation. However, it is unclear if OIP-1 represents a common mediator for IFN-γ and IL-12 inhibition of osteoclast formation. Therefore, we tested the capacity of OIP-1 c-peptide to inhibit osteoclast formation in IFN-γ receptor deficient mouse (IFN-γ R -/-) bone marrow cultures stimulated with RANKL and M-CSF. OIP-1 significantly inhibited osteoclast formation in IFN-γ R -/- mouse bone marrow cultures analogous to non-transgenic control mice. We

further examined IL-12 regulation of OIP-1 expression using cycle-dependent RT-PCR analysis, which demonstrated that IL-12 treatment (24 hr) significantly enhanced OIP-1 mRNA expression in normal human bone marrow cells, but had no effect on highly purified osteoclast precursor cells. To determine the role of IFN-γ and OIP-1 in IL-12 inhibition of osteoclast formation, we tested the capacity of IL-12 to inhibit osteoclast formation in IFN-γ receptor deficient mice (IFN-γ R -/-) bone marrow cultures. Interestingly, in contrast to non-transgenic control mice, IL-12 (20 ng/ml) did not inhibit osteoclast formation in IFN-γ R -/- mice bone marrow cultures stimulated with RANKL and M-CSF. Furthermore, addition of a neutralizing antibody against IFN-γ to IL-12 treated control mice bone marrow cultures, completely abolished IL-12 inhibition of osteoclast formation. However, addition of OIP-1 c-peptide specific neutralizing antibody partially (50%) blocked IL-12's inhibition of osteoclast formation in normal mouse bone marrow cultures. In contrast, a non-specific IgG did not affect IL-12 inhibition of osteoclast formation in these cultures. Furthermore, IL-12 did not inhibit osteoclast formation in normal mouse bone marrow cultures that were depleted of T-cells using a Thy 1.2 antibody. These data suggest that IFN-γ and OIP-1 are responsible for the inhibitory effects of IL-12 on osteoclast formation.

Disclosures: ***S.V. Reddy****, None.*

SU219

gp130-Mediated Signals Play a Role in Osteoclast Differentiation and Activation. H. I. Shin[1], E. K. Park[2], S. Y. Kim[2], T. Kobayashi[3], P. Divieti[3], F. R. Bringhurst[3], H. M. Kronenberg[3]. [1]Department of Oral Pathology, Kyunpook National University, Daegu, Republic of Korea, [2]Skeletal Genome Research Center, Kyunpook National University Hospital, Daegu, Republic of Korea, [3]Endocrine Unit, MGH, Harvard, Boston, MA, USA.

gp130, a common signal-transducing 130 Kd glycoprotein for gp130-associated cytokines such as IL-6, IL-11, LIF, OSM, CNTF, and CT-1, may play an important role in osteoclastogenesis. However, the precise actions of gp130 that influence osteoclast differentiation and activation are not well understood. To address this issue, we analyzed the structural characteristics of osteoclasts in *gp130-/-* fetuses by staining for TRAP activity and by ultrastructural observation. We also studied in vitro osteoclastic induction by PTH(1-34), 1, 25 $(0H)_2$ vitamin D_3 and sRANKL using co-cultures, with analysis of enzymatic activity and resorptional activities for induced TRAP+ multinucleated cells. The *gp130-/-* TRAP+ osteoclasts in fetal tibiae were characteristically larger and more ovoid in shape with more nuclei/cell, than WT TRAP+ osteoclasts, which exhibited a small, flat triangular appearance. Ultrastructurally, the *gp130-/-* osteoclasts showed poor development of ruffled borders, suggesting dysfunctional bone resorbing activity. The *in vitro* induction of osteoclasts by co-culture of *gp130-/-* primary calvarial osteoblasts and *wt* adult bone marrow cells stimulated by either 10^{-7} M PTH(1-34) and 10^{-8} M 1, 25 $(0H)_2$ vitamin D_3 was significantly reduced when compared to cultures using WT calvarial osteoblasts, but the induction was closer to normal in response to exogenous sRANKL treatment. The TRAP+ multinucleated cells induced by *gp130-/-* primary calvarial osteoblasts in co-cultures showed disarrangement of actin filaments and weaker immunoreactivity using antiserum to TRAP and Cathepsin K. Furthermore, they did not form pits on dentin slices. These findings suggest that both PTH(1-34) and 1, 25 $(0H)_2$ vitamin D_3 require gp130-mediated signaling in osteoblast lineage cells to induce adequate RANKL for osteoclastogenesis and that gp130-mediated signaling is important for full osteoclastic activation through regulation of actin ring and ruffled border development, as well as TRAP and cathepsin K production.

Disclosures: ***H.I. Shin****, None.*

SU220

Gamma-Glutamyl Transpeptidase Protein Enhances RANK Ligand Expression and Osteoclastogenesis in Osteolysis. S. Niida[1], T. Kondo*[2], T. Hibi*[1], K. Ikeda[1]. [1]Geriatric Research, National Institute for Longevity Sciences, Obu, Japan, [2]Department of Otolaryngology, Indiana University School of Medicine, Indianapolis, IN, USA.

γ-Glutamyl transpeptidase (GGT) is an ectoenzyme expressed in kidney, pancreas and liver, and used for a marker enzyme for several diseases. We identified GGT as a novel bone resorbing factor by using expression cloning system. Then, we examined the action of GGT in osteoclast formation. Addition of purified GGT (5-625ng/ml) to murine bone marrow cultures dose-dependently induced TRAP-positive cells,which expressed calcitonin receptor and pit forming activity, without $1,25(OH)_2D_3$. Furthermore, we found that inactive form of GGT, whose enzymatic activity was blocked by chemical modification with acivicin, supported osteoclast formation. Taken together, it was demonstrated that GGT stimulated osteoclast formation independently of its enzymatic activity and may involved a putative receptor molecule. Native GGT and inactive GGT induced the expression of RANKL mRNA and protein in bone marow stromal cells, and OPG completely supressed GGT-induced osteoclast formation. In collagen-induced arthritis (CIA) mice and LPS-induced periodontal destruction, GGT expression was markedly increased in synovial tissue and tissue macrophages. Culture of isolated cells from arthritic paws in CIA mice spontaneously appeared the osteoclast-like cells, which was decreased by anti-GGT antibody. These phenomenon suggests that GGT is induced by inflammatory and stimulates osteoclastogenesis through the RANKL expression. Next, we examined effects of recombinat human GGT (rhGGT) on osteoclastogenesis in the co-culture system with bone marrow cells and ST2 cells. rhGGT also induced osteoclast formation and RANKL mRNA expression. Osteoclastogenetic activity of GGT is a novel function of this protein, and further investigation is required to identify the mechanism of induction of RANKL expression.

Disclosures: ***S. Niida****, None.*

SU221

Systemic TNFa Mediates an Increase in Peripheral CD11b-high Osteoclast Precursors by Inducing their Mobilization from the Bone Marrow. P. Li*[1], E. M. Schwarz[2], R. J. O'Keefe[2], L. Ma*[2], R. J. Looney*[2], C. T. Ritchlin*[2], B. F. Boyce[2], L. Xing[2]. [1]Dept. Microbiol. and Immunol., Univ.Rochester, Rochester, NY, USA, [2]Center for Musculoskeletal Res., Univ.Rochester, Rochester, NY, USA.

Chronic exposure to TNFa enhances osteoclastogenesis by increasing the frequency of CD11bhi osteoclast precursors (OCPs) in the periphery. To elucidate the possible mechanism(s) involved (proliferation, survival, differentiation or redistribution from bone marrow), we used TNFa transgenic (TNF-Tg) mice and wild type (wt) mice injected with TNFa. TNF-Tg and wt mice were BrdU labeled for 24 hr and spleen cells were stained with antibodies to CD11b and BrdU. TNF-Tg mice had the expected increase in CD11bhi cells, but no increase in proliferation (% BrdU+ cells in the CD11bhi population was similar in TNF-Tg and wt mice:16.3% vs 18.3%). Next, TNF-Tg and wt splenocytes were analyzed for apoptosis by FACS using antibodies to CD11b, fluorescence-labeled annexin V and 7-AAD. In the CD11bhi population, the % of annexin V+/7-AAD- (apoptotic) cells was similar in TNF-Tg and wt mice (9.6% vs 9.5%). To determine if TNFa induces differentiation of CD11b-/lo cells to CD11bhi OCPs, wt splenocytes were cultured with 10 ng/ml of TNFa for various times. No significant difference was detected in the % of CD11hi cells in control and TNF-treated cultures, determined by FACS after 24h, and no detectable induction of CD11b was found in CD11b mRNA expression by quantitative real-time PCR after 1, 4 and 24h TNF treatment. Similar results were obtained from CD11b- and CD11blo splenocytes sorted by FACS and treated with TNFa for 12h. To examine if TNFa affects the distribution of CD11bhi cells in vivo, wt mice were injected with BrdU for 3d to maximally label bone marrow CD11b+ cells (>96%) and given PBS or TNFa (1mg ip per injection.) either once and sacrificed 4h later or 4x/d for 3d. Bone marrow, spleen, and peripheral blood monocytes (PBMC) were harvested to determine the % of BrdU+/CD11b+ cells by FACS. TNFa caused a rapid release of CD11b+ cells from bone marrow to the blood at 4h (% CD11b+/BrdU+ PBMC increased 4-fold), but significantly altered this population in the spleen only after treatment for 3d to a level similar to that observed in untreated TNF-Tg mice. Correspondingly, this treatment caused an increase in the osteoclastogenic and CFU-M colony-forming potential of the splenocytes from these mice. Thus, we have identified a novel mechanism whereby TNFa causes a marked increase in circulating CD11bhi OCPs in the periphery to account for the increased osteoclastogenesis in patients with inflammatory arthritis: mobilization of OCPs from bone marrow without affecting their proliferation, survival, or differentiation.

Disclosures: ***L. Xing****, None.*

SU222

IL-3 Acts Directly on Osteoclast Precursors and Inhibits RANKL-Induced Osteoclast Differentiation. S. M. Khapli*, L. S. Mangashetti*, S. D. Yogesha*, M. R. Wani*. Laboratory-1, National Center for Cell Science, PUNE, India, India.

Osteoclasts, the multinucleated cells that resorb bone differentiate from hemopoietic precursors of monocyte/macrophage lineage. Interleukins produced by activated T cells, as well as by other cell types regulates osteoclastogenesis. However, it is not clear how osteoclastogenesis is regulated by immune cell-derived cytokines. IL-3, a cytokine secreted by activated T lymphocytes stimulates the proliferation, differentiation and survival of pluripotent hemopoietic stem cells. Although osteoclast differentiate from hemopoietic stem cells the role of IL-3 in osteoclast differentiation is not clear. IL-3 has previously been shown to have both stimulatory and inhibitory action on osteoclast formation in complex culture systems.

In this study, we investigated the role of IL-3 in RANKL-induced osteoclast differentiation. We show here that IL-3 inhibits RANKL-induced osteoclast differentiation by a direct action on early osteoclast precursors. Anti-IL-3 Ab neutralized the inhibitory effect of IL-3 on osteoclast differentiation. In addition, IL-3 inhibits TNF-a-induced osteoclast differentiation in bone marrow-derived macrophages. However, IL-3 has no inhibitory effect on mature osteoclasts. In osteoclast precursors, IL-3 prevents RANKL-induced nuclear translocation of NF-kB by inhibiting the phosphorylation and degradation of IkB. RT-PCR analysis revealed that IL-3 down-regulated c-Fos transcription. Interestingly, the osteoclast precursors in the presence of IL-3 showed strong expression of macrophage markers such as Mac-1, MOMA-2 and F4/80. Furthermore, the inhibitory effect of IL-3 on osteoclast differentiation was irreversible and the osteoclast precursors preincubated in IL-3 were resistant to RANKL action. Thus, our results first time reveal that IL-3 acts directly on early osteoclast precursors and irreversibly block RANKL-induced osteoclast differentiation by diverting the cells to macrophage lineage.

Disclosures: ***M.R. Wani****, None.*

SU223

Tyrosine-757 of gp130 Plays a Critical Role in Osteoclast Formation. J. M. W. Quinn[1], A. Nakamura*[1], B. Jenkins*[2], N. A. Sims[3], M. Ernst*[2], T. J. Martin[1], M. T. Gillespie[1]. [1]Molecular Endocrinology, St. Vincent's Institute of Medical Research, Victoria, Australia, [2]Ludwig Institute of Cancer Research, Victoria, Australia, [3]Dept of Medicine, University of Melbourne, Victoria, Australia.

IL-6 and IL-11 play important roles in controling bone remodeling and both signal via the gp130 receptor subunit. Two major intracellular signaling pathways are activated by gp130: SHP2-mediated MAPK signaling cascade which emanates from the membrane proximal phospho-tyrosine residue 757, and the STAT-1/3 mediated pathways requiring

membrane distal phospho-tyrosines in gp130. These pathways are under reciprocal negative feedback control. gp130deltaSTAT mice, which have a mutation that ablates STAT1/3 signaling, have normal bone mass while gp130Y757F knock-in mutant mice with a phenylalanine substitution of Y757F display osteopenia, with increased osteoclast and osteoblast numbers. This indicates an important role for gp130 tyrosine 757 in the control of bone remodeling.

In M-CSF-dependent colony formation assays and in cultures stimulated by M-CSF plus RANKL, gp130Y757F bone marrow resulted in greater numbers of colonies (180%) and osteoclasts (191%), respectively, compared to wild type bone marrow. Both IL-6 and IL-11 strongly inhibited colony and osteoclast formation from gp130Y757F bone marrow but had little effect on wild type bone marrow. In contrast, none of these effects were observed in gp130deltaSTAT bone marrow cells.

In co-cultures with osteoblasts, gp130Y757F bone marrow showed a strikingly different pattern of osteoclast formation to the M-CSF plus RANKL stimulated cultures. Wild type bone marrow cells formed 264±22, 406±63 and 309±70 osteoclasts/well in co-cultures stimulated by IL-11, PTH1-34 or 1,25 dihydroxyvitamin-D3 plus PGE2 (D3/PG) respectively. In contrast, gp130Y757F bone marrow cells formed fewer than 5 osteoclasts/well in IL-11 and D3/PG stimulated cultures and 69±3 osteoclasts/well with PTH treatment. This altered response of gp130Y757F bone marrow cells was also observed in co-cultures with gp130Y757F osteoblasts.

These results suggest that bone marrow cells from gp130Y757F mice contain more osteoclast progenitors and thus form more osteoclasts (in response to RANKL) relative to bone marrow from wild type mice. However, the co-culture data suggests that osteoblasts may produce an activity that is inhibitory of osteoclastogenesis which is evident when signals emanating from gp130Y757 are ablated.

Disclosures: ***J.M.W. Quinn****, None.*

SU224

The Effect of Calcitonin-Gene-Related-Peptide (CGRP) on Osteoclast-like Cells (OCL) Differentiation and Function. A. C. Demulder[1], E. D. Wittersheim*[1], M. Guns*[1], P. Fondu*[1], P. Bergmann[2]. [1]Laboratory of Hematology, Brugmann University Hospital, Brussels, Belgium, [2]Laboratory of Experimental Medicine, Brugmann University Hospital, Brussels, Belgium.

We have shown previously that CGRP has in vitro a direct and dose dependent inhibitory effect on human OCL precursor that is at least in part mediated by cAMP. The direct action of CGRP on OCL precursors does not exclude an indirect action through the bone marrow microenvironment. The aim of the present study was to assess the effects of CGRP on bone resorption in human bone marrow cultures and to investigate the interaction of CGRP with the bone marrow microenvironment in this setting, particularly with the RANK-L/Osteoprotegerin (OPG) system. For this purpose, we used two different systems of human bone marrow cultures: the long term bone marrow culture (LTBMC) for OCL differentiation and the CFU-GM derived OCL cells cultivated on hydroxyapatite slices for bone resorption experiments. As previously shown, the formation of OCL in LTBMC was decreased when CGRP was added continuously during the first week of culture. This statistically significant inhibitory effect on proliferation was dose-dependent for 10^{-11} to 10^{-6} M concentration of CGRP. The conditioned media of these cultures were frozen for measurements of OPG and RANK-L by ELISA. Levels of OPG were low in control and CGRP treated cultures (100-250 pg/ml) and were not influenced by CGRP concentrations. RANK-L was undetectable in most cultures. Unexpectedly, despite the fact that OPG levels were low and did not differ between treated and controls, the addition of increasing concentrations of a neutralizing antibody directed against OPG (anti-OPG 1/200 to 1/50) restored the formation of OCL in CGRP treated LTBMC and increased OCL formation in control wells. When CFU-GM derived OCL were cultivated on hydroxyapatite slices, RANK-L stimulated bone resorption in a dose dependent manner. CGRP decreased consistently bone resorption by 30-50% when added together with RANK-L 20 ng/ml. In comparison, the addition of OPG at 50ng/ml totally abolished bone resorption. In conclusion, CGRP inhibits both differentiation and function of OCL. These effects do not seem to be mediated through the RANK-L/OPG system and result probably only of a direct action on OCL precursor. The very low or undetectable levels of RANK-L in LTBMC conditioned media could explain why these LTBMC derived OCL are unable to resorb bone.

Disclosures: ***A.C. Demulder****, None.*

SU225

The Relationship between Circulating Osteoprotegerin Levels and Bone Mineral Metabolism of Korean Women. K. Oh[1], E. Oh*[1], S. Moon*[2], D. Lee*[2], W. Lee*[3], K. Baik*[4], M. Kang[4]. [1]Department of Internal Medicine, Miz Medi Hospital, Seoul, Republic of Korea, [2]Department of Family Medicine, Miz Medi Hospital, Seoul, Republic of Korea, [3]Department of Internal Medicine, Sungkyunkwan University School of Medicine, College of Medicine, Seoul, Republic of Korea, [4]Department of Internal Medicine, Catholic University of Korea, College of Medicine, Seoul, Republic of Korea.

Osteoprotegerin (OPG) is a recently identified cytokine that acts as a decoy receptor for the RANK ligand. OPG has been shown to be an important inhibitor of osteoclastogenesis in animal models. The relationship between circulating OPG levels and female bone status in human populations is unclear. Thus, the aim of this study was to investigate the relationship between circulating OPG levels and bone mineral metabolism in Korean women. Subjects were 294 women aged 33–73 (mean age, 51.5 yr). Serum concentrations of OPG were determined by ELISA. Biochemical markers of bone turnover and follicular stimulating hormone (FSH) were measured by standard methods. Bone mineral density at femoral neck and lumbar spine was measured by dual energy x-ray absorptiometry. We observed a significant positive association between circulating OPG levels and urine deoxypyridinoline levels (r=0.125; p<0.05). And, there was a significant positive relationship between circulating OPG levels and urine calcium excretion (r=0.220; p<0.05). We found that mean OPG levels were about 10% greater in postmenopausal women (mean±SD, 1386.9±532.6 pg/mL) than in premenopausal women (1255.8±451.8 pg/mL; p<0.05). There was a significant positive relationship between circulating OPG levels and serum FSH levels (r=0.153; p<0.01). There was a no significant relationship between circulating OPG levels and bone mineral density at femoral neck and lumbar spine. In conclusion, our data shows that the circulating OPG levels are associated with the markers of bone resorption and serum FSH levels in Korean women. These data suggest that OPG may be an important paracrine mediator of female bone metabolism in human populations.

Disclosures: ***K. Oh****, None.*

SU226

TGFβ1 Upregulates CXCR4 Expression in Osteoclasts (OC): A Possible Mechanism to Enhance OC Survival and Bone Resorption. X. Yu, Y. Huang*, P. Collin-Osdoby, P. Osdoby. Department of Biology, Washington University, St. Louis, MO, USA.

Osteoclast (OC) bone resorption is essential for normal bone development and remodeling, but is excessive in skeletal pathologies such as inflammatory bone loss (rheumatoid arthritis, periodontal disease) or tumor osteolysis. OC precursors (pre-OCs) reside in the bone marrow and peripheral circulation, from which they migrate to bone sites for RANKL-mediated development into resorptive OCs. This process involves multiple factors including chemokines which affect cell migration, invasion, activation and survival. Previously, we showed that pre-OCs and OCs expressed the chemokine receptor CXCR4 and that SDF-1, the unique ligand of CXCR4: 1) increased pre-OC chemotaxis, 2) promoted pre-OC transcollagen migration via increasing MMP-9 activity, 3) stimulated the angiogenic-related recruitment of pre-OCs that developed into resorptive cells on bone in vivo, and 4) increased mature OC survival. TGFβ1 has also been found to increase OC survival. Because TGFβ1 induces CXCR4 expression in various cells, we investigated CXCR4 expression as a function of OC differentiation and TGFβ1 levels in two murine OC developmental models. Whereas CXCR4 expression declined during RANKL-induced OC formation from RAW 264.7 cells (RAW-OCs), it increased during OC formation from primary bone marrow cells (MA-OCs). This correlated with 2 to 3-fold higher basal TGFβ1 expression levels in primary MA-OCs (highly purified) compared to RAW-OCs. Addition of TGFβ1 restored CXCR4 levels in RAW-OCs and allowed high CXCR4 levels to persist during RAW-OC formation. In contrast, other factors known to increase CXCR4, such as VEGF, bFGF and IL-6, did not alter RAW-OC CXCR4 expression. Thus, higher TGFβ1 expression by primary MA-OCs may account for higher CXCR4 expression via an autocrine mechanism. Similar findings were observed during OC generation from human blood monocytes by M-CSF/RANKL: CXCR4 declined during human OC development in parallel with decreasing TGFβ1 expression, and the addition of TGFβ1 to differentiated human OCs upregulated their CXCR4 expression. TGFβ1 is both produced and activated by OCs, and is synthesized by many other cell types and released in abundant quantities from bone matrix during normal OC resorption. Furthermore, both TGFβ1 and SDF-1 are reportedly increased in rheumatoid arthritis, and a selective CXCR4 inhibitor efficiently suppresses bone loss in a murine model of this disease. Thus, in addition to promoting angiogenesis and OC recruitment, TGFβ1 may have an important role in regulating OC survival through elevating CXCR4 expression and thereby enhance OC bone resorption and remodeling in both physiological and pathological conditions.

Disclosures: ***X. Yu****, None.*

SU227

RANKL Regulates Fas Expression during Osteoclastogenesis. X. Wu[1], G. Pan*[1], M. A. McKenna[1], J. M. McDonald[2]. [1]Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Department of Pathology, University of Alabama at Birmingham; Veterans Administration Medical Center, Birmingham, AL, USA.

Osteoclast apoptosis is an influential determinant of osteoclast bone resorbing activity. Fas, the death receptor, is important in mediating apoptosis in osteoclasts, and Lpr and Gld mice with non-functional Fas and FasL, respectively, have decreased bone mineral density. Here, we report that RANKL regulation of Fas is biphasic, being increased during the early stage (1 day) of osteoclast differentiation and decreased during the late stage. To examine late stage of differentiation, monocyte-macrophage precursors isolated from C57BL/6 mouse bone marrow were differentiated with M-CSF or M-CSF plus RANKL for 6 days. Cells cultured with both RANKL and M-CSF had 42.7±10.5% (n=5) less Fas mRNA (semi-quantitative Real Time PCR) than cells differentiated in the presence of M-CSF only. Fas protein (Western blotting) was dose-dependently downregulated by RANKL with a maximal decrease of 44.9±17.1% (n=7). Flow cytometry confirmed decreased RANKL-induced surface Fas expression. Similar data were obtained with osteoclasts differentiated from RAW264.7. Both Fas expression and Fas-mediated apoptosis were downregulated by RANKL in a dose-dependent manner. Fas activating antibody induced 51% apoptosis, in the presence of 1.1nmol/L (50ng/ml) RANKL; in contrast, cells differentiated in 3.3nmol/L RANKL were resistant to Fas antibody-induced apoptosis. To investigate the role of RANKL in the regulation of Fas expression during the early stage of differentiation, RAW264.7 cells were cultured with or without RANKL for 1 day. Fas mRNA (Real Time PCR) was increased by 30.4±9.9% (n=3) in cells cultured in the presence of RANKL. Surface expression of Fas was also increased by RANKL, as detected by flow cytometry. Using a dual luciferase report assay, Fas promoter activity was increased 2.4 ± 0.1 fold (n=9) in cells treated with 2.2nmol/L RANKL for 1 day, compared to untreated cells. Also, Fas promoter activity responded to RANKL treatment dose-dependently. Using the transcription inhibitor, actinomycin D, we demonstrated that Fas mRNA from RAW264.7 cells cultured with RANKL was more stable than Fas mRNA from control. These results demonstrate that RANKL upregulates Fas expression at an early stage, thus implicating

RANKL in regulating osteoclast homeostasis by limiting the number of precursors entering the differentiation process. At the late stage of osteoclast differentiation, RANKL downregulates Fas expression and Fas-mediated apoptosis, which acts as a positive regulator for osteoclast function and activity.

Disclosures: ***X. Wu****, None.*

SU228

The Role of PTHrP in Regulation of OPG/RANKL in Cementoblasts. F. Boabaid*[1], J. E. Berry*[2], M. J. Somerman[3], L. K. McCauley[2]. [1]Federal Univ of Sao Paulo, Sao Paulo, Brazil, [2]U of Michigan Dental School, Ann Arbor, MI, USA, [3]U of Washington Dental School, Seattle, WA, USA.

Tooth eruption is a physiological event that involves epithelial-mesenchymal interactions, and PTHrP is well known to be involved in this process. In the tooth germ microenvironment PTHrP is produced by cells of the stellate reticulum (epithelial), and can bind to receptors present on follicle cells, cementoblasts and osteoblasts (mesenchymal). PTHrP has been linked with a variety of activities including recruitment of mononuclear cells, fusion of these cells into multinucleated osteoclasts, resorption of alveolar bone and creation of an eruption pathway. Studies in osteoblasts have shown that PTHrP promotes osteoclastogenesis by inhibiting expression of OPG, a decoy receptor for RANK, and by enhancing production of RANKL. However, little is known regarding the role of PTHrP in regulating cementoblast-mediated osteoclastogenesis or odontoclastogenesis. To analyze the effect of PTHrP on regulation of OPG/RANKL levels and, subsequently, promotion of osteoclastogenesis, three groups of cell cultures were analyzed; 1) OCCM-30 (immortalized murine cementoblasts) alone, 2) RAW 264.7 cells (murine myeloid cell line) alone, or 3) OCCM-30 plus RAW 264.7 cells. These groups were treated with vehicle alone, PTHrP (1-34) (10^{-7}M) alone, RANKL (0.2ug/ml) alone or PTHrP and RANKL combined. Tartrate-resistant acid phosphatase (TRAP) staining for osteoclast detection and ELISA for OPG and RANKL (standardized to protein levels) were performed after 5d. The highest numbers of TRAP positive cells were found in the RAW cell only groups treated with RANKL, and PTHrP did not alter these numbers. In the combination OCCM & RAW cultures there were no TRAP+ cells in the vehicle group. PTHrP-treated cultures had few (6.3 ± 0.9), RANKL had a moderate number (17.0 ± 1.5) and the combination of PTHrP & RANKL had the highest (84.3 ± 8.6) TRAP+ cells/well suggesting a synergistic response between PTHrP and RANKL. OPG levels in cell lysates and secreted in media were highest from OCCM cells and PTHrP significantly decreased these levels. In co-cultures, OPG levels were lower than OCCM alone and the PTHrP effect was diminished. In contrast, RANKL levels were low in OCCM cell lysates and PTHrP increased RANKL (9-fold) but in OCCM & RAW co-cultures, RANKL was high and PTHrP decreased levels (6-fold) in lysates but did not alter secreted RANKL levels. In conclusion, PTHrP can target cementoblasts, inhibit OPG, increase RANKL and consequently promote osteoclastogenesis, but this is dependent on the context of surrounding cells. Osteoclast-promoting actions of PTHrP may be important during the process of tooth eruption, and periodontal development and/or disease.

Disclosures: ***F. Boabaid****, None.*

SU229

MyD88 is Essentially Involved in Osteoclast Differentiation Induced by Lipopolysaccharide, Lipopeptide and IL-1. N. Sato*[1], N. Takahashi[2], K. Suda[3], Y. Kobayashi[2], K. Takeda*[4], S. Akira*[4], K. Shibata*[5], T. Noguchi*[1], N. Udagawa[6]. [1]Periodontology, School of Dentistry, Aichi-Gakuin Univ., Nagoya, Japan, [2]Institute for Oral Science, Matsumoto Dental Univ., Shiojiri, Japan, [3]School of Dentistry, Showa Univ., Tokyo, Japan, [4]Research Institute for Microbial Diseases, Osaka Univ., Suita, Japan, [5]Oral Pathobiological Science, Hokkaido Univ. Graduate School of Dental Medicine, Sapporo, Japan, [6]Biochemistry, Matsumoto Dental Univ., Shiojiri, Japan.

Lipopolysaccharide (LPS) is proposed to be a potent stimulator of bone resorption in inflammatory diseases caused by bacteria. Bacterial lipoprotein/lipopeptides are also pathogen-specific molecular patterns. Recently, toll-like receptor 4 (TLR4) was identified as the signaling receptor for LPS. In addition, TLR6 associates with TLR2, and the complex of TLR6 and TLR2 recognizes diacylated mycoplasmal lipopeptides. The signaling cascade of TLR is believed to be similar to that of IL-1 receptors (IL-1R), because both TLR and IL-1R use myeloid differentiation factor 88 (MyD88) as a common cytoplasmic signaling molecule. However, accumulating evidence also demonstrates the existence of MyD88-independent pathways, which may explain unique biological responses of individual TLR and IL-1R. Using MyD88-deficient (-/-) mice, we explored the involvement of MyD88-mediated signals in osteoclast formation. LPS, synthetic lipopeptide (FSL-1), IL-1α and $1,25(OH)_2D_3$ all stimulated osteoclast formation in co-cultures of primary osteoblasts and bone marrow cells obtained from wild-type mice. Osteoprotegerin, a decoy receptor of RANKL, completely inhibited the osteoclast formation in the co-culture. In contrasts, LPS, lipopeptide and IL-1α failed to induce the osteoclast formation in the co-culture of MyD88 (-/-) mice-derived osteoblasts and bone marrow cells, though $1,25(OH)_2D_3$ stimulated osteoclast formation even in the MyD88 (-/-) co-culture. RT-PCR analysis showed that primary osteoblasts obtained from both wild type and MyD88 (-/-) mice similarly expressed TLR2, TLR4, TLR6 and IL-1R mRNAs. LPS, lipopeptide and IL-1α stimulated expression of RANKL mRNA within 24 hr in primary osteoblasts obtained from wild-type mice but not in those from MyD88 (-/-) mice. Similarly, LPS and IL-1α stimulated phosphorylation of ERK in wild-type osteoblasts but not MyD88 (-/-) osteoblasts. Hemopoietic cells obtained from MyD88 (-/-) mice and those from wild-type mice similarly differentiated into osteoclasts in response to RANKL and M-CSF. These results suggest that the MyD88-mediated signaling pathway is essentially involved in osteoclast formation induced by LPS, lipopeptide and IL-1α through the RANKL expression by osteoblasts.

Disclosures: ***N. Sato****, None.*

SU230

Transcription Factors Involved in Osteoclastogenesis. C. Day*[1], M. Kim*[1], G. Nicholson[2], N. A. Morrison[1]. [1]Health Science, Griffith University, Gold Coast, Australia, [2]Department of Medicine, Geelong Hospital, Geelong, Australia.

Surprisingly, very few transcription factors have been implicated in osteoclast differentiation. Newly discovered upregulated transcription factors are obviously important in understanding osteoclast differentiation. However, we speculate that repression of transcription factor genes may also be necessary for osteoclast differentiation. In particular, we have looked for differential regulation of transcription factors between the two alternative states: macrophage and osteoclast. Two recent papers verified the role of NFATc1 in osteoclastogenesis [1,2]; NFATc1 is required for the production of TRAP positive multinucleated osteoclast like cells (OCLs). We show here that NFATc1 and other transcription factors are strongly induced by RANKL in human osteoclast like cells. NFATc1 regulation steadily increases across time with a peak of 26 fold at three weeks post treatment with macrophage colony stimulating factor (M-CSF) and RANKL. Along with NFATc1 we have observed significant up-regulation of four other transcription factors: GA-binding protein a and b (GABP), early response growth factor 1 (EGR-1), and FUSE binding protein (FBP). The dynamics of regulation of ERG-1 and GABPa and b were identical to that of NFATc1. However FBP regulation peaks earlier and is of great magnitude. These data show that NFATc1 is not the only strongly regulated transcription factor in osteoclast differentiation and is later induced than FBP.

1 Ishida N, Hayashi K, Hoshijima M, Ogawa T, Koga S, Miyatake Y, Kumegawa M, Kimura T, Takeya T. 2002. J Biol Chem 277:41147

2 Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. 2002. Dev Cell 3:889

Disclosures: ***C. Day****, None.*

SU231

RANKL Strongly Induces the GM-CSF Receptor during Osteoclast Differentiation but Continuous Exposure to GM-CSF Represses Osteoclast Formation. M. Kim*, C. Day*, N. A. Morrison. Health Science, Griffith University, Gold Coast, Australia.

The standard model of osteoclastogenesis uses M-CSF and RANKL to differentiate osteoclasts from peripheral blood mononuclear cells (PBMCs). Using real time PCR, we found that RANKL profoundly up-regulates the GM-CSF receptor in this model. This suggests that GM-CSF receptor might represent a target for regulation, whereby osteoclast precursors integrate RANKL and GM-CSF signals to either promote or inhibit differentiation. Exogenous GM-CSF was added to the in vitro osteoclastogenesis model to test these alternative hypotheses. Cells were examined through time, with TRAP staining, morphology and gene array analysis. Continuous exposure to GM-CSF totally represses osteoclast differentiation; and resulted in an alternative cell phenotype induced by GM-CSF in the presence of M-CSF and RANKL when compared to the alternative treatments.

A 19,000 gene microarray was used to compare GM-CSF+RANKL+M-CSF treated cells against osteoclasts. Microarray analysis showed GM-CSF mediated repression of osteoclast differentiation was concurrent with suppression of osteoclast marker genes: cathepsin K; osteoclast specific H+ ATPase; surface marker, CD68; and transcription factors we have shown are up-regulated in osteoclasts, such as NFATc1. The inhibition by GM-CSF of known osteoclast markers indicates an alternative GM-CSF dependent differentiation pathway. Real-time PCR analysis of 7 regulated genes validated the array data (FBP, GABPa, GABPb, ILF3, Kox31, NFATc1 and SCYA2). These data support the hypothesis that GM-CSF receptor up-regulation by RANKL sensitises the cell for inhibition of differentiation. In the cytokine milieu of the bone marrow, the ratio of GM-CSF and RANKL may be an important determinant of osteoclast differentiation.

Disclosures: ***N.A. Morrison****, None.*

SU232

Over-Expression of TBP-2, a Protein Involved in Redox Regulation, Inhibits Osteoclastogenesis. C. J. Aitken*[1], J. M. Hodge*[1], T. Vaughan*[2], D. E. Myers*[1], N. A. Morrison[2], G. C. Nicholson[1]. [1]Clinical and Biomedical Sciences: Barwon Health, The University of Melbourne, Geelong, Australia, [2]Genomics Research Centre, Griffith University, Gold Coast Campus, Australia.

Using gene array analysis, we found that thioredoxin (TRX) binding protein-2 (TBP-2) was down-regulated during osteoclast (OC) differentiation. TBP-2 is a negative regulator of TRX, a small protein with a redox-active dithiol active site. TRX enhances DNA binding of redox-sensitve transcription factors such as NFκB and AP-1.

OC were generated on dentine slices using human CFU-GM precursor cells treated with RANKL and M-CSF. At 4 days of culture, efficient (>80%) infection with adenovirus expressing β-galactosidase (AdLacZ) was achieved. Infection with adenovirus expressing TBP-2 (AdTBP-2) for 14 days resulted in 66% reduction in the total TRAP+ area and 50% reduction in OC numbers as compared to the AdLacZ control. The size of OC formed in the presence of AdTBP-2 was reduced by 66% and they contained fewer nuclei. Resorption of dentine was inhibited by 80%. In mature OC infected with AdTBP-2, RANKL-induced NFκB activation was reduced by 63% and Western analysis demonstrated markedly increased expression of TBP-2 protein. We have shown that the over-expression of TBP-2, a gene down-regulated during OC formation, inhibits OC differentiation and NFκB activation. These results are consistent with the known function of TBP-2 as a negative regulator of TRX and the importance of the redox-sensitive transcription factor NFκB in osteoclastogenesis.

Disclosures: ***G.C. Nicholson****, None.*

SU233

Expression of Regucalcin, a Novel Intracellular Signaling Regulatory Protein, in the Bone Marrow Cells of Normal and Transgenic Rats. N. Sawada*, M. Yamaguchi. Endocrinology and Molecular Metabolism, University of Shizuoka, Shizuoka, Japan.

Regucalcin, which was found by Yamaguchi et al., has been demonstrated to play a multifunctional role as regulatory protein in intracellular signaling process [Life Sci 66:1769-1780, 2000; BBRC 276:1-6, 2000]. The regucalcin gene is highly conserved in human and various vertebrate species [Int J Mol Med 6:191-196, 2000]. More recently, it has been shown that regucalcin is expressed in the femoral-diaphyseal and –metaphyseal tissues of rats, and that bone loss is induced in regucalcin transgenic rats [Int J Mol Med 10:761-766, 2002]. Furthermore, the present study was undertaken to clarify whether regucalcin is expressed in bone marrow cells. We found that regucalcin mRNA is expressed in bone marrow cells of normal rats by RT-PCR and Western blot analyses. The expression of regucalcin in bone marrow cells was enhanced in the transgenic rats. The effect of regucalcin on osteoclastic formation is in progress.

Disclosures: ***N. Sawada****, None.*

SU234

Regulation of c-Fos Protein Expression in Osteoclast Lineage Cells: Involvement of Ubiquitin/Proteasome Pathway. Y. Ito*, D. Inoue, S. Kido, I. Endo*, T. Matsumoto. Department of Medicine and Bioregulatory Sciences, University of Tokushima, Tokushima, Japan.

Gene knockout studies have demonstrated that c-Fos, an AP-1 family transcription factor, is indispensable for osteoclast differentiation. So far, four members of fos family have been identified: fosB, c-fos, fra-1 and fra-2. Rescue experiments with c-fos -/- spleen cells and retroviral expression vectors of fos family members indicated that introduction of not only c-fos itself but also either one of the other three members was able to restore the ability of c-fos -/- spleen cells to differentiate into osteoclasts, suggesting a functional redundancy among the family members. On the other hand, in vivo gene deletion of other fos family members such as fosB and fra-1 did not result in osteopetrosis as observed in c-fos gene-deleted mice, indicating that only the c-fos gene has a unique function that cannot be compensated by the other family member genes. These enigmatic observations led us to hypothesize that specificity of c-fos resides not in its protein function but in its regulatory mechanisms of protein expression from the endogenous gene. In mouse osteoclast precursor cell lines, RAW264 and C7, serum and TPA induced expression of all the fos family members at both the mRNA and protein levels. Similar induction was observed in c-fos -/- primary spleen cells except c-fos, which is absent in these cells. We also confirmed that RANK expression was normal in c-fos -/- spleen cells. However, treatment with TPA in addition to RANKL and M-CSF did not rescue the defective osteoclast differentiation in c-fos -/- spleen cells. In RAW cells, TPA induction of c-Fos protein was well detectable at 3 hr, reached a peak within 8 hrs and was down-regulated by 24 hrs. In contrast, up-regulation of c-Fos protein expression by RANKL was much slower and more prolonged with a peak at 24 hrs or later. These results are compatible with an idea that stable c-Fos expression may be necessary for osteoclast differentiation. Therefore, we examined degradation pathways, especially focusing on the ubiquitin (Ub)/proteasome system. We found that a proteasome inhibitor MG132, but not a protease inhibitor E64, increased the level of c-Fos protein expression in RAW and C7 cells in a time- and dose-dependent manner. Immunoprecipitation with c-Fos antibody and subsequent blot with Ub antibody demonstrated that ubiquitinated c-Fos accumulated in the presence MG132. We observed similar effects on c-Jun, a known target of Ub. We conclude that c-Fos is a target of Ub/proteasome degradation pathway, which may play a role in unique regulatory mechanism of c-Fos expression in osteoclast precursors.

Disclosures: ***Y. Ito****, None.*

SU235

Prostaglandin E2 Stimulation of Osteoclast Formation in Hematopoietic Cell Cultures is Mediated by a Soluble T Cell Factor. H. Kaneko[1], K. Ono[2], S. K. Lee[1], J. A. Lorenzo[1], Y. Toyama*[3], C. C. Pilbeam[1], L. G. Raisz[1]. [1]Medicine, University of Connecticut Health Center, Connecticut, CT, USA, [2]General Medicine, National Defense Medical College, Tokorozawa, Japan, [3]Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan.

Previously we found that PGE2 stimulated osteoclast-like cell (OCL) formation in spleen cell cultures treated with RANKL and M-CSF and that this stimulation was mediated by the EP2 receptor. Since T cells are possible regulators of bone resorption in inflammation, we examined their role in this response. Spleen cells from 6-week-old male C57BL/6 mice were plated at 2.5 x 10^5 cells/well (48 well plate) and cultured in αMEM containing 10% FCS with 10 ng/ml M-CSF and 30 ng/ml RANKL. At 5 to 9 d of culture adherent cells were stained for TRAP. TRAP(+) cells containing 3 or more nuclei were counted as OCL. Since T cells can produce RANKL, we first determined the dose response to RANKL. Spleen cells were cultured with 10 ng/ml M-CSF and 1-100 ng/ml RANKL. 10 to 100 ng/ml RANKL increased OCL number with the peak at 8d. There was no significant difference in OCL number between 30 to 100 ng/ml RANKL. In contrast, the mean effect of 10^{-6} M PGE2 added to 10 ng/ml M-CSF and 30 ng/ml RANKL at 8 d was a 48±9 % increase ($p<0.01$). T cell depletion using mouse pan T (Thy 1.2) immunomagnetic beads abrogated the stimulatory effect of PGE2. To determine whether a soluble product of T cells might mediate the effect of PGE2, we incubated T cells isolated from the spleen with or without 10^{-6} M PGE2 for 6 h, washed the cells, and then collected supernatants for the next 12 h of culture. This conditioned medium was then added to fresh spleen cultures. At 8 d, control cultures without supernatant produced 130±23 OCL, cultures with control T cell supernatant produced 252±20 OCL, and cultures with supernatant from T cells treated with PGE2 produced 353±17 OCL ($p<0.01$). These results suggest that T cells release stimulators of OCL formation and that PGE2 increases this activity. We speculate that the role of PGE2 in the bone loss associated with inflammation may be mediated by its effect on T cells.

Disclosures: ***H. Kaneko****, None.*

SU236

YY1 is Positively Involved in RANKL-Induced Transcription of Tartrate-Resistant Acid Phosphatase (TRAP) Gene. Z. Shi, Y. Liu*, A. Silveira*, P. Patel*, X. Feng. Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Osteoclasts, the principal bone-resorbing cells, are derived from cells of monocyte/macrophage lineage. RANKL is an essential and potent activator of osteoclast differentiation. During RANKL-induced osteoclast differentiation, a variety of genes are activated, including the gene for Tartrate-Resistant Acid Phosphatase (TRAP), which is implicated in osteoclastic bone resorption. However, the molecular mechanism underlying the RANKL-induced TRAP expression has not been completely understood. Our previous characterization of the mouse TRAP promoter identified a 12-bp sequence AGCCACGTGGTG (–1220 to –1198, relative to the translation start site) that regulates RANKL-induced TRAP transcription by utilizing USF1 and USF2. Interestingly, during the course of the previous study, we also found that oligonucleotides (designated as Oligo IV) derived from a 50-bp TRAP promoter region (-1124 to –1074) bound RANKL-induced nuclear proteins from RAW264.7 cells. In the current study, we elucidated the identity of the nuclear protein binding to Oligo IV and further investigated the role of this nuclear protein in RANKL-induced transcription of TRAP gene. Given that RANKL activates NF-kB or AP1, we determined whether the nuclear protein binding to Oligo IV is NF-kB or AP1. Supershift assays with antibodies against p50, p65, c-fos and c-jun demonstrated that Oligo IV binds transcription factors other than NF-kB and AP1. To reveal the identity of the nuclear protein, we decided first to locate the specific sequence in Oligo IV that binds the protein. To this end, we performed EMSA/competition assays with shortened oligos derived from Oligo IV as competitors and these assays identified a 21-bp sequence TTATGATGGC-GAGGGGGAACT (-1097 to –1077) that specifically binds the nuclear protein. Computer analysis revealed that this sequence contains putative sites for both AP-2 and YY1. Subsequent competition assays with AP-2 and YY1 consensus sequences showed that only YY1 consensus sequence efficiently competed off the binding, suggesting that the nuclear protein binding to this sequence is YY1. Finally, supershift assays with antibody against YY1 confirmed that nuclear protein binding to the 21-bp sequence is indeed YY1. Importantly, mutation of the YY1-binding site resulted in a reduction in RANKL-induced TRAP transcription in RAW264.7 cells, indicating that YY1 is positively involved in RANKL-induced TRAP transcription. In summary, our data have not only established a functional role for YY1 in TRAP expression in osteoclasts but also defined a new transcriptional mechanism by which RANKL regulates gene transcription during osteoclast differentiation.

Disclosures: ***Z. Shi****, None.*

SU237

AZT-Associated Bone Loss in AIDS Therapy. G. Pan*[1], X. Wu[1], O. Mamaeva*[1], M. A. McKenna[1], J. M. McDonald[2]. [1]Pathology, Uni. of Alabama at Birmingham, Birmingham, AL, USA, [2]Pathology, Uni. of Alabama at Birmingham; Veterans Administration Medical Center, Birmingham, AL, USA.

Although highly active anti-retroviral therapy (HAART) has made a very significant advance in the treatment of AIDS, a variety of metabolic complications, including osteoporosis, osteopenia, and osteonecrosis, have been reported to be associated with this therapeutic regimen. To determine the effects of Zidovudine (AZT), a nucleoside reverse transcriptase inhibitor, on bone resorption and osteoclastogenesis, we cultured mouse osteoclast precursors, RAW264.7 cells, or mouse primary bone marrow macrophage-monocyte precursors with and without AZT *in vitro*. AZT significantly increases RANKL-stimulated differentiation of RAW264.7 cells, whereas AZT alone fails to increase osteoclastogenesis. The maximum stimulatory effects of AZT, occurring between 10 and 40 μ M, increases TRAP-positive cells 10-fold over that obtained with RANKL alone ($p< 0.05$, t-test). Similarly, AZT increases relative TRAP activity in the primary bone marrow precursors by 2-fold compared to cells cultured without AZT. AZT is also capable of reducing the amount of RANKL required to induce maximal osteoclast differentiation from 50 ng/ml to 12.5 ng/ml, indicating an increased sensitivity of osteoclast precursors to RANKL. To confirm the effects of AZT on TRAP promoter activation, an early key event in osteoclast differentiation, a luciferase reporter assay containing the TRAP gene promoter was performed. AZT increases luciferase activity by up to 10-fold confirming the stimulatory effects of AZT on RANKL-induced osteoclastogenesis. More importantly, an animal model was generated to investigate the effects of AZT on bone *in vivo*. Mice treated with AZT (10mg/day) for 4 weeks show features of osteopenia as measured by DEXA, the bone mineral density (BMD) decreasing from 0.0462 ± 0.0015 g/cm^2 in controls to 0.0416 ± 0.0001 g/cm^2 in the AZT-treated animals (n=3, $p< 0.05$). The average mineralizing surface in the AZT-treated mice is increased from 1.75 to 4.28 mm, and the bone formation rate from 0.59 to 1.43 mm compared to the control ($p< 0.05$). The number of TRAP positive osteoclasts in tibial sections of AZT-treated mice is increased. In summary, AZT increases osteoclastogenesis and decreases bone mineral density by increasing RANK signaling in osteoclast precursors.

Disclosures: ***G. Pan****, None.*

SU238

Activation of Human Microvascular Endothelial Cells by IL-1 or TNF-α Enhances Transendothelial Migration and Chemokine (SDF-1) Recruitment of Precursors from Human Peripheral Blood that Develop into Osteoclasts. L. Blair*, L. Rothe, P. Osdoby, P. Collin-Osdoby. Department of Biology, Washington University, St. Louis, MO, USA.

Increased osteoclast (OC) bone resorption is prevalent in such pathological inflammatory diseases as rheumatoid arthritis and periodontal disease. OC precursors (pre-OC) are present in the bone marrow and peripheral circulation, from which they may be recruited to bone and develop into resorptive OCs. While much is known about osteoblast/stromal cell regulation of marrow OC development and function, little is understood about how circulating pre-OCs are recruited from peripheral blood into bone and to sites of resorption. We hypothesized that normal or pathological pre-OC recruitment may depend on bone microvascular interactions and particular combinations of chemoattractants, cell adhesion molecules, and matrix degradative enzymes. Therefore, we analyzed IL-1 and TNF-α effects on human microvascular endothelial cells (HMVEC) and their interactions with human peripheral blood monocytes (hPBMC) containing pre-OCs. Cell adhesion studies showed that HMVEC pretreatment (24 h) with IL-1 or TNF-α increased hPBMC adhesion to HMVEC (1 h). Consistent with this, microarray analysis of IL-1 treated (6 h) HMVEC revealed upregulated expression of cell adhesion molecules that could mediate such interactions, including ICAM-1, β1 integrin, and CD44. Previously, we showed that the hematopoietic cell chemoattractant and homing signal stromal-derived factor-1 (SDF-1): 1) increased the chemotaxis of hPBMC which subsequently developed into resorptive OCs following their culture with M-CSF/RANKL, 2) increased pre-OC transcollagen migration via increasing MMP-9 activity, and 3) stimulated the angiogenic-related recruitment of pre-OCs that developed into resorptive cells on bone in vivo. Here, we further showed that hPBMC transmigration through a confluent HMVEC monolayer, alone or in response to an SDF-1 gradient, was enhanced by IL-1 or TNF-α pre-activation (24 h) of HMVEC. Importantly, increases in transendothelial migration of hPBMC led to greater OC development following their culture with M-CSF/RANKL. Previously we found that IL-1 and TNF-α stimulate HMVEC release of multiple OC modulatory factors (IL-1, TNF-α, IL-6, M-CSF, IL-8, PDGF, bFGF) and raise RANKL production by HMVEC to promote human OC formation, resorption and survival. Therefore our findings suggest that inflammatory activation of the microvasculature may lead to increased local OC bone resorption through multiple mechanisms involving stimulation of pre-OC recruitment, adhesion to blood vessels, transendothelial migration, and development into bone-resorptive OCs.

Disclosures: ***L. Blair****, None.*

SU239

Osteoclasts Rely on Lipoproteins to Regulate Cellular Cholesterol Levels – Relationship to Survival and Morphology. E. Luegmayr*, H. Glantschnig*, G. A. Rodan, A. A. Reszka. Bone Biology and Osteoporsis Research, Merck Research Laboratories, West Point, PA, USA.

Growing evidence suggests that abnormal lipid metabolism might be associated with the epidemiological correlation between osteoporosis and atherosclerosis. We demonstrate here that osteoclasts (OCL) depend on lipoproteins to maintain cellular cholesterol levels, which in turn control OCL morphology and survival.

Cholesterol was withdrawn from purified OCL maintained in media containing lipoprotein-deficient FBS (LPDS) by treatment with HDL or methyl-beta-cyclodextrin (MBCD). Rapid removal of cholesterol via MBCD induced OCL, but not osteoblast, apoptosis (>10-fold), with nuclear condensation, caspase activation and actin disruption. Meanwhile, cholesterol withdrawal induced HMG-CoA reductase to synthesize cholesterol in osteoblasts, but not in OCL.

Physiological, gradual removal of cholesterol from OCL with HDL was accompanied by gradual induction of apoptosis (up to five-fold, reaching 50% of OCL at 48 hr). In reciprocal experiments, LDL-mediated cholesterol delivery significantly suppressed spontaneous OCL apoptosis at 72 hr. Thus LDL and HDL have opposing effects on the levels of cellular cholesterol and on OCL survival.

We further examined apoptosis in OCL derived from LDL receptor knockout (-/-) mice relative to the (+/+) background strain (C57BL6). Purified -/- OCL were significantly more susceptible to spontaneous apoptosis, associated with an inability to gather LDL-cholesterol present in FBS.

Prior to purification -/- OCL also showed distinct morphological phenotypes associated with a significant 50% reduction in mean area. The number of large OCL decreased, while small multinucleate OCL increased. Interestingly, the morphology of -/- OCL was restored (2-4 fold) to the +/+ phenotype by daily cholesterol pulsing, using cholesterol-saturated MBCD. Reciprocally, +/+ OCL adopted the -/- morphological phenotype when differentiated in media lacking lipoproteins. This is consistent with the uptake of cholesterol as a primary driver for the phenotype.

In summary, these findings suggest an effect of extracellular cholesterol and lipoproteins on the regulation of OCL formation and function. This relationship might play a role in the epidemiological link between osteoporosis and atherosclerosis.

Disclosures: ***E. Luegmayr****, Merck and Co., Inc. 3.*

SU240

RANKL Induced iNOS/NO Acts as a Negative Feedback Inhibitor During Osteoclastogenesis Via an NF-kB and IFN-β-ERK-Stat1 Signaling Pathway. H. Zheng, X. Yu, P. Collin-Osdoby, P. Osdoby. Department of Biology, Washington University, St. Louis, MO, USA.

RANKL is essential for the formation of resorptive osteoclasts (OCs) via interaction with RANK on precursors to trigger multiple intracellular signal pathways (ERK, p38 MAPK, JNK, NF-kB). Recently, we showed that RANKL induces iNOS mRNA and NO production during OC differentiation and this NO functions as an autocrine negative feedback signal to regulate RANKL-mediated OC development from murine RAW 264.7 cells or primary bone marrow cells. Conversely, inhibitors of iNOS/NO enhanced RANKL-induced OC development and bone resorption. Similarly, RANKL-induced OC formation and resorption was greater in marrow cultures from iNOS -/- compared to iNOS +/+ mice. Here, we investigated signal mechanisms by which RANKL induces iNOS/NO to restrain OC development in RAW cells using inhibitors, western blots, and EMSAs. We found that the NF-kB inhibitor PDTC blocked both OC formation and iNOS/NO induction. In contrast, p38 MAPK (SB20358) inhibition blocked both OC formation and iNOS/NO induction, JNK (SP60025) inhibition blocked only OC formation, and ERK1/2 (PD98059) inhibition blocked only iNOS/NO induction which led to enhanced RANKL-mediated OC formation and resorption. Therefore, p38 MAPK, JNK, and NF-kB, but not ERK, are essential for OC formation. More importantly, ERK inhibition selectively abrogated the NO negative feedback signal pathway triggered by RANKL in OC formation, thereby mimicking the actions of iNOS inhibitors or iNOS -/- deficiency to enhance RANKL-induced OC formation. Because IFNβ was recently shown to be a RANKL-induced negative feedback signal during OC development, and IFNβ can induce iNOS/NO in other cells, we investigated if RANKL induction of IFNβ (and downstream phosphorylation of STAT-1) was involved in the RANKL stimulation of iNOS/NO to restrain OC formation. These studies showed that: 1) RANKL induced IFNβ (by 30') and P-STAT-1 (by 4 h) in RAW cells, 2) RANKL stimulation of P-STAT-1 and iNOS/NO (but not IFNβ) required new protein synthesis (CHX^s), was mimicked by exogenous IFNβ, and was inhibited by neutralizing Ab to IFNβ which enhanced OC formation, 3) NF-kB inhibition blocked RANKL induced IFNβ, and 4) ERK inhibition blocked IFNβ stimulated P-STAT-1 and iNOS/NO (but not NF-kB or IFNβ). These and other findings indicate that the novel autocrine negative feedback pathway triggered by RANKL during OC development involves NF-kB activation, IFNβ induction, ERK activation, phosphorylation of STAT-1, and induction of iNOS and NO production. Specifically interfering with RANKL-mediated IFNβ or ERK activation prevents subsequent iNOS/NO induction and thereby enhances RANKL-induced OC formation and bone resorption.

Disclosures: ***H. Zheng****, None.*

SU241

Molecular, Biochemical and Functional Analysis of the Actin-Binding Site in the B Subunit of Vacuolar H+-ATPase. J. Zuo*[1], S. Chen*[1], M. R. Bubb*[2], E. G. Yarmola*[3], J. Jiang*[4], I. R. Hurst*[1], M. Lu*[5], S. L. Gluck*[3], L. S. Holliday[1]. [1]Orthodontics, University of Florida College of Dentistry, Gainesville, FL, USA, [2]Medicine/Rheumatology, University of Florida College of Medicine, Gainesville, FL, USA, [3]Medicine, University of Florida College of Medicine, Gainesville, FL, USA, [4]Endodontics, University of Florida College of Dentistry, Gainesville, FL, USA, [5]Medicine/Nephrology, University of Florida College of Medicine, Gainesville, FL, USA.

Vacuolar H+-ATPase (V-ATPase) binds microfilaments in a regulated manner in osteoclasts suggesting that the interaction has a physiologic role. The B subunit binds actin and isolated V-ATPase can be competed from microfilaments by an excess of B subunit fusion proteins. The actin binding activity of B subunit maps to amino acids 23-67 of the B1 isoform and 29-73 of B2. High confidence models of the B subunit developed using 3D-PSSM (Imperial College, London) indicate that this region is divided into two surface exposed sections in the region of the B subunit furthest removed from the membrane, with a buried intervening fold. The exposed regions are AA 54-60 and 37-42 in B1. Each contains a number of exposed charged residues that are conserved in the B subunits of higher organisms. Replacement of 49-59 in B1, which has similarity to the actin binding site of profilin and which binds to actin as a 13-mer peptide, with sequence from an Archaebacteria, abolishes the actin binding activity of the resulting fusion proteins without disturbing the predicted structure. Modification of cysteine 40 with n-ethyl maleimide reduces the affinity for actin of a fusion protein containing B1 23-67. A polyclonal antibody against amino acids 1-20 of B1 does not interfere with the actin binding activity of the B1 1-106 fusion protein. Full length wild type B1 or B1 with the 49-59 substitution (B1(49-59)) were constructed and incorporated into an adeno-associated virus vector. By this means high level expression of B1 or the B1(49-59) has been achieved in OPGL-stimulated RAW 264.7 osteoclast-like cells and in mouse marrow osteoclasts. Preliminary data indicate that although B1 is not normally expressed in osteoclasts, it is incorporated into the holoenzyme and is transported to ruffled membranes. Efforts are now underway to characterize the phenotype of osteoclasts containing high levels of the actin binding deficient B1(49-59) construct and compare them with osteoclasts expressing similar levels of wild-type B1.

Disclosures: ***J. Zuo****, None.*

SU242

Role of Phosphatidylinositol 3-kinase (p110 alpha) in Osteoclasts. J. Jiang[1], J. Zuo*[2], Y. Gong*[3], I. R. Hurst*[2], L. S. Holliday[2]. [1]Endodontics, University of Florida, Gainesville, FL, USA, [2]Orthodontics, University of Florida, Gainesville, FL, USA, [3]Pharmacy, University of Florida, Gainesville, FL, USA.

Phosphatidylinositol 3-Kinases (PI 3-Kinases) are enzymes that phosphorylate inositol lipids on the 3-position of inositol headgroup. Signal transduction by PI-3 kinases is known to be crucial for regulating osteoclastic bone resorption. We found that members of three distinct classes of PI 3-kinases were expressed in osteoclasts. The classes differ in structure, substrate specificity and targeting elements. To date, few data are available about the precise role of each type of PI 3-kinase in osteoclasts. In the present work, we investigated the role of a subclass of the class I PI 3-kinase (p110α) in regulation of osteoclasts. We produced a recombinant adeno-associated virus serotype 2 (AAV-2) containing CMV promoter, a 300-base pair reversed 5'-coding region of p110α (p110αAS), internal ribosomal entry site (IRES) and green fluorescent protein (GFP) gene (AAV2p110αAS-GFP). The efficiency of AAV2 gene transfer was examined with control virus (GFP AAV2 virus). Raw 264.7 cells stimulated with OPGL and primary murine osteoclasts were transduced with AAV2GFP. 80%-90% of the total TRAP positive cells were GFP positive after three days of transduction. The transduced cells displayed normal actin ring structure when stained with TRITC conjugated phalloidin. Transduction with p110α antisense virus in OPGL-stimulated Raw 264.7 cells reduced the expression of endogenous p110α compared to cells transduced with GFP virus. P110α AS expression dramatically reduced osteoclast survival after 3 day of transduction. Osteoclasts transduced with p110α AS showed disrupted actin rings. These data suggest that p110α plays an important role in osteoclast survival and cytoskeletal organization.

Disclosures: ***J. Jiang****, None.*

SU243

Receptors for RANKL and M-CSF Are Induced during Monocytic Differentiation of Promonocytic U937 Cells via Protein Kinase Cδ- and p38 MAP Kinase-Dependent Pathway. E. Park[1], H. Kang*[1], K. Kim*[1], J. Choi[2], H. Shin[3], S. Kim[4]. [1]Skeletal Diseases Genome Research Center, Kyungpook National University Hospital, Daegu, Republic of Korea, [2]Biochemistry, Kyungpook National University, Daegu, Republic of Korea, [3]Oral Pathology, Kyungpook National University, Daegu, Republic of Korea, [4]Department of Orthopedic Surgery, Kyungpook National University Hospital, Daegu, Republic of Korea.

Osteoclasts share common precursors with monocyte/macrophage lineage cells. However, molecular mechanisms underlying an osteoclastic commitment of precursor cells are not clear. In the present study, we have investigated signal transduction pathway leading to an osteoclastic commitment of promonocytes by analyzing an expression of RANK and c-fms, critical receptors for osteoclastic differentiation. RANK and c-fms are induced during monocyte/macrophage differentiation of U937 cells stimulated with PMA, alone or in combination with vitamin D3. Analysis of PKC isoforms reveals that most PKC isoforms are down-regulated whereas isoforms of PKCγ, δ and μ are sustained throughout PMA stimulation. MAP kinase family members including ERK, p38 MAP kinase and JNK are also activated by PMA stimulation. These results suggest a possible involvement of some PKC isoforms and MAP kinase families in the expression of RANK and c-fms. Further analyses with pharmacological inhibitors revealed that Gö6983, a specific inhibitor for classical PKCs, PKCδ and γ, significantly reduced an expression of RANK and c-fms. Furthermore, Rottlerin, a PKCδ-specific inhibitor, suppressed a PMA-induced expression of RANK and c-fms, but not that of CD11b, suggesting that PKCδ is involved in the PMA-induced expression of RANK and c-fms. While Gö6983 reduces the activities of both ERK and p38 MAP kinase, Rottlerin specifically suppresses the activity of p38 MAP kinase. Moreover, inhibition of p38 MAP kinase reduces an expression of RANK and c-fms, but not CD11b. Taken together, these results demonstrate that receptors for RANKL and M-CSF induced during monocytic differentiation of U937 cells are dependent on both PKCδ and p38 MAP kinase.

Disclosures: ***E. Park****, None.*

SU244

Eosinophil Chemotactic Factor-L (ECF-L) Enhances RANKL Signaling. H. Y. Chung[1], S. J. Choi[1], G. D. Roodman[2]. [1]Medicine-Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Hematology/Oncology, University of Pittsburgh and Department of Veterans Affairs Medical Center, Pittsburgh, PA, USA.

We recently identified ECF-L as a novel stimulator of osteoclast formation. ECF-L induces osteoclast formation independent of RANKL. Importantly, anti-ECF-L antibody blocks RANKL induced osteoclastogenesis, suggesting that ECF-L is a critical cofactor for RANKL induced osteoclast formation. ECF-L is a chemoattractant for osteoclast precursors, but its other effects on osteoclastogenesis are unknown. To further examine the role of ECF-L in osteoclastogenesis, the effects of ECF-L on intracellular signaling events after binding of RANKL to RANK, including the activation of the transcription factor NF-κB and the stimulation of the protein kinase JNK in osteoclast precursors were evaluated. RAW264.7 cells were treated with RANKL or conditioned media from 293 cells transfected with mouse ECF-L cDNA. ECF-L increased NF-κB binding activity by EMSA analysis after 30 minutes compared to basal levels. Furthermore, increased NF-κB binding activity in ECF-L treated RAW264.7 cells was further enhanced in the presence of low concentrations of RANKL (2.5 ng/ml). JNK activities were then measured using immunoprecipitation and Western blotting in human marrow cells. Human ECF-L increased JNK activity and acted synergistically with RANKL to increase JNK activity. In addition, the ECF-L induced DNA binding activity AP-1 was also increased and enhanced by JNK activity. Taken together, these data suggest that ECF-L supports osteoclast formation not only through chemoattraction for OCL precursors but also stimulation of cellular signaling responses necessary for osteoclast formation. In conclusion, these data demonstrate ECF-L is a previously unknown factor that is a potent mediator of OCL formation that enhances RANK signaling.

Disclosures: ***H.Y. Chung****, None.*

SU245

Lipid Rafts Are Important for the Akt Activation by RANK in Osteoclasts. Z. Lee[1], H. Ha*[1], H. Kim[2]. [1]Microbiol. & Immunol., Chosun University College of Dentistry, Gwangju, Republic of Korea, [2]Cell & Developmental Biology, Seoul National University College of Dentistry, Seoul, Republic of Korea.

TRAF6 and Src have been shown to play crucial roles in osteoclast function and RANK signaling. As Src family kinases preferentially segregate to raft microdomains, we sought to address the potential role of membrane rafts for signaling by RANK/TRAF6 in bone resorption function of osteoclasts. Our findings demonstrate that raft expression increases during the osteoclastogenesis and that TRAF6 is recruited to rafts by RANKL stimulation in osteoclasts. Further, we show that disruption of rafts interfere with a variety of parameters required for osteoclast function and differentiation. These include impeded RANK signaling to Akt, defective actin ring formation and resorption activity of osteoclasts, and the reduced survival of osteoclasts. Overall, our findings demonstrate for the first time, a crucial role for membrane lipid rafts in the function, and potentially differentiation, of osteoclasts.

Disclosures: ***H. Kim****, None.*

SU246

A Voltage-gated H^+ Ghannel, a Powerful H^+ Extruding Pathway, Operates in Murine Osteoclasts Stimulated by Phorbol Myristate Acetate in Association with Cell Acidosis. H. Mori*[1], H. Sakai[1], H. Morihata*[1], J. Kawawaki*[2], H. Amano[3], T. Yamano*[4], M. Kuno[1]. [1]Physiology, Osaka City Univ. Grad. Sch. Med., Osaka, Japan, [2]Central Laboratory, Osaka City Univ. Grad. Sch. Med., Osaka, Japan, [3]Pharmacology, School of Dentistry, Showa University, Tokyo, Japan, [4]Pediatrics, Osaka City Univ. Grad. Sch. Med., Osaka, Japan.

Diverse H^+-translocating mechanisms are involved in regulation of osteoclast functions. Voltage-gated H^+ channels are distinct in their surprisingly high H^+ extrusion rate, ~100 times as strong as the H^+ pumps and transporters, and can compensate for pH imbalance rapidly. The H^+ channel changes its activity by sensing the pH gradient across the plasma membrane (ΔpH), but its physiological relevance to osteoclast functions is unclear. Protein kinase C (PKC) is a strong activator for H^+ channels and modifies various osteoclastic activities. To investigate how the channel operates in functional osteoclasts, the effects of phorbol 12-myristate 13-acetate (PMA) on the H^+ channel was examined in murine osteoclasts generated in the presence of M-CSF/CSF-1 and sRANKL. The H^+ currents and intracellular pH (pH_i) in clamped cells were recorded successfully in the permeabilized-patch. The reversal potential (V_{rev}) was a valuable tool for real-time monitoring ΔpH. PMA (10 nM - 1.6 μM) increased the current density and the activation rate, slowed decay of tail currents and shifted the threshold toward more negative voltages. Inward H^+ currents were often recorded, suggesting that H^+ could enter the cell. In addition, PMA caused a negative shift of V_{rev}, indicating intracellular acidification. The PMA-induced cell acidosis was confirmed using a fluorescent pH indicator (BCECF), which recovered quickly in a K^+-rich alkaline solution probably via the activated H^+ channel. Both cell acidosis and activation of the H^+ channel by PMA were inhibited by staurosporine, a PKC inhibitor. In ~ 80% of cells, the PMA-induced augmentation in the H^+ current remained after compensating for the ΔpH changes, so that ΔpH-independent mechanisms also contributed to the channel activation. There was a correlation between V_{rev} and the membrane potential, indicating that the channel could control the membrane potential. PMA shifted the membrane potential shifted toward V_{rev}. These data showed that activation of PKC provided a functional state favorable for utilizing the H^+ channel, by potentiating the channel activity and lowering the threshold, together with cell acidosis. Enhanced H^+ efflux through the activated H^+ channel may contribute to quick regulation of the pH environments and the membrane potential, to maintain osteoclast functions.

Disclosures: ***H. Mori****, None.*

SU247

Estrogen Modulates RANK-Mediated Signaling in the Rabbit Osteoclast. J. Shyu[1], D. Sung*[1], J. Wang*[1], C. Lin*[2], C. Shih[1]. [1]Biology & Anatomy, National Defense Medical Center, Taipei, Taiwan Republic of China, [2]Microbiology & Immunology, National Yang-Ming University, 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-κB), 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. We therefore examined the possibility that estrogen has its effect on osteoclasts by modulating the RANK-mediated signaling. Using rabbit osteoclast primary culture system, the molecules involved in the RANK-activated signaling was analyzed. Immunoprecipitation and Western blot analysis revealed that both RANKL and estrogen induced increased tyrosine phosphorylation of RANK. Estrogen induced transient increased associations between RANK and TNF-associated factor 6 (TRAF6). Estrogen also induced increased phosphorylation of P65 subunit of NF-κB. These effects of estrogen on RANK signaling appeared to have different time course. Both RANK-TRAF6 association and P65 phosphorylation were peaked at 5 min. The RANK-TRAF2 association was also increased after estrogen stimulation, but it appeared later and persisted for several hours. The increase of P65 phosphorylation also persisted for several hours. Both increases of RANK-TRAF6 association and P65 phosphorylation induced by estrogen were inhibited by PD98059 pretreatment. In conclusion, the current study provided evidences that estrogen modulated RANK-TRAF6- NF-κB signaling pathway, probably through Erk, in rabbit osteoclasts.

Disclosures: ***J. Shyu****, None.*

SU248

Osteoclast Apoptosis Is Driven by Mitochondrial Hydrogen Peroxide-Mediated Oxidative Damage and Degradation of PI3K/MEK/ERK Signaling Pathway Components. E. W. Bradley*[1], S. L. Elfering*[2], C. Giulivi*[3], M. J. Oursler[4]. [1]Biochemistry and Molecular Biology, University of Minnesota, Duluth, MN, USA, [2]Chemistry, University of Minnesota, Duluth, MN, USA, [3]Chemistry, Biochemistry and Molecular Biology, University of Minnesota, Duluth, MN, USA, [4]Biology, Medical Microbiology and Immunology, Biochemistry and Molecular Biology, University of Minnesota, Duluth, MN, USA.

Bone resorbtion levels depend primarily on the number of osteoclasts present. Therefore, factors contributing to the survival of osteoclasts increase the amount of bone loss during both normal and pathological bone turnover. We have examined the mechanism of osteoclast survival using an in vitro generated mouse osteoclast model (mOCL). Survival of mOCLs is dependant on the activation of the PI3 kinase/MEK/ERK pathway. Inhibition of this pathway using kinase specific inhibitors as well as inhibition of protein synthesis leads to decreased mOCL survival. In this study, expression of downstream targets of the PI3 kinase/MEK/ERK pathway was examined for roles in osteoclast survival. Activation of Elk-1, a transcription factor for several Bcl-2 pro-survival genes, was detected five minutes after stromal cell removal. Using cDNA arrays, transcript levels for bcl-xL decreased 30 minutes after mOCL purification, whereas transcript levels for mcl-1 increased. Expression of Mcl-1protein increased 15 minutes after mOCL purification, followed by decreased expression within 45 minutes. Bcl-xL expression was detected at time zero and decreased until no expression was detected within 45 minutes after purification. Decreased levels of AKT, MEK, ERK and total cellular protein have also been observed 60 minutes after mOCL purification, suggesting general proteolysis. In addition, a decrease in the mitochondrial membrane potential and an increase in cytosolic cytochrome c were detected in cultured mOCLs. Evidence supports mitochondrial hydrogen peroxide production drives osteoclast apoptosis. These data include apoptosis repression when FCCP addition decreases mitochondrial hydrogen peroxide production and when supplementation with spin traps sequesters oxygen free radicals. We therefore conclude that the production of mitochondrial hydrogen peroxide is driving apoptosis via oxidative damage, leading to a decrease in downstream PI3 kinase/MEK/ERK targets and decreased osteoclast survival.

Disclosures: ***E.W. Bradley****, None.*

SU249

Exposure to Pasteurella Multocida Toxin Reveals an Inhibitory Role for the Small GTPase Rho in Osteoclast Differentiation. N. W. A. McGowan*[1], D. Harmey[2], G. Stenbeck*[3], A. E. Grigoriadis[1]. [1]Craniofacial Development, Kings College London, London, United Kingdom, [2]The Burnham Institute, La Jolla, CA, USA, [3]University College London, Bone and Mineral Centre, London, United Kingdom.

Rho GTPases belong to the ras superfamily of small GTP-binding proteins that are involved in multiple signal transduction pathways. Rho proteins are involved in the regulation of the actin cytoskeleton, and during the process of bone resorption osteoclasts are critically dependent on rapid cytoskeletal re-arrangements, for the maintenance of the F-actin podosomal ring and subsequent ruffled border formation. However, the role of Rho in osteoclast differentiation is unknown.

In this study osteoclast differentiation was examined in the presence of Pasteurella Multocida Toxin (PMT), a bacterially-produced toxin that causes the porcine bone resorbing disease, atrophic rhinitis. In addition to inducing actin rearrangements through Rho, PMT is a potent mitogen and stimulates phospholipase C, leading to activation of protein kinase C, and increases in inositol phosphates and intracellular calcium. PMT also signals indirectly through the Ras/MAP kinase pathway. Previous work by our laboratory has established that PMT inhibits osteoblast differentiation, in part via activation of Rho and Rho kinase (ROK).

In RANKL- and MCSF-based cultures of murine bone marrow cells or human peripheral blood monocytes, PMT dose-dependently inhibited osteoclast formation and resorption, and pulse studies demonstrated that this effect was manifested predominantly in the early stages of culture. To investigate the signalling pathways responsible for the inhibitory effects of PMT, we used inhibitors. Treatment of murine bone marrow cultures with the ROK inhibitor Y-27632 partially rescued the inhibition in the number of TRAP-positive osteoclasts, whereas Y-27632 alone had little or no effect. These data suggest an important inhibitory role for Rho and its downstream effector, ROK, in osteoclast differentiation. Interestingly, the inhibition of differentiation was in contrast to the effect of PMT on differentiated osteoclasts where treatment of human osteoclast cultures after day 10 failed to inhibit resorption. Rather, preliminary experiments suggest that addition of PMT to mature osteoclasts increased the proportion of F-actin ring-containing vitronectin receptor-positive osteoclasts, with a concomitant increase in resorption. Thus, although Rho activation is apparently required for osteoclast activity, this pathway negatively regulates osteoclast precursor differentiation. Current studies using PMT will dissect the signalling pathways used by osteoclasts during differentiation and bone resorption.

Disclosures: ***N.W.A. McGowan****, None.*

SU250

Characterization of an Anti-rat $\alpha_v\beta_3$ mAb which Blocks Differentiation of Osteoclasts. L. An*[1], S. Langermann*[1], J. Suzich*[2], S. Mao[2]. [1]Cell Biology, MedImmune, Inc., Gaithersburg, MD, USA, [2]Molecular Biology/Biochemistry, MedImmune, Inc., Gaithersburg, MD, USA.

$\alpha_v\beta_3$ integrin is highly expressed on osteoclasts and is crucial for mediating their attachment to bone matrix to allow active bone resorption. We have characterized a rat $\alpha_v\beta_3$-specific mAb, VNR149, and tested its effects on rat tumor cell attachment and osteoclast differentiation *in vitro*. VNR149 binds to the $\alpha_v\beta_3$ integrin of rat and mouse, but not human. Replacement of either rat α_v or β_3 subunit by its human homologue prevents binding of the antibody. This property was used for selection so that VNR149 does not cross react with other rat integrins containing either α_v or β_3 subunit. Binding studies showed that the antibody has a high affinity (K_D = 3 nM) for rat $\alpha_v\beta_3$, and binds mouse $\alpha_v\beta_3$ with an affinity 3-fold lower than that for rat analog. In this study, rat osteoclasts were differentiated *in vitro* from bone marrow cells. The precursor cells were cultured with MCSF and RANKL for 7 days in the presence or absence of VNR149. The osteoclasts were identified as multinucleated, TRAP-positive cells. VNR149 inhibited osteoclast differentiation in a dose-dependent manner, with complete inhibition observed at doses greater than 7 nM. In separate experiments, VNR149 was shown to block the attachment of $\alpha_v\beta_3$-positive rat glioma cells to vitronectin. Approximately 35% less cells became attached after 1 hour in the presence of 0.7 nM of the mAb. Taken together, our results indicate that VNR149 is effective at inhibiting osteoclast differentiation and tumor cell adhesion mediated by $\alpha_v\beta_3$ integrin; thus, it is a useful reagent for studying the role of $\alpha_v\beta_3$ integrin in various rodent models of human diseases.

Disclosures: ***S. Mao****, None.*

SU251

P2Y6 Nucleotide Receptors Signal through Nuclear Factor κB in Rabbit Osteoclasts. J. Korcok*, L. N. Raimundo*, X. Du*, S. M. Sims*, S. J. Dixon. CIHR Group in Skeletal Development and Remodeling, The University of Western Ontario, London, ON, Canada.

ATP and UTP are released from cells at sites of inflammation or in response to mechanical stimuli. Extracellular nucleotides can then act through P2 cell-surface receptors, which are subdivided into P2X (ligand-gated cation channels) and P2Y (G protein-coupled receptors) families. Previous studies by others have identified expression of P2Y1 and P2Y2 receptors in osteoclasts. Moreover, ADP acting through P2Y1 receptors stimulates osteoclast formation and resorptive activity (FASEB J 15: 1139, 2001). RANK ligand is a potent activator of osteoclast formation and bone resorption – effects that are mediated at least in part through activation of nuclear factor κB (NF-κB). The aim of this study was to determine whether P2Y receptors also signal through NF-κB in osteoclasts. Osteoclasts were isolated from long bones of neonatal rabbits. In osteoclasts further purified by micromanipulation, transcripts encoding P2Y1, P2Y2 and P2Y6 receptors were identified by RT-PCR. Immunofluorescence was used to detect the p65 subunit of NF-κB, which upon activation translocates from the cytosol to the nucleus. In control samples, 7 ± 2% of osteoclasts demonstrated nuclear localization of NF-κB. Exposure to the P2Y6-selective agonist UDP (10 μM) for 3 h resulted in nuclear translocation of NF-κB in 34 ± 5% of osteoclasts. In contrast, there was no significant response to the highly selective P2Y1 agonist, 2MeSADP (10 μM) or to the P2Y2 agonists ATP or UTP (10 μM). Thus, NF-κB appears to be activated selectively by P2Y6 receptors. To monitor the concentration of cytosolic free calcium ($[Ca^{2+}]_i$), osteoclasts were loaded with the Ca^{2+}-sensitive dye fura-2. All nucleotides tested (10 μM) induced a transient rise of $[Ca^{2+}]_i$, consistent with the presence of functional P2Y1, P2Y2 and P2Y6 receptors. Moreover, the coupling of multiple P2Y receptors to Ca^{2+} signaling indicates that elevation of $[Ca^{2+}]_i$ alone is not sufficient to activate NF-κB. Pretreatment with osteoprotegerin (OPG, a decoy receptor for RANK ligand) inhibited activation of NF-κB by RANK ligand, but not by UDP, establishing that UDP-induced translocation of NF-κB does not involve RANK ligand. These findings demonstrate that osteoclasts express functional P2Y1, P2Y2 and P2Y6 nucleotide receptors and that, of these receptors, NF-κB is activated selectively by P2Y6. Upon release, ATP and UTP can activate P2Y2 receptors and subsequently breakdown to form ADP and UDP, which can then activate P2Y1 and P2Y6 receptors. NF-κB signaling through P2Y6 receptors may contribute to the regulation of osteoclast formation and activity.

Disclosures: ***J. Korcok****, None.*

SU252

Regulation of Bone Resorption by c-Cbl: Increased Bone Resorption in Osteoclasts Expressing v-Cbl, an Oncogenic Form of c-Cbl , and Inhibition by C-Terminal Mutants. C. Itzstein, T. Miyazaki, W. C. Horne, A. Sanjay, R. Baron. Yale University - School of Medicine, New Haven, CT, USA.

Several studies have indicated that c-Cbl, an adaptor protein with no kinase activity, can be both a positive and a negative regulator of bone resorption. c-Cbl$^{-/-}$ osteoclasts exhibit decreased migration and lack of c-Cbl phosphorylation in src$^{-/-}$ leads to a decrease in bone resorption. Thus, as a positive modulator, c-Cbl forms a tri-molecular complex with Pyk2 and Src in osteoclasts (OCs) promoting OC motility and bone resorption. However, and in contrast, c-Cbl also mediates the down-regulation of several tyrosine kinase receptors, including M-CSF, and non-receptor tyrosine kinases such as Src itself, by promoting their ubiquitination. The structure-function relationship that allows c-Cbl to act both as a positive and a negative regulator of signaling events is however still unclear. Structurally, the N-terminal half of c-Cbl includes a tyrosine kinase binding (TKB) domain and a RING finger, responsible for the ubiquitin-ligase activity of c-Cbl, while the C-terminal half contains a proline-rich region and several regulatory tyrosines, responsible for SH2 and SH3 mediated protein interactions. To further investigate the functional roles of various protein binding domains of c-Cbl, we have expressed myc-tagged wild-type (WT) c-Cbl, the C-terminal half of c-Cbl (Cbl-CT) or c-Cbl containing a mutated Src SH3 binding site (Cbl6PA) or a mutated PI3-kinase binding site (CblY731F) in murine OCLs using the adenovirus system, and evaluated their effects on OC activity in a bone resorption assay. Neither WT c-Cbl nor Cbl-CT affected pit formation. However, over-expression of Cbl6PA or CblY731F inhibited bone resorption by 25% ($P<0.05$) and 70% ($P<0.001$) respectively, demonstrating that interaction between Cbl and Src or Cbl and PI3-kinase play positive roles in OC activity. In contrast, v-Cbl (containing only the TKB domain) increased pit formation by 30% ($p<0.05$), an effect that was even more pronounced in c-Cbl$^{-/-}$ OCLs, increasing pit formation by three-fold. These results suggest that v-Cbl acts in a dominant negative fashion by displacing full-length Cbl proteins from phosphotyrosine binding sites on other key signaling protein. This demonstrates that the N-terminal region of Cbl is involved in the down-regulation of bone resorption. Thus, c-Cbl participates both in stimulatory and in inhibitory regulatory events in bone resorption. Binding of Cbl proteins to specific phosphotyrosines in signaling molecules is required for the down-regulation of resorption-induced mechanisms in OCs, whereas SH2 and SH3-mediated interactions with its C-terminal domain are required for their activation.

Disclosures: ***C. Itzstein****, None.*

SU253

Comparison between 60 Matched Pairs of Postmenopausal African American and Caucasian Women: Analysis of Risk Factors Related to Bone Loss. J. E. Ballard[1], L. S. Wallace[2], D. Holiday*[3], K. Wells*[1]. [1]Health and Kinesiology, University of Texas at Tyler, Tyler, TX, USA, [2]Family Medicine, University of Tennessee Graduate School of Medicine, Knoxville, TN, USA, [3]Biostatistics, University of Texas Health Center at Tyler, Tyler, TX, USA.

The purpose of this study was to compare known or suspected risk factors (gynecological, nutritional, and/or medical variables) related to bone loss between 60 African American (AA) and 60 Caucasian (C) postmenopausal women. The two racial groups had been matched one for one on selective anthropometric variables [age (yrs), standing height (cm), and body weight (kg)] in order to equate age and body size between groups. Information on risk factors was obtained from an orally administered questionnaire and body composition variables (in addition to those used for matching) were assessed by anthropometry and total body DEXA. Four skinfold sites (chest, triceps, mid-axillary, and abdomen) were measured with Harpendon calipers and four body circumferences (chest, forearm contracted, waist, and gluteal) were assessed with a Gulick tape. DEXA whole body measurements were obtained on a Hologic QDR-2000 with software version 7.20. The same technician administered the oral questionnaire to all subjects. Results of the questionnaire revealed that the C women reported significantly higher proportions of alcohol use, family history of broken bones, and a greater utilization of hormones, calcium and vitamins than did the AA women. On the other hand, the AA sample reported a higher proportion who had undergone extensive dental work and a greater numbers who had other diseases (i.e., overactive thyroid, diabetes, rheumatoid arthritis, or kidney stones). On the basis of these data, it was concluded that part of the difference often observed in bone density between AA and C postmenopausal women may be due to lifestyle factors.

Disclosures: ***J.E. Ballard****, None.*

SU254

Knowledge about Osteoporosis in the German Population - Perception and Reality. F. Raue[1], J. Scheld*[2], S. Schmitt[2], B. Tischer*[3]. [1]Practice for Endocrinology & Genetics, Heidelberg, Germany, [2]Procter & Gamble Pharmaceuticals, Weiterstadt, Germany, [3]TNS EMNID, Pullach, Germany.

Awareness for and knowledge about specific diseases, their risk factors, outcome and treatment possibilities are strong factors for the patients support for diagnostic interventions, treatment adherence and long term compliance. However, there is little data on the impact of global or local initiatives on awareness of osteoporosis.

Objective of this research executed by an independent market research institute, TNS EMNID, was to assess awareness, knowledge, attitudes and medication habits regarding osteoporosis in the German population and compare it with the awareness of other common diseases.

A telephone survey was performed in an adult German population between 20 and 80 years in January 2003. 22 mainly closed questions were presented to a representative sample of 2309 Germans in an omnibus study. 53% of the participants were female, 33% aged 50+, 6% had been previously diagnosed with osteoporosis.

Unaided awareness of osteoporosis as being a chronic disease was only 3%, significantly lower than awareness for asthma (27%), diabetes (20%) and similar to the awareness of urinary tract infection (3%). Knowledge about osteoporosis was also rather low, even in the population at highest risk: 55% of the men aged 50+ and 63% of the women aged 50+ perceived the progression of osteoporosis after diagnosis as slow. Only 17% of the men aged 50+ and 12% of women aged 50+ associated an increased risk to die with osteoporosis. Information about illness, progression and mortality risk worsens with increasing age. Only 12% of the population aged 50+ feared to suffer osteoporosis in the future, other diseases like cancer (38%) or cardiac infarction (28%) were perceived to be of greater threat. Consistent with this, 71% of the total population assessed themselves not being at risk for osteoporosis. 37% of the total population and 54% of people aged 50+ suffered from any chronic disease, accompanied by regular intake of medication. Regarding the most convenient dosing frequency, 55% of the total population preferred daily intake vs. 35% weekly. However, for a medication with intake instructions like bisphosphonates, 60% preferred weekly medication vs. 32% for daily intake.

We conclude that ignorance and misinformation about osteoporosis is still common both amongst the total population and the higher risk group of elderly aged 50+. An effective communication framework between physicians, patients and their organisations is required to change this situation. Preference for intake of medications depends on the mode of administration and the habits of the respective patients, with preferences for either daily or weekly intake.

Disclosures: ***F. Raue****, None.*

SU255

Osteoporosis Health Belief Scale: Psychometric Properties by Telephone Administration. S. M. Cadarette, D. E. Beaton*, G. A. Hawker. Health Policy, Management & Evaluation, University of Toronto, Toronto, ON, Canada.

The Osteoporosis Health Belief Scale (OHBS) is a 42-item self-administered questionnaire designed to measure 7 constructs: susceptibility, seriousness, calcium benefits, calcium barriers, exercise benefits, exercise barriers and health motivation. Susceptibility measures the perceived risk of developing osteoporosis; seriousness measures the perceived threat from having osteoporosis; benefits focus on the belief in the effectiveness of specific behaviors to prevent the occurrence of osteoporosis; barriers measure beliefs about the negative components of the behaviors required to prevent osteoporosis; and health motivation measures the tendency to engage in healthy behaviors. Understanding current levels of osteoporosis health beliefs in a population of older women who are entering the highest risk for osteoporosis-related fracture may facilitate the development of interventions to improve osteoporosis prevention. Therefore, we conducted this study to examine the psychometric properties of the OHBS by telephone administration in a cohort of older women. Ethical approval for this study was provided by the Health Sciences II Review Committee of the University of Toronto Office of Research Services (protocol reference # 8376). A convenience sample of 425 women aged 61-93 years (mean=73.6) participating in a longitudinal arthritis study was recruited by telephone. Item clarity was evaluated and 22 additional items were considered to supplement or replace existing scale items. Multitrait scaling techniques and exploratory factor analysis were used to test scale structure. A few modifications to the OHBS scale were suggested based on these data, reducing the scale by 5 items, rewording 1 item and moving 1 item to a different subscale. The modified 37-item OHBS had a 7-factor uncorrelated solution explaining 48% of the model variance with internal consistency ranging from 0.73 to 0.88. In summary, this study found that minor changes to the OHBS results in reduced redundancy and improved internal structure of the scale for telephone administration among women over 60 years of age. However, given that a convenience sample of women participating in a longitudinal study of arthritis was used to test the psychometric properties of the scale, the study should be repeated in other cohorts to confirm these findings. Thus, we recommend that the reworded item: "taking in enough calcium cuts down the chances of getting osteoporosis" be added to the end of the current 42-item OHBS in future telephone administration to permit full confirmatory analysis of both the original and the modified scale.

Disclosures: ***S.M. Cadarette****, None.*

SU256

Low Calcium Intake Magnifies the Bone Loss Seen with Low Dietary Protein Intake in Elderly Men and Women. M. T. Hannan[1], K. L. Tucker[2], B. Dawson-Hughes[2], I. Chazaro*[3], D. Kiel[1]. [1]Harvard Med Sch, Hebrew Rehab Ctr for Aged, Boston, MA, USA, [2]USDA HNRC, Tufts Univ, Boston, MA, USA, [3]BUSPH, Boston, MA, USA.

Our prior work showed that low protein intake resulted in greater bone loss in elderly men and women in the Framingham Study, suggesting that protein intake is important in minimizing bone loss. It is unclear if this effect in elders depends on calcium (CA) intake. Thus, we examined the relation between baseline dietary protein and subsequent 4-year change in femoral bone mineral density (BMD) according to CA levels for 392 women and 224 men from the population-based Framingham Osteoporosis Study. BMD was assessed in 1988-89 and in 1992-93 at the femoral neck (FN), trochanter (TR), and Ward's area, using Lunar DPA & DXA densitometers adjusting for shift in technology using published corrections. Dietary protein was determined using the Willett food frequency questionnaire and expressed as percent of energy from protein (%Protein). We also evaluated the ratio of animal to vegetable protein (A:V Ratio). BMD loss over 4-y was regressed on protein adjusting for baseline age, sex, weight, height, smoking, alcohol use, physical activity, total CA intake, total energy intake and for women, estrogen use. Protein was evaluated as a continuous variable and as quartiles for the whole sample, as well as by total CA intake group (<800 mg/d=low CA, 800+ mg/d=high CA) and the group not using supplemental CA (n=490).

Mean age (SD, range) at baseline was 74y (4.4, 68-90), 18% used supplemental CA, mean protein intake was 68 g/d (23.5, 16-152) and %Protein was 16% (3.3, 8-27). Lower %Protein was significantly related to bone loss (all sites p<0.05) and this association was stronger when limited to subjects with low CA (FN, p=0.03; TR, p=0.02). %Protein quartiles for the whole sample and low CA subset showed again subjects with low CA and low protein intakes had the greatest bone loss (see table, model FN p=0.02, TR p=0.003),. Similar results were seen for those not using CA supplements and at other BMD sites. A:V Ratio showed no relation to bone loss for the whole sample (all p>0.24) or subsets of subjects.
Thus, in elderly men and women, low CA, in addition to low protein intake, leads to even greater bone loss than low dietary protein alone. Our results suggest that protein intake may be even more important for elderly persons with low CA intakes than those with high CA intakes to minimize bone loss.

Adjusted Mean FN-BMD (SD, p-value for quartile comparison) by Quartile of Protein Intake

% Protein Quartiles	Whole Sample n=616	Low CA (<800) Group n=368	High CA (800+) Group n=248
Q1-low	-4.44 (.72, p=0.008)	-4.65 (.86, p=0.004)	-3.60 (1.3, p=0.758)
Q2	-3.57 (.71, p=0.070)	-2.98 (.92, p=0.171)	-4.27 (1.2, p=0.399)
Q3	-2.97 (.73, p=0.258)	-2.77 (.91, p=0.226)	-3.51 (1.3, p=0.752)
Q4-high (referent)	-2.03 (.74, ref)	-1.47 (.91, ref)	-3.08 (1.3, ref)

Disclosures: ***M.T. Hannan****, None.*

SU257

Prevalence and Seasonal Variation of Hypovitaminosis D and its Relationship to Bone Metabolism in Healthy Postmenopausal Hungarian Women. H. P. Bhattoa, P. Bettembuk, A. Balogh. Regional Osteoporosis Center, Department of Obstetrics and Gynecology, University of Debrecen, Debrecen, Hungary.

Hypovitaminosis D can result in low bone mass. The prevalence of hypovitaminosis D has public health implications, especially where data are lacking. Since diet and sunlight are the two sources of vitamin D, the results obtained in one geographical region may not be universally applicable.
The aim of this study is to characterize the prevalence and seasonal variation of hypovitaminosis D and its relationship to parathyroid hormone (PTH), dietary calcium intake, biochemical markers of bone turnover and BMD at the L2-L4 lumbar spine (LS) and the femur neck (FN) in healthy community dwelling postmenopausal women in Hungary.
We determined serum levels of 25 hydroxyvitamin D (25-OH-D), PTH, osteocalcin (OC) degradation products of C-terminal telopeptides of type-I collagen (CTx), dietary calcium intake and BMD at LS and FN in 319 ambulatory postmenopausal women.
The prevalence of hypovitaminosis D (serum 25-OH-D ≤ 50 nmol/l) was 56.7%. On comparing the patients with normal and low 25-OH-D, significant difference was found in age (67.3 ± 9.9 years vs. 61.6.3 ± 8.5 years; $p < 0.001$), FN BMD (0.744 ± 0.125 gm/cm^2 vs. 0.802 ± 0.123 gm/cm^2; $p < 0.001$) and dietary calcium intake (607.9 ± 233 gm/day vs. 714.4 ± 199.4 gm/day; $p < 0.001$). Osteoporotic patients had a significantly lower 25-OH-D (37.6 ± 19.8 nmol/l vs. 56.4 ± 24 nmol/l; $p < 0.001$) and dietary calcium intake (781.2 ± 164.3 mg/day vs. 519.2 ± 244.5 mg/day; $p < 0.001$). After controlling for all other variables 25-OH-D was found to be significantly associated with age, the average hours of sunshine in the three months prior to 25-OH-D level determination and dietary calcium intake ($R^2 = 0.190$; $p < 0.001$). For FN BMD significant independent predictors were age, body mass index, 25-OH-D and dietary calcium intake ($R^2 = 0.435$; $p < 0.001$). The prevalence of hypovitaminosis D during spring, summer, autumn and winter was 71%, 46.3%, 49.4% and 56.7%, respectively. There was significant seasonal variation in 25-OH-D, PTH, OC, calcium intake and FN BMD.
There is a high prevalence of hypovitaminosis D in the healthy postmenopausal Hungarian women, and FN BMD is associated with serum 25-OH-D and dietary calcium intake. We believe that universal vitamin D and calcium supplementation, especially in winter, can be beneficial and cost effective at a population level.

Disclosures: ***H.P. Bhattoa****, None.*

SU258

Green Tea Drinking Is Associated with Increased Bone Mineral Density. S. Muraki*[1], S. Yamamoto[2], H. Ishibashi*[2], T. Horiuchi*[2], T. Hosoi*[2], T. Suzuki*[3], H. Orimo[2], K. Nakamura[1]. [1]Orthopaedics, Tokyo University School of Medicine, Tokyo, Japan, [2]Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan, [3]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.

Tea drinking is associated with increased bone mineral density (BMD), as demonstrated by previous studies, suggesting that flavonoids, contained in tea, may partly account for it. However, to the best of our knowledge, this study serves as the first to investigate the relationship between consumption of green tea, which also contains flavonoids, and BMD. The aim of this study is to determine whether green tea drinking is associated with increased BMD. This study included six hundred and fifty-five women aged 60 years and above (mean age 71.6 psul/minus 7.6 years) visiting the Osteoporosis Outpatient Clinic in Tokyo Metropolitan Geriatric Medical Center. At entry to this study, body height and weight were measured, and body mass index (BMI) was calculated. Subjects were interviewed their lifestyles by means of a questionnaire which includes consumption of 10 dietary items such as green tea, milk, cheese, yogurt, fish, vegetable, tofu, natto, meat and coffee as well as their history of smoking, alcohol consumption, their level of physical activity, and the use of osteoporosis medicaton. For each dietary item, subjects were divided into two groups: consuming five or more days per week, and less than five days per week. BMD of lumbar spine, serum calcium, serum phosphorus, serum parathyroid hormone, serum alkaline phosphatase, serum osteocalcin, serum 1,25-dihydroxyvitamin D and urine deoxypyridinoline were measured. Statistical analysis was performed using a non-paired t test and multiple regression analysis. Among six hundred and fifty-five subjects, six hundred (91.6%) consume green tea five days or more per week and their mean BMD was 0.808±0.199 g/cm^2, and fifty-five (8.4%) consume green tea less than five days per week and their mean BMD was 0.738±0.174 g/cm^2. The BMD of the former subjects was significantly higher than the latter. The correlation between green tea drinking and BMD was independent of age, BMI, other dietary items, their history of smoking and alcohol consumption, their level of physical activity and the use of osteoporosis medication. No significant correlation was observed between green tea drinking and any serum or urinary levels. Estrogenic effect induced by flavonoids or apoptosis of osteoclasts induced by (-)-epigallocatechin-3-gallate, one of flavonoids, may account for the significant increase of the BMD. We conclude that green tea drinking is associated with increased BMD among elderly women.

Disclosures: ***S. Muraki****, None.*

SU259

Carbonated Beverage Consumption and Bone Mineral Density. K. L. Tucker[1], L. Troy*[1], K. Morita*[1], L. A. Cupples*[2], M. T. Hannan*[3], D. P. Kiel*[3]. [1]USDA HNRCA Tufts University, Boston, MA, USA, [2]Boston University, Boston, MA, USA, [3]Hebrew Rehab Center for Aged, Boston, MA, USA.

Soft drink consumption has been thought to have negative effects on BMD, but studies have shown mixed results. Carbonated soft drinks often displace milk in the diet and introduce phosphoric acid (H3PO4) without calcium. Since the phosphorus content of regular cola is 44-62 mg, and of diet cola 27-39 mg, per 12 oz serving, while most other carbonated beverages contain no phosphorus, we hypothesize that consumption of these specific soft drinks would be associated with lower BMD in adult participants in the Framingham Offspring Study. Valid dietary data and BMD measurements were collected for 1672 women and 1148 men from 1996 to 2001. BMD was measured at the spine and 3 hip sites using a Lunar® DPX-L. Dietary intake was assessed with a semi-quantitative food frequency questionnaire that specifically queried respondents on the number of servings of cola and other carbonated beverages consumed, and differentiated between regular, caffeine-free and diet beverages. We regressed each BMD measure onto various measures of soft drink consumption, adjusting for BMI, height, age, energy intake, physical activity score, smoking, alcohol use, use of osteoporosis medication, use of calcium or vitamin D supplements, intake of calcium and vitamin D from diet and, for women, menopause status and estrogen use. There were no significant relationships between total carbonated beverage consumption or non-cola carbonated beverage consumption and BMD at any site. Women, but not men, consuming more than three servings of cola (all types)/d had significantly lower BMD at each of the three hip sites, relative to those consuming less than one serving/d: 2.3% lower at the trochanter (p=0.05), 3.3% lower at the femoral neck (P<0.001), and 5.1% lower at Ward's area (P<0.0005). For the spine those with the highest cola intake had BMD 1.2% lower than those consuming less than one serving/d, but this was not significant. This same pattern of results was generally seen for non-caffeinated and diet cola. Adjusting for caffeine did not change results and there was no significant interaction with calcium intake. These results suggest that cola, but not other carbonated soft drink consumption, contributes to lower BMD in adult women, after adjusting for calcium and caffeine intake. Because similar results were seen with diet and non-caffeinated cola, these associations may be due to the phosphoric acid content of cola.

Disclosures: ***K.L. Tucker****, None.*

SU260

Cortical Bone Response to Long-term Smoke and Ethanol Exposure. D. M. Cullen[1], J. Carter*[1], S. Morgan*[1], M. Gentry-Nielsen*[2], M. P. Akhter[1]. [1]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [2]Medical Microbiology, Creighton University, Omaha, NE, USA.

Smoking and drinking behavior often coincide and have been associated with poor bone mass. In human studies, the effects of these activities can not be separated. In a previous study we showed negative effects of 36% EtOH on cortical bone formation. In this study we examined the dose response of EtOH combined with smoking. Young male Sprague-Dawley rats (120g, n=96 or 12/group) were either Nonexposed or Smoke Exposed to mainstream and sidestream smoke for an hour (30 cigarettes) twice/day weekdays and once/day weekends for 12 weeks. For the last 5 weeks, the Nonexposed and Exposed groups were randomized to liquid diets with 0, 16, 26, or 36% of the calories from EtOH. Bone formation was labeled with calcein (6mg/kg). Tibiae were dehydrated, embedded in methylmethacrylate, and sectioned at 70mcm. Tibial periosteal and endocortical surfaces were measured by histomorphometry for mineralizing surface (MS), mineral apposition rate (MAR), bone formation rate (BFR), resorption surface and intracortical bone formation. Difference due to Smoke and EtOH were tested by two-factor ANOVA and reported below as mean (SD).
Smoke effects were seen on the periosteal surface where MS and BFR were elevated in smokers. A similar pattern was seen for intracortical MS in smokers. There were no smoke effects seen on the endocortical surface. Although no differences in periosteal resorption surface were detected, the increase in intracortical MS suggests that elevated formation may be secondary to resorption. Smoking had no effect on periosteal MAR. Alcohol effects were seen only on the endocortical surface. In both smokers and nonsmokers, 36% EtOH suppressed MS, MAR, and BFR. There were no effects of either 16% or 26% EtOH on bone formation parameters. There was no interaction between smoke and alcohol. In conclusion, in young growing male rats smoking stimulated periosteal bone formation, while high alcohol consumption suppressed endocortical formation.

Trt	Peri MS[a]	Peri MAR	Endo MS[b]	Endo MAR[b]
Non- 0%	51 (18)	1.45 (0.28)	31 (14)	1.51 (0.35)
Non-16%	44 (13)	1.38 (0.14)	34 (9)	1.52 (0.26)
Non-26%	47 (14)	1.49 (0.20)	35 (6)	1.61 (0.41)
Non-36%	48 (15)	1.70 (0.32)	21 (10)	1.22 (0.36)
Exp- 0%	56 (18)	1.51 (0.15)	34 (9)	1.77 (0.34)
Exp -16%	62 (14)	1.43 (0.18)	32 (7)	1.68 (0.45)
Exp -26%	56 (13)	1.49 (0.30)	32 (10)	1.68 (0.51)
Exp -36%	46 (20)	1.45 (0.32)	16 (10)	1.22 (0.30)

[a]dif Nonexposed vs Exposed P=0.02; [b]dif Chow vs EtOH P<0.001

Disclosures: ***D.M. Cullen****, None.*

SU261

Bone Density Measurement Positively Influences Postmenopausal Women to Improve Osteoporosis Prevention Habits. C. S. Cowgill, D. Krueger, S. Zeldin*, N. Vallarta-Ast, N. Kaech*, K. Hansen, M. Drezner, N. Binkley. Osteoporosis Clinical Research Center and Research Program, University of Wisconsin, Madison, WI, USA.

Results of the Women's Health Initiative prompted many postmenopausal women to discontinue estrogen, thereby ending effective bone preserving therapy. However, controversy exists regarding the need to measure bone mineral density (BMD) after discontinuing estrogen. We hypothesized that BMD measurement will encourage postmenopausal women to increase bone prevention activities or seek pharmaceutical intervention when appropriate.

Community-dwelling postmenopausal women were invited through newspaper advertising to have a free BMD measurement. Proximal femur and lumbar spine BMD was measured by dual x-ray absorptiometry in 215 women utilizing GE Lunar DPXIQ or Prodigy densitometers. All participants discussed their results with a physician or nurse practitioner immediately after the scan; osteoporosis prevention or intervention was suggested as appropriate. Subsequently, 75 days after their BMD test, a 25-question survey was mailed to the women who agreed to be contacted. Information on what changes, if any, these women implemented is reported from 50 completed surveys received from the 74 women contacted to this time. The survey consisted of questions about osteoporosis related lifestyle and overall health habits before and after their "DXA Day" scan. Of 50 responders, 17 had normal BMD, 26 were osteopenic and 8 osteoporotic. 90% of these women correctly remembered their BMD classification.

When asked why they elected to have a BMD measurement, 73% listed concern for personal health and 48% because they recently stopped estrogen. Lifestyle changes were made by 90%; specific interventions included increasing exercise (48%) increasing calcium or vitamin D (42%/36%) reducing caffeine intake (28%) seeking medical treatment (20%) and discontinuing smoking (4%). Of the 45 women reporting lifestyle changes, 47% made unsolicited comments indicating that the interaction with a clinician was important.

In conclusion, BMD measurement immediately followed by osteoporosis education is effective in motivating 90% of women to favorably alter lifestyle factors which reduce osteoporotic fracture risk. Perhaps osteoporosis education should become part of routine bone mass measurement.

Disclosures: ***C.S. Cowgill****, None.*

SU262

Prospective, 2-Year Study in Postmenopausal Women Reveals Negative Association of Vitamin A and Bone Mineral Density of Various Skeletal Sites. J. Z. Ilich[1], R. A. Brownbill[1], H. C. Furr*[2]. [1]School of Allied Health, University of Connecticut, Storrs, CT, USA, [2]Craft Technologies, Inc., Wilson, NC, USA.

Recent epidemiological studies suggest that higher vitamin A intake is associated with lower BMD and higher risk of hip fractures. It is not clear what level of vitamin A intake becomes detrimental for bone health. The purpose of this study was to evaluate relationship between dietary vitamin A and BMD of various skeletal sites in over 100 postmenopausal women followed for 2 y. Subjects were generally healthy, free of chronic diseases or medications known to affect bone (including HRT) and participants of a larger clinical trial examining the effect of sodium (Na) on bone. BMD at various skeletal sites was measured by LUNAR DPX-MD and dietary intake was assessed by 3-day dietary record, both at baseline and 6,12,18 and 24 months into the study. The diets were analyzed using Food Processor® and mean daily intake, including total energy and all other nutrients was calculated. The intake of supplements was recorded as well, and included in the nutrient analysis. Vitamin A was evaluated both as continuous and categorical variable, the latter one by stratifying subjects below and above the median of intake. Repeated measures ANCOVA (adjusted for age, BMI, energy, and Ca, Na intake) were used to compare groups over time. Multiple regression models were utilized at each time point to assess relationships between vitamin A metabolites and BMD or BMC.

The average intake of vitamin A (diet and supplements) in our population at each visit was above the tolerable upper limits (10000 IU/day), with over 50% of subjects taking vitamin A supplements (from 1000 to 20000 IU/day) throughout the study period. The average cumulative intake at 24 months was 10034±4352 IU/day. The results reveal that higher intakes of vitamin A were significantly associated with decreased BMD of the femoral shaft and total femur, as well as total body and forearm. This trend was noticeable at each time point and as a cumulative effect throughout 24 months of follow-up.

Based on our data, intakes of vitamin A above 9000-10000 IU/day, may be detrimental for bone health and elderly women should use caution when selecting/consuming vitamin A supplements. Several foods, such as milk, cereals, and some juices are currently fortified with vitamin A and their re-evaluation may be warranted.

Disclosures: ***J.Z. Ilich****, None.*

SU263

Relationship of Life Style, Dietary Factors, Serum PTH and 25-Vitamin D_3 Level with Lumbar Spine BMD in Premenopausal and Postmenopausal Women in Korea. S. K. Lee[1], I. S. Yeo*[2], K. S. Jeon*[2], S. W. Kim*[3], H. W. Baik*[1], C. Kim*[1], J. K. Oh*[1], K. S. Park*[1]. [1]Eulji University School of Medicine, Daejeon, Republic of Korea, [2]Eulji University Hospital, Daejeon, Republic of Korea, [3]Food and Nutrition, Joongbu University, Daejeon, Republic of Korea.

Bone mineral density (BMD) is determined by the combined effects of genetic factors and environmental factors such as life style and dietary facotrs. The present study was to evaluate the relationships of life style, dietary factors, serum intact PTH (iPTH) and 25-vitamin D_3 (25-Vit D_3) level to lumbar BMD in Korean women. We investgated 95 premenopausal (PRE) and postmenopausl (POST) women who had no confounding diseases except hypertension, visiting Eulji University Hospital, South Korea in Winter. We used Questionnaire about physical activity, quality of life (QOL), food habit. We measured various blood parameters, serum iPTH & 25-Vit D_3 level and spine L_1-L_4 BMD using DXA. Mean (±SD) of each variable in PRE normal BMD group and PRE osteopenia & osteoporosis group was Age (42±7 vs 43±7 yrs), Height (158±6 vs 153±6 cm), Weight (58±6 vs 54±6 kg), serum iPTH (28.8±9.3 vs 40.8 ±22.7 pg/mL) and 25-Vit D_3 (59.5±18.0 vs 53.5±22.0 nmol/L). There was a significant difference only in height, but no difference in physical activity score, QOL score, food habit score, smoking, alcohol intake, dietary calcium intake, Ca/P ratio in diet between PRE normal BMD and PRE osteopenia & osteoporosis group. Mean (±SD) of each variable in POST normal BMD, POST osteopenia and POST osteoporosis group was Age (56±5 vs 58±6 vs 62±7 yrs), Height (157±4 vs 154±4 vs 151±4 cm), Weight (68±10 vs 62±10 vs 57±9 kg), Fat mass (24±7 vs 21±6 vs 19±6 kg), Menopause Duration (5±5 vs 10±7 vs 13±8 yrs), serum iPTH (35.0±3.1 vs 31.7±11.3 vs 35.9±17.7 pg/mL), serum 25-Vit D_3 (94.0±32.3 vs 78.8±21.8 vs 62.3±20.8 nmol/L). There were significant differences in age, height, weight, fat mass, menopause duration, serum 25-Vit D_3 between POST normal BMD and POST osteoporosis group. However, there were no significant differences in life style & dietary factors including dietary calcium intake, Ca/P ratio in diet among POST three groups. Correlations of BMD with age, height, weight, fat mass, menopause duration, serum 25-Vit D_3 were significant in POST three groups. In conclusion, life style & dietary factors, serum iPTH level had no relationships to lumbar BMD in this limited Korean women. Age, body composition, menopause duration and serum 25-Vit D_3 level had relationships to lumbar BMD only in postmenopausal women. Subjects in this study had relatively low calcium intake, low Ca/P ratio in diet, irrespective of lumbar BMD. Population-based study and genetic study is needed.

Disclosures: ***S.K. Lee****, None.*

SU264

High Vitamin A Intake Is Not Associated with Low Bone Mineral Density in Older Men. K. L. Stone[1], T. Blackwell*[1], E. S. Orwoll[2], J. A. Cauley[3], E. Barrett-Connor[4], D. Sellmeyer[1], S. R. Cummings[5]. [1]University of California, San Francisco, 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, San Diego, CA, USA, [5]San Francisco Coordinating Center, San Francisco, CA, USA.

Vitamin A in high doses stimulates bone resorption and inhibits bone formation. However, epidemiologic studies of the relationship between Vitamin A intake and bone mineral density (BMD) or fracture risk have been conflicting. Similar studies based on serum retinol concentrations are also conflicting.

We tested the relationship between intakes of Vitamin A, retinol and beta-carotene and BMD among older men participating in the multi-center Osteoporotic Fractures in Men (MrOS) Study. During the baseline examination, a 70-item food frequency questionnaire (Block Dietary Data Systems) was administered in 5995 men aged 65 and older. BMD of the whole body, total hip and total spine were assessed by DXA (Hologic QDR 4500W). Food questionnaires were analyzed to obtain estimated daily intake of nutrients from both diet and supplements. 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, health status, calcium and vitamin D did not change the results. As shown in the table below, there is a trend towards increasing total hip BMD across levels of intake of dietary vitamin A and beta-carotene. This trend becomes statistically significant for total intake of Vitamin A (diet + supplement): men with the highest intakes have the highest BMD (p=.003). On the other hand retinol intake shows no relationship with BMD. Results are similar for other BMD sites, or when analyzed as dietary intake excluding vitamin A supplement users.

Adjusted mean total hip BMD (g/cm2) by levels of dietary intake.

Daily Intake	Mean (SD)	Low	Med	High	P(trend)
Vitamin A (IU)	10597 (8567)	0.954	0.958	0.962	0.22
Retinol (ug)	471 (319)	0.958	0.958	0.958	0.99
Beta-carotene(mg)	3828 (3376)	0.954	0.958	0.962	0.19

We found no evidence of lower BMD among men consuming the highest amounts of Vitamin A. Although vitamin A consumption may be lower in our study population than in others, our highest category of intake is similar to others in which lower BMD has been reported. It remains to be seen whether higher vitamin A intakes will be associated with subsequent rates of bone loss, or fracture risk.

Disclosures: ***K.L. Stone****, None.*

SU265

Type 2 Diabetes andNon-Traumatic Fractures in Older White and Black Men and Women. E. Strotmeyer[1], J. Cauley[1], A. Schwartz[2], H. E. Resnick*[3], D. C. Bauer[2], F. Tylavsky[4], N. De Rekeneire*[5], T. Harris*[5], A. Newman*[1]. [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of California, San Francisco, CA, USA, [3]MedStar Research Institute, Hyattsville, MD, USA, [4]University of Tennessee Health Science Center, Memphis, TN, USA, [5]National Institute on Aging, Bethesda, MD, USA.

Older white women with type 2 diabetes may have increased fracture rates despite higher bone mineral density (BMD) but little information exists for men and older black adults. The Health, Aging, and Body Composition Study included white and black, physically able, men and women age 70-79 years, followed for health events including incident non-traumatic fractures verified by radiology reports. Falls in year prior to baseline were assessed by self-report. BMD, total fat and total lean mass were measured with DXA (QDR 4500A, Hologic Inc, Bedford, MA) and visceral fat area by CT scans. Diabetes was defined as reporting diabetes, diabetes medication use, or fasting glucose of ≥126 mg/dl. 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 (mean age 74±3 years; 49% men; 42% black), 566 (19%) had diabetes at baseline. Participants with diabetes were more likely men (57% vs. 47%; p<0.001) and less likely white (43% vs. 62%; p<0.001) than those without diabetes. Diabetic participants had higher total hip BMD than non-diabetic participants (0.96±0.17 vs. 0.87±0.17 g/cm^2, p<0.001). A total 164 non-traumatic fractures were observed in 4.5±1.1 years. Non-traumatic fracture incidence was 11.7 per 1000 person-years for those with type 2 diabetes and 12.3 for those without diabetes (p=0.80). Race and gender specific rates are shown below.

Non-traumatic fracture incidence/1000 person-years

	White men	Black men	White women	Black women
Type 2 diabetes	7.6	4.4	23.5	17.8
No diabetes	8.6	3.9	22.7	9.6

Diabetic black women had almost a two-fold higher incidence of fractures than non-diabetic black women (p=0.07). In cox regression models controlled for gender, race, site, age, hip BMD, falls, weight change history, HbA1C, diabetes and bone-active medications, visceral fat, total fat and lean mass, baseline diabetes was significantly related to non-traumatic fractures (RR=1.6; 95%CI: 1.1-2.5). Type 2 diabetes was independently associated with a 60% increased risk of fracture in this cohort of white and black men and women despite higher BMD in diabetic participants at baseline.

Disclosures: ***E. Strotmeyer,*** *None.*

SU266

Assessment of Women after Colles Fracture for Osteoporosis and Hip Fracture Risk using the 'Black Fracture Score'. L. Dolan*[1], S. Califf*[2]. [1]Rheumatology, Queen Elizabeth Hospital, Woolwich, London SE10, United Kingdom, [2]Rheumatology, Queen Elizabeth Hospital, Woolwich, London, United Kingdom.

Colles Fractures are early low impact fractures and a 'red flag' for identifying osteoporosis. Fracture clinics have been poor at this. We use Fracture Intervention nurses to identify and arrange DEXA on these patients.

This study assesses whether Colles patients are at imminant risk of more severe fractures. The 'Black Fracture Score'[1] is a well validated tool to assess future hip fracture risk, based on age, previous fracture, maternal hip fracture, smoking and inability to rise from a chair.

Method. Sequential women attending the fracture clinic at Queen Elizabeth Hospital were identified by a fracture nurse and referred for DEXA. They completed a risk factor questionnaire. The 'Black fracture score' was calculated.

Results; 118 women with Colles fracture were identified over 6 months. Median age 67 yrs, range 35-83yrs. 97.5% Caucasian. 36.4% had spine BMD T score <-2.5. 37.2% had spine BMD T score <-2.5 or z <-1. 17.7% had hip BMD T score <-2.5. 18.6% had hip BMD T score <-2.5 or z <-1. 40% satisfied hip or spine BMD criteria for treatment. Risk factors were early menopause (33%), low BMI (8.4%), previous falls (8.4%), use of arms to stand from chair (36.4%), smoking (18.6%). Few had previous major fractures (4 hip, 2 pelvic, 4 vertebral) but 26% had other peripheral fractures. Previous use of osteoporosis treatment was low. Black fracture score showed 33 had a raw score of ≥ 5 (28%)(ie 5yr hip# risk of >8.3%); 22 had fracture score including BMD of ≥ 8 (18.6%) (ie 5yr hip # risk of >8.7%) .

Age	**<50**	**50-64**	**65-74**	**>75yr**	**p**
Number of women	5	46	39	28	
Raw Black fracture score > 5	0	5 (11%)	6 (15%)	22 (78%)	<0.0001
Black fracture score inc hip BMD > 8	0	1 (2%)	3 (7%)	15 (53.5%)	<0.0001
Hip T < -2.5	1 (20%)	3 (6%)	5 (13%)	9 (32%)	<0.004
Spine T < -2.5	2 (40%)	12 (26%)	13 (33%)	12 (43%)	ns

P = comparison of >75 yr with rest, using Chi sq test

Comment: Bone densitometry in Colles patients identified a significant number satisfying BMD criteria for treatment (40%) and supports a strategy of case finding by a fracture intervention nurse. Few had hip or vertebral fracture, confirming Colles fracture as an early low-trauma fracture. More women with colles # have reduced spine BMD than hip. The discrepency between the raw score and that with may reflect a tendency to spine osteoporosis, so fewer are at risk of hip fracture. Imminent fracture risk increases with age, whilst low spine BMD incidence is stable.

Reference; [1] D Black, Osteoporosis Int. 2001 12 519-28

Disclosures: ***L. Dolan****, None.*

SU267

Impact of Height Loss Due to Vertebral Fractures on Body Mass Index. K. S. Davison*[1], K. Siminoski[2], C. Chik*[3], H. Jen*[4], R. Warshawski*[4], K. Lee*[4]. [1]Medicine, McMaster/Laval Universities, Hamilton/Quebec City, ON, Canada, [2]Radiology and Medicine, University of Alberta, Edmonton, AB, Canada, [3]Medicine, University of Alberta, Edmonton, AB, Canada, [4]Radiology, University of Alberta, Edmonton, AB, Canada.

Body mass index (BMI; weight in kg divided by the square of height in m) is frequently used to define clinical obesity. Vertebral fracture, most often due to osteoporosis, can result in height loss and a falsely elevated BMI. The objective of this investigation was to determine the effect of vertebral fractures on BMI (1) theoretically, by modeling, and (2) empirically, by assessing height loss attributable to vertebral fracture to determine the actual effect on BMI. Empirical data was derived from the study of 458 women (mean age 56 years) referred for osteoporosis assessment. Height loss was calculated as the difference between recalled tallest height and current height (by stadiometer). Lateral radiographs of the thoracic and lumbar spine were assessed by quantitative morphometry (T4-L4) with fractures defined as reduction in vertebral height ratio of >20%. The amount of height loss attributable to vertebral fractures was determine by linear regression modelling.

The theoretical relationship between percent change in height and percent change in BMI can be described by: ΔBMI% = {(1/[1-(0.01 x Δheight%)]squared)-1} x 100. While the relationship is curved, height loss up to 5% leads to a percentage increase in BMI that is about 2.1 times the percentage loss of height.

Among the study population, 34.3% had one or more vertebral fractures. After correction for height loss due to aging, each fracture was associated with a height loss of 1.0 cm (r = 0.56, p<0.001). Thoracic vertebral fractures led to height loss of 0.76 cm (r = 0.33, p<0.001; corrected for age and presence of lumbar fracture), while each lumbar vertebral fracture produced height loss of 1.5 cm (r = 0.36, p<0.001; corrected for age and presence of thoracic fracture). When the calculated percent height loss per fracture was applied to the average height of the non-fractured patients (164.3 cm), the theoretical percent change in BMI ranged from 1.2% for one fracture to 12.1% for nine fractures. The increase in BMI for a patient with two vertebral fractures would be 2.5% on average, 1.9% if both fractures were thoracic, or 3.7% if both fractures were in the lumbar spine.

We conclude that (1) height loss due to fractures can significantly alter apparent BMI, (2) that the average height loss per fracture is 1.0 cm; per thoracic fracture is 0.76 cm; and per lumbar fracture is 1.5 cm, and (3) patients with known vertebral fractures should have their heights corrected for loss due to fractures, and corrected height should be used to calculate a corrected BMI.

Disclosures: ***K.S. Davison****, None.*

SU268

Evaluation of Mechanical Competence of Radius in Women with Colles Fracture. P. Pludowski*[1], R. Bienkowska*[2], R. S. Lorenc[1]. [1]Dept. of Biochemistry and Exp. Medicine, Children's Memorial Health Institute, Warsaw, Poland, [2]Szpital Kolejowy, Warsaw, Poland.

Distal radius was evaluated with use of pQCT concerning bone mineral content, density, geometry and bone strength aimed to discriminate between fractured and non-fractured individuals. The study population comprised 76 wrist fractured female aged 46-70 years and 144 healthy female the same ages. Using logistic regressions, we tested how well the data approached the probability of a given individual to belong to fractured or non-fractured group. Odds ratios (OR) were derived from the data corresponding to the inflexion points of logistic curves. ROC was performed to calculate AUC and "optimal cut off" values, leading to evaluate the most efficient discriminator of fractured subjects. The results of logistic regressions (Chi-square, p values, OR) and ROC (AUC, cut off values) are presented in decreasing order of discriminatory power in Table 1 and as follows: (pQCT measured; Chi-square; p; OR; AUC%; cut off;) (TotalBMD; 9.8; <0.01; 1.72; 63.2; 347.3; CorticalBMD; 4.5; <0.05; 1.87; 55.2; 571.4; Trabecular area; 1.8; ns; -; 52.9; 129.7; Periosteal circumference; 0.3; ns; -; 46.5; 62.3; Total area; 0.3; ns; -; 46.4; 296.0). As shown, indicators of mineral content ranked higher than respective volumetric bone densities. Within each type of bone (total, trab, cort) the assessments of bone mass provided higher chi-squares indicating better-adjusted logistic curves, higher OR and larger AUC than volumetric density. The geometric indicators, except Cortical area, did not discriminate fractured subjects. The SSI-X, SSI-P ranked higher than mass and density parameters itself (except Trabecular), indicating that compilation of structural distribution (CSMI) and vBMD into single number, as SSI, provided valuable estimation of bone strength. The results of logistic regressions and ROC pointed out, that mass and density indicators of Trabecular compartment were the most efficient discriminators between fractured and non-fractured women. It seems to be surprising, because long bone fractures are caused by stresses on the periosteal surface. However, the large proportion of trabecular bone in distal radius probably influences the mechanical competence of bone by supporting the cortical shell. It can be concluded that pQCT offer significant informations of mechanical status of human radius.

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pQCT measured	Chi-square; p	OR	AUC%	cut off
Trabecular Cont, mg	50.3 ; <0.0001	8.54	77.0	18.9
Trabecular BMD, mg/cm3	46.0 ; <0.0001	5.48	75.3	147.5
SSI-P, mm3	41.2 ; <0.0001	5.35	74.9	350.4
SSI-X, mm3	26.4 ; <0.0001	4.50	70.6	195.6
Cortical Area, mm2	21.2 ; <0.0001	2.28	68.1	136.6
Total Cont, mg	14.4 ; <0.001	1.61	65.7	103.5
Cortical Cont, mg	11.7 ; <0.001	2.75	64.5	79.8

Disclosures: ***P. Pludowski****, None.*

SU269

Nationwide Hip Fracture Survey in Japan. H. Hagino[1], T. Nakamura[2], K. Sakamoto*[3]. [1]Rehabilitation Division, Tottori University, Yonago, Japan, [2]Department of Orthopedic Surgery, University of Occupational and Environmental Health, Kitakyushu, Japan, [3]Department of Orthopedic Surgery, Showa University, Tokyo, Japan.

Purpose: To elucidate the current status of hip fracture incidence and treatment in Japan, Japanese Orthopedic Association (JOA) conducted a tally of hip fractures in JOA related hospitals in Japan.
Patients and Methods: A tally of all hip fractures in patients from 1998 to 2001 was conducted in JOA-authorized hospitals and in Japanese Clinical Orthopedic Association (JCOA) hospitals. There were 2270, 2264, 2312, and 2291 JOA-authorized hospitals and 1529, 1430, 1512, and 1493 JCOA hospitals in 1998, 1999, 2000, and 2001, respectively. Registration forms were sent to these hospitals by mail and registration was performed by doctors at each hospital according to their hospital records.
Results: Response rates were 48.4%, 55.1%, 47.0%, and 53.0% in 1998, 1999, 2000, and 2001, respectively. The survey found a total of 155,216 new hip fractures aged 35 year-old and over during the survey years. The number of women patients was 3.7 times that of men. Dividing by fracture types, 86,558 were trochanteric fractures, 66,880 neck fractures, and 1,778 were unclassified fractures during the three-year survey period. Age- and gender- specific number of patients increased with age and peaked at the age 80-84, then leveled off after 85 years of age. The number of patients with femoral neck fractures exceeded that with trochanteric fractures before 75 years of age and these figures became inverted thereafter. The number of patients per month was highest in January (15,027), and lowest in June (11,004) during the four observation years, showing a significant monthly variation. More left hips were fractured than right in all survey years; however, the difference was not statistically significant. The most common cause of hip fractures was a simple fall, and 73% sustained fractures in-doors. In patients aged 90 years old and over, simple fall was the cause in more than 80%. Ninety-three percent in patients with femoral neck fractures and 94% in patients with trochanteric fractures were treated surgically. About 3/4 were treated with hemi-arthroplasty among patients with femoral neck fractures. The mean hospitalization period was 55.7 days during the observation period.
Conclusion: The data from a survey covering more than 40% of all hip fractures occurring during 1998 to 2001 in Japan showed patient distribution by age and fracture type, cause of fracture, selection of the treatment, and duration of hospitalization.

Disclosures: ***H. Hagino****, None.*

SU270

Prevalence of Vertebral Fractures in Mexico: A Population-Based Study. P. Clark[1], M. Deleze[2], F. Cons-Molina*[3], J. Salmeron*[4], L. Palermo*[5], S. R. Cummings[5], A. The LAVOS Study Group*[6]. [1]Clinical Epidemiology Unit, Centro Medico Nacional, IMSS-Faculty of Medicine UNAM, Mexico City, Mexico, [2]Clinica de Osteoporosis, Puebla, Mexico, Mexico, [3]Unidad de Diagnostico de Osteoporosis, Mexicali, Mexico, [4]Epidemiology & Health Systems Unit, IMSS, Morelos, Mexico, [5]SF Coordinating Center, San Francisco, CA, USA, [6]Clinical Epidemiology Unit, Centro Medico Nacional, IMSS, Mexico City, Mexico.

The rate of vertebral fractures in Latin America has never been studied in population-based samples. We designed the Latin American Vertebral Osteoporosis Study (LAVOS) to determine the prevalence of vertebral fractures in women over 50 years in several countries. We report here preliminarily results from the Mexico survey. An age-stratified sample of 400 randomly selected women from Puebla Mexico was surveyed in a face-to-face interview. A questionnaire to get information on demographics, OP conventional risk factors, and some life styles were applied. BMD in two regions and lateral dorsal/lumbar X rays were obtain in all cases accordingly with international protocols to be able to have cross-national comparisons. Digital Morphometry was used to determine vertebral deformities by Eastell criterion. The overall prevalence of vertebral fractures was 17.5% and increased exponentially with age. Comparing to studies that used very similar methods and criteria, the rate in Mexican women is very similar to that in Caucasian women and higher then the prevalence found in African American and Chinese women. This first population-based study of radiographically confirmed vertebral fractures in a Latin American country that shows indicates that Mexican women have a risk of vertebral fracture that is similar to white US women and greater than the risk of Chinese and African-American women. Treatments to prevent vertebral fracture as important for Mexican as for US women.

Age	SOF-Whites Prev (IC 95%)	SOF- AA Prev (IC 95%)	Beijing Prev (IC 95%)	Mexico Prev (IC 95%)
50-59	-	-	4.1 (0.6-7.7)	8.3 (2.7–13.8)
60-69	14.5* (13.4-15.5)	-	12.6 (7.0-16.2)	12.6 (6.1–19.1)
70-79	22.0 (20.8-23.3)	9.5 (6.3-12.6)	17.5 (10.7-24.3)	18.6 (10.7–26.4)
> 80	33.9 (30.9-36.9)	17.3 (10.9-23.7)	27.1 (15.8-38.5)	37.9 (28.3–47.4)

*> 65 years

Disclosures: ***P. Clark****, None.*

SU271

Irregularity of the Thoracolumbar Curvature Is a Sensitive and Specific Indicator of Structural Failure of the Spine. R. M. D. Zebaze*[1], G. Maalouf*[2], E. Seeman[1]. [1]Endocrinology, Austin and Repatriation Medical Centre, University of Melbourne, Australia, [2]Saint George University Hospital, Beyrouth, Lebanon.

The credibility of comparisons of vertebral fracture (fx) rates between sexes, races, locations, times and clinical trials depends on there being accurate, consistent and reproducible methods of quantifying spinal structural failure. Quantitative vertebral morphometry (QVM) is not an optimal option. It focuses on vertebral body (VB) shape but this varies unpredictably across individuals, sexes, races, and time. Moreover, 'fracture' defined by QVM as loss of VB height is difficult to distinguish from the necessity for there to be differences in anterior and posterior VB heights to form a thoracolumbar curvature (TLC): a biomechanical necessity, critical in providing 'shock absorber' function to maintain cephalic stability during bipedal gait. Resemblance and consistency of an individual's adjacent VB dimensions produces the regularity in the TLC. Rather than considering structure failure of vertebral elements separately as done in QVM, we quantified this regularity as a single continuous mathematical function expressing how well adjacent vertebrae fit a sector of a circle, a function sensitive to structural failure because of the resemblance of adjacent vertebrae, but insensitive to anatomical variation (often called a 'fracture' using QVM). We expressed the deviation from regularity of the TLC as a Spinal Curvature Irregularity index (SCII = 100 - x%). Spine BMD and VB heights were measured using DXA in 697 Lebanese women (20-87 yrs). Deformities (fx by QVM) were assessed by QVM. In premenopausal women, the SCII was independent of age, height and weight but correlated with BMD (r= - 0.22) and had a mean of 10% (range 4-15%). In postmenopausal women, the mean SCII was unchanged but the scatter increased (4-30%). The SCII correlated with height (r = - 0.23), BMD (r = - 0.18), and the number of fx by QVM (0.31-0.60) (all p lower than 0.001). SCII was better correlated with indicators of spinal fragility (age, height loss, BMD) than number of deformities. Only 0.8% of women had an SCII of > 17% (the value defining fx by SCII) before menopause, but this proportion increased exponentially to 15% after age 80 (r = 0.97). They had lower BMD (- 1.01 SD) and height (- 0.49 SD) and 3 to 9 times more fx by QVM than those with no fx by SCII (< 17%). Women with fx by SCII but no fx by QVM had lower BMD and height, whereas women with no fx by SCII but fx by QVM had normal BMD and height. The SCII (i) can be used as a continuous variable to monitor structural failure (ii) is sensitive and specific, and more so than QVM (iii) is easy to compute in an individual (iii) does not require a reference range and (iv) can be a reliable alternative to QVM.

Disclosures: ***R.M.D. Zebaze****, None.*

SU272

What Is a Vertebral Fracture? R. M. D. Zebaze*[1], G. Maalouf*[2], J. Wehbe*[2], A. Nehme*[3], N. Maalouf*[4], E. Seeman[1]. [1]Endocrinology, Austin and Repatriation Medical Centre, Melbourne, Australia, [2]Saint George University Hospital, Lebanon, Lebanon, [3]Centre Hospitalier Rangueil, Toulouse, France, [4]University of Texas Southwestern Medical Center, Texas, TX, USA.

Credible inferences regarding the burden of vertebral fractures (VF) within and between sexes, races, placebo arms of clinical trials, countries and decades cannot be made until there is a globally accepted method of defining 'fracture'. Differences in vertebral heights are essential for there to be a thoracolumbar curvature. These differences do not necessarily reflect structural failure but are used to define 'fracture' based on reductions in intra- or inter- vertebral body (VB) height ratios, by 15 or 20%, or 3-4 SD below the mean. As there is no gold standard to distinguish anatomical variation from fractures, group differences in the VF prevalence may reflect differences in methodology, not differences in fragility.
A quantitative definition of VF was developed based on (i) a new method of defining threshold values and (ii) the requirement for two abnormal height ratios which reduces the chance of false positives ten fold. BMD and vertebral heights were measured using dual X-ray absorptiometry (Lunar Expert-XL) in 697 Lebanese women (age 20-89 years). VF prevalence ranged from 7 to 70 percent using published cut-offs defining 'fracture'. At 3 SD, the prevalence of deformities was 70-80% in older women (aged 50 and over) and women with lower BMD, but ~65% in younger women (aged 20-49) and women with normal BMD; 4 SD or 15% cut offs produced similar high sensitivity and poor specificity. The 20% cut off produced a 40-50% prevalence in older women and women with low BMD, a 25-35% prevalence in young women and women with normal BMD. The 30% cut off produced a low prevalence which decreased with age. The new classification produced prevalence figures of 3.3% in younger and 14% in older women, 20, 10, and 7% in the lower, middle and upper BMD tertials respectively. 'Deformities' detected in younger women were not associated with height loss or low BMD. Subtracting the prevalence of deformities in young from older women resulted in a decrease the disparity from 10 to 2 fold between methods. Current morphometric criteria for VF produce prevalence figures which are unrelated to age or low BMD and are likely to capture anatomical variation rather than structural failure. A standardized definition of deformities is needed before credible comparison across epidemiological studies can be made.

Disclosures: ***R.M.D. Zebaze****, None.*

SU273

Diabetes Mellitus and the Risk of Non-Vertebral Fractures. L. A. Ahmed*[1], R. M. Joakimsen[2], G. K. Berntsen[1], V. Fønnebø[1]. [1]Institute of Community Medicine, University of Tromsø, Tromsø, Norway, [2]Department of Internal Medicine, University Hospital of Tromsø, Tromsø, Norway.

The aim of this study was to assess the relation between diabetes mellitus and non-vertebral fractures. Methods: this is a population based study where we followed up 12 270 persons, who attended both the second (1979/80) and third (1986/87) surveys in the Tromsø Study, from 1988 to 1995 with respect to non-vertebral fractures. At baseline, 1988, the age range for women was between 29 to 58 years, and for men was between 29 to 63 years. Diabetes mellitus cases were defined by self-report, which could be validated by information in medical records. All non-vertebral fractures, which occurred in the follow up period, were registered by computerized search in radiographic archives in the sole provider of radiographic service in the area. This method was shown to identify 97% of all forearm fractures in the original cohort. Adjustment for possible confounding by age, body mass index (BMI) from the third survey, weight loss (between the two surveys), physical activity, smoking (previous and current), milk consumption and self-reported health status was performed. Results: we identified 72 cases of diabetes mellitus, and 935 non-vertebral fracture cases (491 women, 444 men). During the follow up period, 6 out of 22 diabetic women and 3 out of 50 diabetic men had fractures. The crude relative risk (RR) of fracture was 4.7 (95% confidence interval (CI) 2.1-10.4) and 0.9 (95% CI 0.3-2.9) for women and men respectively. Among women, age-adjusted relative risk of fracture was 5.7 (95% CI 1.8-17.8) in type I diabetics, and 1.2 (95% CI 0.2-8.7) in type II diabetics compared to non-diabetics. There was a significant interaction between diabetes and Body mass index (BMI) on fracture risk. Women with BMI less than 25 had a relative risk of 14.3 (95% CI 5.9-34.5). The relative risk was not significant among women with BMI of 25 or more, (RR 1.0 (95% CI 0.1-7.3)) or among men. Adjustment for Age, BMI from the third survey and weight loss gave a relative risk of 10.6 (95% CI 3.9-28.7) and 1.1 (95% CI 0.2-7.7) for diabetic women with BMI less than 25 and more than 25 respectively. For men, the adjusted relative risk was 1.3 (95% CI 0.3-5.1) for those with BMI less than 25, and 0.6 (95% CI 0.1-4.5) for those with BMI of 25 or more. Conclusion: women with type I diabetes and women with diabetes and a BMI less than 25 had increased the risk of non-vertebral fractures. Male diabetics and female diabetics with BMI of 25 or more had no such increase in fracture risk.

Disclosures: ***L.A. Ahmed****, None.*

SU274

Prevalence of Vertebral Fractures in Postmenopausal Glucocorticoid-treated Women: a National Study. A. Angeli[1], G. Capelli*[2], S. Giannini[3], G. Guglielmi[4], M. Bevilacqua*[5], L. Moro[6], L. Sinigaglia*[7], D. de Feo*[8], A. Giustina*[9]. [1]Internal Medicine, University of Torino, Orbassano, Italy, [2]Public Health, University of Cassino, Frosinone, Italy, [3]Internal Medicine, University of Padova, Padova, Italy, [4]Radiology, Scientific Institute Hospital "CSS", Foggia, Italy, [5]Endocrinology, Sacco Hospital, Milano, Italy, [6]Bbcm, University of Trieste, Trieste, Italy, [7]Rheumatology, Gaetano Pini, Milano, Italy, [8]Procter & Gamble, Rome, Italy, [9]Internal Medicine, Brescia University, Brescia, Italy.

Glucocorticoids (GCs) administration in post-menopausal women accelerates bone loss and increases fracture risk. GIOVE (Glucocorticoid-Induced Osteoporosis Vertebral Evaluation) is a national study aimed at measuring the prevalence of vertebral fractures (VFs) in a sample of postmenopausal women treated with GCs. Forty-three University and Hospital centers in Italy participated in the study. A total of 979 ambulatory female subjects (aged 45+ yrs, 1+ yrs in menopause, treated with systemic GCs for at least 6 months, cumulative dosage of GCs > 1.350 mg of prednisone or equivalent) affected by Reumathoid Arthritis (RA), Lupus (SLE), Vasculitis/Connectivitis, Polymyalgia rheumatica (PMR), Asthma/COPD were enrolled. All subjects had undergone an X-ray evaluation of thoracic and lumbar spine. Films were centrally digitized: T4-L4 vertebral heights ratio were measured using a morphometry software (Spine-X Analyzer; CAM Diagnostics, Milan, Italy). The VFs were defined as: mild, moderate or severe, based on a height ratio decrease of 20%-25%, 25%-35% and more than 35% respectively. Currently data on 485 of the women are under revision mainly due to low-quality radiographs. The table below shows the age-adjusted prevalence VFs rates (%) calculated in the women grouped by each major disease.

	RA (n=253)	SLE (n=40)	PMR (n=80)	Vasculitis/ Connectivitis (n=69)	Asthma/ COPD (n=34)	ALL (n=476)
1+ VFs T4-L4	34.8	32.0	44.2	41.4	40.7	37.6
Mild	13.4	22.1	24.2	17.4	14.7	16.6
Moderate	15.2	3.2	16.1	15.0	11.6	14.3
Severe	6.2	6.7	3.9	9.0	14.4	6.7
2+ VFs T4-L4	13.1	11.3	16.1	10.7	20.1	13.9
1+ VFs T4-T12	29.1	27.3	39.0	26.6	37.8	31.0
Mild	12.7	20.5	21.9	13.1	17.6	15.3
Moderate	11.8	3.1	13.5	8.9	8.7	10.9
Severe	4.6	3.7	3.6	4.6	11.5	4.8
1+ VFs L1-L4	8.1	5.1	9.0	19.4	5.8	9.5
Mild	4.8	2.6	7.3	5.8	0.0	4.8
Moderate	1.7	0.0	1.7	7.8	2.9	2.5
Severe	1.6	2.5	0.0	5.8	2.9	2.0

About 38% of patients had one VF and about 14% had more than one VF. PMR, Vasculitis/ Connectivitis and Asthma/COPD affected patients showed the highest rates of VFs prevalence. Even if PMR patients were generally older and SLE patients were younger than others, age adjustment did not significantly change the pattern. This study confirms that the use of GCs is associated with a higher risk of developing single or multiple VFs.

Disclosures: ***S. Giannini****, None.*

SU275

The Influence of Socioeconomic Deprivation on the Incidence of Fractures & on the Uptake of Post-fracture Osteoporosis Assessment by a Fracture Liaison Service. A. R. McLellan[1], M. Fraser*[1], S. J. Gallacher[2], I. Baxter*[3], C. Morrison*[3]. [1]Medicine & Therapeutics, Western Infirmary, Glasgow, United Kingdom, [2]Medicine, Southern General Hospital, Glasgow, United Kingdom, [3]Greater Glasgow NHS Board, Glasgow, United Kingdom.

Socioeconomic deprivation (SED)has implications for disease and health-care access; whether this is true for fractures (fx) is unclear. SED adversely influences access to osteoporosis assessment when fx case-finding involves Primary Care (Gallacher JBMR 2002;17supp1, absF269). A prospective audit of incident fx was undertaken to assess whether SED influences fx incidence and access to assessment by the North Glasgow (NG) Fracture Liaison Service (FLS) that covers a population of 500K. The FLS offers osteoporosis assessment and treatment for the 2^{y} prevention of fx to all >50yr with any new fx (non-RTA); Case-finding of inpatients & outpatients with fx, does not require participation of Primary Care, and is achieved by dedicated nurse practitioners. To investigate the influences of SED, all 3190 incident Fx cases presenting to NGFLS in 2002 were reviewed; deprivation category (depcat)(Carstairs 1991) was allocated to each patient based on their postal code of residence. Depcat is a 7 point score; depcat 1 being most affluent & depcat 7 least affluent. Fx incidence at all sites expressed as age /sex adjusted incidence rate (ASAIR) per 10K by depcat from 1 to 7 was 176.2, 134.8, 93.8, 146.9, 209.6, 170.7 & 190.5 resp. Data for the 4 commonest fx sites are shown in table 1; those for hand/foot(n=364) and all other fx(n=395)are not shown, but trends are similar to ankle and wrist fx resp.. Depcats 2 or 3 have the lowest ASAIRs for fx at any site. Depcat 7 had the highest ASAIR for fx of hip and humerus, while depcat 1 had the highest incidence of fx of ankle and hand/foot.

ASAIR for Fx per 10K by Depcat

Depcat	Wrist(n=925)	Hip(652)	Humerus(476)	Ankle(378)
1	51.2	27.7	25.4	21.6
2	40	32.4	22.9	7
3	31.4	11.4	10.7	16.7
4	42.7	24.3	24.9	21.1
5	64.9	50.4	19.4	11.6
6	47.9	41.6	27.9	11.5
7	52.9	38.2	30.1	7.2

The FLS offers assessment to all fx cases >50yr with fx irrespective of depcat; however, uptake is inversely related to depcat; for depcat 1 to 7, 18%,19%,28%,20%,22%,21% and 31% resp. either decline assessment or do not attend. SED contributes to fx risk at hip and humerus. The FLS model achieves its aim of offering post-fx assessment to all fx, regardless of depcat, but uptake of that offer is lower with SED. For optimal 2^{y} prevention of fx, a FLS must address the disadvantages of SED that contribute to fx risk and that are also barriers to accepting opportunities to reduce future fx risk.<br

Disclosures: ***A.R. McLellan****, None.*

SU276

Risk Factors that Predict New Non-Vertebral Fracture in Postmenopausal Women: The Canadian Multicentre Osteoporosis Study (CaMos). A. Papaioannou[1], G. Ioannidis[1], J. P. Brown[2], C. Berger*[3], D. A. Hanley[4], J. C. Prior[5], L. Joseph*[3], W. P. Olszynski[6], T. M. Murray[7], A. Tenenhouse[3], T. Anastassiades*[8], S. Kirkland*[9], C. Joyce*[10], S. Poliquin*[3], N. Kreiger*[7], K. Siminoski[11], J. D. Adachi[1]. [1]McMaster University, Hamilton, ON, Canada, [2]Laval University, Ste-Foy, PQ, Canada, [3]McGill University, Montreal, PQ, Canada, [4]University of Calgary, Calgary, AB, Canada, [5]University of British Columbia, Vancouver, BC, Canada, [6]University of Saskatchewan, Saskatoon, SK, Canada, [7]University of Toronto, Toronto, ON, Canada, [8]Queen's University, Kingston, ON, Canada, [9]Dalhousie University, Halifax, NS, Canada, [10]Memorial University, St. John's, NF, Canada, [11]University of Alberta, Edmonton, AB, Canada.

The purpose of this study was to determine the association among various potential risk factors and new non-vertebral fractures in a three-year prospective cohort study of menopausal women who were enrolled in CaMos. CaMos is a nation-wide, randomly selected sample of the Canadian population with extensive osteoporosis related data. Participants were classified into three groups according to their new non-vertebral fracture status: those without a new fracture (No-Fx, n=4829), those with a new non-vertebral fracture at the wrist, hip, humerus, pelvis or ribs (Main-Fx, n=163) and those with a new non-vertebral fracture at any skeletal location (All-Fx, n=280) during the study period. Follow-up was calculated as time from baseline examination to the date of the new fracture or three years. We preformed Cox multivariate survival analysis on several potentially important risk factors. Final model selection was conducted using the Bayesian Information Criterion technique. Relative risk and 95% confidence intervals (CI) were calculated. Results indicated

that higher quality of life (measured by the SF-36 physical component summary scale) and lumbar spine and femoral neck BMD was associated with a decrease risk of sustaining a new Main-Fx 0.969 (CI: 0.952, 0.986), 0.162 (CI: 0.034, 0.766) and 0.065 (CI: 0.007, 0.605); and a new All-Fx 0.967 (CI: 0.954, 0.980), 0.161 (CI: 0.046, 0.566) and 0.089 (CI: 0.016, 0.504). In addition, a previous minimal trauma forearm fracture after the age of 50 years (2.078; CI: 1.202, 3.593), and weight loss (1.020; CI: 1.002, 1.037) were associated with an increase risk of sustaining a new Main-Fx. Inflammatory bowel disease (1.683; CI: 1.084, 2.613), kidney disease (3.084; CI: 1.560, 6.099), height (1.039; CI: 1.015, 1.064) and diuretic use (0.550; CI: 0.304, 0.994) was associated with All-Fx. In conclusion, several important historical and medical factors are related with non-vertebral fractures. These should be assessed in osteoporosis management.

Disclosures: ***A. Papaioannou****, None.*

SU277

Participant Characteristics that Predict New Clinically Recognized Vertebral Fracture in Postmenopausal Women: The Canadian Multicentre Osteoporosis Study (CaMos). W. P. Olszynski[1], G. Ioannidis[2], C. Berger*[3], A. Papaioannou[2], J. C. Prior[4], L. Joseph*[3], D. A. Hanley[5], T. M. Murray[6], J. P. Brown[7], A. Tenenhouse[3], T. Anastassiades*[8], S. Kirkland*[9], C. Joyce*[10], S. Poliquin*[3], N. Kreiger*[6], K. Siminoski[11], J. D. Adachi[2]. [1]University of Saskatchewan, Saskatoon, SK, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]McGill University, Montreal, PQ, Canada, [4]University of British Columbia, Vancouver, BC, Canada, [5]University of Calgary, Calgary, AB, Canada, [6]University of Toronto, Toronto, ON, Canada, [7]Laval University, Ste-Foy, PQ, Canada, [8]Queen's University, Kingston, ON, Canada, [9]Dalhousie University, Halifax, NS, Canada, [10]Memorial University, St. John's, NF, Canada, [11]University of Alberta, Edmonton, AB, Canada.

Utilizing participants from CaMos, a nation-wide, random sample of the population, we performed a three-year prospective cohort study in community dwelling menopausal women to examine the relationship among various participant characteristics and clinically recognized new vertebral fractures as reported on the yearly follow-up questionnaire. Participants were classified into two groups according to their new fracture status: those without new fractures during the study period (n=4829), and those with a new minimal trauma vertebral fracture (n=34). Follow-up was calculated as the time from baseline examination to the date of the new fracture or three years. Characteristics examined in the Cox multivariate survival analysis included age, prevalent vertebral fracture status, change in height, current height, change in weight, current weight, body mass index, SF-36 physical component summary score, and baseline lumbar spine and femoral neck bone mineral density (BMD). Final model selection was conducted using the Bayesian Information Criterion technique. Relative risks and 95% confidence intervals (CI) were calculated. The mean (standard deviation) age of the participants was 66 (10) years, and 74 (10) years for those without new fractures and for those with a new vertebral fracture. The relative risk of sustaining a new vertebral fracture was negatively associated with the SF-36 physical component summary score (0.962; CI: 0.930, 0.996) and femoral neck BMD (0.0002; CI: 0.000, 0.010). A prevalent vertebral deformity (2.357; CI: 0.920, 6.039) and a loss of height (1.089; CI: 0.993, 1.194) tended to be positively associated with new fracture status but further evidence will need to be collected to verify these findings. Women with lower physical quality of life measures and femoral neck BMD were at higher risk for sustaining a new vertebral fracture. The identification of postmenopausal women at risk is important given that proven therapies are available.

Disclosures: ***W.P. Olszynski****, None.*

SU278

Most Fractures are Incidental with No Lasting Medical or Social Consequences. A. Hoiseth. Sentrum Roentgeninstitutt, 0155 Oslo, Norway.

To assess the seriousness of fractures, historical fracture data was obtained from 3997 women, consecutively having routine BMD-measurements. Lasting consequences of the fractures, namely pain, social and/or medical consequences were recorded. Mean age of the subjects was 63 years (sd 12). 1780 had had a fracture; 789 at least one fore-arm fracture, 271 at least one vertebral fracture, 179 at least one femoral fracture and 888 at least one "other" type of fracture. 944 reported having had one fracture, 445 two fractures, 188 three fractures and 203 at least four fractures. Only 331 (19%) reported lasting consequences of the fractures; 13% of those with one fracture, 21% with two fractures, 23% with three fractures and 39% with at least four fractures. Further, only 7% with one fore-arm fracture only had som lasting sequel, this increased to respectively 19%, 9%& and 42% in those with one, two and three additional fractures. For those with one vertebral fracture and additional fractures the figures were respectively 17%, 38%, 40% and 43%. But, for those with femoral fractures the values were respectively 43%, 41%, 33%, 53% and for "other" types of fractures, 8%, 21%, 20% and 40%. The majority of fractures seemed to have no lasting or permanent consequences, even in those with just one vertebral fracture the number of patients reporting lasting consequences was small. However, having had at least a femoral fracture or multiple fractures left nearly half of the women with some lasting sequel. This corresponded to the group of women with low BMD in the femur and spine. A rational approach to osteoporosis intervention seems to be BMD assessments to find those at risk of having at least one femoral, more than one vertebral or multiple other fractures. Single, or event two non-vertebral or non-femoral fractures do not seem to be indicate a serious condition and should probably not be used as an indication for medical intervention without a BMD based assessment of the risk of additinal fractures.

Disclosures: ***A. Hoiseth****, None.*

SU279

Prospective Study of Health-Related Quality of Life in Women with Hip Fractures. A. Cranney, D. Coyle*, W. Hopman*, V. Hum*, P. Tugwell*. Medicine, Queen's University, Kingston, ON, Canada.

To prospectively assess Health-related quality of life (HRQoL) in women with recent hip fractures. Forty women over age 50 were identified within one month of their hip fracture. Study interviews were conducted at baseline, 3 and 9 months. Utility values were elicited using direct methods; with the Feeling thermometer (FT), (Current health and 5 osteoporotic health states) and the Standard gamble (SG). Indirect elicitation methods included use of the HUI II. Health status was measured using the SF-36. Baseline measurements were taken in a group of 40 controls without a history of hip fracture. 35 women with hip fractures completed 3 visits; 3 women died, 1 woman completed the initial visit and 1 dropped out after second visit. The mean age of controls and hip fracture patients was 72 and 80 years respectively. Twenty-five percent of hip fracture patients were on osteoporosis medications. The SF-36 scores were lower for the hip fracture patients as compared to controls and age and sex-matched normative Canadian data. Utility values were significantly lower in hip fracture patients when elicited using the HUI II and FT, but not with the SG. There were progressive improvements in the Physical Component Scale, Role Physical, Body Pain and Physical Functioning domains of the SF-36 over the 9-month period. There were similar improvements in the HUI II, and FT, but the SG values did not change over the 9 month period. Health states that included side effects were rated lower than a similar health state without side effects. There was a significant correlation between the FT and HUI results. There was also a significant correlation between the SF-36 scores and the HUI. The standard gamble may not be sensitive to changes in HRQoL in women with hip fractures. Women with hip fractures have lower HRQoL in comparison to non-fracture controls, but show good improvement over time.

Measure	Controls N=40 Mean (SD)	Baseline N=40 Hip fracture Mean (SD)	3 mos post fracture N=38 Mean (SD)	9 mos post fracture N=35 Mean (SD)
SF-36 (PCS)	46.0 (9.5)	21 (5.8)	28.5 (8.9)	31.2 (10.5) **
SF-36 (MCS)	56.5 (8.4)	53 (10.5)	53.5 (11.7)	53.8 (11.0)
HUI- II	0.85 (0.11)	0.51 (0.18)	0.63 (0.20)	0.73 (0.19) **
Feeling Thermometer (Current Health)	0.86 (0.10)	0.61 (0.17)	0.68 (0.20)	0.71 (0.19) *
Standard Gamble	0.95 (0.10)	0.84 (0.22)	0.86 (0.21)	0.91 (0.14)
Health State (vertebral fracture)	0.50 (0.18)	0.42 (0.15)	0.42 (0.12)	0.51 (0.13) **
Health State (hip fracture)	0.62 (0.14)	0.61 (0.15)	0.63 (0.11)	0.67 (0.15)
Health State (Hip fracture with medication side effects)	0. 33 (14)	0.30 (0.13)	0.28 (0.10)	0.37 (0.13)

** significant difference from baseline value P<0.001, * P=0.01

Disclosures: ***A. Cranney****, None.*

SU280

Health Related Quality of Life After Osteoporotic Fractures. I. Hallberg*[1], A. Rosenqvist*[2], L. Kartous*[2], O. Löfman*[3], O. Wahlström*[4], G. Toss[1]. [1]Endocrinology, Medicine and Care, Health Faculty, Linköping, Sweden, [2]Geriatrics, Jönköping, Sweden, [3]Centre for Health Sciences, Public Health, Linköping, Sweden, [4]Orthopedics, Linköping, Sweden.

In order to compare the impact on Health Related Quality of Life (HRQOL) of different types of fractures we examined consecutive women 55-75 years old with a new low-energy fracture of forearm (n=171), proximal humerus (n=37), vertebra (n=55) or hip (n=40). Anti-osteoporotic treatment was given according to local guide-lines. Calcium 500 mg, vitamin D 10 mikrogram and cyclic etidronate were the most common treatment in all fracture groups. HRQOL was evaluated by the SF-36 questionnaire and compared with a large age matched local populations sample. A baseline examination was performed 82 days (59 –103, IQR) after fracture, and a second examination two years later.

At baseline women with forearm fracture had reduced scores for role function (-physical causes), bodily pain, general health and role function (-emotional cause), but not for physical function, vitality or mental health. Two years after forearm fractures all the eight scores were normal. Women with fracture of the proximal humerus showed a similar pattern as the forearm fracture group.

At baseline after hip fracture scores for physical function, role function (-physical cause), bodily pain, vitality, social function and role function (-emotional cause) were lower than after forearm or humerus fractures. After vertebral fracture at baseline scores for physical function and role function (-physical cause) were slightly less reduced than after hip fracture while bodily pain, general health and vitality were more severely reduced. Two years after vertebral fracture improvements were seen for some scores, but all scores were still significantly below normal and lower than after hip fracture. Patients with one or more fracture before the index fracture had lower HRQOL than those with no previous fracture. Patients with osteoporosis had lower scores than those with normal BMD.

In conclusion complete normalization of HRQOL as measured by SF-36 was seen two years after forearm and humerus fractures, while vertebral fractures had a pronounced impact on HRQOL even two years after the fracture, even more than hip fractures. HRQOL should be taken into account when evaluating new treatment methods for fractures and osteoporosis.

Disclosures: ***G. Toss****, None.*

SU281

Relation Between Previous Fracture and Loss of Teeth Independent of Bone Mineral Density: A Population Study of 566 - 70 Year Old Women, The Nordos Study. T. Österberg*[1], U. H. Lerner[2], O. Johnell[3], D. Mellström[1]. [1]University of Gothenburg, Department of Geriatrics, Gothenburg, Sweden, [2]University of Umeå, Department of Oral Cell Biology, Umeå, Sweden, [3]University of Lund-Malmö, Department of Orthopedic Surgery, Malmö, Sweden.

Previous studies have shown a correlation between low bone mineral density (BMD) and loss of teeth. Other studies have indicated a relation between previous fracture and prospective fracture, independent of BMD. The aim of this study was to examine the relation between previous fracture and loss of teeth. The question is if loss of teeth can be an indicator of osteoporosis and fractures?
A population based sample of 566 - 70 year old women were examined with a health interview, including questions about dental status. Bone densitometry was performed using a Hologic 4500A.
Result: In the sample, 12% had no own teeth and 25% reported previous fracture(s). Toothlessness was related to increased risk for previous fracture; odds ratio 1.75 (1.10-2.78). Loss of teeth was not related to BMD in spine or hip, but to BMD in whole body ($p < 0.01$) and manifest osteoporosis; odds ratio 2.07 (1.13-3.78). Women with a normal BMD according to WHO had a lower risk for loss of teeth compared to women with osteoporosis or osteopenia. Loss of teeth was more prevalent in shorter and smoking women. A logistic regression model showed that loss of teeth was an independent predictor for previous fracture; odds ratio 1.25 (1.04-1.50). Other significant predictors for previous fractures in the model were BMD in hip, body height and BMI.
Conclusion: there is a relation between loss of teeth and previous fracture independent of bone mineral density.

Disclosures: ***U.H. Lerner****, None.*

SU282

Risk Factors Related with Quality of Life (QOL) in Postmenopausal Women With Prevalent Vertebral Fractures: The Canadian Database of Osteoporosis and Osteopenia (CANDOO). D. A. Hanley[1], G. Ioannidis[2], R. J. Josse[3], T. M. Murray[3], J. P. Brown[4], R. J. Sebaldt*[2], C. H. Goldsmith*[2], A. Papaioannou[2], W. P. Olszynski[5], A. Petrie*[2], K. S. Davison*[2], J. D. Adachi[2]. [1]University of Calgary, Calgary, AB, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]University of Toronto, Toronto, ON, Canada, [4]Laval University, Ste-Foy, PQ, Canada, [5]University of Saskatchewan, Saskatoon, SK, Canada.

Several risk factors may be related to QOL in patients who have sustained vertebral fractures. Thus, the purpose of this cross-sectional study was to examine a broad variety of general health risk factors associated with QOL in postmenopausal women with vertebral fractures who were registered in CANDOO. CANDOO is a prospective, observational database designed to longitudinally capture a standardized and comprehensive set of clinical information that includes data regarding demographics, medications, illnesses, fracture history, medical and family history, dietary and lifestyle factors, bone mineral density results and laboratory investigations. The mini-Osteoporosis Quality of Life Questionnaire was used to measure QOL. The instrument is self-administered and consists of 10 items (total score) subdivided into five domains (symptoms, physical functioning, emotional functioning, activities of daily living, and leisure). Higher scores indicate improving QOL. A total of 1129 women with a mean (standard deviation) age of 67.2 (11.9) years, height 155.4 (12.5) cm, and weight 64.7 (17.6) kg were evaluated. These women had 2.2 (1.6) vertebral fractures (confirmed by x-ray or medical report). Multivariable linear regression analysis was conducted to examine the relationship between potential risk factors and QOL (total score). Variable selection for the final model was determined using Mallows C(P) statistic. Regression coefficient estimates and 95% confidence intervals (CI) were calculated. Adjusted results indicated that previous surgeries of the hip or spine (-0.94; CI: -1.47, -0.41) and prior cardiovascular diseases (-0.74; CI: -1.15, -0.33) were associated with decreased QOL. The number of hours spent exercising per week (0.08; 0.03, 0.13), those working full time (0.41; CI: 0.04, 0.77), and those with a longer duration between their last non-vertebral fracture and baseline assessment (0.01; CI: 0.00, 0.02) were associated with increased QOL. In conclusion, improving QOL is an outcome of primary importance. In osteoporotic women with vertebral fractures, a comprehensive patient evaluation is necessary to accurately assess QOL.

Disclosures: ***D.A. Hanley****, None.*

SU283

The Human Cost of Fractures in Community Dwelling Older Adults. A. Papaioannou, M. Bedard*, A. Uppaluri*, G. Ioannidis, J. D. Adachi. Medicine, McMaster University, Hamilton, ON, Canada.

The purpose of our study was to examine the potential impact of fractures on pain, functional status and quality of life in the elderly from the epidemiological population based Canadian Study on Health and Aging (CSHA-2).
Data were obtained from the second wave of the CSHA study on dementia. The population included 5393 survivors from the first wave between 1996-97. Cognitive status was determined using the expanded version of Mini-Mental State Examination (3MS). Participants were interviewed regarding their living arrangements, need for supports around the house and transportation, and number of fractures in the year prior to the interview. Fractured cases (n=262) were compared to controls matched on age, gender, cognition, vision problems, living arrangements, use of supports, help with transportation and help around the house. Cases and controls were compared based on activities of daily living (ADL) and pain interference. The McNemar test for dichotomous data was used and continuous data were examined using paired t-tests or analysis of variance.
Mean age (SD) for cases and controls was 81 years (6.0), and 76% of the cohort was female. In the matched sample of 262 cases, a total of 294 fractures were reported for the year prior to interview. Predominant fracture types included: hip (17.3%), wrist (14.6%), rib (9.9%) and ankle (8.5%). Unmatched cases in the sample had 52 fractures and more likely to be older, more cognitively impaired with vision problems. 3MS scores were 86.74 (10.8) for cases and 87.01 (9.98) for controls. Dementia was determined if 3MS scores < 78. Higher dependence was reported for instrumental (IADL) such as shopping (p=0.001) and housework (p=0.007) among the cases as compared to the controls. Individuals with fractures also demonstrated higher dependence in basic activities of daily living (ADL) such as bathing (p=0.001), dressing/undressing (p=0.007) and grooming (p=0.008). The presence of pain was noted in 47% cases and 41% of controls. A higher proportion of cases (31%) reported moderate to severe pain as compared to controls (21%) (p=0.032). Self-reported pain interference measures showed a severe effect on mood in cases as compared to controls (p=0.031). Individuals with fractures have increased pain that interferes with their mood. In addition, they have difficulties with ADL and IADL that persist a year post fracture.

Disclosures: ***A. Papaioannou****, None.*

SU284

Calcium, Multivitamin and Osteoporosis Medication use in Women and Men with Recent Fractures. A. Pro-Risquez*[1], S. S. Harris[2], E. Ross*[3], S. Rudicel*[3], B. Barnewolt*[3], B. Dawson-Hughes[4]. [1]Brookline Associates of Internal Medicine, Brighton, MA, USA, [2]New England Research Institutes, Watertown, MA, USA, [3]Tufts-New England Medical Center, Boston, MA, USA, [4]Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA.

Fractures are associated with a substantial morbidity and mortality and they predict future fractures. For this reason, evaluation and treatment with calcium (Ca), vitamin D (vit D) and prescription medications is important in fracture patients. This study was conducted to assess and compare Ca and vit D intake and bone medication use at the time of an acute fracture and 6 and 12 mo later. We studied 106 patients, 69 female and 37 male, mean age 66.7±10.3 yrs. Medical history and diet questionnaires were administered at enrollment (in an urban hospital) and again 6 and 12 mo later (by telephone). Of 86 patients who could be contacted 6 mo after their fracture, 36.2% of the women and 7.4% of the men had recently discussed osteoporosis with their primary care doctor. At 6 months, 24.2% of the women and 3.6% of the men were taking bone medications (compared with 27.8% and 3.6% before the fracture, ns). At 6 mo, 52.6% of the women and 10.7% of the men indicated that their doctor had recently recommended Ca or vit D. Overall, Ca supplement use rose from 53.4% at baseline to 74.1% at 6 mo (P= 0.004) in the women and from 14.8% to 17.9% in the men (ns). Among the women who had recently been advised by their primary care doctor to use Ca or vit D, supplement use increased from 63.3% to 90.0% (P= 0.021) and dairy food intake increased from 1.5 +/- 1.1 to 2.4 +/- 1.9 servings/d (P= 0.016). Only 3 men received this advice and 2 of them heeded it. Among women and men not receiving this advice, there was no significant increase in calcium supplement use or dairy food intake. There was no increase in multivitamin use after the fracture in the women or the men. Of the 76 patients who could be contacted 12 mo after the fracture, prescription medication and supplement use was not different from use at 6 mo. Of note, 7.9% had sustained another fracture during that year. In conclusion, the occurrence of a fracture did not increase likelihood of pharmacologic treatment for osteoporosis and osteoporosis was rarely discussed with men with fractures. After their fractures, women did increase their intake of calcium supplements and dairy foods particularly when it was recommended by their doctor. This suggests that the primary care physician is well positioned to bring about much needed change in the quality of care of fracture patients.

Disclosures: ***A. Pro-Risquez****, None.*

SU285

Prevalence and Identification of Vertebral Fractures: Comparison of Visual Inspection, Digital Computerized Morphometry and Visual Semiquantitative Assessment. G. Di Fede[1], N. Napoli[1], G. Guglielmi[2], S. Bucchieri*[1], C. Sferrazza*[1], R. Giorgino[3], E. Carmina*[1], G. Rini[1]. [1]University of Palermo, Palermo, Italy, [2]Scientific Insitute Hospital "CSS", San Giovanni Rotondo, Italy, [3]Novartis Pharma, Origgio, Italy.

The aim of this study is to determine how many vertebral fractures elude X-ray reports and to estimate the accuracy of a digital morphometry system used for X-ray analysis of vertebral body heights. We studied 233 postmenopausal women (aged 63.9± 0.5) who underwent clinical observation in a Bone Metabolic Diseases workut setting. A lateral and posterior-anterior spine radiograph of both thoracic and lumbar vertebrae was performed in each subject. Reports from inspection of radiographs showed the presence of at least 1 vertebral fracture in 12% subjects. We analyzed each T4-L4 film by a digital computerized morphometry [DCM] (Spine-X Analyzer, CAM Diagnostics, Milan, Italy), which calculates vertebral height ratios based on a standard 6-point identification method of the 3 vertebral heights. A 20% reduction of any vertebral height ratio was chosen as threshold for vertebral deformity definition. A 46.25% prevalence of fractures was observed. In addition, X-ray films were blindly analyzed by an expert radiologist using the Genant's semiquantitative grading scheme for the assessment of vertebral fractures [VQA]. A 49.75% prevalence of fractures was observed in this phase. Both methods showed that most fractures occurred in the T6 and T9 interval. End-plate fractures were the most common (68.9% by DCM, 58.4%, by VQA). Good agreement (0.839) between VQA and DCM was found using κ score. Sensitivity (0.655) and specificity (0.957) of vertebral fractures identified by VQA, were calculated considering DCM method the gold standard.

The present study shows that in clinical practice vertebral fractures are often undiagnosed and not adequately reported by inspection of radiographs and that both DCM and VQA methods demonstrate the high prevalence of vertebral fractures and represent a valid tools in clinical research.

Disclosures: ***G. Di Fede****, None.*

SU286

Racial and Ethnic Disparities in Length of Stay and Discharge Disposition of Patients with Hip Fracture. S. L. Silverman[1], D. Zingmond*[2]. [1]Cedars-Sinai/UCLA, Beverly hills, CA, USA, [2]UCLA, Los Angeles, CA, USA.

There is a need for understanding the costs of hip fracture in racial and ethnically diverse populations in the United States. The state of California has an ethnically diverse population and has maintained detailed data documenting hip fractures over the past two decades. The greatest cost of hip fracture is the direct cost of hip fracture hospitalization.
Methods: We studied length of stay (LOS) and discharge disposition (DD), two surrogate measures of cost and utilization, associated with hospitalization following hip fracture requiring surgery in California in 2000 as an index year among four different racial/ethnic groups: Nonhispanic White (NHW); African-American (AfrA); Hispanic American (HispA) and Asian American (AsA). We used the California Office of Statewide Health Planning and Development annual hospital Patient Discharge Database (PDD), to identify all hospitalizations for acute hip fracture among individuals at non-Federal California general acute care hospitals from 1983 to 2000. Individual patient race/ethnicity, gender, and age are reported for each hospital discharge. We report length of stay (LOS) since charges are not available for those in Kaiser hospitals, representing care for one quarter of insured Californians. LOS is highly correlated with charges reported at non-Kaiser hospitals.
Results: Mean length of stay in 2000 was 10.2 days for NHW, 10.4 days for HispA, 12.4 days for AsA and 12.9 days for AfrA. In 2000 only 13.2% of NHW were reported to have routine discharge to home, while 18.6% of AsA, 22.7% of AfrA, and 24.4% of HispA were discharged to home. The numbers discharged to home with arranged home health care visits were similar among the four groups: 12.6% of NHW, 13.2% of AfrA, 14% of HispA, and 13.2% of HispA. 60% of NHW were discharged to skilled nursing facilities while 51.4% of AsA, 45% of HispA and 46% of AfrA. Less than 10% of all ethnic groups were discharged to inpatient rehab settings. There were no significant differences in incidence of death during hospitalization among the four groups (overall < 3%). Female patients predominated (72% NHW, 61% AfrA, 65% HispA , and 71% AsA). The median age range of NHW and AsA was somewhat greater (80 to 84 years old) than for AfrA and HispA (75 to 79 years old).
Conclusions: Racial and ethnic disparities do exist in California during hospitalization for hip fracture. Further studies are needed to determine which factors result in the variation in LOS and DD in California ethnic groups. Potential factors include age, prior residential status, comorbidities and socioeconomic status.

Disclosures: ***S.L. Silverman****, Procter and Gamble 2.*

SU287

Hip Fractures in Lebanese Patients: Determinants and Prognosis. H. Hreybe*[1], M. Salamoun*[1], M. Badra*[2], N. Affeich*[2], O. Baddoura*[2], S. Boulos*[2], R. Haidar*[2], S. Lakkis*[2], R. Musharrafieh*[2], A. Nsouli*[2], A. Taha*[2], A. Tayim*[2], G. El-Hajj Fuleihan[1]. [1]Calcium Metabolism and Osteoporosis Program, American University of Beirut, Beirut, Lebanon, [2]Orthopedics Department, American University of Beirut, Beirut, Lebanon.

Due to demographic explosion worldwide and especially in the Middle East, the human and economic toll of osteoporosis will increase substantially. Hip fractures are among the most costly of all osteoporotic fractures, but very little is known about the determinants of this fracture in our region.
We evaluated all hip fracture (hip fx) patients above 50 years of age admitted to our institution from 1992-2002. Patients with osteoarthritis admitted during the same period and with the same age for total hip replacement were used as controls (C). Information on gender, age, type of fracture, co-morbid conditions and medications use was obtained. Patients or their families were called on the average 5±2.5 years post fracture to assess mortality. Numbers are expressed as mean (± SD).
There were a total of 274 hip fx patients and 112 C. The mean age for hip fx patients was 71(4) years and 72(9) for C, 62% of hip fx patients were women compared with 67% of C. Fractures were 59% intertrochanteric, 34% femoral neck and 7% sub-trochanteric, with no gender differences. 13% of hip fx subjects suffered from neurological disorders compared with 4% of C (p=0.01), 6% had renal disorders compared to 1% of C (P=0.04) and 11% had a history of prior fracture compared with 2% of C, p<0.0001.The higher prevalence of neurological and renal disorders among hip fx patients was mostly due to the high prevalence of these disorders in male subjects with hip fx. Less than 10% of hip fx patients received any calcium, vitamin D or osteoporotic therapy, either on admission or upon discharge post- hip fx. Follow up information was available on 1/3 of all subjects. There was no significant difference in the clinical characteristics, including age, gender, co-morbid conditions (except for neurological diseases) between patients with follow-up and those lost to follow up. The mortality rate among hip fx patients was 47% compared to 18% among C, p<0.001. 70% of hip fx subjects who died did so within the first year post fracture, compared with 66% of C, p=0.02. Gender, but not fracture type, was a predictor of mortality post hip fracture. The mortality in male subjects was 73% compared with 28% in female subjects, p=0.0004.
Risk factors for hip fx in Lebanese subjects include gender, history of prior fracture, renal and neurological disorders. Few hip fx subjects received therapy for osteoporosis. Compared with western counterparts, Lebanese patients with hip fx are significantly younger, and experience a higher mortality rate.

Disclosures: ***G. El-Hajj Fuleihan****, Merck Sharp and Dohme 2.*

SU288

Predicting Previous Fracture With The Health Utilities Index (HUI) Mark II and III Systems in Both Women and Men: The Canadian Multicentre Osteoporosis Study (CaMos). G. Ioannidis[1], J. P. Brown[2], C. Berger*[3], D. A. Hanley[4], J. C. Prior[5], L. Joseph*[3], W. P. Olszynski[6], L. Pickard*[1], T. M. Murray[7], A. Tenenhouse[3], T. Anastassiades*[8], W. Hopman*[8], S. Kirkland*[9], C. Joyce*[10], A. Papaioannou[1], A. Cranney[8], O. Johnell[11], E. A. Papadimitropoulos*[12], J. D. Adachi[1]. [1]McMaster University, Hamilton, ON, Canada, [2]Laval University, Ste-Foy, PQ, Canada, [3]McGill University, Montreal, PQ, Canada, [4]University of Calgary, Calgary, AB, Canada, [5]University of British Columbia, Vancouver, BC, Canada, [6]University of Saskatchewan, Saskatoon, SK, Canada, [7]University of Toronto, Toronto, ON, Canada, [8]Queen's University, Kingston, ON, Canada, [9]Dalhousie University, Halifax, NS, Canada, [10]Memorial University, St. John's, NF, Canada, [11]Malmo University Hospital, Malmo, Sweden, [12]Eli Lilly and Company, Toronto, ON, Canada.

The purpose of the study was to examine the relationship between a prior minimal trauma fracture and health related quality of life (HRQL), as measured by the HUI Mark II & III. Participants were included from CaMos, a nation-wide, randomly selected sample of the Canadian population, and were classified into three groups according to their previous fracture history: those with any prior fracture (ANY), those with a prior main fracture at the wrist, hip, clinical spine, pelvis or ribs (MAIN), and those with only a prior wrist/forearm fracture (WRIST). A total of 1598, 760 and 558 women and 535, 210 and 134 men 50 years of age and older had a minimal trauma ANY, MAIN, and WRIST. HRQL was measured using the HUI Mark II & Mark III. The Mark II and III multi-attribute utility scales (global health) are both classified such that the score for 0=dead and the score for 1=perfect health. Multivariable logistic regression analyses were performed to determine the association between prior fracture types and HRQL. All analyses were conducted separately for women and men. Final model selection was conducted using the Bayesian Information Criterion technique. From these analyses, we calculated odds ratios (OD) and 95% confidence intervals (CI) for all parameters. In women, adjusted OD indicated that higher Mark II HRQL was associated with a lower likelihood of having a prior ANY (0.45; CI: 0.27, 0.74), and MAIN (0.45; CI: 0.25, 0.84). Higher Mark III HRQL was associated with a lower likelihood of having a prior ANY (0.49; CI: 0.35, 0.69), and MAIN (0.53; CI: 0.35, 0.81). Higher Mark II (0.51; CI: 0.26, 1.01) and Mark III HRQL (0.66; CI: 0.41, 1.06) tended to lower the likelihood of having a prior WRIST but further evidence will need to be collected to verify these findings. In men, our results had wide CI and are difficult to interpret. In conclusion, HRQL as measured by the HUI was negatively associated with previous fractures.

Disclosures: ***G. Ioannidis****, None.*

SU289

Patterns in Loss to Follow-up Can Influence Estimates of Risk Reduction of Fracture. D. M. Black[1], D. E. Thompson[2], C. Teutsch[2], A. E. de Papp[2]. [1]University of California San Francisco, San Francisco, CA, USA, [2]Merck & Co., Inc., West Point, PA, USA.

When exact times or intervals containing exact time of fracture are known, survival analysis methodology is appropriate to describe and infer about time-to-event data. In clinical trials when two groups are compared, there are three components to the analysis: estimating cumulative incidence within each group, making inference about differences between two cumulative incidence curves, and quantifying difference in terms of relative risk. Each component uses different methodology: Kaplan Meier (KM) estimation of cumulative incidence, log rank test, and Cox's proportional hazard model, respectively. The focus of this paper is to comment on issues that can influence the first component of the analysis, specifically, can patterns in loss to follow-up influence estimates of cumulative incidence derived from KM methodology? KM methodology attempts to estimate proportion of patients with a specific event during the study. It takes into account that not all patients will complete the study. Patients who are lost to follow up (e.g., died) are considered "censored" and are included in the denominator at each event only prior to being censored. When dropouts are low and occur at a uniform rate, difference between KM estimate at study end and observed proportion at study end is small. When dropouts occur late in the study they have little effect on estimates of true cumulative incidence. However, when dropouts are large and occur very early in the study, the KM estimated cumulative incidence in early periods have a much greater carryover effect on cumulative incidence at the end of the study. Specifically, early effects are given more weight than late effects. We examine loss to follow-up in two (FIT I and VERT MN) recent osteoporosis clinical trials and possible effect this loss could have on the calculated fracture risk reduction. In both studies an intention-to-treat analysis was employed. In VERT MN the loss to follow-up was large (approximately 40%) and appeared to be early. KM estimates of the cumulative incidence at study end were 29 and 18% in the placebo and treated groups, respectively. Observed simple %'s which assumes no dropout were 25.7% and 15.4%, respectively. Corresponding relative risk reductions (RRR) were 49% and 39%. In FIT I, loss to follow-up was less than 3% and corresponding RRR were 51 and 52%. If we simulate a 50% loss to follow-up, RRR would be 63%. Loss to follow-up can bias the true relative risk reduction. If there is significant loss to follow-up, particularly early in the trial, the statements that investigators conducted an "intention-to-treat analysis" generally provide little assurance about the accuracy of the risk reduction.

Disclosures: ***D.E. Thompson****, Merck & Co., Inc. 3.*

SU290

Hyperkyphosis Predicts Mortality Independent of Vertebral Osteoporosis in Older Women. D. M. Kado[1], G. A. Greendale[1], L. Lui[2], K. E. Ensrud[3], H. A. Fink[3], T. Hillier[4], S. R. Cummings[2]. [1]Medicine, University of California, Los Angeles, Los Angeles, CA, USA, [2]San Francisco Coordinating Center, San Francisco, CA, USA, [3]University of Minnesota, Minneapolis, MN, USA, [4]Kaiser Center for Health Research, Portland, OR, USA.

Only about half of hyperkyphosis, or excessive curvature of the thorax, is due to vertebral fractures. Vertebral fractures are associated with increased overall mortality. Whether hyperkyphosis, also predicts increased mortality, independently of vertebral fractures, is unclear. To answer this question, we studied a consecutive sample of 610 women, aged 67-93 years, who had kyphosis measured using a flexicurve (Milne and Lauder, Ann Hum Biol, 1974). Prevalent vertebral fractures at baseline were defined by morphometry, and mortality was assessed during an average follow-up of 11 years. In age-adjusted models, each SD increase in kyphosis carried a 1.19-fold increased risk of mortality (95% C.I.: 1.04-1.36, p = .01). Those in the top quartile of kyphosis had an age-adjusted risk of 1.38 (95% C.I.: 1.02- 1.87, p = .04) compared to the lower three quartiles. Adjusting for age, self-reported health, physical activity, weight change, lumbar bone mineral density, and number of prevalent vertebral fractures did not change the relationship between poorer kyphosis and increased mortality (RH per SD increase in kyphosis: 1.19; 95% C.I.: 1.01-1.39, p = .03). We conclude that older women with hyperkyphosis are at increased risk of mortality that is not due to underlying spinal osteoporosis.

Disclosures: ***D.M. Kado****, None.*

SU291

Evaluation of Decision Rules for Referring African-American Women for Bone Densitometry by Dual-Energy X-Ray Absorptiometry. L. Wallace[1], J. Ballard[2], D. Holiday*[3], P. Cussen*[4], L. Turner*[5]. [1]Family Medicine, University of Tennessee Graduate School of Medicine, Knoxville, TN, USA, [2]Health & Kinesiology, University of Texas at Tyler, Tyler, TX, USA, [3]Biostatistics, University of Texas Health Center at Tyler, Tyler, TX, USA, [4]Radiology, University of Texas Health Center at Tyler, Tyler, TX, USA, [5]Health Science, University of Arkansas, Fayetteville, AR, USA.

Osteoporosis is an increasingly extensive, chronic, metabolic bone disease characterized by decreased bone mass and increased susceptibility to fractures. It is widely accepted that bone mineral density (BMD) measurements form the basis for the diagnosis of osteoporosis. The purpose of this study was to assess criterion validity of 6 decision rules--Age, Body Size, No Estrogen (ABONE), National Osteoporosis Foundation (NOF) practice guidelines, Osteoporosis Risk Assessment Instrument (ORAI), Osteoporosis Self-Assessment Tool (OST), Simple Calculated Osteoporosis Risk Estimation (SCORE), and body weight less than 70 kg (Weight Criterion)--for selecting postmenopausal African-American women for dual-energy x-ray absorptiometry (DEXA). Chart abstractions from 174 African-American women (mean age=59.4±12.5 years) with BMD testing results by DEXA were completed. Sensitivity, specificity, 95% confidence intervals (CIs), and area under the receiver operating characteristic (AUROC) were used to measure the overall ability of each decision rule to discriminate between women with varying degrees of low BMD. The percent of women with a BMD T-score less than –1, less than –2, and no more than –2.5 were, 25.3%, 14.9%, and 14.9% respectively. Sensitivity for identifying women with a BMD T-Score of less than –2.0 ranged from 31.2-62.5%, while specificity ranged from 62.9-93.7%. Sensitivity for identifying women with a BMD T-Score of no more than –2.5 ranged from 32.1-61.3%, while specificity ranged from 84.2-96.2% (Table). The AUROC curves were greatest using SCORE, OST, and Weight Criterion for BMD T-Scores of no more than –2.5. The OST and Weight Criterion are easiest to calculate therefore may be most useful in clinical practice.

Sensitivity (SEN) and Specificity (SPC) of Decision Rules by Femoral Neck BMD T-Scores

Decision Rule	BMD less than -2.0 SEN (95% CI)	BMD less than -2.0 SPC (95% CI)	BMD no more -2.5 SEN (95% CI)	BMD no more -2.5 SPC (95% CI)
ABONE	50.0 (32.1-67.9)	85.1 (77.0-93.2)	51.6 (34.0-69.2)	86.3 (78.4-94.2)
NOF	31.2 (21.4-40.9)	92.9 (84.2-100)	32.1 (21.7-42.4)	96.2 (91.8-100)
ORAI	53.7 (38.4-68.9)	93.7 (87.6-99.7)	50.0 (34.1-65.9)	89.4 (82.0-96.8)
OST	62.5 (42.5-77.5)	91.7 (85.3-98.1)	61.3 (44.1-78.4)	90.4 (83.7-97.2)
SCORE	55.0 (33.2-76.8)	82.1 (74.0-90.3)	59.2 (38.6-79.6)	84.2 (76.2-92.1)
WT	37.1 (21.1-53.2)	62.9 (51.5-74.3)	51.1 (36.5-65.7)	88.0 (79.7-96.3)

Disclosures: ***L. Wallace****, None.*

SU292

Prevalence of Osteoporosis in Patients Undergoing Evaluation for Lung, Liver, and Heart Transplantation. P. M. Camacho[1], B. Pisani*[2], S. Bhorade*[3], S. Creech*[4], F. Nabhan*[1], P. Sapountzi*[1], S. Hou*[5], G. Sizemore[1], D. Van Thiel*[6]. [1]Endocrinology and Metabolism, Loyola University of Chicago, Maywood, IL, USA, [2]Cardiology, Loyola University of Chicago, Maywood, IL, USA, [3]Pulmonary and Critical Care Medicine, Loyola University of Chicago, Maywood, IL, USA, [4]Biostatistics, Loyola University of Chicago, Maywood, IL, USA, [5]Nephrology, Loyola University of Chicago, Maywood, IL, USA, [6]Gastroenterology and Hepatology, Loyola University of Chicago, Maywood, IL, USA.

This study aimed to determine the prevalence of osteoporosis and osteopenia among patients undergoing evaluation for liver, lung and heart transplantation at Loyola University Medical Center between 1998-2003. 187 patients with pretransplant DEXA scans out of 314 candidates were identified. The group was comprised of 71 liver , 61 lung, and 55 heart transplant candidates.The World Health Organization criteria was used to define osteopenia (<-1 to -2.4) and osteoporosis (<=-2.5). The mean age in years of the liver group was 51.5 +-11.4, lung was 48.7 +-11.6, and heart was 53.3 +-11.2. Females comprised 32% of the liver, 63% of the lung and 20% of the heart transplant group.

Overall 66 % of patients had osteoporosis or osteopenia before transplantation. Prevalence of osteoporosis was highest in the lung transplant group, followed by the liver and heart groups (24%, 16%, and 11% respectively). The rates of osteopenia in the heart, lung and liver groups were 45%, 44%, and 31% respectively.

Mean lumbar spine bone mineral density (LS BMD) and lumbar spine T scores (LS T) were similar between the groups. Mean femoral neck bone mineral density (FN BMD) (0.866 vs. 0.977 vs. 0.982 gm/cm2, p=<.001) and T scores (FN T) (-1.1 vs. -.49 vs.-.554, p=.015) were significantly lower in the lung than liver and heart transplant groups.

In the liver transplant group, patients with primary biliary cirrhosis had significantly lower LS BMD than those with alcoholic cirrhosis and chronic viral hepatitis (0.965 vs. 1.135 vs. 1.20 gm/cm2, p= .030), as well as lower LS T (-1.93 vs. -.786 vs. -.19, p= .042), FN BMD (0.790 vs. 0.984 vs. 0.977 gm/cm2, p= .003) and FN T (-1.5 vs. -.46 vs. -.58, p= .013) . In the lung transplant group, cystic fibrosis patients had significantly lower LS BMD (0.984 vs.1.109 vs.1.201 gm/cm2, p=.005) than those with emphysema and idiopathic pulmonary fibrosis. LS T, FN BMD and FN T scores were similar in these groups. In the heart transplant group, no significant differences in bone density and T scores were seen between patients with dilated cardiomyopathy and ischemic cardiomyopathy.

Patients undergoing heart, liver and lung transplantation are at increased risk of bone loss. Proper pre-transplant screening is advised in this high risk population.

Disclosures: ***P.M. Camacho****, None.*

SU293

Hip Structural Geometry Changes During and After Lactation. M. A. Laskey*[1], B. C. C. Khoo*[2], R. I. Price[2], T. J. Beck[3], A. Prentice[1]. [1]MRC Human Nutrition Research, Cambridge, United Kingdom, [2]Sir Charles Gairdner Hospital, Perth, Australia, [3]Johns Hopkins University, Baltimore, MD, USA.

Lactation is associated with temporary decreases in hip BMC. This study investigates whether these changes are accompanied by alterations in hip structural geometry that may affect bone strength. DXA measurements were made longitudinally during 65 lactations in 47 breast-feeding women (BF). Nonpregnant nonlactating women (NPNL, n=24) were studied as controls. Women had hip DXA scans (Hologic QDR-1000W) at baseline (2 wk post-partum (pp)), peak-lactation (3mo pp if BF for 6mo), post-lactation (1yr pp or 3mo post-lactation if BF >9mo) and follow-up (at least 2 year pp). Hip scans were analysed using the hip structural analysis (HSA) program which measures BMD and structural geometry at the narrow neck (NN), intertrochanter (IT) and proximal shaft (S).

No significant differences from baseline were observed in HSA measurements at any time-point or hip site in NPNL women. In contrast for BF women, at peak lactation, BMD, cross-sectional area (CSA) and average cortical thickness (AvCT) had decreased from baseline values at NN (-2.8%, P<0.001; -3.0%, P<0.001, -2.9%, P<0.001), IT (-2.7%, P<0.001; -2.0%, P<0.001; -2.7%, P<0.001) and shaft (-1.3%, P=0.01; -0.9%, P=0.09; -1.78%, P<0.01). Bone width was unchanged at all sites but endosteal diameter (ED) increased at S (1.6%, P=0.03) and IT (1.1%, P<0.001). These changes resulted in an increase in buckling ratio (BR, index of cortical stability) at S (2.4%, P<0.01) and IT (3.1%. P<0.001) and a trend to a decrease in section modulus (Z, indicator of bending strength) at NN (-2.4%, P=0.08). By post-lactation most HSA measurements were not significantly differently from baseline. Small nonsignificant increases in HSA measurements were observed at NN and S at follow-up. In contrast, at IT, there were significant increases above baseline in BMD (2.5%, P<0.001), CSA (3.2%, P<0.001), Z (5.0%, P<0.001) and AvCT (2.2%, P=0.01) and a significant decrease in BR (-2.2%, P<0.01).

These results suggest that during lactation decreases in HSA measurements are significant but temporary. The alterations occurred mainly at internal surfaces. As these are close to the neutral axis, the effect on fracture risk is minimal. Changes in cortical stability (BR) may be minimal in young women because BR describes a threshold instability that is unlikely to be exceeded in young women. At follow-up, the positive changes in IT hip geometry,above baseline may reflect the return to the prepregnant state and/or indicate a long-term benefit of childbearing on hip structure.

Disclosures: ***M.A. Laskey****, None.*

SU294

Parkinson's Disease Is Associated with Low Bone Mineral Density in Older Men. H. A. Fink[1], M. A. Kuskowski*[1], K. E. Ensrud[2], E. S. Orwoll[3], J. A. Cauley[4], S. R. Cummings[5]. [1]For the MrOS Research Group, GRECC, VA Medical Center, Minneapolis, MN, USA, [2]University of Minnesota, Minneapolis, MN, USA, [3]Oregon Health Sciences University, Portland, OR, USA, [4]University of Pittsburgh, Pittsburgh, PA, USA, [5]UCSF Coordinating Center, San Francisco, CA, USA.

The high risk for fractures associated with Parkinson's disease (PD) is usually attributed to an increased risk for falls. Limited case-control data suggest a possible association between PD and bone mineral density (BMD).

Utilizing data from MrOS, a multi-center, prospective cohort study of osteoporosis in 5995 men aged ≥65 yrs, we examined the association between PD and BMD. At MrOS baseline, self-reported data collected included demographics, lifestyle factors, medications, and medical history, including report of physician diagnosed PD (n=52). Clinic measurements included anthropometry and physical performance. BMD was measured with DXA at the total hip (TH), femoral neck (FN) and lumbar spine (LS). For each skeletal site, ANOVA and ANCOVA were used to assess the unadjusted, age-adjusted and multivariate-adjusted association between PD and BMD. The multivariate models for all sites included age, height, height loss since age 25, usual walking speed and maximum grip strength. In addition, the LS model included SF-12 physical component summary score, and both hip models included PASE physical activity score.

Results are presented as the mean percentage difference in BMD in men with PD vs. men without PD. Negative values indicate lower BMD in the PD group.

Mean Difference in BMD between Men with PD vs. without PD

	Unadjusted	*Age-adjusted*	*Multivariate adjusted*
Total hip	-7.2% p<.001	-6.5% p=.001	-5.4% p=.006
Femoral neck	-6.2% p=.007	-5.5% p=.015	-4.7% p=.034
Lumbar spine	-4.6% p=.061	-4.8% p=.050	-5.1% p=.034

Older men with PD have substantially decreased BMD at the hip and lumbar spine. The association of PD with low BMD was only modestly altered by adjustment for age and multiple covariates. Our results suggest that older men with PD should be targeted for osteoporosis screening and possibly considered for pharmacologic intervention to reduce their elevated risk for fractures.

Disclosures: ***H.A. Fink****, None.*

SU295

Depression Contributes Substantially to Osteoporosis Among Elderly Men. Results from Mr. Os Hong Kong, the First Prospective Cohort Study of Osteoporosis in Asian Men. S. Y. S. Wong[1], E. M. C. Lau[1], J. Woo*[2], K. Ng*[1], H. Lynn*[1], K. L. Stone*[3], S. R. Cummings*[3], E. Orwoll*[4]. [1]Jockey Club Centre for Osteoporosis Care and Control, School of Public Health, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China, [2]Department of Community and Family Medicine, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China, [3]San Francisco Coordinating Center, California, CA, USA, [4]Oregon Health and Science University, Portland, OR, USA.

Depression causes biological changes, such as decreased testosterone and increased cortisol levels, that may influence bone mass and fracture risk. To test the hypothesis that men with depression have lower bone mass, we used data from the baseline examination of Mr. Os Hong Kong, a prospective study of 2000 Hong Kong men age 65 to 92 recruited from community-based sources. Depression was diagnosed by face-to-face interview, using a validated Chinese version of the Geriatric Depression Scale (GDS), with depression being defined as a cut-off of 8 or more. Bone mineral density (BMD) of the total hip was measured by dual X-ray densitometry (DEXA) using the Hologic QDR-4500W. In the study sample, 8.5% of men were found to be depressed; and the BMD at the hip in these subjects were 2.1% lower than non-depressed subjects (95% CI –4.1 to –0.13); after adjusting for body weight, medical history, calcium intake and physical activity. Depression was associated with a 1.4-fold (95% CI=1.00 to 2.08) relative risk (RR) of osteopenia or osteoporosis. Study subjects from Mr. Os were healthy volunteers, and population-based surveys have found that the prevalence of depression in elderly men in Hong Kong was 29.2%. Based on this figure, we estimated that 9.8% of 'osteopenia and osteoporosis' among elderly Hong Kong men could be attributed to depression (Population Attributable Risk, PAR). We conclude that depression is associated with lower BMD and accounts for a substantial percentage of osteoporosis and osteopenia among elderly Chinese men.

Disclosures: ***S.Y.S. Wong****, None.*

SU296

Risk Factors for Low Bone Mass in Elderly Men – Mr. Os (Hong Kong, the Largest Cohort Study in Asian Men). E. M. C. Lau[1], S. Y. S. Wong[1], H. Lynn*[1], J. Leung*[1], P. C. Leung*[2], K. L. Stone*[3], S. R. Cummings*[3], E. Orwoll*[4]. [1]Jockey Club Centre for Osteoporosis Care and Control, School of Public Health, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China, [2]Department of Orthopaedics and Traumatology, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China, [3]San Francisco Coordinating Center, California, CA, USA, [4]Oregon Health and Science University, Portland, OR, USA.

There has been no comprehensive study of risk factors for low bone mass in Asian men. Mr. Os (Hong Kong) is the largest and only cohort study to address this issue. Two thousand community dwelling and ambulatory Hong Kong Chinese men aged 65 to 92 years were recruited from the community and risk factors for osteoporosis were assessed by face-to-face interviews using a standardized, structured questionnaire. Bone mineral density (BMD) was measured by DEXA (Hologic, Inc). Factors found to be significant in regression was put into a final multiple regression model, which is described below.

Percentage difference in BMD (95% confidence interval) for significant risk factors for hip BMD

Factors associated with BMD (unit)	**% Diff (95% CI)**
Age (5 years)	-0.6(-1.2, 0)
Cigarette smoking (20 pack years)	-0.6(-1, -0.3)
COPD (yes / no)	-2.2(-4, -0.4)
GI surgery (yes / no)	-2.6(-4.4, -0.8)
Inhaled steroid (yes / no)	-7.9(-13.8, -1.9)
Body weight (5 kg)	3.6(3.3, 4)
Grip strength (5 kg)	0.8(0.3, 1.3)
Diabetes mellitus (yes / no)	4.2(2.6, 5.7)
Thyroid disease (yes / no)	4(0.1, 7.8)
	R^2=0.307

Model adjusted for dietary intake of protein and calcium, hypertension and thiazide diuretic intake.

These results suggest that ideal body weight, avoiding smoking and maintaining muscle strength are important in preventing osteoporosis in men. However, lifestyle and anthropometric risk factors could only account for 30% of the individual variability in BMD, and cannot replace BMD measurements.

Disclosures: ***E.M.C. Lau****, None.*

SU297

Risk Factors for Low Bone Mass in Elderly Asian Women – Ms. Os (Hong Kong, a Large Cohort Study in Asian Women). D. T. K. Choy*[1], E. M. C. Lau[1], J. Leung*[1], P. C. Leung*[2], J. Woo*[3], A. Hong*[1]. [1]Jockey Club Centre for Osteoporosis Care and Control, School of Public Health, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China, [2]Department of Orthopaedics and Traumatology, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China, [3]Department of Community and Family Medicine, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China.

Little is known about risk factors for low bone mass in elderly Asian women. Ms. Os (Hong Kong) is a large cohort study to address this issue. Eight hundred community dwelling and ambulatory Hong Kong Chinese women aged 65 to 94 years were recruited and followed up. Risk factors for osteoporosis were assessed by face-to-face interviews using a standardized, structured questionnaire. Bone mineral density (BMD) was measured by DEXA (Hologic, Inc). Factors found to be significant in regression was put into a final multiple regression model, which is described below.

Percentage difference in BMD (95% confidence interval) for significant risk factors for hip BMD

Factors associated with BMD (unit)	**% Diff (95% CI)**
Age (5 years)	-3.2(-4.1, -2.2)
Body weight (5 kg)	4.4(3.8, 5)
COPD (yes / no)	-4.1(-7.8, -0.3)
Current cigarette smoking (yes / no)	-6(-12.1, 0)
Thiazide diuretic (yes / no)	7.9(2.4, 13.4)
Calcium intake (50 mg)	0.2(0.1, 0.3)
	R^2=0.350

Model adjusted for hypertension, GI surgery, Diabetes mellitus, grip strength and walking.

The results suggest that maintaining body weight, avoidance of cigarette smoking and maintaining calcium intake will be important in preventing osteoporosis in elderly Chinese women. However, lifestyle factors only account for 35% of the variability in BMD, and could not replace BMD measurement.

Disclosures: ***D.T.K. Choy****, None.*

SU298

Hyperkyphosis Increases Body Sway, Gait Unsteadiness and Risk of Falls in Osteoporosis. M. Sinaki[1], R. H. Brey*[2], C. A. Hughes*[3], K. R. Kaufman*[3]. [1]Physical Medicne and Rehabilitation, Mayo Clinic, Rochester, MN, USA, [2]Vestibular and Balance Laboratory, Mayo Clinic, Rochester, MN, USA, [3]Motion Analysis Laboratory, Mayo Clinic, Rochester, MN, USA.

This controlled trial was designed to investigate the influence of osteoporosis-related thoracic hyperkyphosis (OP-THK) on gait unsteadiness, body sway and falls.
Thirteen OP-THK women (Cobb angle 50 — 65° measured from spine radiographs) and 13 healthy women serving as controls (C) were enrolled. Mean age of the OP-THK group was 76 (±5), height 160 cm (±4), and weight 60 kg (±8) and C group means were age 71 (±5), height 160 (±4), and weight 68 (±13). Isometric strength data was collected using a Quantitative Muscle Assessment (QMA) System. Gait was monitored during unobstructed level walking and while stepping over an obstacle of four different heights randomly assigned (2.5%, 5%, 10%, and 15% of the subject's height). A ten-camera Real Time system was used to collect 3-D marker trajectory at 60 Hz during gait from 28 reflective markers. A repeated measures ANOVA was used for the statistical analysis with the significance level set at $p < 0.05$. Balance was objectively assessed using Computerized Dynamic Posturography (CDP) consisting of two components, the Sensory Organization Test (SOT), and the Motor Control Test (MCT). For this study, a composite score calculated from 14 scores of SOT was used. Statistical analysis was done using a student's paired t-Test with significance level set at $p < 0.05$.
The OP-THK subjects were significantly weaker than C subjects in all lower extremity (LE) muscle groups ($p < 0.05$) except the right ankle plantar flexors. There was a significant difference in the anteroposterior (A/P) and mediolateral (M/L) displacements and velocities. The OP-THK subjects had less A/P displacement, greater M/L displacement, reduced A/P velocity, and increased M/L velocity when compared to the C subjects. This was true for all conditions of unobstructed and obstructed level walking. There was a significant effect of obstacle height on all center of mass (COM) parameters. Even though COM displacement differences were detected, there was no significant difference in the right or left single support time nor the step width, which are usual indicators of balance problems. The OP-THK subjects had statistically significantly greater balance abnormalities on CDP compared to the C group ($p=0.0006$).
Our data show that thoracic hyperkyphosis plays a significant role in increasing body sway, gait unsteadiness and risk of falls in osteoporosis.

Disclosures: ***M. Sinaki****, None.*

SU299

Relationships Between the 4 Major Risk Factors for Hip Fracture and QUS Parameters from 5 Devices: The OPUS Study. A. Stewart*[1], F. Thomasius[2], D. Felsenberg[2], D. M. Reid[1], R. Eastell[3], C. Roux[4], C. C. Glüer[5]. [1]Osteoporosis Research Unit, University of Aberdeen, Aberdeen, United Kingdom, [2]Free University Berlin, Berlin, Germany, [3]University of Sheffield, Sheffield, United Kingdom, [4]René Descartes University, Paris, France, [5]University Hospital Schleswig-Holstein, Kiel, Germany.

Previous studies have identified 4 major risk factors for hip fractures. We examined these risk factors in the Osteoporosis and Ultrasound Study (OPUS) which is a large European population of 2374 postmenopausal women aged 55 to 79 years who were chosen at random. Each answered a risk factor questionnaire and had QUS scans of the heel (Lunar Achilles + (Ach.), UBIS 5000 (UBIS), OSI/Osteometer DTU-One (DTU), Quidel/Metra QUS-2 (QUS-2)) and of the fingers (IGEA DBM Bone Profiler (IGEA)) measuring broadband ultrasound attenuation (BUA), speed of sound (SOS), amplitude-dependent speed of sound (AdSOS), ultrasound bone profile index (UBPI) and bone transmission time (BTT). Comparing the number of risk factors with the QUS results we found significant relationships for all measurements (all $p<0.01$), with those with no risk factors having higher QUS results compared to those with 4 risk factors. Table 1 shows the results taking each of the risk factors individually. Any previous fracture; yes n = 1016, no n = 1323: all QUS parameters $p < 0.001$. Weight; lowest quartile n = 530, upper three quartiles n = 1844: all QUS measurements ($p<0.01$) except for IGEA BTT and IGEA UBPI. Smoking; ever smoked n=1016, never smoked n=1336: significantly different QUS for smokers for DTU SOS, UBIS SOS, IGEA Ad-SOS and IGEA UBPI. Maternal hip fracture; yes n =242, no n = 2095: none of the QUS parameters were significantly different. In conclusion there does appear to be similar relationships between the 4 major risk factors and QUS parameters in this European population. However the different QUS scanners do not all give the same results.

Z-scores	Previous fracture	Maternal hip fracture	Smoking	Weight
Ach. BUA	-0.18 *	0.03	0.04	-0.70 *
DTU BUA	-0.27 *	-0.03	-0.05	-0.75 *
UBIS BUA	-0.31 *	-0.05	-0.01	-0.71 *
QUS2 BUA	-0.23 *	0.03	-0.06	-0.66 *
Ach. SOS	-0.29 *	-0.05	-0.09	-0.23 *
DTU SOS	-0.31 *	-0.08	-0.13 *	-0.31 *
UBIS SOS	-0.33 *	-0.06	-0.16 *	-0.21 *
IGEA Ad-SOS	-0.29 *	0.06	-0.17 *	0.67 *
IGEA BTT	-0.23 *	0.12	-0.08	-0.04
IGEA UBPI	-0.27 *	-0.04	-0.14 *	0.03
Ach. Stiffness	-0.26 *	-0.02	-0.03	-0.47 *

* $p < 0.05$

Disclosures: ***A. Stewart****, None.*

SU300

Two Types of Osteoporosis and Fracture: "A" – BMD-dependent, and "B" – BMD-independent. A New Insight Into Old Classification. J. E. Badurski. Polish Foundation of Osteoporosis & Center of Osteoporosis and Osteoarticular Diseases, Bialystok, Poland.

Mean age of population of adult polish women in two large epidemiological studies BOS (1) and NEPOS (2) are 59 and 56 respectively. Prevalence of osteoporosis (Hip T-score –2.5) in BOS is15% and 18% in NEPOS (Forearm T-score –2.5). The number of fractures in BOS is twice as much as number of women with Hip T-score –2.5. Mean Hip T-score of women with previous fractures is –1.5. It contrasts with epidemiological studies conducted in older segment of female population (3), but is in accordance with that performed in all-adult ones (4). Comparative analysis of BOS, NEPOS and others (5) with known distinguishing factors shows a possibility of existence of two forms of osteoporosis and fractures, which is a proposal for clinical practice:

Distinguishing features	Type "A" - BMD-dependent Osteoporosis & fractures
Reason of compromised bone strength	Low bone density
Increased bone resorption	YES
BMD Hip T-score	Below –2.5
Age (majority)	Over 70 - 75
Impact of ageing on fracture risk	Principled
Localization of fractures	Vertebrae, hip
Diagnosis	Bone densitometry
Credibility of QUS measurements	+
Effectiveness of antiresorptive therapy	30 – 50 %
Relation to Melton & Riggs Type I & II classification	Closer to senile osteoporosis
Frequency of secondary osteoporosis	Rare
Relation to existing definition of osteoporosis	NIH 2001 WHO 1994 / IOF 2000

Overlap of both types increases risk fracture. Mostly epidemiological and observational data of BMD-dependent and BMD non-dependent fractures allows to see Melton's and Riggs's classification (6) in light of difficulties in evaluation of fracture risk and treatment of women with T-score between –1.0 and –2.0, in which antiresorptive treatment is questionable.
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Disclosures: ***J.E. Badurski****, None.*

SU301

Prevalence of Glucocorticoid Treatment in Kiel, Germany: Results From the Population-based PSIO-D Study. C. C. Glüer[1], C. Nöldeke*[1], R. Barkmann[1], M. G. Glüer[1], W. Timm*[1], D. Felsenberg[2], P. Martus*[3], M. Heller*[1]. [1]Diagnostic Radiology, University Hospital Schleswig-Holstein, Kiel, Germany, [2]Diagnostic Radiology, University Hospital Benjamin Franklin, FU, Berlin, Germany, [3]Medizinische Informatik, University Hospital Benjamin Franklin, FU, Berlin, Germany.

Glucocorticoid (GC)-induced osteoporosis is a widespread skeletal disease with substantial impact on health and quality of life. In Germany as well as in other countries few population-based data are available. The "Prevalence of steroid induced osteoporosis in Germany" (PSIO-D) study was designed to survey use of steroids in a population-based setting (survey phase) and subsequently study skeletal and health status in a subset of subjects undergoing extensive evaluations by state-of-the-art diagnostic tests (clinic phase). Here we report data for the survey phase from one center in Kiel, Germany.
30,876 subjects age 55 to 80, representing about 54% of the population of Kiel in this age segment were contacted by mail. 22,944 (74.3%) responded by returning the survey questionnaire. The response rate was similar for men and women and across age groups.
4,458 subjects, i.e. 19,4% (16% for men, 22% for women) reported current or previous GC treatment excluding topical applications. The rate slightly decreased with age ranging around 20% at age 55 down to 16% at age 80. In a random subset of 2,091 respondents that had reported use of GC, details of the kind of GC use was assessed. 49% of these subjects reported a history of oral GC treatment, 25% with a duration of at least 3 months. For current oral treatment the percentages decreased to 11% and 10% (of which 75.3% reported a daily dose of at least 2.5mg prednisolone equivalent), respectively. Based on these data, 4.7% of the general population reported a history of oral GC use exceeding 3 months, with a point prevalence of 1.9% for current use of GC exceeding 3 months, and of 1.4% for current medication of at least 2.5mg/day for at least the previous 3 months. Of the 2,091 individuals, 18% stated that they had been diagnosed with asthma (20% for men, 17% for women) and 19% with rheumatic disorders (14% for men, 22% for women). A diagnosis of osteoporosis was reported by 8.3% of those that stated that they never had taken GC treatment, by18% (6% for men, 24% for women) of subjects with a history of GC use, and 20% if GC use exceeded 3 months.
In conclusion, if our data from Kiel are representative we estimate a point prevalence of 1.4% in the general German population age 55-80 for continuous oral use of GC with a duration of at least 3 months and a daily dose of at least 2.5 mg prednisolone equivalent.

Consequently, approximately 300,000 subjects of age 55-80 in Germany may be at increased risk for osteoporotic fracture.

Disclosures: ***C.C. Glüer,*** *Procter&Gamble Pharmaceuticals 2.*

SU302

Prevalence and Predictors of Osteoporosis and Fractures in Type 1 and Type 2 Diabetes Mellitus. S. Finger*[1], T. Bruckner*[2], J. Schneider*[1], C. Scheidt-Nave*[2], C. Kasperk[1], R. Ziegler[1], G. Leidig-Bruckner[1]. [1]Internal Medicine, University Heidelberg, Heidelberg, Germany, [2]Clinical Social Medicine, University Heidelberg, Heidelberg, Germany.

The study aimed to investigate the prevalence and possible predictors of osteoporosis and related fractures in type 1 and 2 diabetes.

We investigated 398 consecutive patients from the outpatient clinic (diabetes type 1: 79 men (42 +/- 12 years) , 76 women (46 +/- 13 years); diabetes type 2: 115 men (63 +/- 9 years), 128 women (63 +/- 9 years)). All patients received a standardized questionnaire on diabetes history, risk factors for osteoporosis and insufficieny fractures. Diabetes related vascular complications were documented from the patients records and actual HbA1c was determined. Bone mineral density (BMD) was measured using DXA (Hologic 4500) at the lumbar spine (LS) and femoral neck (FN). Relationship between BMD/osteoporosis and possible risk factors (age, duration of diabetes, body mass index (BMI), HbA1c and vascular complications) was assessed by correlation analyses and logistic regression models.

Osteoporosis (t-score <-2.5 SD) at the LS (FN) was found in 5 (11) % of men and 5 (9) % of women with type 1 and in 6 (13) % of men and 9 (22) % of women with type 2 diabetes. Osteopenia (t-score between -1 and -2.5 SD) at the LS (FN) was found in 32 (44) % of men and 30 (41) % of women with type 1 and in 30 (36) % of men and 27 (31) % of women with type 2 diabetes mellitus. 12% of all patients suffered from 1 or more insufficiency fractures (13% in type 1 and 11% in type 2). FN-BMD was negatively correlated to duration of diabetes in type 1 (men: R=-0.25, women: R=-0.3) but not in type 2 diabetes. FN-BMD was positively correlated to BMI in type 1 (men: R=0.28, women: R=0.23) and in type 2 diabetes (men: R=0.37, women: R=0.44), but no relationship was found between BMD and HbA1c. The presence of vascular complications from diabetes was not significantly related to BMD or risk of osteoporosis. Multivariate analysis revealed only BMI to be predicitve in respect to occurrence of osteoporosis in type 1 and type 2 diabetes.

The prevalence of osteoporosis and fractures was similar in type 1 and type 2 diabetes without sex difference, although type 1 patients were about 20 years younger. Our data indicate that osteoporosis is a clinical significant and commonly underestimated problem especially in patients with type 1.

Disclosures: ***S. Finger,*** *None.*

SU303

Body Weight per se can Be used in Case-Finding for Bone Density Measurement in Asian Men – Findings from Mr. Os (Hong Kong). H. Lynn*[1], E. M. C. Lau[1], S. Y. S. Wong[1], P. C. Leung*[2], P. Mannen*[3], S. R. Cummings*[3], E. Orwoll*[4]. [1]Jockey Club Centre for Osteoporosis Care and Control, School of Public Health, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China, [2]Department of Orthopaedics and Traumatology, Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China, [3]San Francisco Coordinating Center, California, CA, USA, [4]Oregon Health and Science University, Portland, OR, USA.

The National Osteoporosis Foundation had clear guidelines for bone mineral density in older women. However, this does not apply to Asian men. The objectives of the current study were to evaluate if body weight could be used to identify older men for BMD measurement, and ascertain the value of adding other risk factors for case-finding. Mr. Os (Hong Kong) is the largest cohort study on osteoporosis in older Chinese men. Two thousand community-dwelling and ambulatory men aged 65 to 92 were recruited. Life style factors, anthropometry and BMD (DEXA, Hologic, Inc) were obtained by standard procedures.

Risk factors were identified by logistic regression, and ROC analysis was performed on a training sample of 1300 and a validation sample of 700. Using body weight less than 62 kg as a case-finding criteria for a BMD T-score of $\leq$ -2.5 at the hip or spine, a sensitivity of 82%, specificity of 57% and an area of 0.77 under the ROC curve was obtained in the validation sample. When cigarette smoking (for 40 years or more) was added, the sensitivity was 87%, specificity was 51%, and the area under the curve was 0.79. In comparison, when history of wrist fracture was added the sensitivity was 83%, specificity was 57%, and the area under the curve was 0.78.

We conclude that body weight provides a simple and satisfactory case-finding criteria for bone density measurement in Asian men.

Disclosures: ***H. Lynn,*** *None.*

SU304

Risk Factors for Osteoporosis in Postmenopausal African American Women. G. C. Woodson. Atlanta Research Center, LLC, Decatur, GA, USA.

Although postmenopausal (PM) African American (AA) women are at lower risk for all categories of osteoporosis-related fragility fractures compared with Caucasian (CA) women, osteoporotic fractures in AA women are associated with significantly higher morbidity and mortality. Therefore the early diagnosis and treatment of osteoporosis (OP) in this population is at least as important as for other ethnic groups and is worthy of the attention of their primary care physician, non-profit voluntary health organizations, and governmental agencies responsible for healthcare policy.

This retrospective case control study investigates risk factors (RF) for OP in population of PM AA women having bone density testing at an outpatient community-based OP specialty center between 1992 and 2002. The subjects were either self-referred or referred by their physician for testing. Spine and hip DXA (Hologic, Bedford, MA) bone density testing was performed on each subject. Patient medical history, family history, past and present pharmaceutical use, and dietary and exercise habits were collected using a patient self-administered osteoporosis RF questionnaire. The DXA manufacture's AA normative database was used for calculation of the patient's t-score and the diagnostic class was determined using the WHO's t-score based OP classification system as applied to either the L1-L4 PA lumbar spine or the total hip sites.

Results showed that 56 patients found to be osteoporotic, 99 were osteopenic, and 46 were diagnosed as having normal BMD. RFs more commonly found in the osteoporotic group compared to the normal group are displayed in table 1. Factors or conditions that were more common in the normal group versus the osteoporotic group are found in table 2.

Results Table 1

Factors Increasing Risk for Osteoporosis	p value
Sedentary lifestyle	<0.03
Family history of osteoporosis	<0.03
Low BMI	<0.05
Bilateral oophorectomy	<0.03
Premature menopause	<0.043

Results Table 2

Factors Decreasing Risk for Osteoporosis	p value
Osteoarthritis	<0.001
Consumption of 2 or more soft drinks daily	<0.03
Premenopausal use of oral contraceptives	<0.004
Postmenopausal use of estrogen replacement therapy	<0.05

In summary, while the incidence of osteoporotic fractures is 60% lower for PM AA women compared with their CA peers, the health consequences of these fractures is significantly greater. In this study, RFs for OP in PM AA women using a race specific normative database were found to be similar to those reported for PM CA women. These common RF can be used to help select AA women appropriate for bone density testing using central DXA. Treatment of PM OP diagnosed by DXA in AA women using US FDA registered therapies is indicated to prevent the consequences of this disease.

Disclosures: ***G.C. Woodson****, None.*

SU305

Relationship between Bone Mass and Cognitive Function in Postmenopausal Women. R. A. Brownbill[1], J. Z. Ilich[2]. [1]School of Allied Health, University of Connecticut, Storrs, CT, USA, [2]School of Allied Health, University of Connecticut, Storrs, CT, USA.

It has been suggested that bone loss and cognitive decline are co-occurring disease states, likely due to their relationship with estrogen. Few studies have examined the relationship between cognitive function and bone mineral density (BMD), a possible marker of cumulative estrogen exposure. We therefore were interested in determining if BMD and bone mineral content (BMC) measured from both cortical and trabecular sites, are positively related to cognitive function in 97 healthy postmenopausal women (ages 59.4-85.0 years). BMD and BMC from the whole body, lumbar spine, femur and forearm were measured with a Lunar DPX-MD instrument. Cognition was assessed using the mini mental state examination (MMSE). The MMSE is a widely used screening tool to assess cognitive impairment. It consists of various graded questions and tasks totaling to a maximum of 30 possible points, with 27 as a cut-off for normal, and less than 27 indicating impaired cognitive abilities. Subjects MMSE scores ranged from 22-30, indicating all subjects had either mild cognitive impairment or no cognitive impairment. Subjects were also divided into two groups, above and below the mean MMSE score (27.9). MANCOVA (adjusted for age, BMI, physical activity and lifetime exposure to smoking) reveled a significant moderate effect of MMSE on BMD, $p<0.05$, indicating 22% of generalized variability in BMD from the whole body, spine, hip and forearm was accounted for by differences in MMSE scores. ANCOVA follow-up tests did not reveal significant group differences for individual bone sites, though Group 2 (>27.9 score) had higher adjusted means for all sites except for the femoral neck, Ward's and trochanter. We hypothesize statistical significance was not reached because the average score on the MMSE of 27.9 was considered normal cognitive function based on MMSE criteria, indicating on average our subject population was not cognitively impaired. ANCOVA follow-up tests to MANCOVA did become significant when BMI was removed as a covariate. Groups were found to be significantly different for both total body BMC ($p=0.018$) and right shaft BMC ($p=0.01$). In conclusion, it appears mild degrees of cognitive impairment may be associated with lower levels of cortical bone specifically in the hip and whole body regions. Individuals with cognitive impairment should be screened for potential bone loss.

Disclosures: ***R.A. Brownbill****, None.*

SU306

Womens' Knowledge about Osteoporosis Does Not Influence Health-related Behavior. B. J. Edwards[1], S. Desai*[2], J. Feinglass*[1]. [1]Medicine, Northwestern University, Chicago, IL, USA, [2]Medicine, University of Illinois, Chicago, IL, USA.

Methods: 102 women seen at the McGaw Medical Center admitted with minimal trauma (MTF) hip fracture (n=44, 43%), or outpatients at the Geriatrics (n=19, 18%), or Osteoporosis Clinics (n=39, 38.2%) were interviewed. 58 (57%) had a history of prior fracture, 81 (79%) were on calcium and vitamin D, and 69 (69%) had a prior bone density test (DEXA). Subjects received education about osteoporosis risk factors, and osteoporosis diagnosis and treatment. Subsequently they underwent a survey exploring prior knowledge about osteoporosis and attitudes towards benefit of osteoporosis treatment.
Analysis: descriptive statistics, and correlations were performed.
Results:

Measures of Osteoporosis Knowledge and Attitudes Toward Benefit of Treatment

Knowledge Items	All Mean (SD)	Prior Fracture Mean (SD)	No Prior Fracture Mean (SD)
Understand Medications	0.96 (17)	0.96 (0.19)	0.97 (0.15)
Medication Compliance	0.97 (0.03)	1.00	0.90 (0.29)
Long-term benefit of medication	0.98 (0.29)	0.90 (0.29)	0.88 (0.29)
Overall Knowledge	4.90 (1.45)	4.70 (1.49)	5.13 (1.36)
Motivation to change scale	2.90 (1.61)	2.79 (1.62)	3.11 (1.65)
Importance of Osteoporosis	2.86 (1.13)	3.09 (1.06)	2.51 (1.15)
Overall Attitude	8.52 (1.65)	8.69 (1.63)	7.90 (1.57)

Overall Knowledge is assessed on a scale (1-6) based on respondents knowledge and understanding of medications.
Motivation to change scale is calculated by a number of lifestyle changes respondents are willing to make (Scale 0-4)
Importance of Osteoporosis Scale is calculated by reversing the rankings given to osteoporosis by respondents (scale 1-5)
Overall attitude/behavior is assessed by combining the scales of benefits gained from the information and importance of osteoporosis for the respondents (Scale 2-10)

Predictors of Knowledge and Attitude Levels

Predictors	High Knowledge Level (n=52) Percent (%)	High Knowledge Level . Odds Ratio	Positive Attitude level (n=28) Percent (%)	Positive Attitude Level (n=28) Odds Ratio
Mean Age	67.29	0.91	69.55	1.00
Osteoporosis Clinic	63	1.75	65.5	2.72
Geriatric Clinic	1.9	0.05	20	1.17
MTF Hip Fracture	60	2.31	13.8	0.24
Medication use	69	2.46	77	1.26
Prior DEXA	56	0.66	89	1.50
Prior Fracture	46	0.67	82	1.74

High knowledge is defined as respondents indicating yes to all six knowledge items.
Subjects attending the Ostoporosis Clinic exhibited positive attitude level as compared to other patient groups. Subjects with hip fractures exhibited high knowledge but low positive attitudes/behavior with regard to osteoporosis. The lowest knowledge and attitude/behavior toward osteoporosis was seen in Geriatric patients.
Conclusions: Our results indicated that attitudes toward osteoporosis are not greatly influenced by knowledge about this disease. In addition patients at high-risk for fracture (Geriatrics & MTF hip fractures) exhibit low health-related behavior about osteoporosis. Greater patient awareness is necessary to address the growing problem of osteoporotic fractures.

Disclosures: ***B.J. Edwards****, Merck 2, 5; Procter & Gamble 2, 5; Eli Lilly 8; Wyeth 8.*

SU307

Thyroid Functions, Bone Mass and Body Composition in a Population Study of 482 70-Year Old Women. The Nordos Study. M. Stenstrom*[1], E. Nystrom*[2], S. Jansson*[3], D. Mellstrom[1]. [1]Geriatric Medicine, Goteborg university, Goteborg, Sweden, [2]Dep of Endocrinology, Goteborg university, Goteborg, Sweden, [3]Dep of Endocrine Surgery, Goteborg university, Goteborg, Sweden.

Excess thyroid function increase bone turnover and the risk for osteoporosis. The question is if thyroid function within the normal reference range are related to bonemass and boby composition. An other question is if thyroxin treatment is related to low bone density in a representative population?
A random sample of 482 70 years old women participated in the study. Bone mass was measured with Hologic 4500A in hip, spine, forearm and whole body. Blood samples were taken in the morning under fasting conditions. Free thyroxineand TSH.
S FT_4 was not related to BMI r 0.05 but was related to fat mass r 0.125 $p < 0.01$ and indirectly to lean mass r -0.100 $p< 0.02$ and indirectly to whole body BMD r -0.095 $p < 0.02$. FT_4 wasrelated to s-calcium, r 0.18 and s-alcaline phosfatase activety r 015 $p < 0.01$. After exclusion of women treated with thyroxin and controling for BMI these correlations were strengthen further and s FT_4 correlated inversily to hip BMD r -0.083 $p < 0.05$. Women who were treated with thyroxin, 13.1 per cent, had a non significant trend to higher BMD in spine and hip compared to non-treated in spite of a significant higher s FT_4 and a significant lower TSH. There was not any difference in prevalence of previous fracture between thyroxin treated and controls. In conclusion we found a direct correlation between sFT_4 and fat mass and a indirect correlation to lean mass and whole body BMD. Thyroxin treated women did not had lower bone mass in spite of significant higher s FT_4 and lower TSH.

Disclosures: ***M. Stenstrom****, None.*

SU308

Awareness, Knowledge, Risk Factors and Current Treatment of Osteoporosis in a Brazilian Cohort of Elderly Subjects. R. Restituti*, C. H. M. Castro, M. M. Pinheiro*, V. L. Szejnfeld. Rheumatology, Universidade Federal de Sao Paulo, Sao Paulo, Brazil.

We evaluated awareness, knowledge, risk factors and current treatment of osteoporosis in 54 consecutive seniors attending an educational/social program for elderly people and 32 elderly subjects living in a nursing home, in Sao Paulo, Brazil. Our objective was to determine differences between the two cohorts and detect differences between men and women, as well. Subjects included were 70 women and 16 men, with an average age of 76 years. All participants answered an interviewer-administered questionaire regarding osteoporosis awareness, risk factors and treatment. Most of the subjects from the nursing home (44%) had finished high school, while the majority of seniors attending the educational program (55.5%) had not completed elementary school. Ninety six percent of all subjects were aware of osteoporosis and 49% gave the correct definition. In spite of the different education backgrounds, awareness of osteoporosis and its correct definition were not different between the two groups, neither between men and women. Television, friends and physicians were identified as the main source of information, especially among women. Most of the subjects (78% and 79%, respectively) were aware that osteoporosis could affect men and that diet was important. In all, 80% knew that osteoporosis could be prevented, and this was less in the nursing home group ($p<0.001$). Women believed that they could get osteoporosis more than men ($p<0.001$) and, in fact, a previous diagnosis of osteoporosis was more prevalent among women(40%) compared to men(6%; $p<0.05$). Besides old age and estrogen deficiency associated with menopause, the most prevalent risk factors included familial history of fractures-FHF (19%), past smoking (30%) and previous fractures (13%). FHF and previous fractures were both more common among seniors attending educational program than between subjects from a nursing home ($p<0.05$) and among women compared to men ($p<0.05$). Only 25% percent of the subjects were in use of specific treatment for osteoporosis and were taking calcium supplements. Calcium supplements use was more prevalent among women (30%) than men (6%; $p<0.01$). Our results demonstrate a high level of awareness and accurate definition of osteoporosis in a Brazilian cohort of elderly people. The study also shows that educational programs can provide specific information for elderly people that might influence the management of osteoporosis. Specific therapy and prevention measures for osteoporosis were inappropriately low for this group of subjects at high risk of osteoporosis.

Disclosures: ***C.H.M. Castro****, None.*

SU309

Response of Cancellous Bone to Estrogen Depletion in Three Strains of Mice. U. T. Iwaniec, D. Yuan*, R. A. Power, T. J. Wronski. Physiological Sciences, University of Florida, Gainesville, FL, USA.

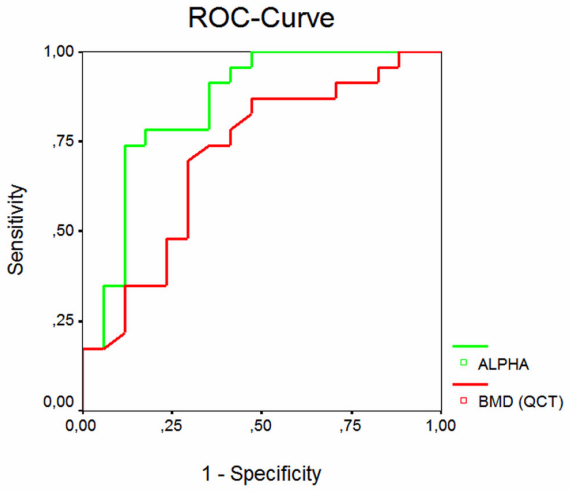
The purpose of this study was to characterize the skeletal response to estrogen depletion (ovariectomy) in three strains of mice (129P3, C57BL/6, and B6129PF2) commonly used in transgenic and/or knockout studies. The mice were ovariectomized (ovx) or sham-operated at 4 months of age. At this time, femora were collected from baseline control groups to determine strain-specific bone mass and turnover prior to estrogen depletion. The remaining mice were sacrificed at 1 or 3 months postOVX. Distal femora were processed undecalcified for collection of histomorphometric data, including cancellous bone volume, and osteoclast, osteoblast, and osteoid surfaces. The data are expressed as mean ± SD.
The 3 strains of mice differed in cancellous bone mass at baseline. Cancellous bone volume (BV/TV) was significantly greater in 4 month old 129P3 (12.9±4.0%) mice than in 4 month old C57BL/6 (5.2±1.3%) and B6129PF2 (5.5±1.4%) mice. Differences in osteoclast surface (Oc.S) were not detected among the 3 murine strains at baseline. However, osteoblast surface (Ob.S) and osteoid surface (OS) tended to be greater ($P<0.1$) in C57BL/6 and B6129PF2 mice than in 129P3 mice, suggesting increased bone turnover in the former 2 strains. Ovariectomy decreased bone volume and there was also a significant strain by ovx interaction. At 1 month postOVX, BV/TV was approximately 40% ($P<0.05$), 50% (P0.1) lower in ovx 129P3, C57BL/6, and B6129PF2 mice compared to age-matched sham mice, respectively. At 3 months postOVX, BV/TV was approximately 60% ($P<0.05$), 40% ($P>0.1$), and 60% ($P>0.1$) lower in ovx 129P3, C57BL/6, and B6129PF2 mice compared to age-matched sham mice, respectively. Although Oc.S, an index of bone resorption, increased with ovx and there was a significant strain by ovx interaction, Oc.S was significantly higher in ovx 129P3 mice than in sham 129P3 mice at 1 month postOVX only and significant differences in Oc.S were not detected between ovx and sham C57BL/6 or B6129PF2 mice. Ob.S and OS also tended to increase with ovx by approximately 20%. However, there was no significant strain by ovx interaction and significant differences in Ob.S and OS following ovx were not detected in any of the 3 strains. In conclusion, 129P3 and C57BL/6 mice lose bone following ovx, while only a trend for postOVX bone loss occurs in the B6129PF2 strain. In comparison to mice, rats ovx at 3 months of age lose approximately 80% of cancellous bone in the proximal tibia in the first 3 months postOVX and exhibit a 7-fold increase in Ob.S at 5 weeks postOVX (Wronski et al., CTI 43:179, 1988). Therefore, estrogen depletion appears to induce a greater increase in cancellous bone turnover in the rat skeleton than in the mouse skeleton.

Disclosures: ***U.T. Iwaniec****, None.*

SU310

Structure Analysis of the Distal Radius Using the Scaling Index Algorithm in the Prediction of Osteoporotic Spine Fractures. D. Mueller*[1], T. M. Link[1], R. Monetti*[2], J. Bauer*[1], E. J. Rummeny*[1], C. W. Raeth*[2]. [1]Department of Radiology, Technische Universitaet Muenchen, Muenchen, Germany, [2]Institut fuer extraterrestrische Physik, Max-Planck-Society, Garching, Germany.

Purpose: In this study we are using a newly developed 3D-based scaling index method in comparison with standard 2D-techniques to analyze High resolution magnetic resonance (HR-MR) images of the distal radius to investigate the trabecular structure in patients with and without osteoporotic spine fractures.
Methods and materials: Axial HR-MR images of the distal radius were obtained at 1.5 T in 46 women (22 postmenopausal women with osteoporotic spine fractures and 24 postmenopausal controls). A three-dimensional gradient-echo sequence was used with a slice thickness of 500 µm and in plane spatial resolution of 195x195 µm. Structure analysis was performed using algorithms based on a local 3D scaling index method as well as morphological 2D parameters. In addition BMD measurements of the spine using quantitative CT (QCT) and dual energy X-ray absorptiometry (DXA) were obtained in all patients.
Results: Significant differences between both patient groups were obtained using structure analysis and spine BMD ($p<0.05$). Receiver operating characteristics (ROC) analyses were used to determine the diagnostic performance in differentiating both groups. In comparison with BMD of the spine (Area under curve (AUC) =0.72) and the 2D based structure parameters (AUC up to 0.70) the best results were found for the local 3D scaling index method (AUC=0.85, Fig. 1).



Conclusion: The results of our study show that trabecular structure measures derived from HR-MRI of the radius using a newly developed algorithm based on a local 3D scaling index method can improve the diagnostic performance in differentiating postmenopausal women with and without osteoporotic spine fractures.

Disclosures: ***D. Mueller****, None.*

SU311

Bone Material Properties and Hip Fracture: Regional Decreases in Hardness and the Association with Bone Remodelling. E. Follon*, N. Loveridge, J. Power*, B. Bonfield*. University of Cambridge, Cambridge, United Kingdom.

Although differences in bone mass and structure between cases of intracapsular hip fracture and controls have been documented, there is little data on the material properties of the femoral neck bone or its relationship to either habitual load or the degree of bone remodelling. A micro-indentation technique was used to determine the regional hardness of the femoral neck cortex in cases of hip fracture (3F 1 M; ages 60-95y) in comparison with age/gender matched controls (4F, 1M; 52-91y).
After embedding, biopsies were ground and polished then indented using a Mitutoyo MVK-H2 microhardness tester with a Vickers tip. A 50g load was applied for a period of 10s. Each section was indented 200 times, with 50 indents in each of the four regions (anterior (A), inferior (I), posterior (P), and superior (S)); 30 in osteonal bone and 20 in interstitial bone. Data were converted to GPa and analysed using regression modelling in which region, bone type (interstitial/osteonal) and case/control status were considered as independent variables. Cortical remodelling was assessed on histological sections as the proportion of canals with osteoid (forming) or crenellated (resorbing) surfaces.
Interstitial bone was harder than osteonal bone (+10%; $p<0.0001$) and hardness was related to the proportion of canals with new bone formation (Osteonal: GPa=0.63-0.025*ln%osteoid; Adj.r2=0.20, p=0.005; Interstitial: GPa=0.7-0.026*ln%osteoid; Adj.r2=0.22, p=0.003). There were marked regional differences in the hardness of both osteonal and interstitial bone (Osteonal: P: 0.61±0.011 and I: 0.60±0.010 > A: 0.56±0.008 and S: 0.55±0.006. Interstitial: P: 0.68±0.011 and I: 0.66±0.009 > A: 0.062±0.007 and S: 0.62±0.004). Fracture cases had significantly lower hardness values in the anterior (-3.9%, p=0.005) and inferior (-2.5% p=0.049) regions for osteonal bone and in the anterior region (-5%, p=0.0005) for interstitial bone. After adjustment for the indices of remodelling the case-control differences were non-significant but the regional differences remained.
In conclusion, there are marked variations in the hardness of bone in the femoral neck, both between osteonal and interstitial bone and between cortical regions subjected to varying habitual strain modes. Bone habitually loaded in compression (I) was harder than that loaded in tension (S), and interstitial bone was harder than osteonal bone. Finally, hardness was lower in the anterior regions of the cases of hip fracture for both bone types, and lower in the inferior for osteonal bone, associated with increased remodelling.

Disclosures: ***E. Follon****, None.*

SU312

Periosteal Bone Turnover at the Femoral Neck in Non-Human Primates. M. Bliziotes[1], J. Sibonga[2], R. Turner[2], E. Orwoll[1]. [1]Oregon Health and Science University, Portland, OR, USA, [2]Mayo Clinic, Rochester, MN, USA.

Bone size is an important determinant of bone strength, and modeling at the periosteal surface alters bone dimensions in adults as well as during growth. Nevertheless, periosteal biology remains poorly understood.
Therefore, the proximal femurs of 16 adult non-human primates (Rhesus (Macaca mulatta, n=9) and Japanese Macaque (Macaca fuscata, n=7)), were analyzed after double-labeling with two discrete intervals of tetracycline (250 mg bid for 3 days separated by a 14 or 15 day interlabeling period). Necropsy was performed 2-7 days following the last administration of tetracycline. Histomorphometric measurements of fluorochrome labels and of stained sections complied with standardized procedures, formulae and nomenclature. Statistical comparisons between multiple groups were analyzed by 1-way ANOVA followed by a Fisher PLSD post-hoc test after determination of significance.
Both bone resorption and formation were present on the femoral neck periosteal surface. Multinucleated, acid phosphatase positive osteoclasts were present in typical Howship's lacunae. This osteoclastic activity was not the result of the emergence of intracortical tunneling at the bone surface. The periosteal eroded surface of the femoral neck was significantly greater than in the marrow compartment ($p<0.0001$) or on the femoral shaft periosteum ($p<0.0001$), although osteoclast number was similar at all three surfaces. Mineral apposition rate on the periosteal surface of the femoral neck was greater than on the shaft but less than on cancellous surfaces. Femoral neck periosteal bone formation rate was 2.5% of tissue volume per year, approximately 10-fold more than observed in the femoral shaft.
Thus, not only have we documented intramembranous periosteal bone formation in the femoral neck in a series of non-human primates, we have also found that the tissue level bone formation rate was sufficient to add substantively to femoral neck size over time. We also documented active periosteal bone resorption at the femoral neck. The turnover rate in the femoral neck periosteum was intermediate between femoral shaft periosteum and femoral neck cancellous bone. In conclusion, bone formation and resorption are active in the femoral neck periosteum and have the potential to exert meaningful effects on femoral neck size. The modeling rate at the femoral neck is different from femoral shaft periosteal and cancellous bone sites, suggesting distinct regulatory influences.

Disclosures: ***M. Bliziotes****, None.*

SU313

Regional Trabecular Anisotropies Suggest a 'Two-Domain' Loading Regime in the Proximal Femur. J. G. Skedros. Ortho, U of Utah, Salt Lake City, UT, USA.

Conventional wisdom teaches that the human proximal femur is adapted for transmission of tension/compression stresses associated with habitual bending. This appears to be embodied as arched trabecular patterns. However, recent 3-D finite-element analyses (FEAs) of physiologic loading suggest an alternative interpretation for stress transfer across this region [Lotz et al., 1995, Osteopor. Int.]. These analyses suggest that the 'expanded' trochanteric region allows for the appropriate mechanical advantage and attachment site for muscles rather than for structural integrity of the bone itself. Studies of patterns of predominant collagen fiber orientation (CFO) in cortical bone suggest that prevalent bending occurs in the subtrochanteric region, and prevalent torsion/compression at mid-neck. These data suggest that the trochanteric region may separate two loading 'domains' (subtrochanteric and femoral neck (FN)), each with important differences in stress distribution and transfer. This issue is relevant in understanding normal loading conditions across the hip and bone mass/quality changes that occur with age and implantation of intramedullary prostheses, and may have implications for age-related changes in the prevalence of FN vs. intertrochanteric fractures. Rigorously examining this 'two-domain' hypothesis is difficult because it requires the application of in vivo strain gauges. In this study we examined this question using patterns of trabecular architecture. 5mm thick sections of 15 Caucasian human femora (age range 20-70) were obtained in the plane of anteversion. Using radiographs of each specimen, obvious arched trabecular tracts were traced in the FN and lesser trochanteric (LT) regions. Cartesian data of each tract (superior & inferior in FN; lateral & medial in LT) were fit to linear or non-linear equations ($r^2 > 0.95$). Intersection angles at 'arch' apices were measured. Results (Table) demonstrate that the LT trabecular tracts are symmetric, and FN tracts are non-symmetric. The LT region exhibits intersections that are nearly orthogonal, in contrast to the non-orthogonal intersections in the FN ($p<0.05$). These data are consistent with the 'two-domain' hypothesis, as suggested previously by CFO data and FEAs showing that non-orthogonal, non-symmetric trabecular tracts are optimal for habitual torsion [Pidaparti and Turner, 1997; J Biomech].

Equations	Sup. FN	Inf. FN	Lat. LT	Med. LT
y^(-1)=a+b/x	93.8%	37.5%	100%	100%
y=a+bexp(-x/c)	6.2%	56.3%	0%	0%
y=ax+b	0%	6.2%	0%	0%
Angle of Intersect	69+/-12 deg		92+/-6 deg	

Disclosures: ***J.G. Skedros****, None.*

SU314

Structural and Biomechanical Basis for Differences in Vertebral Fragility in Chinese and Caucasians. Y. Duan[1], X. Wang*[1], C. H. Turner[2], C. Fong*[1], E. Seeman[1]. [1]Endocrinology, Austin & Repatriation Medical Centre, The University of Melbourne, Melbourne, Australia, [2]The Biomechanics and Biomaterials Research Centre, Indiana University, School of Medicine, IN, USA.

We hypothesized that the structural abnormalities predisposed to vertebral fracture are similar in Chinese and Caucasians, accounting for the similar vertebral fracture rates between races. We studied 687 healthy Chinese (449 females) and 1088 healthy Caucasians (738 females) aged 18 to 92 yrs. Vertebral body (VB) cross-sectional area (CSA) and volumetric BMD (vBMD, excluded posterior elements) were measured using dual x-ray absorptiometry by postero-anterior and lateral scanning. We calculated VB stress (load/CSA) and FRI (load/strength) during bending forward. In young adulthood, VB stress did not differ by race in either sex because the lower load (10-14%) in Chinese was distributed on a proportionately lower CSA (13-14%) than in Caucasians. However, vBMD was 9-13% higher in Chinese than Caucasians, conferring 12-19% lower FRI in Chinese men and women. Ageing was associated with increased CSA in both Chinese and Caucasian men and women. However, racial differences in periosteal expansion were minimal, increasing by 8.7% and 11.8% in elderly Chinese and Caucasian men, and increasing by 8.6% and 5.7% in elderly Chinese and Caucasian women (both no significant different to each other). VB stress decreased similarly in Chinese and Caucasian men (13.3% vs 13.7%) but decreased more in Chinese than Caucasian women (10.0% vs 5.5%, $p < 0.01$). Net decline in vBMD was greater in elderly Chinese than Caucasian women (33% vs 27%, $p < 0.01$) but similar in Chinese and Caucasian men (11% vs 12%). These structural changes were captured by FRI; a similar proportion of elderly Chinese and Caucasians men (5% vs 6%) and women (25% vs 29%) had the FRI >= 1. The results are consistent with the notion that vertebral fractures occur more commonly in women than in men but similar proportions of Chinese and Caucasians (of either sex) sustain fractures.

Disclosures: ***Y. Duan****, None.*

SU315

Structural Basis for Differences in Femoral Neck Fragility in Chinese and Caucasians. X. Wang*[1], Y. Duan[1], T. J. Beck[2], E. Seeman[1]. [1]Endocrinology, Austin & Repatriation Medical Centre, The University of Melbourne, Melbourne, Australia, [2]Radiology, The Johns Hopkins University, School of Medicine, Baltimore, MD, USA.

We hypothesized that structural characteristics may be better maintained in Chinese than Caucasians in old age, accounting for the lower hip fracture rates reported in epidemiological studies. A faster rate of periosteal apposition maintains bending strength, while a slower rate of periosteal expansion with a slower rate of endocortical resorption should reduce the increased risk of buckling with age. We measured femoral neck (FN) dimensions and bone mass using DXA, estimated endocortical diameter, cortical thickness, section modulus (a measure of bending strength), and buckling ratio (subperiosteal radius/cortical thickness) in 738 Chinese (490 females) and 1181 Caucasians (788 females) aged 18 to 93 years. In young adult women, after adjusting for racial differences in height and weight, FN axis length and diameter remained 4-8% lower in Chinese, while cortical thickness and vBMD were no different by race. Thus, growth produced racial differences in FN geometry; the same cortical thickness was distributed further from the FN neutral axis conferring 22.3% greater bending strength in Caucasians than Chinese. However, buckling ratio was 5.2% lower in Chinese than Caucasian women. In young adult men, bending strength was 12.5% lower while buckling ratio was no different in Chinese compared to Caucasians. From young (~30yrs) to old age (~70yrs), FN periosteal diameter (height and weight adjusted) increased less in Chinese than Caucasian men (1.0% vs. 9.1%), but increased similarly in Chinese and Caucasian women (4.6% vs. 3.3%). Endocortical diameter also increased less in Chinese than Caucasian men (2.6% vs. 12.5%), but similarly in Chinese and Caucasian women (8.5% vs. 6.5%). Consequently, bending strength decreased by 6.9% in Chinese men but maintained in Caucasian men, while bending strength decreased similarly in Chinese and Caucasian women (4.0% vs 6.9%). Buckling ratio increased less in Chinese than Caucasian men (14.5% vs 28.4%) but increased similarly among Chinese and Caucasian women (28.8% vs 31.2%). These changes produced 17.4-25.0% lower bending strength and 6.9-8.7% lower buckling ratio in elderly Chinese than Caucasians in both sexes. We concluded that despite the smaller FN diameter and lower bending strength, the relatively thicker cortex and narrower diameters in elderly Chinese suggest a lower risk of structural failure by local buckling than Caucasians. These structural differences in Chinese and Caucasians are likely to be established during both growth and aging.

Disclosures: ***Y. Duan****, None.*

SU316

Longitudinal Changes in 3D Microarchitecture of Human Iliac Crest Bone Biopsies: Transmenopausal versus Postmenopausal. J. J. Zhao[1], Y. Jiang[1], R. R. Recker[2], M. W. Draper[3], E. F. Eriksen[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 compares true longitudinal transmenopausal (TransM) changes with longitudinal postmenopausal osteoporotic (PostM) changes in 3D trabecular (Tb) structure. This 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 given that the mechanical competence of Tb bone is a function of its apparent density and 3D distribution. During aging and osteoporosis, Tb plates are perforated and connecting rods are dissolved, with a continuous shift from one structural type to the other, which cannot be evaluated by 2D histological sections. There is debate among histomorphometrists about whether Tb thinning, or rather Tb disappearance occurs with aging and/or menopause based on 2D sections using the parallel plate model. We examined 39 paired iliac crest bone biopsies (78 specimens). For 20 TransM women, the 1st biopsy was from normal, premenopausal women, mean age 49 (±SD, ±3) years (yrs), and the 2nd biopsy from the same group of women, but 1 yr postmenopausal, occurring 5 (±2) yrs) after the 1st biopsy. From 19 PostM women with osteoporotic vertebral fractures and the hip/lumbar BMD at least 1 SD below the mean value in normal young women, the 1st biopsy was taken at age 68 (±7) yrs, 20 (±9) yrs since menopause, while the 2nd was 19 (±5) months later. The specimens were scanned using a Scanco micro CT with isotropic resolution of 17 µ. 3D Tb structural parameters were directly measured without stereological model assumption. Structure model index 0 represents an ideal plate structure and 3 represents rod structure. There was a significant change between paired bone biopsies in 3D Tb bone volume fraction (TransM versus PostM, -4.0%/yr vs. –3.3%/yr), Tb number (-1.2%/yr vs. –0.1%/yr), Tb thickness (-2.7%/yr vs.-0.5%/yr), Tb separation (2.4%/yr vs. 0.5%/yr), structure model index (8.0%/yr vs. 4.6%/yr), degree of anisotropy (-0.6%/yr vs. -0.1%/yr), and connectivity density (-1.1%/yr vs. –9.2%/yr). The percentage change was greater in 3D Tb thickness than in Tb number and Tb separation. Thus, 3D Tb microstructure rapidly deteriorates in the iliac crest, which is more pronounced in TransM women than in PostM women with osteoporotic fracture, while loss in Tb connectivity density is much greater in PostM women than in TransM women. Tb thinning does occur and trabeculae dramatically shift from a plate-like structural type to a rod-like pattern, and become more isotropic.

Disclosures: ***J.J. Zhao****, None.*

SU317

Performance of In Vivo Micro-CT Analysis of Mouse Lumbar Vertebral and Knee Trabecular Bone Architecture. P. L. Salmon, E. Buelens*, A. Sasov*. Skyscan N.V., Aartselaar, Belgium.

The knee and lumbar vertebra are commonly studied trabecular bone sites in mice. There are obvious advantages to in vivo microCT analysis of trabecular bone, such as the possibility of sequential analysis of a bone site in a single animal. However there are several factors limiting the quality of microCT images of trabecular bone obtainable in vivo. Xray image contrast is reduced by the presence of surrounding soft tissue causing additional attenuation. The source and detector geometry imposed by an immobile subject limits pixel sizes attainable compared to ex vivo microCT systems. The need to rotate the source and detector array, and small animal movements during scanning, further limit image resolution. Bone morphometric parameters measured by microCT are presented for knee and vertebra both in vivo and ex vivo, using the Skyscan 1076 and 1072 scanners respectively. Measurements of aluminium phantoms are also presented. Sensitivity of morphometric parameter measurement to factors such as pixel size and thickness of surrounding x-ray absorbing material is assessed. Different histomorphometric parameters show different sensitivity to varying image resolution and contrast. Basic structural parameters such as bone volume and surface are relatively less sensitive. However parameters indicating connectedness or dissociation of structures, such as trabecular pattern factor and Euler connectivity, are acutely sensitive to image resolution. We demonstrate that despite the above mentioned constraints on in vivo microCT image resolution, useful and representative architectural parameters of mouse trabecular bone can be obtained by microCT in vivo, with pixel size of 8.9 micron and 10 percent MTF resolution below 15 micron.

Disclosures: ***P.L. Salmon****, Skyscan N.V. 3.*

SU318

Sex and Strain-Dependent Variation in Vertebral Cortical and Trabecular Bone Traits in Inbred Mice. M. L. Bouxsein[1], M. Russ*[1], M. Shea[2], E. S. Orwoll[2], R. F. Klein[2]. [1]Orthopedic Biomechanics, Beth Israel Deaconess Med Ctr, Boston, MA, USA, [2]Oregon Health & Sciences University, Portland, OR, USA.

Bone density and structure are strongly influenced by genetic factors. To date, most genetic studies have assessed BMD, bone size or strength characteristics that reflect either cortical bone, or a combination of cortical and trabecular bone, with few focusing exclusively on genetic determinants of trabecular bone density. We previously evaluated female mice from the C57Bl/6J-C3H/HeJ F2 intercross to show that vertebral trabecular bone traits are highly heritable, and we identified several QTL associated with these traits. In this study we used µCT to evaluate sex- and strain-dependent differences in vertebral trabecular and cortical bone traits in 4 mo-old male (M) and female (F) mice from 8 inbred strains: 129S1, Castaneus (Cast), BALB/cByJ (Balb/c), C57Bl/6J (B6), C3H/HeJ, DBA/2J (D2), A/J and AKR/J (n=13-20/gr). Narrow-sense heritability estimates for weight-corrected BV/TV, trabecular number (TbN) and separation (TbSp) were high in both M and F ($H2 = 0.74$ to 0.83), but somewhat lower for trabecular thickness (TbTh, $H2 = 0.33$ for M, 0.53 for F). Effects of strain, sex, and the interaction of strain x sex were highly significant for all vertebral traits ($p<0.0001$ for all). For example, trabecular BV/TV was highest in 129S1 (33.1±1.3%), intermediate in A/J, AKR, Balb/c, B6 and DBA (22 to 28%) and lowest in Cast (16.5±0.6%)($p<0.0001$ for strain). Interestingly, in 6/8 strains, TbN was higher in M than F ($p<0.0001$), whereas TbTh was consistently higher in F (7/8 strains, $p<0.0001$). Connectivity varied over 3-fold between strains, and was on average, 21% higher in M (6/8 strains, $p<0.0001$). Trabecular bone surface to volume ratio was 50% greater in Cast than in AKR and Balb/c ($p<0.0001$), with M having, on average, 14% higher values than F (7/8 strains, $p<0.0001$). The thickness of the anterior cortex was highest in AKR (150±2.3 µm) and lowest in Cast (59±0.9 µm), with F having consistently higher values than M (7/8 strains, $p<0.0001$). Across strains there was no correlation between cortical thickness and trabecular traits. These data confirm strong genetic regula-

tion of vertebral bone density and architecture in inbred mouse strains, with pronounced sex-specific patterns for trabecular microarchitecture. Additional studies of the genetic and sex-specific factors regulating vertebral morphology may provide key information regarding the etiology of vertebral fragility fractures.

Disclosures: ***M.L. Bouxsein****, None.*

SU319

Effects of Physical Activity and Lifestyle on Evolution of Hip Bending Resistance in Men and Women over 65: The Population-Based EPIC-Norfolk Cohort Study. S. Kaptoge[1], N. Dalzell*[1], R. Jakes*[1], N. Wareham*[1], K. Khaw*[1], T. Beck[2], J. Reeve[1]. [1]Cambridge University, Cambridge, United Kingdom, [2]Johns Hopkins University School of Medicine, Baltimore, MD, USA.

That a bone's fracture resistance is more directly related to its resistance to bending than to its areal bone mineral density (BMD) follows from the mechanical principles developed by Galileo and Euler. BMD is used in the investigation of hip fracture without regard to its relationship to bending resistance but little is known of the determinants of changing bending resistance in the proximal femur.

We analyzed data from years at recruitment to test the effects of aging, anthropometry and physical activity on section modulus (SM), a measure of bending resistance, and BMD. All subjects (680M, 666F, 67-76y) had hip Hologic 1000W DXA scans taken at baseline and at 2.9 years (476M, 446F) and 5.4 years (343M, 314F) later. Hip structural analysis was used to calculate BMD and SM on 3 regions; narrow neck (NN), intertrochanter (IT) and shaft (S). Linear mixed modeling was used to test effects of the predictors on both outcomes while adjusting for correlation between regions. Interactions with region and age were tested to determine if the effect of a predictor was similar in all regions and if it modified the effect of aging.

There was significant between-region correlation of outcomes in both genders (coefficients: 0.68 – 0.84 for BMD, 0.46 – 0.62 for SM). Hypothesis tests accounting for this between-region correlation indicated that the effects of most predictors varied significantly between regions. SM in women increased with increasing age, weight, height and grip strength and all had a significant interaction with region ($P < 0.003$). Weekly recreation time (WRT) and lifetime physical activity score were positively associated with SM and the coefficients were similar in the 3 regions ($P < 0.015$). SM was lower in women with history of any fracture and differences varied by region ($P<0.0001$). An interaction between age and weight indicated that low body weight was associated with lower SM that increased faster with age ($P = 0.001$). The principal discordance seen in contrasting BMD with SM was that BMD declined instead of rose with age, due to subperiosteal expansion.

In men SM increased with aging, weight, height and lifetime physical and sporting activity scores; the size of effect differed by region ($P < 0.029$). WRT had a positive effect on SM that was similar in the 3 regions ($P=0.011$).

We conclude that there are important effects of aging, physical activity and anthropometric variables on the evolution of hip bending resistance in men and women. The extent to which BMD can be validly used as a surrogate for SM in the epidemiology of hip fractures remains to be determined.

Disclosures: ***S. Kaptoge****, None.*

SU320

Impact of Spinal Mobility on Quality of Life in Patients with Postmenopausal Osteoporosis. N. Miyakoshi[1], E. Itoi*[1], M. Kobayashi*[1], H. Kodama*[2]. [1]Orthopedic Surgery, Akita University School of Medicine, Akita, Japan, [2]South Akita Orthopedic Clinic, Akita, Japan.

Previous studies have shown that progression of spinal osteoporosis with vertebral fractures results in a progressive decline in the quality of life (QOL). It has also been reported that patients with osteoporotic spinal deformities have a markedly limited range of motion (ROM) in lumbar extension. However, it has not been documented whether or not spinal mobility in patients with osteoporotic spinal deformities affects the QOL. The objective of the study was to evaluate the impact of spinal mobility on the QOL in patients with spinal osteoporosis. A total of 157 postmenopausal women with osteoporosis aged over 60 years was divided into five groups according to their spinal deformities: round back (RB, n=41), hollow round back (HRB, n=33), whole kyphosis (WK, n=40), lower acute kyphosis (LAK, n=18), and normal posture (NP, n=25). The QOL was evaluated using the Japanese Osteoporosis QOL Questionnaire (JOQOL) proposed by the Japanese Society for Bone and Mineral Research. This questionnaire contains six domains with higher scores indicating higher levels of QOL. The number of vertebral fractures, angles of thoracic kyphosis and lumbar lordosis, and spinal ROM during maximum flexion and extension were measured with radiographs. The total QOL score in RB, HRB, WK, and LAK groups was significantly lower than that in NP group ($p<0.05$). Among the groups with spinal deformities, WK group showed the lowest QOL score ($p<0.05$). All the groups with spinal deformities, but not NP group, showed significant positive correlations between the QOL and spinal ROM ($0.52<r<0.75$, $p<0.05$). In total of 157 patients, the total QOL score showed significant correlations with age, number of vertebral fractures, lumbar lordosis angle, and spinal ROM. Multiple regression analysis revealed that the spinal ROM best correlated with the total QOL score. We conclude that the QOL in patients with osteoporosis is impaired not only by the spinal deformities, but also by the spinal mobility.

Disclosures: ***N. Miyakoshi****, None.*

SU321

Binge-Like Alcohol Exposure of Adult Male Rats Alters Bone Collagen Degradation and Bone Mineral Density in Femur and Lumbar Spine: Effects Abolished by Risedronate. J. J. Callaci*, D. Juknelis*, N. Frost*, F. H. Wezeman. Orthopaedic Surgery, Loyola University Medical Center, Maywood, IL, USA.

Chronic alcohol consumption reduces bone mass and strength, increasing fracture risk for alcohol abusers. Mechanisms underlying this vulnerability involve modulation of bone remodeling. Direct effects of alcohol on bone formation are documented, those on bone resorption are less well studied. The skeletal effects of exposure to high blood alcohol concentrations (BAC's) attained during binge drinking have not been studied to date. In this report we examine the effects of repeated high dose alcohol treatment on bone collagen degradation, a marker of bone resorption and bone mineral density in an adult rat model of binge-like alcohol exposure. Intraperitoneal (IP) injections were used to administer a 20% (vol/vol) ethanol/saline solution (3g/kg, 1X/day) to rats on four consecutive days for 1, 2 or 3 weeks. Effects of treatment with the anti-resorptive agent risedronate (35 mg/kg, 1X/week) were also evaluated. Peak BAC's averaged 308.5 + 12 mg/dL, with an average BAC of 258.6 + 28.7 mg/dL at time of euthanasia. No significant effects of treatment were observed after 1 or 2 weeks of binge alcohol exposure. After 3 weeks of binge-like alcohol treatment, total serum deoxypyridinoline (Dpd) a crosslink of bone type I collagen released during the resorption process was significantly increased (205%, $p < 0.05$) over control levels. Bone mineral density (BMD) in cancellous bone of the distal femur and lumbar spine were significantly decreased (34 and 21% respectively, $p < 0.01$) also after 3 weeks of alcohol binge treatment. Significant effects of risedronate treatment were noted in reversing both ethanol-induced Dpd increases ($p < 0.01$) and BMD decreases ($p < 0.001$) of binge-treated rats to control levels. These findings suggest that BAC's attained during alcoholic binge drinking may effect the skeleton in part by stimulating bone resorption, an effect that is mitigated by risedronate.

Disclosures: ***J.J. Callaci****, None.*

SU322

Factors Responsible for Pain in Osteoporosis and Degenerative Joint Disease Based on Electroalgometry Using Fall of Skin Impedance in Response to Exercise Load. T. Fujita[1], M. Ohue*[1], S. Nakamura*[1], Y. Fujii[2], A. Miyauchi[3], Y. Takagi[3]. [1]Katsuragi Hospital, Kishiwada, Osaka, Japan, [2]Calcium Research Institute, Kishiwada, Osaka, Japan, [3]National Sanatorium Hyogo Chuo Hospital, Hyogo, Japan.

Accuracy and efficiency of electroalgometry (EAM) measuring skin impedance was improved by using portable Prep Check Electrode Impedance Meter Model EIM 105-T1 (General Devices, NJ) and Vitrode L (Nihon Kohden, Tokyo). Measurement range was 100 to 2000 k ohms with an accuracy of ± 3 ohms using 9μA, 5Hz test current. Seasonal variation was minor and insignificant in an air-conditioned test room. Basal impedance tended to rise with age in both males and females probably reflecting drying and coarsening of the skin, but the response to exercise load was independent of the basal value. Responses as pain expressed by visual rating scale (VRS) and fall of skin impedance measured by EAM were significantly correlated on knee extension and flexion (knee pain), spine extension and flexion (back pain) and walking (overall pain). Back pain expressed in VRS and EAM was significantly correlated with relative cortical volume in radius measured by pQCT ($P=0.0007$ and 0.0368) but not with lumbar bone mineral density (LBMD) measured by DXA, radial trabecular BMD measured by pQCT, degree of spondylotic changes in X-ray and intra-individual coefficient of variation of LBMD used as a measure of spondylotic deformity. Spinal compression fracture was significantly correlated with back pain expressed in EAM ($p= 0.0214$) but not subjective pain expressed in VRS. Back pain induced by spinal extension and flexion appears to be mainly influenced by vertebral instability dependent on the strength of cortical bone, the major supporter of the skeletal resistance to external force. Back and joint pain in osteoporosis and osteoarthritis improved in response to bisphosphonates, active absorbable algal calcium (AAA Ca) and collagen, probably through improvement of spinal stability by inhibition of osteoclastic and chondroclastic resorption and collagenase activity, according to the sensitive and objective pain measurement by elctroalgometry utilizing the fall of skin impedance as an index.

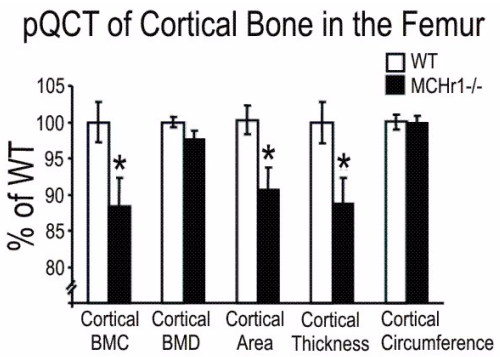
Disclosures: ***T. Fujita****, None.*

SU323

Osteoporosis in Melanin Concentrating Hormone Receptor 1 (MCHR1) Deficient Mice. M. Bohlooly-Y*[1], M. Mahlapuu*[1], H. Andersén*[1], A. Åstrand*[1], S. Hjorth*[1], L. Svensson*[1], J. Törnell*[1], M. R. Snaith*[1], D. G. Morgan*[1], C. Ohlsson[2]. [1]AstraZeneca R&D Mölndal, Mölndal, Sweden, [2]Centre for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden.

Osteoporosis is a metabolic bone disease characterized by low bone mass, leading to an increase in fracture risk. However, the mechanisms that lead to osteoporosis are still poorly understood. Recent studies indicate that the hypothalamus plays an important role in the regulation of bone mass. Melanin concentrating hormone (MCH) is highly expressed in the hypothalamus and has been implicated in the regulation of energy homeostasis (MCH). We investigated if MCH receptor 1 (MCHR1) -signalling is involved in bone homeostasis. Here we present data demonstrating that MCHR1-signalling is involved in a tonic stimulation of bone mass. Mice with an inactivated MCHR1 had osteoporosis, caused by a clear reduction in the cortical bone mass due to decreased cortical bone thickness. Serum levels of c-telopeptide, a marker of bone resorption, were increased in *MCHR1*$^{-/-}$ mice, demonstrating that the *MCHR1*$^{-/-}$ mice have a high bone turnover osteoporosis. We conclude that

the MCHR1 is a novel drug target for osteoporosis.



Disclosures: ***C. Ohlsson****, None.*

SU324

Six Minute Walk Test: A New Powerful Predictor of Functional Dysfunction in Osteoporotic Patients with and without Vertebral Fractures. J. T. Lin*[1], S. Backus*[2], E. Myers*[3], M. Peterson*[4], J. M. Lane[5]. [1]Physiatry/Metabolic Bone, Hospital for Special Surgery, New York, NY, USA, [2]Rehabilitation Medicine, Hospital for Special Surgery, New York, NY, USA, [3]Biomechanics, Hospital for Special Surgery, New York, NY, USA, [4]Statistics, Hospital for Special Surgery, New York, NY, USA, [5]Orthopedics/Metabolic Bone, Hospital for Special Surgery, New York, NY, USA.

Osteoporotic patients with vertebral compression fractures (VCF) are known to have increased morbidity and mortality. While it is recognized that VCF impair function, specific functional deficits have not been clearly identified. The purpose of our study was to identify functional deficits using kinesiologic tests performed in the motion analysis laboratory. 89 osteoporotic patients both with and without VCF underwent functional evaluation in the motion analysis laboratory, including timed up and go, functional reach, six minute walk, and bilateral single limb testing. Age of patients and number of compression fractures was recorded. Patients without VCF (n=41) versus patients with VCF (n=48) had an average age of 74.58 ± 8.86 vs. 73.39 ± 6.99 years, functional reach of 11.68 ± 2.94 vs. 10.81 ± 2.60 inches, six minute walk of 388.03 ± 127.99 vs. 315.20 ± 140.89 meters, average right single limb stance of 16.12 ± 19.26 vs. 15.27 ± 18.14 seconds, and average left single limb stance of 16.10 ± 18.93 vs. 13.40 ± 16.44 seconds. Patients with VCF versus patients without VCF were found to have statistically significant differences in the ability to perform six minute walk (Levene's equality of variances = 0.332, 2-tailed t test p=0.013). Analysis of our data shows trends suggesting that while all of the kinesiologic tests used may be useful in identifying functional deficits, the 6 minute walk is clearly efficacious. Previous authors have identified other balance instruments such as the get up and go and functional reach tests as useful predictors of functional limitation. Based on our data, we propose that the six minute walk test be used to identify dysfunction in osteoporotic patients. The six minute walk, a test of endurance and balance, may be a better predictor of the ability to perform instrumental activities of daily living such as banking and grocery shopping. Our data did not show direct correlations between number of VCF and functional limitations, suggesting that other factors, such as pain and kyphosis, may play important roles in dysfunction.

Disclosures: ***J.T. Lin****, None.*

SU325

The Effect of Age on the Expression of Nerve-Related Genes During the Healing of Femoral Fractures in the Rat. M. H. Meyer, W. Etienne*, R. A. Meyer. Department of Orthopaedic Surgery, Carolinas Medical Center, Charlotte, NC, USA.

Bone formation to bridge the fracture gap following femoral fracture slows with age in both humans and rats for unknown reasons. Abnormalities in the innervation of the fracture site will slow skeletal healing clinically. We explored whether abnormal nerve behavior in the older rats might contribute to this slowing of skeletal repair. Simple, transverse, mid-shaft, femoral fractures with intramedullary rod fixation were induced in female Sprague-Dawley rats at 6, 26 and 52 weeks of age. At 0, 0.4, 1, 2, 4, and 6 weeks after fracture a bony segment, one-third the length of the femur, centered on the fracture site, including the external callus, cortical bone, and marrow elements, was harvested from each subject. Total RNA was extracted from each segment. RNA was pooled between two rats of the same age and time after fracture. cRNA was prepared and hybridized to 18 Affymetrix U34A microarrays (1/age/time point). Of the 8,700 genes on each array, an average of 3,300 were scored as present. 240 genes were related to neural function. The mRNA expression of most of these responded to fracture. About 70 were increased by fracture, such as galanin (J03624), pleiotrophin (AI102795), and glia maturation factor B (Z11558). 52 decreased after fracture at all three ages, such as synuclein (AF007758), neurogranin (L09119), and neuropeptide Y (M15880). There was some differential effect with age. A few genes, 12, had a stronger response to fracture in young rats than in older rats, such as PCTAIRE 2 (AB005540) and 3 (AB005541) and synaptic scaffolding molecule (AF034863). More genes, 42, had a greater rise after fracture in the older rats than in the younger rats, such as glial-derived neurotrophic factor (L15305), thrombospondin 4 (X89963), and postsynaptic density protein (PSD-95) (M96853). In conclusion, mRNA of genes related to neuronal function is found in bone and in the fracture callus. Most of these genes respond to fracture with altered mRNA gene expression. Differential expression with age may reflect altered nerve cell function at the fracture site that may be related to the slowing of fracture healing.

Disclosures: ***M.H. Meyer****, None.*

SU326

The Effect of Age on the Expression of Mitochondrial Genes During the Healing of Femoral Fractures in the Rat. R. A. Meyer, M. H. Meyer. Department of Orthopaedic Surgery, Carolinas Medical Center, Charlotte, NC, USA.

In both humans and rats, bone formation to bridge the fracture gap after femoral fracture slows with age for unknown reasons. Cellular energy production is important to fracture repair. We had noted earlier transient decreases in the expression of mitochondrial genes following femoral fractures in the rat (JBMR 17 (Suppl. 1): S326, 2002). In this study, we have explored these genes in greater detail. In particular, we asked whether abnormal mitochondrial gene expression might contribute to the slowing of skeletal repair in the older rats. Simple, transverse, mid-shaft, femoral fractures with intramedullary rod fixation were induced in female Sprague-Dawley rats at 6 (young), 26 (adult) and 52 (old) weeks of age. At 0, 0.4, 1, 2, 4, and 6 weeks after fracture a bony segment, one-third the length of the femur, centered on the fracture site, including the external callus, cortical bone, and marrow elements, was harvested from each subject. Total RNA was extracted from each segment. RNA was pooled between two rats of the same age and time after fracture. cRNA was prepared and hybridized to 18 Affymetrix U34A microarrays (1/age/time point). Of the 8,700 genes on each array, an average of 3,300 were scored as present. 74 genes related to mitochondrial function were studied. The mRNA expression of these genes fell into one of three patterns. First, genes located within the mitochondrial DNA showed no decline in gene expression with age in unfractured bone. Fracture induced a moderate decrease in gene expression with recovery to baseline activity at 4 weeks after fracture in young and adult, but no recovery in the oldest rats. Examples are ND1 (M35826) and ND5 (S46798). Second, some autosomal genes for mitochondrial function showed reduced activity in unfractured bone in the oldest rats. Fracture led to a profound transient decrease in mRNA levels with recovery by week 2 in young and adult rats but no change in expression from their low basal values in the oldest rats following fracture. Examples include ATP synthase (D13123) and adenylate kinase 1 (D13376). Third, other autosomal genes for mitochondrial function also had a decrease in mRNA levels with age in unfractured bone. But, for all three ages, fracture caused a decrease in gene activity with subsequent recovery to baseline activity which was slower in the oldest rats. Examples include rhodanese (X56228) and creatine kinase (X59736). In conclusion, mRNA of mitochondria-related genes decrease after fracture. The prolonged down-regulation of some of these genes in the oldest rats may be related to the slowing of fracture healing.

Disclosures: ***R.A. Meyer****, None.*

SU327

Beta-blocker Use, BMD and Fractures in the Study of Osteoporotic Fractures (SOF). I. R. Reid[1], G. D. Gamble*[1], A. B. Grey[1], D. C. Bauer[2]. [1]Department of Medicine, University of Auckland, Auckland, New Zealand, [2]University of California, San Francisco, San Francisco, CA, USA.

The central nervous system has been demonstrated to regulate bone mass in mice, and recent work suggests that this is mediated by beta-adrenoreceptors on osteoblasts (Takeda et al, Cell 111:305, 2002). Beta-blockers have been shown to increase bone mass in mice. Since these agents are widely used therapeutically, it is possible that they may influence fracture epidemiology in humans, and they are a potential therapy for osteoporosis. We have studied their association with BMD and fracture rates in SOF.

Beta-blocker use was recorded at the 4th visit (94-96), and this information was available in 8412 women, of whom 1099 were users. Users had significantly higher weight, less smoking, more thiazide use, more HRT use, less glucocorticoid use, more statin use, and more hypertension than non-users. Total hip BMD at the 4th visit was higher in the beta-blocker users (0.746 vs 0.735 g/cm2, P=0.02), but adjustment for weight alone, or together with these other variables, eliminated this difference (P=0.62). There was no effect of beta-blocker use on loss of hip BMD over a mean follow-up of 4 years (0.69% per yr in users vs. 0.62% in non-users, P=0.25). Os calcis BMD at visit 4 was also higher in those taking beta-blockers (0.385 vs 0.375 g/cm2, P=0.005). Once again, weight-adjustment eliminated this difference (P=0.14).

The prevalences of hip, wrist or any fracture (since age 50 and prior to visit 4) were similar in users and non-users. However, over a mean follow-up of 7yr after visit 4, the incidence of hip fractures was 5.8% and 7.8% for users(n=1028) and non-users(n=6577), respectively, (P=0.03). Adjustment for weight and other factors previously shown to influence hip fracture incidence in this cohort, eliminated this effect (OR 0.95, 95%CI 0.64-1.40). There were 353 incident wrist fractures, but no effect of beta-blocker use was found (adjusted OR 1.30, 95%CI 0.91-1.84). The same pattern was observed for non-vertebral fractures, which were not different between-groups after adjustment for weight.

Thus, there are not major associations between beta-blocker use and either BMD or fracture rates in this cohort. The differences between these findings and those in mice may be related to differences in study design, in doses or durations of drug use, or in selectivity of beta-blockers studied. It is concluded that the use of these drugs is not associated with a reduced risk of osteoporosis in older women.

Disclosures: ***I.R. Reid****, None.*

SU328

Status of 25(OH)D in Korean Postmenopausal Women and its Relations to the Bone Metabolism. Y. Rhee*[1], Y. Kim*[1], Y. Won[2], S. Lim[1]. [1]Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea, [2]Internal Medicine, College of Medicine, Kwandong University, Seoul, Republic of Korea.

Active vitamin D is one of the key bone-related hormone. Vitamin D deficiency causes osteomalacia, and even its insufficiency would bring irreversible bone loss and increased risk of fracture. We evaluated the concentration of 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone(PTH) level in 172 Koreans postmenopausal women who visited our hospital during October 2001 to March 2002. For further analysis of the factors affecting the vitamin D status and relations with bone mass, Among them, 83 women were studied ; demographic characteristics, bone mineral density(BMD), and the bone markers. The mean serum 25(OH)D level was 14.0 ng/ml(34.8 nM). The prevalence of vitamin D insufficient patients defined as 25(OH)D less than 12 ng/ml was 23.8% which is quite high prevalence concerning that of the other countries. Serum 25(OH)D was inversely related to serum PTH level (r= -0.187, p=0.016) and also negatively associated with aging(r= -0.143, p=0.06). Eighty three postmenopausal women had taken the DXA(Hologic QDR 4500A), then they were divided into 3 groups; vitamin D sufficient group(S)[25(OH)D>12 ng/ml], vitamin D insufficient group(I)[12>25(OH)D>6ng/ml], vitamin D deficient group(D)[25(OH)D<6ng/ml]. In the D group, women were older and had significantly lower BMD at the lumbar vertebrae, the femoral neck, femoral trochanter and total hip($p<0.05$) than the S group. There were more patients with vertebral fracture in the D group than in I and S group(75% vs. 18.9%, 16.0%, p=0.046). Ten patients with low bone mass had been treated with alfacalcidol for 1 year and BMD increased 5.8%(p=0.04) at the lumbar spine and 2.0%(p=0,04) at the total hip respectively, We found out that the status of vitamin D expressed in 25(OH)D in Koreans postmenopausal women were much worse and were affecting bone negatively. However, we also had the positive result with the 1 year administration of active vitamin D against further bone loss. Therefore we need to be alarmed at this finding since this surely will affect the bone health especially in the elderly and to consider the adequate replacement of vitamin D in these patients.

Disclosures: ***Y. Rhee****, None.*

SU329

Trabecularization of Femoral Neck Cortex through Fenestration of Osteonal Walls: Association with Endocortical Thinning and Hip Fracture. J. Power*, N. Loveridge, A. Lyon*, J. Reeve. University of Cambridge, Cambridge, United Kingdom.

Femoral neck fractures are associated with increased intra- and endo-cortical remodelling and reduced wall thickness of endocortical packets. Furthermore, giant cortical canals develop in association with clustered intracortical remodelling. We hypothesised that these two characteristic phenomena of disordered remodelling may be driven by the same pathophysiological processes (eg. secondary hyperparathyroidism or mechanical underloading). If this were true they should be statistically associated.

Femoral neck fracture biopsies (female, n=12, mean age±SE = 81.3±1.3) and age/gender matched post-mortem controls (n=12; 81.4±1.8) were fixed and embedded in PMMA. Sections were stained with solchrome cyanine R for quantification of osteoid bearing canals and the extent of endocortical osteoid surface (%OS/BS). Osteonal wall thickness (W. Th.) and endocortical bone packet W.Th. were measured under polarised light. Eroded canals and endocortical surfaces (%ES/BS) were quantified on Goldner's stained sections.

There was a significant correlation between cortical and endocortical %OS/BS over the whole biopsy (P=0.041; Spearman-Rho test) and in the anterior (P=0.026), inferior (P<0.001) and posterior (P=0.047) regional quadrants. For %ES/BS, only in the inferior region was there a significant correlation (P=0.031) between compartments. Endocortical W.Th. data was compared with cortical osteon W.Th. where the canals were catagorised into simple (canal surrounded by a single undisrupted cement line) and composite systems in which the osteon is comprised of at least 2 separate bone packets. Endocortical mean W.Th. (31 microns ± 0.4) was identical to that in composite osteons (31 microns ± 0.9); and this was markedly lower (P<0.0001) than seen in simple osteons (46 microns ± 1.8), however there was no significant case/control difference within the regression model (P=0.33).

In conclusion, remodelling indices significantly associated between the intra- and endocortical envelopes are consistent with both being controlled by common regulatory processes. Furthermore, W.Th. of endocortical bone packets was the same as that in composite osteons. These results suggest the following scheme for cortical thinning: remodelling, regulatory osteonal canals merge to form giant canals through osteoclastic fenestration of their walls. Bone formation in these canals is reduced to the level in the endosteum. As the composite canals enlarge and the marrow compartment advances, trabeculae develop out of the residue of cortical bone. Bending resistance declines, while at the same time risk of femoral neck buckling during a fall onto the trochanter increases.

Disclosures: ***J. Power****, None.*

SU330

The Effects of Hindlimb Unloading and Alcohol on Cancellous Bone Histomorphometry and Gene Expression. T. E. Hefferan, G. L. Evans*, M. Zhang*, A. M. Kennedy*, R. T. Turner. Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

Alcoholism, a known risk factor for osteoporosis, can occur in combination with other risk factors. In this study we examined the effect of disuse and alcohol consumption on cancellous bone. The hindlimb unloading model was used to study 6 month-old intact male Fisher 344 rats for a period of 4 weeks. The treatments were baseline, control or alcohol diet; normal weight bearing, control or alcohol diet; and hindlimb unloaded, control or alcohol diet, with alcohol contributing 35% of the calories. Histomorphometry and molecular biology measurements were done on the tibia and femur metaphyses respectively. A two-way ANOVA was used to determine the effects of unloading and alcohol, as well as the interaction between treatments. Unloading resulted in a significant decrease in bone volume, while double-labeled surface was significantly decreased with unloading and alcohol consumption. Trabecular thickness and number were significantly decreased with unloading, while trabecular separation was increased. Mineral apposition rate was significantly decreased in the unloaded animals with significant interaction between unloading and alcohol. Indices of bone formation, mineralizing surface/bone surface, bone formation rate/bone surface and volume were significantly reduced with both unloading and alcohol treatments. There was a significant interaction between unloading and alcohol. Northern blot analysis and ribonuclease protection assay were used to measure mRNA levels of matrix proteins and cytokines, IL-1β, IL-6, IFN-γ, TGF-βs, and TNF-α. Type I collagen and osteonectin gene expression was significantly decreased with unloading, 4.5 fold and 3 fold. Osteocalcin gene expression was significantly decreased 4 fold with unloading and 2 fold with alcohol and there was a significant interaction between the two variables. IL-1β was significantly increased 0.8 fold with unloading and decreased 1.25 fold with alcohol treatment. In summary, histomorphometric and gene expression analysis show significant detrimental skeletal changes with unloading and alcohol consumption. Specifically, reductions in indices of bone formation were noted with both treatments. However, the detrimental effects of alcohol and unloading were not additive suggesting that they share, in part, common cellular pathways.

Disclosures: ***T.E. Hefferan****, None.*

SU331

Endothelial Nitric Oxide Synthase Expression Decreases in the Growth Plate but Increases in the Metaphyses of Bones from Lactating Rats in Association with Reduced Endochondral Growth and Increased Resorption and Osteoid Deposition. J. I. Aguirre[1], S. U. Igal*[1], A. Larsen*[1], A. Quiroga*[1], N. Lausada*[1], M. Petruccelli*[1], C. Perfumo*[1], S. Wimalawansa[2]. [1]Facultad de Ciencias Veterinarias, Univ. Nacional de La Plata, La Plata, Argentina, [2]Endocrinology, Robert Wood Johnson Medical School, New Brunswick, NJ, USA.

Lactation is a physiological condition in which a significant reduction in bone mineral density and loss of cancellous and cortical bone mass are observed. Like postmenopause or after ovariectomy, it represents an estrogen-deficient state due to the absence of estrous cycle. Previous studies demonstrated that estrogen regulates endothelial nitric oxide synthase (eNOS) expression and that nitric oxide is one of the local mediators by which estrogen regulates bone cell activity. Based on this, we hypothesized that the reduction in bone mass that occurs during lactation is mediated, at least in part, by a reduction in eNOS expression in the bone microenvironment as a result of estrogen deficiency. To examine this possibility, we studied bone metabolism in lactating rats by histomorphometry of the distal femur, proximal tibia and the first lumbar vertebra metaphyses and by using biochemical serum markers of bone turnover. In addition, we analyzed the pattern of eNOS expression by immunohistochemistry. Osteocalcin and crosslaps were significantly increased in the serum of lactating rats compared to age-matched, postpartum and non-lactating rats controls. Moreover, lactating rats showed a reduction in endochondral growth (EG), a decrease in bone volume (BV/TV), trabecular thickness (Tb.Th.) and mineralized surface (MS/BS) and an increase in osteoid surface (OS/BS) and eroded surface (ES/BS). In contrast, in ovariectomized (OVX) rats, EG was normal but the MS/BS was increased compared to sham operated rats. Interestingly, eNOS expression dramatically decreased in hypertrophic chondrocytes of the growth plates of lactating rats compared to their respective controls, but remained unchanged in OVX rats compared to sham. On the other hand, the immunoreactivity of eNOS increased particularly in osteoblasts in the trabecular surface of the secondary spongiosa in lactating rats. Taken together these data indicate that the estrogen deficiency state during lactation causes a reduction in bone growth, an increase in osteoclast resorption and in osteoid deposition without mineralization; and that these changes are associated with decreased eNOS expression in the chondrocytes of the growth plates and increased expression in osteoblasts in bone metaphyses suggesting a critical role of eNOS in lactation-induced bone loss.

Disclosures: ***J.I. Aguirre****, None.*

SU332

Long-Term Treatment with Risedronate Preserves Bone Quality. E. Paschalis[1], R. J. Phipps[2]. [1]Hospital for Special Surgery, New York, NY, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

It is now well established that bone quality, in addition to bone microarchitecture and bone mineral density (BMD), is an important determinant of bone strength and fracture risk. Treatment of postmenopausal osteoporotic subjects with risedronate reduces vertebral and nonvertebral fractures while concomitantly preserving bone microarchitecture and increasing BMD. In this analysis we compared the effects of up to 5-yr treatment with placebo or risedronate on two parameters of bone quality, mineral crystallinity and collagen cross-link ratio (pyr/deH-DHLNL), via Fourier transform infrared microscopic imaging (FTIRI).

Paired iliac crest biopsies were obtained from postmenopausal osteoporotic subjects at baseline and after 3-yr treatment with placebo (n=8) or risedronate (5 mg/day po; n=11). Biopsies were also obtained after 5-yr treatment with risedronate from 8 of these 11 subjects. Biopsies were embedded in methylmethacrylate, and the trabecular bone region was analyzed by FTIRI in ~4 um thick sections for mineral crystallinity (bone mineral crystallite size in the crystallographic c-axis) and collagen cross-link ratio. Analysis was focused

on trabeculae devoid of resorbing surfaces. Three images per section were acquired (each image 400 x 400 um^2 area or >2000 pixels with a spatial resolution of 7 um). Crystallinity and cross-link ratio (mean ± standard deviation) were compared before and after treatment (*P<0.05).
Subjects treated for 3-yr with placebo had statistically significant increases in both mineral crystallinity and collagen cross-link ratio, a pattern consistent with untreated osteoporosis. In contrast, 3-yr and 5-yr treatment with risedronate preserved mineral crystallinity and collagen cross-link ratio of trabecular bone.
This lack of an increase in both mineral crystallinity and collagen cross-link ratio coupled with increased BMD and preservation of microarchitecture suggests that risedronate suppresses osteoclastic activity relatively more than osteoblastic activity. These results contrast to previously reported increases in mineral crystallinity seen with other antiresorptive therapies, including ibandronate and estrogen.

	Crystallinity		Collagen Cross-Link Ratio	
Time	Placebo	Risedronate	Placebo	Risedronate
Baseline	0.92 ± 0.06	1.00 ± 0.11	1.40 ± 0.20	1.61 ± 0.40
3-yr	1.22 ± 0.04*	0.93 ± 0.06	1.90 ± 0.04*	1.61 ± 0.86
5-yr		0.93 ± 0.04		1.64 ± 0.52

Disclosures: ***E. Paschalis****, None.*

SU333

LS BMD Increase Accounts for only a Fraction of the Vertebral Fracture Reduction at 1 Year: Results from the VERT and HIP Trials of Risedronate. N. B. Watts[1], T. D. Johnson*[2], Z. Li*[2], C. Kasibhatla[2]. [1]University of Cincinnati, Cincinnati, OH, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

The relationship between BMD increase and vertebral fracture risk reduction is not linear. The proportion of risk reduction explained by BMD increase varies amongst different studies. The proportion of vertebral fracture risk explained by LS BMD increase at 1 year has been investigated for osteoporotic women taking risedronate and the results are reported here.
The analysis included patients from the VERT and HIP studies, who were enrolled either on the basis of low LS BMD (T-score < -2.0) and at least one prevalent vertebral fracture (VERT-NA) or two prevalent vertebral fractures (VERT-MN) or FN T-score < -4 or –3 with at least one nonskeletal risk factor for hip fracture (HIP studies). Patients were randomized to receive either placebo or risedronate 2.5 mg or 5 mg daily. In addition, patients also received vitamin D, if baseline levels were low, and1000 mg/day calcium supplementation. Cox regression analysis was used to estimate the overall treatment effect and the treatment effect explained by LS BMD at 1 year. Only patients who had a post-baseline BMD measurement prior to 1 year and a known fracture status were included in the analysis. There were 70 patients who had an incident vertebral fracture, out of a total of 1739. The overall risk reduction over 1 year was 60% (CI: 54-65). The proportion of fracture risk reduction explained by LS BMD increase over 1 year was estimated to be 7%.In conclusion, the increase in lumbar spine LS BMD only weakly contributes to vertebral fracture risk reduction at 1 year.

Disclosures: ***N.B. Watts****, None.*

SU334

Only a Small Proportion of Non-Vertebral Fracture Risk Reduction is Explained by BMD Increases. N. B. Watts[1], D. Felsenberg[2], T. D. Johnson*[3], Z. Li*[3], R. Eastell[4]. [1]University of Cincinnati, Cincinnati, OH, USA, [2]Department of Radiology, University Hospital Benjamin Franklin, Berlin, Germany, [3]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [4]University of Sheffield, Sheffield, United Kingdom.

The objective of the present study was to determine the contribution of BMD to osteoporosis-related nonvertebral fracture risk reduction in postmenopausal osteoporotic women. The analysis included patients from the two risedronate VERT studies who were enrolled in the trials on the basis of two prevalent vertebral fractures or low LS BMD (T-score < -2.0) and at least one prevalent vertebral fracture. Patients were randomized to receive either risedronate 5 mg or placebo daily. Patients also received vitamin-D, if baseline levels were low and 1000 mg/day calcium supplementation. Risedronate 5 mg daily reduced the risk of nonvertebral fractures by 35% over 3 years (95% CI=11%, 52%). The mean BMD percent increases over placebo at endpoint were 4.6% at lumbar spine and 2.6% at femoral neck. There was a non-linear relationship between BMD increases and nonvertebral fracture risk reductions. The proportion of nonvertebral fracture risk reduction explained by the BMD increases over 3 years (5mg vs. placebo) was estimated to be 12.2% (95% CI=5.7%, 26.1%) for lumbar spine and 5.5% (95% CI=2.6%, 11.9%) for femoral neck. The relationship between BMD increases and nonvertebral fracture risk reduction is non-linear. BMD increases observed during risedronate therapy only explain a small portion of the reduction in risk of non-vertebral fractures.

Disclosures: ***N.B. Watts****, None.*

SU335

Anti-Fracture Efficacy of Risedronate Is Greater than Nasal Calcitonin and Alendronate in an Observational Setting. N. B. Watts[1], K. Worley*[2], J. Doyle*[3], R. Sheer*[2], M. Steinbuch*[2]. [1]University of Cincinnati, Cincinnati, OH, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [3]Aventis Pharmceuticals, Bridgewater, NJ, USA.

To compare the anti-fracture efficacy of risedronate with other osteoporosis therapies in an observational setting, we used an administrative claims database to conduct a cohort study of patients age ≥ 45 years initiating bisphosphonate or nasal calcitonin therapy (93% women, mean age 69 years). Patients with prescriptions in the prior 6 months for a bisphosphonate, nasal calcitonin, or raloxifene were excluded. All risk estimates were adjusted for age, sex, estrogen use, prior fragility fractures, and a general morbidity indicator (number of concomitant medications). The incidence of nonvertebral fractures (clavicle, humerus, wrist, pelvis, hip and leg) was compared for patients receiving risedronate, alendronate, and nasal calcitonin.
The first analysis consisted of six-month fracture assessment in 7081 individuals with a new "index" prescription for risedronate (5 mg daily or 30 mg weekly), alendronate (5/10 mg daily, 35/70 mg weekly) or nasal calcitonin between July 2000 and December 2001. A second analysis examined 12-month fracture incidence in 5024 patients with an index prescription between July 2000 and June 2001. Compared with patients receiving nasal calcitonin, patients receiving risedronate showed statistically significant reductions in risk of nonvertebral fractures at 6 and 12 months (see table). Compared with patients receiving alendronate, risedronate-treated patients had a lower risk of nonvertebral fractures at 6 months that attained borderline statistical significance, and a significant reduction in nonvertebral fractures at 12 months (see table).

	Relative risk (95% confidence limits)			
	Risedronate vs. Alendronate		Risedronate vs. Calcitonin	
	6 months	12 months	6 months	12 months
Nonvertebral fractures	0.46† (0.20,1.06)	0.41* (0.18,0.94)	0.31* (0.12,0.81)	0.25** (0.10,0.64)

†p=0.067, *p<0.05, **p<0.01
In conclusion, in a large administrative claims database, patients initiating therapy with risedronate had a significantly lower risk of nonvertebral fractures compared with both alendronate and nasal calcitonin during the first 12 months of therapy. This study demonstrates substantial and rapid anti-fracture efficacy for risedronate in an observational setting, consistent with the fracture reductions observed at 12 months in clinical trials.

Disclosures: ***N.B. Watts****, None.*

SU336

Economic Evaluation of Gastrointestinal Events in Osteoporotic Patients Receiving Bisphosphonate Therapy in a Managed Care Setting. N. N. Borisov*[1], J. J. Doyle*[2], C. P. Brezovic*[1], R. L. Sheer*[1]. [1]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [2]Aventis Pharmaceuticals, Bridgewater, NJ, USA.

The objective of this study was to compare resource utilization and associated direct medical costs of GI-related events for both alendronate and risedronate patients utilizing an integrated administrative, medical and pharmacy claims database.
A retrospective cohort study was conducted among 4259 women and men (aged 65+) with a new prescription for risedronate (5mg/day), or alendronate (5mg/day, 10mg/day, 35mg/week or 70mg/week) between November 1, 2000 and February 28, 2002 (16-month capture period). GI-related medical resource utilization and direct medical costs were assessed for a 4-month period following initiation of the bisphosphonate therapy. GI-related events and/or medications were assessed to select only patients with no GI events in the 6-month pre-treatment period. Utilized GI-related resources (pharmacy, outpatient, and inpatient) were valued using 2002 US dollars.
There were 623 (15%) patients treated with risedronate, and 3636 (85%) patients treated with alendronate. The mean age in both cohorts was 75 years and 94% were women. In the first four months after initiating bisphosphonate therapy, the average GI-related direct medical per-member-per-month (PMPM) cost was significantly lower for risedronate patients compared to alendronate patients ($2.15 vs. $6.00, p = 0.0001). Risedronate patients had almost three times fewer inpatient visits (per 100 patients per month) than alendronate patients (0.24 vs. 0.60, p = 0.0157). Also, risedronate patients averaged half the number (per 100 patients per month) of outpatient visits incurred by alendronate patients (0.88 vs. 1.53, p = 0.0273).In a patient population aged 65+ years receiving osteoporosis treatment, risedronate was associated with markedly lower GI-related medical costs compared to alendronate. The main source of the GI related cost difference between risedronate and alendronate was inpatient care utilization, suggesting that alendronate patients were experiencing clinically more severe GI events.

Disclosures: ***N.N. Borisov****, None.*

SU337

An Observational Study of the Incidence of GI Events Among Patients Receiving Treament with a Bisphosphonate. K. Worley*[1], J. J. Doyle*[2], R. L. Sheer*[1], M. Steinbuch*[1]. [1]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [2]Aventis Pharmaceuticals, Bridgewater, NJ, USA.

The purpose of the present study is to evaluate potential differences in the occurrence of gastrointestinal (GI) events between patients taking risedronate and alendronate, two oral bisphosphonates for the prevention and/or treatment of osteoporosis, in an observational setting.

A retrospective cohort study was conducted among 835 risedronate patients and 4828 alendronate patients, aged 65 years and older, in the Protocare Sciences administrative claims database. Women and men initiating therapy with daily risedronate (5 mg), daily alendronate (5/10 mg), or weekly alendronate (35/70 mg) between November, 2000, and February, 2002, were included in the analysis. In order to evaluate only newly starting bisphosphonate patients, individuals were excluded from the analysis based on one or more prescriptions for risedronate or alendronate in the 6 months prior to the index prescription. A GI event was defined as a GI-related primary ICD-9 diagnosis code (e.g., esophagitis, heartburn, gastric ulcer). The incidence of GI events during the 4 months after initiation of therapy was compared for alendronate and risedronate patients. The occurrence of GI events in the 6 months prior to therapy was also evaluated for the two groups so as to capture any potential differences in GI risk prior to initiation of treatment.
Alendronate patients exhibited a 44% higher risk of incurring a GI event compared to risedronate patients during the treatment period. This difference in risk was statistically significant ($p = 0.01$), adjusting for age, sex, and GI-related events in the 6 months prior to initiating treatment. The two dosing regimens of alendronate were combined in the analysis since the weekly users did not notably differ from the daily users with respect to incidence of GI events ($RR = 1.08$). Patients initiating alendronate were 20% less likely to have a history of GI events compared to those initiating risedronate ($RR = 0.80$, $p < 0.05$), suggesting a possible preference by physicians for prescribing risedronate among high-risk GI patients.
This study shows that among patients aged 65 years of age and older, alendronate users are at a significantly higher risk of GI events compared to risedronate patients during the first 4 months of therapy. The elevated risk cannot be attributed to age, sex, or preexisting GI problems

Disclosures: ***K. Worley****, None.*

SU338

Bone Histology and Histomorphometry in a 2-Year Risedronate Study Comparing Daily and Weekly Dosing. R. R. Recker[1], E. W. Sod[2], Z. Li*[2], A. A. Chines[2]. [1]Creighton University, Omaha, NE, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

The risedronate once-a-week efficacy study compared daily 5 mg to 35 and 50 mg once-a-week treatments in postmenopausal osteoporotic women. The primary endpoint of the non-inferiority study was lumbar spine BMD percent change at 1 year with safety evaluated over 2 years. Both weekly doses were non-inferior to 5 mg daily in lumbar spine BMD. Iliac crest biopsies were obtained in a subset of patients at baseline (n=109) and year 2 (n=86). Histology and histomorphometry data were collected from the biopsy samples as part of the safety evaluation. 193 of the biopsy samples were suitable for evaluation.
No histological abnormalities were observed in any of the risedronate-treated groups, suggesting normal bone quality even at the highest dose of 50 mg once- a-week. All endpoint biopsies had double tetracycline labeled surfaces.
Bone structural parameters were generally unchanged and similar among the treatment groups. Bone turnover by histomorphometry was significantly and similarly reduced in all groups by approximately 65%. This was consistent with observed decreases in the resorption marker NTx (50-60% reduction). All groups showed increased formation periods (FP) with significant changes in the 5 mg daily and 35 mg weekly groups. Consistent with previous 5 mg/d data, median mineralizing surface was approximately 1.5% in all groups after 2 years of therapy. Median change in mineral apposition rate (MAR) was positive in all groups and reached statistical significance in the weekly dose groups. Osteoid measures (surface, thickness, and volume) decreased in all treatment groups. Although wall thickness was unchanged in all groups, increased MAR and FP is suggestive of an anabolic effect of risedronate.
This is the first study demonstrating daily and weekly risedronate regimens produce similar results at the bone tissue, BMU, and cellular levels. The histology observations and histomorphometry data support the safety of the daily and once-weekly doses tested. These bone biopsy results, in combination with the non-inferiority of lumbar spine BMD in both weekly regimens, supports the selection of 35 mg once-a-week as an alternative to 5 mg daily risedronate.

Disclosures: ***R.R. Recker****, None.*

SU339

A New Dosing Concept for Bisphosphonate Therapy: Rationale and Design for the Monthly Oral iBandronate in LadiEs (MOBILE) Study. R. R. Recker[1], J. Y. Reginster[2], P. D. Delmas[3], K. Coutant*[4], B. Bonvoisin[4]. [1]Creighton University, Omaha, NE, USA, [2]University of Liège, Liège, Belgium, [3]Claude Bernard University and INSERM Research Unit 403, Lyon, France, [4]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

Due to stringent dosing recommendations, oral daily and weekly bisphosphonate regimens may not be fully acceptable to some patients, potentially compromising adherence and therapeutic outcomes. Simplified, less frequent regimens may help optimize patient management. Ibandronate is a highly potent, nitrogen-containing bisphosphonate with proven fracture efficacy when administered as an oral daily (2.5mg; vertebral fracture risk reduction: 62%) or novel oral intermittent (20mg every other day for 12 doses every 3 months; vertebral fracture risk reduction: 50%) regimen. An oral monthly formulation of ibandronate is currently undergoing clinical assessment and is expected to provide an optimal combination of efficacy, tolerability and patient convenience. A randomized, double-blind, parallel-group study (Monthly Oral iBandronate In LadiEs: MOBILE) is exploring the non-inferiority of once-monthly oral ibandronate (100mg or 150mg) to the proven oral daily ibandronate (2.5mg) regimen in terms of lumbar spine (L2–L4) BMD change in postmenopausal women (aged 55–80 years, TSM ≥5 years) with osteoporosis (lumbar spine BMD [L2–L4] T-score <–2.5 and ≥–5.0). Approximately 1,600 patients were randomized to one of four treatment groups for 24 months: 2.5mg oral daily ibandronate; 100mg oral monthly ibandronate (given on a single day); 100mg oral monthly ibandronate (given as separate 50mg doses on two consecutive days); 150mg oral monthly ibandronate (given on a single day). All patients receive the same medication schedule, with placebo replacing active medication as required for blinding. Patients receive oral daily calcium (500–1500mg) and vitamin D (400IU). The primary endpoint is the relative change in lumbar spine (L2–L4) BMD after 12 months. Secondary endpoints include the relative change in lumbar spine (L2–L4) BMD after 24 months, relative change in hip BMD (all sites) after12 and 24 months and biochemical markers of bone turnover after 3, 6, 12 and 24 months. Adverse events, including clinical vertebral and non-vertebral fractures, are monitored throughout the study. Non-inferiority analysis is an accepted and widely used methodology for demonstrating therapeutic equivalence between alternative regimens (e.g. weekly vs daily alendronate, weekly vs daily risedronate). Thus, in the MOBILE study, likely vertebral fracture efficacy will be demonstrated if the study shows the non-inferiority of a once-monthly regimen to the proven oral daily regimen in terms of spinal BMD change.

Disclosures: ***R.R. Recker****, None.*

SU340

Effect of Risedronate on Bone Mass and Remodeling in Orchidectomized Male Wistar Rats. M. Diaz-Curiel[1], C. Garcia-Moreno*[2], M. Lefort*[2], C. De la Piedra[2]. [1]Internal Medicine, Fundacion Jimenez Diaz, Madrid, Spain, [2]Bone Pathophysiology Lab, Fundacion Jimenez Diaz, Madrid, Spain.

The purpose of this study was to determine the ability of risedronate to prevent and/or treat orchidectomy-induced osteoporosis in male rats.
A total of 108 male Wistar rats, age 10 weeks old, weight 269,1 +/- 22,5g were operated (sham operated or orquidectomized). Two studies have been performed: prevention of orchidectomy-induced bone loss, and treatment of established orchidectomy-induced bone loss.
- Prevention study: Sham (n=13): rats sham operated, treated with placebo and sacrificed at 16 weeks old. OQX (n=14):orquidectomized rats treated with placebo and sacrificed at 16 weeks old. OQX + RSD (n=14): orquidectomized rats, treated with risedronate and sacrificed at 16 weeks old.
- Treatment study: Sham1 (n=9): rats sham operated sacrificed at 3 months after orchidectomy. OQX1 (n=9): orquidectomized ratas sacrificed at 3 months after orchidectomy. Sham2 (n=9): rats sham operated treated with placebo starting 3 months after operation. OQX2 (n=14): orquidectomized rats treated with placebo 3 months after operation.OQX2+RSD (n=14): orquidectomized rats treated with risedronate starting 3 months after operation.
Risedronate (0,5 mg/kg/day) and placebo (saline) were administered via oral gavage for 6 weeks. After sacrifice non-consecutive sections of lumbar vertebrae stained with von Kossa were used for Trabecular Bone Volume (BV/TV %) calculations. Osteocalcin (BGP), as bone formation marker, and carboxyterminal telopeptide of collagen Type I (CTX), as bone resorption marker, were respectively measured by ELISA (Rat-MID Osteocalcin ELISA and RatLaps ELISA, Osteometer Bio Tech A/S, Denmark) in rat serum.

GROUPS	BV / TV (%)	BGP (ng / ml)	CTX (ng / ml)
Sham	37.4 +/- 5.6	307.01 +/- 39.56	90.17 +/- 16.07
OQX	33.8 +/- 4.7**	397.11 +/- 49.72***	102.55 +/- 6.71*
OQX+RSD	32.7 +/- 4.4***	285.05 +/- 69.49[a]	99.31 +/- 13.8
Sham1	32.4 +/- 5.1	221.64 +/- 99.38	81.78 +/- 21.11
OQX1	25.3 +/- 4.4***	313.96 +/- 47.62	75.95 +/- 24.38
Sham2	32.5 +/- 4.3	123.73 +/- 63.68	82.54 +/- 26.62
OQX2	27.5 +/- 5.6***	191.84 +/- 86.46	68.01 +/- 33.55
OQX2+RSD	35.6 +/- 4.6*c	79.94 +/- 45.47[a]	90.35 +/- 28.23

*p<0.05 vs. their respective Sham; **p<0.01 vs. their respective Sham; ***p<0.001 vs. their respective Sham; ap<0.01 vs. their respective OQX; cp<0.001 vs. their respective OQX (Mann-Whitney test).
Risedronate treatment was able to avoid the increase in bone remodeling produced by orchidectomy and to restore trabecular bone volume in male rats with established osteopenia due to orchidectomy.

Disclosures: ***M. Diaz-Curiel****, Aventis 2.*

SU341

Effects of a 12 Months Risedronate Treatment on Biochemical Markers of Bone Rurnover and on Serum Osteoprotegerin and Soluble Receptor Activator of NF-kB Ligand in Postmenopausal Osteoporotic Women. R. Truniger*, A. W. E. Popp, R. Perrelet*, A. Noesberger*, K. Lippuner. Osteoporosis Unit, University Hospital, Berne, Switzerland.

Biochemical markers of bone turnover are used to assess effects of therapeutic agents in osteoporotic patients. Serum bone-specific alkaline phosphatase (BSAP), and osteocalcin (OC) - both reflecting bone formation -, as well as serum C-terminal telopeptides of type I collagen (CTx) - reflecting bone resorption -, are widely used parameters. However, little is known about the utility of serum C-terminal peptide of type I procollagen (PICP), and serum tartrate-resistant acid phosphatase isoform 5b (TRAP-5b) in monitoring treatment effect of risedronate. Furthermore, it is unknown, whether serum osteoprotegerin (OPG) and soluble receptor activator of NF-kB ligand (sRANKL) are influenced by antiresorptive treatment.
All parameters were measured using ELISA technique (Metra TM, Osteometer TM, Suomen Bioanalytiikka TM, Biomedica TM) before, after 6, and after 12 months of daily treatment with risedronate (5mg) plus calcium (500mg) and vitamin D (400 IU) in 13 post-

menopausal women (mean age 68.7 yrs, range 65-78).
Results are given as means (±SEM) of the absolute values, and as percent changes from baseline (±SEM).

Parameter	M0	M6 (%change)	M12 (% change)
BSAP (U/L)	26.5 ±2.5	19.2 ±1.4 (-24.9±4.4%)	18.5 ±1.1 (-26.6±4.6%)
OC (ng/ml)	11.0 ±0.9	8.6 ±0.4 (-17.6±7.2%)	8.7 ±0.5 (-17.3±5.8%)
PICP (ng/ml)	94.8 ±8.6	59.1 ±4.8 (-35.4±3.8%)	66.7 ±6.1 (-24.4±8.6%)
CTx (pmol/L)	3910 ±313	1856.9 ±130.9 (-49.0±5.3%)	1757.7 ±145.9 (-50.9±6.2%)
TRAP-5b (U/L)	1.8 ±0.1	0.7 ±0.1 (-64.9±6.2%)	1.2 ±0.2 (-33.2±9.4%)
OPG (pmol/L)	4.9 ±0.4	4.6* ±0.3 (-1.5±5.2%)	4.6* ±0.4 (-1.2±7.4%)
sRANKL (pmol/L)	0.7 ±0.06	0.6* ±0.03 (-7.0±4.8%)	0.6* ±0.03 (-10.9±6.0%)

All changes vs baseline were significant (paired t-test p< 0.05) except for OPG and sRANKL (*marked). While significant decreases in all parameters of bone formation and bone resorption were detected during treatment, no changes were observed in OPG and sRANKL.

Disclosures: ***K. Lippuner****, None.*

SU342

Effects of Intravenous Zoledronic Acid on the Degree of Mineralization of Bone in Post-Menopausal Osteoporosis: a Quantitative Microradiographic Analysis of Transiliac Biopsies after One Year. G. Boivin[1], M. Arlot[1], U. Trechsel[2], P. J. Meunier[1]. [1]Faculté de Médecine R. Laennec, INSERM Unité 403, 69372 Lyon Cedex 08, France, [2]Zoledronic Acid Study Group, Novartis Pharma, Basel, Switzerland.

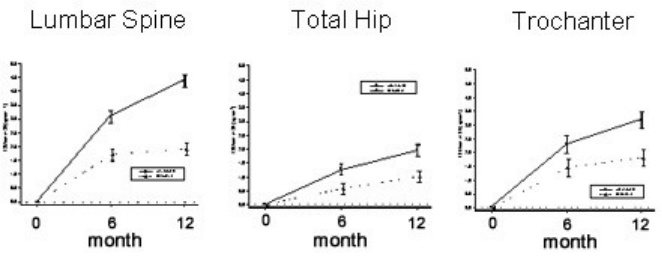
The bisphosphonate zoledronic acid (Z) is a very potent antiresorptive agent. To assess its effects on human bone "minerality" i.e. the degree of mineralization of bone (DMB), intravenous bolus zoledronic acid was given to post-menopausal women with a DXA T score <=-2 and no more than one osteoporotic vertebral fracture in a Phase II study (Reid et al. 2002, NEJM 346:653-61). Patients received placebo or zoledronic acid given either every 3 months (0.25, 0.5, 1 mg), or every 6 months (2mg) or only once (4 mg) for one year. All patients received 1g of calcium daily. At the end of one year treatment, transiliac bone biopsies were obtained from 38 patients and embedded in methyl-methacrylate. Thick sections were ground to a uniform thickness of 100 µm and then microradiographed with an aluminum step-wedge (Boivin & Meunier 2002, CTI 70:503-11). DMB was measured in total bone (compact+cancellous) and expressed as g mineral/cm3 of bone. The mean DMB (SD) was 1.09 (0.14) in the 6 Placebo, 1.11 (0.08) in the 5 Z 4x0.25, 1.05 (0.12) in the 5 Z 4x0.50, 1.08 (0.06) in the 8 Z 2x2, 1.13 (0.04) in the 5 Z 4x1, and 1.14 (0.10) in the 9 Z 1x4. When compared to placebo, DMB of patients treated with zoledronic acid increased only in the 2 last groups by 3.9% and 5.2%, respectively. No dose effect was detected but the highest increases in DMB were noted in the groups 4x1 and 1x4. When compared to the other groups, the distribution of the DMB in patients from the groups 1x4 or 4x1 showed a shift toward the high values of DMB. When these two groups were pooled, the gain in DMB was 4.75% versus placebo. Mean DMB and distribution of DMB values in this mixed group were similar to those showing that alendronate (10mg/day), after 2 years of treatment, increased bone strength by increasing the DMB in osteoporotic women (Boivin et al. 2000, Bone 27:687-94). The antiresorptive effect of zoledronic acid has been shown histomorphometrically with a marked reduction in the activation frequency of new remodeling units (Arlot et al. 2002 JBMR 16 suppl 1:S284, Reid et al. 2002, NEJM 346:653-61). As previously shown with alendronate, the increase in BMD measured by DXA at the lumbar spine level (+4.3 to +5.1%) during the year of treament with zoledronic acid, was not induced by modifications of bone mass and microarchitecture. The modifications of DMB under treatment represent once again the main mechanism responsible for the gain in BMD. In conclusion, one year treatment with zoledronic acid markedly increased the "minerality" of bone in post-menopausal osteoporotic women.

Disclosures: ***G. Boivin****, None.*

SU343

Efficacy of Fosamax vs Evista Comparison Trial (EFFECT): Results of a Randomized, Multicenter Study. M. Luckey[1], S. Greenspan[2], H. Bone[3], R. D. Kiel[4], R. Kagan[5], J. Sackarowitz*[6], E. Chen*[6], A. E. de Papp[6]. [1]St. Barnabas Osteoporosis & Metabolic Bone Disease Center, Livingston, NJ, USA, [2]Osteoporosis Prevention and Treatment Center, Pittsburgh, PA, USA, [3]Michigan Bone & Mineral Clinic, Detroit, MI, USA, [4]Beth Israel Deaconess Medical Center, Boston, MA, USA, [5]Foundation for Osteoporosis Research and Education, Oakland, CA, USA, [6]Merck & Co., Inc., West Point, PA, USA.

In order to compare the efficacy and tolerability of once-weekly (OW) alendronate (ALN) (70 mg), and daily raloxifene (RLX) (60 mg) for the treatment of postmenopausal osteoporosis, we performed a 12 month, head-to-head, double-blind trial of these two therapies in 456 postmenopausal women with low bone mineral density (BMD) (lumbar spine or total hip T score ≤ -2.0), recruited from 52 centers in the U.S. Patients were randomized 1:1 to OW ALN 70 mg with daily RLX placebo or daily RLX 60 mg with OW ALN placebo. Efficacy measurements included lumbar spine (LS) and femoral ("total hip" and trochanter) BMD at 6 and 12 months, and biochemical markers of bone turnover. The primary endpoint was LS BMD at 12 months, as measured on an intention-to-treat basis. The mean age was 64.2 years, and the mean time since menopause was 17.5 years. The mean baseline PA lumbar spine T-score was –2.49. The mean change from baseline at month 12 in the PA lumbar spine BMD was 4.4% for ALN and 1.9% for RLX ($p < 0.001$). A significant difference in BMD was seen as early as six months. The proportion of patients who either maintained or increased LS BMD at 12 months was 94 % for ALN and 75% for RLX ($p < 0.001$). Greater BMD increases with ALN (—) versus RLX (····) were also seen at the femoral sites as depicted below. The mean change from baseline at 12 months for urinary N-telopeptide (NTx) was -48% for ALN and -26% for RLX ($p < 0.001$). The mean change from baseline for bone-specific alkaline phosphatase (BSAP) was -40% for ALN and -21% for RLX ($p<0.001$). The proportion of patients discontinuing from the study due to clinical adverse experiences (AEs) was similar for both groups (11.2% ALN, 10.3% for RLX). Additional safety and tolerability results, including upper gastrointestinal and vasomotor AEs will be available for presentation at the meeting. Conclusion: OW ALN 70 mg produced significantly greater BMD increases, a greater percentage of responders, and greater suppression of markers of bone turnover than did daily RLX.



Disclosures: ***A.E. de Papp****, Merck & Co., Inc. E.*

SU344

Adynamic Bone Disease during Bisphosphonate Therapy: Should We Be Concerned? C. V. Odvina[1], D. S. Rao[2], C. Y. C. Pak[1], N. Maalouf[1], J. E. Zerwekh[1]. [1]Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA, [2]Bone and Mineral Metabolism, Henry Ford Hospital, Detroit, MI, USA.

Bisphosphonates (BPs) have been shown to increase bone density and decrease fractures. Although available data suggest that these agents are safe, concerns have been raised over whether these agents may oversuppress bone turnover and thereby compromise bone quality. We report herein four patients who, despite years of BP therapy continued to sustain atraumatic fractures. Two possibilites were considered: treatment failure or oversuppression of bone turnover. To further clarify and to determine the best approach to manage such patients, we advised them to undergo transiliac bone biopsy for histomorphometry. All 4 patients were postmenopausal women (55-76 years), and had been on BP for 3-7 years. One patient had been on estrogen for 12 years, and one on Prednisone for fibromyalgia for 9 years. All 4 were on calcium and three were also taking vitamin D. Spontaneous pelvic fractures were reported in 2 patients, sacral fracture in 2, and proximal femur in 1. Other fractures reported were at the metacarpal and metatarsal bones and rib. Fracture healing was delayed in 2 patients until BP was discontinued..

Histomorphometric findings

Parameters	Patient 1	Patient 2	Patient 3	Patient 4
BV/TV (%)	9.4	8.9	14.3	15.2
OV/BV (%)	0	0.05	0.42	0.66
Oc.S/BS	0	0.12	1.0	0.3
ES/BS (%)	0.85	1.7	9.5	9.3
Ob.S	0	0.19	1.7	0
dLS/BS(%)	0	0	0	0
sLS/BS(%)	0	0.3	0.58	0

Histomorphometric findings in cancellous bone are summarized in the table. Measures of bone formation were markedly diminished with low osteoblast surface and absence of double tetracycline label in all specimens. Single tetracycline label was either absent or diminshed. Osteoclast surface was decreased in 3 and eroded surface in 2. The same trend was noted in the cortical bone. Our preliminary results suggest that BPs may cause hypo- or adynamic bone disease and potentially increase the risk of fractures especially if given chronically or in combination with other agents which can also suppress bone turnover. At present, the prevalence and pathogenetic determinants of this complication are not fully known. This study does not dispute the important role of BPs in the management of osteoporosis. Rather, it emphasizes the need for awareness that such complication can occur, especially among patients who develop fractures during long-term BP therapy.

Disclosures: ***C.V. Odvina****, Procter and Gamble 5.*

SU345

Risedronate Treated Patients with Reduced Renal Function Show No Significant Increase in Renal Function-Related AEs as Compared to Placebo. P. D. Miller[1], I. P. Barton*[2], L. E. Dunlap*[3], D. E. Burgio*[3]. [1]Colorado Center for Bone Research, Lakewood, CO, USA, [2]Procter & Gamble Pharmaceuticals, Egham, United Kingdom, [3]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

The objective of this analysis was to investigate the influence of renal function on the incidence of renal function-related AEs and changes in serum creatinine in a population of osteoporotic women taking risedronate.

The analysis included osteoporotic women enrolled in the placebo-controlled phase III clinical trials who were randomized and received either placebo or risedronate 5mg daily, in addition to 1000 mg/day calcium supplementation and vitamin D if baseline levels were low. For each patient, creatinine clearance was estimated using the Cockcroft and Gault method based on baseline serum creatinine, body weight and age. The incidence of renal function-related AEs and changes in serum creatinine were summarised for each treatment group based on FDA criteria for patients considered to have Mild (≥50-<80mL/min), Moderate (≥30-<50mL/min) or Severe (<30mL/min) renal impairment.

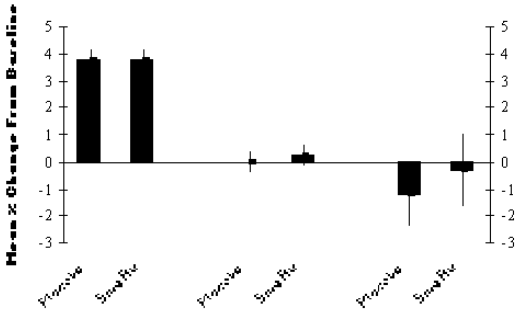
8996 patients were classified as having at least mild creatinine clearance at baseline (Placebo: n=4500, Risedronate 5mg: n=4496). The average duration of study exposure, per patient, across the phase III clinical program was 2-years. The incidence of renal function-related AEs was similar between treatment groups within and across the three renal impairment patient subgroups. Likewise, the incidence of any AE was similar between treatment groups and across the three renal impairment subgroups. The average serum creatinine for both treatment groups was similar within and across the three renal impairment subgroups.

In conclusion this analysis shows, based on phase III clinical trial experience, that patients taking risedronate with mild to severe renal impairment exhibit no significant increase in renal function-related AEs, incidence of any AEs, or serum creatinine compared to patients taking placebo.

Incidence Of Renal Function-Related AEs

Creatinine Clearance	Placebo	5mg Risedronate
≥50-<80mL / min	396 / 2192 (18%)	376 / 2161 (17%)
≥30-<50mL / min	363 / 2037 (18%)	363 / 2034 (18%)
<30mL / min	55 / 271 (20%)	57 / 301 (19%)

Serum Creatinine % Change From Baseline At Endpoint [Mean ± SE]



Disclosures: ***P.D. Miller****, None.*

SU346

Rationale for Intermittent Intravenous Ibandronate Injections in Postmenopausal Osteoporosis. P. D. Miller[1], R. R. Recker[2], S. Adami[3], B. Bonvoisin[4], R. C. Schimmer[4]. [1]Colorado Center for Bone Research, Lakewood, CO, USA, [2]Creighton University, Omaha, NE, USA, [3]Centro Ospedalerio Clinicizzato di Valeggio, Verona, Italy, [4]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

Acute renal failure has been observed following the rapid intravenous (i.v.) administration of several bisphosphonates. This serious adverse event was shown to be due to the rapid accumulation of high molar concentrations of bisphosphonates in the renal tubules in the period immediately post-dose, resulting in the formation of insoluble bisphosphonate-calcium complexes. Thus, parenteral administrations of current bisphosphonates must be given as extended infusions, with a recommended dosing rate of 200mg/hour or 4mg/min. Although a proven mode of delivery, prolonged i.v. infusions are inconvenient and frequently associated with thrombotic complications and infections. Moreover, as specialized equipment and skilled personnel are required to facilitate delivery, i.v. infusions must be performed in the hospital or clinic setting. Bisphosphonate dosing by i.v. injection could provide a convenient alternative to i.v. infusion, which is suitable for use in the primary care setting and avoids the many complications associated with prolonged venous access. Ibandronate is a highly potent, nitrogen-containing bisphosphonate, which, unlike lower potency bisphosphonates, can be administered as an i.v. injection in regimens with extended between-dose intervals. Initial studies of i.v. ibandronate showed that high doses (6mg) of ibandronate could be administered at decreasing infusion rates (60 to 15 minutes) without detrimental affects on renal function. A reduced dose of ibandronate (3mg) given as a short i.v. injection (60–120 seconds) was also shown to be effective, without adversely effecting renal function. The efficacy and tolerability of i.v. ibandronate (dose range: 0.25mg–2mg) injections (15–30 seconds) given once every 3 months has been demonstrated in approximately 3,500 women with postmenopausal osteoporosis (PMO) in the clinical trial setting. Importantly, no indicators of renal toxicity have been reported. A randomized, double-blind, parallel-group, multicenter study (Dosing IntraVenous Administration [DIVA]) is ongoing to further assess the influence of dose and dosing interval on the efficacy and safety of i.v. ibandronate injections in PMO. Intermittent i.v. ibandronate injections are expected to provide an efficacious, convenient and well-tolerated alternative to i.v. infusions in PMO.

Disclosures: ***P.D. Miller****, None.*

SU347

Differences in Early Dynamics of Serum Bone Markers in Women with Postmenopausal Osteoporosis Treated by Alendronate or Risedronate. E. Carmina*[1], G. Di Fede*[1], C. Sferrazza*[1], M. C. Pandolfo*[1], G. Vitale*[1], N. Napoli[1], R. Villareal[2], G. B. Rini*[1]. [1]Department of Clinical Medicine, University of Palermo, Palermo, Italy, [2]Bone and Mineral Division, Washington University, St Louis, MO, USA.

Bisphosphonates increase bone density mostly by reducing bone resorption and some studies have shown that, 3 months after initiation of bisphosphonate therapy, there is a significant reduction of circulating bone markers. However, the exact dynamics of serum bone markers after bisposphonate therapy are unclear and earlier changes of serum bone markers have not been evaluated. In this study, in 50 women with postmenopausal osteoporosis (L1-L4 t score < 2.5 DS), with a mean age of 55 ± 1 years (range 50-64 years, mean years from menopause 4 ± 1), serum levels of osteocalcin (OCT), bone alkaline phosphatase (BAP) and C-telopeptides (CTX) were determined. 50 postmenopausal women with similar mean age (55 ± 1 years) and similar menopausal age (mean years from menopause 4 ± 1), but normal bone density (L1-L4 t score between + 0.5 and – 0.9 DS) were used to determine normal values of serum bone markers. Compared to control postmenopausal women, women with postmenopausal osteoporosis presented significantly ($p<0.01$) higher levels of CTX (5775 ± 514 vs 2400 ± 168 pmol/L), OCT (21.6 ± 1.4 vs 12.7 ± 0.7 ng/ml) and BAP (24.3 ± 1.3 vs 17.5 ± 1 μg/ml). Women with postmenopausal osteoporosis were randomized to treatment with alendronate (AL, 70 mg once a week) or risedronate (RE, 5 mg/day). The two groups had similar age, bone density and serum bone markers. Serum samples were obtained for bone markers after 15, 30, 60 and 90 days. AL treated patients (n=25) presented a progressive decrease of serum CTX with a nadir after 90 days (15days: -20.6%; 30days: -39.3%; 60days: -51%; 90days: -56%) while RE treated patients (n=25) showed a rapid decrease of serum CTX (15days: -57.5%; 30days: 59.3%; 60days: -61%; 90days -59%). The nadir values of CTX after treatment (AL treated: 2340 ± 210 and RE treated: 2303 ± 140 pmol/L) were similar in the two groups and similar to the values of normal postmenopausal women. In AL treated pts, markers of bone formation were almost unchanged until 60 days of therapy but decreased significantly ($p<0.01$) after 90 days (OCT -35.5%, BAP -20.5%). In RE treated pts, markers of bone formation started to decrease after 30 days (OCT -20%, BAP -19.8%) and reached the nadir after 60 days of therapy (OCT -37%, BAP -28%, $p<0.01$). In both groups, the nadir values of OCT and BAP were similar to those of normal postmenopausal women. In conclusion, our data show that RE normalizes bone turnover more rapidly than AL. It may be useful in some osteoporotic patients with high fracture risk.

Disclosures: ***E. Carmina****, None.*

SU348

Effect of Alternate-day Administration of Alendronate on Postmenopausal Osteoporosis Patients in Japan. T. Kato, K. Katase*, Y. Hirai*. Gynecology, Cancer Institute Hospital, Tokyo, Japan.

Alendronate (ALN), the drug for osteoporosis, is usually administered daily in Japan. In the present study, the effects of ALN were investigated in patients who wished to take the drug on alternate days. The bone mineral density (BMD) of the lumbar spine was measured by the dual energy X-ray absorptiometry (Lunar DPX-L) and 23 women diagnosed with osteoporosis were eligible for the study. Before administration, the BMD was 0.699 ± 0.071 g/cm2 (mean ± SD). The patients were given ALN 5 mg, which is the dose approved in Japan, on alternate days and precipitated calcium carbonate 500 mg daily. No one dropped out. The lumbar spine BMD was measured at 6 months after the start of administration of ALN and the percent change was calculated. After 6 months of administration, the percent change in BMD from baseline was 5.9 ± 4.0%. The change in BMD showed a biphasic distribution, so the patients were classified as responders and non-responders. Four patients were non-responders and the change in BMD was from -3% to 1.4%, while 19 patients were responders and the change in BMD ranged from 3.6% to 16.4%. When the characteristics of these two groups were investigated, the non-responders were all participants in the clinical trial of another bisphosphonate. The mean change in BMD was 7.2 ± 3.2% in the 18 patients who had not received administration of a bisphosphonate before. Three patients experienced a stomach discomfort. In the evaluation about the therapeutic effect of ALN administered on alternate days for 6 months, an increase in BMD was 6% beyond our expectations. The incidence of adverse reactions was 13% and the reactions were mild, indicating no change of safety. The reason why no improvement of BMD was observed in the patients who had participated in the clinical trial of another bisphosphonate should be investigated. Administration on a daily basis may be required when no effect is observed in such patients taking the drug on alternate days, or the bone mass response may be poor despite daily administration. Since our hospital specializes in treating cancer, a percentage of the patients with breast cancer or uterine cancer, which is a contraindication to estrogen replacement therapy, would be higher than that of other hospitals. As the prognosis of breast cancer or uterine cancer improves, to maintain the bone mass by bisphos-

phonate therapy can be considered as one aspect of QOL. In the future, we hope to extend the administration period and determine the therapeutic effect in Japanese patients. We found that in women with osteoporosis, who continued to take ALN on alternate days for 6 months, increased BMD by 5.9% and the effectiveness was proved.

Disclosures: ***T. Kato****, None.*

SU349

Statins Prevent the Acute Phase Response to Bisphosphonates in an *In Vitro* Model. K. Thompson, M. J. Rogers. Bone Research Group, Medicine & Therapeutics, University of Aberdeen, Aberdeen, United Kingdom.

An acute phase response is the major adverse effect of intravenously-administered nitrogen-containing bisphosphonates (N-BPs) such as zoledronic acid (ZOL). We have recently proposed that this effect is due to inhibition of FPP synthase, an enzyme in the mevalonate pathway. Inhibition of this enzyme causes the accumulation and release of upstream isoprenoid lipids such as IPP (known antigens for γ,δ-T cells) that then directly activate γ,δ-T cells. By this mechanism, N-BPs stimulate cytokine release and the proliferation of γ,δ-T cells in human PBMC cultures in a manner consistent with the potency for inhibition of FPP synthase (ZOL>alendronate~ibandronate>pamidronate).

The mevalonate pathway is also inhibited by cholesterol-lowering statins, that act on HMG-CoA reductase (the most proximal enzyme of the pathway). Statins could therefore prevent the accumulation of IPP that occurs in PBMCs following N-BP treatment. To test this, human PBMC cultures were treated with 1μM N-BP in the absence or presence of mevastatin. The stimulatory effect of N-BPs on proliferation of $CD3^+$ γ,δ-T cells was abrogated by 1μM mevastatin, and reduced by concentrations as low as 10nM mevastatin. This abrogative effect of mevastatin was not due to cytotoxicity, since 1μM mevastatin did not affect the proliferation of γ,δ-T cells in response to 100nM BrHPP (a synthetic agonist of γ,δ-T cells) or to anti-CD3 antibody. Furthermore, 1μM mevastatin did not affect the stimulation of TNFα release in response to BrHPP treatment, but completely inhibited TNFα release following treatment of PBMCs with ZOL for 48 hours. As well as inhibiting HMG-CoA reductase, statins can interfere with lymphocyte adhesion and co-stimulation via LFA-1. However, 1μM des-oxo-lovastatin (an analogue of lovastatin that prevents LFA-1 binding but does not inhibit HMG-CoA reductase) had no effect on ZOL-induced γ,δ-T cell proliferation, whereas 1μM lovastatin significantly decreased γ,δ-T cell proliferation following ZOL treatment.

These observations demonstrate that statins can prevent N-BP-induced γ,δ-T cell proliferation and release of proinflammatory cytokines in human PBMC cultures, through inhibition of HMG-CoA reductase and a subsequent decrease in the accumulation of isoprenoid lipids upstream of FPP synthase in the mevalonate pathway. Co-administration of a statin with intravenous N-BP may therefore provide a novel and effective means for preventing the acute-phase response to bisphosphonate therapy *in vivo*.

Disclosures: ***K. Thompson****, Novartis Pharma AG 2.*

SU350

Oral Bisphosphonates Induce Aortic Root Inflammation and Plaque Rupture in an Apo E Knock Out Mouse. M. Shimshi*[1], E. Abe[1], E. Fisher*[2], M. Zaidi[1], J. T. Fallon*[2]. [1]Medicine, Mount Sinai School of Medicine, New York, NY, USA, [2]Cardiology, Mount Sinai School of Medicine, New York, NY, USA.

The apolipoprotein E (Apo-E) knockout mouse is known to develop arterial plaques with age. We examined the effect of alendronate and risedronate on arterial plaque formation, particularly because bisphosphonates have been implicated in reducing calcification. Alendronate and risedronate were administered by oral gavage to 2 week-old wild type and ApoE null mice at 6 and 9 μg twice weekly for 8 weeks. Arteries were dissected, embedded for sectioning, stained with hematoxylin and eosin, and the lesions were scored by a blinded examiner. To our surprise, we found that both bisphosphonates caused aortic root inflammation with or without plaque rupture. Five out of 17 mice had severe inflammation with plaque rupture, while 4 and 3 mice, respectively, showed severe and mild inflammation, but without plaque rupture. Only few mice were spared. The effect of the two drugs was essentially similar, and both were without effect in wild type mice. Morphometric analysis showed no changes in aortic root plaque size. To evaluate the mechanism of action of the two bisphosphonates in causing arterial plaque rupture, we performed immunocytochemistry to detect capase 3, an enzyme involved in arterial plaque apoptosis, as well as metalloproteinases 1 and 9. No differences in staining of the enzymes could be detected in inflammed or ruptured plaques. Finally, we confirmed the bioactivity of the administered bisphosphonates by measuring bone mineral density (BMD) changes using a small animal densitometer (Piximus). There was a time-dependent increase in BMD over the 8 weeks of risedronate administration ($p < 0.01$ from 4 weeks onward, Student's t-test with Bonferroni's Correction for Inequality). At 8 weeks, there was no difference between the BMD at any sites (lumbar spine, tibia, femur and total body) between alendronate and risedronate treated groups. There are no reliable models to study arterial plaque rupture – our discovery of bisphosphonate action on arterial plaques will likely be utilized to further characterize the mechanism of plaque rupture. The study also demonstrates close parallels between bone and vascular biology, particularly as osteoprotegerin, a bone-active cytokine, has been implicated in atherosclerotic plaque formation, and that osteoclast-like cells identified within plaques may in fact remodel these structures. Finally, we are unaware at this time of any implications of our work on ApoE null mouse vis-à-vis the use of bisphosphonates as the mainstay for osteoporosis therapy in post menopausal women.

Disclosures: ***M. Shimshi****, None.*

SU351

Discordances in Vertebral Fracture Reduction in Terms of Age and Fracture Location in Response to Calcitonin Nasal Spray: Results from the PROOF Study. M. Olson*[1], S. L. Silverman[2], L. Mindeholm*[1], M. Azria[1], C. Chesnut[3]. [1]Novartis Pharma, Basel, Switzerland, [2]Cedars-Sinai/UCLA, Los Angeles, CA, USA, [3]University of Washington, Seattle, WA, USA.

The PROOF study demonstrated vertebral fracture(VF) efficacy of 200 IU calcitonin nasal spray (NS-CT). Oleksik (2001) and Silverman (2002) have shown greater loss in health related quality of life with lumbar VF in the MORE trial. Fink (2003) has shown greater number of disability days following clinical lumbar VF in FIT. Data from QUEST(Chesnut, 2003)have shown site-specific changes in response to antiresorptive therapy. Johnell(2001) has shown lesser efficacy of an antiresorptive therapy in reducing VF in terms of age. We performed a secondary analysis of thoracic (T) and lumbar (L) vertebral fracture efficacy in PROOF with stratification by age, using a generalized estimating equation model treating the patient as the grouping unit and the fracture location as the blocking unit. Covariance of treatment and baseline number of fractures were used in the model.

Using this model there is a 38% relative risk reduction (RRR) (p=0.01) in VF in PROOF using 200 IU NS-CT. In PROOF there was a RRR of 44% (p=0.03) for L VF and a RRR of 35% (p=0.04)for T VF. The RRR in VF in women>=70 was 41% (odds ratio 53%), p=0.04. Secondary analyses in this elderly population >=70 revealed that the RRR was 68% for L VF (p=0.003) and 22% for T VF (p=NS), suggesting greater protection for the lumbar spine in this age group. Secondary analyses in the women <70 revealed that the RRR was 16% for L VF (p=NS) and 51% for T VF (p=0.02), suggesting greater protection in the thoracic spine in this age group. These analyses of the PROOF data therefore suggest that efficacy of antiresorptive therapies may vary based on fracture location and age of the patient. We hypothesize that the differences seen between thoracic and lumbar vertebral fracture efficacy may represent local differences in bone turnover, in bone quality such as microarchitecture, or in biomechanical variables that may be confounded or influenced by age.

Disclosures: ***M. Olson****, Novartis 3.*

SU352

Efficacy and Safety of a Novel Oral Formulation of Salmon Calcitonin (SMC021) in Postmenopausal Women. L. B. Tankó[1], Y. Z. Bagger[1], J. Y. Reginster[2], J. P. Devogalar*[2], L. Mindeholm*[3], R. Chick*[3], H. Benmammar*[3], M. Azria*[3], C. Christiansen[1]. [1]CCBR A/S, Ballerup, Denmark, [2]WHO Collaborating Center for Public Health Aspects of Rheumatic Disease, University of Liege, Liege, Belgium, [3]Novartis, Basel, Switzerland.

Results of a 3-month study demonstrating effective oral delivery of a macromolecule polypeptide are reported. The purpose was to investigate the efficacy and safety of a novel oral formulation of salmon calcitonin (CT) in healthy elderly women.

This phase II dose-ranging trial used a randomized, double-blind, placebo (PBO)-controlled, and parallel group design, and enrolled 278 women (55-85 years). They received CT, combined with a caprylic acid derivative carrier, orally either QD (0.15, 0.4, 1.0, or 2.5 mg) or 1.0 mg every other day, or PBO for 3-months. All received 1 g Ca plus 400 IU of vit. D QD. Efficacy parameters were biochemical markers of bone resorption (serum and urinary CTx) and bone formation (osteocalcin (OC), bone specific alkaline phosphatase (BSAP)). 24-hour profile and cumulative changes were assessed at baseline (BL), after the first dose, and at month 1 and 3.

94% of patients completed the study. After oral intake, CT was rapidly absorbed, resulting in dose-dependent serum CT increases with Cmax at 30 min. The day 1 24-hour profile of sCTx showed marked CT-dose-dependent decreases, with nadirs at 3h, ranging from –61.2 to –82.7%. Values remained decreased after 24 hours at levels of –4.0 to –27.6%. At 3 months, there were little differences in the nadirs of all but the lowest dose (-72.8 to -75.7%). The pre-dose least-square mean values at 3 months ranged from -9.5 to -19.5% compared to BL (median -11.1 to -35.7%), with the 1.0 mg/day dose reaching statistical significance vs. BL and PBO (p=.0016). All doses but the lowest showed similar decreases in uCTx/Cr at all time points.

At Month 1, the cumulative changes in OC evoked by the 0.4 mg/day dose indicated a a slight but statistically significant increase in bone formation. At higher doses (1.0 mg/day), a trend toward slight decreases in formation markers was seen which only for BSAP reached statistical significance at 3 months. 24-hour profiles of serum PTH showed transient increases. No drug related serious adverse events occurred. The most frequent adverse events were gastrointestinal (nausea, vomiting, diarrhea) occurring mainly at higher doses.

In summary, the innovative oral formulation of CT is effectively absorbed and provide a strong cyclic inhibition on bone resorption. As the oral formulation may enhance compliance to CT treatment, these findings warrant further exploration.

Disclosures: ***L.B. Tankó****, None.*

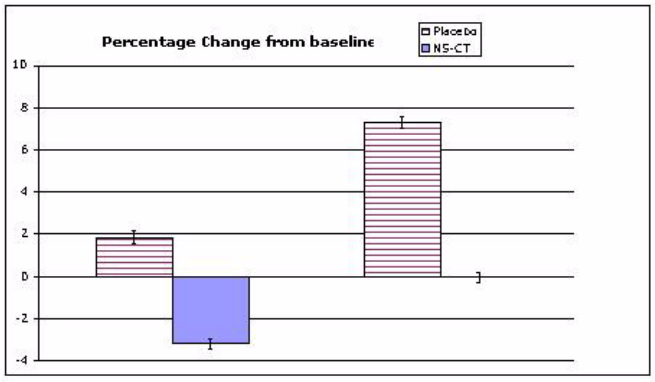
SU353

The Impact of Salmon Calcitonin Nasal Spray (CT-NS) on Trabecular Bone Density Distribution as Measured by Magnetic Resonance (MR) T2* Times in the Proximal Femur in a 2 Year Study. S. Majumdar[1], C. Chesnut[2], A. Shields*[2], D. Newitt*[1], E. Laschansky*[2], A. Kriegman*[3], M. Olson*[4], F. Hornig*[4], M. Azria*[4], P. Richardson*[4], L. Mindeholm*[4]. [1]Radiology, University of California San Francisco, San Francisco, CA, USA, [2]University of Washington, Seattle, WA, USA, [3]Novartis Pharma, Hanover, NJ, USA, [4]Novartis Pharma, Basel, Switzerland.

Calcitonin-NS 200 IU has shown significant fracture reduction, with modest impact on bone mineral density and turnover. In order to identify additional factors, such as bone

quality, that contribute to the fracture reduction effect of CT-NS, a 2 year study was performed. MR based T2* relaxation times have been shown to reflect not only trabecular bone density but also spatial distribution, orientation of trabeculae hence the term trabecular bone density distribution (tb-BDD) is used to represent the characteristics that T2* measures. T2* is inversely related to tb-BDD, i.e. a decrease in T2* reflects an increase in tb-BDD.

In this double-blind, placebo controlled randomized study in 91 postmenopausal osteoporotic women, T2* was measured from MR images. All subjects received 500 mg of Ca; the treatment cohort received CT-NS 200 IU. For the assessment of T2* single slice, water saturated, coronal MR images (resolution 1.875 x 1.875 x 10 mm) were obtained at 1.5 Tesla, at 12 different echo times, ranging from 4 to 40 msec. Using the set of images obtained at different echo times, T2* was calculated in four regions of interest (femoral neck, Wards triangle, upper trochanteric, and lower trochanteric regions). Different regions of the proximal femur show different magnitudes of change. In the upper trochanteric region there was a decrease of 4.4% seen in the treated group (n=34) in comparison with placebo (n=38) (p=0.06) with respect to the mean percent change from baseline in T2* relaxation time. The corresponding decrease in the lower trochanteric region compared with placebo was 6.3% (p=0.008). In Wards triangle and at the femoral neck the decreases compared with placebo were 0.5% and 2.3% (ns) respectively. In conclusion, NS-CT decreased hip T2* by 0.5-6.3%% at all measured hip sites compared with placebo as evaluated by the non-invasive MRI technique, equivalent to an increase in tb-BDD, a composite of bone density and microarchitecture.



Disclosures: ***S. Majumdar****, Pfizer Pharmaceuticals 2; Merck 8.*

SU354

Oral Delivery of Salmon Calcitonin in Humans. J. P. Gilligan[1], W. Stern*[1], N. Mehta[1], A. Sturmer*[1], A. Malootian*[1], J. Symons[2]. [1]Unigene Laboratories Inc, Fairfield, NJ, USA, [2]Pfizer Inc, Ann Arbor, MI, USA.

Salmon calcitonin (sCT) is a 32 amino acid amidated peptide hormone that is currently marketed in injectable and nasal dosage forms for the reduction of vertebral fractures and bone pain in osteoporotic patients, as well as for the treatment of Paget's disease and hypercalcemia. An orally delivered, solid dosage form containing recombinant sCT (rsCT) that is produced in a high-level bacterial expression system has been developed and tested in animals and humans. This enteric-coated solid dosage form contains excipients that modulate intestinal proteolytic activity and enhance peptide transport. In a phase I study, 10 healthy volunteers were given a single enteric-coated capsule containing 500 µg of rsCT. Plasma samples were collected at several time points and assayed by an sCT-specific immunoassay. Significant levels of rsCT were detected in 9 of the 10 volunteers. The mean Cmax was greater than 300 pg/ml, which is twice that obtained with a standard 50 IU injectable dose, and approximately 30 times greater than the Cmax obtained with a standard 200 IU dose of nasal sCT. In a Phase II dose-ranging study, 191 healthy postmenopausal women were given daily an enteric-coated tablet containing 50, 100 or 200 µg of formulated rsCT for a period of 8 weeks. A linear, dose-dependent pharmacokinetic response was obtained, with mean Cmax values ranging from 13 to 40 pg/ml. The bioavailability of rsCT in these oral studies relative to a subcutaneous dose was approximately 1%. This bioavailability is high enough to allow the development of a cost-effective oral formulation for rsCT. The availability of an orally delivered tablet or capsule will afford greater patient convenience and should lead to a higher level of long-term compliance for this important antiresorptive therapy.

Disclosures: ***J.P. Gilligan****, Unigene Laboratories Inc 1, 3.*

SU355

Pharmacokinetics of a Modified, Oral Calcitonin Product (Oratonin™) in Healthy Volunteers. C. M. Chin, PhD*, J. G. Still, MD, PhD, G. Kosutic, MD*. Nobex, Research Triangle Park, NC, USA.

Objectives: The objectives of this Phase 1 exploratory study were to assess the preliminary pharmacokinetics, pharmacodynamics, and tolerability of single oral doses of a chemically modified salmon calcitonin product (Oratonin™) in healthy volunteers (HV).
Methods: Twelve HV (age, 22-44 years) were enrolled in this 5–way crossover study. Subjects were administered a single oral dose of Oratonin tablets on 5 separate days with a 72–hour washout period between doses. The 5 treatments varied in dose (160 & 320 µg) as well as formulation (Form) excipients (Form A, B, & C). Serial blood samples were collected for determination of plasma Oratonin, total serum calcium, and ionized serum calcium concentrations during the 4–hour postdose period. Plasma Oratonin concentrations were determined using a validated radioimmunoassay. Oratonin pharmacokinetics were evaluated using noncompartmental methods.
Results: The data presented herein describe results from 4 of 5 dosing days, in which subjects received 160 & 320 µg of Oratonin in Form A & B. The data indicated that Oratonin was rapidly absorbed from the gastrointestinal tract following oral administration. Mean peak plasma concentrations of 51-110 pg/mL were observed between 36 & 54 minutes after administration (Fig. 1). The mean terminal elimination half–life ranged from 54-76 minutes. There was a trend for mean C_{max} & AUC_{0-t} values to increase with dose. Precision of the derived pharmacokinetic parameter estimates, $AUC_{0-\infty}$, $t_{½}$, CL/F, & V_d/F, was limited by the sensitivity of the bioanalytical method, which did not allow for complete characterization of the pharmacokinetic profiles. Single oral doses of 160 & 320 µg Oratonin were pharmacologically active, inducing a slight decrease in total and ionized serum calcium concentrations. **Conclusions:** Single doses of Oratonin were absorbed into the systemic circulation following oral administration and were well–tolerated in HV at doses up to 320 µg. The results from this Phase 1 exploratory study suggest that oral delivery of salmon calcitonin is feasible, and these data support the further clinical investigation of Oratonin as a treatment for osteoporosis.

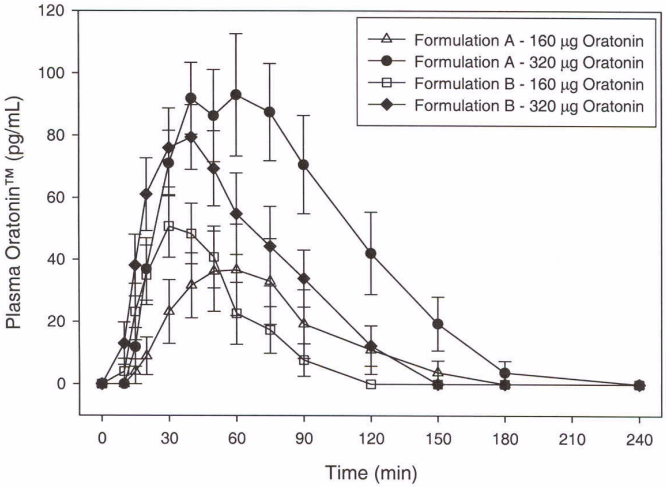


Figure 1: Concentration vs. time profiles of Oratonin after single oral doses of 160 µg & 320 µg of Oratonin (Form A & B) in HV. Data represent means ± SEM (n = 12 for each dose and for each timepoint).

Disclosures: ***J.G. Still, MD, PhD****, Nobex Corporation 3.*

SU356

Characteristics of Glucocorticoid Treated Patients in 2003: Poor Calcium Supplementation and High Prevalent Fractures. N. Lane[1], S. Goldring[2], R. Kane*[3], J. Stewart*[3], S. Morris[3]. [1]Department of Medicine, UCSF, San Francisco, CA, USA, [2]Chief of Rheumatology, Beth Israel Deaconess Medical Center, Boston, MA, USA, [3]Medical Research, Aventis, Bridgewater, NJ, USA.

The ACTIVATE study is designed to determine if providing patients with information on vertebral fractures using instantaneous vertebral assessment (IVA) and bone turnover markers (BTM) will affect compliance with daily risedronate therapy in 350 patients taking oral glucocorticoids (GC) (oral prednisone ≥7.5 mg/day or equivalent). The study recruited 2 groups of patients on GCs: those taking daily GC for 6 mos or greater (TREAT) and those initiating GC therapy (GC for < 8 weeks; PREV). Study exclusion criteria included the use of bisphosphonates within 1 year months of the time of enrollment. Currently, 139 patients are enrolled (40% of total): the majority of patients are in the TREAT group (106 patients; 76%). Mean age of the TREAT group was lower than the PREV group (61.4 vs 67.7 years, p<0.006) and mean GC dose was higher in the PREV group then the TREAT group (17 mg vs 13 mg prednisone, p<0.06). No significant gender difference between the two groups was found. Among the multiple disease states for which GC were prescribed, a significantly higher prevalence was found in the PREV group for polymyalgia rheumatica (45.5% vs 19.6%, respectively, p<0.004); in contrast, the prevalence of rheumatoid arthritis (40%) was essentially equal in both groups. Both body weight (kg) and body mass index (kg/m^2) were higher in the TREAT group than PREV group (85.5 kg and 30.1 kg/m^2 vs 76.9 kg and 27.8 kg/m^2, both at p<0.04). T score for the spine in the TREAT group was (-0.79) and significantly lower than for the PREV group (-0.09; p<0.04). T score for total hip was not significantly different for the 2 groups (-0.71 TREAT vs -0.80 PREV). Using IVA, we found that 23/106 patients in the TREAT group had at least 1 vertebral fracture (22%), and 6/33 patients in the PREV group had at least 1 vertebral fracture (18%). The lack of a difference in prevalent vertebral fractures between the 2 groups, despite the use of chronic GC, may reflect a difference in the clinical features of patients on GC, particularly in light of more recent developments in the therapy of RA. Despite the established efficacy of oral calcium supplements in individuals on GC therapy, ≤ 50% of individuals in both TREAT and PREV group were taking calcium supplements at enrollment. Despite normal lumbar spine and hip BMD, about 20% of subjects in both TREAT and PREV have prevalent vertebral fractures at study entry. Therefore, physician and patient education is still needed concerning the use of supplemental calcium in prevention of GC induced osteoporosis.

Disclosures: ***S. Morris****, Aventis 3.*

SU357

Olive Oil Prevents Inflammation-Induced Bone Loss in the Ovariectomized Rats, Used as a Model for Senile Osteoporosis. C. Puel*[1], J. Mathey*[1], C. Obled*[2], A. Mazur*[3], M. Davicco*[1], P. Lebecque*[1], P. Pastoureau[4], V. Coxam[1]. [1]Groupe Ostéoporose, INRA, Saint Genès-Champanelle, France, [2]Unité nutrition métabolisme protéique, INRA, Saint Genès-Champanelle, France, [3]Unité des maladies métaboliques, INRA, Saint Genès-Champanelle, France, [4]Servier, Suresnes, France.

Aging and estrogenic deficiency lead to inflammatory and oxidant conditions, which are involved in the pathogenesis of osteoporosis. Mediterranean diet is associated with a lower incidence of chronic diseases. Because olive oil, used as a staple in such a diet, contains specific fatty acids, a series of phenolic « minor components » and vitamins, exhibiting anti-oxidant and anti-inflammatory properties, the present study was designed to evaluate the potential protective effect of olive oil on experimentally induced bone loss in ovariectomized rats under inflammatory conditions.

Forty eight female Wistar rats (6 months old) were ovariectomized (OVX) or sham-operated (SH) as controls. OVX rats were randomly allocated to two groups receiving, for three months, either a control diet (OVX) or a diet with 5% olive oil (HO). SH rats were given the same control diet. After 2 months, subcutaneous inflammation was ellicited by injection of 3.2 g sterile talc (sigma) in one half of each group.

At necropsy, the success of ovariectomy was checked by the atrophy of uterine horns (g) (OVX : 0.032 ± 0.003 vs 0.221 ± 0.017 in SH ; p<0.0001). Olive oil didn't exhibit any uterotrophic activity. Alpha 1 glycoprotein concentration (µg/ml), an indicator of the acute phase response of inflammation was increased in OVX rats with talc injection (OVX inf : 35.9 ± 7.5 vs 22.6 ± 4.3 in OVX ; p< 0.05). Olive oil restored this level (HO inf : 22.8 ± 1.7). Concerning bone parameters, osteopenia in OVX assessed by a decreased metaphyseal and total femoral density (g/cm2) (T-BMD : in OVX : 0.2243 ± 0.0031 vs 0.2378 ± 0.005 in SH ; p<0.001) was exacerbated by inflammation (T-BMD in OVX inf : 0.2109 ± 0.0018 vs 0.2243 ± 0.0031 in OVX ; p<0.01) and prevented by olive oil diet (HO inf : 0.2249 ± 0.0038). Olive oil consumption had no effect either on osteocalcinemia (a marker for osteoblastic activity) or on urinary deoxypiridinoline excretion, a marker for bone resorption.

In conclusion, olive oil can prevent inflammation-induced osteopenia in OVX rats. This results suggest implication of specific fatty acids or micronutrients with antioxidant properties such as polyphenols, vitamines.

Disclosures: ***V. Coxam****, None.*

SU358

Dietary Fructooligosaccharides Improve Soy-Osteopenia Prevention in the Ovariectomized Rat. J. Mathey*[1], S. Katicoulibali*[2], C. Puel*[1], C. Bennetau*[3], P. Lebecque*[1], M. Davicco*[1], M. Horcajada*[1], J. Garel[4], V. Coxam[1]. [1]Ostéoporose, U3M, INRA, Ceyrat, France, [2]UFR Biosciences, Abidjan, Cote d'Ivoire, [3]ENITA, Bordeaux, France, [4]INSERM U349, Paris, France.

Dietary phytoestrogens deserve special mention due to evidence of their estrogenic effects in postmenopausal women experiencing bone loss. Concurrent dietary intake, in particular high dietary fibers, may exert an influence on isoflavones (IF) metabolism. Fructooligosaccharides (FOS), a mixture of indigestible and fermentable sugars, may affect their bioavailability, as well, by modulating large bowel microflora. The aim of the present study was thus to investigate if FOS may have a potential promise for improving the dose-dependant bone sparing effect of long term IF consumption in ovariectomized rats. In that purpose, 96 3-month old Wistar rats were randomly assigned to either castration (OVX=80) or sham surgery (SH=16). Animals were fed a soy protein free semi purified diet for 90 days containing IF (Prevastein®HC, Eridania Beghin-Say) at 0 (OVX and SH), 10 (IF10), 20 (IF20), 40 (IF40) or 80 (IF80) µg of total IF/g body weight per day. FOS (Actilight®, Beghin-Meiji) were orally given to the half of the groups, (OVX FOS), (IF10 FOS), (IF20 FOS), (IF40 FOS), (IF80 FOS) and (SH FOS) at 2.5% the first week, 5% the 2nd week, then 7.5% for the last 10 weeks. Animals were killed on day 91.

Ovariectomy induced a decrease in femoral bone mineral density (BMD) (g/cm2) (OVX : 0.2178 ± 0.0062 vs 0.2399 ± 0.0051 in SH) and femoral failure load (N) (OVX : 111 ± 6 vs 123 ± 8 in SH). Isoflavones exhibited a bone sparing effect as soon as consumption reached 20 µg/g/d, whereas only the highest dose induced a weak uterotrophic activity. Indeed, total femoral BMD was significantly enhanced (compared to that of OVX rats), as was the metaphyseal compartment. Bone strength was also improved. As far as the FOS diet is concerned, prebiotics addition significantly raised efficiency of the IF protective effect on both femoral BMD and mechanical properties. The trend towards higher BMD levels (g/cm2) with the lowest IF dose (IF10) even reached a significant level when FOS were added. (IF10FOS : 0.2366 ± 0.0026 vs IF10 : 0.2252 ± 0.0049 ; IF20 FOS : 0.2314 ± 0.0022 vs IF20 : 0.2311 ± 0.0024 ; IF40 FOS : 0.2469 ± 0.0026 vs IF40 : 0.2361 ± 0.0025 ; IF80 FOS : 0.2555 ± 0.0023 vs IF80: 0.2343 ± 0.0031). In each case, this effect could be explained by a reduced bone resorption (drop in urinary DPD).

Thus, daily IF consumption prevented ovariectomy-induced osteopenia by decreasing bone resorption, when given at 20, 40, or 80 µg (total isoflavones) /g body weight /d. Simultaneous FOS consumption improved this IF-protective effect on the skeleton, probably through enhancement of IF bioavailability.

Disclosures: ***V. Coxam****, Beghin-Meiji 2.*

SU359

Altered Mineral Absorption and Bone Turnover in Postmenopausal Women Treated with Oligofructose plus Inulin. L. Holloway[1], K. Kent[1], S. Moynihan*[1], S. A. Abrams[2], A. Friedlander*[1]. [1]VAPAHCS, Palo Alto, CA, USA, [2]Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA.

The deficiency of estrogen at menopause leads to a decrease in calcium absorption by the intestine and a corresponding increase in urinary calcium excretion, producing a negative calcium balance that contributes to bone loss. Magnesium deficiency has also been associated with bone loss. In this study, we attempted to positively affect the absorption of both minerals with treatment with non-digestible oligofructose plus inulin (NOF+1). Fifteen postmenopausal women were treated with (NOF+1) or placebo for six weeks in a crossover fashion. Fractional calcium and magnesium absorption were measured by dual isotope before and after treatment. Markers of bone turnover were also measured at baseline, 3 weeks, and 6 weeks. As expected, there was an increase in both calcium and magnesium absorption after six weeks of treatment with the active compound (p=ns). Bone resorption, as measured by deoxypyridinoline crosslinks (D-pyd), initially decreased after 3 weeks of active treatment. However, by 6 weeks, bone resorption had rebounded to level greater than that at baseline (p<0.05). Bone formation, measured by osteocalcin, was non-significantly increased at 3 weeks and continued to increase throughout the six weeks of treatment (p<0.05).

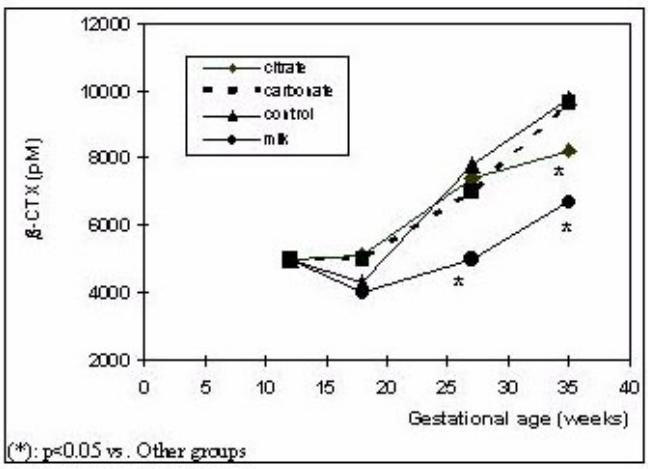
Closer examination showed variation in response within the subject population. Five out of fifteen subjects showed a decrease in absorption following treatment. Differences in response were not attributable to either initial absorption levels or supplementation. Comparison of calcium and magnesium intake levels showed no significant difference between the responder groups. There was a strong positive correlation between bone resorption at baseline and the degree of change in magnesium absorption in actively treated subjects (R^2 = 0.6, p<0.001). There was no correlation between baseline D-pyd and changes in calcium absorption. Subjects who had an increase in magnesium absorption following treatment had a significantly lower lumbar spine bone mineral density (DXA) than those who had a decrease in absorption (0.840 ± .119 g/cm^2 vs. 1.051 ± .112, p<.01). Subjects who had a positive calcium absorption response tended to have lower lumbar spine BMD (positive 0.886 ± .108 vs. negative responders 1.000 ± .229). There was no significant difference between the positive and negative responders in any hip parameter as measured by DXA. When stratified by response in absorption, it became apparent that the changes in bone turnover markers occurred only in the subjects that had a positive response to NOF+1 treatment.

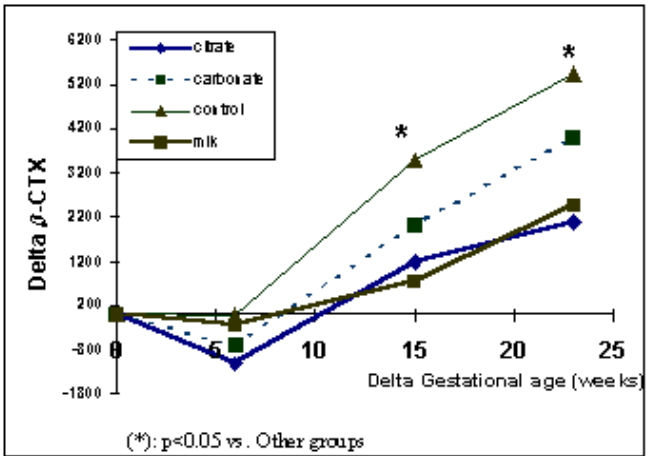
Disclosures: ***L. Holloway****, None.*

SU360

Effect of Calcium Supplementation on Bone Remodeling in Pregnant Women with Usually Low Calcium Intake. S. Zeni[1], S. Fleischman*[2], A. Lazzari*[3], L. López*[3], M. Suarez*[3], J. Somoza*[1], C. Ortela Soler*[3], M. L. de Portela*[2]. [1]Sección Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Cátedra de Nutrición, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, [3]Hospital, Buenos Aires, Argentina.

During pregnancy maternal calcium and skeletal homeostasis adapt to meet the calcium demands of growing fetus. Previously we found that calcium intake (CaI) lower than Adequate Intake (1000mg/d) affects bone remodeling. The present report evaluates the effect of different sources of calcium supplementation on bone remodeling during pregnancy. A group of 40 pregnant women (22±5 years) with an initial CaI of 446±206 mg/day were studied in a suburban hospital of Buenos Aires outskirts. Women were divided in four groups: G1: receiving no supplementation and informed of the beneficial effect of consuming dairy products. The other groups received 500mg Ca/d from G2: milk, G3: citrate and G4: carbonate. Bone remodeling throughout the gestational period was assessed by bone specific alkaline phosphatase (b-AL) and serum carboxy-terminal C-telopeptide cross-linked of type I collagen (b-CTX).





Results: Whereas groups showed no differences in b-AL, the women receiving milk supplementation exhibited the lowest increase in b-CTX (figure 1). Similarly, no differences were found among groups as regards Delta b-AL, although women not receiving supplementation has the highest delta b-CTX values (figure 2). Delta b-CTX during the third trimester presented a high negative correlation with CaI when the data were grouped according to ranges of CaI (r=-0.90, p<0.001). Conclusion: An adequate CaI may control the increment in bone resorption, specially during the third trimester of pregnancy.

Disclosures: ***S. Zeni****, None.*

SU361

Short Term Effect of Milk, Calcium Carbonate and a Calcium Fortified Food Drink on Bone Turnover of Postmenopausal Women. K. Karnik[1], S. Aston*[1], M. E. Barker*[1], R. Eastell[2], A. Blumsohn[2]. [1]Centre for Human Nutrition, University of Sheffield, Sheffield, United Kingdom, [2]Bone Metabolism Group, Division of Clinical Sciences, University of Sheffield, Sheffield, United Kingdom.

The extent to which the skeletal effects of milk can be attributed to increased calcium intake is not certain. Several other components of milk (e.g. proteins, phosphate) may have beneficial or deleterious effects on the skeleton. A variety of calcium supplemented food products are also available, but the skeletal benefits of these supplements cannot necessarily be assumed from their calcium composition. Useful information about the acute and chronic effects of dietary supplements on the skeleton can be obtained using biochemical markers of bone turnover.
In this study, we examined the short term (7 days) anti-resorptive effect of three different modes of calcium administration in 68 healthy postmenopausal women with low habitual dietary calcium intakes (mean 561 mg/day, SEM 15.9 by food frequency questionnaire and 24-hour recall). Participants were randomised to receive 1000 mg of calcium either as 1) fresh semi-skimmed milk 2) calcium carbonate 3) a malt- and dairy- based food drink fortified with calcium, Horlicks®. All supplements were administered twice a day for 7 days in an identical volume (800 ml per day). Participants kept a weighed record of food intake for 2 days before supplementation and during supplementation to assess displacement. The response to intervention was assessed by measurement of NTX in 24 hour urine samples, and βCTX and PTH in serum.
There was no significant displacement of non-supplemented dietary calcium intake during the supplementation period (p>0.05).
Mean (S.E.) % change between baseline and one week:

	24hr NTX	βCTX	PTH
Calcium carbonate	-27.6 (3.1)	-31.6 (4.1)	-24.3 (3.8)
Food drink	-18.4 (4.9)	-28.2 (4.3)	-20.4 (4.1)
Milk	-15.4 (3.5)	-30.8 (3.3)	-14.0 (3.8)

There was significant decrease in all three analytes in all 3 groups over the period of the study (p<0.001). The decrease in NTX and PTH was less following milk than following calcium carbonate supplementation (p<0.05). Calcium fortified food drinks may provide a useful means to increase calcium intakes.
Glaxo SmithKline provided calcium fortified Horlicks.

Disclosures: ***K. Karnik****, None.*

SU362

A Mixed Herbal Extract Prevents Bone Loss in Ovariectomized (OVX) Rats. H. Ha[1], H. Yang*[1], D. Jung*[1], H. Lee*[2], D. Jung*[1], H. Lee*[1], K. Song*[3], C. Kim[1]. [1]Drug Research and Development Team, Korea Institute of Oriental Medicine, Seoul, Republic of Korea, [2]Wuli Oriental Hospital, Seoul, Republic of Korea, [3]College of Medicine, Chung-Ang University, Seoul, Republic of Korea.

Estrogen replacement therapy is indeed effective in preventing bone loss caused by menopause, but it is also accompanied by some adverse effects, such as uterus bleeding and breast cancer. This study was focused on development of nutraceuticals from herbal extracts, to prevent and treat osteoporosis after menopause without any side effects. KUNBO, a mixed herbal extract including Astragali Radix and Rhynchosiae nulubilis Semen, was tested for analysis their therapeutic efficacy on osteoporosis. The proliferation of osteoblast like cell (Saos-2) induced by KUNBO was analyzed using a tetrazolim salt (MTT) and alkaline phosphatase (ALP) activity. The inhibition on osteoclast was studied using the coculture method of mouse bone marrow cells and mouse stromal cells (ST-2). Adult OVX SD rats (10 weeks old) were divided into four groups; sham, control, 17beta-estradiol (E2; 1 microg/kg/day), and KUNBO (5 g/kg/day). Animals in each group were administrated daily dosage for 9 weeks. After complete blood cells counts and biochemical analysis in plasma as biomarkers, tibia and lumbar were isolated and then trabecular bone areas (TBAs) of tibia and lumbar were measured by bone histomorphometry. In results, KUNBO induced cell proliferation (120.2% of control) on Saos-2. The TBAs of tibia in KUNBO group were increased 142.1% of control. KUNBO did not induce estrogenic side effects in uterus. In conclusion, KUNBO prevents OVX-induced cancellous bone loss for 9 weeks in OVX rats. (Supported partially by a grant, #02-PJI-PG11-VN04-SV04-0004, from Health Technology Planning & Evaluation Board, Korea)

Disclosures: ***C. Kim****, None.*

SU363

Alpha-ketoglutarate (AKG) Inhibit Osteoporosis Development in Postmenopausal Women. A. Tocaj*[1], R. Filip*[2], B. Lindergård*[3], J. Wernerman*[4], T. Studzinski*[5], K. Öhman*[1], S. Pierzynowski[6]. [1]Gramineer International AB, R&D, Lund, Sweden, [2]Osteoporosis Outpatient Dept., Institute of Agricultural Medicine, Lublin, Poland, [3]Department of Nephrology, Lund University Hospital, Lund, Sweden, [4]Intensive Care Medicine, Karolinska Institutet, Huddinge, Sweden, [5]Department of Animal Physiology, Lublin University, Lunblin, Poland, [6]Department of Cell and Organism Biology, Lund University, Lund, Sweden.

It is known that the stabilisation of collagen is dependent on hydroxylation of peptide-bound proline to hydroxyproline catalysed by prolyl hydroxylase. This process requires Fe^{2+}, AKG, molecular O_2 and ascorbate as co-substrates. In preclinical studies we have shown that enteral AKG enhance gut proline synthesis and improve bone mineralization in pig, turkey and rats. Thus, the question arises – can AKG inhibit osteoporoses development in humans?
The study (phase II) was a randomised, double-blind, placebo-controlled 6-month trial. AKG + calcium (active treatment) or calcium (Ca) alone (reference treatment) was to be taken before main meals (3 x 2 tablets per day). The daily dose of AKG was 6.0 g and of Ca 1.68 g. The primary end-points were changes in serum levels of biochemical bone turnover markers: 1) type I collagen C-telopeptide (CTx) and 2) osteocalcin (OC). Bone mineral density (BMD) was chosen as secondary end point due to the short duration of the study.
Results are presented in table 1. The CTx level in the Ca group remained unchanged whereas CTx decreased with 35 % in the AKG + Ca group. No statistically significant difference was observed in serum OC levels for either treatment. The BMD of the lumbar spine increased by 1.6 %, (p=0,038) in the group receiving AKG + Ca, whereas no statistically significant difference was observed in the Ca group.
Conclusions; enteraly administered AKG is useful in preserving the bone mass as well as lowering bone turnover in postmenopausal women. AKG treatment increased BMD in postmenopausal women suffering from osteopenia and we believe that AKG has the metabolic capability to improve BMD also in patients with established osteoporoses.

Table 1. Bone mass density, and CTx / OC serum levels (mean ± SD).

Treatments	BMD (g/cm^2)		CTx (pmol/L)		OC (ug/L)	
	baseline	6 months after	baseline	6months after	baseline	6 months after
Ca n = 29	.9729[a] ± .0308	.9788[a,b] ± .0526	5946[a] ± 4093	4980[a] ± 2613	22.7 ± 18.3	20.8 ± 18.3
AKG + Ca n = 32	.9721[a] ± .0454	.9873[b] ± .0609	5924[a] ± 3307	3727[c] ± 1714	22.3 ± 16.4	23.1 ± 21.0

Different letter given with results describe statistical differences where p<.05

Disclosures: ***A. Tocaj****, None.*

SU364

Effects of Soy, Fructooligosaccharide, and their Combination on Reversal of Bone Loss in Ovariectomized Osteopenic Rats. L. J. Hammond*, D. A. Khalil, L. Devareddy*, D. Y. Soung, E. A. Lucas, B. J. Smith, S. Juma, J. Johnston*, B. H. Arjmandi. Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA.

The study examined the efficacy of soy and fructooligosaccharide (FOS), a non-digestible carbohydrate known to support the growth of beneficial bacteria in the gut, on reversal of bone loss in ovariectomized (ovx) osteopenic rats. Sixty-four 9-mo old Sprague-Dawley rats were either sham-operated (sham) or ovx and fed a standard casein-based diet for 90 days during which time the ovx rats significantly lost bone as verified by whole body bone mineral density (BMD) and content (BMC) using dual-energy x-ray absoptiometry. Thereafter, the ovx rats were randomized into 4 treatment groups: ovx (control), ovx + soy, ovx + 5% FOS, ovx + soy + 5% FOS and treatments continued for 125 days. Treatment effects on BMD and BMC of the tibiae and the lumbar vertebrae are presented below.

	Left tibia		Third lumbar		Fourth lumbar	
Group	BMC	BMD	BMC	BMD	BMC	BMD
Sham	0.3584[a]	0.1993[a]	0.1279[a]	0.2264[a]	0.1465[a]	0.2342[a]
Ovx	0.3103[c]	0.1825[c]	0.1071[c]	0.1986[c]	0.1189[c]	0.2055[c]
Soy	0.3423[ab]	0.1908[b]	0.1135[bc]	0.2046[bc]	0.1273[bc]	0.2124[bc]
FOS	0.3269[bc]	0.1892[b]	0.1201[ab]	0.2100[b]	0.1341[b]	0.2183[b]
Soy + FOS	0.3221[bc]	0.1887[b]	0.1147[bc]	0.2088[b]	0.1331[b]	0.2181[b]

Values in a column not sharing a superscript letter are significantly different from each other (P<0.05).

Although the above data indicate that soy and FOS each improve tibial and vertebral BMD and BMC, their combination do not exert a greater bone protective effects.

Disclosures: ***L.J. Hammond****, None.*

SU365

Effects of Soy Supplementation on Bone in Postmenopausal Women. B. H. Arjmandi[1], D. A. Khalil[1], C. Hardin*[1], L. J. Hammond*[1], L. Devareddy*[1], E. A. Lucas[1], B. J. Smith[1], J. McDonald*[1], A. B. Arquitt*[1], M. E. Payton*[2], A. Babaknia*[1]. [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Department of Statistics, Oklahoma State University, Stillwater, OK, USA.

Reports suggest that soy protein may reduce the risk of osteoporosis in peri- and postmenopausal women. The objective of this study was to examine if soy containing foods (60 mg isoflavones and 25 g protein provided by consuming any two serving of three food products) exerts beneficial effects on bone in postmenopausal women. Eighty eligible women were randomly assigned to consume soy or control foods daily for one year. Subjects in both groups received similar amounts of energy, carbohydrates, protein, fat, fiber, calcium, and vitamin D. Sixty-two subjects completed the one-year long study. Bone mineral density (BMD) of the whole body, spine, and hip at baseline and after one year were measured using dual energy x-ray absorptiometry (Hologic QDR 4500 Elite). Blood and urine markers of bone metabolism were also assessed. The following table represents selected BMD values:

	Control		Soy	
BMD	Percent Change	*P*	Percent Change	P
Whole body	-1.4	0.002	-1.0	0.009
Fourth Lumbar	-0.5	0.119	+0.4	0.966
Right hip Total	-0.8	0.347	+1.3	0.106
Left hip Total	-0.6	0.316	-0.6	0.211

Values represent mean ± (SD).

The initial data analysis suggest that subjects who consumed soy products: 1) had lower degree of bone loss (whole body and left hip total BMD); 2) maintained the 4th lumbar BMD; and 3) had slightly higher right hip total BMD in comparison with women who consumed milk protein. However, soy supplementation at the level used in this study was not able to completely prevent bone loss at all sites. This marginal, but positive effect, may be due to insufficient isoflavone content of the soy products consumed by the subjects in the present study as recent data suggest that 90 mg soy isoflavones are required to detect a significant effect on bone. Moreover, both protein sources positively affected markers of bone formation and bone resorption as indicated by increased serum bone-specific alkaline phosphatase activity and osteocalcin and a tendency to decrease urinary deoxypyridinoline. These findings infer that additional protein intake, regardless of the source, does not negatively effect skeletal health. A higher dose of soy protein and/or soy isoflavones may be necessary for complete prevention of bone loss in postmenopausal women.

Disclosures: ***B.H. Arjmandi****, None.*

SU366

Effects of Soy Isoflavones on Bone Biomechanical and Micro-architectural Properties in an Aged Orchidectomized Rat Model of Male Osteoporosis. D. Y. Soung[1], D. A. Khalil[1], L. Devareddy*[1], L. J. Hammond*[1], E. A. Lucas[1], B. J. Smith[1], D. D. Marlow*[2], M. P. Akhter[3], B. H. Arjmandi[1]. [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA, [3]Creighton University Osteoporosis Research Center, Omaha, NE, USA.

Among the dietary approaches for improving skeletal health, soy protein and its isoflavones have received considerable attention. However, research on soy and its isoflavones has been directed primarily towards postmenopausal osteoporosis. The purpose of this study was to examine whether soy isoflavones improve the bone biomechanical and micro-architectural properties in aged orchidectomized (ORX) male rats, a model of male osteoporosis. Seventy-two, 13-month old F344 rats were either sham-operated (sham) or ORX and divided into six treatment groups (n= 12). Rats in the sham and one ORX group were fed a casein-based control diet and the remaining four ORX groups were fed one of two doses of isoflavones (593 or 1186 mg/kg diet) in the context of either casein or soy protein for 180 days. Biomechanical properties of 4th lumbar (n=12/group) as well as micro-architectural properties of the left tibia (n=7/group) were determined. Treatment effects on select biomechanical properties and on the structure model index (SMI) are presented in the following table:

Protein Source	Isoflavones (mg/kgdiet)	Ultimate Load (N)	Ultimate Stress (N/mm^2)	SMI
Sham+Casein	-	210.21 ± 14.20[a]	31.067 ± 2.312	1.347 ± 0.081[d]
ORX+Casein	-	156.62 ± 11.22[b]	23.912 ± 1.828	1.628 ± 0.081[abc]
ORX+Casein	593	159.28 ± 13.54[b]	23.167 ± 2.205	1.844 ± 0.081[ab]
ORX+Casein	1186	163.73 ± 14.20[b]	23.272 ± 2.312	1.862 ± 0.081[a]
ORX+Soy	593	175.32 ± 14.20[ab]	24.845 ± 2.312	1.603 ± 0.081[bc]
ORX+Soy	1186	190.13 ± 14.97[ab]	27.215 ± 2.437	1.508 ± 0.081[cd]

Values are mean ± SE. Means in a column that do not share the same superscript letter(s) are significantly different from each other (P<0.05)

The data suggest that soy isoflavones in the context of soy protein, but not casein, were able to preserve SMI, an index of trabecular plate morphometry, due to gonadal hormone deficiency. Furthermore, it appears that soy protein irrespective of its isoflavones can moderately prevent the loss of structural properties as evident by the observed effect on ultimate load.

Disclosures: ***D.Y. Soung****, None.*

SU367

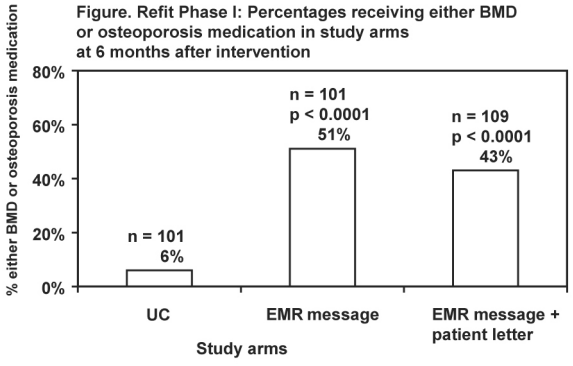
Results of a Randomized, Controlled Trial to Improve the Management of Osteoporosis After Fracture. A. C. Feldstein*[1], P. J. Elmer*[1], D. H. Smith*[1], M. Aickin*[1], M. K. Herson*[2], E. S. Orwoll[3], C. Chen*[1], M. C. Swain*[1]. [1]Center for Health Research, Portland, OR, USA, [2]Kaiser Permanente Northwest, Portland, OR, USA, [3]Oregon Health & Science University, Portland, OR, USA.

Prior work found that 54% of women do not receive guideline-recommended BMD measurement or medication for osteoporosis after a fracture. This analysis reports on Phase I of the Re-Fracture Intervention Trial (REFIT), which was designed to evaluate methods to increase the proportion of these patients who receive BMD measurement or a medication for osteoporosis.

The study enrolled 311 female Kaiser Permanente Northwest members aged 50-89 who had recently suffered an osteoporotic fracture but who had not received a BMD measurement or medication for osteoporosis. We collected baseline data from a questionnaire (64% return rate) and our electronic databases and then randomly assigned the participants to usual care or one of the two interventions: 1) patient-specific clinical guideline advice to the primary care provider (PCP) delivered through an electronic medical record e-mail message (EMR message) or 2) an EMR message to the PCP plus an advisory letter with educational materials mailed to the patient. We used logistic regression to evaluate the response to the interventions.

At 6 months, EMR messaging to the PCP alone resulted in 51% of patients receiving a BMD measurement or an osteoporosis medication as compared to 6% in usual care (p<0.0001). EMR messaging to the PCP plus communicating with the patient resulted in 43% receiving a BMD measurement or an osteoporosis medication as compared to 6% in usual care (p< 0.0001). The effect of the EMR message to the PCP alone was not significantly different from the EMR message to the PCP combined with the patient mailing (p= 0.23). Age, weight <127 lb, chronic disease score, and diagnosis of osteoporosis were not significantly associated with response to the intervention.

REFIT Phase I demonstrated that patient-specific clinical guideline advice to the PCP through an EMR message following a fracture is highly effective for increasing use of BMD measurement and osteoporosis medication. As EMRs become more widely adopted, this intervention could significantly improve the management of osteoporosis for many post-fracture patients. The finding that including the patient in the intervention did not increase the effect should be a topic of further study.



Disclosures: ***A.C. Feldstein****, Merck & Co., Inc. 2.*

SU368

Hospital-based Fracture Care: A Comparison of Fracture Clinics and Emergency Departments in Ontario, Canada. S. B. Jaglal[1], G. Hawker[2], S. M. Cadarette[3], L. Jaakkimainen*[4], H. Kreder*[5], E. Bogoch*[5], J. Carroll*[4], W. McIsaac*[4], D. Davis*[4]. [1]Rehabilitation Science, University of Toronto, Toronto, ON, Canada, [2]Medicine, University of Toronto, Toronto, ON, Canada, [3]Health Policy, Management and Evaluation, University of Toronto, Toronto, ON, Canada, [4]Family and Community Medicine, University of Toronto, Toronto, ON, Canada, [5]Surgery, University of Toronto, Toronto, ON, Canada.

A provincial telephone survey of individuals responsible for hospital-based fracture care was conducted between June and October 2002. The survey was part of a larger study to develop recommendations for an evidence-based best practice model for integrated post-fracture care in Ontario, Canada. The objective of the hospital survey was to more fully understand and describe the current processes and patterns of post-fracture care for hip and wrist fracture patients, including barriers and facilitators, patterns of referral, communication and follow-up. Surveys were completed for all 178 eligible hospitals in Ontario (100% response rate). Ninety-five hospitals (53%) had Emergency Departments only, while 83 hospitals (47%) had a Fracture Clinic. Most non-surgical fracture patients (95%) receive their x-ray, treatment and follow-up at the presenting hospital (by either an Orthopaedic Surgeon or Emergency Department Physician) whereas surgical fracture patients may receive treatment and follow-up at more than one hospital depending on hospital resources (i.e., availability of an Orthopaedic Surgeon). Emergency Department and Fracture Clinic notes are usually used to notify family physicians of a fracture. However, notification is not routine. Other mechanisms for notification include the X-ray report, follow-up letter and verbal communication. Most fracture patients (93%) are referred to outpatient rehabilitation for post-fracture care and are provided educational information (90% wrist fracture, 53% hip fracture), primarily on fracture care and mostly in written format. In comparison, few hospitals provided information about osteoporosis. Over 40% did not know under what circumstances fracture patients should be investigated for osteoporosis, but most felt that, where indicated, it should be the responsibility of physicians in the Emergency Department or Fracture Clinic to make the referral for osteoporosis investigation. This survey demonstrated a need to raise awareness among both health care professionals and fracture patients regarding the link between fragility fracture and osteoporosis, and identified possible areas for intervention on the continuum of fracture care.

Disclosures: ***S.B. Jaglal****, None.*

SU369

Impact of a Management Plan for Patients Admitted with Osteoporotic Fractures on Complinace to Osteoporosis Drug Therapy. P. Dagenais*[1], J. Beauchemin*[2]. [1]Medicine, University of Montreal, Montreal, PQ, Canada, [2]Medicine, University of Sherbrooke, Sherbrooke, PQ, Canada.

In order to improved quality of care for patients admitted to the rheumatology ward with fragility vertebral fractures due to osteoporosis (OP), we instituted an OP management plan. This plan offers a multidisciplinary approach in which teaching of favourable behaviours to prevent recurrences of OP fractures is an important part. This teaching insists on compliance and persistence to OP treatments i.e. anti-resorptive therapies and the use of calcium and vitamin D. In order to measure the impact of this management plan on compliance to OP drug treatments, we decided to conduct a case-control study comparing the compliance to OP treatment of elderly (≥ 65 y. old) patients exposed to the OP management plan on the rheumatology (R) ward, with unexposed patients admitted to the geriatric (G) ward from January 1998 to December 2000. One hundred and thirty four patients (75 in R, 59 in G) were eligible for the study. Dead patients (n=34) were excluded (20 in R, 14 in G) and 32 patients refused to answer questions or could not be retraced (15 in R, 17 in G). Intention to treat analysis of the data was used for this study. There was no statistical differences between ages (77.4 ± 6.9 in R vs. 82.9 ± 7 in G), gender distribution, and co-morbidities, using a validated categorial scale of sickness severity, between the 2 groups. A higher proportion of R patients were prescribed anti-resorptive therapy (92.5%) compared to G patients (67.8%) (p = 0.02). A higher proportion of R patients (67.5%) were still on these treatments vs. G patients (42,8%) (p = 0.04) 1 to 3 y. after initiation of treatment. There was no differences between the rate of prescription and persistence of calcium and vitamin D use between the two groups. The small number of patients in the two groups did not allowed us to see a difference between R and G patients on persistence to any OP therapy over time. In summary OP management plan had no impact on compliance and persistence to anti-resorptive therapy. It may have played a role in the higher prescription rate of anti-resorptive therapy in patients admitted on the rheumatology ward for vertebral fractures due to OP.

Disclosures: ***P. Dagenais****, Procter&Gamble 2, 5; Merck Frosst 5; Eli Lilly 5.*

SU370

Quality of Life Following Percutaneous Vertebroplasty. F. E. McKiernan[1], T. Faciszewski[2], R. Jensen*[2]. [1]Center for Bone Diseases, Marshfield Clinic, Marshfield, WI, USA, [2]Department of Orthopedic Spine Surgery, Marshfield Clinic, Marshfield, WI, USA.

Percutaneous vertebroplasty (PV) relieves pain in patients with symptomatic osteoporotic vertebral compression fractures (VCFs) that have failed non-operative treatment. No study has reported PV outcome with an instrument validated for use in osteoporosis. We report quality of life following PV using a disease-specific health related quality of life (HRQL) outcome instrument.
This is an IRB approved, prospective study of patients that underwent PV at a tertiary referral center. At enrollment and 1 year later subjects completed the Osteoporosis Quality of Life Questionnaire (OQLQ), a validated, 30-item, 5-domain instrument that measures HRQL in osteoporotic women with back pain due to VCF. 2 wk, 2 mo and 6 mo postoperatively subjects completed the mini-OQLQ, a 10-item, 5-domain, validated OQLQ extraction. 2 gender-neutral activities of daily living (ADL) questions were added to balance 2 gender-biased OQLQ ADL questions. Pain was rated (VAS1-10) 1d postop and at each other evaluation point. 6 mo results are reported.
44 consecutive subjects (31F,13M) underwent 47 PVs to treat 64 VCFs. Average age was 74.2 yrs. 55% were ever- and 25% current-glucocorticoid users. 68% were ever- and 20% current-smokers (average 55 pack-years). Average T- and Z-scores were –2.0/-0.8 (lumbar spine) and -2.26/-0.84 (femoral neck) respectively. 59% had sustained prior fragility fracture. 59% were on osteoporosis treatment at entry. 1d after PV pain rating fell from 7.8 to 2.8 (p<0.001). Mean pain rating increased 1.3 from postoperative day 1 to month 6 (p=0.041). 4 of 5 HRQL domains (symptoms, physical function, ADL, leisure) and the 2 gender neutral ADL questions improved significantly at postop wk 2 (p=<0.001). The fifth HRQL domain (emotional function) improved but not significantly (p=0.058). All HRQL domains remained significantly improved through month 6. There were 7 asymptomatic cement leaks and no serious adverse events (SAE). Subsequent vertebral fracture rate was not greater than expected.
This is the first prospective outcome analysis of HRQL in osteoporotic subjects following PV using a validated disease-specific instrument. Contrary to the natural history of VCF, fracture pain and quality of life quickly improve after PV and improvement is maintained through 6 mo. Adverse events are infrequent and SAE are rare. Concern that PV may accelerate subsequent vertebral fracture rate was not confirmed in this study. Quality of life improves immediately following percutaneous vertebroplasty and remains improved through 6 months.

Disclosures: ***F.E. McKiernan****, None.*

SU371

Determinants of Early Outcome after Percutaneous Vertebroplasty. F. E. McKiernan[1], T. Faciszewski[2], R. Jensen*[2]. [1]Center for Bone Diseases, Marshfield Clinic, Marshfield, WI, USA, [2]Department of Orthopedic Spine Surgery, Marshfield Clinic, Marshfield, WI, USA.

Percutaneous vertebroplasty (PV) relieves pain and is thought to improve quality of life in patients with symptomatic osteoporotic vertebral compression fractures (VCFs) that have failed non-operative treatment. Using a disease-specific, health related quality of life (HRQL) outcome instrument we investigated the determinants of early outcome of PV.
This is an IRB approved, prospective study of patients that underwent PV at a tertiary referral center. At enrollment and 1 year later subjects completed the Osteoporosis Quality of Life Questionnaire (OQLQ), a validated, 30-item, 5-domain (symptoms (Sx), physical function (Pf), activities of daily living (ADL), leisure (Le), emotional function (Ef)) instrument that measures HRQL in osteoporotic women with back pain due to VCF. 2 wk, 2 mo and 6 mo postoperatively subjects completed the mini-OQLQ, a validated 10-item, 5-domain OQLQ extraction. 2 gender-neutral ADL questions were added to balance 2 gender-biased OQLQ ADL questions. Pain was rated (VAS1-10) 1d postop and at each other evaluation point. Early outcome (1d, 2 wk) results are reported.
44 consecutive subjects (31F,13M) underwent 47 PVs to treat 64 VCFs. Average age was 74.2 yrs (29.9 yrs past menopause). Average T- and Z-scores were –2.0/-0.8 (lumbar spine) and -2.26/-0.84 (femoral neck) respectively. 59% had sustained prior fragility fracture. 1d following PV pain rating fell from 7.8 to 2.8 (p<0.001) and increased slightly (+1.3) through 6 mo (p=0.041). Four of five HRQL domains (Sx, Pf, ADL and Le) and the 2 gender neutral ADL questions improved significantly at postoperative wk 2 (p=<0.001) and then did not change significantly through 6 mos. Ef improved but did not reach statistical significance (p=0.058). Multivariate analysis showed that pain relief 1d postop was unrelated to baseline BMD, past or current cigarette or steroid use or the presence of an intravertebral cleft but was strongly correlated with preoperative level of pain (p<0.0001), age >75 yrs (p<0.02) and correlated inversely with vertebral height restoration (p<0.05). 2 wk postoperative improvements in ADL, Sx, Ef and Le were negatively correlated with preoperative ADL (p<0.01), Sx (p<0.001), Ef (p<0.0001) and Le (p<0.005) respectively. 2 wk postoperative improvements in ADL were correlated with vertebral height restoration (p<0.05). There were no preoperative correlations with postop Pf at 2 wks.
Early outcome following PV is better in subjects > 75 yrs of age, with greater preoperative pain and poorer preoperative quality of life. Vertebral height restoration may be associated with greater pain 1d postop and better ADL score 2 wks postop.

Disclosures: ***F.E. McKiernan****, None.*

SU372

4 Month Evaluation of the Osteoporosis Exemplary Care Program at St Michael's Hospital. E. R. Bogoch[1], V. Elliot-Gibson*[2], D. E. Beaton*[2], S. A. Jamal[3], R. G. Josse[3], T. M. Murray[3]. [1]Department of Surgery, St. Michael's Hospital, University of Toronto, Toronto, ON, Canada, [2]Mobility Program Clinical Research Unit, St. Michael's Hospital, University of Toronto, Toronto, ON, Canada, [3]Department of Medicine, St. Michael's Hospital, University of Toronto, Toronto, ON, Canada.

Fragility fractures are followed by further fragility fractures in the absence of intervention. Current osteoporosis (OP) treatment rates for fragility fracture patients are reported to range from 0 -35%. St Michael's Hospital, a large university teaching hospital in Toronto, Ontario, has implemented an Osteoporosis Exemplary Care Program (OECP) to improve OP recognition and intervention rates in patients who have sustained a fragility fracture, and to identify barriers to OP care. Criteria for patient selection: male ≥ 50; female ≥ 40; fragility fracture resulting from low trauma; fracture site: distal radius, proximal humerus, proximal femur, vertebrae. Program coordinator role is to: identify orthopaedic inpatients and fracture clinic outpatients daily utilizing criteria; provide individual education related

to OP and nutrition; consult with orthopaedic surgeons and residents regarding nutritional supplementation with calcium and vitamin D_3 +/- treatment with a bisphosphonate; arrange densitometry and referral to the Metabolic Bone Disease Clinic (MBDC) or inpatient consultation for OP. Data presented is from the period December 1, 2002 to March 31, 2003.

	Inpatients*		Outpatients*	
	Female n = 50	Male n = 11	Female n = 103	Male n = 37
mean age	80	75	70	66
Fracture Site: Hip	39	10	33	7
Wrist	-	-	44	15
Shoulder	5	-	19	13
Vertebra / Other#	6	1	7	2
Previous Dx / Rx of OP	17	4	51	4
Referred to MBDC / GP	11	3	48^	31
No referral	22	4	7	2

**includes 2 inpatients and 3 outpatients with high trauma fracture and OP risk factors; # other fracture sites at discretion of attending surgeon; ^ 3 patients with OP referred to MBDC for further consultation.*

Inpatients were not referred to the MBDC because of death (3), co-morbidities/lack of support to facilitate attendance (10), refusal (3), language barrier (3), awaiting BMD results (2); and other reasons (5). Outpatients were not referred to the MBDC because of refusal (4), language barrier (1), and other reasons (4). Six-month follow-up will be completed in those patients who agreed to be contacted. The OECP implemented a care pathway that effectively identifies fragility fracture patients requiring OP intervention. The expectation is to detect 500 to 600 fragility fracture patients per year. Barriers to treatment in an urban hospital population are identified.

Disclosures: ***V. Elliot-Gibson****, None.*

SU373

Relative Utility of New Serum Markers of Bone Turnover for Monitoring Raloxifene Therapy. J. A. Clowes, N. F. Peel, R. A. Hannon, F. Gossiel*, R. Eastell, A. Blumsohn. Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

The practical use of biochemical markers of bone turnover to monitor raloxifene therapy has not been explored in any detail. Reliable serum markers of bone resorption are likely to provide a better indication of therapeutic response than urinary markers. In this study we examined the relative ability of four serum markers of bone turnover to detect change in bone turnover following combined therapy with raloxifene and calcium. 47 patients were randomised to receive either placebo (n=25) or raloxifene (n=22; 60 mg/day with calcium 500 mg/day). Fasting morning blood samples were collected at –1,0,1, 2, 4,8, 12,24 and 25 weeks in the raloxifene arm or at –1,0,12,24,25 in the placebo arm. We measured serum C-terminal telopeptide of type I collagen (βCTX, Roche Elecsys), osteocalcin (OC, Roche), procollagen N-terminal propeptide of type I collagen (PINP, Roche) and TRACP5b (Suomen Bioanalytiikka Oy). Samples were assayed in replicate. Measurement imprecision over 6 months in the placebo group was partitioned into analytical variability (CVa), and within subject variability after accounting for CVa (CVi) using nested ANOVA on data replicated at each level.

At two weeks of therapy there was a 22.7%±4.9SEM decrease in βCTX and a 13.2%±2.6 decrease in TRACP5b (both P<0.01). At this early time-point there was no significant decrease in either marker of bone formation (PINP 0.38%±3.4; OC 5.1%±3.2 decrease). Response to therapy at 25 weeks (%Ch25), CVa, CVi, and signal to noise ratio at 2 weeks and 25 weeks (%Ch25/CVi) are shown (Table). TRACP5b showed larger within subject variability than previously reported over a short time-scale. This is likely to be due to the probable long half-life and serial correlation of this analyte. The optimum choice of marker is likely to depend on the interval since starting therapy. Serum PINP is likely to be the most reliable marker to monitor therapy at times after 12 weeks, but sβCTX or TRACP5b may be more efficient at earlier times.

	CVa	CVi	%Ch25±SEM	S / N 2w	S / N 25w
OC	2.0	11.9	24.8±3.7	0.42	2.1
PINP	1.6	13.2	37.8±5.0	0.03	2.7
TRACP5b	1.8	16.4	17.6±2.1	0.8	1.1
βCTX	4.7	22.9	37.8±8.4	1.0	1.7

Disclosures: ***A. Blumsohn****, None.*

SU374

Bone-Targeting Nanocapules. N. K. Vail, H. Dixon*, J. Trevino*. Nanomaterials and Pharmaceutical Development, Southwest Research Institute, San Antonio, TX, USA.

Maintenance of skeletal health is one of the more challenging current public health issues. We hypothesized that nanocapsules designed to target the mineralized phase of bone can deliver therapeutic agents to treat bone maladies and improve bone health. Our initial focus is the delivery of bone anabolic agents to treat osteoporosis. Anabolic agents are not selective to bone and their therapeutic-toxic window may be narrow. Targeted delivery has significant opportunity to increase the efficacy of anabolic agents while reducing dosage requirements and mitigating systemic side effects. We identified several bone-binding ligands and selected methylene bisphosphonate (MBP) as a model targeting ligand. Amino-MBP was synthesized and the structure confirmed by ^{1}H NMR. The ligand was linked via sulfhydryl conversion of the amine to a maleimide-functionalized PEGylated phospholipid (DSPE-PEG_{2000}). The conjugation was monitored by changes in the sulhydryl content by UV-Vis spectroscopy. MALDI-TOF/MS confirmed the functionalized lipid (m/z ~3010) increased uniformly in mass, and without peak broadening, by an amount equivalent to the mass of the MBP ligand (m/z ~280). Long-circulating liposomes were prepared by controlled hydration and sized by extrusion to typically 120nm. ± 20 nm. The lipid-ligand conjugate was inserted into the preformed liposomes by the method of post-insertion wherein the conjugate transfers to the external leaflet of liposomes during mild co-incubation. Modified liposomes were purified by size exclusion chromatography and the ligand content on the liposomes quantified by complexing the MBP with ^{99m}Tc and performing gamma counting. MBP ligand contents could be varied between 0 and 4 mol % of the total lipid. In vitro studies showed that MBP ligands conjugated to the surfaces of nanocapsules convey preferential targeting to hydroxyapaptite matrices. Fluorescent analysis of disrupted carboxyfluorescein-containing liposomes indicated liposomes with no targeting ligands had no significant affinity for the HAp substrate while modified liposomes accumulated significantly on the HAp substrate. Adherence to the HAp substrate was confirmed by gamma counting ^{99m}Tc-chelated ligand-containing liposomes. This study showed that specifically formulated bone-targeting nanocapsules can target and adhere to bone-like surfaces. This technology has tremendous potential in the treatment of musculoskeletal diseases and disorders.

Disclosures: ***N.K. Vail****, None.*

SU375

Prevention of Osteoporosis in Patients Treated Chronically with Oral Glucocorticoids: Comparison with Guidelines. R. A. Adler, M. I. Williams*, M. Gordon*, V. I. Petkov*. Endocrinology, McGuire Veterans Affairs Medical Center, Richmond, VA, USA.

Prevention of glucocorticoid-induced osteoporosis (GIOP) remains challenging. We have recently suggested guidelines for GIOP for patients cared for by the Department of Veterans Affairs (VA), the largest health system in the U.S. In order to provide baseline information for an intervention trial, we examined factors associated with initiation of preventive practices in veteran patients exposed to oral glucocorticoids (GC) of more than 675 mg (equivalent of 7.5 mg/ 3 months).

Electronic search of pharmacy records over a 15-month period identified 1475 patients with prednisone prescriptions. The cumulative GC dose was calculated for each patient based on tablet strength, duration of therapy, and recorded refills. A total of 750 patients received more than 675 mg. In order to identify patients with adequate preventive measures, electronic prescription files were linked with files containing bone mineral density test (DXA) requests and prescriptions for calcium, vitamin D/multivitamins, and bisphosphonates.

The mean cumulative dose was 2,984mg/ 15 months (range 680 to 28,380). One–third of the patients received more than 7.5 mg/d for 15 months and 8.4% more than 15 mg/d for 15 months. The proportion of patients who had a BMD was 38.5%. Use of vitamin D and/or multivitamins was recorded in 18.3%. Only 26.4% were prescribed calcium. Bisphosphonates were prescribed for 18.1% and 9.6% had all 4 interventions. About 12% were prescribed both calcium and vitamin D/multivitamins. Even patients receiving an average daily dose $\geq$ 15mg/15 months were inadequately treated: 30% were prescribed bisphosphonates, 38% calcium and 25% vitamin D/ multivitamins, and 14% had all medications plus a BMD. By t-test all of the preventive measures were significantly associated with higher cumulative doses of GC. Patients treated with an average dose $\geq$ 7.5mg/d were somewhat more likely to have DXA (OR 2.1 [1.5, 2.9]), prescribed bisphosphonates (OR 2.7 [1.8, 3.9]) or calcium (OR 1.8 [1.3, 2.6]. Nonetheless, the care of few patients met published guidelines or the in press VA guidelines.

Other variables to be assessed at baseline include: age, gender, race, weight/body mass index, history of fractures, GC treatment duration, condition for which GC have been prescribed, provider specialty, number of co-morbidities, and concomitant medications.

Disclosures: ***R.A. Adler****, Eli Lilly 2, 8; Novartis 2; Merck 2, 5, 8; Procter & Gamble 2, 5, 8.*

SU376

Economic Burden of Osteoporosis Among Women. M. Rousculp*[1], A. Sasser*[2], H. Birnbaum*[2], E. Oster*[2], E. Lufkin[1], D. Mallet*[3]. [1]Eli Lilly and Company, Indianapolis, IN, USA, [2]Analysis Group, Inc., Boston, MA, USA, [3]Ingenix, New Haven, CT, USA.

The purpose of this study is to estimate the financial burden of osteoporosis among women, age 50 to 64 years, in terms of direct health care and indirect work-loss costs to an employer.

Medical claims data from seven large employers (n>600,000) were analyzed between 1998 and 2000. Patients were identified as female employees, age 50 to 64 years, enrolled in either a fee-for-service or managed indemnity health plan. Osteoporosis diagnosis was defined as "strict" (ICD-9-CM 733.X, n=1,493) and "loose" (733.X or CPT for fracture, n=2,313) Both treatment samples were compared to a random sample of women, age 50 to 64 years (n=6,170), in terms of average annual direct and indirect costs during the three-year period. Multivariate regression techniques were used to control for demographic characteristics and co-morbid conditions.

Between 1998 and 2000, the average annual number of medical claims per patient was 21.9 for the random sample, compared to 34.5 for "strict" osteoporosis patients (p<0.001) and 35.9 for "loose" osteoporosis patients (p<0.001). During this period, the "strict" sample had average annual healthcare care costs that were $1,960 greater than those for the random sample ($5,582 versus $3,622, p<0.001). For the "loose" sample, the difference was $2,636 (p<0.001). In both cases, outpatient services accounted for approximately one-third of the total difference. Average annual work-loss costs, including absenteeism and

disability, were $885 and $1,395 greater than the random sample for the "strict" and the "loose" samples respectively. Using multivariate regression to control for demographics and co-morbid conditions reduced the differences in both direct healthcare and indirect work-loss costs between both patient samples and the random sample. Controlling for these factors, incremental average annual costs were 89% higher for direct healthcare costs and 43% higher for indirect work-loss costs for the "strict sample." For the "loose" sample, direct healthcare costs and indirect work-loss costs were 105% and 64% higher respectively. We conclude that women treated for osteoporosis impose a significant financial burden on employers. This burden is due to both greater healthcare utilization as well as higher work-loss prevalence compared to a random sample of similar employees. Controlling for demographic characteristics and co-morbid conditions reduces the differences in both direct healthcare costs and indirect work-loss costs. However, even controlling for these factors, the differences between the patient samples and the random sample continue to be large and significant.

Disclosures: ***M. Rousculp****, Eli Lilly 3.*

SU377

Patterns of Osteoporosis Therapy Use: Disease Severity, Patient Knowledge and Socioeconomic Factors. R. L. Ohsfeldt*[1], J. N. Liberman*[2], M. Rousculp*[3], M. Draper[3]. [1]University of Iowa, Iowa City, IA, USA, [2]IMR, Inc., An AdvancePCS Company, Hunt Valley, MD, USA, [3]Eli Lilly and Company, Indianapolis, IN, USA.

The association between osteoporosis drug therapy selection and indicators of disease severity, patient knowledge and attitudes about osteoporosis, and patient socioeconomic characteristics, among postmenopausal women is examined. A random sample of 898 women aged 55-84 who were insured by a U.S. national health plan and filled an initial prescription for alendronate or raloxifene between 3/1/1999 and 2/28/2001 was obtained. Alendronate and raloxifene were selected because they were used for prevention and/or treatment of osteoporosis.. Women in the sample were asked to participate in a telephone survey conducted from July to September 2001. Of these, 9.2% declined to participate and 25.3% were ineligible to participate. Another 6.6% only partially completed the survey, resulting in a final sample of 300 raloxifene and 288 alendronate users. The survey instrument collected information about self-reported indication for therapy (prevention or treatment), the patient's knowledge and attitudes about osteoporosis, prior fractures, past BMD tests, past health care utilization, age, race, educational attainment, employment status, and family income. Step-wise logistic regression models were used to identify variables most associated with the initial osteoporosis drug selected. The results indicated that women using therapy for prevention of osteoporosis were more likely to use raloxifene than alendronate (OR = 2.5, CI: 1.7, 3.6). Women tended to be less likely to use raloxifene if they had a family history of osteoporosis (OR =0.8, CI: 0.6, 1.2) or prior BMD tests (OR = 0.3, CI: 0.15, 0.5). Women in the highest family income and highest educational attainment categories were less likely to use raloxifene than women in the lowest income and education categories. Osteoporosis knowledge, attitudes, prior all-cause physician visits, age, race, smoking status, and employment status were not statistically associated with patterns of use. Data do not allow us to assess the clinical appropriateness of therapy use. In U.S. women prescribed antiresorptive agents, the selection of initial osteoporosis therapy appears to be explained mainly by clinical factors. Women with self-reported, less severe osteoporosis are more likely to use raloxifene than alendronate. Education attained and income also may have some impact on the initial drug used. Patient knowledge and attitudes do not appear to affect initial drug selection significantly.

Disclosures: ***M. Rousculp****, Eli Lilly and Company 3.*

SU378

Does Use of Electronic Monitoring Increase Pill Taking Compliance? D. Travers Gustafson, J. M. Lappe, G. Haynatzki, R. P. Heaney, R. R. Recker. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

One of the major challenges of a medication intervention is to assure compliance with pill taking. It has been suggested that use of electronic monitoring devices increases compliance with medication regimens. Thus, we included use of the electronic Drug Exposure Monitor (eDEM™) bottle cap in our population-based study of the anti-fracture efficacy of calcium and calcium plus vitamin D in postmenopausal women. The purpose of this analysis is to determine whether study supplement compliance is enhanced for participants who use the eDEM.

We randomly selected and enrolled 1180 healthy women from a nine county area of rural Nebraska into the prospective study. All participants were given either calcium supplementation or placebo. We randomly assigned participants to eDEM or usual bottle cap for their calcium/placebo pills.

The eDEM monitoring system consists of a cap containing a microelectronic circuit that records opening and closing of the system. Medication event information is transferred from the electronic memory cap to a computer. The compliance report includes: dosing chronology, total number of doses taken, missing doses, drug holidays (three or more days between doses), and percentages of both number of prescribed doses taken and prescribed doses taken on schedule. We explained these eDEM functions to those participants assigned to its use. At each semiannual study visit the eDEM caps are "read". We also assessed compliance by pill bottle weights in each group. To do this we use the ACAI Precision Counting Function of the EK-H series Precision Scale manufactured by A & D Company. After two years of follow up, we compared the pill counts in women assigned to eDEM with those assigned to a usual bottle cap. We compared compliance rates for two different groups: 1. Per Protocol = compliance per protocol while on study and 2. Active = compliance while on study, may not take the supplement per protocol.

For either group, we found no significant difference in compliance by bottle weight between those participants who were assigned the eDEM cap and those who were not.

We conclude that use of an electronic monitoring device does not improve compliance in older healthy women taking nutritional supplements.

Group	eDEMS	# in group	average compliance
Per protocol	yes	569	78%
Per protocol	no	570	79%
Active	yes	520	76%
Active	no	515	78%

Disclosures: ***D. Travers Gustafson****, None.*

SU379

Osteoporosis Education at the Time of Bone Densitometry and Quarterly Phone Follow-up for One Year Improves Calcium Intake and Frequency of Exercise, but not Anti-Resorptive Drug Use. J. T. Schousboe[1], C. R. DeBold*[1], L. S. Kuno*[2], K. Delaney-Mroz*[1], T. W. Weiss[3], Y. Chen[3], S. Brenneman[3], T. A. Abbott[3]. [1]Park Nicollet Clinic, Minneapolis, MN, USA, [2]Park Nicollet Institute, Minneapolis, MN, USA, [3]Merck and Co, Inc., Rahway, NJ, USA.

Adherence to anti-resorptive drug therapy (rx) has been shown to be poor in a few studies. We performed a prospective trial to determine if Nurse Care (nurse education at the time of a bone density (BMD) test plus a follow-up appointment with the primary care provider (PCP), and quarterly phone follow-up with the nurse educator) might improve drug initiation & persistence in women at high risk of osteoporosis. We randomized 310 women (age ≥ 55, not on estrogen or any anti-resorptive drug, SCORE Questionnaire score ≥ 8) to Nurse Care (n = 158) or to Usual Care (BMD test plus a follow-up appointment with PCP; n = 152). Self-reported outcomes, including initiation & persistence with anti-resorptive drug rx, increased calcium intake & increased exercise frequency were compared between the two groups one year later in 287 completers. Odds ratios (OR) and 95% confidence intervals (CI) were computed for each outcome using logistic regression controlling for age, BMD, personal & family history of fracture, glucocorticoid use, actual follow-up with the PCP, & height loss.

Baseline characteristics were comparable between the two groups. Compared to Usual Care, Nurse Care improved self-reported calcium intake (OR = 2.11; 95% CI = 1.27-3.51) & exercise frequency for one year (OR = 1.82; 95% CI = 1.05-2.13), but had no effect on self-reported initiation (OR = 1.06; 95% CI = 0.53-2.13) of and persistence with (OR = 0.89; 95% CI = 0.43-1.83) anti-resorptive drug rx. The presence of osteopenia (T score –1.0 to –2.49) & especially osteoporosis (T-score ≤ -2.5) were highly associated with drug initiation & persistence in both groups. In the subset of women with osteoporosis (n = 66), only 41 (62%) started drug therapy & only 26 (39%) persisted with drug rx for one year. Nurse Care also appeared to have no effect on initiation or persistence with drug rx in the subset with osteoporosis, but our power to detect a benefit from Nurse Care in this subset was quite limited. Further studies are needed to investigate determinants of non-initiation of and non-persistence with drug rx & to develop strategies to improve persistence with drug rx among those with osteoporosis.

Disclosures: ***J.T. Schousboe****, Merck and Co. 2.*

SU380

Soybean Isoflavones and Raloxifen in Women with Osteoporosis. A. Bazarra*. Health Sciences, University of La Coruña, La Coruña., Spain.

Statement of purpose: determining if there are diferences in using soybean isoflavones and raloxifen on bone mass loss in postmenopausal women.

Statement of method: we studied for 12 months 38 women who were 45 to 68 years old at base line, were within 1 and 18 years of menopause, and had a bone mineral density at the lumbar spine between 160 mg/cc and 77 mg/cc measured by the QBMAP system with a spiral CT Picker PQ-S densitometer at L2, L3, L4 and L5. Of all the women, 20 were assigned to therapy with soybean isoflavones 80 mg and 18 were treated with raloxifene HCL 60 mg. The SPSS programme was used for statiscal analysis.

Summary of results: The characteristics of the women recruited for both groups were similar. Mean mineral bone density at the lumbar spine was between 1 and 4 DS below the mean value for 30 years old normal premenopausal women. After a treatment statistically significant difference was found among the groups as for the bone mineral density at the lumbar spine.

Conclusions: it is necessary to carry out a wider study but it seems that raloxifene HCL contribute advantages versus isoflavones therapy to decrease the bone mass loss in postmenopausal women at least at lumbar spine.

Disclosures: ***A. Bazarra****, None.*

SU381

Raloxifene Is Not Associated with Biologically Relevant Changes in Hot Flushes in Postmenopausal Women Receiving Treatment for Prevention or Treatment of Osteoporosis. S. Palacios*[1], M. L. Farias[2], H. Luebbert*[3], G. Gomez*[4], J. A. Yabur*[5], D. C. Quail*[6], C. Turbi*[6], M. J. Kayath[6], M. J. Almeida*[6], E. Moennig*[6], T. N. Nickelsen[6]. [1]Instituto Palacios, Madrid, Spain, [2]HUCFF, Rio de Janeiro, Brazil, [3]Medical Department, UKBF, Freie Universitaet, Berlin, Germany, [4]Hospital Universitario del Valle, Cali, Colombia, [5]Hospital de Clinicas, Caracas, Venezuela, [6]Medical Department, Eli Lilly & Co, Indianapolis, IN, USA.

The selective estrogen receptor modulator (SERM) raloxifene is an established drug for the treatment and prevention of postmenopausal osteoporosis. Previous studies have described a raloxifene-associated increase in hot flushes in postmenopausal women, reported as adverse events. In the present randomized, double-blind, placebo-controlled study, we evaluated in detail the hot flushes observed during treatment for 8 months with raloxifene, administered either in a dose of 60 mg every other day for the first 2 months followed by 60 mg/d (slow dose escalation, SDE) or at 60 mg/d throughout (RLX), or placebo (PL) in 487 unselected postmenopausal women for whom raloxifene was considered appropriate therapy. Data on the number, duration, intensity, severity and impact of hot flushes were collected using standardized, validated methods. During treatment, the mean number of hot flushes was low. It increased by <1 hot flush/week in the RLX and SDE groups, and decreased by <1 hot flush/week with PL. There was a high proportion of asymptomatic patients at baseline (~60%), which had increased further at the end of treatment in all groups. The number of women whose preexisting hot flushes abated during the study was significantly greater with SDE (p=0.005) and PL (p=0.050), but not with RLX, than the number of women with treatment-emergent hot flushes. Statistical analysis of the categorical distribution of the number of hot flushes revealed no significant overall differences between the groups and no pairwise differences at baseline or after 2 months of treatment. At endpoint, the only significant difference was between RLX and PL (p=0.035), indicating that RLX, but not SDE, was associated with more hot flushes than PL. There were no statistically significant differences among the 3 groups in the proportion of women requesting symptomatic treatment or stopping the study early due to vasomotor symptoms. In conclusion, the overall effect of raloxifene on hot flushes is low. Previous studies using adverse event reports may have overestimated the importance of hot flushes in postmenopausal women during treatment with raloxifene. SDE may be a useful strategy for some patients starting raloxifene therapy.

Disclosures: ***T.N. Nickelsen****, Eli Lilly and Company 1, 3.*

SU382

Association of the Calcitonin Gene (CA) Polymorphism with Bone Mass and Bone Responsiveness to Hormone Replacement Therapy in Postmenopausal Korean Women. J. Kim, Y. Choi*, S. Kim*, S. Ku*, S. Moon*. Dept. of Obsterics & Gynecology, Seoul National University Hospital, Seoul, Republic of Korea.

Osteoporosis is considered to be a polygenic disorder, in which a number of different genes play a significant role. Many genes, believed to be involved in bone mineral density (BMD), have been investigated, and these studies have produced conflicting data in various population. Calcitonin is a polypeptide hormone which interacts with a specific G-protein-coupled receptor on the osteoclast surface and thereby inhibits bone resortption.. Recently, the presence of a polymorphic microsatellite composed of variable (CA) repeats at the human calcitonin locus has been reported. The aims of the present study were to test an association between the (CA) polymorphism in the calcitonin gene and BMD in postmenopausal Korean women and to investigate whether this polymorphism affects bone responsiveness to hormone replacement therapy (HRT). Calcitonin (CA) polymorphism, serum calcitonin, and BMD at the lumbar spine and proximal femur were determined in 430 postmenopausal Korean women. One hundred and eighty-one women were treated with sequential HRT for 2 years. Four major calcitonin alleles were present with a frequency greater than 5 %: 122 bp 61.3 %, 108 bp 25.1 %, 110 bp 7.0 %, and 124 bp 6.2 %. There were no differences in the BMD at the lumbar spine and proximal femur in postmenopausal women with zero, one, or two copies of major alleles. Serum calcitonin levels in women with two copies of the 108bp allele were significantly higher than those in women with zero or one copy of the 108 bp allele. The annual rate of positive change of BMD at the femoral neck after HRT was significantly higher in women homozygous for the 108 bp allele than in women with zero or one copy of the 108 bp allele, but the number of copies of the major calcitonin alleles was not significantly associated with HRT-respon The calcitonin gene (CA) polymorphism is not associated with bone mass, but one of genetic factors which may affect change in bone mineral density at the femoral neck after hormone replacement therapy in Korean women

Disclosures: ***J. Kim****, None.*

SU383

Changes in Healthcare Provider Attitudes and Behavior Concerning Estrogen Therapy for the Management of Osteoporosis. E. M. Lewiecki, L. A. Rudolph, J. R. Chavez*. New Mexco Clinical Research & Osteoporosis Center, Albuquerque, NM, USA.

Background: Data from the prematurely terminated arm of the Women's Health Initiative (WHI) on estrogen plus medroxyprogesterone in the treatment of postmenopausal women showed that the risks of therapy were greater than the benefits in the study population. Publicity generated from the study may have major consequences in the future role of hormone therapy (HT) and estrogen therapy (ET) in postmenopausal women. Objectives: This study was undertaken to evaluate the impact of the WHI study on attitudes and prescribing behavior of healthcare professionals for HT/ET in the management of postmenopausal osteoporosis. Methods: A three-page survey was mailed to 1144 New Mexico healthcare professionals in selected specialties about five months after publication of the WHI study. Surveys returned within six weeks were analyzed. Results: The survey response rate was 16% (186/1144). The greatest response was from OB-GYN (41%, 47/116), and 84% (157/186) of responders were OB-GYN, family practice, or internal medicine. Forty-nine percent (91/186) felt that the WHI findings applied only to Prempro or equivalent, while 33% (61/186) believed that the findings applied to treatment with ET alone. As a result of WHI, prescription writing for HT has changed for 86% (160/186) of providers, and for ET by 67% (125/186) of providers. Family practitioners were twice as likely to initiate changes in HRT/ERT as OB-GYNs. Thirty-seven to 45% of responders felt that HT/ET still had role in the prevention and treatment of postmenopausal osteoporosis. Conclusion: Major changes in attitude and behavior of healthcare providers toward HT/ET have occurred since the publication of the WHI study. The magnitude of change varies according to the specialty. Less than half felt that HT/ET should be used in the management of postmenopausal osteoporosis.

Disclosures: ***E.M. Lewiecki****, None.*

SU384

Changes in Patient Perceptions of Estrogen Therapy for the Management of Osteoporosis. E. M. Lewiecki, L. A. Rudolph, J. R. Chavez*. New Mexico Clinical Research & Osteoporosis Center, Albuquerque, NM, USA.

Background: Data from the prematurely terminated arm of the Women's Health Initiative (WHI) on estrogen plus medroxyprogesterone in the treatment of postmenopausal women showed that the risks of therapy were greater than the benefits in the study population. Publicity generated from the study may have major consequences in the future role of hormone therapy (HT) and estrogen therapy (ET) in postmenopausal women. Objectives: This study was undertaken to evaluate the impact of the WHI study on perceptions of postmenopausal women on HT/ET in the management of postmenopausal osteoporosis. Methods: A two-page survey was sent to offices of volunteer New Mexico healthcare professionals in selected specialties about five months after publication of the WHI study. Surveys returned within six weeks were screened, with analysis of data confined to those who were aware of the WHI study. Results: Surveys were returned by 121 patients, of whom 77 (64%) were "aware" of the WHI study. Of these, 5 (6.5%) heard of WHI exclusively from the healthcare provider, while the rest heard from other sources. Eventually, 43 (52%) discussed WHI findings with provider, 9 (21%) of whom chose to stop HT/ET, while 34 (79%) remained on HT/ET. Forty-six (60%) of aware patients felt the HT/ET increased the risk of breast cancer, while 12 (16%) felt that it decreased the risk of vertebral fractures. Conclusion: Patients surveyed were predominately aware of the WHI study through sources other than their healthcare providers. When women taking HT/ET discussed the WHI study with their healthcare providers, they were most often advised to change or stop therapy, but only 21% actually discontinued therapy. Most felt that HT increased the risk of breast cancer, but did not feel that it reduced vertebral fracture risk. The WHI study has had major impact on perceptions of HT/ET in postmenopausal women.

Disclosures: ***E.M. Lewiecki****, None.*

SU385

Bazedoxifene+Conjugated Estrogens: A Balanced Combination to Provide Optimal "Estrogenic" Safety and Efficacy. B. S. Komm, Y. Kharode, P. Bodine, F. Bex. Osteoporosis Research, Wyeth Research, Collegeville, PA, USA.

Tissue selective activity is key to the functional application of SERMs for the prevention and treatment of osteoporosis. Optimally, a SERM would exhibit the positive attributes associated with hormone therapy, which include skeletal protection, reduction in hot flushes, and a reduction in vulvar/vaginal atrophy. However, the optimal SERM would not stimulate the uterus as seen with classic estrogen replacement therapies. While the currently available SERM, raloxifene (RAL) partially achieves these goals, by no means has an optimal SERM profile been obtained. We hypothesized that it may not be feasible for one compound to meet these stringent requirements, yet perhaps, in combination with estrogens with less pronounced selective characteristics, would provide a balanced "estrogenic" response. Bazedoxifene acetate (BZA), a newer generation SERM currently in Phase III clinical trials, was combined with Premarin (conjugated estrogens=CE). After establishing the efficacious dose of CE (1-2.5mg/kg) to maintain bone mass in an ovariectomized (ovx) rat model of osteopenia, CE was combined with BZA to determine the efficacy of BZA to antagonize the CE stimulation of the uterus. In both a 3-day immature and 6-week ovx rat model, it was determined that approximately 7-10 fold the bone efficacious dose of BZA (0.3mg/kg) was required. Ovx rats co-dosed with 2.5mg/kg CE and 3.0 mg/kg BZA demonstrated BMDs equivalent to sham operated animals via DEXA of the L4 vertebra and pQCT of the proximal tibia (PT). Histologic analysis of the PT revealed the cancellous bone compartment to be indistinguishable from the sham-operated controls. The uteri from these animals were not different from the ovx, untreated controls. This dose of BZA did not antagonize the reduction in vasomotor control elicited by CE in a rat "hot flush" model and potently inhibited (IC50<2.0nM) CE-stimulated proliferation of a human breast cancer cell line. These data strongly support our hypothesis that BZA+CE provides a balanced, acceptable "estrogenic" profile, and early clinical data correlate well with these preclinical findings.

Disclosures: ***B.S. Komm****, Wyeth Pharmaceuticals 1, 3.*

SU386

Combining a SERM with Conjgated Estrogens(CE) to Improve the SERM Profile: Not all SERMs may Succeed. B. S. Komm, Y. Kharode, P. Bodine, F. Bex. Osteoporosis, Wyeth Research, Collegeville, PA, USA.

The need to intervene with effective therapeutics to maintain bone mass in postmenopausal women has been become clear both from a health and economic perspective. As alternatives to hormone therapy (HT), the bisphosphonates(Bps) and SERMs have risen to the top as acceptable bone sparing pharmaceuticals. While the Bps exclusively target the skeleton they are generally prescribed to women from age 65 and beyond. The SERMs target a younger population, which is increasing based on changes in the philosophy of using HT. The currently available SERM, raloxifene (RAL), while modestly effective at preventing and treating osteoporosis, does not achieve the same BMD effect as HT, nor alleviate other menopausal symptoms such as hot flush and vaginal dryness. Two new SERMs, bazedoxifene (BZA) and lasofoxifene (LAS), in Phase III evaluation for the treatment of osteoporosis, in animal models protect the skeleton like raloxifene, although their potencies are different. BZA combined with CE results in a SERM/estrogen balance providing an excellent bone profile plus positive vasomotor and vaginal responses without uterine or mammary gland stimulation. Using a 6-week ovariectomized rat model of osteopenia we compared the combination BZA, RAL, and LAS with CE. Two doses of each SERM were combined with 2.5 mg/kg of CE (bone efficacious dose). The SERM doses chosen were the bone effective and up to a 10-fold higher dose (BZA=1.0mg/kg, LAS=0.1mg/kg, and RAL=1.0mg/kg). After 6 weeks of oral, the animals' proximal tibia (PT) was evaluated by pQCT and lumbar spine by DEXA. In addition, total cholesterol and uterine wet weight was analyzed. PT trabecular density was equal between all groups and not different than CE alone. The same can be said for total density. The same generalized conclusion can be drawn for their effect on the lumbar spine at L4. Total cholesterol was positively affected by all combinations without statistical differences between themselves, but all different from ovex controls and CE alone, suggesting a possible synergistic effect. However, when uterine wet weights were analyzed only BZA was able to antagonize the CE stimulation sufficiently to abolish a statistical difference with untreated animals. This correlates well with data that have shown that LAS and RAL significantly stimulate the uterine endometrium in animal models, while BZA does not at its bone sparing dose of 0.3-1 mg/kg. It does appear that combining these two classes of "estrogens" results in a blend of activities; however the critical inhibition of CE-stimulated endometrial changes is most likely SERM specific, and it should not be assumed that all SERMs will provide the same safety margin when co-dosed with CE or other estrogen receptor agonists.

Disclosures: ***B.S. Komm****, Wyeth Research 1, 3.*

SU387

Comparison of the Effects of Bazedoxifene, Raloxifene, Lasofoxifene and Risedronate Co-Treatment on hPTH-Induced Reversal of Established Osteopenia in Ovariectomized Rats. Y. P. Kharode, P. D. Green*, J. T. Marzolf*, R. J. Murrills, P. V. N. Bodine, B. S. Komm, F. J. Bex. Women's Health Research Institute, Wyeth Research, Collegeville, PA, USA.

The osteogenic activity of intermittent administration of hPTH has been well characterized in both intact and ovariectomized rats. In this study we have evaluated the effects of hPTH alone and co-administered with the SERMs, bazedoxifene (BAZ), raloxifene (RAL), lasofoxifene (LAS) or with the bisphosphonate, risedronate (RIS), on restoration of bone loss in ovariectomized (ovx) rats. Rats were ovx at 12 weeks of age and 4 weeks post-ovx were found to have developed significant osteopenia when compared to age matched sham ovx rats as judged by proximal tibial (PT) bone mineral density (BMD) using pQCT (total BMD 521 ±4 mg/gm^3 for ovx vs. 654 ± 5mg/cm^3 for sham, $p<0.01$ and trabecular BMD 331 ± 6 mg/cm^3 for ovx vs. 552 ± 18 mg/cm^3 for sham, $p<0.01$). The osteopenic ovx rats were randomly divided into six groups and received daily treatment for four weeks as follows: (1) Vehicle; (2) hPTH (1-34) 10µg/kg, sc (PTH); (3) PTH + BZA, 0.3 mg/kg, po; (4) PTH + RAL, 3.0 mg/kg, po; (5) PTH + LAS, 0.1 mg/kg, po; and (6) PTH + RIS, 1.0 mg/kg, po. After four weeks of treatment (eight weeks after ovx) the total and trabecular BMD (570±7 mg/cm^3 and 269± 13 mg/cm^3, respectively) were significantly lower in the vehicle group compared to the sham group. Total and trabecular BMD in ovx + PTH group (673 ± 15 mg/gm^3 and 461 ±16 mg/gm^3, respectively) were significantly ($p<0.01$) higher than ovx + vehicle group. Total and trabecular BMD in all co-treatment groups were not only significantly ($p<0.01$) greater than the corresponding vehicle group but they were 3 to 10 % higher than the PTH alone group. Compared to the vehicle group, the uterine wet weights were significantly higher in the PTH + LAS group (94±4 mg for vehicle vs. 145 ± 7 mg, $p<0.01$). The uterine weights did not change significantly in the PTH and the other co-treatment groups. Total cholesterol was significantly decreased in PTH + SERM groups but not in PTH alone and PTH +RIS group. We conclude that (1) Co-treatment of PTH with BZA, RAL, LAS or RIS maintained or enhanced the osteogenic effects of PTH in ovx rats with established osteopenia; (2) BZA, RAL and LAS but not RIS co-treatment significantly reduced serum cholesterol; and (3) BAZ, RAL and RIS co-treatment did not cause uterine stimulation but co-treatment with LAS significantly increased uterine weights of ovx rats. Based on non-skeletal end points, these results suggest that careful design of combination therapy may be beneficial in the management of osteoporosis.

Disclosures: ***Y.P. Kharode****, Wyeth Research 3.*

SU388

CHF 4227, a New Selective Estrogen Receptor Modulator (SERM), Prevents Estrogen-Dependent Bone Loss in Mature Rats with a Unique Efficacy Profile. R. Armamento-Villareal[1], N. Napoli[1], S. Sheikh[1], A. Nawaz*[1], C. Muller*[1], M. Brodt*[1], M. Silva*[1], E. Galbiati*[2], P. Caruso*[2], M. Civelli*[2], R. Civitelli[1]. [1]Bone and Mineral Diseases, Washington University, St. Louis, MO, USA, [2]Chiesi Farmaceutici, Parma, Italy.

We tested the therapeutic and safety profile of a new SERM, CHF 4227, in mature female rats in comparison to 17α-ethinlyestradiol (E), raloxifene (RLX) and lasofoxifene (LFX). Six-months-old female Sprague Dawley rats were ovariectomized (ovx) and randomized to one of the following treatment groups (n=10 each): Vehicle (Veh), CHF at doses of 1.0, 0.1, 0.01 and 0.001 mg/kg body weight (bw), E 0.1 mg/kg bw, LFX 0.1 mg/kg bw, and RLX 1.0 mg/kg bw. Drugs were given daily by gavage 5 days/week for 4 months. By DEXA, a significant bone mineral density (BMD) loss occurred in the lumbar spine (-8.5±1.5%), proximal femur (-7.4±1.4%), and total femur (-4.6±1.5%) in the Veh animals at 4 months. Bone loss in the spine was prevented by CHF 0.1 and 1.0 (-1.7±1.2 % and -1.2±1.5%, respectively), LSX (-1.9±2.1%) and RLX (-1.0±1.2%), with lesser effects by lower CHF doses. At the proximal femur, positive changes in BMD were observed after treatment with CHF 1.0 (+3.4±1.5%), and to a lesser degree in rats treated with E (+1.3±2.4%), LFX (+1.3±1.6%), and RLX (+1.6±1.8%). Volumetric BMD obtained by pQCT ex-vivo confirmed prevention of bone loss in the lumbar spine by CHF, LFX and RLX. In the proximal femur, CHF 1.0 and 0.1 prevented trabecular bone loss (-1.3±5.0%, -4.9±5.7%, respectively) relative to Veh (-13.4±10.9%), while LFX (-8.9±5.7%) and RLX (-3.9±5.4%) were less powerful. Likewise, bone histomorphometry showed maintenance of bone volume, trabecular thickness, number and separation by CHF 1.0 and 0.1, LFX and RLX against a >50% loss of bone volume and trabecular number observed in the Veh group. Intriguingly, CHF did not show any trends toward suppression of bone formation rate, which increased after ovx, whereas LFX, E, and RLX (to a lesser extent) were inhibitory. By contrast, urine Dpd-Pyd excretion was suppressed, dose-dependently, by CHF and E, whereas the other SERMs were less powerful inhibitors. Finally, there were no significant differences in mean uterine weight in any of CHF treated rats compared to Veh, RLX and LFX treated rats. However, CHF 1.0, 0.1 and E decreased body fat, while RLX and LSX only prevented ovx-induced increase in body adiposity. In conclusion, CHF 4227 at a doses of 0.1-1.0 mg/kg bw prevents ovx-induced bone loss in the spine and proximal femur in the rat and decreases bone resorption without suppressing bone formation. This new SERM exhibits a unique and perhaps more beneficial efficacy profile relative to RLX and LFX.

Disclosures: ***R. Civitelli****, None.*

SU389

Raloxifene May Reduce All-Cause Mortality in Heavier Older Women. The MORE Trial. K. Ensrud[1], T. Blackwell[2], E. Barrett-Connor[3], S. Cummings[2]. [1]VA Medical Center and U of MN, Minneapolis, MN, USA, [2]University of California, San Francisco, CA, USA, [3]University of California, San Deigo, CA, USA.

Raloxifene (RLX) modulates the action of estradiol in many tissues by binding to the estrogen receptor. Endogenous estradiol levels are correlated with body weight and other measures of adiposity. Thus, a woman's level of estradiol or degree of adiposity may alter the effects of RLX on disease outcomes. For example, RLX reduces breast cancer risk in older women to a greater degree in heavier women or those higher estradiol levels. To examine whether the effect of RLX on all cause mortality in older women is dependent on body weight, we utilized data from the MORE trial that randomly assigned 7705 women with osteoporosis to RLX (60 or 120 mg) or placebo (PBO) for 4 yrs and compared the effect of RLX vs. PBO on all cause mortality within a priori specified subgroups defined at baseline by quartiles of body weight or BMI. All comparisons were by intention-to-treat analyses. 5692 (74%) completed the 4th annual exam during which time 100 deaths were confirmed. Overall, there was no effect of RLX on all cause mortality (RH 0.88;CI 0.58, 1.32). However, among the heaviest women (highest quartile of BMI [≥27.5 kg/m^2]), those women assigned to RLX had a 63% lower mortality rate compared with those assigned to PBO (0.6% vs. 1.7%; RH 0.37; CI 0.15 to 0.92). Among the thinnest women (lowest quartile of BMI [<22.5 kg/m^2]), there was no effect of RLX on mortality (RH 1.31; CI 0.61 to 2.82). There was some evidence of a treatment *BMI interaction (P=0.054). Substitution of body weight for BMI or 3 yr mortality for 4 yr mortality did not alter our results. Reduction in mortality could not be attributed to any one cause of death. In conclusion, RLX may reduce overall mortality in older heavier women, but these preliminary findings require confirmation in other ongoing RLX trials.

Disclosures: ***K. Ensrud****, Merck 2; Eli Lilly 2; Pfizer 2; Berlex 2.*

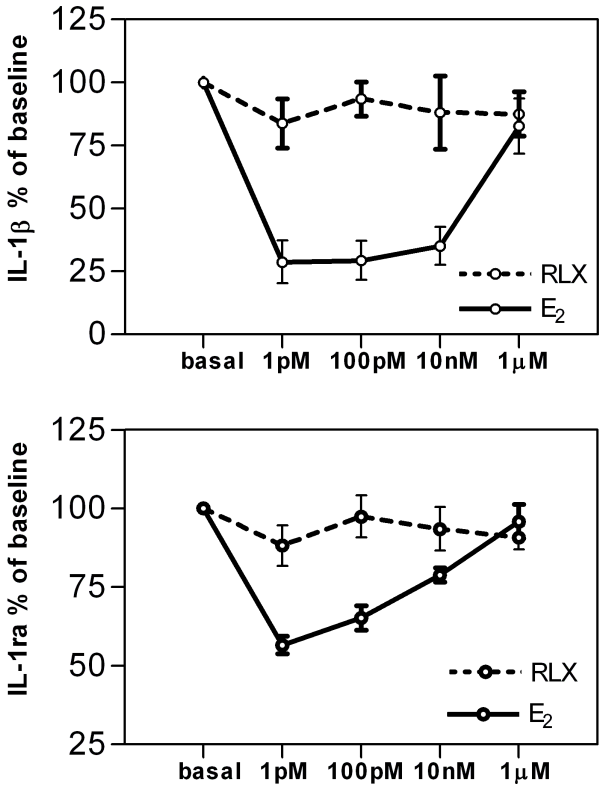
SU390

Comparison of the Effects of Raloxifene and Estradiol, *In Vitro* and *In Vivo,* on Cytokine Production in Peripheral Whole Blood Cultures. A. Rogers, J. A. Clowes, C. A. Pereda*, R. Eastell. Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Raloxifene (RLX), a selective estrogen receptor modulator, has a smaller effect on lumbar spine BMD compared to treatment with estradiol (E_2). The reasons for this may relate to its ability to modulate the production of locally produced pro-inflammatory cytokines, in the red marrow of the spine. The aim of this study was to determine the effect of RLX and E_2 both *in vitro* and *in vivo* on the production of interleukin-1β (IL-1β) and interleukin-1 receptor antagonist (IL-1ra).

We obtained samples of peripheral blood from a) 10 healthy postmenopausal women (ages

53 to 72 years, mean 61 years), b) 15 postmenopausal women (ages 52 to 72 years, mean 63 years) at baseline and after 6 months of RLX therapy (60 mg/day) and c) 10 postmenopausal women (ages 60 to 75 years, mean 64 years) at baseline and 6 months after a single E_2 implant (25 mg). Cultures of whole blood from the untreated women were incubated with RLX or 17β-E_2 at 1pM, 100pM, 10nM and 1μM concentrations for 24 hours. Whole blood cultures from the treated women were stimulated with 500ng/ml lipopolysaccharide for 24 hours. IL-1β and IL-1ra were measured by ELISA in the conditioned media. All samples from each individual were assayed in the same analytical run.
There was no significant change in IL-1β or IL-1ra in response to RLX (both P>0.05, graph). There was a significant dose-dependent decrease in IL-1β and IL-1ra compared to control in response to 17β-E2 (both P<0.0001, ANOVA, graph).



There was no significant change in either IL-1β or IL-1ra after 6 months RLX therapy (+20±14% and +12±10%, both P>0.05). There was a significant decrease in IL-1β (-36±8%, P=0.01) and an increase in IL-1ra (+29±20%, P=0.3) in the women receiving E_2 implant respectively (paired t-test).We conclude that RLX does not modulate the cytokines IL-β and IL-1ra using whole blood culture methods and that this may partly account for the differences in the effect of RLX compared to E_2 on lumbar spine bone density.

Disclosures: ***A. Rogers****, None.*

SU391

Long-term Effects of Hormone Replacement Therapy on Bone Loss in Post-Menopausal Women - a 23-Year Prospective Study. H. G. Ahlborg*, O. Johnell, M. K. Karlsson. Dept of Orthopaedics, Inst of Orthopaedics, Malmo, Sweden.

The purpose of the study was to determine the long-term effect of hormone replacement therapy (HRT) on bone loss after menopause.
Twenty-eight women taking HRT and 196 women not taking HRT during the follow-up was observed in this prospective observational study over 23 years. We evaluated the rate of forearm bone loss measured by single-photon absorptiometry (SPA) every second year between the age of 48 and 72 years.
Women taking HRT, for a median of 17 years (range 4 - 26), had 8.7 percentage points (95% CI 3.8 - 13.5) lower rate of BMD loss compared to the women not taking HRT during the same period. Each year of HRT reduced the rate of BMD loss by 0.8 percentage points (95% CI 0.2 - 1.4).
The use of HRT seems to reduce the rate of bone loss in the postmenopausal period over a period of 23 years, and the longer the duration of the therapy, the less bone loss.

Disclosures: ***M.K. Karlsson****, None.*

SU392

FGF9 Treatment of Ovariectomized Rats. R. S. Cooper*, J. A. Spinelli*, C. A. Blanton*, A. Houghton, Y. O. Taiwo, M. W. Lundy. Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Our previous experiments investigating the role of FGFR3 in osteoblasts have demonstrated that FGF9 affects osteoblast proliferation, differentiation and bone formation in various in vitro and in vivo local administration models. In order to determine whether FGF9 could induce bone formation when administered systemically, we repeated a previously reported study which demonstrated the ability of FGF2 to increase bone matrix formation in rats (Liang,H., Endocrinology, 140:5780, 1999). Briefly, Sprague-Dawley rats were ovariectomized (OVX, or sham surgery) at 3 months of age, allowed to lose bone for 60 days, then treated for 14 days via jugular catheters with vehicle or 0.1 or 0.3 mg/kg FGF9. Vehicle and FGF9 treated groups were euthanized on day 14 (v/14 or 0.1/14 or 0.3/14, respectively). Two additional groups that had been treated with 0.1 or 0.3 mg/kg FGF9 continued without additional treatment for 7 more days (0.1/14/7 or 0.3/14/7). Ovariectomy significantly decreased vertebral bone volume and distal femur BMD compared to the final sham-vehicle group. FGF9 at either dose did not significantly change vertebral bone volume or femoral BMD compared to the final OVX group (14 or 14/7). However, mid-femur BMD was significantly increased by both 0.1/14/7 and 0.3/14/7 mg/kg FGF9. Histomorphometry showed increases in osteoid in the proximal tibial metaphysis (0.3/14), which decreased after 7 days without treatment (0.3/14/7). The bone resorbing surface decreased after FGF9 treatment (0.1/14 and 0.3/14). Bone formation rate/bone surface increased initially (0.3/14) then decreased when treatment was discontinued (0.3/14/7). In contrast, in the marrow of the tibial diaphysis there were large amounts of osteoid and mineralized bone at 14 days (0.1/14 and 0.3/14) which continued to increase without concurrent treatment (0.1 and 0.3/14/7). These results indicate that systemic administration of FGF9 increases bone mass in rats, with the largest increases seen in the cortical region of the femur. Furthermore, this new bone formation was dissimilar to that reported to be induced by FGF2 where mineralization of the new bone matrix was only observed after cessation of treatment.

Disclosures: ***M.W. Lundy****, Procter & Gamble Pharmaceuticals 3.*

SU393

Characterization of AC-100, a New Anabolic Agent with Potent Osteoblast Stimulating Properties. D. E. Nagel*[1], S. Khosla[1], D. M. Rosen[2], Y. Kumagai[2], B. L. Riggs[1]. [1]Endocrinology, Mayo Clinic, Rochester, MN, USA, [2]Acologix, Inc., Emeryville, CA, USA.

Anabolic agents are required to enhance bone mass substantially in osteoporotic patients. The most potent known osteoblast-differentiating agent is bone morphogenetic protein-2 (BMP-2). However, BMP-2 cannot be used therapeutically because it converts soft tissue into bone at the site of injection. Here we report initial studies with a new anabolic agent, AC-100, and show that it is equipotent to BMP-2 with respect to osteoblast proliferation and differentiation. AC-100 is a 2.5 kD peptide that forms the central core of MEPE (matrix extracellular phosphoglycoprotein), a 56.6 kD protein that was isolated from tumors associated with hypophosphatemic osteomalacia and that is expressed in normal human bone marrow. We made a side-by-side comparison of the effects of AC-100 (0 – 1000 ng/ml) and BMP-2 (0 – 1000 ng/ml) on normal bone marrow stromal cells (obtained from Clonetics, Inc.) that have the potential to differentiate into multiple lineages, depending upon culture conditions. When cultured in MEM, 10% FBS, 10^{-8}M dexamethasone, 10^{-8}M calcitriol, 100 mM ascorbate and 10 mM β-glycerol phosphate, the Clonetic cells differentiate along the osteoblast lineage pathway. As assessed by ^{3}H-thymidine incorporation, AC-100 increased cell proliferation over control by 1.99 ±0.08 fold (P<0.005) whereas BMP-2 increased it by 1.83 ±0.04 fold (P<0.005). As assessed by real time RT-PCR, AC-100 increased type I procollagen over control by 3.12 ±0.14 fold (P<0.005), alkaline phosphatase (AP) by 21.8 ±0.4 fold (P<0.001), and osteocalcin by 75.3±18.3 fold (P<0.001). Comparable fold increases for BMP-2 were 1.8 ±0.1 (P<0.005), 11.7±0.3 (P<0.005), and 14.6±1.6 (P<0.01). AC-100 also increased mineralized nodule formation by 4.08±0.30 fold (P<0.001) over control whereas BMP-2 increased it by 3.5±0.3 fold (P<0.001). Both agents also increased AP and procollagen protein production. All increases occurred in a dose-dependent and time-dependent manner. In all studies, osteoblast stimulation with AC-100 was statistically similar to that of BMP-2. In the Clonetic cells, AC-100 increased the expression of the early differentiation gene Runx2 (Cbfa1) by 6.91±1.18 fold (P<0.01), as assessed by real time RT-PCR. The possibility that AC-100 action is mediated by BMP-2 was eliminated by showing that AC-100 treatment did not increase BMP-2 gene expression. Because AC-100 has comparable osteoblast stimulating properties to BMP-2 and because unpublished data show that AC-100 can be injected subcutaneously to normal persons without adverse local effects, AC-100 may have potential as a potent anabolic agent for the treatment of osteoporosis.

Disclosures: ***B.L. Riggs****, Acologix, Inc. 2.*

SU394

Inhibition of c-src Promotes Bone Formation *In Vivo*. M. R. Moalli[1], L. C. Pan[2], T. A. Owen[2], M. L. Volberg*[1], D. Brees*[3]. [1]Comparative Physiology and Medicine, Pfizer Global Research & Development, Groton, CT, USA, [2]Cardiovascular and Metabolic Diseases, Pfizer Global Research & Development, Groton, CT, USA, [3]Pathology, Pfizer Global Research & Development, Groton, CT, USA.

It has been widely recognized that c-src is essential for osteoclast function and, more recently, a new role for src in osteoblast activity has been proposed. It has been demonstrated that osteoblast cultures from c-src null mice or from mice treated with anti-sense c-

src had higher levels of alkaline phosphatase and greater levels of mineralized nodule formation, indicating increased osteoblastic differentiation. We have refined a mouse model of ectopic ossicle formation (Schneider et al., ASBMR, 2002) to test the feasibility of src kinase inhibition on bone formation *in vivo*. In this model ossicles formed within the subcutaneous space of scid mice by differentiation of transplanted bone marrow stromal cells cultured from either donor CD-1 mice or Sprague-Dawley rats over a 3 week period. In a first study, gelfoam™ discs were loaded with murine stromal cells and implanted subcutaneously into 4 sites along the backs of recipient scid mice. In a second study, scid mice received gelfoam™ discs loaded with rat stromal cells. In both studies, on days 5-12 post-implantation, one group of mice received a daily injection of the c-Src kinase inhibitor PP2 at each implant site, while the control group received equivalent injections of the inactive analog PP3. The mice were euthanized at post-implantation days 12, 18, and 24. A third group of untreated mice were euthanized at corresponding time-points for comparison and to evaluate the temporal progression of ossicle formation. Formalin-fixed specimens were processed for paraffin embedding and 5 μ sections were evaluated for histologic evidence of bone formation by routine H & E staining. In all specimens, there was gradual infiltration of fibroblastic cells from the periphery of the implant, followed by the appearance of cell-associated osteoid-like material. The PP2-treated groups had multiple areas of new bone formation with areas of mineralized matrix and marrow-like spaces that included cells with morphologic characteristics of mature osteoblasts and osteoclasts. In contrast, the PP3-treated and untreated groups contained only woven, immature tissue. The rat stromal implants were equally well tolerated by the scid recipient mice and differentiated over a similar timecourse. These data provide the first *in vivo* demonstration of accelerated bone formation in response to local administration of PP2. Furthermore, they demonstrate the feasibility of this *in vivo* model to test the effect of novel src kinase inhibitors on bone turnover from both rat and mouse derived bone tissue.

Disclosures: ***M.R. Moalli****, Pfizer Global Research & Development 3.*

SU395

A Comparison of the Bone Anabolic Effects of Basic Fibroblast Growth Factor Administered Subcutaneously and Intravenously in Ovariectomized Rats. U. T. Iwaniec, D. Yuan*, N. Mitova-Caneva*, T. J. Wronski. Physiological Sciences, University of Florida, Gainesville, FL, USA.

Systemic treatment of rats with basic fibroblast growth factor (bFGF) involves intravenous (iv) injection of the peptide in nearly all studies to date. The purpose of the current study was to characterize the skeletal response to subcutaneous (sc) injection of bFGF, and to compare this response to the bone anabolic effects of iv injection of bFGF. Female Sprague Dawley rats were ovariectomized (ovx) or sham-operated (sham) at 3 months of age and left untreated for 2 months to establish cancellous osteopenia in the ovx group. The sham rats (n=10) and one group of ovx rats (n=8) were then injected sc with vehicle alone for 3 weeks. An additional group of ovx rats was injected sc with bFGF (n=10) for 3 weeks at a daily dose of 1 mg/kg. In a previous study with ovx rats of the same age and time post-tOVX, bFGF was administered iv at a daily dose of 200 ug/kg for 2 weeks (Pun et al., Bone 28:220, 2001). Proximal tibiae were collected at the end of both studies and processed undecalcified for quantitative bone histomorphometry. For each study, data were analyzed with the Kruskal-Wallis test followed by a nonparametric posthoc test. The data are expressed as mean ± SD.

Cancellous bone volume was lower and cancellous bone turnover was higher in vehicle-treated ovx rats than in vehicle-treated sham rats in both studies. Subcutaneous treatment of ovx rats with bFGF for 3 weeks resulted in a 5-fold increase in osteoblast surface and an 8-fold increase in osteoid surface in comparison to vehicle treatment of ovx rats. Osteoid volume was also markedly increased in ovx rats treated sc with bFGF (4±3%) in comparison to ovx rats treated sc with vehicle (<0.1%). Osteoclast surface, an index of bone resorption, was lower in ovx rats treated sc with bFGF (1.2±1.4%) than in vehicle-treated ovx rats (2.3±0.8%). Intravenous treatment of ovx rats with bFGF for two weeks resulted in a 15-fold increase in osteoblast surface and a 21-fold increase in osteoid surface, and an osteoid volume of 8±4% in the proximal tibia. Osteoclast surface was lower in ovx rats treated iv with bFGF (0.3±0.3%) than in ovx rats treated with vehicle (3.3±1.7%). Although iv administration of bFGF at 200 ug/kg/day in the previous study had a stronger bone anabolic effect than sc administration of bFGF at 1 mg/kg/day in the current study, the possibility exists that an even higher dose of bFGF administered sc may induce a skeletal response similar in magnitude to that of iv administration of the peptide. Nevertheless, our findings indicate that sc administration of bFGF is very effective for stimulating bone formation in ovx rats.

Disclosures: ***U.T. Iwaniec****, None.*

SU396

The Effects of Growth Hormone and Bisphosphonate on Intact Bones. T. T. Andreassen*, A. Bruel*, H. Oxlund. Department of Connective Tissue Biology, University of Aarhus, Aarhus C, Denmark.

In humans and rats, growth hormone (GH) treatment enhances bone turnover by inducing a substantial increase in bone formation and bone resorption. Bisphosphonate (Bisph) treatment reduces bone resorption substantially even when the resorption is pronounced. The effects of GH and Bisph on rat bone formation, resorption, mass and mechanical strength have been studied when GH and Bisph were given either separately or in combination.

Two-year-old female Wistar rats were divided into the following groups: baseline; vehicle-injected (Veh); GH-treated; Bisph-treated; GH+Bisph-treated. Doses: hGH 2.7 mg/kg b.w. daily, Bisph (Risedronate) 5 microg/kg b.w. twice weekly. Treatment period was 9 weeks. The animals were labeled with fluorochromes twice during the experiment. The femoral diaphysis and the vertebral body were analyzed mechanically and by use of static and dynamic histomorphometry (mean values with SEM are given; * = significantly different from Veh).

GH increased bone resorption, and Bisph decreased bone resorption (urinary deoxypyridinoline excretion [10nmol/day]: Veh 7 (1); Bisph 3 (1)*; GH 19 (1)*; GH+Bisp 10 (1)*). Femur diaphysis: GH and GH+Bisph increased ultimate load by 56%* and 39%*. GH and GH+Bisph increased cortical bone cross-sectional area by 11%* and 13%*. GH and GH+Bisph increased periosteal bone formation rate ([10^3microm^3/day]: Veh 0.0 (0); Bisph 0.4 (0.3); GH 26 (3)*; GH+Bisph 27 (3)*). Vertebral body: GH and GH+Bisph increased ultimate load by 56%* and 59%*. GH and GH+Bisph increased bone mass by 29%* and 35%*. GH and GH+Bisph increased cancellous bone volume/total volume by 38%* and 23%*.

Conclusion: GH increased bone resorption substantially, whereas GH+Bisph only induced a modest increase in bone resorption. GH increased bone formation, and enhances both vertebral body and femoral diaphysial bone mass and mechanical strength. GH+Bisph also increased bone formation, bone mass, and mechanical strength, and these responses seemed to be almost identical whether GH was given alone or in combination with Bisph.

Disclosures: ***T.T. Andreassen****, None.*

SU397

The Effects of a Group of Novel Anabolic Peptides on Bone Density and Bone Strength in Adult Rats. G. B. Schneider[1], M. J. Askew*[2], K. J. Grecco*[1], J. Hsu*[1], E. Mugler*[2], D. A. Noe*[2]. [1]Division of Basic Medical Sciences, Northeastern Ohio Universities College of Medicine, Rootstown, OH, USA, [2]Musculoskeletal Research Laboratory, SUMMA Health System, Akron, OH, USA.

We previously demonstrated that a 14 amino acid fragment from the third domain of the human serum protein, vitamin D binding protein (DBP), when delivered systemically, elicited anabolic effects on bone. Intermittent subcutaneous injections of these 14 a.a. peptides to intact, adult rats resulted in significant increases in total bone density in the long bones with just two weeks of treatment (Schneider et. al., JBMR, 16:S231). In the current study we evaluated peptide fragments, ranging from 3-13 a.a. in length, created by single a.a. deletions from the c-terminal end of our original peptide. Adult, intact female rats were given subcutaneous injections of saline or peptide (0.4 ng/g body wt.) every 48 hrs. for two weeks; two days after the final injections the animals were euthanized, the femurs and tibias analyzed by peripheral quantitative computerized tomography (pQCT) and by three-point bending, biomechanical testing. Specific slices through the metaphysis and mid-shaft of each bone were analyzed from the scans. A number of the peptide fragments elicited responses which included highly significant ($p < 0.001$) increases in total bone density, significant increases in cortical/subcortical bone density, little change in trabecular bone density, decreases in total surface area, and decreases in periosteal and endosteal circumferences. All of the peptide-treated and control bones were subjected to 3-point bending at the mid-shaft, to take advantage of the geometrical data generated by the pQCT analysis, which was subsequently used in the biomechanical analyses. All of the specific peptide fragments which demonstrated highly significant increases in total bone density also demonstrated highly significant increases in bone strength. None of the peptide treatments affected the modulus of the bones as compared to controls. There was a very significant correlation between bone density and strength in the various peptide-treatment groups. The correlation coefficient was 0.60 ($p = 0.002$). In conclusion, those peptide fragments which enhanced bone density also enhanced bone strength, suggesting that the use of these novel peptides results in the generation of superior quality bone.

Disclosures: ***G.B. Schneider****, None.*

SU398

Long-Term Exposure to Strontium Ranelate Dose-Dependently Increases Intrinsic Bone Quality. P. Ammann[1], B. Robin*[2], R. Rizzoli[1]. [1]Department of Geriatry and Internal Medicine, Division of Bone Diseases, Geneva-14, Switzerland, [2]Institut de Recherches Internationales Servier, Courbevoie, France.

Recent clinical studies have demonstrated that strontium ranelate (SR) reduces the risk of vertebral and non-vertebral fracture in women with postmenopausal osteoporosis. We previously demonstrated that SR treatment dose-dependently increased ultimate strength without affecting stiffness in adult female rats. To further characterize these changes in mechanical properties, we investigated energy to failure in seven-week old intact female rats. Four groups of 30 rats were fed ad libitum a diet containing SR at a daily dose of 0 (control), 225, 450 or 900 mg/kg/day, for 104 weeks. From the load deflection curve, obtained by compression of vertebral body and using a three-point bending test of midshaft femur, yield point, total energy (E), elastic and plastic energy were measured. Values tabulated are means ± SEM and were obtained at the level of the vertebral body; * indicates $p<0.05$, ** $p<0.01$ vs control by ANOVA.

	Control	SR-225	SR-450	SR-900
E (N.mm)	101.8±7.9	118.9±10.2	130.4±11.7	157.3±15.0*
E elastic (N.mm)	68.9±5.9	71.3±7.6	80.8±7.3	86.6±10.1
E plastic (N.mm)	30.0±3.3	49.4±7.4	49.7±8.7	70.7±10.0**
Yield (N)	242.3±10.1	241.3±19.4	272.1±17.0	274.4±17.0

The increases of energy to failure (+54.5%, $p<0.05$) achieved with SR treatment at 900 mg/kg/d, was essentially due to an increment of plastic energy (+136 %, $p<0.05$), without significant influence on elastic energy (+ 26 %, ns), and no variation of bone stiffness. These results strongly suggest that bone formed under SR treatment is able to withstand greater deformation before fracture while possessing similar elastic properties than normal bone. Similar results were observed at the level of the midshaft femur. Such modifications observed under strontium ranelate treatment are in good agreement with an improvement of intrinsic bone quality leading to greater bone resistance.

Disclosures: ***P. Ammann****, None.*

SU399

Strontium Is an Agonist of the Extracellular Calcium-Sensing Receptor (CaR) in CaR-Transfected Human Embryonic Kidney Cells. S. J. Quinn*[1], O. Kifor*[1], C. Ye*[1], N. Chattopadhyay*[1], B. Robin*[2], E. M. Brown[1]. [1]Endocrine-Hypertesion Division, Brigham and Women's Hospital, Boston, MA, MA, USA, [2]Institut de Recherches Internationales Servier, Courbevoie, France.

Strontium ranelate (SR) has been shown to be effective in reducing fracture risk in women with postmenopausal osteoporosis. Its cellular mechanism of action has not yet been clearly elucidated. As the atomic and ionic structures of strontium and calcium are similar, extracellular strontium (Sr2+o), similarly to extracellular calcium (Ca2+o), could exert its biological actions, in part, via the extracellular calcium-sensing receptor (CaR). The goal of this study was to evaluate whether Sr2+o directly activates the CaR by assessing changes in the following intracellular transduction pathways and biological responses, such as elevations in the cytosolic calcium concentration (Ca2+i), accumulation of inositol phosphates (IPs), activation of mitogen-activated protein kinase (MAPK) and stimulation of the activity of a non-selective cation channel (NCC). These pathways were tested in CaR-transfected HEK293 cells, with non-transfected HEK293 cells acting as controls. Raising the level of Ca2+o produced a dose-dependent activation of the CaR in CaR-transfected HEK293 cells as assessed by increases in Ca2+i, enhanced accumulation of IPs, activation of MAPK, and increased activity of the NCC. The EC50s for the activation of these four parameters by Ca2+o were in the range of 2.5-3.4 mM. Sr2+o, at concentrations of 0.25 mM and above, also dose-dependently activates the CaR in transfected HEK293 cells. Its efficacy is similar to that of Ca2+o for activation of the NCC and MAPK, and about 30% lower for stimulating increases in Ca2+i and accumulation of IPs. Neither Sr2+o nor Ca2+o had any effect on these four parameters in non-transfected cells. Small increases in Sr2+ may preferentially stimulate CaR-expressing cells where the receptor activates the MAPK pathway in preference to the PLC/calcium transduction systems. Thus Sr2+o is a full agonist of the calcium-sensing receptor in CaR-transfected human embryonic kidney cells and could exert some of its biological actions in vivo via this receptor

Disclosures: ***E.M. Brown****, Institut de Recherches Internationales Servier 2.*

SU400

New Formulation for Transdermal Delivery of Lovastatin. G. Gutierrez[1], D. Lalka*[2], I. R. Garrett*[1], B. McCluskey*[1], G. Rossini*[1], R. E. Fourquet*[1], P. Rivas*[1], G. R. Mundy*[1]. [1]OsteoScreen, Ltd., San Antonio, TX, USA, [2]West Virginia University School of Pharmacy, Morgantown, WV, USA.

There is a continuous search for new anabolic therapies for prevention of bone loss and restoration of bone mass, preferably that are inexpensive and have a satisfactory toxicity profile.

In recent years, it has been shown that statins, drugs that have been available for more than 10 years as cholesterol-lowering drugs (through inhibition of HMG-CoA reductase), are also bone anabolic agents, causing substantial increases in bone formation in rodents in vitro and in vivo. Statins are subject to extensive first-pass metabolism by cytochrome P450 enzymes in the liver, and it has been shown in rats that a simple transdermal formulation of lovastatin in hydrophilic petrolatum yields higher, less variable and more sustained plasma levels of HMG CoA reductase inhibitory activity (HMG-CoARIA) than comparable oral doses. This dermal formulation also causes substantially greater effects on bone than those resulting from oral administration. The purpose of this current study was to identify a formulation for transdermal delivery that allowed higher and more sustained plasma levels than hydrophilic petrolatum. In the present study, we determined plasma lovastatin concentrations by measuring HMG-CoARIA in the plasma of rats and found that topical lovastatin in a hydro-alcoholic gel based on carbopol 940 provides 2-4 times the area under the plasma concentration-time curve (AUC) of HMG-CoARIA of the petrolatum preparation over the dosage range of 6.25 to 25 mg/kg. When dosed at 6.25 mg/kg, AUC was 603 hr ng/ml vs. 244 hr ng/ml for the gel and petrolatum formulations respectively, with the Cmax at 132"20 ng/ml for the gel vs. 48"33 for petrolatum. To exclude hepatic and neuromuscular toxicity, we also measured liver and muscle enzymes (ALT, AST, CPK, and Alk Phos), in the serum of rats treated with lovastatin gel, 1 and 5 mg/kg/day after 35 and 5 days respectively, and found all the levels to be in the normal range. This gel was chosen because a highly lipophilic drug such as lovastatin should partition more rapidly into skin allowing faster absorption of the drug. The principal goal of developing a transdermal formulation of a potential anabolic agent that could be used for the treatment of osteoporosis and other metabolic diseases is to achieve better delivery to the skeleton following systemic administration, while maintaining a toxicity profile similar to placebo. Based on these results, we propose this gel as a vehicle for lovastatin administration.

Disclosures: ***G. Gutierrez****, OsteoScreen, Ltd. 1.*

SU401

Strontium Ranelate Treatment Preserves Bone Crystal Characteristics and Dissolution Properties of Bone Apatite. R. Z. LeGeros*, S. Lin*, J. P. LeGeros*. College of Dentistry, New York University, New York, NY, USA.

Strontium ranelate (SR) treatment has shown its efficacy in preventing vertebral and nonvertebral fractures in postmenopausal women with osteoporosis. The purpose of the present study was to determine the effect of SR treatment on characteristics (e.g., crystallinity, composition) and dissolution properties of the bone apatite. Female *Macaca fascicularis* monkeys were separated into 4 groups receiving 0, 200, 500, 1250 mg/kg/d of SR, respectively. At the end of 52 weeks of treatment, 4 animals were sacrificed in each group, and 2 animals were sacrificed after a 10-week reversibility period. Bone samples of humeral diaphysis were powdered (granulometry of 10-15 μm) and analyzed before and after dissolution in the acidic buffer (acetate, 0.1M, pH 5, 37ºC, 60 min), using X-ray diffraction to determine crystal size and Fourier transform infrared spectroscopy for the evaluation of organic/inorganic phases and carbonate/phosphate ratios. Ca, Sr, Mg, and P ions' bone powder contents before and after the dissolution, and their release in the buffer during dissolution were also determined using inductive coupled plasma emission spectrophotometry. The Ca ions' release onto the buffer with time was also monitored using a Ca-ion selective electrode.

At the end of a 52-week treatment with SR, Sr was taken up in bone in a dose-dependent manner up to 0.021 mmol/100g of powder. Ten weeks after the end of the treatment, the maximal decrease in Sr content was -49% with the SR dose of 500 mg/kg/d. SR treatment, however, had no effect on Ca, Mg and P contents, nor on the mineral crystal characteristics (composition, organic/inorganic and carbonate/phosphate relative ratios, crystal size). SR treatment had no effect on the bone dissolution rate, nor on the amounts of Ca, Mg and P ions released in the acidic buffer. The release of Sr ions in the acidic buffer was dependent on the bone Sr content. After exposure to acid buffer, the Sr concentration in bone powder was reduced up to 0.012 mmol/100g of powder and still dose-dependent in the 52-week treated groups. No modification of the mineral crystal characteristics was observed after dissolution. Thus low bone Sr content had no effect on the dissolution of natural apatites (non-stoichiometric and slightly substituted) which makes it difficult to establish comparisons with other results obtained with highly substituted synthetic stoichiometric apatites.

These data demonstrate the safety of SR in preserving characteristics (composition, crystallinity) and dissolution properties of bone apatite, after long-term treatment up to 40 times the human therapeutic dose of 2 g/day.

Disclosures: ***R.Z. LeGeros****, None.*

SU402

Osteopontin-deficiency Enhances Prostaglandin E EP4 Receptor-dependent Increase in Cancellous Bone Mass in Mice. N. Kato[1], K. Kitahara[1], K. Tsuji[1], S. R. Rittling[2], 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.

To increase bone mass in osteoporosis patients, not only bone resorption inhibitors but also stimulators for bone formation would be required. In addition to PTH, it has also been known that PGE_2 induces bone formation *in vivo*. Administration of PGE receptor EP4-selective agonist prevents bone loss in ovariectomized rats and thus EP4 signaling is responsible for the enhancement of bone formation. However, the molecular mechanisms for such EP4 signaling is not fully understood. Osteopontin (OPN) is a non-collagenous bone matrix protein and cytokine, expressed in both osteoblasts and osteoclasts. OPN-deficiency induces cortical bone formation by intermittent PTH administration. In this paper, we examined the effects OPN on EP4 receptor-agonist induced bone formation using OPN-deficient mice. EP4 agonist (ONO-AE1-329, 30μg/kg) was injected three times per day, 5 days a week for 4 weeks subcutaneously into either wild type or OPN-deficient mice. EP4 agonist treatment did not alter BMD at the distal end of the femora in wild type mice. In contrast, OPN-deficient mice indicated significant increase in BMD after the treatment with EP4 agonist. Micro CT analysis on cancellous bone within the distal end of the femora revealed that EP4 agonist did not alter the levels of cancellous bone volume per tissue volume (BV/TV) in wild type mice. OPN-deficiency, however, enhanced the levels of cancellous BV/TV after the treatment with EP4. EP4 agonist did not alter total cross-sectional area, cortical area and cortical thickness as well as bone marrow area regardless of the genotype. Bone marrow cells obtained from wild type did form mineralized nodule in culture. However, EP4 agonist treatment did not reveal any modulation on the levels of nodule formation. On the other hand, EP4 agonist treatment enhanced bone nodule formation in the cultures of bone marrow cells obtained from OPN-deficient mice. TRAP-positive osteoclast cell numbers in the bone marrow cells cultured in the presence of vitamine D_3 and dexamethazone were similarly increased after 9 days culture in wild type mice as well as in OPN-deficient mice. EP4 agonist did not alter the levels of TRAP-positive cell number in culture regardless of the genotype. These observations indicated that OPN-deficiency enhanced anabolic effects of the EP4 agonist on the cancellous bone volume, possibly via the increase in osteoblastic progenitor cell population in the bone marrow.

Disclosures: ***N. Kato****, None.*

SU403

Long-term Treatment with Strontium Ranelate Increases Histomorphometric Indices of Bone Formation in Ovariectomized Rats. S. Bain[1], V. Shen[1], H. Zheng*[1], C. Liu[1], P. Hara*[1], I. Dupin-Roger*[2]. [1]SkeleTech, Inc., Bothell, WA, USA, [2]Laboratories SERVIER, Courbevoie, France.

The skeletal response to ovariectomy and to the effects of a preventive treatment with Strontium Ranelate (SR) were evaluated using static and dynamic bone histomorphometry. Experimentally, 6-month old Sprague-Dawley rats were either ovariectomized (OVX) or received sham (SHAM) surgeries. Beginning 1 day post-ovariectomy, 3 groups of OVX rats were treated daily for 52 weeks with 125, 250, or 625 mg/kg of SR (SR125, SR250, and SR625, respectively) and one received vehicule. Vehicle-treated OVX and SHAM animals served as controls. At the end of the 1-year treatment, bone histomorphometry of the proximal tibia showed a 72% reduction in cancellous bone volume (BV/TV) in OVX rats vs. SHAM (see Table). The reduced BV/TV was accompanied by a 70% lower trabecular number (Tb.N) and a 394% increase in trabecular spacing (Tb.Sp). Similar findings were also observed in the lumbar vertebra (data not shown). Compared to OVX controls, preventive therapy with SR showed positive, dose-dependent effects on all parameters, increasing BV/TV and Tb.N, and decreasing Tb.Sp. Bone formation rates (BFR/BS) were also maintained at levels equivalent to those observed in OVX rats treated with vehicle. Furthermore, trabecular thickness (Tb.Th) increased 19% to 23% in SR-treated rats. More-

over, a comparison of the Tb.Th values with a Baseline control group indicated that an age-related decline in the Tb.Th was prevented by treatment with SR. There was no modification of the osteoid tissue and of the mineral apposition rate, confirming the absence of any mineralization defect under strontium ranelate treatment. These results indicate that treatment of OVX-induced bone loss with SR improves bone balance and exerts positive effects on bone structure via a pathway that involves the stimulation of bone formation.

Bone Histomorphometry of Proximal Tibia

Parameter	SHAM	OVX	OVX		
			SR125	SR250	SR625
BV/TV (%)	9.2 ± 3.6**	2.6 ± 2.5	4.5 ± 2.9**	4.6 ± 3.4**	5.6 ± 4.5**
Tb.Sp (μm)	420 ± 232**	2073 ± 1628	1242 ± 1157*	992 ± 570**	984 ± 748**
Tb.N (#/mm)	2.74 ± 1.2**	0.83 ± 0.63	1.22 ± 0.67	1.29 ± 0.71*	1.36 ± 0.69**
Tb.Th (μm)	32.1 ± 5.2	30.3 ± 6.2	36.2 ± 10.8	33.8 ± 8.3	37.3 ± 12.3
BFR/BS (μm3/μm2/y)	23.4 ± 19.9**	59.5 ± 31.7	59.4 ± 36.7	50.3 ± 29.0	65.9 ± 39.7

Means ± SD. *: P<0.05 vs. OVX; **: P<0.01 vs. OVX.

Disclosures: ***S. Bain****, None.*

SU404

Dried Plum Promotes Bone Recovery Comparable to Parathyroid Hormone in Osteopenic Rats Following Hind Limb Unloading. B. J. Smith[1], A. Ethriedge*[1], E. A. Lucas[1], D. Bellmer*[2], B. J. Stoecker[1], B. H. Arjmandi[1]. [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Oklahoma Food and Agriculture Products Research and Technology Center, Oklahoma State University, Stillwater, OK, USA.

Dried plum has been shown to both prevent and reverse bone loss in animal models of ovarian hormone deficiency as well as having a positive effect on bone biomarkers in postmenopausal women. The objectives of this study were to: 1) examine the dose-dependent effects of dried plum in a animal model of skeletal unloading, i.e. the hind limb unloaded rat (HLU); and 2) compare the effects of dried plum on bone recovery to that of parathyroid hormone (PTH). Six month old female virgin Sprague-Dawley rats were either HLU (n=70) or kept ambulatory (AMB=16) for twenty-one days to induce osteopenia. At the end of the suspension period, a HLU (n=9) and AMB (n=9) group was sacrificed to confirm that significant bone loss had occurred. Animals were then randomly assigned to dietary treatments: standard semi-purified diet with either LD=5%, MD=15%, or HD=25% (w/w) dried plum, or administered PTH (80 μg/kg bw s.c.; 3 x wk; Bachem, Inc.) for 90 days. Following the recovery period, tibial bone mineral content (BMC) and density (BMD) were enhanced significantly by the HD compared to animals on the control diet, although not to the level of PTH treated animals. Assessment of distal femur microarchitecture using micro-computed tomography (Scanco Medical μCT 40) revealed bone volume (BV/TV) of rats in the HD group was similar to the PTH group and significantly higher than those on the control diet. Trabecular thickness (TbTh) was significantly increased by all doses of dried plum, although not to the level of PTH. Trabecular space (TbSp) and number (TbN) were not altered by either dried plum or PTH. Biomechanical data suggest that there was no difference in the structural and material properties of the femur mid-shaft due to dietary treatments or PTH. Biomarkers of bone metabolism indicate that urinary deoxypyridinoline excretion, a marker of bone resorption was unaltered, however, serum alkaline phosphatase was significantly increased with HD dried plum. We conclude that bone recovery is enhanced by dried plum in the osteopenic rat to a greater extent than observed with animals on the control diet and to a comparable level to that of PTH. Additionally, our findings suggest that these positive effects on bone are associated with enhanced bone formation and not inhibition of bone resorption.

Disclosures: ***B.J. Smith****, None.*

SU405

Alterations in Vertebral Bone with Hind Limb Unloading and the Effects of Dried Plum and Parathyroid Hormone During Recovery. A. Ethriedge*, E. Lucas, B. J. Stoecker, C. I. Wei*, B. H. Arjmandi, B. J. Smith. Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA.

This study was designed to: 1) examine the response of vertebral bone to hind limb unloading (HLU); and 2) evaluate the effects of dried plum and parathyroid hormone (PTH) on vertebral bone during recovery. Six month old female virgin Sprague-Dawley rats were either hind limb unloaded (HLU=70) or remained ambulatory (AMB=16) for twenty-one days to induce bone loss. At the end of the 21 day period, one HLU group (n=8) and one AMB (n=8) was necropsied to confirm the occurrence of significant osteopenia. Animals (1 AMB group and 5 HLU groups) were then randomly assigned to the following treatments for 90 days: a standard semi-purified diet with either control, LD=5%, MD=15%, or HD=25% (w/w) dried plum or PTH (80 μg/kg bw; 3 x wk Bachem, Inc.). In response to HLU a significant decrease in L5 bone mineral content (BMC) and bone mineral density (BMD) was observed using dual energy x-ray absorptiometry (DXA). Micro-computed tomography analyses (Scanco Medical μCT 40) indicated that L4 bone volume (BV/TV) and trabecular thickness (TbTh), were significantly reduced with unloading, but there were no changes in trabecular number (TbN) and trabecular space (TbSp). Following the 90 day recovery period, BMC for all doses of dried plum was similar to that of the PTH treated group (Table.). BV/TV was restored in all treatment groups to that of the AMB control, but only the MD dried plum brought bone volume back to the level of the PTH group. Although no dose of dried plum increased TbTh to a greater extent than the control diet, the MD did result in a similar TbSp to that of the AMB-control and PTH groups. These findings support the notion that HLU produces significant vertebral bone loss, but dietary consumption of dried plum may not only restore bone to that of the AMB animals, but have an anabolic effect on the vertebra similar to PTH. (OCAST HR01-133)

	BMC	BV/TV	TbTh	TbSp	TbN
AMB- Control	0.1684[c,d]	0.4328 [b,c]	0.0940[b]	0.2198[a,c]	4.2859
HLU-Control	0.1683[c,d]	0.3848[c]	0.0888[b,c]	0.2302[a]	4.1276
HLU-LD	0.1763[b,c]	0.4199[b,c]	0.0946[b]	0.2217[a,b]	4.2128
HLU-MD	0.1760[b,d]	0.4626[a,b]	0.0945[b]	0.1939[c]	4.7042
HLU-HD	0.1886[a]	0.4453[b,c]	0.0975[b]	0.2144[a]	4.3174
HLU-PTH	0.1883[a,b]	0.5216[a]	0.1119[a]	0.2004[b,c]	4.4381
P-Value	*<.0001*	*<.0001*	*<.0001*	*0.0152*	*0.1172*

Disclosures: ***A. Ethriedge****, None.*

SU406

Development of Magnetic Retroviral Vectors for Site-Specific Targeting in Skeletal Gene Therapy. S. T. Chen*[1], M. F. Tai*[2], K. M. Chi*[3], M. C. Wen*[2], C. H. Rundle*[1], K. H. W. Lau[1], D. J. Baylink[1]. [1]Musculoskeletal Disease Center, J.L. Pettis VAMC, Loma Linda, CA, USA, [2]Physics, National Chung Cheng University, Chia-Yi 621, Taiwan Republic of China, [3]Chem. & Biochem., National Chung Cheng University, Chia-Yi 621, Taiwan Republic of China.

Gene therapy for bone fractures requires accurate delivery of therapeutic genes to the fracture site. We recently showed that the direct injection of a moloney leukemia virus (MLV)-based vector expressing BMP4 to the rat femoral fracture callus enhanced bone formation. However, the technical difficulties related to injection frequently led to site-inaccurate injections, resulting in extraperiosteal bone formation. We sought to develop a magnetic retroviral vector (MRV) system for site-specific delivery of viral vector to fracture callus to improve the safety and efficacy of the therapy, because: 1) magnetotransfection significantly enhanced viral transduction efficiency, and 2) a magnet can be used to accurately target delivery MRV to fracture sites. To generate MRV, we synthesized magnetic nanoparticles of γ-Fe_2O_3 by a high-yield reduction-oxidation lyothermal method at high temperature under argon gas. Iron pentacarbonyl precursors were reduced to form iron nanoparticles and then oxidized by trimethylamine. High-resolution transmission electron microscopy (HRTEM) analysis revealed a narrow distribution of particle size of 4 ± 0.8 nm. The resulting magnetic nanoparticles were then coated with polycationic, polyethyleneimine (PEI) by sonication. PEI is a cationic polymer capable of binding retroviral vectors. The average size of PEI-coated magnetic nanoparticles was 100-200 nm, determined by HRTEM. To synthesize the MRV complex, the PEI-coated particles were mixed with various concentrations of MLV vectors. To test the transduction efficiency of our MRV system, we monitored the transduction efficiency of our MRV system expressing green fluorescent protein in HT-1080 cells and found that application of the magnetic field markedly increased transduction efficiency by 3- to 5-fold ($p<0.001$) with an MOI between 0.1 to 1. We have made two important observations in our preliminary application of our MRV to the rat femoral fracture: 1) the magnetic nanoparticles are not cytotoxic; and 2) with the β-galactosidase reporter gene, we demonstrated that the transduction of MRV was directed to fracture callus with the aid of a magnet, with little or no transduction outside the magnetic field. In conclusion, we have developed a MRV system that provides efficient site-specific targeted delivery of viral vectors to specific bone sites. We anticipate that this MRV system will be extremely useful in site-specific targeting in skeletal gene therapy.

Disclosures: ***S.T. Chen****, None.*

SU407

Post Lower Extremity Fracture Study. C. G. Horn*, M. C. Gibson*, B. B. Heil*, B. A. Tracewell*. The Foundation for Osteoporosis Research and Education, Oakland, CA, USA.

Fractures related to osteoporosis are an important cause of increased morbidity and mortality in the elderly. The aim of this 1-year study was to examine the effect of a post-rehabilitation community-based exercise program on the overall physical function, quality of life and bone density (BD) of a cohort with recent low trauma, below waist fracture. A group of 51 subjects, (47) women and (4) men, were randomized into one of three groups: 6-month exercise (19), 12-month exercise (20) or control (12) Data collection included a battery of 24 evaluation tools to measure strength, balance, gait, quality of life and confidence levels, administered according to accepted standards of physical therapy practice. Bone density testing of the hip and spine was done pre-entry and at the conclusion of study participation. All subjects were given a daily 1000 mg supplement of elemental calcium with Vitamin D. Activity levels of control subjects were not programmed. The exercise subjects attended twice weekly, hour-long sessions made up of strength, flexibility and balance activities. Exercises were chosen specifically to strengthen all anti-gravity lower extremity muscle groups. These exercises were done both standing and sitting. Strength dynamometry was used to determine appropriate initial weight levels for resistive exercises. Exercise progression began with subjects completing 8 repetitions of each exercise, increasing to 16 repetitions, then adding hand or ankle weights and returning to 8 repetitions. Subjects were instructed to add weight as tolerated but not by more than one pound per week, per site. At 3 months a weighted vest and lunge program was added. At 4 months a step-up program was initiated. Compliance varied between subjects, however we found all exercising participants gained significant strength, and reported improved Quality of Life based on response to the "QUALEFO" quality of life questionnaire. Of special note is that at the start of the program 22 of the 39 exercise participants scored 45 or below (High Risk for Falls category) on the Berg Balance Scale and all but 3 scored over 45 at 6

months. Each participant has maintained or made slight (not statistically significant) increases in bone density. In addition to the physical benefits of the study, the social aspect was important in keeping participants interested and attending regularly. We conclude that a post-rehabilitation community-based group exercise is a viable mechanism to optimize long-term physical function and quality of life in persons who have sustained low trauma, below waist fractures.

Disclosures: ***C.G. Horn****, None.*

SU408

Lack of Seasonal Variations in Urinary Calcium/Creatinine Ratio in School Age Children. M. Stuart-Hilgenfeld, M.D.*[1], S. Simon, Ph.D.*[2], D. Blowey, M.D.*[1], D. Cokely, Ph.D.*[2], W. Richmond, B.S.*[1], U. S. Alon, M.D.[1]. [1]Pediatric Nephrology, Children's Mercy Hospital, Kansas City, MO, USA, [2]Medical Research, Children's Mercy Hospital, Kansas City, MO, USA.

Hypercalciuria is a common pediatric problem known to cause hematuria, voiding dysfunction, abdominal pain and urolithiasis. Random urine calcium/creatinine ratio (U Ca/Cr) is routinely used to diagnose hypercalciuria. Normal U Ca/Cr values are well established for age, ethnicity and geographic location. Seasonal variations in urinary calcium excretion have been observed in adults and are thought to be due to seasonal changes in sunlight exposure which results in fluctuation of blood concentration of 25(OH) Vitamin D.

We studied the possible effect of season on U Ca/Cr in healthy school children. 65 healthy Caucasian children aged 5.1 to 14.6 years (mean $\pm$ SD 9.61$\pm$ 1.96, median 9.67) from a local suburban private school volunteered. Random, nonfasting urine samples were collected from each participant between the 1st and 5th days of the month for 16 successive months and tested for calcium and creatinine using standard laboratory methods. U Ca/Cr was then calculated for each specimen. Meteorological data (average monthly temperature and average monthly UV index) were also collected during the study period.

A mathematical model describing the monthly mean values of U Ca/Cr as a sine function was used to determine the presence of seasonal variation. The observed maximum mean U Ca/Cr of 0.092 mg/mg occurred in the late summer months (August - September) and minimum mean U Ca/Cr of 0.083 mg/mg occurred in mid-winter (January - February), reflecting an infinitesimal and statistically insignificant difference. Moreover, both values were well within the previously establsihed normal range for U Ca/Cr of $\leq$ 0.20 mg/mg. The pattern seen in the last four months of the study repeated that seen in the first four months. Mean temperature in July of the first year was 27.1 $\pm$ 2.6, January 1.8 $\pm$ 5.4 and July of the second year 27.0 $\pm$ 2.2 degrees C. Mean UV index in July of the first year 7.61 $\pm$ 1.01 was not different from that in July of the second year 7.73 $\pm$ 0.86, and both were statistically much higher than that in January 1.37 $\pm$ 0.44 ($p<0.001$).

Evidently, in spite of significant changes in seasonal meterological indices of sunlight, no significant seasonal variations in U Ca/Cr were observed in a cohort of randomly selected healthy school children. Thus the current U Ca/Cr value used to diagnose hypercalciuria does not require a seasonal adjustment.

Disclosures: ***U.S. Alon, M.D.****, None.*

SU409

Bone Mineral Changes in AIDS Associated Lipodystrophy. L. Rosenthall*[1], J. Falutz*[2]. [1]Radiology, McGill University Health Center, Montreal, PQ, Canada, [2]Medicine, McGill University Health Center, Montreal, PQ, Canada.

The use of highly active antiviral therapy (HAART) for HIV+ patients has brought about an increase in the frequency of the body fat redistribution. Specifically, this HIV/HAART-associated lipodystrophy (HAL), includes a loss of subcutaneous fat in the lower and upper limbs, buttocks and face, and an increase in abdominal adiposity in some of these affected individuals along with dorsocervical fat pads (buffalo hump). Alterations in bone turnover and mineral depletion have been reported prior to the introduction of HAART, but its frequency has increased since. There are numerous studies demonstrating the influence of body weight on bone mass, but the principal determinant of this bone mass dependence, i.e., lean mass or fat mass, varies in the literature from primarily lean mass, to both lean and fat mass, to primarily fat mass. The question was addressed in this report by total and regional DXA body composition studies in two groups of HIV+ men receiving HAART who were classified clinically as HAL-ve (N=26) or HAL+ve (N=43). Age=49±8.3 yr.

*Controls consisted of 79 nonAIDS males with minimum t-score <=-2, age matched (50.6±9.4 yr). From Table 1, it is noted that there is no significant difference in the lower limb lean mass between HAL-ve and HAL+ve patients, but the fat mass content is significantly lower in the HAL+ve group. The BMC and the fat/lean ratio are significantly lower in HAL+ve patients. Similar results were obtained for the upper limbs and trunk. From Table 2, it is noted that the BMD t-scores of the lumbar spine and femoral neck are lower in the HAL+ve than HAL-ve patients. Furthermore the mineral depletion is significantly greater in the femoral neck than the lumbar spine in both groups, whereas in the control group the opposite prevails.

Fat mass decrease is a significant factor in the skeletal mineral loss in male HIV+ patients and it occurs in the absence of lean fat mass loss. The mineral loss is greater in the extremities than centrally, corresponding to the evolution of fat atrophy.

Table 1 LOWER LIMB

	HAL-ve	HAL+ve	Probabilty
Fat mass(kg)	4.51(1.89)	2.53(1.86)	0.000
Lean mass(kg)	18.83(3.14)	18.00(2.99)	0.306
BMC(gm)	1.10(0.21)	0.97(0.14)	0.009
Ratio Fat/Lean	0.235(0.087)	0.142(0.096)	0.000

Table 2

	Lumbar spine t-score(SD)	Femoral neck t-score SD)	Paired t-test Probability
HAL-ve	-0.68(0.99)	-1.51(1.25)	0.000
HAL+ve	-1.32(0.76)	-2.28(0.95)	0.000
Probability HAL-ve vs HAL+ve)	0.007	0.008	
Controls*	-2.58(0.94)	-2.17(0.95)	0.007

Disclosures: ***L. Rosenthall****, None.*

SU410

A Possible Role for Extracellular Calcium in the Inflammatory Process. S. Castro*[1], R. Garofalo*[1], E. M. Brown[2], G. L. Klein[1]. [1]Pediatrics, University of Texas Medical Branch, Galveston, TX, USA, [2]Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA.

Olszak et al (*J Clin Invest* 2000; 105:1299) demonstrated that extracellular calcium(Ca) alone or in combination with the chemokine monocyte chemoattractant protein(MCP)-1 enhances monocyte chemotaxis. The aim of our study was to determine if extracellular Ca could stimulate peripheral blood mononuclear cell (PBMC) chemokine production. We obtained blood from normal adult volunteers, separated the PBMC on a Ficoll gradient,and performed 4 separate incubations in triplicate-sextuplicate incubating 1 million cells/well inRPMI with 10% fetal calf serum and penicillin-streptomycin for 24h. Ca concentration in the medium was 0.35 mM. Wells were incubated with Ca chloride ranging from 0-5 mM. Two additional experiments were done in triplicate in which PBMC were incubated with an antibody cocktail to isolate CD14+ monocytes (CD14). These were incubated at 500,000 cells/well under the same conditions. The media from all wells was analyzed by ELISA for the chemokines macrophage inflammatory protein (MIP)-1 alpha and RANTES. For the PBMC, natural log (ln) transformation of the mean concentration of MIP-1 alpha/ Ca concentration demonstrated a significant regression against Ca concentration, r= 0.732, $p<0.005$ (n=18). Non-parametric rank correlation coefficient for RANTES with Ca concentration also showed a direct relationship, r=0.684, $p<0.005$, n=14. For CD14 cells, ln transformation of the mean MIP-1 alpha concentration per Ca demonstrated r= 0.722, $p<0.025$ (n=8). For one experiment with RANTES (n=4), the relationship between RANTES and extracellular Ca concentration was r=0.928. These data support a role of extracellular Ca in chemokine production and suggest that Ca liberated from bone following inflammation-induced osteoclastic resorption may feed the inflammatory reaction leading to further bone loss, a possible scenario in a condition such as rheumatoid arthritis.

Disclosures: ***G.L. Klein****, None.*

SU411

Longitudinal Evolution of Bone Mineral Density in Human Immunodeficiency Virus-Infected Patients. S. Azriel*[1], M. Torralba*[2], E. Jódar*[1], G. Martínez*[1], R. Rubio*[2], F. Hawkins[1]. [1]Endocrinology, Hospital Universitario 12 de Octubre, Madrid, Spain, [2]HIV Unit, Hospital Universitario 12 de Octubre, Madrid, Spain.

HIV infection and HAART (highly active antiretroviral therapy) have been associated with the development of numerous acute and long-term toxicities. Recently, several bone disorders, including osteopenia (op) and osteoporosis (OP) have been detected. The underlying mechanisms for these metabolic complications have yet to be defined. Our goal was to estimate bone mineral density (BMD) in HIV-infected patients treated with antiretroviral therapy, its relationship with anthropometrics parameters and infection activity markers, and evaluate the longitudinal evolution of BMD during a 12 months time period.

Subjects and Methods: We studied 52 HIV-infected subjects: 35 males and y 17 premenopausic females. Mean age of the group was 41 years (SD 9); the current weight: 68,2 kg (11,7) and the BMI: 24,2 kg/m^2 (3,5). Mean current CD4 cell count was 592 cel/ml (SD 265). Ninety five percent of the cohort had an undetectable virus load, and the mean duration of HIV infection was 42 months (SD 20). The majority of the patients (78,8%) were taking HAART regimens and 21,2% bitherapy. Dual X Ray Absorptiometry (DXA, Hologic QDR 4500) of the lumbar spine (L1-L4; LS) total hip (TH) and distal third radius (DTR) was performed to determine bone mass at baseline and 12 months follow-up (variation coeficient: <2%).

Results: Patients showed reduced axial BMD : *z score* LS: -0,82 (SD:1,02), $p<0,001$; TH: -0,49 (SD:0,85), $p<0,001$; DTR: -0,02 (SD:1,03), p=0,85. Males (vs females) and HAART-treated patients (vs bitherapy) showed greater op: -0,99 (SD:1,04) vs -0,48 (SD: 0,93); -0,94 (SD:1) vs –0,37 (SD:1), $p<0,1$. LS BMD (*z score*) was related to age (r=0,293, $p<0,05$). TH BMD was related to weight (r=0,312, $p<0,05$) and to BMI (r=0,330, $p<0,05$), and DTR BMD was related to duration of therapy (r=0,31, $p<0,05$). No significant changes in BMD were detected after one year of follow-up: %/year; LS: -0,16 (SD: 2,54); TH: -0,06 (SD: 2,84); DTR: 0,41 (SD: 2,53); p>0,2.

Conclusions: HIV-infected patients on antiretroviral therapy show a mild osteopenia without increased bone loss during a 12 months period of follow-up. Male patients and those treated with HAART seems to be more affected. Long-term prospective studies are warranted to confirm these results.

Disclosures: ***S. Azriel****, None.*

SU412

Effects of Levothyroxine on Bone Mineral Density, Muscle Strength and Bone Turnover Markers: A Prospective Cohort Study. R. Schneider*, P. Schneider, C. Reiners*. Clinic for Nuclear Medicine, University of Wuerzburg, Wuerzburg, Germany.

Studies on levothyroxine effects on bone mineral density (BMD) provided conflicting results. Many studies were descriptive only, cross-sectional and mainly included postmenopausal women. The purpose of this controlled longitudinal study was to evaluate effects of levothyroxine therapy on BMD, bone and muscle strength, markers of bone, calcium and thyroid metabolism in men and women.

This prospective cohort study followed 3 patient groups (mean age, 40 years) for a mean of 1.1 years. Patients with differentiated thyroid cancer (28 men; 46 premenopausal women) receiving suppressive levothyroxine doses (mean TSH, 0.05 mU/l; mean dose, 186 ug/day) and 23 premenopausal women with nontoxic goiter on replacement doses (mean TSH, 1.09 mU/l; mean dose, 105 ug/day) were compared to 102 controls matched for sex, age and body mass index. Serum thyroid hormones, sex hormone binding globuline, a thyroid metabolism marker at the tissue level, markers of bone and calcium metabolism were assessed. BMD and bone strength index (BSI) at the ultradistal radius were measured by peripheral quantitative computed tomography (pQCT), BMD at the femoral neck, hip and lumbar spine by dual X-ray absorptiometry (DXA) and maximum grip strength by dynamometer.

All 3 patient groups had lower calcitonin levels, but no significant differences compared to controls in BMD, BSI and grip strength at base-line. At follow-up, calcitonin levels were lower in male and female cancer patients versus controls. Female cancer patients had lower grip strength, goiter patients had higher lumbar spine BMD and BSI. Annualized mean changes or percent changes from base-line differed from controls in women, only. In female cancer patients the mean increase in phosphate was 6.0% higher, the decrease in total BMD at the radius was 1.8% greater and the rise in calcitonin of 0.2 pg/ml was smaller. In goiter patients the mean decrease in grip strength was 4.4% greater. Male cancer patients had a significant within-group reduction in BSI of 4.4%. Within-group differences were also seen in the whole patient group, a reduction of 2.2% in BSI and of 1.3% in total BMD, and surprisingly a 1.1% increase in trabecular BMD at the radius.

In conclusion, BMD in men is not affected by suppressive doses, but men are at risk of impaired bone strength. In women negative effects of levothyroxine on muscle strength seem to be irrespective of dose. In female cancer patients reduced total BMD of radius is associated to suppressive doses. By contrast, beneficial effects of replacement therapy on trabecular BMD and BSI are suggested in goiter patients. Low or normal calcitonin levels or reduced muscle strength must be taken into account as confounders.

Disclosures: ***R. Schneider****, None.*

SU413

Osteopenia in Anorexia Nervosa. Effect of Duration of Amenorrhea and Low Body Weight. G. Martinez*[1], M. García*[2], L. García*[1], M. Muñoz*[2], E. Jódar*[1], J. Argente*[2], F. Hawkins[1]. [1]Endocrinology Service, Hospital Universitario 12 de Octubre, Madrid, Spain, [2]Endocrinology Service, Hospital Universitario Niño Jesús, Madrid, Spain.

Background: Low bone mass is a common finding in anorexia nervosa (AN). In these patients, amenorrhea, either primary or secondary, reflects low estradiol levels, whereas low body weight is the result of restrictive dietary habits. The purpose of our study was to investigate the relative contribution of these factors in the development of osteopenia. Methods: eighty Spanish female adolescents (age 15.3 ± 1.7 years) with diagnosis of AN (DSM-IV criteria) were classified according to different stages of their illness: Group I (n=16), low body weight (defined as BMI < - 1 SD) and amenorrhea > 1 year; Group II (n=15), normal body weight (BMI > -1 SD) and amenorrhea > 1 y; Group III (n=18), BMI > -1SD and spontaneous recovery of menstrual cycles >6 months; Group IV (n=12), BMI < -1SD and amenorrhea > 6 months (recent diagnosis); and Group V, primary amenorrhea. Tanner stage and BMI (expressed as Z–score) were assessed in all patients. Lumbar spine BMD and the percentage of total body fat were measured with an Hologic QDR 4500 densitometer.

Results: Age and pubertal development were similar in groups I to IV. Patients in group V were younger and with less pubertal development. BMD was significantly reduced in Group I and V (see table); in these groups percentage of osteopenic patients (Z-score< -1SD) was 69% and 63%, respectively. Thirty-three percent of the patients were osteopenic in groups III and IV, and 53% in group II.

	Group I	Group II	Group III	Group IV	Group V	ANOVA
Lumbar Z-score	-1.4	-1.14	-0.55	-0.55	-1.39	P=0.047
BMI Z-score	-1.66	-0.53	-0.39	-1.51	-1.26	P<0.001
Body Fat%	18.2	22.3	25.4	19.5	19.7	P=0.001

Conclusion: total time of amenorrhea (a surrogate marker of hypoestrogenism) and low body weight contribute to low bone mass in AN patients. However, our data suggest that the former plays an outstanding role in BMD compared with body weight or body fat. This effect seems to be independent of the pubertal stage.

Disclosures: ***G. Martinez****, None.*

SU414

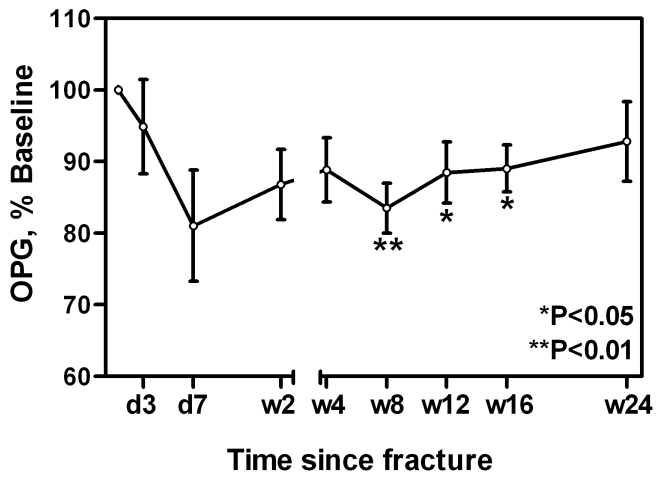
Change in Serum OPG after Tibial Shaft Fracture in Adults. A. Rogers[1], R. Beaumont*[1], S. Veitch*[1], S. Findlay*[1], A. Hamer*[2], B. M. Ingle[1], R. Eastell[1]. [1]Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom, [2]Northern General Hospital, Orthopaedic Department, Sheffield, United Kingdom.

Osteoprotegerin (OPG) inhibits bone resorption by blocking the RANK/RANKL signalling pathway and has been implicated in the regulation of bone remodelling. A study of simple transverse fractures in mouse tibias has demonstrated changes in OPG expression during fracture healing which may indicate a role for OPG in the fracture repair process.

The aim of the study was to determine change in serum OPG with time following traumatic fracture of the tibial shaft in otherwise healthy adults.

We studied 18 subjects (16 male, 2 female, ages 16 to 78, mean 32 years) who had sustained an isolated mid third tibial shaft fracture. Non-fasting blood samples were taken within 48 hours of fracture. Subsequent fasting samples were taken at 3 and 7 days and at 2, 4, 8, 12, 16 and 24 weeks. Serum OPG was measured using an in house ELISA. Bone formation markers osteocalcin, PINP, Bone ALP, PIIINP and bone resorption marker sβCTX were assayed at all time points. Serum OPG was also measured at day 1 and at 52 weeks following fracture in 14 individuals. Results were analysed by repeated measures ANOVA and between time contrasts were corrected for multiple comparisons.

There was a significant decrease in serum OPG from the time of fracture, which remained below day 1 at week 24 (graph, overall P<0.0001).



There was no significant difference in serum OPG at day 1 and week 52 in 14 subjects (-6±7%, P=0.2). Bone turnover increased following fracture (PINP +72±21%, P<0.0001, Bone ALP +99±22%, P=0.004 and sβCTX +105±23% P<0.0001, all at 24 weeks). There was a smaller 33 ±10% increase in osteocalcin at 24 weeks (P=0.03). PIIINP concentration peaked at week 8 (+57±9% P< 0.0001). We observed a significant negative correlation between day 1 measurements of serum OPG and osteocalcin (r=-0.54, P=0.02) and PINP (r=-0.49, P=0.05) but not with other bone turnover markers or changes in bone turnover.

We conclude that serum OPG is significantly decreased during fracture healing and that OPG may be involved in the bone repair process following fracture.

Disclosures: ***A. Rogers****, None.*

SU415

T Lymphocyte Subsets Modulate the Dynamics, but Are not Critical for Tibial Fracture Healing. J. M. Weaver*[1], R. J. O'Keefe[2], J. E. Puzas[2], R. N. Rosier[2], E. M. Schwarz[2]. [1]Microbiology and Immunology, University of Rochester, Rochester, NY, USA, [2]Orthopaedics, University of Rochester, Rochester, NY, USA.

The interaction of T lymphocytes with bone cells has been implicated in many bone diseases, such as Rheumatoid Arthritis, Fibrous Dysplasia Ossificans Progressiva, and Osteomyelitis but there has been no direct evidence of their role in these diseases or during skeletal repair. Activated T cells have been shown to produce RANKL (Receptor Activator of NFκ-B Ligand), a potent cytokine in initiating osteoclastogenesis in the resorptive phase of fracture healing. TGFβ-1, a potent immune suppressor, and osteoclastogenic and chondrogenic potentiator, is produced by T_H3 and Treg1 subsets of T lymphocytes. The possibility that T lymphocytes have a major role in regulating the fracture healing process makes them good targets for studying bone phenotypic effects.

We investigated the potential effects T lymphocytes may have on fracture healing. Using a murine tibial fracture model, we observed the focal interaction of CD3+ cells at the fracture site of CBAxB/6 wild-type mice starting at day 4 until day 14 post tibial fracture. We further observed the normal healing of RAG2-/-, CD4-/-, CD8-/- mice compared to CBAxB/6 wild-type mice up to 28 days post fracture. Despite normal healing, small modulations were observed in each of the different knockout strains. RAG2-/- mice showed decreased Mineral Apposition and Bone Formation Rates of long bone (humerus) as compared to native mice, but no differences were observed in the calvaria. Osteoclast numbers were increased in CD8-/- mice, but not altered in any other strain examined. In quantification of the fracture callus, there was an increase in percent cartilage formation in the CD4-/- compared to all other strains, but no differences were found in the percent mesenchyme

while CD8-/- mice showed a decrease in the percent of bone in comparison to wild-type. Overall these experiments conclude that T lymphocytes are not essential for normal fracture healing process.

Disclosures: ***J.M. Weaver****, None.*

SU416

Carotid Artery Plaque Thickness Is Associated with Increased Serum Calcium Levels: The Northern Manhattan Study. M. R. Rubin, T. Rundek*, H. S. Lee*, R. L. Sacco*, S. J. Silverberg. Columbia University College of Physicians & Surgeons, New York, NY, USA.

Symptomatic primary hyperparathyroidism (PHPT) is associated with vascular calcification and cardiovascular (CV) disease. In a large Swedish population study, higher serum calcium (CA) levels even within the normal range were found to be associated with increased CV mortality. However, it is unknown whether high-normal CA levels are associated with adverse vascular effects in a US population. Carotid atherosclerosis is an early marker of CV disease and predicts an increased risk of CV events. We investigated the association between CA levels and carotid atherosclerosis in a community-based population (The Northern Manhattan Study).

We studied 1,350 stroke-free subjects (age 67±9 years; 59% women; 55% Caribbean-Hispanic, 23% African-American, 22% Caucasian). CV risk factors included: hypertension (72%), diabetes mellitus (22%), pre-existing heart disease (20%), hypercholesterolemia (21%), current smoking (14%), and non-moderate alcohol consumption (63%). Atherosclerosis was measured by high-resolution B-mode ultrasonography as maximum carotid artery plaque thickness (MCPT). Mean CA was 9.09 ± 0.43 mg/dl (range 6.9-10.4; Q1 8.8, median 9.1, Q3 9.4). Eight subjects with elevated CA (>10.4 mg/dl) were excluded. 55% of subjects with normal CA had carotid plaque detected by ultrasound. CA level was greater among subjects with than without plaque (9.12 ± 0.43 vs. 9.06 ± 0.42, p=0.025). Greater MCPT was found in those in the highest quartile of CA compared to the lowest (1.18 vs. 0.83 mm; P<0.0001). In a multivariate regression model, each 1 mg/dl increase of CA was significantly associated with 0.16 mm increase in MCPT, after adjusting for age, gender, race, education and CV risk factors (p<0.01). There was no interaction between CA and other CV risk factors. When MCPT was treated as a categorical variable in which the highest quartile of >1.6 mm was defined as an "advanced premature atherosclerosis," the highest CA quartile vs. the lowest was more likely to be associated with advanced premature atherosclerosis (OR 1.6, 1.2-2.1).

In summary, higher levels of CA within the normal range are associated with increased carotid plaque thickness in a community dwelling elderly population. Although the role of CA in the development of atherosclerosis is unclear, possible mechanisms include vascular calcification, loss of arterial wall distensibility, and a weakening of the interface between the arterial wall and fibrous material. Further studies are needed to confirm the clinical correlates of this finding, and to determine the relevance of these observations to patients with mild hypercalcemia, such as those with asymptomatic PHPT.

Disclosures: ***M.R. Rubin****, None.*

SU417

Risk of Fluorosis in a Patient Treated with Fluoride for Otosclerosis. M. S. Parisi*, A. Bagur, B. Oliveri. Sección Osteopatías Médicas, Hospital de Clínicas, Univeridad de Buenos Aires, Buenos Aires, Argentina.

Since 1967 fluoride therapy has been used to arrest the progression of hearing loss in patients with cochlear otosclerosis. Long-term fluoride ingestion has been associated with a high incidence of dyspeptic symptoms. We describe the case of a 71 year old man who developed signs of fluorosis after 4 years of fluoride treatment, without receiving calcium supplement, for otosclerosis.

On presentation the patient presented high levels of alkaline phosphatase (ALP). He underwent surgical excision of prostatic adenoma 6 years prior to consultation. He was receiving 18mg/day of fluoride for treatment of Otosclerosis. Mean dietary calcium intake was about 500mg. No calcium supplement was administered. Laboratory tests revealed high levels of ALP(310IU/L, normal value: 68-240), bone ALP (BALP), urinary crosslaps (uCTX) and osteocalcin (BGP). Determination of prostatic specific antigen, testosterone, thyroid hormones and other parameters of bone mineral metabolism were normal. Bone scintigraphy showed an unspecific pattern. Plain radiographs evidenced coarse trabeculations. Lumbar spine BMD (LUNAR-DXA) was 1.178g/cm2 (t-score: -0.5, z-score: 0.0) and total skeleton BMD was 1.066g/cm2 (t-score: -1.9, z-score: -1.4). One month later the patient suffered a traumatic fracture of the tibia. Bone biopsy could not be performed. Immediate interruption of fluoride administration was indicated. Laboratory tests performed 6 months after interrupting fluoride treatment revealed normalization of laboratory test of bone metabolism.

This case report draws attention to the risk of fluorosis in patients treated with fluoride compounds without adequate calcium association.

Normal value	BALP (IU/L) 31-95	uCTX (ug/mmolCr) 10-400	BGP (ng/ml) 11-46
On presentation	145	1240	76
6 months after fluoride interruption	78	467	42

Disclosures: ***M.S. Parisi****, None.*

SU418

Bone Regeneration in Osteonecrosis of Rat Femoral Head. H. Kitahara*[1], K. Tokunaga[1], T. Ito*[1], M. Ito*[1], N. Amizuka[2], K. Oda*[3], N. Endo[1]. [1]Orthopedic surgery, Niigata University, Niigata City, Japan, [2]Oral Anatomy, Niigata University, Niigata City, Japan, [3]Biochemistry, Niigata University, Niigata City, Japan.

We established a rat osteonecrosis model by disrupting the blood supply to the femoral head. Pyknotic and eccentric osteocyte nuclei were observed and showed positive staining for TUNEL on day 2 after the induction of osteonecrosis. The osteocytic lacunae that contained these apoptotic cells became empty by day 21. These results suggest that apoptosis precedes osteonecrosis. On day 21, additional bone formation was identified in metaphyseal and neck regions. Cuboidal osteoblasts, lining newly formed bone, showed an intense signal for type I collagen mRNA and alkaline phosphatase (ALP) protein, whereas the mRNA expression of osteopontin and osteocalcin was weak, indicating that these cells were immature osteoblasts. Some cells intercalating into newly formed bone were immature osteoblasts, since they still expressed type I collagen mRNA. Additional bone formation was observed in all necrotic lesions by day 42, and the amount of newly formed bone increased with time. On day 42, the expression of osteopontin and osteocalcin mRNAs on plump osteoblasts was more intense than on day 21. These results suggest that additional bone formation is achieved mainly by immature osteoblasts. Osteoclasts accumulated at the boundary of viable and necrotic bone on day 7. Osteoclasts were detected only at newly formed bone surfaces but never at necrotic ones throughout the osteonecrotic development and regeneration. Osteoclasts possibly require bone surfaces to be covered with viable osteoblastic cells for resorption.

Disclosures: ***K. Tokunaga****, None.*

SU419

Impaired Bone Formation during Distraction Osteogenesis in ZDF Rats. Z. Liu*, J. Aronson*, E. C. Wahl*, L. Liu, D. S. Perrien, P. A. Kern*, J. L. Fowlkes, K. M. Thrailkill, R. C. Bunn*, L. J. Suva, R. A. Skinner*, C. K. Lumpkin. Pediatrics, Orthopedics and Physiology, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Increasing evidence suggests that impairment in bone formation and/or turnover is associated with metabolic problems in type 2 diabetes mellitus (T2DM). However, there are no studies that address bone regeneration and repair in T2DM reported in the literature. Using Zucker Diabetic Fatty (ZDF) rats, a model of T2DM, for tibial distraction osteogenesis (DO), we hypothesize that bone formation within the distraction gap will be significantly impaired. Male 10 week old ZDF rats (ZDF/Gemi-fa/fa, 360 g) and Zucker lean rat controls (ZDF/Gemi-lean, 280 g) (n=10 per group) were used in the study. All rats were examined for body weight, glycosuria and glycosemia to confirm the diabetic condition during the study. The rats received placement of the external fixators and osteotomies on the left tibia. Distraction was initiated the following day (1 day latency) at 0.2mm bid and continued for 14 days. The lengthened tibiae were harvested after sacrifice and distraction gaps were radiographically and histologically measured. Blood glucose levels in lean rats remained within the physiologic range at an average value of 108.9±5.1mg/dL. ZDF rats exhibited glycosuria (1000~2000 mg/dL) during the study but did not develop ketonuria (<5 mg/dL). Blood glucose levels for ZDF rats were above the physiologic range at a value of 346.7 ±21.2 mg/dL. Weight gain during the procedure was not significant in either group. The radiographic analysis showed a significant decrease in the area of mineralization of distraction gaps in ZDF rats when compared to the lean rats (31±4.1% vs 61±3.5%)(p<0.001), and also in the density of mineralization as well (25±1.8% vs 34±2.8%)(p<0.05). The histological analysis demonstrated that a significant deficiency of bone formation was found not only in endosteal bone formation (7.3±3.2% vs 42.2±7.7%)(p<0.001), but also in periosteal bone formation (5.3±1.8% vs 16±2.9%)(p<0.001), in ZDF rats versus the lean rats. The ZDF is an inbred rat model that spontaneously develops T2DM characterized by hyperglycemia, hyperinsulinemia, insulin resistance, and obesity in all males. Recent studies have revealed that skeletal complications have been found in ZDF rats, including periodontal disease and diabetic osteopenia. Our study has demonstrated significant decreases in new bone formation in the distraction gaps of ZDF rats, both radiographically and histologically. The mechanism of impaired bone formation in T2DM might be elucidated with further studies using this model.

Disclosures: ***Z. Liu****, None.*

SU420

Bone Mass and Mineral Metabolism in HIV Infected Postmenopausal Women. M. Yin*, J. Dobkin*, C. Becker, K. Brudney*, J. Zadel*, V. Addesso, M. Manandhar*, R. B. Staron*, E. Shane. Medicine, Columbia University, College of Physicians & Surgeons, New York, NY, USA.

Osteoporosis (OP) is a recently recognized complication of HIV infection and treatment. Hypothesizing that prevalence of OP is higher in HIV+ postmenopausal (PM) women, we evaluated 32 PM HIV+ women with respect to bone density (BMD) by DXA assessment at the lumbar spine (LS) and total hip (TH), established risk factors for OP, biochemical indices of mineral metabolism and serological makers of HIV infection. BMD was compared to data from 192 age and ethnicity matched PM women screened for OP on the same DXA machine. Univariate analysis was performed with Chi square and T tests and multivariate analysis with linear regression. Data are reported ± SD.

HIV+ subjects were aged 56±8 Y and PM by 10±8 Y. Duration of HIV was 8±3 Y. 72% were Hispanic and 25% African American. 88% had been exposed to antiretroviral therapy (ART) for 5±3 Y. By WHO criteria, OP at the LS was present in 41% of HIV+ and 22% of HIV- (p=.03). OP at the TH was present in 9% of HIV+ and 1% HIV- (p<.01). HIV status

remained a significant predictor of BMD after controlling for age, BMI, and ethnicity. Among HIV+, vitamin D insufficiency (10-20 ng/dl) and deficiency (<10 ng/dl) were present in 44% and 16%, respectively. Mean PTH level was normal (42±3 pg/ml), but 13% had frankly elevated PTH (>65 pg/ml) which was inversely correlated with 25-OHD (r=-.4, p=.02). Serum osteocalcin and NTX were elevated in 19% and 16% of subjects, respectively, and were highly correlated (r=.9, p<.01). Weight, time since menopause, history of PM use of HRT, and multivitamin use were all significant predictors of LS BMD, while duration or class of ART, nadir CD4, AIDS diagnosis, steroid use, and vitamin D deficiency were not.

In summary, prevalence of OP is considerably higher in HIV+ Hispanic and African American PM women than in age and ethnicity matched controls. Established OP risk factors were more important in predicting BMD than factors associated with HIV infection and ART. Vitamin D insufficiency and secondary hyperparathyroidism were common, but their role in HIV associated OP is unclear. Long-term management of the female HIV population should include evaluation for vitamin D deficiency and OP.

Disclosures: ***E. Shane****, None.*

SU421

Mast Cell -Release of Platelet-Derived Growth Factor -A in Patients with Paratrabecular Fibrosis. J. D. Sibonga[1], C. Y. Li*[2], R. T. Turner[1]. [1]Orthopedic Research, Mayo Clinic, Rochester, MN, USA, [2]Hematopathology, Mayo Clinic, Rochester, MN, USA.

A preclinical report suggests that some of the skeletal effects of hyperparathyroidism (HPT) – particularly paratrabecular fibrosis – are mediated by the induction of platelet-derived growth factor A (PDGF-A) in mast cells by continuously elevated parathyroid hormone (PTH). Reports dating more than 20 years have documented increased mast cells in patients with renal disease and in animal models for secondary HPT. Patients diagnosed with Systemic Mastocytosis may manifest skeletal effects seen in HPT patients. The skeletal impact of Systemic Mastocytosis may include presence of osteosclerotic lesions, osteoporosis and bone marrow fibrosis. These variable effects can be attributed to specific bone factors released by mast cell such as prostaglandin D2, histamine, heparin and PDGF-A. Thus, we investigated the similarities between mast cells in Systemic Mastocytosis and HPT by localizing PDGF-A in mastocytosis patients with bone marrow fibrosis. The availability of paraffin-embedded bone marrow biopsies in the Tissue Registry of the Mayo Clinic facilitated this investigation. For 87 cases of diagnosed mastocytosis from a period between 1994-2002, hematopathology, radiology, and serum total calcium assays were surveyed. For 26 reports of serum calcium, ten were hypocalcemic (<8.9 mg/dl) with the balance in the normal range (8.9-10.1 mg/dl). Immunohistochemical staining for PDGF-A was performed with a polyclonal antibody (Santa Cruz Biotechnology, CA) on 7 cases diagnosed with fibrosis or myelofibrosis. There was specific PDGF-A staining of mast cells in patients with paratrabecular fibrosis as confirmed by co-localization of the PDGF-A positive cells with tryptase-positive cells in serial sections. PDGF-A is a fibrogenic cytokine whose gene expression can be induced in mast cell cultures by phorbol esters or anti-IgE. In the rat model for HPT, peritrabecular fibrosis can be abrogated by inhibition of the PDGF-A receptor. The described clinical observation that mast cell-release of PDGF-A is associated with paratrabecular fibrosis in mastocytosis, as well as in models for HPT, suggests that the increase in mass cell number plays a role in the development of fibrosis in both diseases.

Disclosures: ***J.D. Sibonga****, None.*

SU422

Hind Limb Unloading Decreases Bone Formation in the Primary and Secondary Spongiosa of the Proximal Tibia in Young Male Rats. Y. Jia*. Faculty of Dentistry, University of Toronto, Toronto, ON, Canada.

Several lines of evidence indicate that the decrease in bone mass observed in humans and rats following short duration space flight or mechanical unloading is primarily caused by a disruption in the development of cells of the osteoblast lineage. Using the NASA model of hindlimb unloading (HU), we have previously shown that HU decreases the number of osteoprogenitors and cells with osteogenic potential in cell populations isolated from the proximal femur but has no effect on these parameters in the humerus, which is normally loaded in this model. To further evaluate the effects of HU on bone formation *in vivo* and the activity of osteoblasts and osteoclasts *in situ*, we conducted a 14-day HU experiment with 6 week old male Fisher rats (n=5/group) and analyzed the changes histomorphometrically and by using *in situ* hybridization to quantitate osteocalcin (OCN) and bone sialoprotein (BSP) mRNA in individual osteoblasts. In the proximal tibia four areas were analyzed: the primary spongiosa and three areas of the secondary spongiosa (I, II, and III respectively), each 1 mm in length, measured starting from the beginning of the secondary spongiosa. In HU rats, bone volume (BV/TV) and trabecular number (Tb.N) were decreased in both primary spongiosa (26% and 14% respectively, p<0.05) and secondary spongiosa I (48% and 34% respectively, p<0.05). No changes in BV/TV and Tb.N were observed in secondary spongiosa II and secondary spongiosa III. Trabecular thickness (Tb.Th) was decreased in the secondary spongiosa I only (22.5%, p<0.01). Trabecular separation (Tb.Sp) was increased only in the primary spongiosa (140% of control, p<0.01). No significant changes in osteoclastic parameters were seen in any of these areas. Using *in situ* hybridization, the amount of BSP mRNA per osteoblast (measured in the primary spongiosa) and of OCN mRNA per osteoblast (measured in endosteal cortical osteoblasts) did not change as a result of HU. In conclusion, we have shown that HU induces a decrease in bone formation parameters in the primary and secondary spongiosa and that unloading did not alter osteoclastic parameters or activity of individual osteoblasts. This indicates that the reduced amount of bone in the unloaded tibia resulted from a decrease in osteoblast numbers, which is consistent with the results from cell culture experiments that showed reduced CFU-O and CFU-AP numbers following HU.

Disclosures: ***Y. Jia****, None.*

SU423

Nicotine may not Be Detrimental to Lumbar Spinal Fusion. S. D. Sylvester*, M. M. Buchanan*, M. Scheel*, M. Veale*, E. S. Ackerman*, V. L. Kish*, N. B. Clovis*, J. C. France*, T. L. Norman*, N. Mukherjee. Orthopedics, West Virginia University, Morgantown, WV, USA.

Intertransverse process, lumbar spine fusion with autologous iliac crest bone using a posteriolateral approach (ITP-LSF-PL) is commonly performed to correct spinal instability in patients. An increased rate of bony non-union in ITP-LSF-PL is associated with patients who smoke (nicotine in blood: 10-70 ng/ml for smokers; in saliva- up to 1.56mg/ml for chewers). We developed a rabbit model in which the effects of nicotine on the outcome of ITP-LSF-PL were studied. 10.5 mg Nicotine patches affixed to the ear of New Zealand white rabbits were able to maintain blood levels of nicotine at 78±49 ng/ml for up to 5 weeks. Twenty rabbits (1 year, 3.9±0.5 kg) were randomly divided into two groups of 10: control and Nicotine. Both groups underwent a single level ITP- LSF- PL (Boden, *J Bone Joint Surg Am,* 1995). Five weeks later, the spines were harvested and assessed for fusion rate (blinded, manual palpation, 2 surgeons) and radiographic assessment (Silcox, *Spine*, 1998). The fusion rates of the nicotine group were significantly greater than control (66.0± 7.2% vs. 37.5± 12.5%, p<0.05) while they scored higher, but not significantly on the radiographic assessment scores (1.91±0.33 vs. 1.56 ±0.46). To explore this result further, we performed an in vitro study to assess the effects of nicotine on rabbit osteoblastic cells. Precursors of osteoblastic cells were harvested from the bone marrow of rabbits and induced to differentiate along the osteoblastic pathway using minimum essential medium supplemented with 40 ng/ml dexamethasone, 2.8e-4M Ascorbic Acid and 10 mM beta- glycerophosphate. After 1 week, nicotine was added (20, 40 and 80 ng/ml, and 10, 100, 250 μg/ml). Alkaline phosphatase activity was assessed at 1, 2, 3 and 4 weeks and von Kossa staining for deposited calcium was assessed at week 4. A 2 way (dose and time) ANOVA analysis revealed that 100 μg/ml of nicotine significantly enhanced both alkaline phosphatase activity (% area stain of 30.77±2.0 vs. 17.81±2.0, p<0.05) and von Kossa staining (% area stain of 16.6 ± 1.5 vs. 8.1±1.5, p<0.05) over controls. The other groups were not statistically different from control. The results of both the in vivo and in vitro studies suggest that nicotine alone may not be detrimental, even beneficial, to the outcome of ITP-LSF-PL in rabbits via stimulation of osteoblasts. Other factors in tobacco smoke may be responsible for the poor outcomes reported previously. Heeshcen, *Nat Med*, 2001 demonstrated that nicotine stimulates angiogenesis and this might also enhance bony fusion in ITP-LSF-PL. However, more data is needed before we can definitely say that nicotine enhances bone fusion in ITP-LSF-PL.

Disclosures: ***N. Mukherjee****, None.*

SU424

Bone Mass of Type I Iodothyronine 5´Deiodinase-Deficient Mice. C. R. Zaitune*[1], A. C. Bianco*[2], C. H. A. Gouveia[1]. [1]Department of Anatomy, University of Sao Paulo, Sao Paulo, Brazil, [2]Department of Medicine, Brigham and Women´s Hospital and Harvard Medical School, Boston, MA, USA.

C3H/HeJ (C3H) and C57BL/6J (B6) mice differ in their capacity to activate thyroxine (T4) to triiodothyronine (T3). C3H mice have lower mRNA expression, concentration and activity of type I iodothyronine 5´deiodinase (D1), one of two enzymes that convert T4 to T3. An increased serum T4 (due to reduced T4 clearance) compensates for the decrease in the T4 to T3 fractional conversion rate, resulting in normal serum T3 and gross phenotype. In order to investigate the bone mass development of C3H mice in situations of thyroid hormone (TH) deficiency and excess, 4-week old C3H and B6 female mice were grouped (n=6/group) as control (CON); hypothyroid [HYPO, induced by $NaClO_4^-$ and metimazole (P+MM)]; 1xT4 and 10xT4, P+MM-treated mice receiving ~1 physiological dose of T4 (1 μ g/100 g BW) or 10 times this dose/day for 16 weeks, respectively. Bone mineral density (BMD) of total body (TB), femur, tibia and lumbar spine (LS) and femoral and tibial lengths (FL and TL) were measured by DEXA every 4 weeks; BW was measured every week and body length (BL), every four weeks. C3H and B6 HYPO groups presented significantly lower BW, BL, FL and TL from the first to the 16th week of treatment when compared to CONs. 1xT4 and 10xT4 groups of C3H and B6 mice presented BW, BL, FL and TL values that were significantly lower than CON groups and significantly higher than HYPO groups practically during the whole study. In C3H animals (Fig.1A), hypothyroidism (hypo) impaired TB BMD gain and 1xT4 normalized it only after 16 weeks of treatment. In B6 animals (Fig. 1B), hypo limited BMD gain only after 12 weeks, which was normalized by treatment with 1xT4. The same pattern of BMD changes was seen in the other skeletal sites of both strains, except in LS of 1xT4 C3H animals, where the BMD was always significantly lower than CON. 10xT4 was deleterious to the BMD of all skeletal sites of both strains starting at four weeks of treatment. However, in C3H animals, 10xT4 treatment was deleterious to vertebral BMD only after two months treatment. These results show that the skeletons of C3H and B6 animals respond differently to TH excess and deficiency. In spite of the different genetic background of these two strains, these results suggest an important role of iodothyronine deiodination for bone mass development.

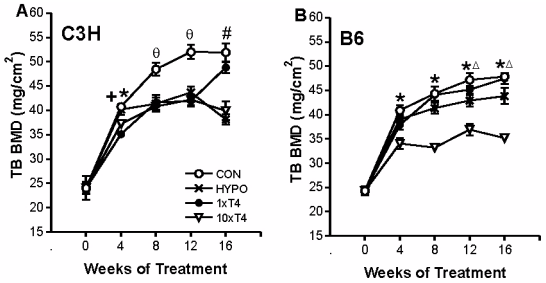


Fig 1. TB BMD of C3H (A) and B6 (B) mice. p<0.05 by Student-Newman-Keuls. ★, 10xT4 vs. all groups; Δ, HYPO vs. CON; ✚, 1xT4 vs. CON and HYPO; θ, CON vs. all groups; # CON and 1xT4 vs HYPO and 10xT4.

Disclosures: ***C.H.A. Gouveia****, None.*

SU425

Infra Red Spectroscopic Analysis of Osteomalacic Bones. D. Faibish[1], G. Boivin*[2], A. L. Boskey[1]. [1]Mineralized Tissue Laboratory, Hospital for Special Surgery, New York, NY, USA, [2]Faculte de Medecine, INSERM Unite 403, Lyon, France.

Osteomalacia is a pathological bone condition in which there is deficient primary mineralization of the matrix, leading to an accumulation of osteoid tissue and reduced mechanical strength. The collagen type and composition of collagen of osteomalacia patients is normal (Aust J Exp Biol Med Sci 62:309,1984). To test the hypothesis that there are qualitative and quantitative differences in osteomalacic bone tissue components, we examined 2 groups of PMMA embedded iliac crest biopsies using FTIR imaging (FTIRI). Controls were 7 female subjects, aged 36-57, without apparent bone disease. The experimental group were 3 female patients diagnosed with osteomalacia, aged 37-72. Two μm-thick sections were scanned using a FTIRI spectrometer. Parallel sections were stained using the von Kossa method for comparison. The spectroscopic parameters examined were previously established as sensitive to bone quality: mineral to matrix peak area ratio, 1660/1690 cm^{-1} peak ratio (collagen maturity) and the 1030/1020 cm^{-1} peak ratio (mineral crystallinity). Bone histomorphometry was examined on parallel sections using solochrome cyanin R stained 5 μm-thick sections and the degree of mineralization of bone (DMB) was measured on 100 μm-thick sections using quantitative microradiography (Calcif Tissue Int, 70:503, 2002; J Musc & Neur Int, 2:538, 2002).
In osteomalacic sections, calcified BV/TV and mineralization rate were decreased, while osteoid parameters were increased, revealing defective primary mineralization. In those sections, DMB measured in old bone (calcified tissue) was slightly higher (1.12 g/cm^3) than in 4 of the controls (1.00 g/cm^3), reflecting the normal evolution of the secondary mineralization of bone tissue. The FTIRI results were expressed both as color-coded images and as histograms of pixel population distribution. Pixel population means were compared with unpaired t-tests. Small differences were found in mineral/matrix ratios of whole bone surfaces in the cortical regions, 3.64±0.39 vs 3.02±0.50. Significant differences were found in the trabecular regions, 3.76±0.61 vs 2.06±0.79 (p=0.012), consistent with histological data. No differences in mineral content, crystallinity, or collagen maturity parameters were found when 400μ x 400μ areas of mineralized regions in osteomalacic and control specimens were compared. These findings agree with previous studies revealing no differences in the mineral quality of osteomalacic bone, and support the hypothesis that the inferior mechanical properties of osteomalacic bone originate in the delayed pattern of primary mineralization, and the resulting smaller amount of mineralized tissue.

Disclosures: ***D. Faibish****, None.*

SU426

Mechanism of Hypomineralization in Growth Plates of Alkaline Phosphatase Deficient Mice. H. Clarke Anderson[1], J. E. Sipe*[1], R. Dhanyamraju[1], N. Camacho[2], L. Hessle*[3], J. L. Millan[3]. [1]Pathology & Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, USA, [2]Hospital for Special Surgery, New York, NY, USA, [3]The Burnham Institute, La Jolla, CA, USA.

The presence of skeletal hypomineralization was confirmed in knockout mice lacking a functional tissue non-specific alkaline phosphatase (TNAP). In this study, a detailed characterization of the ultrastructural localization, the relative amount and crystallinity of mineral was carried out in tibial growth plates and metaphyseal bone of 10 day old TNAP knockout mice, for the first time. Alizarin red staining and micro-computerized tomography (micro-CT) confirmed a markedly decreased mineral density in the cartilage and bone matrix of TNAP-deficient mice. Transmission electron microscopy (TEM) showed diminished mineral in growth plate cartilage and in newly formed bone matrix. Although, mineral crystals were initiated normally within matrix vesicles (MVs) of growth plate and bone matrix of TNAP deficient mice, mineral crystal proliferation was reduced in the matrix surrounding MVs, as is the case in human hereditary hypophosphatasia. The failure of nascent mineral crystals to self nucleate and to proliferate beyond the protective confines of the MV membrane in TNAP deficiency may be caused by a peri-matrix vesicle build-up of mineral-inhibiting pyrophosphate (PPi). In normal cartilage and bone matrix, the adequate concentration of PPi is maintained by the hydrolytic activity of TNAP on the outer surfaces of matrix vesicles.

Disclosures: ***H. Clarke Anderson****, NIH NIDCR 2.*

SU427

Abnormal Translational Regulation of Renal 25-Hydroxyvitamin D-1α-hydroxylase Activity in the *Hyp*-Mouse. B. Yuan*, Y. Xing*, R. Veber*, M. K. Drezner. Medicine, University of Wisconsin, Madison, WI, USA.

Abnormally regulated renal 25-hydroxyvitamin D-1α-hydroxylase (1-OHylase) activity is a characteristic abnormality in *hyp*-mice, the murine homologue of X-linked hypophosphatemia. This defect manifests as normal enzyme activity in the basal state, despite marked hypophosphatemia, and limited responsivity of 1-OHylase activity to parathyroid hormone (PTH) stimulation. Although, we previously reported that the abnormal regulation of enzyme activity does not result from errant transcriptional regulation of 1-OHylase mRNA, the post-transcriptional defect causing aberrant enzyme function remains unknown. Therefore, we extended our studies to investigate if abnormal regulation of 1-OHylase translation underlies the dysfunctional enzyme activity. We compared 1-OHylase activity with mRNA (RNAse protection assay) and protein (Western blot) expression in renal homogenates from 6-8 week old normal and *hyp*-mice. As documented previously, renal 1-OHylase activity in the normal and *hyp*-mice in the basal state is no different (4.8±0.7 vs 5.3±0.8 fmoles/mg/min), while, in accord with hypophosphatemia, mutants manifest significantly enhanced renal 1-OHylase mRNA (4.5±0.4 vs 2.7±0.3 PSL U; p<0.01). However, despite the increase in mRNA, in the present study Western blots reveal that 1-OHylase protein in the *hyp*-mouse kidney is no different from that in normals (0.9±0.3 vs 1.0±0.8 RDU). In accord, as previously reported, we found that PTH stimulation of normal and *hyp*-mice similarly increases 1-OHylase mRNA expression, compared to that in unstimulated corresponding animals, (3.2±0.5 vs 2.9±0.3 fold) but failed to enhance enzyme activity in the mutants to levels achieved in normals (6.9±0.5 vs 15.3±0.9 fmoles/mg/min; p<0.001). As shown in the present study, the disparate enzyme responsivity reflects the inability of PTH to stimulate 1-OHylase protein in *hyp*-mice (0.9± 0.3 vs 0.9±0.2) compared to the significant enhancement observed in normal mice (1.08±0.8 vs 2.3±0.4; p<0.05). Collectively, these observations indicate that the failure of hypophosphatemia and PTH to normally enhance 1-OHylase in *hyp*-mice is the result of aberrant translational control of enzyme function, likely due to the abnormal phosphate-depleted intracellular milieu in the proximal convoluted tubule.

Disclosures: ***B. Yuan****, None.*

SU428

Self-actualized Perceptions of X-Linked Hypophosphatemia Suggest a Pro-active role for Patient-Support Networks in Managing Patients with this Rare Metabolic Bone Disorder. L. Winger*, J. Reed*, S. Schmitz*, E. M. Jacobson. The XLH Network, Bowie, MD, USA.

A survey of families with XLH(1) indicated that 1/3 have little comprehension of the ramifications of their disorder. An internet-based patient-support network has since developed, which seeks with timely information to enfranchise and empower people affected by XLH. Enquirers join if a family member is professionally diagnosed with XLH. We asked whether this network (recognizing the potential for bias towards severely affected cases) could help to characterize a population of adults with XLH that is beyond the assessment capacity of major metropolitan medical centers. In a rapid email poll, 121 responses of self-identified adults with XLH were elicited and grouped according to age, gender and height. By contrast, other studies assessed 57(1), 22(2), or 16(3) XLH adults. Average (modal, normal distribution, 30 respondents) male height in the XLH Network poll was 5'5" (UK national average 5'9") while average female height (91 respondents) was 5'0" (UK national average 5'4"). Of the females, 1/3 were at or below 4'8". We observed that profoundly short stature was not reported by respondents aged 18-25 years, though the number in this cohort was low (10 female, 5 male). This poll may reinforce the perception of members that short stature is a manifest consequence of XLH, while subtly emphasizing, to parents of newly diagnosed children, the potential for improved height provided by optimal therapeutic compliance (calcitriol and phosphate) since the '80s. A separate poll revealed that 26 of 49 adult respondents were not taking medication for XLH; clinical observations (2,3) that osteomalacia with bone pain may be alleviated by a return to these medications, are regularly discussed in the XLH Network. Continuing anecdotal presentation of problems associated with XLH in adulthood: spinal stenosis; Meniere's disease; enthesopathies; knee and joint pain; provides clear evidence that XLH is not only a childhood disorder, but can have important ramifications throughout adulthood, which may require a pro-active approach with clinicians.
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Disclosures: ***L. Winger****, None.*

SU429

The Phosphaturic Effect of Secreted Frizzled Related Protein-4 (sFRP-4) Is Independent of Parathyroid Hormone. T. Berndt*[1], T. A. Craig*[1], A. E. Bowe*[2], J. Vassiliadis*[2], D. Reczek*[2], R. Finnegan*[2], S. M. Jan De Beur[3], S. C. Schiavi[2], R. Kumar[1]. [1]Medicine, Mayo Clinic, Rochester, MN, USA, [2]Receptor Ligand Therapeutics, Genzyme Corporation, Framingham, MA, USA, [3]Division of Endocrinology, Johns Hopkins University, Baltimore, MD, USA.

Patients exhibiting tumor-induced osteomalacia (TIO) elaborate a circulating factor known as "phosphatonin" which increases phosphate excretion. Recent studies have identified sFRP-4 as a potential tumor derived phosphaturic factor. The present studies were performed to determine whether the phosphaturic effect of sFRP-4 is PTH-dependent. Normal or acutely thyroparathyroidectomized (TPTX) rats were anesthetized and prepared for clearance studies. After a two-hour equilibration period, either vehicle or sFRP-4 (0.3

micrograms/kg/hr) was infused intravenously. After a one-hour equilibration period, another clearance was taken. In vehicle infused rats with intact parathyroid glands, the fractional excretion of phosphate (FEPi) was stable (FEPi 14 ± 3% to 18 ± 2%, n=7, NS.) Infusion of sFRP-4 resulted in a significant threefold increase in the FEPi from 14 ± 2% to 34 ± 5%, (n=10, p<0.05). Urinary cAMP excretion or calcium excretion did not change in either group. In TPTX rats, effective TPTX was confirmed by a significant reduction in basal FEPi, an increase in basal FECa and hypocalcemia. FEPi was stable throughout the experiment in the vehicle treated animals (FEPi 0.4 ± 0.1 to 0.4 ± 0.2% (n=6). Infusion of sFRP-4 resulted in a threefold increase in the FEPi from 1.0 ± 0.3% to 3.8 ± 1.2% (n=10, p<0.05). Sodium and calcium excretions did not change. Thus, sFRP-4 does not increase phosphate excretion via parathyroid hormone dependent pathways. We conclude that sFRP-4, which is over expressed in tumors associated with renal phosphate wasting, specifically increases phosphate excretion in vivo in a PTH independent manner and has the biological properties of "phosphatonin".

Disclosures: ***T. Berndt****, Genzyme Corporation 2.*

SU430

FGF-23 in Chronic Kidney Disease and Post Renal Transplantation. S. Pande*[1], C. S. Ritter*[1], S. C. Schiavi[2], A. J. Brown[1]. [1]Renal division, Washington University School Of Medicine, St louis, MO, USA, [2]Dept of Applied Genomics, Genzyme, Farmingham, MA, USA.

FGF-23 is a 30 kDa-secreted protein believed to directly inhibit phosphate transport through a PTH independent pathway. Little is known about its role in normal phosphate homeostasis or the factors regulating this proposed phosphatonin. We hypothesized that the hyperphosphatemia seen in chronic kidney disease (CKD) could elevate FGF-23 levels, and that post transplant hypophosphatemia in renal transplant patients is driven by persistent elevation in FGF-23 levels. To test these hypotheses, we examined FGF-23 levels in patients with chronic kidney disease and renal transplant patients. In patients with CKD (n=41), FGF–23 levels, creatinine clearance, serum creatinine, phosphorus and PTH were measured. In renal transplant patients (n=10) FGF-23, seum creatinine and phosphorus were measured just before and 4-5 days after transplantation.

In the CKD patients, there were significant correlations between FGF-23 levels and creatinine clearance (r = -0.5537, P=0.0002), and between FGF-23 and serum phosphorus (r = 0.5885, P value< 0.0001). FGF-23 levels were in the normal range in CKD patients with creatinine clearance >50 ml/min. No correlations were found between FGF-23 and PTH or serum calcium. In kidney transplant patients, by 4-5 days post transplant there were substantial decreases in FGF-23 levels (-88.8 +/- 5.4%), serum phosphorus (-64 +/- 10.2%) and serum creatinine (-76.6 +/- 11.5%). In those patients with post-transplant hypophosphatemia (serum phosphorus < 2 mg/dl), there was a significant correlation between serum phosphorus and FGF-23 (r = -0.8128, P < 0.05).

In conclusion, an increase in FGF-23 levels in CKD patients was associated with decreasing creatinine clearance and increasing serum phosphorus. Despite dramatic decreases in FGF-23 levels in all post transplant patients, persistently elevated levels of FGF-23 were observed in the hypophosphatemic patients that correlated with serum phosphorus. This suggests a role of FGF-23 in post-transplant hypophosphatemia.

Disclosures: ***S. Pande****, None.*

SU431

Mice Transgenic for Fibroblast Growth Factor 23 Exhibit Growth Retardation, Osteomalacia and Disturbed Calcium/Phosphate Homeostasis. T. E. M. Larsson*[1], R. Marsell*[2], E. Schipani[3], C. Ohlsson[4], H. S. Tenenhouse[5], Ö. Ljunggren[1], H. Jueppner[3], K. B. Jonsson[2]. [1]Medical Sciences, University of Uppsala, Uppsala, Sweden, [2]Surgical Sciences, University of Uppsala, Uppsala, Sweden, [3]Endocrine Unit, MGH, Boston, MA, USA, [4]Internal Medicine, University of Gothenburg, Göteborg, Sweden, [5]Montreal Children's Hospital, Montreal, PQ, Canada.

Current evidence indicates that Fibroblast Growth Factor 23 (FGF-23) plays an important role in the regulation of phosphate homeostasis and vitamin D metabolism. In order to investigate the effects of FGF-23 on the bone mineralization process, we generated mice transgenic for human wild-type FGF-23 and for FGF-23 containing the R176Q mutation identified in patients with autosomal dominant hypophosphatemic rickets. FGF-23 expression was driven by the human alpha I collagen promoter directing expression to osteoblasts.

Transgenic mice expresssing the wild-type FGF-23 cDNA showed retarded growth potential (male body weight at eight weeks 17.5±1.4 vs 24.2±1.7 g). At eight weeks, serum phosphate levels in mutant animals were reduced compared to those in normal littermates (phosphate 1.64±0.18 vs 2.75±0.22 mmol/l). Urinary phosphate levels relative to urinary creatinine levels were increased (Pi/creatinine ratio 11.4 vs 7.2). PTH levels were also raised (346±49 vs 159±56 ng/l) indicating development of secondary hyperparathyroidism. However, no difference in serum $1,25(OH)_2VitD_3$ (234±12 vs 261±22 ng/l) or calcium was observed (2.01±0.07 vs 2.17±0.10 mmol/l).Protein and mRNA expression of the renal phosphate transporter Npt2a, which is believed to be responsible for the bulk of tubular phosphate reabsorption, were reduced. Furthermore, Npt1 and Npt2c renal mRNA levels were also reduced, as was expression of the PTH/PTHrP receptor. Renal expression of the 24-hydroxylase mRNA was increased whereas 1-hydroxylase mRNA levels were unchanged. Histological analysis of tibiae revealed a highly disorganized and widened growth plate ; in addition the histological findings were consistent with a picture of increased osteoblast and osteoclast activity. DXA and pQCT analysis revealed severely reduced BMD. R176Q transgenic mice exhibited a more severe phenotype than the wild-type FGF-23 transgenes and had reduces fertility. Interestingly, circulating levels of intact FGF-23 were higher compared to those of wild-type FGF-23 transgenes, possibly due to increased resistance to proteolytic cleavage.

This transgenic model will provide further opportunity to study phosphate/calcium homeostasis and to determine whether the ostomalacia is caused only by the observed changes in mineral ion homeostasis or is a consequence of local bone effects of FGF-23.

Disclosures: ***T.E.M. Larsson****, None.*

SU432

Osteoid Water Content and Porosity Increased in Hypomineralized Cortical Bone in an Animal Model of Osteomalacia. M. A. Fernandez-Seara*[1], A. C. Wright*[1], S. L. Wehrli*[2], P. Saha*[1], F. W. Wehrli[1]. [1]Radiology, University of Pennsylvania Medical Center, Philadelphia, PA, USA, [2]NMR Core Facility, Children Hospital, Philadelphia, PA, USA.

Osteomalacia is a defect of osteoid mineralization resulting from inadequate calcium or phosphorus deposition onto the matrix. We previously showed in a rabbit model of osteomalacia that the decrease in mineral is paralleled by an increase in osteoid water as measured by nuclear magnetic resonance (NMR). Further, we found strong inverse correlations between osteoid water and bone mechanical properties (ultimate strength and Young's modulus), suggesting that water content could potentially be used as a predictor of bone intrinsic strength. The purpose of this study was to investigate whether the increased water content in osteomalacia is paralleled by increased cortical porosity. We used a subset of the bone samples from a previous study, in which hypomineralization of the skeleton was induced in a group of rabbits (treatment, TR) via a low phosphorus diet (0.09%). A control (CO) group received the diet supplemented with sodium phosphate to normal phosphorus levels (0.5%). After 8 wks the animals were sacrificed and cortical specimens were cut form the tibial mid-shaft. Five of the 11 specimens of the prior study (3 TR and 2 CO) were imaged at 17 μm (isotropic resolution) using a micro-CT specimen scanner (EVS Corp MS-9; scan parameters: 80 kVp voltage with a 40 mil Al filter, 360° acquisition with 721 views at 0.5° increments, 3 averages, scan time = 3.5 hrs). Although not fully resolved, the images clearly showed the presence of pores. Both BMD and porosity were quantified in the center of the samples over a circumscribed cubic volume (~1 mm^3). For BMD, gray scale values were calibrated using a bone-equivalent phantom. Porosity was measured using a two-steps algorithm. After correcting for intensity inhomogeneity, an expected bone intensity distribution was computed by fitting a Gaussian whose mean was estimated as the mode of the deshaded intensity histogram and the standard deviation was derived from the intensities higher than the mean, since these intensities had maximum probability of representing pure bone voxels. Porosity was computed for every voxel as 1 minus the probability of a voxel to contain bone, calculated from this gaussian distribution. As expected, osteomalacic bone had lower BMD than normal bone (1168.7±43.1 vs. 1360.1±65.7 mg/cc, p=0.03). Porosity was higher in the TR than in the CO group (38.0±6.1 vs. 24.5±0.7 %, p=0.06). There was a strong correlation between porosity and NMR-derived water volume fraction (r^2=0.83, p=0.03). Finally, an inverse correlation was found between cortical porosity and bone's ultimate strength (r^2=0.79, p=0.04).

Disclosures: ***F.W. Wehrli****, None.*

SU433

Pamidronate: First Choice Treatment for Hypercalcemia in Neonatal Subcutaneous Fat Necrosis. N. Alos*[1], M. Fillion*[1], J. Powell*[2], G. Chabot[1]. [1]Pediatrics, Hôpital Sainte-Justine, Montreal, PQ, Canada, [2]Dermatology, Hôpital Sainte-Justine, Montreal, PQ, Canada.

Background: Subcutaneous fat necrosis (SCFN) of the newborn is an uncommon disorder which occurs in the first weeks of life in full term neonates after fetal distress. It is characterized by firm blue/purple skin nodules and is sometimes accompanied by potentially life-threatening hypercalcemia. Treatments have included hydration, furosemide and corticosteroids. To date, only one report has described the use of intraveneous bisphosphonates for this condition. In newborns with osteogenesis imperfecta (OI), pamidronate has been safely administrated. We suggest that low dose pamidronate could be the first choice therapy for hypercalcemia in SCFN.

Patients and results: Three full-term newborns presented with SCFN in 2001-2002. Two were born via caesarean section for severe fetal distress. The severity of the clinical presentation was variable but likely related to the delay in diagnosis; exquisitely tender subcutaneous lesions (case 1), non-tender subcutaneous lesions with failure to thrive (case 2) and non-tender subcutaneous lesions, failure to thrive and renal failure (case 3). Despite treatment with IV fluids and furosemide for 48-72 h, Ca levels remained high in the 3 cases. The patients were given 3 to 4 doses of pamidronate over a period of 5-20 days. Ca levels decreased within 12-24h. Further use of furosemide or corticosteroids was not needed. Low calcium diet did not change the evolution of the hypercalcemia. No significant nephrocalcinosis was observed.

Case	Sex	Onset of lesions	Onset of hypercalcemia	Peak Ca tot./ ionized (mmol/l)	First dose of pamidronate	Dose (mg/kg)
1	F	Day 1	Day 13	2.76/1.56	Day 18	0.25x2 ; 0.5x2
2	F	Day 1	Day 6	3.49/1.72	Day 33	0.25x3
3	M	Day 35	Day 42	3.98/2.28	Day 45	0.25x4

Conclusion: In our experience, low dose pamidronate is effective, well tolerated and obviates the need for prolonged treatment with furosemide and corticosteroids. Newborns with fetal distress are at risk for the development of SCFN with hypercalcemia. They should be carefully monitored for this potential complication. However, further studies are needed to evaluate the impact of pamidronate on the natural history of SCFN and on its physiopathological mechanisms.

Disclosures: ***N. Alos****, None.*

SU434

Spinal Changes Are Common in Children Assessed for Secondary Osteoporosis. O. Makitie*[1], F. Henriques*[1], A. Doria*[2], S. Compeyrot*[3], W. G. Cole*[4], D. Gilday*[2], R. Laxer*[3], E. Silverman*[3], A. Daneman*[2], E. Sochett[1]. [1]Division of Endocrinology, The Hospital for Sick Children, Toronto, ON, Canada, [2]Diagnostic Imaging, The Hospital for Sick Children, Toronto, ON, Canada, [3]Division of Rheumatology, The Hospital for Sick Children, Toronto, ON, Canada, [4]Division of Orthopaedics, The Hospital for Sick Children, Toronto, ON, Canada.

Osteoporosis is increasingly reported in chronically ill children, who because of medications, nutrient and hormone deficiencies, and decreased physical activity may be predisposed to impaired bone health. The significance of subnormal bone mineral density (BMD) in many of these patients is not clear due to impaired growth and delayed bone maturation. Furthermore, bone quality and microarchitecture may be disturbed even in the presence of normal BMD.

The aim of this study was to assess whether spinal radiographs could provide additional information on bone quality and help to determine the significance of reduced BMD in chronically ill children.

Patients assessed at the Osteoporosis clinic, The Hospital for Sick Children, Toronto, between January 2002 – February 2003 for suspected secondary osteoporosis were eligible for inclusion. Data on previous fracture history and back pain were collected from hospital records. BMD (GE-Lunar Prodigy) z-scores for the lumbar spine (L2-L4) were used for the analysis. Lateral spinal radiographs were reviewed independently by two pediatric radiologists for the presence of vertebral body deformities; these were classified as mild, moderate or severe by the Kleerekoper radiographic method modified for pediatric use.

Thirty-two patients (17 males), median age 13.5 years (5.0 – 17.8 yrs) were included. The median BMD z-score for the 32 patients was -2.7 SDS (-8.0 – -0.8 SDS). Thirteen patients (41%) had compression fractures (=moderate to severe spinal changes), 17 patients (53%) mild spinal changes and only two patients (6%), normal spinal X-rays. The characteristics of these patients are shown in Table 1.

The results suggest that 1) clinically significant secondary osteoporosis is common among chronically ill children, 2) compression fractures are frequent in this patient population, 3) BMD, clinical symptoms or previous fracture history are not good predictors of spinal changes, and that 3) earlier pharmacological intervention may be justified.

Characteristics of the patients with spinal changes

Radiographic findings	No or mild spinal changes	Moderate to severe spinal changes
N	19 (59%)	13 (41%)
median BMD	-2.7 SDS	-2.9 SDS
BMD < -3.0 SDS	7 (37%)	5 (38%)
BMD > -2.0 SDS	3 (16%)	2 (15%)
Back pain	4 (21%)	2 (15%)
Previous non-vertebral fractures	3 (16%)	1 (8%)

Disclosures: ***O. Makitie****, None.*

SU435

Low Serum Levels of 25-OH-Vitamin D in Adult Thalassaemic Patients. E. Carmina*[1], G. Di Fede*[1], R. Malizia*[2], M. Capra*[3], N. Napoli[1], G. Cusumano*[1], G. B. Rini*[1]. [1]Department of Clinical Medicine, University of Palermo, Palermo, Italy, [2]Department of Hereditary Blood Diseases, Villa Sofia Hospital, Palermo, Italy, [3]Department of Hereditary Blood Diseases, Children's Hospital, Palermo, Italy.

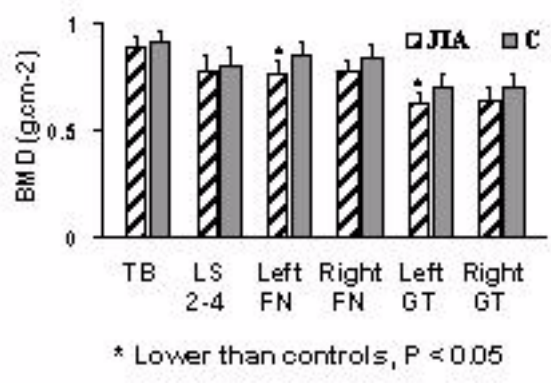
Adult thalassaemic patients present a reduced bone mass that is determined by several different mechanisms affecting bone turnover. To evaluate if a deficiency of vitamin D may contribute to the low bone mass of adult thalassaemic subjects, we studied serum 25-OH-vitamin D in 71 adult patients with thalassaemia major (mean age 27.2 ± 1 years, range 21 and 41 years). 50 normal subjects of similar age and BMI were used as controls. The thalassaemic patients had been put on regular transfusions within the age of 2 years and, compared to controls, presented increased ($p< 0.01$) serum ferritin, glutamic oxalacetic transaminase, glutamic piruvic transaminase and low ($p< 0.01$) bone density (L1-L4 z score – 2.07 ± 0.2). Mean 25-OH-vitamin D was significantly ($p<0.05$) lower in thalassaemic patients (23.1 ± 0.9 ng/ml) than in normal controls (26.4 ± 0.9 ng/ml). An absolute (<mean -2DS of control values, serum levels less than 17.2 ng/ml) deficiency of 25-OH-vitamin D was found in 24% of thalassaemic patients but not in normal controls. A relative (< mean -1DS of control values, serum levels less than 21.8 ng/ml) deficiency was found in 15% of thalassaemic patients but also in 10% of normal controls. Totally, 39% of adult thalassaemic patients presented a deficiency of 25-OH-vitamin D. In thalassaemic patients, but not in normal subjects, 25-OH-vitamin D correlated negatively with age ($p<0.05$) and with serum ferritin ($p<0.05$). In conclusion, adult thalassaemic patients frequently present a reduced hydroxylation of vitamin D that may be consequence of the progressive liver damage determined by iron overload.

Disclosures: ***E. Carmina****, None.*

SU436

Low Bone Mineral Density in Children with Juvenile Idiopathic Arthritis might Be Related to Disuse and Be Prevented by Weight-bearing Exercise. N. J. Farpour-Lambert*[1], L. M. Keller-Marchand*[1], D. Hans[2], R. Rizzoli[3], M. F. Hofer*[4]. [1]Pediatrics, University Hospital, Geneva, Switzerland, [2]Nuclear Medicine, University Hospital, Geneva, Switzerland, [3]Bone Diseases, University Hospital, Geneva, Switzerland, [4]Multisite Center for Pediatric Rheumatology, Geneva and Lausanne, Switzerland.

Juvenile idiopathic arthritis (JIA) is associated with low bone mineral density (BMD). Physical activity during childhood is generally thought to increase bone mass. The purpose of this study was to determine areal BMD (aBMD), heel speed of sound (SOS) and broadband ultrasound attenuation (BUA) in children with JIA, and the relationships to severity of disease, physical activity and fitness, muscle strength, calcium intake, and corticosteroid use. This case-control study included 2 groups of 24 subjects aged 5 to 16 years (10.13 +/- 2.70 SD): JIA children and controls, matched for gender, age, height, weight and pubertal stage. Measures included total body (TB), lumbar spine (L2-4), left and right femoral neck (FN) and greater trochanter (GT) aBMD by DXA (Lunar Prodigy); heel SOS and BUA by quantitative ultrasound (Lunar Achilles +); physical activity level and aerobic fitness (VO2peak); knee flexion and extension peak torque; body composition (DXA); calcium intake; joint count, pain and disease activity; erythrocyte sedimentation rate (ESR) and serum C-reactive protein level. There were no significant differences among groups for age, height, weight, pubertal stage, body composition, physical activity, VO2peak, muscle strength or calcium intake. Only one of them was treated by systemic steroids. In JIA subjects, the left side was more often affected than the right one. Twelve (50%), 4 (17%) and 8 (33%) children had arthritis at the left, right and both legs, respectively. BMD results are shown in the figure below.



Left FN and GT aBMD, and left SOS were significantly lower in JIA, compared to controls. In JIA subjects, aBMD at all sites correlated positively ($P<0.05$) with physical activity, VO2peak, muscle strength and lean tissue mass, and negatively with joint count, ESR, pain and disease activity. Results indicated a lower BMD on the side more affected by the joint disease, in relation with pain and altered joint function. The positive correlation with physical activity would suggest to envisage weight-bearing exercise intervention in this population.

Disclosures: ***N.J. Farpour-Lambert****, None.*

SU437

Body Composition and Bone Characteristics After Completion of Intensive Chemotherapy in Children with Acute Lymphoblastic Leukemia (ALL). J. L. Quick*, R. VanOrden*, C. Bruggers*, L. J. Moyer-Mileur. Pediatrics, University of Utah, Salt Lake City, UT, USA.

Background: Diminished lumbar spine bone mineral density (BMD, mg/cm2) has been reported during therapy in children with ALL. Measurement of bone characteristics during the early stages of cancer therapy, however, has been limited to dual energy x-ray absorptiometry (DXA). DXA measurement in children does not account for bone size or shape or the child's height and weight. Peripheral quantitative computed tomography (pQCT)accounts for bone size and geometry, measures true volumetric density (vBMD, mg/cm3) and has been validated in children. Objective: To evaluate bone characteristics measured by pQCT and DXA in children with standard risk ALL following intensive chemotherapy. Design/Methods: A cross-section study of 8 children ages 4-9 y (4F/4M) with standard risk ALL was performed. Subjects were recruited form the Hematology/Oncology Clinical, Primary Children's Medical Center, Salt Lake City, UT. Measurements were obtained at the completion of intensive therapy. Duration of intensive treatment was 10.6+/-3.5 months (7-17 months). Measurements of the tibia by pQCT (XCT2000, Orthometrix) to assess cortical and trabecular bone compartments, bone size and strength; whole body, hip, and spine by dual energy x-ray (DXA, Hologic QDR4500A) for body composition and bone characteristics were obtained. A regional healthy reference (n=68, 38M/30F) was used for comparison. Anthropometrics and medical, diet, and physical activity histories were also collected. ANCOVA controlling for age, gender, and body size was used for comparison of ALL to reference values. Results: Tibia trabecular and cortical BMC (mg)and vBMD (mg/cm3), cortical thickness (mm), total hip areal BMD (g/cm2) and BMAD (g/cm3/2), and whole body BMC in relation to fat-free mass were significantly lower in ALL children ($p<0.03$). (Table) Body weight, height, tibia bone strength and lumbar spine BMD were not significantly different. Conclusions: Children treated for ALL experience alterations in both trabecular and cortical bone compartments. Overall, bone

size and mineral deposition was diminished at the completion of intensive chemotherapy in children with standard risk ALL.

ALL vs. Reference

	ALL (n=8)	Reference (n=68)	p
Trab vMBD (mg/cm3)	148.5 +/- 37.2	260.0 +/- 52.1	0.000
Cort BMC (mg)	88.1 +/- 23.1	140.5 +/- 41.3	0.04
Cort Thickness (mm)	1.9 +/- 0.3	2.2 +/- 0.5	0.002
Hip BMAD (g/cm2/3)	0.065 +/- 0.016	0.071 +/- 0.013	0.02
WBBMC:FFM	0.024 +/- 0.007	0.028 +/- 0.005	0.03

Disclosures: ***J.L. Quick****, None.*

SU438

A Model for the Assessment of Bone Development in the Juvenile Rat. C. Liu[1], J. E. Ridings*[2], R. Leininger*[1], V. Shen[1], D. Clerkin*[2], S. Iqbal*[2]. [1]SkeleTech, Inc., Bothell, WA, USA, [2]Covance Laboratories, Harrogate, United Kingdom.

Toxicology studies in juvenile rats are often used to support the development of new pharmaceuticals for pediatric use. However, most of these investigations focus on major organ systems and generally do not account for adverse pharmacological effects on skeletal growth and development. Furthermore, while techniques for the evaluation of the adult skeleton are well established, little is known of the application of these techniques to juvenile animals. The objective of this study was to evaluate the utility of standard research techniques to the assessment of bone development in the juvenile rat.

Eight rats with offspring at 3 days of age were obtained for this study. Each parental female and litter were housed individually and given access *ad libitum* to water and food. Offspring within each litter were allocated to study groups on Day 10 *post partum* using a split litter design with representatives of each group and sex in each litter. Day 10 is the earliest age deemed acceptable for experimentation in terms of evaluating normal bone development. Five males and five females each were sacrificed and bone tissues collected on 10, 17, 24 and 31 days *post partum*. Longitudinal bone growth, strength, density and morphology were evaluated.

The findings are: 1) Femoral length increased almost linearly as animals aged. Longitudinal growth rate, as evaluated by calcein labels, began to decrease only after weaning on Day 21 *post partum*. 2) Bone mineral content was very low at Day 10 *post partum*. Bone area and density at both the trabecular bone-rich and cortical bone-rich sites of the femur increased as the animals aged. At the cortical bone-rich site, periosteal expansion was greater than endosteal expansion leading to an increase in cortical thickness. 3) Extrinsic mechanical strength of the femoral shaft reflected the increases seen in bone mineral content, area and density. 4) Epiphysis of the tibia was under developed early *post partum* and only assumed an adult appearance at Day 31 *post partum*. The height of growth plate remained constant, but the site of new bone formation, primary spongiosa of the proximal tibia, increased continuously in the first month of animal life. 5) Timing of the development at the metaphysis was similar to the epiphysis. 6) Overall, there appeared to be no sex differences in any of the measured parameters, with the exception of the trabecular bone volume at the metaphysis of the tibia. Male animals approached a plateau at Day 17 *post partum*, while the females continued to accrue trabecular bone. Our results suggest currently available techniques can be used to evaluate bone growth in juvenile rats from 10 days of age with an adult appearance being evident at 31 days of age.

Disclosures: ***C. Liu****, None.*

SU439

Bone Health in Children with Alagille Syndrome. I. E. Olsen[1], R. F. Ittenbach*[2], A. J. Rovner*[1], M. B. Leonard[1], A. E. Mulberg*[1], V. A. Stallings*[1], D. A. Piccoli*[1], B. S. Zemel[1]. [1]GI and Nutrition, The Children's Hospital of Philadelphia, Phila, PA, USA, [2]Biostatistics and Epidemiology, The Children's Hospital of Philadelphia, Phila, PA, USA.

AGS is an autosomal dominant disorder defined by bile duct paucity and an array of additional features that include chronic cholestasis and skeletal abnormalities. Growth failure and bone fractures are common in children with AGS. Because of altered body size, clinical evaluation of bone health by DXA in these children is difficult. This study's primary goal was to evaluate DXA measures of bone health in children with AGS compared to healthy controls, adjusting for short stature. Pre-pubertal children with AGS and similarly aged controls were evaluated at The Children's Hospital of Philadelphia. Whole body (WB) and AP spine (SP) scans were obtained on a Hologic QDR 2000 DXA (Bedford, MA) in array mode for assessment of total bone area (BA) and bone mineral content (BMC). Z-scores for weight (WAZ) and height (HAZ) were based on CDC growth charts. Comparisons of growth and bone status were made between groups using Student's t-test and chi-square test of significance. Multiple linear regression models were developed using the natural logs of BA, BMC and height. Additional measures of disease severity (e.g. selected lab tests, coefficient of fecal fat absorption) were also tested as predictors of bone status in the AGS group, as available. Twenty-eight children with AGS and 80 controls were similar for age (8.0 ± 2.4 vs 8.8 ± 2.5 yr) and maturation (all Tanner Stage 1 or 2); 90% were Caucasian. The AGS group had lower HAZ (-1.97 ± 1.30 vs 0.18 ± 0.83) and WAZ (-1.64 ± 1.68 vs 0.24 ± 0.94) than controls ($p < 0.001$). All bone measures (BA and BMC for WB and SP scans) were lower in the AGS group compared to controls (all $p < 0.03$). After adjusting for height, WB-BA and WB-BMC in the AGS group remained lower than controls ($p < 0.0001$). In the AGS group, the strongest predictor of WB and SP-BMC was height. In addition, after adjusting for height, the disease-related factors that showed strong inverse associations with WB and SP-BMC included: degree of fat malabsorption ($p < 0.01$) and cholestasis as indicated by serum bilirubin ($p < 0.005$) and cholesterol ($p < 0.05$), but not measures of synthetic function such as albumin or prothrombin time. Overall, the bones of children with AGS were smaller and less mineralized than those of healthy children, even after adjusting for short stature. Although our results were limited by the sample size, this approach to evaluating pediatric DXA data, which adjusts for body size and then tests for the potential impact of disease-related factors on bone, suggests a new model for the assessment of bone health in children with chronic disease, such as AGS.

Disclosures: ***I.E. Olsen****, None.*

SU440

Quantitative Ultrasound and Bone Mineral Density in Children with Osteogenesis Imperfecta Before and During Bisphosphonate Therapy. L. A. DiMeglio[1], L. Ford*[2], C. McClintock*[2], M. Wang*[2], M. Peacock[2]. [1]Pediatric Endocrinology, Indiana University, Indianapolis, IN, USA, [2]Medicine, Indiana University, Indianapolis, IN, USA.

Quantitative ultrasound (QUS) offers the ability to monitor serially bone responses to treatment of pediatric osteoporoses without radiation exposure. We assessed QUS & bone mineral density (BMD) in children with Osteogenesis Imperfecta (OI) before & during bisphosphonate therapy. In 18 patients (age range 3-18 yr, 11F) we measured bilateral calcaneal QUS broadband attenuation (BUA) and speed of sound (SOS) & BMD of the total body (T) and lumbar spine (LS) by DXA. 12 patients were ambulatory; 6 were primarily wheelchair-bound. Precision errors of repeated baseline measurements were: TBMD 1% (N=13), LSBMD 4% (N=13), BUA 3% (N=19), & SOS 0.5% (N=19). QUS of both heels were averaged.

At baseline, TBMD & LSBMD were significantly correlated to BUA (r=0.58 & =0.66, p<0.02) but not SOS (r=-0.29 & =-0.12) (Fig1A). Children were randomized to receive either IV pamidronate, 3 mg/kg over 3 days 4 monthly or oral alendronate 1 mg/kg, from a minimum of 10 mg to a maximum of 20 mg daily. At the last visit (range 4 months to 3 years on treatment) all had increased TBMD (mean increase 0.100 g/cm^2 (range 0.02 to 0.17)) & LSBMD (mean increase 0.226 g/cm^2 (range 0.04 to 0.46)). Corresponding mean changes in QUS were: BUA 7.7 dB/MHz (range –7.7 to +21.3) & SOS 1.7 m/s (range –25 to +36). 13 of 14 patients had concurrent increases in TBMD & BUA (Figure 1b), whereas only 8 increased SOS with treatment. However, there were no significant correlations between QUS & TBMD (v. BUA r=0.47, p=0.08, v. SOS r=-0.16, p=0.59) or LSBMD (v. BUA r=0.31, p=0.27 , SOS r=-.12, p=0.69). Changes in QUS did not differ in non-ambulatory & ambulatory patients.

BUA but not SOS reflects the degree of low bone mass in OI & response to bisphosphonate treatment, but it should not be used as a surrogate for BMD. Correlation with bone turnover & fracture incidence will be necessary to determine if serial QUS measures have utility in treatment monitoring.

FIGURE 1A

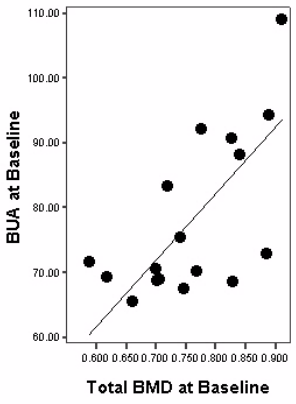
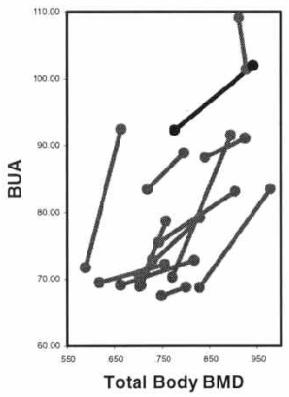


FIGURE 1B



Disclosures: ***L.A. DiMeglio****, None.*

SU441

Chronic Recurrent Multifocal Osteomyelitis Mimicked in Two Patients with Childhood Hypophosphatasia. M. P. Whyte[1], Z. Mughal[2], A. J. Freemont[2], M. Geimer*[3], R. Whitehouse*[2], E. Baildam*[2], S. Mumm[3], W. H. McAlister*[3]. [1]Shriners Hospitals for Children, St. Louis, MO, USA, [2]University of Manchester, Manchester, United Kingdom, [3]Washington University School of Medicine, St. Louis, MO, USA.

Hypophosphatasia (HPP), an inborn-error-of-metabolism characterized biochemically by subnormal serum levels of alkaline phosphatase (ALP), manifests with rickets or osteomalacia and premature loss of deciduous teeth. Deactivating mutations in the gene which encodes the tissue-nonspecific ALP isoenzyme (TNSALP) have been documented in patients worldwide. Radiographs in "childhood" HPP typically show "tongues" of lucency that project from physes into metaphyses in major long bones. There can also be patchy areas of metaphyseal osteosclerosis. Adults often have chondrocalcinosis, sometimes suffer pyrophosphate arthropathy including pseudogout, and rarely have calcific periarthritis. Affected children can be troubled by one or a few painful, swollen joints where the pathogenesis is unclear.

We report two unrelated children with HPP who were also investigated for possible chronic

recurrent multifocal osteomyelitis (CRMO), primarily because of periarticular pain and edema together with metaphyseal radiolucencies and osteosclerosis.
Patient #1, a 13-year-old American boy without premature loss of deciduous teeth, has had a peculiar, stiff-legged gait life-long as well as recurrent joint pains — especially in his ankles (with considerable weight gain over 2 years). There has also been remarkable distal tibial metaphyseal expansion over 2 years that we have not encountered in our care for ~100 children with HPP. Periarticular soft tissue swelling and marked metaphyseal expansion are not typical of childhood HPP. Biopsy of a painful radius at age 5 yielded no evidence of CRMO. Study of *TNSALP* showed Asp361Val, a mutation that can manifest with dominant inheritance.
Patient # 2, a 10-year-old English girl with HPP, developed worsening pain in her left ankle after minor trauma. Inflammatory markers (CRP & ESR) were low, although the affected metaphyseal area was warm to touch with significant bone pain. Gram, PAS, and other stains for pathogens were negative. She was empirically treated with broad-spectrum antibiotics and pamidronate intravenously, but these measures did not improve symptoms or mobility. Soft tissue swelling in the left distal tibia and fibula together with metaphyseal sclerosis and lucencies was thought to possibly represent superimposed CRMO.
Suspicion of CRMO often leads to extensive investigation and therapy. Here, we document that childhood hypophosphatasia can mimic the symptoms and radiographic changes of CRMO.

Disclosures: ***M.P. Whyte****, None.*

SU442

Expansile Ankylosing Skeletal Disease: A New Disorder. D. Wenkert[1], J. Aronson*[2], W. H. McAlister*[3], R. Weinstein[2], S. Mumm[3], M. P. Whyte[1]. [1]Shriners Hospitals for Children, St. Louis, MO, USA, [2]University of Arkansas, Little Rock, AR, USA, [3]Washington University School of Medicine, St. Louis, MO, USA.

We report a 12-year-old boy with a unique, sporadic, skeletal disorder featuring recurrent fractures, exuberant callus formation, remarkable expansion and then fusion of his pelvis to all major long bones of his lower extremities, and contractures of large joints. His prenatal ultrasound was remarkable for short arms and legs and a small chest cavity. At age 2½ months, he sustained his first fracture (right tibia). Numerous fractures occurred subsequently with expansive new bone formation crossing and ankylosing large joints. He has expanded bone overlying both elbows and knees. Biochemical parameters of bone and mineral homeostasis are remarkable for persistent hypercalciuria and, marked increases in serum alkaline phosphatase activity following significant fractures. Expansion of bones has been associated with almost complete loss of subcutaneous soft tissue and muscle mass in his lower limbs. Now, bone scanning shows multiple foci of increased uptake. On CT, major long bones appear as cystic structures filled with fat. Histological studies have revealed woven bone, and cartilage on long bone surfaces. The pathogenesis seems to evolve from disruption of osseous tissue repair after fracture leading to bone expansion and replacement by adipose tissue. Alendronate for 5 months improved the x-ray appearance of his hands, without diminution of his pain, but he developed new, painful, nonmalignant lumps of bone on his thighs which have continued to appear over 17 months. "Type V osteogenesis imperfecta" was considered due to presence of wormian bones as well as calcification of forearm interosseous membranes, however, his exuberant callus formation occurs not in one limb, but in all extremities and in his pelvis, causing fusion of six joints. Familial expansile osteolysis (FEO) and juvenile Paget disease (JPD) were considered with PCR and sequence analysis of all exons and splice sites of the genes encoding Receptor Activator of Nuclear Factor-κ B (RANK) and osteoprotegerin (OPG), respectively, but no mutations were found. Expansile ankylosing skeletal disease is a new metabolic bone disorder.

SU443

Alendronate Treatment of Brtl Mouse Model for Osteogenesis Imperfecta Increases Bone Strength by Increasing Bone Volume but Fails to Improve Femoral Brittleness or Mineralization. J. C. Marini[1], K. M. Kozloff*[2], T. E. Uveges*[1], J. M. Ty*[1], G. Gronowicz*[3], F. Ledgard*[3], S. A. Goldstein[2]. [1]Sect Connective Tiss Disd, NICHD/NIH, Bethesda, MD, USA, [2]Ortho Res Labs, U Mich, Ann Arbor, MI, USA, [3]U Conn Health Ctr, Farmington, CT, USA.

Bisphosphonate drugs are widely administered to children with osteogenesis imperfecta (OI), but their effects on OI bone tissue containing abnormal type I collagen have not been directly examined. The Brtl mouse model for type IV OI has a glycine substitution (G349C) knocked-into one COL1A1 allele. We treated Brtl and wt offspring of Brtl x CD-1 matings from 2-14 weeks of age with alendronate (Aln) (0.219 mg/kg/wk, gift of Merck) or saline placebo. Brtl mouse weight and femoral length were significantly smaller than wt and unchanged by Aln. Whole bone BMD of femurs and lumbar vertebrae, measured using a Lunar Piximus, were significantly increased in both treated Brtl and wt; treated Brtl samples attained average untreated wt BMD. Micro CT data suggest these differences in Brtl are due to increases in bone volume rather than mineralization. Distal femoral BV/TV doubled with treatment in both Brtl and wt due to increased TbN. Diaphyseal Ct.Th. increased by periosteal bone deposition in Brtl and wt femurs. In treated Brtl femurs, overall geometry was reshaped to a more rounded structure. Mechanical properties of femurs were tested in 4-point bending. Aln treatment increased yield load (wt, 23.5±4.8 to 30.9±4.0, p=.009; Brtl, 20.1±4.1 to 26.5±2.9, p=.007), ultimate load (wt, 38.0±7.3 to 47.9±9.0, p=.024; Brtl, 29.3±5.0 to 36.0±9.5, p=.05) and stiffness (wt, 311.5 ±60.3 to 440.4±67.1, p=.001; Brtl, 255.1±54.8 to 329.7±57.7, p=.023) in both Brtl and wt. However, Aln failed to improve the brittleness of the Brtl femoral bone; post-yield displacement remained significantly lower in treated Brtl than wt. Increased cortical shattering during longitudinal sectioning supports greater brittleness of treated Brtl bones. Osteoclast surface was increased 60% in both wt and Brtl femurs. Osteoblast surface was significantly increased in wt but was unchanged in Brtl. In Aln treated Brtl, fewer plump cuboidal Ob were seen; many Ob had an intermediate morphology with enlarged Golgi suggesting functionally exhausted cells. Our interpretation is that Aln treatment of Brtl increases the amount of new periosteal and trabecular bone, leaving synthetically depleted Ob at 14 weeks. Because of the increased bone volume post-treatment, Brtl femurs can withstand a larger load before undergoing permanent deformation. However, femur brittleness is not improved and may be exacerbated. After the yield point is reached, Brtl femurs will fracture with similar additional load and deformation as if untreated.

Disclosures: ***J.C. Marini****, None.*

SU444

Bone Density Changes and Particular Anthropometric Parameters in Children Treated with Inhaled Corticosteroids. V. Vyskocil[1], J. Varvarovska*[2]. [1]Osteocenter,the IInd Department of Internal Diseases, Charles University Hospital, Plzen, Czech Republic, [2]Department of Pediatric, Charles University Hospital, Plzen, Czech Republic.

24 asthmatic children (10 girls, 14 boys) have been studied since 1996 i.e. in average 7 years. Their mean age was 12.7 years (in girls 12.2 years, in boys 12.4 years). The studied group was extended up to 50 children within the following 2 years, their shortest period of follow-up was 5 years. Patients were treated with inhaled corticosteroids: beclomethasone, budesonide,fluticason as Becodisk, Pulmicort or Seretide, all forms twice a day. The patients were divided into groups according to the dose of applied drug: the first group treated with more than 0.35 mg hydrocortisone/ kg/day (46 % patients of the total population) and the second group treated with a dose lower than 0.35 mg/kg/day (54% patients of the total population). The first group had mean age 12.1 years at the beginning of treatment and the period of corticosteroid application was 35 months, spine BMD was 89% of standard expressed in percentage of Z score. Forearm BMD was 85% of standard and the difference between ultradistal and middle shaft BMD was 69.8%. The second group had patients´mean age 14,3 years and the period of corticosteroid treatment lasted 42 months. Spine BMD was 94% according to Z score. Forearm BMD was 91% of standard and the difference between ultradistal and middle shaft only 38%. After 5 or 7 years of inhaled corticosteroid treatment, the first group with higher dose of hydrocortisone (> 0.35 mg hydrocortisone/kg/day) had minimal increase of BMD (from 0.607 to 0.627) in spite of simultaneous supplementation with Ca and vitamin D. The second group with hydrocortisone dose lower than 0.35 mg/kg/day had BMD increase from 0.757 to 0.875g/cm^2.
When the same group was subdivided according to the dose of prednisone higher or lower than 3mg/day, then the patients with higher dose (> 3mg prednisone daily) had BMD altered from 0.657 to 0.719 and in patients with lower dose their BMD had turned to increased value (0.706 to 0.817 g/cm^2). Negative deviation from normal height followed in patients with lower dose of hydrocortisone was – 4.5 cm and – 6 cm in the group with higher dose of hydrocortisone. In the group treated with the dose of prednisone lower than 3 mg/day, there was a negative deviation from normal height – 4 cm and in the second group with higher dose of prednisone was – 6 cm. Height decrease was verified by means of index arm size : real height. In higher doses of corticosteroids the index was significantly lower /0.99 versus 0.96/. The authors did not find a correlation between cummulative dose of corticosteroids and BMD value. BMD DXA is significantly lower when compared with reference values (p<0.003).

Disclosures: ***V. Vyskocil****, None.*

SU445

Bone Density and Renal Tubular Function in Pediatric Patients with Idiopathic Hypercalciuria. S. Skalova*[1], V. Palicka*[2], S. Kutilek*[3]. [1]Department of Pediatrics, Medical Faculty, Charles University, Hradec Kralove, Czech Republic, [2]Department of Biochemistry and Centre for Metabolic Bone Disease, Medical Faculty, Charles University, Hradec Kralove, Czech Republic, [3]Department of Pediatrics, 1st Medical Faculty, Charles University, Prague, Czech Republic.

Background: Idiopathic hypercalciuria (IH) is defined as hypercalciuria that persists after correction of dietary inbalances and has no detectable causes. Defective reabsorption of calcium by the renal tubule is considered as a likely mechanism of IH. N-acetyl-beta-D-glucosaminidase (NAG) is a lysosomal enzyme which is abundantly present in the cells of the proximal tubule and is considered as a very sensitive marker of renal tubular impairment. Currently available reports give conflictive results regarding the urinary excretion of NAG and values of bone mineral density (BMD) in patients with IH. Our aim was to assess tubular function by means of urinary NAG evaluations and BMD by dual energy x-ray absorptiometry (DXA) in patients with IH. **Patients, Materials, Methods:** 18 patients (12 boys and 6 girls, mean age 10.3 ±5.5 years) with IH (urinary calcium excretion>0.1 mmol/kg/24 hours) had their urinary NAG/creatinine ratio (U-NAG) and 24-hour urinary calcium excretion (U-Ca) assessed. Spinal BMD was measured by DXA in a subgroup of 11 children with IH. The obtained results were expressed as Z-scores, compared to previously reported reference values and mutually correlated. **Results*:*** The values of calciuria were significantly increased in comparison to reference values (p<0.0006). The U-NAG values were significantly higher and BMD was significantly lower when compared to the reference values (p<0.006 and p<0.001, respectively). There was no correlation between U-NAG and U-Ca (r = 0.2). We found inverse and significant correlation between BMD and U-Ca (r = -0.8, p<0.01), and there was no correlation between U-NAG and U-Ca (r = -0.3). **Conclusions: 1.** Tubular impairment is highly probable in children with IH, however, there seems to be poor relationship with the degree of calcium leakage. **2.** The pediatric patients with IH had a lower spinal BMD which was inversely related to the urinary calcium loss. **3.** Further studies are necessary for more detailed clarification of these issues.

Disclosures: ***S. Kutilek****, None.*

SU446

Accuracy of Self-Reported Pubertal Stage in Children. J. C. Desmangles*, J. M. Lappe, G. Lypaczewski*, G. Haynatzki*. Osteoporosis Research Center, Creighton University School of Medicine, Omaha, NE, USA.

Objectives: Previous studies looking at the accuracy of self-reported Tanner stages in pediatric patients have given conflicting results, some reporting good others reporting poor concordance between self-reported pubertal staging and staging done by an endocrinologist. We are reporting on the reliability of self-reported Tanner stage in non-obese, healthy children.
Methods: We studied 93 children (58 girls, 35 boys mean ages 11.4 ± 3.5 and 12.3 ± 2.5 years, respectively): The girls reported on breast and pubic Tanner stages whereas the boys reported on pubic Tanner stage. A pediatric endocrinologist or a trained nurse who was not aware of the subject self-assessment examined each subjects after self-rating, and we compared self-rated to actual Tanner stage.
Results: 27.6% of the girls rated their breast Tanner stage incorrectly (62.5 overestimated and 37.5 % underestimated); they were also incorrect in 24.1% of the cases regarding pubic stage (50% overestimation and 50% underestimation); 38% of the girls rated both breast and pubic stage correctly. 68.5% of the boys self-rated their pubic stage correctly, whereas 31.4% self-reported incorrectly (81.8% overestimated and 18.1% underestimated). We performed a Student t-test to compare the ages of the different groups studied and found that there was no statistical difference between the mean ages of the groups who self rated correctly and incorrectly.
Conclusions: The results of this analysis suggest that self-rated Tanner pubertal stage is not a reliable method of assessing actual Tanner; age does not influence the accuracy of self reported Tanner stage in children.

Disclosures: ***J.C. Desmangles****, None.*

SU447

Determining the Reliability and Usability of Bone Density Outcome Measures for Children with Spina Bifida. B. C. Craven[1], P. Johnson*[2], M. Ross*[2], C. E. Webber[3], J. Wright*[4], B. Oddson*[5], D. Biggar*[2]. [1]Department of Medicine Toronto Rehab, University of Toronto, Toronto, ON, Canada, [2]Bloorview MacMillan Children's Centre, Toronto, ON, Canada, [3]Hamilton Health Sciences, Hamilton, ON, Canada, [4]Hospital for Sick Children, Toronto, ON, Canada, [5]University of Toronto, Toronto, ON, Canada.

Children with Spina Bifida (SB) are prone to fragility fractures of their lower extremities. Identifying bone mineral density (BMD) outcomes for these children is a critical step in reducing fracture burden. This study was conducted to identify the reliability and usability of dual energy x-ray absorptiometry (DEXA) and peripheral quantitative ultrasound (QUS) in children with Spina Bifida. A convenience sample of sixty children with SB (50% male) between 6-18 years of age were recruited from the Bloorview MacMillan Children's Centre. Demographic, anthropometric, mobility and impairment data were recorded. Each participant had DEXA measurement of BMD of the total body, lumbar spine, and hip region using the Hologic 4500A densitometer. The distal femur and proximal tibia BMD was measured using the Hologic AP spine software with modifications (Moreno & Craven 2001). The width of the epiphysis was used to estimate bone length and assign the region of interest. QUS of the distal tibia was performed using the Sunlight Omnisense ultrasound. Two investigators reviewed DEXA results for quality and region of interest placement before analysis. Test-retest reliability was determined for repeated measures. The usability questionnaire completed by the participants and technologist was based on the international standard ISO-13407 (1999). DEXA scans of the whole body, hip and distal femur were obtained for all participants. The protocol for measuring BMD of the tibia was inadequate due to the software's inability to assign a region of interest for participants with narrow tibial epiphyses, difficulties detecting very low BMD and problems with the face validity of the region of interest assignment. The reliability of DEXA scanning was high. Accommodations were made with positioning the children due to scoliosis, kyphosis, and lower extremity contractures, however this did not impede DEXA scan acquisition. QUS values were derived for 34 participants. QUS was reliable but not usable for 1/3 of this sample due to peripheral edema, severe lower extremity atrophy or a body mass index greater than 30. The usability of QUS and DEXA of the proximal tibia are currently inadequate for children with SB. There appears to be no major problems with the usability and reliability of DEXA measures of BMD of the whole body, hip and distal femur regions.

Disclosures: ***B.C. Craven****, None.*

SU448

The Relations between Minimum Joint Space Width in the Knee and Bone Mineral Density in the Spine, Hip, Distal Femur and Proximal Tibia. K. A. Beattie*[1], P. Boulos*[2], J. Duryea*[3], E. Jurriaans*[4], C. Gordon[5], D. Inglis*[5], A. Papaioannou[6], J. D. Adachi[2], C. Webber[7]. [1]Dept. of Medical Sciences, McMaster University, Hamilton, ON, Canada, [2]Dept. of Rheumatology, McMaster University, Hamilton, ON, Canada, [3]Dept. of Radiology, Brigham and Women's Hospital, Boston, MA, USA, [4]Dept. of Radiology, St. Joseph's Healthcare, Hamilton, ON, Canada, [5]McMaster University, Hamilton, ON, Canada, [6]Dept. of Geriatrics, McMaster University, Hamilton, ON, Canada, [7]Dept. of Nuclear Medicine, McMaster University, Hamilton, ON, Canada.

Objective: To investigate the relation between medial tibiofemoral minimum joint space width (mJSW) in the knee, a surrogate measure of knee osteoarthritis, and bone mineral density (BMD) in the lumbar spine, hip, distal femur and proximal tibia in healthy individuals.
Methods: Men and women (20-69 years) participated provided they did not suffer from knee pain, had never sustained a knee injury or been diagnosed with a bone or joint disease. Volunteers consented to have a single, fixed-flexion knee radiograph and a DXA scan of the corresponding femur, tibia, hip and lumbar spine. Femur and tibia BMD scans were acquired using a lumbar spine scanning protocol. Radiographs were graded according to the Kellgren-Lawrence (K-L) scale. Films were digitized and evaluated for medial tibiofemoral mJSW using an automated computer algorithm.
Results: Of 45 volunteers, 1 case was excluded (lumbar spine T score <-2.5 = osteoporosis). Of the remaining 44 cases, 32 were women and 12 were men, mean age (SD) 42.0 years (13.5) and BMI 25.7 (4.0) kg/m^2. Linear regression analyses controlling for age, sex and BMI together with each of lumbar spine, femoral neck, trochanter and total hip BMDs revealed that BMI, lumbar spine, femoral neck and total hip were significant predictors of medial mJSW (β coeff.: mean BMI -0.385, $p<0.02$, lumbar spine 0.346, $p<0.05$, femoral neck 0.390, $p<0.02$ and total hip 0.426, $p<0.02$). Linear regression analyses where sex, BMI and age were entered as independent variables examined the predictive value of BMD in the distal femur, proximal tibia and subchondral femoral and tibial regions as they relate to medial mJSW. Age was found to consistently and significantly predict medial mJSW (β coeff. -0.359, $p<0.05$). Total proximal tibial BMD also significantly predicted medial mJSW (β coeff. 0.432, $p<0.005$). Neither total distal nor subchondral femoral BMDs nor subchondral tibial BMD were significant predictors of medial mJSW.
Conclusions: In healthy men and women, lumbar spine, femoral neck, total hip and total proximal tibial BMDs are significant predictors of medial mJSW. Total distal femoral and subchondral femoral and tibial BMDs are not significant predictors of mJSW.

Disclosures: ***A. Papaioannou****, None.*

SU449

Changes in the Serum OPG levels after Bone Marrow Transplantation: Association with Bone Mineral Metabolism. M. I. Kang[1], K. H. Baek*[1], W. Y. Lee*[2], H. J. Tae*[1], B. Y. Cha*[1], K. W. Lee*[1], H. Y. Son*[1], S. K. Kang*[1]. [1]Internal Medicine, The Catholic University of Korea, College of Medicine, Seoul, Republic of Korea, [2]Internal Medicine, Sungkunkwan University, College of Medicine, Seoul, Republic of Korea.

The loss of bone mass is usually detected after bone marrow transplantation (BMT), particularly during the early post-transplant period. We recently reported that enhanced bone resorption following BMT is related to both the steroid dose and the increase in IL-6. It was also suggested damage of the marrow stromal microenvironment by the myoablation and changes of bone growth factors contribute to post-BMT bone loss. The present study was designed to clarify the changes of osteoprotegerin (OPG) after BMT and its correlation with markers of bone metabolism. We prospectively investigated 110 patients undergoing allogenic BMT and analyzed 36 patients (32.4±1.3 years, 17 men and 19 women) who had DEXA performed before the BMT and 1 year after the BMT. The Serum bone turnover marker levels were measured before the BMT and at 1, 2, 3, 4 and 12 wks, 6 Ms, and 1 yr after the BMT. The Serum OPG levels were measured in all patients before the BMT and 1, 3, and 12 wks after the BMT. The mean bone loss in the lumbar spine and the total proximal femur, which was calculated as the percent change from the baseline to the level at 1 yr, was 5.2% ($p<0.01$) and 11.6% ($p<0.01$), respectively. The mean serum ICTP, a bone resorption marker, increased progressively until 3 and 6 months after the BMT. Thereafter, it decreased gradually to reach the basal values after 1 year. The serum osteocalcin levels decreased progressively until 3 wks after the BMT. It then increased transiently at 3 and 6 Ms but had returned to the basal level by 1 yr. The serum OPG levels increased significantly on week 1 and 3 compared with the baseline level ($p<0.01$), then decreased at 3 months, but still significantly higher than baseline level ($p<0.01$). There was a tendency for OPG levels to be associated positively with ICTP, but statistically not significant. Our study implies that a rapid impairment of bone formation and an increase in bone resorption occurs during the immediate post-BMT period and dynamic changes in the OPG levels occur during the same period. The increased serum concentration of OPG may reflect a compensative response to enhanced osteoclastic bone resorption.

Disclosures: ***M.I. Kang****, None.*

SU450

Osteoporosis in Heart-transplant Recipients: Preliminary Data of a Multicenter Study. V. Germoni*[1], S. Giannini[1], S. Adami[2], C. Marcocci[3], C. Marzari*[1], F. Cobelli*[4], A. Gambino*[5], L. Dalle Carbonare*[1], V. Braga*[6], E. Vignali*[3], S. Sella*[1], G. Crepaldi[1]. [1]Dept. of Medical and Surgical Sciences, University of Padova, Padova, Italy, [2]Department of Osteo-articular Rehabilitation, University of Verona, Verona, Italy, [3]Institute of Endocrinology, University of Pisa, Pisa, Italy, [4]Medical Center for Rehabilitation, Fondazione Salvatore Maugeri, Montescano, Italy, [5]Dept. of Cardiovascular Surgery, University of Padova, Padova, Italy, [6]Dept. of Osteo-articular Rehabilitation, University of Verona, Verona, Italy.

Osteoporosis is one of the most important complications in organ transplantation. However, there are no systematic studies that assess ethiopathogenesis and the dimension of the problem on a large scale. We are carrying out a multicenter study on heart transplantation (HTx) with the participation of 4 operative units (Padova, Pavia, Pisa e Verona). We enrolled 180 pts (Padova: 40, Pavia: 118, Verona: 22), 156 m and 24 f, aged 16-72 yrs (mean age: 53.3 ± 13.1). Pts transplanted less than 10 years before were included and subjects with multi-organ transplantation were excluded. HTx mean period was 47 ± 33 months. All subjects underwent clinical evaluation including Barthel's index and questionnaire on vertebral pain. Blood and urine samples were obtained for the main bone metabolism determinants. Vertebral and femoral DXA as well as spine X-ray were performed for the morphometric evaluation. 27% of pts were of average weight, 62% overweight, and 11% underweight. Primitive cardiopathy was subdivided into three groups from an etio-

logic point of view: ischemic cardiopathy (35%), idiopathic dilated cardiopathy (40%), and other cardiopathies (25%). 50% of the subjects had a daily calcium intake below 800 mg/day. At transplantation, 55% of pts was self-sufficient, while the other 45% experienced different degrees of dependency. Tx corrected this fact in almost all the cases (dependency: 0.6%, self-sufficiency: 99.4%). At the time of the interview, 24% of the pts had been suffering from almost continuous back pain in the previous week. Of them, 16% reported pain for the whole day and mild/severe intensity in 23% of the cases. Bone densitometry showed osteoporosis in 24% of the cases at spine and in 13% at femoral neck. Osteopenia was present in 42% of the pts at spine, and in 51% of the pts at femoral neck. 179 of 180 subjects had been under corticosteroid treatment. 40% of pts had taken a prednisone cumulative dose ≥ 10 grams, which was associated with a significant reduction of bone density (OR: 2.25, 95% CI: 1.14 - 4.41). Steroid therapy has been shown to be a risk factor for osteoporosis after HTx. Further analyses will allow a better definition of severity and pathogenetic factors of this disease.

Disclosures: ***V. Germoni****, None.*

SU451

Prevalence of Osteoporotic Fractures in Long-term Kidney Transplant Patients with Preserved Renal Function. J. W. R. Braga*[1], R. M. S. Neves*[2], M. M. Pinheiro*[1], R. E. Heymann*[1], A. B. Carvalho[2], V. L. Szejnfeld[1]. [1]Rheumatology, Universidade Federal de Sao Paulo, Sao Paulo, Brazil, [2]Nephrology, Universidade Federal de Sao Paulo, Sao Paulo, Brazil.

End-stage renal disease is associated with multiple bone and mineral disorders and increased risk of fractures. Kidney transplantation(KT) can revert many of the metabolic abnormalities, however bone disease may persist longer after KT leading to low bone mineral density(BMD) and fractures. In this study we evaluate the prevalence of low BMD and osteoporotic fractures in KT patients and determine risk factors associated with osteoporotic fractures in this population. One hundred ninety one patients with KT for 3 years or more presenting serum creatinine levels lower than 2.5 mg/dl were included. Clinical risk factors studied were cause of end-estage renal disease, duration of pretransplant dialysis, time since transplantation, diabetes mellitus, time since menopause, kind of graft, cumulative dose of steroids, cyclosporin and azathioprine. BMD and fracture risk were determine by using DXA (Lunar, DPX) at multiple sites (spine, femur and total body) and quantitative ultrasound of the calcaneous. Vertebral fracture was surveyed by X-ray and defined using Rigg's method. Twenty four percent (46) of all patients had either vertebral (29/46) or appendicular (17/46) fractures following KT. Using WHO definition, we found osteoporosis in 3-13% of KT patients and osteopenia was observed in 30-37%, according to gender or skeletal site. Main predictors of fractures in women were diabetes mellitus, time since menopause, high dose of steroids, low femoral neck BMD, low Stiffness index and high body mass index. For men, the main risk factors for fractures included long duration of dialysis, low lumbar spine BMD and low stiffness index. Our results demonstrate high prevalence of low BMD and osteoporotic fractures in KT patients. Postmenopausal women and patients with diabetes mellitus were at particularly higher risk for fracture after KT.

Disclosures: ***J.W.R. Braga****, None.*

SU452

β-Arrestin Mediates Desensitization but Not Internilization of CASR Expressed in HEK293 Cells. M. Pi, R. F. Spurney, L. D. Quarles. Medicine, Duke University, Durham, NC, USA.

G protein coupled kinases (GRK) and β-arrestins are important regulators of G-protein coupled receptor (GPCR) desensitization. The phosphorylation of agonist-stimulated GPCRs by GRK leads to the binding of β-arrestins and GPCR internalization resulting in disruption of signal transduction. CASR is a GPCR belonging to the metabotropic glutamate receptor family. CASR is predominantly expressed in parathyroid glands and kidneys where it respectively regulates PTH secretion and renal calcium excretion. The role of GRKs and β-arrestins in regulating CASR function has not been examined. We co-expressed rat CASR with either β-arrestins-1 and -2, GRK-2 and -5 or combinations of β-arrestins and GRKs into HEK293 cells. CASR activation was assessed by measuring luciferase activity in cells transfected with an SRE-luc reporter construct, by assessing ERK activity using an anti-phospho-ERK antibody, and by measurement of inositol monophosphate synthesis following stimulation of CASR with 5 mM extracellular calcium. Calcium stimulated luciferase activity, ERK activity and inositol production in CASR expressing HEK cells. The addition of β-arrestin-1 and -2, as well as GRK-2, but not GRK-5, blocked calcium-mediated increase in luciferase activity, ERK activity and inositol production, consistent with desensitization of CASR. Combinations of GRK-2 with either β-arrestin-1 or -2 resulted in greater inhibition of CASR activation as measured by luciferase activity. We also found that CASR stimulation of ERK activation was partially inhibited by β-arrestin-1 and -2. In addition, the ERK inhibitor PD98095 blocked calcium-mediated SRE activation in CASR expressing HEK293 cells. To determine if GRK and β-arrestin inhibition of CASR signaling is due to receptor internalization, we used FACS to evaluate the percentage of cell surface receptors before and after agonist stimulation. Using the β-2 receptor as a positive control, we found that isoproterenol resulted in loss of approximately 9% of the receptors, consistent with prior reports of internalization in HEK293 cells, whereas CASR failed to internalize. Direct interactions between CASR and β-arrestin-1 were demonstrated by co-immunoprecipitation. Using a mammalian two-hybrid system, we also established that β-arrestin-1 interacts with the C-terminus of CASR (877-1079) but not the CASR (636-805) fragment containing intracellular loops 1, 2 and 3. These data suggest that β-arrestin-dependent desensitization involves binding to the C-terminus of CASR but not receptor internalization.

Disclosures: ***L.D. Quarles****, Amgen 2, 5, 8.*

SU453

The Extracellular Calcium-Sensing Receptor (CaR) Is Indispensable for Expression of Alkaline Phosphatase (ALP) and Osteocalcin (OC) as well as Mineralization in Mouse Osteoblastic MC3T3-E1 Cells. M. Yamauchi*, T. Yamaguchi, H. Sowa, S. Yano, H. Kaji, T. Sugimoto, K. Chihara*. Division of Endocrinology/Metabolism, Neurology and Hematology/Oncology, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan.

We have previously shown that the extracellular calcium-sensing receptor (CaR) is expressed in various bone marrow-derived cell lines and plays a possible important role in stimulating their proliferation and chemotaxis. It has also reported that the CaR-deficient mice revealed mineralized abnormalities in bone in spite of hypercalcemia by excess of parathyroid hormone (PTH), and that the CaR modulated matrix production and mineralization in chondrogenic cells. However, it remains unclear whether or not the CaR plays any role in regulating osteoblast differentiation. We hypothesize that the CaR participates in physiological processes occurring at osteoblast differentiation, such as bone matrix production and mineralization. In this study, we used the mouse osteoblastic MC3T3-E1 cell line. This cell line increased mineralization detected by Alizarin Red stain or von Kossa stain when it was exposed upon high calcium (2.8 and 3.8 mM) or a specific CaR activator, NPS R467 (1-3 μM), for 10 days after reaching confluency. Next, we stably transfected MC3T3-E1 cell with either a CaR antisense (AS) vector (AS clone) or a vector inserted with the inactivating R185Q-variant of CaR that has previously been shown to exert a dominant negative (DN) action on the wild type receptor (DN clone). The ALP activities in both AS and DN clones were decreased compared with those in empty vector-transfected clones (the control). In AS and DN clones, the levels of type I procollagen and osteopontin (OPN) mRNA detected by Nothern blotting were almost the same as the control. On the other hand, the expression of osteocalcin (OC), which was expressed at a later stage of osteoblastic differentiation, reduced in both AS and DN clones compared with the control. Mineralization detected by Alizarin Red stain or von Kossa stain was also decreased in both clones. In conclusion, this study showed that in mouse osteoblastic cells, the stimulation of the CaR enhances their mineralization and the abolishment of the CaR function results in diminishing ALP activity, OC expression and mineralization. It suggests that the CaR expression may be indispensable for osteoblastic differentiation.

Disclosures: ***M. Yamauchi****, None.*

SU454

Calcium Sensing Receptor (CaR) Activation Stimulates Parathyroid Hormone Related Protein (PTHrP) Secretion in Prostate Cancer Cells: Role of Transactivation of Epidermal Growth Factor Receptor (EGFR). S. Yano, R. J. Macleod*, N. Chattopadhyay*, J. Tfelt-Hansen*, O. Kifor*, R. R. Butters*, E. M. Brown. Division of Endocrinolgy, Diabetes and Hypertension, Brigham and Women's Hospital, Boston, MA, USA.

We have reported that high extracellular Ca stimulates PTHrP release in human prostate and breast cancer cell lines as well as H-500 rat leydig cancer cells, actions mediated by the CaR. Activating the CaR leads to phosphorylation of mitogen-activated protein kinases (MAPKs) that also participate in PTHrP synthesis and secretion. Since the CaR is a G protein-coupled receptor, it is likely to transactivate the epidermal growth factor receptor (EGFR) or the platelet-derived growth factor receptor (PDGFR). In this study, we hypothesized that activation of the CaR transactivates the EGFR and PDGFR, and examined whether transactivation affected PTHrP secretion in PC3 human prostate cancer cells. After overnight serum starvation, subconfluent cells were preincubated with or without 0.7 μM AG1478 (EGFR kinase inhibitor) or 1 μm AG1296 (PDGFR kinase inhibitor) for 30 min. Cells were incubated with 0.5, 3.0, or 7.5 mM Ca for 6 hrs, and medium was collected to measure PTHrP. We confirmed that high Ca stimulates PTHrP release from PC3 cells (1.5-2.5 and 3-4 fold increases in 3.0 and 7.5 mM Ca, respectively). When cells were preincubated with AG1478, PTHrP secretion was significantly inhibited at basal as well as high Ca conditions, while AG1296 had no effect on PTHrP secretion. Preincubation with 5 μg/ml of an antihuman heparin binding EGF (HBEGF) antibody resulted in similar findings as with AG1478. In contrast, addition of EGF significantly increased PTHrP secretion. GM6001, a matrix metalloprotease (MMP) inhibitor, also suppressed basal and high Ca-induced PTHrP secretion. By Western analysis, an acute increase in extracellular Ca led to delayed activation of extracellular signal-regulated kinase (ERK) in PC3 cells. AG1478 and GM6001 inhibited the high Ca-induced phosphorylation of ERK1/2. Taken together, these findings indicate that activation of the CaR transactivates the EGFR, but not the PDGFR, leading to phosphorylation of ERK1/2 and promotion of PTHrP secretion. This transactivation is most likely mediated by activation of MMP and cleavage of proHBEGF to HBEGF.

Disclosures: ***S. Yano****, None.*

SU455

High Extracellular Ca2+ Enhances the Differentiation of Mouse Growth Plate Chondrocytes. W. Chang, L. Rodriguez*, C. Tu*, Y. Oda*, D. Shoback. Endocrine Research Unit, VAMC, University of California San Francisco, San Francisco, CA, USA.

Our previous studies showed that high extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$) inhibited proteoglycan synthesis and the expression of early chondrogenic markers and enhanced the expression of markers of terminal differentiation and mineral deposition in the rat chondrogenic cell line RCJ3.1C5.18, potentially via the activation of Ca^{2+}-sensing receptors (CaRs) (Chang et al, Endocrinology, 141:1467, 2002). To determine whether high $[Ca^{2+}]_o$ mediates similar changes in growth plate chondrocytes, we tested the effects of different $[Ca^{2+}]_o$ on proteoglycan synthesis by alcian green staining and matrix mineralization by alizarin red

staining in cultured mouse growth plate chondrocytes from wild-type mice (CaR+/+) and mice in which the expression of full-length CaR was disrupted (CaR-/-) (Ho et al, Nature Genetics, 11:389, 1995). In CaR+/+ chondrocytes, raising $[Ca^{2+}]_o$ from 0.6 to 2.9 mM dose-dependently suppressed proteoglycan accumulation and increased matrix mineral deposition (ED_{50}'s ≈ 1-2 mM Ca^{2+}). Increasing $[Ca^{2+}]_o$ from 0.5 to 5.0 mM produced a transient followed by a sustained increase in intracellular $[Ca^{2+}]$ in CaR+/+ chondrocytes by fura-2 microflurimetry. Similar effects of high $[Ca^{2+}]_o$ on the deposition of matrix minerals were seen in cultured CaR-/- chondrocytes. Although full-length wild-type CaRs were not expressed in CaR-/- chondrocytes, RT-PCR and sequence analysis of products amplified from CaR-/- growth plate RNA revealed a truncated variant CaR missing exon 5. This exon encodes amino acids Val-460 to Glu-536 in the extracellular domain of the CaR. The expression of this splice variant CaR in CaR-/- growth plate was further demonstrated by immunocytochemistry with CaR antibodies. Taken together, these studies suggest that high $[Ca^{2+}]_o$ promote the differentiation of mouse growth plate chondrocytes. The effects of high $[Ca^{2+}]_o$ to promote differentiation of CaR-/- chondrocytes could be mediated via this truncated CaR or an alternate extracellular Ca^{2+}-sensing mechanism. Further studies are in progress to distinguish between these possibilities.

Disclosures: ***W. Chang****, None.*

SU456

Hypercalcemia in a Women with Breast Cancer Misdiagnosed as Hypercalcemia of Malignancy: An Unusual Presentation of Familial Hypocalciuric Hypercalcemia. C. Marcocci, F. Cetani, S. Borsari*, E. Pardi*, G. Dipollina*, E. Ambrogini*, L. Cianferotti, G. Viccica*, A. Pinchera*. Endocrinology, University of Pisa, Pisa, Italy.

Hypercalcemia of malignancy (HCM) is a serious complication of cancer patients, especially those with advanced lung, breast, and hematological tumors.

We describe a 45-yr-old woman with metastatic breast cancer in whom the finding of hypercalcemia was initially attributed to HCM. She was treated elsewhere with bisphosphonates without significant changes of serum Ca^{2+} levels. When the patient was seen at our outpatient clinic a careful evaluation (Ca^{2+} 2.75 mmol/L; PTH 3.7 pmol/L, 24h urinary calcium 4.0 mmol; Ca/Cr Clearance 0.007) challenged the diagnosis of HCM and suggested the diagnosis of familial hypocalciuric hypercalcemia (FHH), which was confirmed by evaluating other family members and genetic studies. Four out of 6 relatives had mild hypercalcemia (2.68-2.79 mmol/L) and detectable serum PTH (2.7-32 pmol/L). The entire coding region of the calcium sensing receptor (CaR) gene was sequenced in the proband and the region of interest was sequenced in family members. A novel heterozygous I212T missense mutation in exon 4 was found. The same mutation was identified in affected, but not in unaffected family members, and in any of the 50 unrelated Italian controls. Expression of the WT CaR in COS-7 cells conferred a 4.5-8 fold increase in total IPs at high levels of Ca^{2+}, with an EC_{50} value of 3.7 mM±0.4. On the other hand, transfection of the mutant I212T CaR resulted in no response of IPs to any Ca^{2+} concentration. Nontransfected cells showed no effect in IPs production at any concentration of Ca^{2+} tested. The Ca^{2+} dose-response curve of the coexpressed receptors (WT/I212T) was lower than that of the WT receptor alone, suggesting that the mutant receptor interferes with the function of the WT receptor. Western analysis of membrane from COS-7 cells transiently transfected with the WT or the mutant CaRs showed that the quantity of the expressed WT CaR protein was comparable to that of the mutant CaR. This finding indicates that the abnormal functional responses of the mutant CaR was due to a dominant-negative effect rather than to a lack of receptor expression on the cell membrane.

In conclusion, we described a case of FHH, due to a novel CaR mutation, whose hypercalcemia was initially attributed to HMC simply because the patient had metastatic breast cancer. This report tells us that nothing can be taken for granted in medical practice and that in cancer patients other causes of hypercalcemia should be excluded before concluding that it is due to HCM.

Disclosures: ***C. Marcocci****, None.*

SU457

Regulation of ERK Activity and DNA Synthesis in a PTH-Resistant Subclone of the Proximal Tubule-like OK Cell. J. A. Cole. Medical Pharmacology and Physiology, University of Missouri, Columbia, MO, USA.

In OK cells, PTH regulates cell function by occupying receptors coupled to adenylyl cyclase (AC) and phospholipase C (PLC). AC and PLC activation mimic PTH regulation of ERK activity and DNA synthesis, but the consequences of AC- and PLC-mediated ERK activation are not known. To characterize signal-specific responses to ERK activation, we compared PTH responses in OK and OKH cells (a PTH-resistant subclone of OK cells). OKH cells express PTH receptors and AC activation comparable to OK cells, but lack PTH-induced IP_3 production and intracellular Ca^{2+} mobilization and do not respond to PTH with decreases in phosphate transport. In OK and OKH cells, PTH and EGF cause time-dependent ERK activation that is mimicked by the PKC activator PMA and the AC activator FSK. In OK cells, PTH, PMA and FSK cause longer-lived ERK stimulation (≥ 60 min) than EGF (peak ~5 min). In OKH cells, ERK activity is elevated ≥ 2 h by EGF, FSK and PMA and ~ 1h by PTH. All 4 compounds cause dose-dependent ERK activation and PMA is the most effective activator in both cell lines. The Ca^{2+} ionophore ionomycin and the intracellular Ca^{2+} releaser thapsigargin increase ERK activity in OK and OKH cells. Chelating extracellular Ca^{2+} with EGTA or intracellular Ca^{2+} with EGTA-AM blocks Ca^{2+}-induced ERK activation in OK and OKH cells and reduces ERK activation by PTH in OK cells. However, chelating Ca^{2+} has no effect on PTH-induced ERK activation in OKH cells indicating this response is cAMP-dependent. To determine if the loss of PTH/Ca^{2+}-induced ERK activation alters cellular responses, we examined the effect of PTH on DNA synthesis. In OK and OKH cells, EGF and PMA stimulate and FSK inhibits DNA synthesis. In OK cells, low PTH doses (1 pM -1 nM) cause PMA-like proliferation while high doses (10 nM-1 μM) cause cAMP-like inhibition of DNA synthesis. In OKH cells, PTH does not effect DNA synthesis at low doses but inhibits DNA synthesis at high doses. Since PTH does not activate PLC in OKH cells, this suggests that PLC/Ca^{2+} signals mediate ERK-induced proliferative responses to the hormone. OKH cells should provide a good model system for evaluating the PTH/AC signaling pathways leading to ERK activation and the cellular consequences of AC-dependent increases in ERK activity.

Disclosures: ***J.A. Cole****, None.*

SU458

Differential Regulation of Parathyroid Hormone (PTH) Activity at the PTH Receptors Type I and II. D. Manen*[1], A. Bisello*[2], D. Pierroz[1], T. Usdin*[3], R. Rizzoli[1], S. L. Ferrari[1]. [1]Geriatrics and Internal Medicine, Division of Bone Diseases, Geneva, Switzerland, [2]Div. of Endocrinology and Metabolism, Dept. of Medicine, University of Pittsburgh, Pittsburgh, PA, USA, [3]Unit on Cell Biology, NIH, Bethesda, MD, USA.

We have previously reported that PTH-stimulated cAMP signaling and residence of PTH-bound PTH/PTHrP receptor (PTH1Rc) at the cell surface is regulated by ß-arrestins (ßarr). The PTH receptor Type II (PTH2Rc) is also activated by a tubero-infundibular peptide of 39 amino-acids, TIP39. Due to marked differences in the nature and structure of both agonists and receptors, we hypothesized that selective mechanisms of regulation for agonist activity operate at the two PTH receptor types.

We investigated the cellular trafficking of ßarr1- and ßarr2-GFP and of a series of PTH2Rc-GFP mutants using real-time fluorescence microscopy, and their related cAMP signaling profile in HEK293 cells.

As previously observed with PTH1Rc in response to PTH(1-34) and PTHrP, PTH2Rc activation by TIP39 recruited cytoplasmic ßarr1 and 2 to the cell surface, followed by internalization of both ßarr and PTH2Rc on endosomes. In contrast, PTH(1-34) at concentrations up to 1μM did not promote ßarr mobilization nor ligand (PTH-rhodamine) or receptor endocytosis in cells expressing PTH2Rc. In contrast, I125-PTH rapidly dissociated from PTH2Rc. Accordingly, the profile of PTH2Rc-mediated cAMP signaling markedly differed in response to PTH and TIP39. Whereas PTH(1-34) induced a peak of cAMP accumulation which returned to baseline within 5 min. and could be maximally restimulated by PTH after only 30 min., TIP39-stimulated cAMP accumulation decreased more progressively and remained significantly desensitized after 2 hrs. This agonist-specific response was found to reside in PTH2Rc N-terminus (N-term) and extracellular loop 3 (EC3), as PTH-stimulated recruitment of ßarr and internalization of ligand and receptor were restored when chimeric PTH2Rc carried N-term and EC3 of PTH1Rc, but not when it carried intracellular loop 3 (IC3) and/or C-terminus (C-term) of PTH1Rc. In contrast, by further studying deletion and Ser/Ala substitution mutants in PTH2Rc C-term and IC3, we identified the PTH2Rc C-term to be responsible for ßarr recruitment and desensitization of cAMP signaling in response to TIP39.

In conclusion, whereas PTH and TIP39 trigger similar mechanisms of regulation for agonist activity at the PTH1Rc and PTH2Rc, respectively, PTH activity is differentially regulated at the PTH2Rc, due to structural differences in the receptors extracellular domains. These results suggest that PTH and TIP39 are unlikely to have similar physiologic effects at the PTH2Rc.

Disclosures: ***D. Manen****, None.*

SU459

The Role of Calcium Channels and a Competent IP3 Receptor Complex for the Expression of PTH-dependent Phospholipase C Activity. Q. Sun*[1], R. W. Katz[2], J. P. Bilezikian[1]. [1]Division of Endocrinology, College of Physicians and Surgeons, Columbia University, New York, NY, USA, [2]School of Oral and Dental Surgery, Columbia University, New York, NY, USA.

We reported previously that maximal activation of PLC isoforms in a cell line (EW29) transfected with rat PTH type 1 receptor requires functional plasma membrane calcium channels. In this study, we examined aspects of the signaling pathway needed to gate these membrane channels. Nifedipine (N), an L-type calcium channel blocker, suppressed PTH-dependent PLC activity by ~35%, while A23187, a calcium ionophore, completely rescued PTH-dependent PLC activity from the nifedipine block. A231987 did not increase PLC activity in the absence of PTH stimulation. CoCl2 , a more global calcium channel blocker, inhibited PTH-dependent PLC activity to a much greater extent than N (>80%), suggesting that other calcium channels account for the much of the effects of PTH on calcium influx. 2-APB, a specific IP3 receptor inhibitor, greatly suppressed PTH-dependent PLC activity as measured by formation of labeled inositol phosphate in response to PTH (PTH-IP). This highlights the importance of IP3R control of store depleted calcium channel gating function for the maximal activation of PLCs in response to PTH. Since PTH1R activation of PLC-beta isoforms would be unaffected by this treatment, the 2-APB treatment decrease in PTH-IP suggests two explanations. Either activation of other PLC isoforms are suppressed by IP3R block or calcium modulation of PLC-beta may be responsible for much of the inositol phosphate accumulation. Thapsigargin, which promotes opening of store depleted membrane calcium channels, increased PTH-IP production over untreated control groups but did not significantly increase basal values. By western blotting, we demonstrated the presence of IP3 type II and III receptors but not type I receptors, consistent with the neuron-specificity of IP3R type I and constitutive expression of type II and III. These observations highlight: 1) the importance of IP3R regulation of membrane channel calcium gating to the effects of PTH in PTH1 receptor-enriched cells, 2) the importance of intracellular and transcellular calcium flux to PTH modulation of PLC activity, and 3) the point that PTH stimulation of PLC activity requires IP3R functions independent of their effect on membrane calcium channels. The results are consistent with a model in which IP3R function and transmembrane calcium flux are essential and distinct mechanisms involved in PTH-PTH1R dependent PLC responses.

Disclosures: ***R.W. Katz****, None.*

SU460

NHERF-1 Is Required for Phospholipase C-dependent Calcium Influx Induced by Parathyroid Hormone in Opossum Kidney Cells. M. J. Mahon*, G. V. Segre. Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

Na+/H+-exchanger regulatory factors (NHERFs) are PDZ-domain proteins that interact with the carboxy-terminus of the parathyroid hormone receptor 1 (PTH1R) and promote parathyroid hormone (PTH) signaling via phospholipase C beta (PLCbeta). Here we examine the role of NHERF-1 in PTH-mediated signaling in opossum kidney (OK) cells. In a sub-clone of the OK cell line, OKH, PTH robustly activates adenylyl cyclase (AC), but it fails to increase total inositol phosphates, inhibit phosphate uptake via the sodium phosphate co-transporter 4, or increase in intracellular calcium ([Ca2+]i). OKH cells are deficient in NHERF-1 expression as determined by immunoblotting. Stable expression of NHERF-1 in OKH cells (OKHN1) restores the PTH-mediated rise in [Ca2+]i, as determined in cells preloaded with the fluorescent calcium indicator, fura-2. The PTH-elicited rise in [Ca2+]i is rapid, robust, and transient. It begins within seconds, reaches maximal concentrations of 500 nM, and returns to baseline levels after one minute. In nominally Ca2+-free medium, PTH fails to induce a rise in [Ca2+]I, suggesting that this increase is due entirely or at least in large part to an influx via a Ca2+-channel. The PTH-mediated influx of Ca2+ in OKHN1 cells is not inhibited by the Ca2+-channel blocker nifedipine, but is markedly attenuated by the Ca2+-channel blockers SKF-96365 and 2-amino-diphenyl borate. Pertussis toxin and the PLCbeta inhibitor, U73122, also block the [Ca2+]i response mediated by PTH, suggesting that bg subunits released from Gai/o activate PLCbeta, which then activates a Ca2+-channel. The protein kinase A inhibitor, H-89, and the protein kinase C inhibitor, calphostin C, do not block the PTH-elicited increase in [Ca2+]i. Treatment of OKHN1 cell with either forskolin or phorbol esters alone does not elicit an [Ca2+]i response. However, acute treatment with phorbol esters (5 min) blocks the PTH-mediated increase in [Ca2+]i. Furthermore, PTH1R tagged with the green fluorescent protein (GFP) co-localizes with NHERF-1 in apical patches of OKHN1 cells, as assessed by confocal microscopy. These data indicate that in response to PTH, NHERF-1-assembled PTH1R signaling complexes stimulate Gi/o, that activate PLC, and which then open a Ca2+-channel to increase the entry of calcium into the cell.

Disclosures: ***M.J. Mahon****, None.*

SU461

Down-regulation of Parathyroid Hormone in EW29 Cells Requires PKC but not PKA Function. Q. Sun*[1], R. W. Katz[2], J. P. Bilezikian[1]. [1]Division of Endocrinology, College of Physicians and Surgeons, Columbia University, New York, NY, USA, [2]School of Oral and Dental Surgery, Columbia University, New York, NY, USA.

Desensitization of the PTH/PTHrP receptor occurs rapidly in the presence of PTH due to an uncoupling of the PTH receptor-G protein complex by competition between G proteins and arrestins for the cytoplasmic portion of the PTH receptor. Later, receptor internalization and sequestration as well as decreases in PTH1R mRNA act to reduce cellular levels of membrane PTH1R. To understand post-transcriptional down-regulation of PTH-induced phospholipase C signaling, we used a cell line (EW29) stably transfected with a constitutively expressed rat PTH type 1 receptor. This cell line demonstrates 4-6 fold increases in labeled inositol phosphate accumulation in response to 1 μM PTH(1-34). The hypothesis is that maximal down-regulation of PTH dependent inositol phosphate production (PTH-IP) requires both PKA and PKC activity. We have previously shown that PTH(1-29) is a poor stimulator of PLC activity, while PTH(1-34) and PTH(1-29) equally stimulate maximal adenylyl cyclase activity. Treatment of EW29 cells with 1 μM PTH(1-34) for 3 hours decreases PTH- IP production by 75%. Maximal suppression (85-95%) occurred after overnight exposure. Exposure of cells to 1 μM PTH(1-29) for 3 hours reduced subsequent responsiveness to PTH(1-34) by only 20%. Incubation with 10^{-6} M PTH(1-34) for 3 hours greatly reduced PTH receptor number on the cell surface as measured by ([^{125}I]PTH(1-34) specific binding (~15% of control binding). Exposure to PTH(1-29) had an insignificant effect on membrane receptor number (~90% of control binding). A PKA inhibitor (H89) did not block down-regulation of PTH-IP induced by prior PTH(1-34) exposure. Go-6983, a PKC inhibitor, and staurosporine, a non-specific kinase inhibitor, reversed much of the PLC suppression induced by long term exposure to PTH(1-34) (PTH-IP control=12,920; 3 hour PTH pretx.=3,342; 3 hour PTH pretx + Go-6983=8,738; 3 hour PTH pretx+staurosporine=12,230). But Go-6983 and staurosporine did not reverse the decrease in membrane receptor binding of hot PTH to an equivalent extent (PTH pretx=~15% of control PTH-IP; + staurosporine=~40% of control PTH-IP). Our results indicate that chronic PTH(1-34) down-regulates the IP response of cells to PTH in part by promoting receptor internalization and sequestration. The process of receptor down-regulation appears to distinguish between PKC functions-which are involved- and PKA functions- which do not appear to be involved. PKC activation has some effect on receptor internalization and sequestration but this alone does not explain PKC functions which reverse PTH receptor down-regulation.

Disclosures: ***Q. Sun****, None.*

SU462

hPTH-(1-34)NH_2 and Ostabolin™ Family PTH Peptides are differently Endocytosed in HKRKB7 Porcine Cells Expressing human PTHR1 Receptors and Rat ROS 17/2 Osteosarcoma Cells--A Confocal Microscopy Study. C. M. Allen*, J. R. Barbier*, R. Monette*, S. Maclean*, G. E. Willick*, J. F. Whitfield. Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, Canada.

Signaling from the PTHR1 receptor occurs on two levels - first are the primary signal mechanisms adenylyl cyclase (AC) and/or phospholipase-β/PKC and second are the signalers brought into the cytoplasm and nucleus by the endocytosis of the PTH•PTHR1•arrestin complex. However, endocytosis and therefore the influence of the second-tier signalers depends on the primary signaling. Members of the Ostabolin™ family of AC-stimulating, 31-amino acid PTH peptides are equally effective anabolic agents as hPTH-(1-34) and hPTH-(1-34)NH_2, but differ from the larger peptides in not inducing hypercalcemia when injected intermittently into rodents, monkeys and humans. Could this difference be due to Ostabolin™ family members not being endocytosed? While they invariably stimulate AC, they can stimulate PKC in some cells such as HKRKB7 porcine kidney cells, but not in others such as ROS 17/2 osteosarcoma cells and hFOB human fetal osteoblasts.

To explore this possibility we have compared the endocytosis of Alexa 488-conjugated Ostabolin™ (hPTH-(1-31)NH_2, Ostabolin-C™ ([Leu^{27}]cyclo(E^{22}-K26)hPTH-(1-31)NH_2) and hPTH-(1-34)NH_2 by HKRKB7 cells expressing 950,000 hPTHR1 receptors/ cell and ROS 17/2 cells. All of these peptides stimulate both AC and PKC in the pig cells and all three were endocytosed and collected around the cell nucleus at 37^{0}C but remained on the cell surface at 23^{0}C. Removing the N-terminus to produce hPTH-(13-34)NH_2 which cannot stimulate AC, but can stimulate PKC, did not prevent the AC-stimulating peptide from binding to HKRKB7 cells, but was only marginally endocytosed at 37^{0}C .

Interestingly, the dye-conjugation did not prevent the peptide from activating the receptor as indicated by a robust AC stimulation, *none* of the AC-stimulating peptides were endocytosed by ROS 17/2 cells at 37^{0}C. Remarkably, instead of being evenly distributed on the cell surface as in HKRKB7 cells, they appeared to collect mainly on ca.2.5-μm rod-shaped bodies at both 23^{0}C and 37^{0}C. The nature of these PTH-binding bodies warrants further study.

Disclosures: ***C.M. Allen****, None.*

SU463

Expression of GFP-PTH/PTHrP Receptor Constructs in LLC-PK1 (Clone 46) Cells Restores Responsiveness to hPTH (1-34). E. K. Patterson*[1], P. H. Watson[2], L. E. Canaff*[3], G. N. Hendy*[3], R. Bringhurst[4], A. B. Hodsman[2], L. J. Fraher[1]. [1]Biochemistry, University of Western Ontario, London, ON, Canada, [2]Medicine, University of Western Ontario, London, ON, Canada, [3]Calcium Research Laboratory, McGill University, Montreal, PQ, Canada, [4]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

Previous studies have shown nuclear localization of the type I parathyroid hormone/ parathyroid hormone-related peptide receptor (PTHR) in cells of rat liver, kidney, uterus, ovary and gut as well as in cultures of the osteoblast-like cells UMR106, MC3T3-E1, ROS 17/2.8, and SaOS-2. We had identified an apparent, bipartite nuclear localization sequence (NLS) that we hypothesized controls trafficking of the PTHR to the nucleus. We began studies on the function of the identified NLS by directed mutagenesis of the PTHR tagged with green fluorescent protein (GFP). The PTHR-GFP constructs were transfected into LLC-PK1 (Clone 46) porcine renal cells, which are reported to have no biochemical response to PTH. However when employing five separate PTHR-specific antibodies to untransfected cells, we detected nuclear staining but no evidence of the presence of the PTHR in the cell membrane. RT-PCR directed against three different regions on the PTHR cDNA, residues 171-488, 297-656 and 980-1221 also confirmed the presence of the message for PTHR in LLC-PK1 cells. We further hypothesized that transfecting LLC-PK1 cells with either a wild type or a GFP tagged PTHR would restore a function membrane-bound receptor as well as cAMP signalling in response to PTH 1-34. Wild-type PTHR transfected cells showed intense fluorescence within the cytoplasmic membrane with a ring around the nucleus and occasional bright juxta-nuclear foci. Additionally, cells transfected with the wild-type PTHR show a 10-fold increase in cAMP accumulation when treated with 2.5nM hPTH (1-34), for 15 minutes thus indicating they now respond to parathyroid hormone, suggesting the untransfected cells express a non-functional PTHR that is not membrane bound.

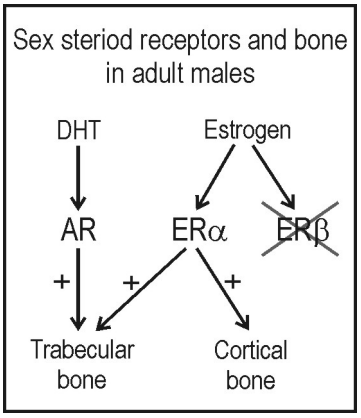
Disclosures: ***E.K. Patterson****, None.*

SU464

Characterization of Sex Steroid Receptor Specificity for the Regulation of Trabecular and Cortical Bone in Male Mice. M. Lindberg[1], S. Movérare[1], K. Venken*[2], A. Eriksson[1], N. Andersson[1], S. Skrtic*[1], P. Salmon*[3], R. Bouillon*[4], J. Å. Gustafsson[5], D. Vanderschueren*[2], C. Ohlsson[1]. [1]Centre for Bone Research at the Sahlgrenska Academy (CBS), Department of Internal Medicine, Göteborg University, Göteborg, Sweden, [2]Laboratory for Experimental Medicine and Endocrinology, Katholieke Universiteit Leuven, Leuven, Belgium, [3]Skyscan N.V., Aartselaar, Belgium, [4]Laboratory for Experimental Medicine and Endocrinology, Katholieke Universiteit Leuven, Leuven, Sweden, [5]Department of Biosciences at Novum & Department of Medical Nutrition, Karolinska Institute, 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 estrogen and, thereafter, via stimulation of the estrogen receptors (ERs). There are two known ERs, ERα and

ERβ. The aim with this study was to directly compare the effect of AR-, ERα- and ERβ-activation on trabecular and cortical bone in orchidectomized (orx) male mice. Treatment with the non-aromatizable androgen 5α-dihydrotestosterone (DHT) was used to specifically activate the AR while 17βestradiol was used to activate the ERs and the estrogen receptor specificity was determined by using ER-inactivated mice. Nine-months-old male wild type (WT), ERα$^{-/-}$, ERβ$^{-/-}$ and ERα$^{-/-}$β$^{-/-}$ mice, were orchidectomized (orx) or sham-operated and then treated for four weeks with DHT, 17β-estradiol or vehicle. 17βestradiol increased the trabecular BMD in WT and ERβ$^{-/-}$ but not in ERα$^{-/-}$ and ERα$^{-/-}$β$^{-/-}$, while DHT increased the trabecular BMD in orx mice of all four genotypes. Thus, both ERα- and AR-activation but not ERβ-activation has the capacity to increase the trabecular BMD in orx male mice. Micro-CT analyses demonstrated that ERα but not AR activation increased the trabecular thickness while AR activation only increased the number of trabeculae. pQCT measurements of cortical bone in the tibial diaphysis demonstrated that 17βestradiol via ERα but not DHT increased cortical bone parameters including cortical bone mineral content (BMC), thickness and cross-sectional area in orx adult male mice. In conclusion, ERα and the AR are redundant in the stimulation of the trabecular bone while only ERα stimulates the cortical bone in adult male mice.



Disclosures: ***M. Lindberg****, None.*

SU465

Antiestrogens and Mechanical Loading both Stimulate Transcriptional Activity of the BMP-6 Promoter in Osteoblasts in an ERα-Dependent Manner. D. B. Ong*[1], H. L. Jessop*[2], S. M. Colley[1], M. R. Norman*[1], R. Kitazawa*[3], S. Kitazawa*[3], L. E. Lanyon[2], J. H. Tobias[1]. [1]University of Bristol, Bristol, United Kingdom, [2]The Royal Veterinary College, London, United Kingdom, [3]Kobe University, Kobe, Japan.

Although antiestrogens are known to stimulate target genes in certain tissues such as bone, the molecular basis for their tissue selective action is unknown. To investigate this further, we examined whether estrogens and antiestrogens regulate the bone morphogenetic protein 6 (BMP-6) promoter differently in distinct cell types. We generated a reporter construct with a 4.3Kb fragment of the BMP-6 promoter controlling expression of the luciferase gene (BMP-6-luc). Cells were transiently transfected with the reporter construct and an expression plasmid for human estrogen receptor alpha (ERα), and exposed to 10^{-7}M ligand. Luciferase activity was corrected for transfection efficiency following analysis of β-gal activity. ICI 182,780 (ICI) significantly stimulated BMP-6-luc activity in osteoblast-like cells (ROS 17/2.8, MG63 and SaOS-2) (P<0.0001, One-Way ANOVA), whereas no response was observed to 17β-estradiol (E2). In contrast, human MCF-7 and T47D breast cancer cells showed no response to ICI but significant stimulation following E2 treatment (P<0.0001, One-Way ANOVA). Using different ER constructs, we found that BMP-6-luc activation was dependent on ERα as opposed to ERβ, and required the presence of the ERα AF-1 activation domain. However, in contrast to the stimulatory response of MCF-7 cells to E2, the response of MG63 cells to ICI was AF-2 independent. These results suggest that antiestrogens stimulate BMP-6 reporter activity in osteoblasts via a bone-specific ERα-dependent mechanism, which may reflect the fact that unlike estrogen, antiestrogens activate ligand independent, AF-1 dependent, ERα pathways which mediate the response of osteoblasts to mechanical strain. To address this possibility, we examined whether mechanical strain shares the ability of antiestrogens to stimulate BMP-6 reporter activity in osteoblasts in an ERα-dependent manner. ROS 17/2.8 cells were transiently transfected with BMP-6-luc and ERα expression plasmid and then exposed to cyclical dynamic strain (peak 3400 με, 1Hz, 600 cycles). Strikingly, mechanical strain significantly stimulated BMP-6-luc activity following transfection with ERα, to a similar extent to that observed following ICI (P<0.01, One-Way ANOVA). We conclude that in osteoblasts, BMP-6 reporter activity is stimulated in an ERα-dependent manner by ICI and mechanical strain, consistent with the hypothesis that antiestrogens exert their tissue-selective effects on bone by activating ERα-dependent mechanical response pathways.

Disclosures: ***D.B. Ong****, None.*

SU466

Effects of Oral Contraceptives on Circulating Osteoprotegerin and Soluble RANKL Serum Levels in Healthy Young Women. M. Schoppet*[1], M. Christ*[1], P. Schueller*[1], V. Viereck[2], L. C. Hofbauer[1]. [1]Philipps-University, Marburg, Germany, [2]Georg-August-University, Goettingen, Germany.

Osteoprotegerin (OPG) represents a secreted cytokine which regulates bone mass by blocking receptor activator of nuclear factor-kappaB ligand (RANKL), the principal regulator of osteoclast function. In vitro, OPG production is up-regulated by estrogens in osteoblastic lineage cells, a mechanism that has been discussed as a protective paracrine mechanism of estrogens on the skeleton. Since OPG serum measurement is increasingly employed as biochemical marker of bone turnover, we evaluated OPG and soluble RANKL (sRANKL) serum levels in healthy young women with or without oral contraceptives in order to define the effects of estrogens on the RANKL/OPG system in vivo. Serum levels of OPG and sRANKL were prospectively assessed in a cohort of healthy young women with (n = 30) or without (n = 25) combined estrogen-progestin based oral contraceptives. OPG and sRANKL serum levels were determined by enzyme-linked immunosorbent assays. In women using oral contraceptives, OPG serum levels were significantly higher (2.69 ± 1.44 pmol/L) compared to non-users (1.35 ± 1.07 pmol/L; P = 0.0004), whereas sRANKL serum levels did not differ between both groups (P = 0.33). This resulted in an increased OPG-to-sRANKL ratio (P = 0.004) in women on oral contraceptives. During the ovarian cycle, OPG and sRANKL serum levels were unchanged in women without oral contraceptives. In conclusion, intake of oral contraceptives is associated with increased OPG levels, but not sRANKL levels, resulting in a higher OPG-to-sRANKL ratio which may contribute to the beneficial effects of estrogens on the skeleton. Furthermore, the status of current use of oral contraceptives has to be considered when assessing components of the RANKL/OPG system in healthy young women.

Disclosures: ***L.C. Hofbauer****, None.*

SU467

Impaired Bone Accrual in Maturing Androgen Deficient (hpg) Mice Is Normalized by Replacement by a Non-aromatizable Androgen. N. A. Sims[1], K. Brennan*[2], J. Spaliviero*[3], D. J. Handelsman*[3], M. J. Seibel[3]. [1]Dept of Medicine at St. Vincent's Hospital, The University of Melbourne, Melbourne, Australia, [2]ANZAC Research Institute, University of Sydney, Sydney, Australia, [3]ANZAC Research Institute, The University of Sydney, Sydney, Australia.

Testosterone is known to play an important role in skeletal maturation, and in the maintenance of bone mass in adult humans and rodent models. To further elucidate the role of androgens in the regulation of trabecular bone structure and turnover during growth, normal (wt) and androgen-deficient (hpg) mice were analyzed by bone histomorphometry at 3 and 9 weeks of age. Sub-groups of mice were orchidectomized and / or treated with testosterone (T) or dihydrotestosterone (DHT) (s.c. silastic implants) for full androgen replacement from 3 weeks, and tissues were collected at 9 weeks of age.

At 3 weeks of age, there was no difference in bone length, width, or trabecular bone volume (BV/TV) between wt (3.1± 0.4%) and untreated hpg mice (3.4 ± 0.4%), confirming that androgens do not determine prepubertal bone size or trabecular bone mass. By 9 weeks of age, however, while BV/TV had increased in wt (5.1± 0.8%, p<0.05 vs 3 week old wt), untreated hpg mice had significantly lost BV/ TV (0.8± 0.2%; p<0.05 vs. 3 week old hpg and 9 week old wt). Furthermore, the increase in cortical thickness (CoTh) associated with normal skeletal maturation, but not the increased bone length, was partially blocked in hpg mice. Although bone volume was normal at 3 weeks of age, indices of bone formation, including osteoid volume and thickness, and osteoblast surface, were all significantly lower in hpg than in wt mice (ObS/BS: 29.1± 2.9% vs. 40.8± 1.3%), while osteoclast surface was significantly higher in hpg than in wt mice (32.3± 3.3% vs. 23.2± 1.6%). Thus, impaired bone formation and excessive bone resorption determine the low BV/TV observed in hpg mice at 9 weeks. Notably, BV/TV in 9 week old hpg mice was equivalent to levels in 9 week old wt mice orchidectomized at 3 weeks of age (0.5± 0.2%).

In hpg mice, treatment with both T and the non-aromatizable androgen DHT maintained BV/TV (9.3±1.2% and 10.3±1.1%, respectively) and CoTh by reducing endosteal bone turnover as well as increasing periosteal bone formation.

In conclusion, prepubertal hpg mice have structurally normal bones, but impaired bone turnover, leading to pronounced bone loss during maturation. Treatment with T and DHT normalized bone turnover and bone size, indicating that androgens are able to maintain normal bone metabolism and accrual without aromatization.

Disclosures: ***N.A. Sims****, None.*

SU468

Estrogen Modulates Megakaryocyte Synthesis of Osteoprotegerin and RANKL and Megakaryocyte Stimulated Osteoblast Expression of Collagen and Osteoprotegerin. S. Bord[1], E. Frith*[2], D. C. Ireland*[1], M. A. Scott*[2], J. A. I. Craig*[2], J. E. Compston*[1]. [1]Medicine, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom, [2]Haematology, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom.

Increasing evidence suggests that megakaryocytes (MKs) play a role in bone remodelling, possibly by their interactions with cells at the bone surface.

To investigate factors and mechanisms by which MKs may influence remodelling, CD34 positive cells were isolated from human cord blood and cultured for 6, 9 and 12 days in a collagen-based system plus or minus 100nM 17beta estradiol. MKs were immunolocalised for osteoprotegerin (OPG), RANKL and CD61, a marker of early megakaryocyte matura-

tion. To investigate direct effects of MKs on osteoblasts, maturing MKs were added to cultures of human osteoblasts and assessed for procollagen and OPG. Protein expression was quantitatively assessed by image analysis and mRNA by RT-PCR.
In MK cultures at 6 days, when immature MKs were evident, OPG expression was suppressed 3-fold ($p<0.01$) in the E-treated cultures whilst RANKL remained at basal levels compared to untreated cells. At 9 days in the E-treated cultures with maturing MKs there was a 2.5-fold ($p<0.01$) higher level of OPG expression compared to controls. RANKL levels were reduced 0.3-fold ($p<0.02$) in cells cultured in the presence of E. Maximal OPG expression was seen at 12 days with a 3-fold induction of expression ($p<0.001$), whilst RANKL levels were further suppressed by 0.5-fold compared to controls ($p<0.01$). Osteoblasts cultured alone showed high levels of expression of procollagen with 74% (± 7%) of cells staining positively. When cultured with MKs the number of positively staining cells remained similar but the intensity of expression was significantly increased 1.54-fold ($p< 0.02$). OPG was expressed by 32% (± 6.3) of osteoblasts increasing to 51% (± 5.5) when cultured with MKs ($p<0.01$) with a 1.63-fold increase in intensity of expression ($p<0.01$). In E-treated cultures MKs significantly increased collagen and OPG expression in osteoblasts compared to controls without MKs. mRNA data supported the protein findings with a 3.1-fold increase in Col 1A1 expression in the MK treated cultures compared to controls ($p<0.02$). Low-level OPG mRNA expression was detected in the osteoblasts; this was increased 8.14-fold in osteoblasts cultured in the presence of MKs ($p<0.01$).
These results demonstrate that in vitro maturing MKs show increased OPG and suppressed RANKL expression with E treatment. In addition, MKs stimulate the expression of collagen type 1 and OPG by osteoblasts. MKs thus have the potential to affect both the resorption and formation of bone.

Disclosures: ***S. Bord****, None.*

SU469

Estrogen and Tamoxifen Conjugates of Porphyrins Selectively Kill Breast Cancer Cells. R. Ray*[1], N. Swamy*[1], A. Fernandez-Gacio*[1], C. Fernandez Marcos*[1], A. Purohit*[2], G. Jones*[2]. [1]Endocrinology, Diabetes and Nutrition, Department of Medicine, Boston University School of Medicine, Boston, MA, USA, [2]Northeastern University, Boston, MA, USA.

Nuclear receptors are well-known molecular targets for cancer-drug discovery. For example, estrogen receptor (ER) has been exploited profitably to develop several estrogens and antiestrogens as breast cancer drugs. Estrogens have also been explored as carriers of cytotoxins and radioisotopes to target ER in tumors by exploiting the over-expression of ER in breast tumor cells. However, anti-estrogens, with potentially less harmful side effect have remained largely unexplored for this purpose. In the present study we combined the ER-binding property of estrogens and anti-estrogens with the photo-toxic and tumor-localizing properties of porphyrins to develop porphyrin-conjugates of estrogen and tamoxifen. We observed that a C17-estradiol-alkynyl conjugate of chlorin e6-dimethyl ester (a porphyrin) (E_2-POR) and a 4-hydroxytamoxifen conjugate of pyropheophorbide (a porphyrin) (TAM-POR) bound ER in a ligand-specific manner. The estrogen conjugate (E_2-POR) was selectively taken up by ER-positive MCF-7 breast cancer cells, but not by ER-negative breast cancer cells, strongly suggesting ER-mediated uptake of the conjugate. Furthermore, incubation of MCF-7 cells with E_2-POR followed by exposure to red light killed the cells. But similar treatment of ER-negative MDA-MB-231 cells produced significantly lower cell-kill. Furthermore, there was significantly lower light-exposed cell-kill with unconjugated porphyrin (chlorin e6-dimethyl ester) in either of the cells. Similarly, light-exposure of MCF-7 cells, incubated with TAM-POR, resulted in strong cell-kill, but there was significantly lower cell-kill when the cells were incubated with the unconjugated porphyrin (pyropheophorbide). These results strongly suggested that the natural tumor localizing property of porphyrins could be strongly enhanced by coupling them with estrogens and anti-estrogens for ER-targeted photodynamic therapy of malignancies in organs where ER is expressed significantly.

Disclosures: ***R. Ray****, None.*

SU470

Targeted Overexpression of Androgen Receptor in Osteoblasts Results in Complex Skeletal Phenotype in Growing Animals. K. Wiren[1], X. W. Zhang[1], A. Toombs*[1], S. Harada[2], K. Jepsen[3]. [1]Portland VA Medical Center, OHSU, Portland, OR, USA, [2]Merck Research Laboratories, West Point, PA, USA, [3]Mt. Sinai School of Medicine, NY, NY, USA.

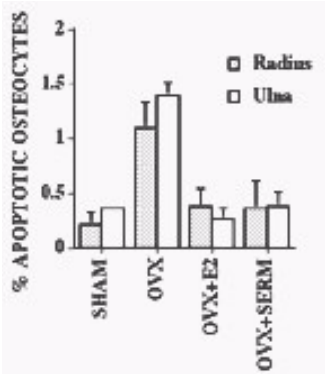
Non-aromatizable androgens have significant effects on skeletal homeostasis independent of conversion to estradiol, but the mechanisms are not established. The goal of this study was to use targeted overexpression of androgen receptor (AR) in osteoblasts as a means of elucidating the specific role(s) for AR in osteoblast function in vivo. Rat AR cDNA was cloned downstream of a rat 3.6-kb α1(I)-collagen promoter fragment, used to create AR-transgenic (AR-TG) mice in two independently derived lines. This promoter was chosen since it is known to control downstream gene expression throughout the osteoblast lineage including the periosteum. Quantitative real-time RT-PCR analysis of AR transgene expression showed bone targeting with highest levels observed in calvaria but ~100-1000 fold lower expression in muscle, heart, intestine and other tissues. Serum estrogen and testosterone levels were not different between AR-TG and wild-type (WT) (of each gender), but serum osteocalcin was significantly reduced in male AR-TG mice ($P<0.001$) and slightly reduced in female AR-TG ($P<0.05$). Bone quality was assessed at 2 months of age. Consistent with the known stimulatory effects of androgens on periosteal bone growth, fluorescent images at the distal femur demonstrated periosteal labeling around the entire circumference in male AR-TG but not female AR-TG or WT controls. Increased calvarial thickness was also noted. Interestingly, labeling was dramatically lacking at the endocortical surface of male AR-TG mice. Radiographic imaging of distal femur demonstrated bulging at the metaphysis in male AR-TG, suggestive of an inhibition of resorption that is consistent with the decline in osteocalcin levels. Although male AR-TG mice were indistinguishable from WT at birth, they progressively demonstrated a reduction in body weight ($P<0.001$), reduced nose-rump length ($P<0.01$), and reduced femur length ($P<0.05$). Biomechanical analysis demonstrated reduced strength only in the male AR-TG, with trends for a reduction in maximum adjusted load ($P=0.08$), adjusted stiffness ($P=0.2$) and increased brittleness as measured by postyield deflection. Thus, overexpression of AR throughout the osteoblast lineage results in a complex phenotype in growing male AR-TG animals likely resulting from its effects on both bone formation and resorption. These findings may offer valuable insight into the role of androgen in bone metabolism, and provide proof of principle for direct androgen actions mediated by osteoblastic expression of AR.

Disclosures: ***K. Wiren****, None.*

SU471

A Selective Estrogen Receptor Modulator (SERM) Inhibits Osteocyte Apoptosis During Estrogen Loss. Implications for Bone Quality Maintenance. S. Collishaw*[1], J. Mosley[1], C. Taylor*[1], J. Reeve[2], B. S. Noble[1]. [1]Edinburgh University, Edinburgh, United Kingdom, [2]CambridgeUniversity, Cambridge, United Kingdom.

Ideally, Selective Estrogen Receptor Modulators should demonstrate all the positive bone associated effects of estrogens without their soft tissue side effects. Estrogens are associated with positive effects not only on the quantity of bone, but also it's quality including the maintenance of osteocyte populations through the inhibition of their apoptosis. Here we have used a rat model of ovariectomy (OVX) to determine whether the osteocyte sparing effect of estradiol can be mimicked by the SERM LY117018. Sixteen 12-week-old rats (Weight, 263) were divided into 4 treatment groups: sham operated (SHAM) (n = 4), OVX (n = 4), and OVX + 17b-estradiol (E2) (0.125mg/kg/day) (n = 4,), OVX + SERM (3mg/kg/day) (n =4). After 7 days treatment, radius and ulna were removed and the percentage of apoptotic osteocytes determined using an in situ nick-translation method. The success of ovariectomy was assessed by measurement of uterine weight. The proportion of apoptotic osteocytes present was five times higher in the OVX compared with the SHAM groups in both radius (1.09% vs. 0.21%, respectively; $p < 0.01$) and ulnae (1.40% vs. 0.36%, respectively; $p = 0.001$). Addition of estradiol to the OVX animals completely abrogated the increase in osteocyte apoptosis in both the radius (0.38%) and ulna (0.26%) (see figure). Addition of the SERM to the OVX animals abrogated the increase in apoptosis to the same extent as estradiol in both the radius (0.35%) and ulna (0.38%) (see figure). Nuclear morphology and electrophoresis of DNA confirmed the presence of apoptotic cells in the samples. In conclusion, estradiol and SERM are equally good at preventing the increase in osteocyte apoptosis engendered by OVX. These data point to the potential benefits of SERM's in the maintenance of both the quantity and the quality of bone in the postmenopausal individual.



Disclosures: ***B.S. Noble****, None.*

SU472

Muscle Anabolic Action of Androgens, Involves Selective Targeting of the IGF-1 Signaling Pathway. K. Chen*[1], T. L. Moore*[1], M. Liu*[1], Y. Wang*[1], M. A. Esterman*[2], X. Ruan*[1], H. A. Bullock*[1], A. G. Geiser*[1], G. Krishnan[1]. [1]Gene Regulation, Bone & Enabling Biology, Eli Lilly and Company, Indianapolis, IN, USA, [2]TADR and Chemistry Information Technology, Eli Lilly and Company, Indianapolis, IN, USA.

Male Wistar rats (4 months old) from Harlan Labs were used to surgically isolate the levator ani muscle tissue. Cell aggregates was digested, and plated followed by immortalization with a retrovirus containing the human telomerase gene. Cells aggregates were selected for single clones over the next 2 to 3 weeks and were screened for expression of AR. 20 such single clones were verified for the presence of muscle specific actin (MF-20) and evaluated for AR antigen using confocal microscopy. A single clone LA-20 was subsequently used for all the studies. Treatment of the LA-20 cell line with 10 nM R1881 (methyl-trienolone) for 6 hr resulted in a robust increase in nuclear AR protein levels as measured by immunofluorescence and western blot analysis. In addition, an increase in IGF-1 mRNA was observed in these cells, after 24 hr of treatment with 10 nM R1881 treatment. The increase in IGF-1 expression led to a small change in phosphorylated Akt levels as measured by Akt and p-Akt specific antibodies. Due to the high levels of PTEN protein found in these cells, we observed a minimal change in p-Akt, in spite of this robust increase in IGF-1 mRNA. To expand on this observation made in the LA-20 cells, we treated the rat L6 mature skeletal muscle cell line, mouse fibroblast C3H10T1/2 cell line, and the multipotent mouse C2C12 myoblast cell line with 10 nM R1881 for various time

periods. All these cells express AR mRNA in response to R1881 treatment. However, upon R1881 treatment, IGF-1 mRNA was only increased in the striated muscle LA-20 cell line. These results seem to indicate that cell context is important for IGF-1 to be a target of AR signaling. To examine this positive feedback on AR mRNA and the increase in IGF-1 mRNA, in vivo, we treated 18 week gonadectomized ICR mice with 2mg/Kg-day Testosterone Enanthate, or vehicle for 14 days. In contrast, to the levator ani tissue IGF-1 levels, serum IGF-1 protein did not increase significantly in these animals. Collectively, results from both the LA-20 cell line and the in vivo experiments, point to a prominent role for IGF-1 in mediating muscle anabolic actions of androgens in a cell and tissue-specific manner.

Disclosures: ***K. Chen****, Eli Lilly and Company 1, 3.*

SU473

The Non-genotropic Synthetic Ligand Estren (4-estren-3α17β-diol) Is an Androgen Receptor Agonist. H. Bullock*[1], R. J. Barr*[2], K. Chen*[1], I. Cohen*[3], M. A. Esterman*[4], Y. Wang*[1], C. Montrose-Rafizadeh*[2], J. A. Dodge*[5], M. Liu*[1], P. K. Shetler*[1], H. U. Bryant[1], G. Krishnan[1]. [1]Gene Regulation, Bone & Enabling Biology, Eli Lilly and Company, Indianapolis, IN, USA, [2]Lead Optimization Biology & Business Operations, Eli Lilly and Company, Indianapolis, IN, USA, [3]Lead Optimization & Investigative Toxicology, Eli Lilly and Company, Indianapolis, IN, USA, [4]TADR & Chemistry Information Technology, Eli Lilly and Company, Indianapolis, IN, USA, [5]Discovery Chemistry Research, Eli Lilly and Company, Indianapolis, IN, USA.

We have examined the non-genotropic synthetic ligand, Estren, for transcriptional activity mediated by human AR. AR binding properties of Estren were tested in a competitive binding assay using full-length human AR protein overexpressed in 293 cells bound to ^{3}H-methyl trienolone. In addition, Estren was found to transactivate the ARE reporter construct in LA20 cells. To extend these observations in reporter constructs to an endogenous target of AR signaling we utilized a prostate specific antigen (PSA) ELISA. Estren was also found to increase the secretion of prostate specific antigen (PSA) in a dose-dependent manner in human LnCAP cells. This increase in PSA was blocked by co-treatment with 4-OH flutamide and bicalutamide in a dose-dependent manner. In contrast, co-treatment with ER antagonist ICI-182780, or ERK inhibitor U0126, failed to completely reverse the effects of Estren in this assay. The LA20 muscle cells were used to assess the direct translocation of endogenous AR protein from its cytoplasmic location to the nucleus, in a time-dependent manner. Addition of Estren to these cells induces endogenous expression of AR as quantified by confocal microscopy in 6hr and this increase in AR lasts for 48 hr. In addition, one observes a robust translocation of the AR signal as detected by an AR-specific antibody conjugated to Alexa 488 fluoroprobe. Finally, 16 week old ICR mice, gonadectomized for 8 weeks, (body weight matched) were treated with vehicle, Estren or TE, dissolved in EtOH and sesame oil, s.c, at 0.1, 1.0 and 10 mg/Kg-day for 14 days. Organ weights from the levator ani, prostate and seminal vesicle are clearly indicative of Estren's actions on the androgen receptor. In addition, female SD rats were ovariectomized and treated with 2.5 or 5.0 mg/Kg-day with Estren (s.c. injection) and serum LH levels was found to be reduced to that of sham levels. This effect of Estren may also represent its in vivo androgen receptor agonist activity. Collectively, in addition to the previously reported non-genotropic action of Estren via ERα/ERβ, this ligand harbors some potentially useful transcriptional activity via the androgen receptor.

Disclosures: ***G. Krishnan****, Eli Lilly and Company 1, 3.*

SU474

Sex-specific Effects of Estrogen Are via PKC-dependent ERK1/2 MAP Kinase. Z. Schwartz[1], J. McMillan*[1], V. L. Sylvia*[2], B. D. Boyan[1]. [1]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [2]Orthopaedics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Estrogen (E2) regulates cells via traditional nuclear estrogen receptors (ER), as well as via membrane-mediated actions that are rapid and include calcium ion flux. Studies using ER+ and ER- breast cancer cells indicate that rapid responses to E2 involve protein kinase C (PKC)-dependent signaling. Similar findings in rat and human chondrocytes show that E2's effect on PKC is sex-specific and is found only in cells from female donors. Inhibition of PKC blocks many of the physiological responses to E2, indicating that genomic regulation occurs. PKC-dependent physiological responses are mediated by mitogen activated protein kinases (MAPK) in other systems, suggesting that they play a role in E2 signaling as well. To test this, confluent cultures of growth plate chondrocytes that had been isolated from the costochondral cartilages of male and female rats were used as the model. Cells were treated with 17-beta estradiol. E2 caused a dose dependent increase in MAPK specific activity that was greatest at 10-8 M. The effect was evident within 9 minutes and gone at 270 minutes and was observed only in cells from female rats. MAPK activation was dependent on PKC, since specific inhibition of PKC with chelerythrine (10 μM) blocked the effect of E2. The mechanism involved activation of phosphatidylinositol-specific phospholipase C (PI-PLC) but not phosphatidylcholine-specific PLC (PC-PLC). When the cells were treated with E2 in the presence of the PI-PLC inhibitor U73122, MAP kinase activation was blocked. In contrast, the PC-PLC inhibitor D609 had no effect. The ERK family of MAPKs is responsible for at least part of the effect of E2 on MAPK specific activity. Specific inhibition of ERK1/2 using PD98059 (1-100 μM) reduced [35S]-sulfate incorporation due to E2 in a dose-dependent manner. However, ERK1/2 was not involved in the regulation of alkaline phosphatase specific activity by E2 since PD98059 did not alter the estrogen-dependent stimulation of this enzyme activity. RT-PCR confirmed that the RGP cells expressed ERK1 and ERK2 mRNAs and western blot confirmed that the protein was present in the cells. This study supports the hypothesis that E2 regulates the physiology of female RGP cells via rapid membrane-mediated signaling pathways, and some, but not all, of the response to E2 is via the ERK family of MAPKs. Moreover, this is the first study to show that MAP kinase signaling can be regulated in a sex-specific manner. Because ERs, PKC and ERK1/2 are present in both male and female cells, the sex-specificity of the response may be conferred by the membrane receptor or coupling factor.

Disclosures: ***Z. Schwartz****, None.*

SU475

Dose-Dependent Effect of Isoflavone and Vitamin E on Lipid Parameters in Rats. E. A. Lucas, L. J. Hammond*, D. A. Khalil, B. J. Smith, L. Devareddy*, D. Y. Soung, S. C. Chai*, C. Wei*, B. H. Arjmandi. Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA.

Both isoflavone and vitamin E have been shown to influence the skeletal and cardiovascular systems in ovarian hormone deficiency. The purpose of this study was to examine the dose-dependent effects of isoflavones and vitamin E individually and in combination on lipid parameters of ovariectomized rats. Twelve-month old female Sprague-Dawley rats were ovariectomized and randomly assigned to one of 12 treatment groups (n =12). For a period of 120 days, all rats were maintained on a semi-purified casein-based diet (AIN-93M). Thereafter, rats were placed on the different dietary treatments: one of the three doses of soy isoflavones (0, 500, or 1000 mg/kg diet) in combination with one of the four doses of vitamin E (75, 300, 525 or 750 mg vitamin E per kg diet) for 100 days. Significant reductions in serum total- and HDL-cholesterol concentrations were observed with the highest dose of isoflavones (Table). However, the highest dose of vitamin E significantly increased HDL-cholesterol concentrations, but had no effect on total cholesterol. Both vitamin E and isoflavones significantly reduced non-HDL cholesterol concentrations. These findings suggest that there is no synergy between vitamin E and isoflavones in improving lipid profile.

	Vitamin E *(mg/kg diet)*				Isoflavones *(mg/kg diet)*		
Dose	T-Chol *(mg/dL)*	HDL *(mg/dL)*	Non-HDL *(mg/dL)*	Dose	T-Chol *(mg/dL)*	HDL *(mg/dL)*	Non-HDL *(mg/dL)*
75	159	59^{b}	100^{a}	0	164^{b}	66^{a}	98^{a}
300	155	62^{b}	94^{ab}	500	156^{b}	65^{a}	94^{a}
525	150	64^{ab}	86^{bc}	1000	139^{a}	60^{b}	79^{b}
750	149	70^{a}	79^{c}				
	P value				*P* value		
Vit E	0.240	0.007	0.0022	Iso	<0.0001	0.046	0.0009
Vit E*Iso	0.925	0.496	0.911				

*Since there were no significant interactions between vitamin E and isoflavones data are not presented. In each column, values that do not share a common superscript are significantly different from each other.

Disclosures: ***E.A. Lucas****, None.*

SU476

Down Regulation of Molecules Critical for Calcium Reabsorption in the Kidney of Aromatase Deficient Mice. O. K. Oz[1], J. Zerwekh[2], A. Hajibeigi*[1], R. J. M. Bindels*[3], C. Y. C. Pak*[4]. [1]Radiology, UT Southwestern Medical Center, Dallas, TX, USA, [2]Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA, [3]Cell Physiology, University of Nijmegen, Nijmegen, Netherlands, [4]Mineral Metabolism, UT Southwestern Medical Center, Dallas, TX, USA.

The incidence of renal stones increases in women after menopause. This increase may be related to changes in calcium handling in the kidney. To determine if estrogen deficiency is sufficient to change molecular factors important for calcium reabsorption in the kidney, we compared expression of key factors in aromatase deficient (ArKO) mice compared to wild-types (Wt). Kidneys were harvested from female Wt, ArKO or estradiol treated ArKO mice for immunohistochemistry, RNA and protein preparation. Estradiol treatment consisted of 20ug/mouse 3x/week for 3 weeks. The levels of calbindin-D28k and ECaC-1 and 2 were compared between groups using immunohistochemistry, real-time PCR, and/or western blotting. Real-time PCR, immunohistochemistry, and western blotting revealed decreased message and protein levels of renal calbindin-D28k in ArKO mice. Estradiol therapy reversed the changes. Compared to Wt, ECaC-1 and 2 message levels were decreased in RNA samples prepared from ArKO kidneys. The change in ECaC-1, the major ECaC isoform in kidney, was reversed by estradiol therapy. This therapy did not change ECaC-2 levels. These results show that estrogen deficiency is sufficient for changing levels of molecules key to calcium rabsorption in the kidney. The data raise the potential for a renal leak of calcium in the estrogen deficient state.

Disclosures: ***O.K. Oz****, None.*

SU477

Syntheses and Biological Activities of 2α-Substituted 20-*epi*-vitamin D_3 Derivatives. S. Honzawa[1], Y. Suhara*[1], N. Saito*[1], S. Kishimoto*[1], T. Fujishima*[1], M. Kurihara*[2], T. Sugiura*[1], K. Waku*[1], H. Takayama[1], A. Kittaka[1]. [1]Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Japan, [2]National Institute of Health Sciences, Setagaya, Japan.

1α,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2VD_3$) is the physiologically active metabolite of vitamin D_3, and an epimerization at the 20-position on the side chain was found to result in higher cell differentiation activity and lower calcium metabolism compared with 1,25-$(OH)_2VD_3$. Recently, we have shown that 2α-methyl and 2α-(3-hydroxypropyl)-substituted 1,25-$(OH)_2VD_3$derivatives showed 3 to 4-fold higher vitamin D receptor (VDR) binding affinity with superagonistic activities. This time we planed to demonstrate the syntheses and biological evaluation of 2α-alkyl and 2α-(ω-hydroxy)alkyl substituted 20-*epi*-1,25-$(OH)_2VD_3$derivatives to examine the effect of 2α-substituent on 20-*epi*-1,25-$(OH)_2VD_3$. In the course of our synthetic study, an efficient scheme for introducing the 2α-alkyl group was developed.

Syntheses of analogues were conducted by a convergent method, the CD ring moiety was derived from vitamin D_2 and the A ring part from D-glucose. 2α-Alkyl groups were introduced by Grignard reaction of the known epoxide intermediate. It was noted that, in this reaction, the use of toluene gave higher chemical yield and shorter reaction time than those in conventional ethereal solvents. According to this new synthetic methodology, we could prepare eight 20-*epi*-analogues, that is, 2α-methyl, ethyl, propyl and butyl-substituted ones and their ω-hydroxylated ones.

Biological evaluation of the analogues was performed by VDR binding and human promyelocytic leukemic HL-60 cell differentiation assays. VDR binding affinity of the analogues was measured by competitive displacement of radioactive 1,25-$(OH)_2VD_3$ on the bovine thymus VDR. We found that 2α-methyl substituted analogue had the highest affinity (12-fold over 1,25-$(OH)_2VD_3$, 3-fold over 20-*epi*-1,25-$(OH)_2VD_3$). Cell differentiation assay was performed using NBT reduction method. In this assay, these analogues also showed similar tendency as in the binding assay, and the activity increased more potently than the analogues having the natural type side chain. In the 2α-alkyl series, the longer the alkyl chain, the less the affinity to VDR and the differentiation activity, which is in accordance with the results of the analogues having the natural type side chain, as already reported from our laboratory. In contrast, in the 2α-(ω-hydroxy)alkyl series, the increases in these activities were modest, and 2α-(3-hydroxypropyl)-substituted analogue was found to be most potent in this series (3.7-fold over 1,25-$(OH)_2VD_3$, 1.9-fold over 20-*epi*-1,25-$(OH)_2VD_3$).

Disclosures: ***S. Honzawa****, None.*

SU478

Vitamin D Antagonist, TEI-9647, Is Potent Inhibitor of Bone Resorption Induced by 1,25-(OH)2D3 with Osteoclasts Derived from Pagetic Bone Marrow Cells. S. Ishizuka[1], N. Kurihara[2], Y. Oue*[1], D. Miura*[1], K. Takenouchi*[1], J. Cornish[3], T. Cundy[3], S. V. Reddy[2], G. D. Roodman[2]. [1]Teijin Institute for Bio-Medical Research, Tokyo, Japan, [2]Division of Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [3]Department of Medicine, University of Auckland, Auckland, New Zealand.

Although Paget's disease is the most flagrant example of disordered bone remodeling, little is known about its pathogenesis. Recently we reported that measles virus infection may play a major role in pathogenesis of Paget's disease including contributing to the hypersensitivity of osteoclast precursors to 1,25-(OH)2D3 through increasing expression of TAFII-17, a potential coactivator of VDR in pagetic osteoclast precursors. Pagetic osteoclast precursors require at least 10 to 100 times less 1,25-(OH)2D3 to form osteoclasts than that required for normal bone marrow cells. These results suggest that in Paget's disease bone resorption might be enhanced by physiologic levels of 1,25-(OH)2D3. If this is correct, vitamin D antagonists that suppress the function of 1,25-(OH)2D3 could be potential agents to inhibit the enhanced bone resorption in patients with Paget's disease. To test this hypothesis, we investigated if the vitamin D antagonist, TEI-9647, inhibited bone resorption induced by 1,25-(OH)2D3 with osteoclasts produced by RANKL/M-CSF from normal bone marrow cells and measles virus nucleocapsid gene transduced early osteoclast precursors (MVNP transduced CFU-GM cells). TEI-9647 alone never caused bone resorption even at 10-6M, but dose-dependently (10-9M to 10-6M) inhibited bone resorption induced by 10-9M 1,25-(OH)2D3. Furthermore, TEI-9647 dose-dependently (10-9M to 10-6M) inhibited osteoclast formation induced by 10-10M 1,25-(OH)2D3 from MVNP transduced CFU-GM cells and bone marrow cells from patients with Paget's disease. At the same time, TEI-9647 dose-dependently suppressed TAFII-17 gene expression induced by 10-9M 1,25-(OH)2D3.

These results suggest that low concentrations of vitamin D antagonist, TEI-9647, acts directly on osteoclast precursors and osteoclasts, and suggest that TEI-9647 may be a novel treatment to suppress the excessive bone resorption and osteoclast formation in the patients with Paget's disease.

Disclosures: ***S. Ishizuka****, None.*

SU479

Photosynthesis and Biologic Evaluation of Suprasterols. D. P. Jamieson*, K. S. Persons*, M. F. Holick. Medicine, Section of Endocrinology, Diabetes and Nutrition, Boston University Medical Center, Boston, MA, USA.

Vitamin D has long been viewed as a hormone important for calcium homeostasis and bone metabolism. Its activated form,1,25-dihydroxyvitamin D_3 is recognized as a potent regulator of cellular proliferation and terminal differentiation. During exposure to sunlight, 7-dehydrocholesterol in the epidermis is photolyzed to previtamin D_3. Once formed to previtamin D_3 in its cis, cis conformation it rapidly undergoes a thermally induced rearrangement of its triene system to form vitamin D_3. When vitamin D_3 is exposed to ultraviolet radiation it undergoes isomerization of its conjugated double bonds to form several photoproducts that are collectively known as suprasterols. Little is known about the biologic function of these unique vitamin D_3 photoisomers. Vitamin D_3 was dissolved in n-hexane and exposed to UV radiation generated from a medium- pressure mercury arc lamp. The irradiation mixture was chromatographed on an Agilent 1100 series normal-phase high performance liquid chromatograph equipped with diode array and evaporative light scattering detectors. A total of 6 different suprasterols were isolated, purified, and quantified. Each of the suprasterols were evaluated for their antiproliferative activity in cultured human keratinocytes. A ^{3}H-thymidine assay was performed to determine the antiproliferative activity of the suprasterols. Neonatal keratinocytes were grown in a 24 well plate and 8 wells each received either control vehicle or one of the suprasterols at either 10^{-8},10^{-7}, or 10^{-6} M. 1,25-Dihydroxyvitamin D_3 was used as a positive control. Suprasterols 3 and 4 showed marked antiproliferative activity when incubated with cultured human keratinocytes. In a dose-dependent fashion, suprasterol 3 and suprasterol 4 decreased ^{3}H-thymidine incorporation into cultured human keratinocytes by approximately 18, 25, and 82% at concentrations of 10^{-8}, 10^{-7}, and 10^{-6} M, respectively. 1,25$(OH)_2D_3$ also demonstrated marked antiproliferative activity in a dose-dependent fashion with 24, 62, and 83% inhibition at 10^{-8}, 10^{-7}, and 10^{-6} M concentrations, respectively. The other 4 suprasterols had no antiproliferative activity when incubated with cultured human keratinocytes. Thus, at least two of the photoproducts of vitamin D_3 have potent antiproliferative activity in cultured human skin cells. These results suggest that the photoisomerization of vitamin D_3 in human skin may have an important physiologic role in regulating epidermal proliferation .

Disclosures: ***M.F. Holick****, None.*

SU480

2-Methylene-19-Nor-(20S)-1,25-Dihydroxyvitamin D3 Potently Stimulates Gene-Specific DNA Binding of the Vitamin D Receptor in Osteoblasts. H. Yamamoto, N. K. Shevde, A. C. Warrier*, L. A. Plum, H. F. DeLuca, J. W. Pike. Biochemistry, University of Wisconsin-Madison, Madison, WI, USA.

The cellular actions of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) are mediated through the vitamin D receptor (VDR), a ligand-induced transcription factor that binds to promoter regions of target genes and initiates additional events that culminate in altered gene expression. Studies over the past decade have indicated that the VDR binds to specific DNA sequences as an RXR heterodimer, and that this complex recruits additional factors termed comodulators that exhibit intrinsic enzymatic activities essential for transcriptional modulation. 2-Methylene-19-nor-(20S)-1,25-dihydroxyvitamin D3 (2MD) is a highly potent analog of 1,25(OH)2D3 whose actions are also mediated through the VDR. In this report, we have replicated this increased potency of 2MD in vitro using osteoblastic cells, and explored its underlying molecular mechanism. 2MD stimulates the expression of several vitamin D-sensitive genes including 25-hydroxyvitamin D3-24 hydroxylase (Cyp24), osteopontin and receptor activator of NFkB ligand and suppresses the expression of osteoprotegerin at concentrations two logs lower than that for 1,25(OH)2D3. 2MD is also more potent in stimulating transfected chimeric reporter genes under either Cyp24 or the osteocalcin promoter control. Enhanced potency is retained regardless of medium serum content. Interestingly, the uptake of both 1,25(OH)2D3 and 2MD into cells in the absence of serum is similar, as is their rapid association with the vitamin D receptor (VDR). This indicates that comparable levels of occupied VDR do not elicit equivalent levels of transactivation. Using chromatin immunoprecipitation (ChIP), however, we observed a strong correlation between DNA-bound receptor and the level of induced transcription suggesting a 2MD-induced increase in affinity of the VDR for DNA. Additional studies using a mammalian two-hybrid system and ChIP indicate that 2MD is also more potent in promoting interaction with RXR and the coactivators SRC-1 and DRIP205. Finally, protease digestion studies revealed the existence of a unique VDR conformation in the presence of 2MD. These studies indicate that the molecular mechanism of 2MD potency is likely due to its ability to promote enhanced levels of specific DNA binding and complex formation by the VDR on target gene promoters and suggest possible explanations for the tissue- and gene-selective actions of 2MD.

Disclosures: ***H. Yamamoto****, None.*

SU481

Enhanced Transcriptional Activity Of Novel Gemini Analogs Of 1,25 Dihydroxyvitamin D_3: Cellular Actions Suggest Enhanced Metabolic Stability. F. A. A. Weyts*[1], P. Dhawan*[1], N. Tibrewal*[1], X. Zhang*[2], J. E. Bishop[2], M. Uskokovic*[3], H. Maehr*[3], A. W. Norman*[2], S. Christakos[1]. [1]Biochemistry, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA, [2]Biochemistry, University of California, Riverside, CA, USA, [3]BioXell Inc., Nutley, NJ, USA.

Gemini analogs of 1,25 D3 contain two 6 carbon side chains, combining a C-20 normal with a C20-epi side chain (Uskokovic, MR, Manchand, PS, Peleg, S and Norman, AW Proceedings of the 10th Workshop on Vitamin D, 1997). We studied the transcriptional potential of Gemini analogs combining the double side chain with a 23-triple bond and a C-26,27 hexafluoro substitution (in either the 20S or 20R configuration) and/or deletion of C-19 (19-nor). All four Gemini analogs tested [20S and 20R-21-(3OH-3-methylbutyl) 23-yne-26,27-F6-19-nor 1,α25$(OH)_2D_3$ ('20R-19-nor' and '20S-19-nor') and 20S and 20R-21-(3OH-3- methylbutyl)-23yne-26,27-F6 1,α25$(OH)_2D_3$ ('20S' and '20R')] were more potent (5-50x) than 1,25D3 in inducing transcriptional activity from the osteocalcin or 24-hydroxylase promoters. The 20S analog was 2 to 7 times more potent than the 20R analog (on the 24OHase and OC promoters, respectively), whereas 20S-19-nor was only found to be more potent (8 times) than 20R-19-nor on the 24OHase promoter. Increased potency

was confirmed in vivo: the 20S analogs induced higher levels of calbindin-$_{9K}$ mRNA levels in intestine following i.p. injection in vitamin D-deficient mice than 1,25D3 or 20R. The increased activity of these Gemini analogs did not correlate with ligand-receptor binding affinity. Binding to VDR was reduced (2-5 fold compared to 1,25D3), without significant differences between 20S and 20R analogs. Binding to the plasma vitamin D binding protein (DBP) was dramatically reduced (10 –20 fold compared to 1,25D3), without significant differences between 20S and 20R analogs. GST DRIP205 and GST GRIP pull down assays performed with nuclear extracts of VDR transfected and Gemini treated (10^{-8} – 10^{-10}M for 24h) COS-7 cells correlate with transcriptional potential; the 20S analogs being (2-5 fold) more potent than the 20R analogs and all four analogs being (up to 10 fold) more potent than 1,25D3. However, when in vitro translated ^{35}S-VDR was used in the pull downs, the Gemini analogs showed equivalent or even attenuated cofactor recruitment compared to 1,25D3. From these data we conclude that the enhanced potency of these novel Gemini analogs is at least partly due to enhanced metabolic stability of the analogs, resulting in increased cofactor binding and increased transcription.

Disclosures: ***F.A.A. Weyts****, None.*

SU482

Distribution and Metabolism of 2MD, an Analog of 1-alpha,25(OH)2D3 with Potent Anabolic Activity in Bone. L. A. Plum*[1], P. Grzywacz*[2], X. Ma*[1], E. Lake*[1], M. Clagett-Dame*[2], H. F. DeLuca[2]. [1]Biochemistry/Deltanoid Pharmaceuticals, University of WI-Madison, Madison, WI, USA, [2]Biochemistry, University of WI-Madison, Madison, WI, USA.

The distribution and metabolism of 2-methylene-19-nor-(20S)-1α,25(OH)2D3 (2MD) in rats was studied to help understand the potency and bone selective action of this vitamin D analog. Radiolabeled 2MD (174 Ci/mmol) was prepared by substituting the six hydrogen atoms located in the 26 and 27 positions with tritium. Male and female adult Sprague-Dawley rats were given a single dose of 3H-2MD or 3H-1α,25(OH)2D3 either by oral gavage or tail vein injection and blood was collected after 0.5, 1, 2, 4, 6, 8, 16, 24 and 48 hours. Urine and feces were collected after 24 and 48 hours. Compared to 3H-1α,25(OH)2D3, there is very little radioactivity that appears or remains in the blood of animals given 3H-2MD. Oral administration of 3H-2MD resulted in less than 1% of the total radioactivity detectable in the serum 0.5-8 hours after the drug was given, whereas 4-6% of the total radioactivity was detectable in 2-8 hours in rats given 3H-1α,25(OH)2D3 orally. Intravenously administered 3H-2MD was cleared rapidly from blood demonstrating that the low amount of radioactivity in blood after an oral dose is not due to inefficient intestinal absorption. Rapid cellular uptake of 2MD in target tissues is the most likely explanation as urine and fecal analyses can not account for the lack of radioactivity in the blood. This idea is supported by in vitro studies using bone cell cultures in which 2MD is more rapidly taken up compared to 1α,25(OH)2D3 (J.W. Pike, personal communication). Total radioactivity and metabolites in classic target tissues of vitamin D - bone, kidney, intestine, and parathyroid gland - are currently being analyzed.

Disclosures: ***L.A. Plum****, None.*

M001

Does Calcium from Milk Minerals Enhance Bone Mass Accrual and Alter Bone Dimensions during Growth Better than Calcium Carbonate. S. Iuliano-Burns[1], A. Evans*[2], X. Wang*[1], S. Matthews*[3], E. Seeman[1]. [1]Medicine, University of Melbourne, A&RMC, Heidelberg, Australia, [2]Bone Density, Austin & Repatriation Medical Centre, Heidelberg, Australia, [3]Endocrinology, Austin Hospital, Heidelberg, Australia.

Only one study in girls, using calcium derived from a milk extract, reported a residual benefit to bone mass and bone size following cessation of supplementation[1]. To test the hypothesis that calcium from milk minerals, but not calcium carbonate will enhance bone mineral content (BMC) accrual and alter bone dimensions, 75 pre-pubertal children were randomly assigned to receive either 800 mg/d calcium from milk minerals, calcium carbonate, or a placebo, for 9 months, in a double blind fashion. Bone dimensions & BMC were assessed pre- and post-supplementation using DXA. Dietary calcium intake was measured pre- mid- and post-intervention using 3-day weighed food diaries. Anthropometry and hours of weight bearing exercise were recorded pre- and post-intervention. Differences between groups were determined using ANCOVA, adjusting for baseline values. Data was analysed using Statview (version 4.51).
Despite randomisation the placebo group was older and larger than the two calcium groups. The calcium carbonate group accrued more BMC (%) at the ulna-radius (20 ± 1 v 15 ± 2, $p < 0.05$) and showed a tendency to accrue more bone (%) at the humerus than the placebo group (16 ± 2 v 12 ± 2). The milk mineral group accrued more BMC (%) at the tibia-fibula than the placebo group (27 ± 2 v 22 ± 2, $p < 0.07$). No differences were reported between groups for % BMC gain at the femur. There was a non-significant trend towards a greater gain in periosteal width (%) in the milk mineral group compared to the placebo group (7 ± 2 v 4 ± 3). The effect of both forms of calcium was evident at the peripheral than distal appendicular skeleton. However, no differences were reported between the two forms of calcium. The effect of calcium supplementation on bone size, and the long-term benefits of calcium supplementation remain to be determined.
1. Bonjour et al. J Clin. Invest. 1997, 99:1287-94

Disclosures: ***S. Iuliano-Burns****, None.*

M002

Effect of Inulin and Oligofructose from Chicory on Bone Mineral Density in Rats. A. T. Nzeusseu*, D. H. Manicourt*, G. Depresseux*, D. Dienst*, J. P. Devogelaer. Rheumatology Unit, St-Luc University Hospital, Brussels, Belgium.

Intestinal calcium absorption in rats can be increased by addition of non-digestible oligosaccharides to their diet.The aim of this study was to assess the role of inulin (IN) and oligofructose (OF) from chicory on BMD acquisition in growing rats.Thirty-nine Whistar male rats,aged 6 weeks and weighing on average 150-175 g were fed with AO4 diet containing 0.6% phosphorus and 1% calcium in order to provide sufficient alimentary calcium.They were divided in 3 groups : Group 1 served as controls (C).Group 2 received OF with a degree of polymerization (DP) between 2 and 20 . Group 3 received IN with DP between 2 and 60 (average DP of minimum 8) . Dosages of soluble fibres from IN,OF in diet amounted to 5%.Treatment duration was 3 months ,when the rats were sacrificed. Whole Body BMC (WBBMC)was measured by DXA (QDR-1000W,Hologic, Inc,Bedford,MA)at the start and after 3 months.pQCT(research XCT,Norland,Fort Atkinson,WI)of L3,of left mid-femur and of left tibia was used to measure BMD after sacrifice.The weight gain was a little greater after 3 months in group 2 .WBBMC increased significantly in group 2 as compared to other groups (287 % in group 2 , vs 272,and 265 % in C and group 3, respectively).pQCT measurements showed a WBBMD ,trabecular BMD and cortical + subcortical BMD of L3 significantly higher in group 3 (648, 262 and 963 g/cm3,vs 616,233,929 and 642,258,956 g/cm3 in C and group 2,respectively).The BMD of total mid-femur was also the highest in group 3 (1002 vs 985 and 977 g/cm3 in group 2 and C and ,respectively). At mid-tibia and metaphyseal proximal region,BMD was also higher in group 3 (1048,625;vs 1006,567 and 1012,589 g/cm3 as compared to C and group 2 , respectively) . In conclusion , a positive,significant effect of IN,OF feeding was observed particularly on BMD of peripheral bones in growing rats , which should encourage to test these natural products in humans.

Disclosures: ***J.P. Devogelaer****, Work supported by a grant of Cosucra Group Warcoing, Belgium. 2.*

M003

The Adaptation of the Pre-Pubertal Skeleton to High Intensity Exercise: A Study of Bone and Muscle Geometry. K. A. Ward[1], J. E. Adams[1], R. L. Ashby*[1], S. Roberts*[1], Z. Mughal[2]. [1]University of Manchester, Manchester, United Kingdom, [2]Pediatric Medicine, Saint Mary's Hospital, Manchester, United Kingdom.

A bone's strength is determined by its size, shape and amount of mass contained within the periosteal envelope. Therefore, to get an indication of a bone's adaptation to high intensity exercise, measurements other than bone mineral density should be made; baseline peripheral quantitative computed tomography (pQCT) data from an ongoing trial have been analysed to investigate these adaptations. We hypothesise that compared to sedentary controls (n=42) gymnasts (n=44) will have larger bones with thicker cortices and therefore greater stress-strain indices (SSI, related to bone bending strength), in the radius (R) and tibia (T). Muscle cross sectional area (CSA), an indicator of muscle force, will also be greater in the gymnasts.
Bone and muscle measurements were measured at 50% R and 65% T using pQCT (XCT-2000, Stratec, Germany); loop analysis was used to measure bone mineral content (BMC), cortical thickness, SSI, periosteal and endosteal circumferences. Bone age was assessed from hand radiographs (Greulich & Pyle).
Natural logs of bone and muscle variables were taken. Results are given as mean difference (ratio controls: gymnasts), p=. After adjustment for sex, weight and height gymnasts had higher cortical bone area (R:0.89, 0.002; T:0.92, 0.002), mineral content (R:0.90, 0.006; T: 0.92, 0.009) and thicker cortices (R:0.93, 0.07, T:0.92, 0.01) in both the R and T than controls. Consequently their SSI was higher in both bones (R:0.85, 0.001, T:0.92, 0.007). Compared to controls the gymnasts also had greater muscle CSA (R:0.82, <0.001, T:0.94, 0.01) and grip strength (0.87, 0.01).
The bones of gymnasts have higher SSI than sedentary controls. This is likely to be achieved by an increase in cortical BMC and area; the increase in area itself reflects deposition of bone on the periosteal surface (NS). These extremely small adaptations have a highly beneficial effect on the strength of the bone thus allowing the appendicular skeleton of pre-pubertal gymnasts to withstand the forces it is subjected to by muscles during activity.

Disclosures: ***K.A. Ward****, None.*

M004

Vitamin D Stores, PTH and VDR Taq1 Genotype are Associated with Gain in Whole Body Bone Mass in Pubertal Children. F. Tylavsky, K. Ryder, R. Li*, V. Park*, L. Carbone. University of Tennessee The Health Science Center, Memphis, TN, USA.

Controversies exist as to the level of Vitamin D stores (25-OH D) necessary to suppress PTH and preserve bone mass and the role of Vitamin D Receptor (VDR) Taq1 gene in this process. The objective of this study was to evaluate the effect of 25-OH D, PTH and VDR Taq1 genotyping on the accumulation of whole body bone mass. There were 69 children (11% black, 44% male) with completed measurements at baseline and 24 months after enrolling in a calcium supplementation trial. Bone area (BA), bone mineral content (BMC) and bone mineral density (BMD) of the whole body were obtained in duplicate at the two measurement times using a QDR 2000 (Hologic, Inc.). Serum measures of 25-OH D, PTH and osteocalcin and urine deoxypyridoline (DpD) were analyzed using standardized methods. Genotyping of the VDR TaqI polymorphism (TT, Tt, tt) was performed by real-time

PCR with allele-specific fluorescent probes. The Hardy-Weinberg equilibrium principle was held for the genotype data of this sample. Serum 25-OH D stores were divided into 3 groups for analyses: low (those who consistently fell into the lower quartile, <17 ng/ml), medium (the middle two quartiles, 17-33 ng/ml) and high (the upper quartile, >33 ng/ml). There were no differences among the 25-OH D groups with respect to calcium intake, urinary calcium excretion, DpD and osteocalcin. Those with low vitamin stores had larger percent gain in BA, BMC and higher PTH levels at baseline and 24 months than those in the high group, adjusting for tanner stage, race, sex and treatment modality.

Adjusted means and SE for the dependent variables:	Percent Gain over 2 years						24 mo PTH, ng / ml	
	BA		BMC		BMD			
25-OH D groups	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Low (n=16)	31	2	42	3	9	1	45	4
Moderate (n=37)	27	2	40	2	9	1	35	3
High (n=16)	21	2	32	3	7	1	27	4
ANOCOVA, p value	0.003		0.010		0.10		0.004	

Similar results were obtained when the analyses were confined to white children. Compared with those who had the TT genotype (N=13), those with at least one 't' allele (Tt and tt genotypes, N=43)) had higher acquisition of BMD (5.6% vs. 8.9%, p<0.005), and BA(22% vs. 28%, p=0.02) and statistical trend for greater acquisition of BMC (34.4% vs. 39.8%, p=0.06) independent of 25-OH D stores and PTH levels. While these associations have been reported on cross sectional data, this is the first study to show that higher PTH levels associated with low 25-OH D stores may be beneficial to accruing larger bone size and BMC. Whether these differences persist throughout puberty remains to be determined.

Disclosures: ***F. Tylavsky****, None.*

M005

Muscle Mass Predicts Peak Bone Mass and Osteocyte Density in the Femora of Lean and Obese Mice. M. Hamrick, C. Pennington*, D. Xie*, C. Isales. Medical College of Georgia, Augusta, GA, USA.

Body mass is one of the strongest predictors of bone mass in humans; however, there is considerable debate as to whether fat mass or muscle mass is a better predictor of peak bone mass. Leptin is a hormone secreted by adipocytes that may contribute to the positive effects of fat mass on bone mass, although several studies have also suggested that leptin deficiency is associated with a high bone mass phenotype. We investigated the relative effects of muscle mass and fat mass on bone mass in a mouse model of obesity, the leptin deficient (ob/ob) mouse, and in lean controls. Ten adult mice, six months of age, per group were included for analysis. DEXA densitometry (PIXImus system) was used to measure bone mass, expressed as bone mineral content (BMC), and bone mineral density (BMD) from the femora of each animal. Histological sections from the distal femur were prepared and cortical thickness, cortical area, and osteocyte lacuna population density measured from thin sections. Sections were also stained with an antibody to TGF-beta 1, since the amount of TGF-beta 1 in bone is frequently correlated with bone mass in experimental animals. Results show that body weight of the obese mice is approximately double that of the lean mice, but the quadriceps muscles of the obese mice are 40% smaller (P<.001) than those of the lean mice. The lean mice also show significantly greater femoral BMC (P<.001) and femoral BMD (P=.001) compared to the obese mice, and femoral BMC exhibits a strong, positive correlation (r=.85, P<.001) with muscle mass among the animals included in this study. The lean animals also have significantly greater osteocyte lacuna population density compared to the obese mice, suggesting an increased rate of osteoblast differentiation, and osteocyte density is positively correlated with muscle mass (r=.66, P=.001). Osteocytes in the bones of both strains stained positively for TGF-beta and the higher total osteocyte number in the lean animals is associated with a higher total number of TGF-beta positive osteocytes. These results suggest that low bone mass in the femora of obese, leptin-deficient mice is due not only to an absence of leptin signaling but also to a marked decrease in muscle mass. Moreover, the greater bone mass of the lean animals is associated with increased osteocyte density, supporting the hypothesis that osteocyte number is an important determinant of BMC and BMD and may be mediated in part by muscle mass.

Disclosures: ***M. Hamrick****, None.*

M006

Bone Mass of Spine and Femur is Reduced in Healthy Term Infants of Multi-Parous Women. H. Kovacs*, U. McCloy*, J. Schelenberg*, R. Veitch*, H. Weiler*. Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada.

Throughout pregnancy a mother will transfer approximately 30 g of calcium to the growing fetus. The relationship between parity and infant bone mineral content (BMC) at birth is not clear. The objective of this study was to describe the association between maternal parity and the BMC of their infants at birth (n=59). Maternal parity, age, height and weight grain during pregnancy, multivitamin use and smoking status were obtained from hospital records. Infants' were measured for anthropometry then BMC of spine and femur using dual energy x-ray absorptiometry (DXA). On average the postnatal age at the DXA visit was 1.5±1.0 weeks. Regression analysis revealed that parity (0.98±1.20) significantly predicts BMC of spine [2.21±0.49 g; y=0.392-0.472(Asian)+0.583(birth weight)-0.0667(parity>3); R=0.781, p=0.001] and femur [2.80±0.58 g; y=0.478+0.698(birth weight)-0.911(parity>3); R=0.581, p=0.002]. Gender, parental heights, smoking, maternal age, prenatal vitamin use, weight gain during pregnancy and the First Nations and black races did not contribute significantly to the regression models. These results may be due to differences in socioeconomic status or activity levels during pregnancy as observed in other studies. Further research is required to elucidate the cause of reduced BMC in infants of multi-parous women, the long-term consequences and whether there are special nutritional needs of multi-parous women during pregnancy.

Disclosures: ***H. Kovacs****, None.*

M007

BMC Growth in Black and White Children. S. L. Hui[1], A. J. Perkins*[2], M. Peacock[1], C. Longcope*[3], C. C. Johnston[1]. [1]Medicine, Indiana University, Indianapolis, IN, USA, [2]Regenstrief Institute, Indianapolis, IN, USA, [3]Ob/gyn, University of Massachusetts, Worcester, MA, USA.

A longitudinal study was conducted to compare the growth in BMC between Black and White boys and girls, and to investigate factors related to their bone growth.

Total body and spine BMC were measured annually on 52 white boys, 39 black boys, 50 white girls and 47 black girls over 1 to 4 (mean= 2.6) years. Their initial ages ranged from 5 to 15 years (mean=10.4±2.7(SD)). Annual measurements were also made on Tanner stage, anthropometrics, body composition, sex hormone levels, and biochemical markers of bone metabolism. Consecutive measurements on individuals were used to calculate an annualized rate of change in BMC (ΔBMC) and other measurements. Mixed-effects models were used to compare ΔBMC between black and white children, separately for boys and girls. Both the mean and the change of each predictor variable were considered as potential covariates in a comprehensive model.

The mean initial ages were almost identical between the black and white children, but the black children were higher in mean Tanner stage. The mean ΔBMC was 224 g/year in the total body and 4.5 g/year in the spine. The rates at both skeletal sites had a curvilinear relationship with age and with Tanner stage, peaking around age 13-14 and Tanner Stage IV for boys, and age 11-12 and Tanner Stage III for girls. Controlling for age and Tanner stages, black children had lower ΔBMC, the difference being more significant in the spine (1.2 g/year, p<0.001 for girls, 0.9 g/year, p<0.1 for boys) than in the total body (32 g/year, p>0.1 for boys, 26 g/year, p=0.04 for girls).

With all significant covariates in the model, change in lean body mass was the most strongly and consistently associated with ΔBMC in both total body and spine, for both boys and girls (p<0.0001), while change in height predicted ΔBMC only in girls. Larger biacromial width was also associated with greater ΔBMC in the spine (but not total body) for both boys and girls.

Among the sex hormones, only the increase in testosterone was correlated with greater ΔBMC at both sites and only for the boys. Only two biochemical markers were significant in the models. ΔBMC was negatively correlated with the change in osteocalcin in boys and with the change bone-specific alkaline phosphatase in girls. After adjusting for all the covariates, black children still have lower ΔBMC than white children of the same age.

In summary, black children do not accrue bone any faster than white children of the same age and Tanner stage. However, black children are generally more sexually mature so they have higher BMC than white children of the same age.

Disclosures: ***S.L. Hui****, None.*

M008

Reported High Strain Physical Activity Is Associated with Cortical Bone Dimensions and Strength in School Age Children. B. S. Zemel, A. Buison*, A. Vresilovic*, J. Tetlak*, R. F. Ittenbach*, V. A. Stallings*, M. B. Leonard. Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA, USA.

In order to develop strategies to prevent osteoporosis later in life, it is important to identify the modifiable factors in childhood that are associated with fracture risk. Cortical bone dimensions and the strain-strength index (SSI) are strong indicators of fracture risk. Little is known about the effects of modifiable factors on bone dimensions and strength in children. Peripheral quantitative computed tomography (Stratec, XCT2000) was used to measure midshaft (distal 20%) tibia cortical area (CRT_A) and content (CRT_C), and SSI in 123 healthy children (77 girls) ages 7 to 10 years of age. BMI z-score (BMIZ) was calculated based on the CDC growth charts. Pubertal development, calcium intake, physical activity, and fracture history were assessed by questionnaire. Peak strain score, based on ground reaction force, was calculated for each reported activity on the physical activity questionnaire. Cumulative strain score for the previous year was calculated. In addition, children were also classified as those with no high strain activities vs. any high strain activities, e.g., jumping rope, basketball, gymnastics (ANY_STR). Continuous variables were tested for skewness and log-normalized when appropriate. Multiple regression models were developed to identify appropriate covariates (tibia length, BMIZ, gender, puberty status), so that the effect of physical activity and calcium intake on pQCT bone measures could be assessed. Tibia length and BMIZ were strong predictors of CRT_A, CRT_C and SSI. Gender was an important additional predictor for CRT_A and CRT_C, but not SSI. Calcium intake (percent of the recommended adequate intake) and fracture history were not associated with any bone measures. Cumulative strain score was not associated with bone measures. Fifty-nine percent of children reported any high strain activities. ANY_STR was significantly associated with greater CRT_A (p = 0.02), CRT_C (p< 0.01) and SSI (p< 0.01) after adjusting for tibia length, BMIZ, gender, and puberty. Overall, R^2 values were 0.73, 0.72 and 0.75 for CRT_A, CRT_C and SSI, respectively. These findings demonstrate (a) the importance of adjusting for growth and BMI effects on bone dimensions and strength, and (b) even small amounts of high strain physical activity in children can significantly improve cortical bone strength.

Disclosures: ***B.S. Zemel****, None.*

M009

Reference Values for Bone Mineral Density Measurement in Healthy 12-18 Year Old Females Categorized by Race, Weight, and Chronologic Age. B. Cromer[1], D. Ziegler*[2], R. Harvey*[2], S. Debanne*[3]. [1]Pediatrics, Director of Adolescent Medicine, Cleveland, OH, USA, [2]Pediatrics, MetroHealth Medical Center, Cleveland, OH, USA, [3]Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH, USA.

The purpose of this study was to establish normative values for bone mineral density in adolescent females. Four hundred and thirty nine healthy females, aged 12 - 18 years, recruited into a large cohort study from four primary care clinics located in a large metropolitan setting. Each subject received bone mineral density measurements at baseline using dual x-ray absorptiometry. Sixty-three percent of the study sample were black; mean chronologic age was 15.6 (±1.6 SD) years; mean gynecologic age was 3.5 (±1.8 SD) years; mean body weight was 66.8 (±17.5 SD) kg; and, mean body mass index was 25.5 (±8.2 SD). Significant predictors of bone mineral density included race, chronologic age, gynecologic age, body weight and body mass index using linear regression. Because of colinearity between gynecologic age and chronologic age and between body weight and body mass index, only race, chronologic and body weight were kept in the model. In addition, these variables were selected for clinician ease of use. First, separate tables of bone mineral density values were established for black and nonblack girls and for each of the two anatomic sites measured, L1 - L4 of the spine and the total hip. Histograms were produced to examine the distribution of the data for chronologic age and body weight. Using mixture method models, the distribution of body weight comprised two distinct normal components with a natural cut off point at the 75th percentile; chronologic age was normally distributed. We collapsed the bone mineral density values into two categories: 12 - 15 and 16 - 18 years, reflective of the predicted deceleration of bone accrual at the end of puberty. In conclusion, we developed normative value guidelines for bone mineral density in adolescent females for clinician use based on race, chronologic age and body weight.

Disclosures: ***B. Cromer,*** *National Institute of Health 2.*

M010

Dairy Foods, Beverages and Activity Predict Bone Mineral Content in Children. J. M. Eichenberger Gilmore*[1], T. A. Marshall*[1], B. Broffitt*[1], S. M. Levy*[1], K. F. Janz*[2], T. L. Burns*[3], J. C. Torner*[4], M. C. Willing[5]. [1]Preventive and Community Dentistry, University of Iowa, Iowa City, IA, USA, [2]Health and Sports Studies, University of Iowa, Iowa City, IA, USA, [3]Biostatistics, University of Iowa, Iowa City, IA, USA, [4]Epidemiology, University of Iowa, Iowa City, IA, USA, [5]Pediatrics, University of Iowa, Iowa City, IA, USA.

Milk and other dairy foods have been associated with higher bone mineral content (BMC), while sugared beverages are hypothesized to reduce BMC. Our objective was to describe associations among BMC, dairy foods, beverages and physical activity in participants of the Iowa Fluoride Study. BMC and bone area of the total body (TB), lumbar spine (S), total hip (H), trochanteric hip (TH) and femoral neck (FN) were measured by dual-energy X-ray absorptiometry in 122 boys aged 4.7-6.5 years and 138 girls aged 4.7-6.8 years. Median energy intakes (kcal) and intake/energy ratios (g/kcal) of milk, other dairy foods, 100% juice, other sugared beverages, water and other sugar-free beverages, sweets and fruit were estimated from 3-day diet records at 5 years of age. Vigorous activity (min/d) was estimated from 4-day accelerometry readings. Television viewing (hr/d) was reported by parents. Weight and height, respectively, were 20.5 ± 3.5 kg and 112.8 ± 5.2 cm for boys and 19.8 ± 3.7 kg and 111.2 ± 5.5 cm for girls. Multiple linear regression models were developed to predict TB-BMC, S-BMC, H-BMC, TH-BMC and FN-BMC from dietary and activity exposures. All models were adjusted for scan age, height and bone area. Higher milk intakes predicted higher TB-BMC in boys ($p < 0.05$); none of the dietary or activity exposures predicted TB-BMC in girls. Higher energy and lower water intakes predicted higher S-BMC in boys ($p < 0.05$); none of the dietary or activity exposures predicted S-BMC in girls. Lower other sugared beverage intakes predicted higher TH-BMC in boys and lower 100% juice intakes and less television viewing predicted higher TH-BMC in girls ($p < 0.05$). None of the dietary or activity exposures predicted H-BMC or FN-BMC in boys or girls. The collective effects of diet and physical activity and television viewing predict BMC differently in young boys and girls. In addition, effects on BMC at individual sites are different, possibly due to the varying proportions of bone type in the regions examined. These data suggest that multi-region analyses are useful in understanding the impact of dietary factors on bone health

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Disclosures: ***J.M. Eichenberger Gilmore****, None.*

M011

Transmenopausal Changes in Activation Frequency. R. R. Recker, J. M. Lappe, M. Davies, R. P. Heaney. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Increased bone remodeling rates may be associated with increased skeletal fragility independent of bone mass, thus partially accounting for the age-related increase in fracture risk in women that is independent of bone loss. Thus, we examined bone remodeling rates before, during and after menopause by measurements of activation frequency (Ac.f, #/yr) in transilial bone biopsies. We recruited 75 women >46 years old who had premenopausal estradiol and gonadotropin levels, and regular menses. During 9.5 years of observation, 51 experienced normal menopause and had 2 transilial bone biopsies after tetracycline labeling, one at the beginning of observation and the second 12 months after the last menses when serum FSH was >75 MIU/ml and serum estradiol was <20 pg/ml. Ac.f and urine hydroxyproline/creatinine ratio (nmol/mmol, HPRO) were examined in this group, and compared with the values we found in a group of older healthy postmenopausal women studied earlier by the same biopsy (Bx) protocol.

The Ac.f was significantly greater at the second Bx, (P<.0001), and was still greater in the older normals (P<.007). Hypro was significantly greater at the time of the second Bx (P<.0001). The increase in Ac.f between biopsies was 93%. The increase in the bone fraction of urine Hypro was 83%, assuming 50% bone fraction at baseline and all the increase occurring in the bone fraction at the second Bx. Ac.f correlated significantly with urine Hypro at the first Bx (r=.354) and the second Bx (r=.523). Conclusion: Bone remodeling rates double at menopause and increase still more later in life, and this may be related to continuing increases in age-related skeletal fragility independent of bone loss.

Summary of Results

	Bx 1	Bx 2	Older Nrmls
	Mean (SD)	Mean (SD)	Mean (SD)
N	51	51	34
Age	49.4 (1.9)	54.6 (2.2)	60.0 (7.6)
Ht (m)	1.646 (.051)	1.649 (.049)	1.624 (.052)
Wt (kg)	68.14 (12.17)	71.4 (13.28)	73.83 (12.24)
Hypro (nmol/mmol)	14.9 (4.7)	21.1 (7.2)	
Ac.f	.15 (.11	.29 (.19)	.44 (.27)

Disclosures: ***R.R. Recker,*** *Wyeth 2, 5; Merck 2, 5; Procter & Gamble 2, 5; Lilly 2, 5.*

M012

Decreased Endocortical Bone Formation and Marrow Osteogenesis Persist with Aging but Do not Impair Periosteal Bone Formation in the SAMP6 Model of Senile Osteoporosis. M. J. Silva, M. D. Brodt*, M. Ko*, Y. Abu-Amer. Orthopaedic Surgery, Washington University, St. Louis, MO, USA.

The senescence accelerated mouse, strain P6 (SAMP6) is a clinically relevant model of senile osteoporosis that exhibits (relative to the control strain SAMR1) reduced areal bone mineral density (BMD), reduced marrow osteogenesis, reduced trabecular bone formation and reduced whole-bone strength. Our objective was to address several unresolved questions regarding the SAMP6 mouse: What is the link between impaired marrow osteogenesis and endocortical bone formation? Does the phenotype resolve with age? Does the defect in endocortical bone formation affect periosteal bone formation? We measured *in vitro* marrow osteogenic potential and *in vivo* endocortical and periosteal bone formation in femora and tibiae from male SAMP6 and SAMR1 (control) mice at 2, 4, 6 and 12 months (n = 10-11/group). Cultures of adherent stromal cells derived from whole bone marrow confirmed a relative deficit in marrow osteogenesis in SAMP6 mice. We measured a 25% reduction in alkaline phosphatase (ALP, day 14) and alizarin red (ALIZ, day 28) staining in femoral cultures, and a 20% reduction in ALP staining in tibial cultures ($p < 0.05$). The differences between strains persisted with age. In both strains, peak marrow osteogenesis occurred at 2 months, followed by progressive reductions at 4 and 6 months and partial recovery at 12 months. Histomorphometric analysis indicated that on the endocortical surface, SAMP6 mice had a deficit in osteoblast number consistent with their marrow deficit, but no deficit in osteoblast function. Endocortical mineralizing surface (eMS/BS) was reduced by 45% in SAMP6 bones compared to SAMR1 ($p < 0.05$), whereas mineral apposition rate (eMAR) was not different between strains. These findings were consistent from 2-12 months. The endocortical deficit observed in SAMP6 bones was reversed on the periosteal surface. Periosteal mineralizing surface (pMS/BS) was increased an average of 70% in SAMP6 versus SAMR1 ($p < 0.05$), while pMAR was not different between strains. These differences are consistent with previous reports of increased periosteal diameter in SAMP6 long bones. In summary, long bones from SAMP6 mice have a deficit in marrow osteogenesis and a corresponding deficit in endocortical mineralizing surface, and these deficits do not resolve with age. Mineral apposition rate is not impaired in SAMP6 mice, suggesting normal osteoblast function despite reduced numbers. Finally, SAMP6 mice have enhanced bone formation periosteally, indicating that a deficit in the ability of the marrow to support osteogenesis does not impair periosteal bone formation.

Disclosures: ***M.J. Silva****, None.*

M013

Central Obesity Promotes Bone Loss from the Hip in Postmenopausal Women through Acceleration of Atherosclerosis. L. B. Tankó, A. Moelgaard, Y. Z. Bagger, C. Christiansen. CCBR A/S, Ballerup, Denmark.

Several lines of evidence suggest that bone mineral density (BMD) in the hip is associated with atherosclerosis by common pathomechanisms. The aim of the present study was to investigate whether body fat mass and its distribution is among the linking factors.

The simultaneous influence of baseline central fat mass (CFM) and peripheral fat mass (PFM) on aortic calcification (AC) and bone loss from the hip was investigated in 105 postmenopausal women aged 62.8 +/- 5.3 years, who were followed for 7.5 years. Body composition and BMD at the hip were measured by DEXA, calcification in the lumbar aorta was graded semi-quantitatively on lateral radiographs. Questionnaires gathered information on traditional risk factors.

Baseline body weight and the CFM/PFM ratio were both significantly correlated with the corresponding measures at the follow-up ($p<0.001$). Baseline CFM, independently of PFM, showed a significant positive correlation, whereas PFM, independently of CFM, was inversely correlated with the progression and follow-up severity of AC ($p<0.05$). Baseline CFM/PFM ratio showed a significant positive correlation with the progression of AC, whereas it was inversely correlated with the changes in hip BMD ($p<0.05$). Changes in AC during the observation period showed significant negative correlation with the simulta-

neous changes in hip BMD (p<0.01).
In conclusion, there is a complex interaction between body fat distribution, atherosclerosis, and bone loss from the hip. Predominant central obesity augments bone loss from the hip through the acceleration of peripheral atherosclerosis. PFM seems to exhibit protective effects in this regard.

Disclosures: ***A. Moelgaard****, None.*

M014

Hip and Radial, but not Lumbar Spine Bone Mass Declines in the Postmenopausal Period in South African Black Women. J. M. Pettifor, D. Basu*, T. M. Sibiya*, S. Mohamed*. MRC Mineral Metabolism Research Unit, Paediatrics, University of the Witwatersrand, Johannesburg, South Africa.

The incidence of minimal trauma fractures in South African black postmenopausal women is markedly reduced compared to that of their white peers. A previous cross-sectional study of bone mass in black and white postmenopausal women <65 years of age revealed that black women had higher BMD at the hip but similar BMD at the spine and mid-shaft radius to white women, which might explain the reduced incidence of hip fractures, but not of forearm or vertebral fractures. We therefore studied longitudinally an age-stratified cohort of 320 randomly selected postmenopausal black women (age >60 yrs, mean 71 ± 7 yrs, range 61 – 95 yrs) from a community in Soweto over a period of 2 years. BMD was measured at the radius, hip and spine by DXA (Hologic QDR 4500A). In the subjects at entry into the study, BMD fell over the 35-year age range by 10.7% at the hip (p=0.002) and by 11.5% at the radius (p=0.003), but remained constant at the lumbar spine (-3.9%, p=0.65). Similar results were found in the 2-year longitudinal study (hip –3.4%, p<0.001; radius –3.4%, p<0.0001; spine –0.6%, p=0.8). Because of the possibility that BMD of the AP spine might be spuriously maintained through the development of arterial calcification or osteophytes, lateral DXA of L2-4 was performed, which confirmed no significant change in mid-lateral BMD with age in the cross-sectional study (r=-0.09, p=0.15). It thus appears that trabecular bone mass is preserved in the postmenopausal period in black South African women, which may account of the low incidence of vertebral fractures in that population. The mechanisms for the preservation of bone mass are unclear, but are currently under investigation.

Disclosures: ***J.M. Pettifor****, None.*

M015

Association between Vascular Compliance and Bone Density in Pre and Post Menopausal Women. L. Graves[1], P. Cagle*[2], L. Hays*[1], M. Hall*[3], B. P. Lukert*[1]. [1]Internal Medicine, University of Kansas School of Medicine, Kansas City, KS, USA, [2]Physical Therapy and Rehabilitation Sciences, University of Kansas School of Allied Health, Kansas City, KS, USA, [3]Kansas Cancer Institute, University of Kansas School of Medicine, Kansas City, KS, USA.

Previous studies have suggested an association between osteoporosis and vascular disease. We hypothesize that low bone mass is associated with a loss of vascular compliance. A loss of vascular compliance is seen with atherosclerotic disease or may be seen earlier than demostrable atherosclerotic changes. Sevnety-three previously healthy women between the ages of 30-65 were recruited for study. Subjects were excluded if they had known previous vascular disease, required medication known to affect vascular compliance or had a BMI > 27. Individuals with known liver disease, renal dysfunction, metabolic bone diseases other than osteoporosis or previous use of glucocorticoids were excluded. Systolic, diastolic and mean arterial blood pressure and pulse rate was determined simultaneously by oscillometry over the left brachial artery. These cardiovascular parameters were measured using the HDI/Pulse Wave Research Cardiovascular Profile instrument (Hypertensive Diagnostics, Eagan, MN). Large artery and small artery elasticity index; cardiac ejection time, stroke volume, systemic vascular resistance, and total vascular impedance was derived from arterial pulse wave contour analysis recorded by applanometry over the right radial artery in the supine position. Bone density of the lumbar spine and hip was performed by dual energy x-ray absorptiometry (Prodigy, GE-Lunar).The mean age of the premenopausal group was 42 (range 30-52) and postmenopausal group was 54 (range 44-70). Consistent with previous studies, systolic and diastolic blood pressure was higher in the postmenopausal group. Also consistent with previous work, large artery elastic index, small artery elastic index, and total vascular impedance were higher in the postmenopausal group compared to premenopausal subjects.Within the postmenopausal subjects Small Artery Elastic Index (SAEI) significantly correlated with lumbar bone mineral density (g/cm^2) r = 0.42,(p=0.0487), the mean hip BMD (g/cm^2) r = 0.60 (p=0.0027), and the femoral neck BMD (g/cm^2) r = 0.61 (p=0.0024).
In conclusion, within this group of individuals at low risk for vascular disease, a lower bone density was associated with a loss of vascular compliance. Further study is needed to investigate factors which may contribute to both low bone mass and loss of vascular compliance.

Disclosures: ***L. Graves****, Merck 8.*

M016

Predictors of Persistent High Bone Mineral Density Among Perimenopausal Women. J. H. Pesonen*[1], J. Sirola*[1], M. T. Tuppurainen[1], R. Honkanen[2], E. M. Alhava*[3], H. Kröger[3]. [1]Bone and Cartilage Research Unit, Kuopio University, Kuopio, Finland, [2]Public Health, Kuopio University, Kuopio, Finland, [3]Surgery, Kuopio University Hospital, Kuopio, Finland.

Early postmenopausal bone loss has been studied quite intensively but studies regarding persistent high bone mineral density (PHBMD) are sparse. In the population based Kuopio Osteoporosis Study a random sample of 2,025 women were selected for BMD measurement by Lunar DPX in 1990-1991 and in 1995-1997. In all 1,873 women underwent both measurements. The mean age was 53.5 years (range 48.0 – 59.6) at the baseline. The initial study groups were constructed as follows: The PHBMD group included 199 women with both lumbar (L2-L4) and femoral neck BMD in the highest quartile at both measurements (baseline lumbar BMD > 1.24 g/cm2; femoral BMD > 1.01 g/cm2, and 5-year follow-up lumbar BMD > 1,22 g/cm2; and femoral BMD > 0.98 g/cm2, respectively). The control group consisted of the rest of 1,674 women. In all, 248 women were excluded because of BMD measurement errors, deformities, calcifications etc: 41 from PHBMD group (20.6%) and 207 (12.4%) from the control group. Two-sided Student's t-test and multivariate logistic regression analyses were used for statistical analyses.
The predictors of PHBMD in multivariate regression analysis were as follows: hormone replacement therapy (HRT) during the follow-up for over 2 years (OR 2.06, 95% CI: 1.29 – 3.30) and overweight [BMI > 25 kg/m2] at baseline (OR 3.97 , 95 % CI: 2.39 – 6.59). Also high physical activity during 11-18 years of age predicted PHBMD (OR 1.56, 95% CI: 1.00 – 2.43). Calcium intake at baseline had a weak positive effect on BMD.
In conclusion, overweight, HRT use and high physical activity as a teenager seemed to be predictors of persistent high BMD in early postmenopausal women. The risk of osteoporosis is low in these women.

Disclosures: ***J.H. Pesonen****, None.*

M017

Bone Resorption in Postmenopausal Women Using ^{41}Ca Technology. J. M. K. Cheong*[1], G. S. Jackson*[2], B. R. Martin[1], D. Elmore*[2], J. R. Nolan*[3], M. Peacock[4], G. P. McCabe*[3], C. M. Weaver[1]. [1]Foods and Nutrition, Purdue University, West Lafayette, IN, USA, [2]PRIME Lab, Purdue University, West Lafayette, IN, USA, [3]Statistics, Purdue University, West Lafayette, IN, USA, [4]School of Medicine, Indiana University, Indianapolis, IN, USA.

High rates of bone resorption are associated with bone loss in the elderly. Quantitating bone resorption has been hampered by lack of direct methods for measurement. The purpose of this study was to assess a novel method using ^{41}Ca, a rare isotope with a half life of 10^5 years, for assessing bone resorption in 18 healthy postmenopausal women aged 59.8 ± 4.7 years (13.7 ± 6.9 years postmenopausal). Approval for the study was obtained from the Purdue University Institutional Review Board for all procedures. The underlying hypothesis is that the excretion of ^{41}Ca reflects the skeletal resorption rate in a skeleton that has been prelabeled with ^{41}Ca 100 days after an intravenous (IV) dose of ^{41}Ca. Urine samples were collected every 10 days for measurement of ^{41}Ca/^{40}Ca ratio by Accelerator Mass Spectrometry. Bone resorption as determined by ^{41}Ca was inversely related to body weight, body mass index, and bone mineral density. Rates of bone resorption using ^{41}Ca were compared to resorption measured by biochemical markers, as well as calcium retention and bone resorption determined by ^{45}Ca kinetic analysis. Urinary ^{41}Ca comes directly from bone, and with a single IV dose of 100 nCi, bone resorption can be monitored for decades. In contrast, ^{45}Ca kinetics produce only estimates of bone resorption because the skeleton is not labeled and biochemical markers of bone resorption are variable and reflect matrix rather than bone. We conclude that ^{41}Ca is a powerful tool for assessing bone resorption and can be used to determine effectiveness of interventions in the same subjects over many years.

Disclosures: ***J.M.K. Cheong****, None.*

M018

Bone Mineral Density (BMD) and Rate of Decline in BMD with Aging Among Older Black and White Men and Women: The Health, Aging and Body Composition Study. J. M. Zmuda[1], J. A. Cauley[1], A. B. Newman*[1], F. A. Tylavsky*[2], M. C. Nevitt*[3], D. M. Black[3], S. M. Rubin*[3], M. Visser*[4], T. B. Harris*[5]. [1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA, [2]University of Tennessee, Memphis, TN, USA, [3]University of California, San Francisco, CA, USA, [4]VU University Medical Center, Amsterdam, Netherlands, [5]National Institute on Aging, Bethesda, MD, USA.

Black women and men experience lower fracture rates than whites, a difference that has been attributed in part to the greater BMD among blacks. Little is known, however, about ethnic differences in the rate of change in BMD with aging. To address this, we analyzed BMD and the rate of change in BMD with aging among 2,109 well-functioning white and black men and women (39% black, 49% men) age 70-79 years who were enrolled in the Health, Aging and Body Composition study. Hip BMD was measured at baseline and an average of 4 years later using dual-energy X-ray absorptiometry (Hologic QDR 4500A). We excluded persons (n=191) taking oral corticosteroids or osteoporosis drugs from analyses. Gender-specific models (analysis of covariance) were used to evaluate race differences in baseline BMD and the annual absolute and percentage rate of change in BMD. Baseline total hip BMD was 8% greater among black men and 11% greater among black women compared to white men and women, respectively, adjusted for age, height, weight and clinic site (P<0.001). Despite their higher initial BMD, black men and women experienced significantly greater rates of decline in hip BMD than white men and women (Table). These results were independent of age, initial BMD, baseline body weight and height, weight change during follow-up, physical activity, smoking, overall health status, coronary heart disease, diabetes, arthritis, lung disease, cancer, use of calcium and vitamin D supplements, thiazide diuretics, anti-inflammatory drugs and oral estrogen, and clinic site. Similar results were observed for the annual absolute rate of change in hip BMD. In conclusion, older black men and women appear to experience accelerated rates of decline in hip BMD compared to white men and women. Additional studies are needed to better understand the mechanisms underlying interethnic differences in skeletal aging.

Table. Rate of Change (%/yr) in Total Hip BMD in Older Black and White Men and Women

	Men	Women
White	-0.39±0.95	-0.20±1.05
Black	-0.56±1.03	-0.35±1.19
P Value	≤0.01	≤0.05

Disclosures: ***J.M. Zmuda****, None.*

M019

Is Significant Loss of Alveolar Bone with Age Inevitable and Associated with Osteopenia Elsewhere in the Aging Skeleton? B. Shemirani*[1], H. Goodis*[2], E. A. Krall[3], R. Garcia*[3], C. McCulloch*[4], A. Lazar*[4], A. Kahn[1]. [1]Growth and Development, UCSF, San Francisco, CA, USA, [2]Preventive and Restorative Dental Sciences, UCSF, San Francisco, CA, USA, [3]Health Policy and Health Services Research, Boston University, Boston, MA, USA, [4]Epidemiology and Biostatistics, UCSF, San Francisco, CA, USA.

It is widely believed that there is a significant and inevitable loss of alveolar bone in aging humans. It is also often assumed that such bone loss is associated with the generalized osteopenia that occurs elsewhere in the aging skeleton. In the present study, we have tested these assumptions using materials collected as part of the Dental Longitudinal Study (DLS), taking care to exclude alveolar bone loss resulting from severe periodontal disease. Serial periapical and bitewing radiographs of bicuspid teeth from 32 men enrolled in the DLS were scanned, digitized and analyzed for the loss of alveolar bone. Alveolar bone loss was taken as the ratio of the distance from the alveolar crest to the cemento-enamel junction (CEJ) to the distance from the root apex to the CEJ (a fixed dimension in normal teeth). Sites were excluded from measurement if the loss of bone during a single recall interval (~3 years) exceeded 4 mm; a radiological indicator of bone loss due to periodontal disease. Bone mineral density (BMD) of metacarpals was determined from scans of hand-wrist radiographs on the same subjects taken at matching recall intervals. At baseline, the subjects were from 32 to 60 yrs; at conclusion, they were 61-88 years. The data were analyzed using fixed effects and multivariate models with $p < 0.05$ considered statistically significant. We found that both age and BMD, alone, were significant predictors of a decline in ratio ($p = <0.0001$), i.e., a loss in alveolar bone. However, the magnitude of loss as a function of age (0.016 in ratio/decade) was small and clinically insignificant. More importantly, when adjusted for BMD, age continued to be a highly predictive of bone loss ($p=0.0025$) while BMD, after adjustment for age, was not ($p=0.37$). These findings document that in the absence of overt radiological evidence of periodontal disease, very little alveolar bone is lost with time in aging men. They also indicate that while the loss of alveolar bone and metacarpal BMD are both age-sensitive events, they are independent and not associated.

Disclosures: ***A. Kahn****, None.*

M020

Cartilage Maturation In Vitro Is Influenced By TGF-beta 1 and T3. M. A. Mello, R. S. Tuan*. Cartilage Biology and Orthopedics Branch, NIH/NIAMS, Bethesda, MD, USA.

Impairment in cartilage maturation leads to skeletal development defects as well as abnormalities in fracture healing. The focus of this investigation is the role of transforming growth factor-beta 1 (TGF-beta1) and the thyroid hormone triiodothyronine (T3) in this process. We hypothesized that cartilage maturation is a balance between proliferation and apoptosis and that TGF-beta1 and T3 modulate this balance.

The in vitro system used for this investigation was high density micromass cultures of chick embryonic mesenchyme. The medium was supplemented with ITS and 100 pM of recombinant human TGF-beta1 and 10nM T3 as follows: TGF-beta1 only, TGF-beta1 and T3 together, T3 only, and non-treated controls. Culture morphology was analyzed by light microscopy of sections stained with H/E and with alcian blue. Cell proliferation was accessed by BrdU incorporation; cell hypertrophy was investigated on the basis of increase in cell size; expression of type X collagen by immunohistochemistry and immunoblot analysis; alkaline phosphatase by enzyme assay; calcification by 45Ca uptake, and apoptosis by terminal deoxynucleotidil transferase-mediated dUTP labeling (TUNEL) and electrophoretic analysis of DNA degradation. For statistical analysis, paired t-test was used; statistical significance was considered at $p<0.05$.

Morphologically, at day 21, the cells treated with T3 only and the controls displayed the columnar organization similar to the growth plate in vivo, showing that TGF-beta1 inhibits column formation. Cell proliferation was stimulated by TGF-beta1 at day 14 and 21 and inhibited dramatically by T3 at day 21. Cell size increased significantly in the cultures treated with T3 only or in combination with TGF-beta1. Type X collagen was detected by IMH and immunoblot in all the cultures since day 7. Alkaline phosphatase activity was strongly stimulated by T3. Calcification, however, was not stimulated by T3, indicating different pathways for these effects. Apoptosis, as detected by TUNEL was present in all cultures, but it was significantly stimulated by T3. TGF-beta1 had a small effect preventing the T3 mediated stimulation of apoptosis in the cultures treated with both factors at day 21. Electrophoresis of DNA showed internucleosomal DNA degradation in the cultures treated with T3 only or T3 and TGF-beta1 in combination.

In conclusion, the results have shown that TGF-beta1 and T3 have iterative effects in cartilage maturation, with TGF-beta1 stimulating chondrocyte proliferation and inhibiting hypertrophy and T3 stimulating hypertrophy and apoptosis. T3 and TGF-beta1 are naturally occurring substances normally found in the growth cartilage, suggesting that in vitro findings may have direct in vivo relevance.

Disclosures: ***M.A. Mello****, None.*

M021

The Chondroprotective Effect of Cynodon Dactylon on Altered Proteoglycan Structure. P. Kailash*[1], C. Arungunram*[2], H. Chegu*[3], N. Ramamoorthy*[4]. [1]Lab. Med and Pathology, UOM, Eden Prairie, MN, USA, [2]Dept.of Rheumatology, Madras Medical College, Chennai, India, [3]Dept.of Biochemistry, Sri Ramachandra Medical College & Research Institute, Chennai, India, [4]Dept.of Virology, King Institute of Preventive Medicine, Chennai, India.

Osteoarthritis (OA) is the most common articular disorder among elderly people. Proteoglycans are major constituents of an articular cartilage which contribute to the resiliency of the tissue. Repair of full thickness joint cartilage defects is within the reach of clinical practice. However, the quality of regenerating hyaline cartilage is difficult to assess. The application of technical advances to clinical studies of chondrocytes and cartilage tissue metabolism will provide important new insights concerning the pathophysiology of OA and identify new therapeutic strategies to regulate and inhibit the degenerative process of OA. The purpose of the present study is to observe the effect of Cynodon dactylon, a herb (Graminaea family), on the degenerated chondrocyte cells in restoring the proteoglycan structure. The degeneration of the chondrocyte cells was achieved by the incorporation of papain, the proteolytic enzyme which affects the cartilage by liberating chondroitin sulphate from the matrix and weakens the attachment of the cartilage to the subchondral plate and selectively degrades proteoglycan as well. Induction of OA by papain is a well known animal model. In the present pilot study, articular cartilage was excised from the knee joints of guinea pigs for the *invitro* tissue culture of chondrocytes. Papain ,0.5 ml (1g in 100ml of isotonic saline) was added to the cultured chondrocytes to observe the damage caused to the chondrocytes. To find out the chondroprotective effect of Cynodon dactylon, 0.5 ml (5g of the dried herb powder in 100ml of distilled water) was added to the papain treated chondrocytes. The effects of both the papain and the papain followed by Cynodon dactylon on the proteoglycan structure was monitored by Scanning Electron Microscopy (SEM). The SEM picture showed that there is deformation in the proteoglycan structure in the papain added chondrocytes. Whereas, in the case of papain followed by Cynodon dactylon, there was regeneration of proteoglycan structure with the presence of prominent hyaluronic acid, chondroitin sulphate, keratan sulphate, link and core proteins. The results were comparable with that of control chondrocytes. Hence, this study throws some light about the chondroprotective nature of Cynodon dactylon and further research has to be carried out in finding the active components involved. In the future, this study can also be extrapolated in clinical practice.

Disclosures: ***P. Kailash****, None.*

M022

PI3K Signaling Pathway Regulates Cartilage-Specific Alternative Splicing of Type II Collagen Pre-mRNA. T. Nishiyama*, G. Sarkar*, M. E. Bolander. Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

ATDC5, a mouse carcinoma-derived cell line undergoes chondrocytic differentiation in culture when stimulated by insulin. The differentiation parallels cartilage-specific alternative splicing of collagen 2 pre-mRNA involving exon2 such that the ratio of Col2B (lacking exon2) to Col2A (includes exon2) mRNAs increases as a function of the differentiation process. We investigated if activated phosphoinositide 3-kinase (PI3K) pathway underlie the mechanism regulating insulin-dependent alternative splicing of Col2 pre-mRNA. We used the PI3K inhibitor, LY294002, that blocks phosphorylation of the splicing factor SRp40 to evaluate the contribution of the PI3K pathway on the splicing process. ATDC5 cells were cultured in the presence of the inhibitor with or without insulin for 14 days, a time that optimally demonstrates cartilage-specific alternative splicing of the Col2 pre-mRNA. The extent of cartilage –specific alternative splicing was measured by the ratio of Col2B over Col2A mRNA by employing reverse transcription-polymerase chain reaction (RT-PCR) of RNA extracted from the cells at desired conditions. At 0 and 5μM LY294002 concentration, a 74-fold Col2B over Col2A mRNA synthesis was observed. However, at 10, 20- and 50-μM LY294002 concentrations, Col2B over Col2A mRNA ratio observed were 1.67, 1.73 and 1.35 respectively clearly indicating that at 10 μM concentration or higher the PI3K inhibitor virtually abolishes the inductive effect of insulin on the alternative splicing process. Interestingly, we also show that the effect of insulin in the alternative splicing process could not be demonstrated at early times of culture (up to 48 hours) although the mRNA concentration of SRp40 appear to remain unchanged up to 21 days of cell culture. Taken together, our data indicates a regulatory role of the PI3K pathway on the alternative splicing of Col2 pre-mRNA involving exon-exclusion process but the regulation of the process appears to differ from the known mechanism.

Disclosures: ***T. Nishiyama****, None.*

M023

Enhanced Vascularization in Posterior Spinal Fusion Model byHydrogel Incorporated With Vascular Endothelial Cell Growth Factor. H. Y. Yeung*[1], J. C. Y. Cheng*[1], X. Guo[2], K. M. Lee*[1], Y. M. Chiu*[1], C. W. Chan*[1], P. Y. Chow*[3], Y. Tabata*[4]. [1]Department Orthopaedics & Traumatology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong Special Administrative Region of China, [2]Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Kowloon, Hong Kong Special Administrative Region of China, [3]Department of Anatomy, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong Special Administrative Region of China, [4]Department of Biomaterial, Institute for Frontier Medical Science, Kyoto, Japan.

In posterior spinal fusion, proper source of cells responsible for osteogenesis and good blood supply to the fusion site components are important for successful fusion. Decortication of osseous elements is a standard procedure in spinal fusion surgery. The role of decortication is to provide osteoprogenitors and to initiate vascularization from the marrow of decorticated bone. In the present study, the vascularization effect of fusion bed by VEGF-incorporated hydrogel in non-decorticated posterior spinal fusion was investigated. New Zealand white rabbits underwent bilateral non-decorticated posterolateral spinal fusion at L5 and L6. The animals were divided into 3 groups using different dosages of VEGF incorporated to the hydrogel (0 μg[control], 20 μg, 40 μg). Immunohistochemistry of endothelial cell surface marker CD31 was preformed on the paraffin section to quantify the endothelial cells and blood vessel formation at the fusion site. At week 3 post-operation, the number of endothelial cells in the hydrogel was significantly increased in a dose dependent manner. The blood vessels formation at the hydrogel was also increased significantly at the experimental groups. At week 7 post-operation, more bone was formed around the transverse processes in VEGF-incorporated hydrogel groups but no bony bridging was observed in all groups. In conclusion, the hydrogel was an effective delivery system of VEGF in posterior spinal fusion model for the enhancement of vascularization of fusion site. Additional osteoinductive factor may require to promote the bone formation after the enhancement of vascularization.

Disclosures: ***H.Y. Yeung****, None.*

M024

Induction of Connective Tissue Growth Factor/ Hypertrophic Chondrocyte-Specific 24/ CCN2 Gene by Dexamethasone in Human Chondrocytic Cells: Mechanism and Biological Outcome. S. Kubota*, N. H. Moritani*, H. Kawaki*, H. Mimura*, M. Minato*, M. Takigawa. Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine & Dentistry, Okayama, Japan.

The endochondral ossification is a complex and integrated biological process which is accomplished by interactive actions of a number of cytokines and hormones. Among them, connective tissue growth factor/hypertrophic chondrocyte-specific gene 24/ccn family member 2 (CTGF/Hcs24/CCN2) is known to be a critical factor for the growth and differentiation of growth plate. In the present study, we analyzed the interaction of the CTGF/Hcs24/CCN2 gene and other factors involved in endochondral ossification and found glucocorticoid-mediated induction of the CTGF/Hcs24/CCN2 gene for the first time in a chondrocytic cell line, HCS-2/8. Steady-state mRNA levels of ctgf/hcs24/ccn2 were drastically increased after the treatment with 50 nM dexamethasone, as confirmed by Northern blotting and quantitative real-time PCR analysis. Corresponding to the increase in mRNA, production of ctgf/hcs24/ccn2 protein was remarkably enhanced, following a time course up to 6 h. The observed increase in mRNA can be ascribed to transcriptional enhancement, since the stability of ctgf/hcs24/ccn2 mRNA was not affected by the same concentration of dexamethasone, as determined by an mRNA degradation assay. However, unexpectedly, the prototypic ctgf/hcs24/ccn2 promoter was totally irresponsible to the dexamethasone treatment. Furthermore, evaluation with another CTGF/Hcs24/CCN2 promoter fragment with an extended upstream region of 310 bp did not reveal accountable response to dexamethasone stimulation. Also, repressive effect of the 3'-untranslated region of the ctgf/hcs24/ccn2 gene was not affected by dexamethasone treatment. As such, locating glucocorticoid-responsive element is still currently underway. In contrast, enhancement of the prototypic promoter activity by dexamethasone was observed in murine fibroblastic cells, demonstrating the complexity of the regulatory mechanism of ctgf/hcs24/ccn2 gene expression. Of importance, dexamethasone at the same concentration significantly stimulated proteoglycan synthesis in HCS-2/8 cells up to the same levels as exogenously-added CTGF/Hcs24/CCN2 did. These findings represent a novel effect of physiological doses of glucocorticoid on the production of CTGF/Hcs24/CCN2 by chondrocytic cells, and indicate that CTGF/Hcs24/CCN2 may mediate the stimulative effect of dexamethasone on chondrocytic phenotypes. The mechanism of such cell type-specific induction of the ctgf/hcs24/ccn2 gene is interested and will be discussed as well.

Disclosures: ***S. Kubota****, None.*

M025

Characterization of Glucocorticoid Receptor Binding in the ATDC5 Clonal Chondrocyte Cell Line. L. Alcocer*[1], C. Farquharson[2], M. Panarelli*[3], S. F. Ahmed[4]. [1]Yorkhill Royal Hospital for Sick Children, Bone & Endocrine Research Group, Glasgow, United Kingdom, and University of California, San Francisco Medical School, San Francisco, CA, USA, [2]Department of Integrative Biology, Roslin Institute, Edinburgh, United Kingdom, [3]Deptartment of Clinical Biochemistry, Stobhill Hospital, Glasgow, United Kingdom, [4]Bone & Endocrine Research Group, Yorkhill Royal Hospital for Sick Children, Glasgow, United Kingdom.

Glucocorticoids (GC) are extensively used in the treatment of a variety of childhood diseases at the expense of adverse growth and skeletal development. The murine ATDC5 clonal chondrocyte cell line mimics the *in vivo* process of longitudinal bone growth undergoing the characteristic multistep differentiation events and GC are known to deleteriously affect this temporal process. The aim of this study was to characterize the GC receptor (GR) in the ATDC5 cell line during the initiation of the chondrocyte phenotype. ATDC5 cells were grown in multiwell plates in insulin-containing medium for 10 days at which time they expressed collagen type II but not collagen type X. GR binding characteristics were determined in a whole cell assay using labelled dexamethasone (DEX) as ligand. An initial equilibrium curve established that the receptor is fully saturated following 3 hours incubation at 24 C. The radioligand whole cell binding assay demonstrated a single class of GC binding component. The dissociation constant (Kd) reflected high affinity binding at 0.21 +/- 0.036 nmol/L (N=6) for DEX. The number of binding sites (Bmax) was 2072.4 +/ - 323.5 sites/cell (N=6). Competition studies indicated that the DEX binding site was GC specific and the competitive hierarchy confirmed previous data reported in the literature with high competition for RU-486 and DEX.
This is the first study to characterize GC binding kinetics for the ATDC5 cell line and it should facilitate an improved understanding of the influence of GC on chondrocyte maturation and the endochondral ossification process.

Disclosures: ***L. Alcocer****, None.*

M026

Nicotine Activates Signaling and Enhances Chondrogenesis in Mesenchymal Stem Cell Cultures. J. F. Baden*, L. Curylo*, E. M. Schwarz, R. J. O'Keefe, R. N. Rosier, J. E. Puzas, M. J. Zuscik. Orthopaedics, University of Rochester, Rochester, NY, USA.

Although it has been established that cigarette smoking has a negative impact on skeletal healing following long bone fracture or spinal fusion surgery, the mechanisms that mediate these effects remain unknown. To address the question of mechanism, we tested the novel hypothesis that one of the components of cigarette smoke, nicotine, affects the healing process by activating nicotinic acetylcholine receptors (nAChRs) expressed by mesenchymal stem cells (MSCs). More specifically, we predict that activation of the nAChR in stem cells that are recruited to participate in the healing process accelerates their commitment to chondrogenesis thus inducing abnormal accretion of cartilage and delayed remodeling into bone. As a culture model of stem cell commitment to chondrogenesis, we employed the mesenchymal stem cell model derived from stage E11.5 mouse limb buds. Using this model, we examined nAChR expression and signaling as well as formation of cartilage in cultures following nicotine treatment. Receptor expression level was determined via saturation binding analysis using the selective nAChR radioligand ^{3}H-epibatadine. Specific binding (B_{max} =97 fm/mg protein) was detected in membranes derived from MSC monolayer cultures. To correlate binding with function, experiments were then performed using the Ca^{2+}-sensitive fluorescent dye Fura-2 and intracellular Ca^{2+} was monitored via fluorescence microscopy. Consistent with the established Ca^{2+} channel function of these receptors, 1 μM nicotine treatment of MSCs induced a robust intracellular Ca^{2+} response that was inhibited by the nACHR-selective antagonist mecamylamine (10 μM). Since downstream signaling via CREB has been reported following nicotine treatment in other cell models, we also monitored CREB signaling in MSCs transfected with the CREB-luciferase reporter. We found that 1 μM nicotine induces a 9-fold increase in signaling via this pathway. Finally, to determine if nicotine affects commitment of stem cells to the chondrocyte lineage, MSCs plated in a micromass were exposed to varying doses of nicotine for 7 days followed by alcian blue staining to quantify cartilage nodule formation. A dose dependent effect was observed, with 1 μM nicotine inducing a 2-fold increase in total nodule area compared to un-treated controls. These findings indicate that nicotine exerts control over MSC fate determination, establishing that these cells are a target for nicotine action following skeletal injury or surgery. Thus, therapeutic strategies can be envisioned that utilize nAChR antagonists to block the impact of nicotine on MSCs during skeletal healing in smokers.

Disclosures: ***M.J. Zuscik****, None.*

M027

IGF-I Reverses the Growth Inhibitory Effects of Glucocorticoids on Bone Growth. T. Mushtaq*[1], F. Ahmed[2], C. Farquharson[1]. [1]Integrative Biology, Roslin Institute, Edinburgh, United Kingdom, [2]Bone & Endocrine Research Group, Royal Hospital for Sick Children, Glasgow, United Kingdom.

Glucocorticoids (GC) are used extensively as anti-inflammatory therapy and in immunosuppressive regimes in children but their use, over relatively short periods, can lead to growth retardation. This may be a consequence of direct interactions between GC and growth plate chondrocytes and involve disruption of the chondrocyte IGF-I signaling pathway. In this present study we have used embryonic mouse metatarsals to study the effects of dexamethasone (Dex) on bone growth and to determine if any adverse effects can be ameliorated by IGF-I. 18-day-old fetal metatarsals were cultured in pentuplicate for up to

12 days in serum-free medium supplemented with either Dex 10-6 M, IGF-I (100ng/ml) or both. Total metatarsal growth was recorded every second day as a percent change from their initial length at harvesting (day 0). Dry weight, and tritiated-thymidine uptake were determined on days 5 and 12.
Dex caused a decrease in metatarsal length of 22% and IGF-I an increase of 23% compared to control bones ($p<0.05$), which increased by 84% over the 12-day period. In addition, IGF-I treatment showed a significant acceleration in linear growth from day 2 (40% compared to 24% in controls)($p<0.05$), whereas Dex treatment caused a reduction in longitudinal growth from day 10 ($p<0.05$). The combination of Dex and IGF-I ameliorated all negative growth effects of IGF-I and resulted in a faster bone growth rate than with IGF-I alone. This suggests a synergistic action between Dex and IGF-I. At 12 days, Dex had no effect on dry weight (82 ± 5.3 ug); whereas IGF-I and IGF-I/Dex treated bones were significantly heavier (152 ± 5 ug and 148 ± 2.3 ug, respectively) than controls (84 ± 2.0 ug)($p<0.05$). A similar trend was noted after 5 days (Con: 34 ± 3.2 ug; Dex: 33 ± 1.9 ug; IGF-I: 48 ± 1.6 ug; IGF/Dex: 59 ± 4.7 ug). Thymidine uptake was significantly reduced with Dex ($p<0.001$)at both 5 and 12 days of culture and this may explain the reduced linear bone growth. However, a stimulation of thymidine uptake by IGF-I and IGF/Dex was only noted at day 5 (Con: 98.6 ± 6.7; Dex: 58.2 ± 8.7; IGF-I: 140.5 ± 9.8; IGF/Dex: 154.9 ± 3.7 - all dpm x 1000) and possibly reflects the slowing of growth at day 12 compared to the controls. During the phase of rapid growth (day 5 cultures) a synergistic action between Dex and IGF-I is also suggested by the dry weight and thymidine uptake data.
The results indicate that Dex and IGF-I had opposite effects on longitudinal bone growth. The amelioration of Dex induced growth retardation by IGF-I may offer potential as a therapeutic approach to counter GC induced growth retardation in children.

Disclosures: ***T. Mushtaq****, None.*

M028

Characterization of Subchondral Bone Remodeling in the Rat Surgically-induced Osteoarthritis Models. M. Pickarski*[1], T. Hayami*[1], G. A. Wesolowski[1], Y. Zhuo*[1], A. Bone*[2], J. Destefano*[2], G. A. Rodan[1], L. T. Duong[1]. [1]Dept. of Bone Biology and Osteoporosis Res., Merck Res. Labs., West Point, PA, USA, [2]Laboratory Animal Resources, Merck Res. Labs., West Point, PA, USA.

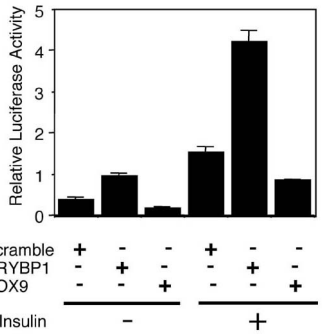
Osteoarthritis (OA) is a chronic joint disease characterized by cartilage destruction, subchondral bone sclerosis, and osteophyte formation. Subchondral bone stiffness has been proposed to initiate and/or contribute to cartilage deterioration in OA. The purpose of this study was to examine subchondral bone changes in the progression of OA in two surgically-induced OA models. Ten week-old male rat knee joints were subjected to either anterior cruciate ligament transection (ACLT) or transection of both ACL and medial collateral ligament and resection of medial meniscus (ACLT+MMx). Changes at 1, 2, 4, 6, and 10 wks post-surgery were compared to sham-operated animals. Articular cartilage changes were evaluated using a modified Mankin's histological score. Subchondral bone volume and osteophyte area were measured using histomorphometric analysis. In addition, changes in markers of cartilage degradation (MMP-13, aggrecanase) and bone resorption (TRAP, cathepsin K, MMP-9) in articular region of surgical and contralateral tibiae were evaluated using real-time quantitative Taqman PCR. Immunostaining was performed with anti-MMP-9, MMP-13, and cathepsin K antibodies. Mankin's scores showed time-dependent OA development in both models, with slower progression of OA in ACLT than in ACLT+MMx model. Both models showed early subchondral bone loss (at 2 wks) and subsequent increases in subchondral bone volume (at 10 wks) relative to sham animals ($p<0.05$). Obvious osteophyte formation was observed in ACLT at 10 wks and in ACLT+MMx at 6 wks post-surgery. From Taqman analysis, cartilage degradation markers, including MMP-13 (2-fold) and aggrecanase (4-fold), were upregulated at 1 wk post-surgery, compared to sham. Bone resorption markers significantly increased (~2-fold) within 2-4 wks in both OA models. These increases in mRNA expression levels were absent in the contralateral joints. Using immunohistochemistry, MMP-13 positive cells were hypertrophic chondrocyte-like cells near damaged cartilage lesions in both OA models. Cathepsin K and MMP-9 positive multinucleated cells were observed invading the articular cartilage from the subchondral region. In summary, the two rat OA models mimic the disease pathogenesis in humans, demonstrating articular cartilage degradation, subchondral bone changes, and osteophyte formation. The two rat OA models characterized here in detail, can be useful in evaluating the effects of bone resorption inhibitors, including Alendronate, in the progression of osteoarthritis.

Disclosures: ***M. Pickarski****, Merck Res. Labs. 3.*

M029

Oligonucleotide Decoy Mimicking aA-Crystallin Binding Protein 1 Binding Site on Col2a1 Enhancer Stimulates Transcription from the Adjacent Col2a1 Promoter in Chondrogenic ATDC5 Cells. H. Yamagiwa[1], Y. Yamada*[2], M. E. Bolander[1], G. Sarkar*[1]. [1]Orthopedic Surgery, Mayo Clinic, Rochester, MN, USA, [2]National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA.

A 48 bp sequence element in intron 1 of type II collagen gene (Col2a1) acts as an enhancer of Col2a1 transcription, which contains binding sites for the transcription activator SOX9, and repressor aA-crystallin binding protein 1 (CRYBP1). We hypothesized that an ability to abrogate CRYBP1 binding should increase transcription from a promoter associated with the Col2a1 enhancer. We tested this hypothesis by co-transfecting an oligonucleotide (ODN) decoy for CRYBP1 and a luciferase-based reporter vector under the transcriptional control of the Col2a1 promoter linked to the 100 bp enhancer (Tanaka et al, Mol Cell Biol 2000) in chondrogenic ATDC5 cells. As a control, we used decoy ODN corresponding to SOX9 binding site. Transfection with CRYBP1 decoy increased luciferase activity by 2.6 and 2.9 fold in the absence or presence of insulin whereas SOX9 decoy ODN decreased luciferase activity by 0.57 and 0.53 fold in the absence or presence of insulin. In addition, the repressive effect of IL-1 on Col2a1 transcription, through decreasing SOX9 mRNA expression and increasing CRYBP1 mRNA expression, was counteracted by CRYBP1 decoy ODN. These results have implications for gene therapy in degenerative joint diseases by elevating Col2a1 expression in chondrocytes.



Disclosures: ***H. Yamagiwa****, None.*

M030

Hypoxia Activates Chondrogenic Gene Programs in Bone Progenitor Cells. J. C. Robins*[1], N. Akeno[2], B. J. Aronow*[3], P. Koopman*[4], T. L. Clemens[2]. [1]Dept. of Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH, USA, [2]Dept. of Medicine, University of Cincinnati, Cincinnati, OH, USA, [3]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA, [4]The University of Queensland, Institute for Molecular Bioscience, Brisbane, Australia.

The formation of a cartilage template during skeletal morphogenesis and following long bone fracture is carried out by chondrocytes which arise from pluripotent mesenchymal stem cells. This process takes place during an avascular period in a hypoxic environment suggesting that oxygen tension might dictate mesenchymal cell fate by modulating the expression of genes associated with chondrogenesis. To test this idea, microarray analysis was performed on replicate RNA samples isolated from mouse ST2 bone stromal cells maintained in either 1% or 21% oxygen over a 48 h period using the Incyte GEM1 mouse chip. The expression of 719 genes was significantly ($p<0.01$) altered in response to hypoxia. Subsets of genes that exhibited distinct expression patterns over time were annotated. Several known genes expressed in cells of the chondrocyte lineage were upregulated and verified by real time PCR. These included connective tissue growth factor, lysyl oxidase, annexin 2 and 5, chondroitin 4-sulfonotransferase, lectin galactose binding protein 3, laminin receptor 1, and Gli-Kruppel family protein. Sox-9, a master regulator of chondrocyte differentiation, was upregulated 10 fold. To determine the effect of hypoxia on Sox-9 transcription, stromal cells were transiently transfected with a Sox-9 promoter construct fused to luciferase and then treated with 1% or 21% oxygen for 24 hours. Hypoxia increased promoter activity by approximately 10 fold over basal levels. Cotransfection of the Sox-9 promoter, which contains three consensus Hif-1 binding sites, with a construct encoding a Hif-1alpha cDNA increased both basal and hypoxia inducible Sox-9 promoter activity. The upregulation of chondrocyte-specific gene expression in hypoxic ST2 cells was associated with the appearance of the chondrocyte phenotype as indicated by increased proteoglycan accumulation. In summary, exposure of mesenchymal stromal cells to hypoxia leads to induction of genes in the chondrocyte differentiation pathway and the appearance of the chondrocyte phenotype. We propose that the ambient level of oxygen is a critical factor in bone progenitor cell gene programming.

Disclosures: ***J.C. Robins****, None.*

M031

Oxygen Sensitive Maturation of Epiphyseal Growth Plate Chondrocytes: the HIF-PHD Axis. S. P. Terkhorn*, M. Kanyama*, E. Koyama*, M. Ohashi*, C. S. Adams, I. M. Shapiro, V. Srinivas*. Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA, USA.

Previous work has shown that the transcription factor, HIF-1 is required for cell survival in the oxygen limited epiphyseal growth plate. To test the hypothesis that prolyl hydroxylase (PHD) mediated regulation of HIF function serves to control the maturation and activity of the chondrocytes in the epiphyseal growth plate, we have modulated the expression and activity of HIF-1 and the PHDs. To perform this study, we initially examined human epiphyseal growth plate for the expression of PHD and HIF by *in situ* hybridization and immunohistochemistry. Results were related to the differentiation status of the growth plate as evidenced by expression of collagen type II and X. The studies indicated that the PHDs were expressed predominantly in the hypertrophic zone of the growth plate with lower level of expression in the maturing and proliferative zones. Expression was then evaluated in a mouse chondrocyte cell line, N1511, which undergoes ordered maturation when treated with BMP-7. Using RT-PCR we demonstrate that these cells express all 3 PHD isoforms. Furthermore, we show by Western blot analysis that HIF-1 is expressed under hypoxic conditions. Interestingly, the BMP-7 treated cells exhibited an elevated level of PHD expression when compared to untreated cells. Inhibition of PHD enzymatic activity by a non-metabolizable substrate also elevated HIF-1 levels and activity as evidenced by an increase in the expression of HIF-1 target genes. Thus, these studies support the importance of the HIF-PHD axis in regulating the ordered maturation of chondrocytes in the mammalian growth plate.

Disclosures: ***V. Srinivas****, None.*

M032

IGF-1, IGF-1 Receptor, IGFBP-4 and PAPP-A mRNA Levels are Regulated by TGF-beta during in vitro Periosteal Chondrogenesis. C. Gonzalez*[1], K. G. Auw Yang*[2], J. H. Schwab*[1], V. R. Clemens*[1], J. S. Fitzsimmons*[1], D. B. F. Saris*[2], G. G. Reinholz[1], C. A. Conover*[3], S. W. O'Driscoll*[1]. [1]Department of Orthopedic Surgery, Mayo Clinic, Rochester, MN, USA, [2]Cluster of Orthopedics, University Medical Center, Utrecht, Netherlands, [3]Endocrine Research Unit, Mayo Clinic, Rochester, MN, USA.

Periosteum contains mesenchymal cells with the potential to form cartilage or bone. In order to study periosteal chondrogenesis and the effects of growth factors on this process, we have established an in vitro culture model in which periosteal tissue can be stimulated to form cartilage. Previously we demonstrated that in vitro periosteal chondrogenesis is enhanced by TGF-beta and IGF-1, and an additive effect is observed when periosteum is treated with both factors. The purpose of this study was to examine the effect of TGF-beta on IGF-1, IGF-1 receptor, IGFBP-4 and PAPP-A mRNA levels during in vitro periosteal chondrogenesis. Periosteal explants were harvested from two-month old New Zealand white rabbits. The explants were cultured in DMEM, 10 % FBS and 1 % agarose with or without TGF-beta (10 ng/mL) during the first 48 h. Explants were cultured for 3, 5, 7, 10, 14, 21, 28, 35, and 42 days. Total RNA was isolated from the explants, reverse transcribed and the mRNA levels of IGF-1, IGF-1 receptor, IGFBP-4 and PAPP-A were determined by quantitative real-time PCR and normalized using GAPDH. TGF-beta treatment resulted in decreased IGF-1, IGF-1 receptor, and IGFBP-4 mRNA levels throughout periosteal chondrogenesis. However, TGF-beta treatment increased PAPP-A mRNA levels at day 7 ($p<0.0006$) and day 10 ($p<0.002$) followed by a decrease on day 14 ($p<0.005$). These results suggest that TGF-beta regulates the expression of multiple genes involved in the IGF-1 autocrine/paracrine axis during periosteal chondrogenesis. However, it is not clear what the net effect of these changes may have on overall IGF-1 signaling. Nevertheless, these results demonstrate that communication between these two important signaling pathways occurs during periosteal chondrogenesis. Currently, we are examining the effects of TGF-beta on PAPP-A protein levels and its IGFBP-4 protease activity between days 7 and 10. It is possible that a transient increase in PAPP-A activity is an important mechanism for controlling IGF-1 availability during this early stage of chondrocyte differentiation. The IGF-1 autocrine/paracrine axis is complex and involves several genes in addition to those examined in this study. Continued examination of this signaling pathway during periosteal chondrogenesis will increase our understanding of fracture healing and may augment the use of periosteal tissue for articular cartilage repair.

Disclosures: ***C. Gonzalez****, None.*

M033

The Regulation of Human Chondrocyte and Osteoblast Differentiation by Activin and Inhibin. L. J. Suva[1], D. Bibbs*[1], S. E. Bledsoe*[1], R. A. Skinner*[1], N. S. Akel*[1], T. Mon-Foote*[1], M. L. Jennings*[2], D. Gaddy[2]. [1]Orthopaedic Surgery, UAMS, Little Rock, AR, USA, [2]Physiology and Biophysics, UAMS, Little Rock, AR, USA.

The chondrocytic differentiation of human bone marrow-derived mesenchymal stem cell (MSC) cultures requires the addition of TGF beta, whereas osteogenic differentiation of the same precursor cell population requires the addition of ascorbic acid and betaglycerol phosphate. Accumulating evidence suggests an important role for both activin and inhibin in the regulation of skeletal development. The goals of the current study were to determine if activin and inhibins regulated human chondrocyte and osteoblast differentiation. Inhibin and activin effects on chondrocyte development were assessed using human MSC cultured in standard chondrogenic micromass cultures stimulated to differentiate by treatment with inhibin A or B, activin A, with or without the addition of TGF beta. Chondrogenic differentiation was evaluated at days 7, 14, 21 and 28 of culture by histological evaluation and immunostaining for type II collagen or the chondrocyte-specific diastrophic dysplasia sulfate transporter (DTDST), as well as real time PCR analysis of chondrocyte marker gene expression. Interestingly, although TGF beta directly stimulated chondrocyte development, activin A was unable to reproduce the effect. In addition, inhibin A or inhibin B markedly suppressed all MSC proliferation and chondrocyte differentiation. In competition experiments, inhibin suppression was not overcome by TGF beta treatment, suggesting competition for the same receptor. The dominance of inhibin over TGF beta in chondrocyte formation suggests a unique role for inhibin in these cultures. Osteogenic differentiation from the same MSC pool was determined on day 8 by measuring expression of alkaline phosphatase (AP) and on day 21 by staining mineralized extracellular matrix with alizarin red. Both inhibin A and inhibin B suppressed osteoblastogenesis; and the suppression of OBL development by inhibins was maintained even in the presence of exogenous activin. The lack of activin antagonism of inhibin specific effects are similar to those we have reported previously in murine MSCs. These data demonstrate that human chondrocytes and OBL progenitors are direct targets of inhibin and activin regulation. We hypothesize that the regulation of inhibin/activin tone in the bone marrow may be an important factor controlling the differentiation of human progenitors toward multiple mesenchymal cell fates, including bone, cartilage, fat and muscle.

Disclosures: ***L.J. Suva****, GlaxoSmithKline 8; Procter and Gamble 5; Wright Medical 2.*

M034

Perlecan Domain I as a Growth Factor Delivery System. W. Yang*, R. R. Gomes*, M. C. Farach-Carson, D. D. Carson. Biological Sciences, University of Delaware, Newark, DE, USA.

Tissue regenerating capacity is an essential characteristic of biomaterials and scaffolds designed for use in tissue engineering. Growth factors (GFs) play an important role in the process of tissue regeneration, both naturally and therapeutically. Numerous polypeptide growth factors bind to the heparan sulfate (HS) proteoglycan, perlecan (Pln), with high affinity. It is hypothesized that the HS chains attached to Pln domain play a key role in modulating the activity of many GFs. Pln is thought to sequester GFs, prevent degradation, and, in some cases, enhance GF binding to cell surface receptors. We hypothesize that the attachment of recombinant Pln domain I (PlnDI) to scaffolds commonly used in tissue engineering, will enhance GF binding and serve to modulate GF release thereby enhancing their activity and use in tissue engineering. The objective of the present investigation was to characterize the binding of GFs (FGF-2, VEGF and BMP-2) involved in bone and cartilage regeneration to PlnDI/type I collagen complex. Dot blot analysis indicates that PlnDI binds FGF-2, VEGF and BMP-2. The binding is competitively inhibited by heparin, but not by chondroitin sulfate. PlnDI digested with heparinases failed to bind FGF-2, VEGF and BMP-2. Thus, GF binding to PlnDI is HS dependent. Experiments employing biotinylated PlnDI demonstrated that procollagen type I specifically binds PlnDI. PlnDI binding to procollagen type I is not HS dependent; however, the fibrillar form of collagen type I binds PlnDI in a HS dependent fashion. Experiments testing the ability of the PlnDI/procollagen type I complex to bind FGF-2 suggest that the PlnDI/procollagen type I complex retains more FGF-2 than procollagen type I alone. Thus, PlnDI maybe a useful tool for modulating GF bioavailability in applications of tissue engineering.

Disclosures: ***W. Yang****, None.*

M035

Identification of Precursor Proteins for Potential Secreted Bioactive Peptides Following a Genome-wide Search. M. R. John[1], A. Bruengger*[2], K. Seuwen*[1]. [1]Bone Metabolism, Novartis Institutes of Biomedical Research, Basel, Switzerland, [2]Oncology Research, Novartis Institutes of Biomedical Research, Basel, Switzerland.

G protein-coupled receptors (GPCRs) such as the calcium-sensing receptor or the parathyroid hormone receptor are excellent targets for drug development. In addition, GPCR peptide ligands themselves have become important prescription medicines. Examples are calcitonin 1-32 or parathyroid hormone 1-34. Approximately 160 non-olfactory human GPCRs are still considered orphan and it is estimated that 30-50 represent candidate receptors for known or yet to be discovered peptide ligands. Peptide ligands are typically processed from precursor proteins that have the following features: presence of a signal peptide, short to moderate length, occurrence of mainly dibasic protease cleavage sites, absence of otherwise classifying protein motifs, and non-uniform expression pattern. Importantly, there is usually a high degree of conservation between most human and mouse ligand precursors. By incorporating these features into a bioinformatics analysis pipeline we aimed to discover potential novel members of the group of non-chemokine GPCR peptide ligands from a genome-wide search.
Following analysis of the structure and features of 59 known human and mouse GPCR ligand precursor proteins we have designed a specific bioinformatics pipeline which we applied to the list of predicted proteins as provided by Celera Inc. for the human genome.
Celera provided 32117 gene predictions (release R26f), including most known chemokines and 55 of 59 non-chemokine GPCR ligands. In total, these known ligands represented 0.31% of all predicted genes. Following application of our feature analysis pipeline we identified a conservative list of 18 novel *bona fide* ligand precursors. Considering possible dibasic cleavage sites and neglecting other potential processing modes, the 18 precursors may give rise to 61 different peptides, 46 showing >50% identity between human and mouse, 33 showing > 80% identity. Among those, our analysis identified two known ligand molecules for which receptors are unknown at present: Lect2 and Neuritin. If the known non-chemokine GPCR ligands had not been excluded during the analysis, 30 of them would have co-segregated with the final pool, indicating significant enrichment of such molecules by our analysis. In conclusion, we have developed a stringent bioinformatics pipeline capable to filter out potential ligand precursor proteins from genomic data.

Disclosures: ***M.R. John****, None.*

M036

The Temporal and Spatial Expression of HIF-1α and Selected Angiogenic Factors During Bone Regeneration. D. E. Komatsu, M. Hadjiargyrou. Biomedical Engineering, SUNY Stony Brook, Stony Brook, NY, USA.

One of the major sequelae of bone fracture is the generation of hypoxic microenvironments, both in the hematoma, due to destruction of the normal vasculature, and in the subsequent callus, as a result of rapid avascular tissue production (e.g. cartilage). Hypoxia Inducible Factor-1α (HIF-1α) is the inducible component of the heterodimeric transcription factor HIF-1, which is activated by hypoxia and responsible for the regulation of numerous genes involved in angiogenesis. A series of experiments was performed to examine the transcriptional and post-translational activity of HIF-1α and a selection of its target angiogenic related genes during fracture repair. Fixed rat femoral fractures were generated and both the fractured and unfractured contralateral (control) femurs were harvested on post-fracture days (PFD) 3, 5. 7. 10, 14 and 21 and processed for either extraction of total RNA and protein or histology and immunohistochemistry. To assay relative mRNA expression, Real Time PCR was performed using gene specific primers. For quantification, all raw values were normalized to Cyclophillin A, and fold changes were calculated relative to control (intact bone). Western blot analysis was performed to determine temporal protein levels using an anti-HIF-1α monoclonal antibody (mAb). The same mAb was used to analyze callus sections to determine spatial expression of HIF-1α. Real Time PCR experiments revealed elevated HIF-1α expression at all time points, with a peak of ~6-fold at PFD 10. Vascular Endothelial Growth Factor (VEGF) and CYR61 expression mirrored that of HIF-1α, with peak fold changes of 2.2 and 7.2 at PFD 10, respectively. Intense activation of inducible Nitric Oxide Synthase (iNOS) mRNA was also detected at PFD 10 (6.8-fold) while all other time points showed slight down-regulation. In contrast, Heme Oxygenase demonstrated peak expression at PFD 3 (4.5-fold) with no significant differ-

ences noticed at all other time points. Western analysis for HIF-1α showed a steady increase in protein from none in intact bone, to a peak in PFD 10 callus. Further, immunohistochemical analysis of PFD 10 callus sections showed strong HIF-1α staining in proliferating chondrocytes but none in further differentiated hypertrophic chondrocytes. Osteoblasts lining the woven bone also stained highly for HIF-1α. These experiments demonstrate for the first time that HIF-1α is up-regulated at both transcriptional and post-translational levels in the fracture callus. Peak expression of HIF-1α, VEGF, CYR61 and iNOS at PFD 10 indicate that this is a key angiogenic time point in callus development. Further work is ongoing to fully characterize the functional significance of HIF-1α in bone regeneration.

Disclosures: ***D.E. Komatsu****, None.*

M037

Lymphoid Enhancer-Binding Factor (LEF)1 Delays Osteoblast Differentiation. R. A. Kahler[1], J. J. Westendorf[2]. [1]Graduate Program in Microbiology, Immunology and Cancer Biology, University of Minnesota, Minneapolis, MN, USA, [2]Orthopaedic Surgery and University of Minnesota Cancer Center, University of Minnesota, Minneapolis, MN, USA.

Osteoblast differentiation is a highly regulated process that requires organized expression and activity of many factors, including the transcription factor Runx2 (Cbfa1). Runx2 is required for both osteoblast and chondrocyte development and regulates tissue-specific gene expression by binding the DNA sequence, TGPuGGTPu, to either activate or repress transcription. We are interested in the molecular mechanisms that regulate Runx2 activity in osteoblasts. We have previously identified a novel interaction between Runx2 and LEF1, which leads to the downregulation of the osteocalcin promoter. LEF1 is a downstream target of the Wnt/ß-catenin signaling pathway. Activation of this pathway increases osteoblast numbers and bone mass. Our previous data showed that ß-catenin enhances LEF1-mediated repression of the osteocalcin promoter. Moreover, LEF1 levels declined during MC3T3-E1 osteoblast differentiation. Based on these data, we hypothesized that LEF1 expression delays osteoblast differentiation and that loss of LEF1 expression would accelerate differentiation. To test this hypothesis, LEF1 was suppressed in MC3T3-E1 osteoblasts by RNA interference. LEF1 short hairpin RNA (shRNA) relieved LEF1-mediated repression of Runx2 transcriptional activity and increased the basal activity of the osteocalcin promoter in MC3T3-E1 cells.
To determine the role of LEF1 in osteoblast differentiation, stable MC3T3-E1 cell lines that express shRNAs for either firefly luciferase or LEF1 were designed. LEF1 shRNA reduced LEF1 levels in undifferentiated MC3T3-E1 cells, but the control firefly luciferase RNAi did not affect LEF1 levels. MC3T3-E1 cells were placed in differentiation media (ascorbic acid and ß-glycerol phosphate) and monitored for up to 3 weeks for alkaline phosphatase activity and/or matrix mineralization. The appearance of these markers of osteoblast differentiation was accelerated by approximately 3 days in MC3T3-E1 cells containing LEF1 shRNAs. These data are consistent with the hypothesis that LEF1 plays a role in immature osteoblast expansion and its loss of expression or activity may be required for optimal osteoblast differentiation and bone formation.

Disclosures: ***R.A. Kahler****, None.*

M038

Gene Microarray Analysis of PDGF and Cortisol Treated Adult Human Bone Cells. S. Whitson[1], J. Allen*[2], S. McCommas*[2], M. Whitson*[1]. [1]Department of Growth, Development and Structure, Southern Illinois University, School of Dental Medicine, Alton, IL, USA, [2]Department of Biological Sciences, Southern Illinois University, Edwardsville, IL, USA.

The goal of this research was to compare the effects of platelet-derived growth factor (PDGF), cortisol, and their combination on the expression of human angiogenesis genes in cultured adult human bone cells, using gene microarray analysis of 109 genes. PDGF (20 ng/ml) or cortisol (10 nm), or the combination of the two, was added to confluent cultures of cells derived from the trabecular bone of the distal femur in mineralization medium containing 50 micograms/ml ascorbic acid, 2.4 mM calcium, 100 nm insulin, and 10 mM beta-glycerol phosphate. The cultures were sampled on treatment days 4 and 14. Ten genes showed significant changes in expression under these conditions. In general, the expression of these genes on day 14 was similar to but greater than the day 4 expression. Fibronectin expression increased substantially in cultures treated with PDGF and cortisol together. The expression of FIGF (c-fos induced vascular endothelial cell growth factor, VEGF-D) increased the most dramatically under combined PDGF and cortisol treatment. ID3 (inhibitor of DNA binding-3), a protein that binds several transcription factors and inhibits differentiation toward either fat or muscle tissue, increased slightly during the combination treatment. Osteonectin expression, which had been high in cells cultured on standard growth medium, began a gradual decline that continued through day 14 in the presence of mineralization medium, no matter which of the experimental factors was added. Thrombospondin 1 expression also decreased in the presence of PDGF. The conclusions reached in this study are that adult human bone cells in culture differ significantly in their expression of several genes when treated with PDGF and/or cortisol. The relatively slow rate of differentiation of the cultured cells into functional bone cells and the absence of visible mineralization suggest that the result of 14 days of treatment represents an intermediate stage of bone cell differentiation.

Disclosures: ***S. Whitson****, None.*

M039

Using Laser Capture Microdissection to Examine Gene Expression in Functionally Distinct Populations of Growth Plate Chondrocytes. Y. Y. Shao*[1], L. Wang*[1], M. A. Kral*[2], D. G. Hicks*[2], R. T. Ballock[3]. [1]Department of Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH, USA, [2]Department of Anatomic Pathology, The Cleveland Clinic Foundation, Cleveland, OH, USA, [3]Departments of Biomedical Engineering and Orthopaedic Surgery, The Cleveland Clinic Foundation, Cleveland, OH, USA.

The purpose of this study was to determine if the technique of laser capture microdissection could be used to examine the expression of genes in functionally and maturationally distinct populations of growth plate chondrocytes in vivo. Neonatal growth plate cartilage was isolated from the distal femora of two day-old Sprague Dawley rats and the tissue embedded in OCT medium. Frozen sections of five micron thickness were cut on a cryostat, fixed in 70% ethanol and attached to microscopic slides containing a thin foil membrane pre-treated with 0.1% poly-L-lysine (Leica). Following staining with hematoxylin and eosin, hypertrophic and non-hypertrophic zones of growth plate cells (100-400 cells per section) were outlined and microdissected by laser ablation (Leica DM LMD). The microdissected tissues were captured into Eppendorf tubes containing DEPC-treated water mounted beneath the microscope stage. RT-PCR was performed on RNA extracted from the growth plate tissue sections using intron-spanning primers for cDNA species encoding alkaline phosphatase, type X collagen and cbfa1(runx2) genes. The results demonstrate that alkaline phosphatase and type X collagen PCR products were successfully amplified from tissue sections containing as few as 100 cells from the hypertrophic zone of the growth plate, and were not amplified from tissue sections containing similar numbers of non-hypertrophic cells. The transcription factor cbfa1(runx2) was successfully amplified from both hypertrophic and non-hypertrophic zone tissue. We conclude from these experiments that the technique of laser capture microdissection is a powerful and sensitive technique that can be used to examine gene expression in functionally and maturationally distinct populations of growth plate chondrocytes in vivo.

Disclosures: ***R.T. Ballock****, None.*

M040

Expression Profiling of Regenerate after Bone Marrow Ablation. S. Kuroda, A. S. Virdi, D. R. Sumner. Department of Anatomy and Cell Biology, Rush University, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, USA.

The sequence of events following bone marrow ablation is similar to the process of bone formation seen during development. The tissue regeneration is characterized by clot formation and inflammation, followed by the closely regulated process of osteogenesis and subsequent resorption. In this study, we determined patterns of gene expressions during tissue regeneration within the marrow cavity following marrow ablation. Sprague Dawley rats were divided into seven groups. Six groups received unilateral femoral ablation followed by saline irrigation and were sacrificed at 1, 3, 5, 7, 10 and 14 days post operation. The seventh group served as an intact control. At prescribed time points, femurs were harvested, denuded of soft tissue and homogenized for RNA extraction using a standard protocol. Total RNA was reverse-transcribed to yield cDNA which served as template for real-time PCR to determine the level of expression of 40 specific genes. All data were normalized with GAPDH and results presented as fold change over intact control. In general, the expression profile reflected the regeneration phases throughout the time period studied. Between 1d and 3d, inflammation stage exhibited TNFα and COX-2 up-regulation. This was followed by increase in Cbfa1, TGFβs and BMPs expression between 3d and 10d. Greatest changes were seen in BMP4 and BMP7 with relatively small change in BMP2. Interestingly, Noggin expression mirrored BMP4 suggesting self-regulation of BMP4 mediated regeneration. Alkaline phosphatase and collagen I expression was elevated from the start and remained high throughout. VEGF was seen to peak at 7d with subsequent decline indicating development of angiogenesis required for new bone formation. Collagen II and X were relatively unaltered which suggests predominantly intramembranous ossification. Down-regulation of RANK is an indicator of suppressed remodeling up to 14d. In summary, the data demonstrates a complex interplay of a number of genes during bone regeneration. This is an ideal model for studying the effect of exogenous factors on osteogenesis.

Disclosures: ***S. Kuroda****, NIH AR 042862; NIH AR 043187 2.*

M041

Gene Expression Profile of Human Chondrosarcoma Cell Line HCS 2/8 by High-throughput EST Sequencing Analysis. Y. Jung*[1], H. Kim*[1], H. Si*[1], H. Ryoo[1], S. Kim[1], E. Park[1], M. Takigawa[2], R. Park*[1], I. Kim*[1], G. S. Kim[3], J. Choi[1]. [1]Biochemistry & SDGRC, School of Medicine, Kyungpook National University, Daegu, Republic of Korea, [2]Biochemistry & Molecular Dentistry, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan, [3]Division of Endocrinology and Metabolism & SDGRC, Asan Medical Center, College of Medicine, Ulsan University, Seoul, Republic of Korea.

Large-scale, single-pass sequencing of cDNA clones randomly picked from library has proven to be a powerful approach to discovering genes and novel members of gene families as well as an expressed gene profile. A clonal cell line, HCS-2/8 was derived from a human chondrosarcoma and has been known as a good model for studying of chondrocyte differentiation. We used high-throughput DNA sequencing analysis and generate 3350 single-pass sequencing reactions obtained from the 5` ends of cDNA clones from HCS 2/8 library and analyzed the sequences. Results of BLAST search showed that the sequences represented 1927 unigene clusters. The BLAST search also showed the identified ESTs that have close homology to known genes, which suggests that these may be newly recog-

nized members of known gene families. The gene expression profile of this cell type was revealed by analyzing both the frequency with which a message was encountered and the functional categorization of expressed sequences. It is expected that high-throughput EST sequencing and data mining analysis will greatly promote our understanding of gene expression in these cells and differentiation of chondrocyte and it will be valuable information in the field of chondrocyte research.

Disclosures: ***Y. Jung****, None.*

M042

Novel Bioinformatic Analysis of Tet-Regulated Bone Formation In Vivo Indicates Novel Cross Talk Between WNT and BMP2 Signalling Pathways. H. Aslan*[1], O. Ravid*[2], A. Cohen*[1], L. Tzur*[1], G. Pelled*[1], G. Turgeman*[1], Y. Zilberman[1], Z. Gazit*[1], B. Clancy*[3], E. Domany*[2], D. Gazit[1]. [1]Skeletal Biotechnology Laboratory, Hebrew University-Hadassah Medical Centre, Jerusalem, Israel, [2]Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot, Israel, [3]Wyeth Pharmaceuticals, Divisions of Musculoskeletal Science and Drug Safety Metabolism, Cambridge, MA, USA.

Endochondral bone formation is a complex process that involves chondrogenic, osteogenic, and angiogenic genes. Several genes were described to function during endochondral bone formation; however, additional pivotal genes might have an important role as well. Micro-arrays and bio-informatics offer the promise of rapid and accurate measurement of gene expression in various experimental conditions. The coupled two-way clustering (CTWC) analysis is a novel unique method that is based on a natural algorithm and enables clustering in two steps, therefore, minimizing the background. The CTWC is especially designed to analyse complex multi-step processes such as endochondral bone formation (Getz et al., 2000). We hypothesized that a gene discovery strategy, which combines Tet-inducible rhBMP2 in vivo and the CTWC, will enable the identification of novel pivotal genes and signalling pathways crucial for bone formation. We utilized genetically engineered pluripotent C3H10T1/2 cells expressing the rhBMP-2 in a Tet-regulated system implanted ectopically in mice, which were kept in two groups: + Tetracycline, rhBMP-2 is not expressed: BAS, and -Tetracycline, rhBMP-2 is expressed: EXP. GeneChip analysis was applied on total RNA isolated from the implants. CTWC analysis was used to analyse the gene expression data. Endochondral ossification was detected in the EXP implants harvested at: 1, 3, 5, 7, 10, 15, and 20 days, while no differentiation was detected in the BAS implants. We have identified several distinct clusters with low background that included a number of genes with obvious role in cartilage/bone formation, genes without known function in the process such as MEST, DKK3, Capn6, and novel genes (ESTs). One of the clusters included 3 genes that encode for inhibitors of the WNT signalling pathway. The up-regulation of WNT inhibitors indicates potential cross-talk between the BMP2 and the WNT pathways that may be crucial in regulating bone differentiation in vivo. We conclude that the combination of Tet-inducible system in vivo and CTWC computational analysis of gene expression can be utilized for studying genomic events in osteogenesis in vivo. This approach led us to the identification of novel pathways/genes crucial for developing new insights and clinical therapies in skeletal disorders.

Disclosures: ***H. Aslan****, None.*

M043

Activation of Wnt Signaling Pathway during Bone Regeneration. N. Zhong*, R. Gersch*, D. Komatsu*, M. Hadjiargyrou. Biomedical Engineering, SUNY, Stony Brook, NY, USA.

The Wnt signaling pathway is crucial for the development of tissues and organs in multicellular organisms. A series of genes involved in the Wnt signaling pathway (including target genes) were previously identified as activated in our studies on transcriptional profiling of bone regeneration. To further investigate these observations, quantitative, real-time PCR was utilized to confirm the temporal expression pattern of the identified genes, as well as other selected genes known to be key players or target genes in the Wnt signaling pathway. Using the rat femoral fixed fracture model, RNA was isolated from calluses at post-fracture days (PF) 3, 5, 7, 10, 14 and 21 and the contralateral unfractured femurs (control). For each time point, RNA samples were pooled (n=4) and processed for real-time PCR analysis (n=3). All results were normalized to cyclophillin A. The 17 genes selected for this study were categorized into four groups: 1) Wnts and Frizzled: Wnt4, Wnt5A, Wnt5B, Frizzled2 ; 2) Intracellular Wnt signaling molecules: Disheveled, CK1A1, CK2A1, Beta-Catenin, LEF1, TCF1; 3) Target genes (transcriptional factors): Engrailed1, OSF2, PPARD; 4) Target genes (adhesion molecules and others): Connexin43, Fibronectin, CD44, retinoic acid receptor gamma. The overall temporal trends for most genes were consistent with our previous microarray data. Although the magnitude of increase in expression varied from 0.3 to 19 fold, all genes except LEF1 showed up-regulation with a peak at either PF day 5, 7, or 10, coinciding with the biological processes of intramembranous ossification and chondrogenesis in the fracture callus. Almost all signaling pathway genes showed peak expression at PF day 5, while the target genes, especially the cell adhesion molecules had the highest expression levels at PF day 7 or 10. It is important to note that in contrast to other Wnt signaling genes that exhibited a smaller than 4 fold increase in expression, Frizzled2 had an 18 fold increase at PF day 7, indicating that Frizzled2 is an important member in the Frizzled family which is involved in the early stages of fracture healing, and its activation is probably regulated at the mRNA level. Initially, LEF1, a recently identified transcriptional co-repressor of Runx2-dependent expression, showed a 9 fold decrease at PF day 3, but then its expression gradually increased to intact bone level. This pattern of expression suggests that LEF1 down-regulation could be required for osteoblast differentiation/proliferation during callus intramembranous ossification. Based on these data, we conclude that Wnt signaling plays a critical role in the development of the fracture callus, presumably in the differentiation and proliferation of osteoblasts and chondrocytes.

Disclosures: ***M. Hadjiargyrou****, None.*

M044

Use of Chromatin Immunoprecipitation (ChIP) to Evaluate Interactions between Runx2 and the Osteocalcin Gene in Intact Cells. H. Roca*, M. Phimphilai*, R. T. Franceschi. Periodontics, Prevention and Geriatrics, University of Michigan School of Dentistry, Ann Arbor, MI, USA.

The Runx2 transcription factor is necessary for osteoblast and hypertrophic chondrocyte differentiation. Several lines of evidence indicate that Runx2 must undergo some form of activation before it becomes transcriptionally active. This activation may involve increases in MAP kinase-dependent phosphorylation and/or changes in levels of nuclear coactivators or inhibitors. Runx2 activation of gene expression involves binding to specific enhancer sequences. Two such sequences were identified in the murine osteocalcin gene 2 promoter at -131 and -602. Cell culture and transgenic analysis indicated that both sites were required for maximal osteocalcin expression with the downstream site having the higher activity (Frendo et al, JBC 273:30509, 1998). We used ChIP assays to measure interactions between Runx2 and upstream and downstream sites in intact MC3T3E1 clone 4 cells grown in control or differentiation medium (i.e. ascorbic acid). Intact cells were cross-linked with formaldehyde and purified chromatin was immunoprecipitated with anti-Runx2 antibody or preimmune IgG. Specific PCR primers were used to detect the presence of upstream and downstream enhancers in immunoprecipitates. Chromatin containing both enhancer regions specifically interacted with the Runx2 antibody. However, more chromatin was immunoprecipitated from differentiated cells suggesting that Runx2 is preferentially associated with its cognate enhancer when it is transcriptionally active. In contrast, a putative Runx2 binding site at ?1335 bp in the bone sialoprotein promoter was not immunoprecipitated under the same conditions consistent with our previous finding that this site is not required for the bone-specific expression of this gene. This study shows that ChIP analysis can be used to detect functional in vivo interactions between Runx2 and target genes and may be useful as a means of identify new Runx2 targets.

Disclosures: ***R.T. Franceschi****, None.*

M045

An Expression Profile Comparison of Human Mesenchymal Stem Cell-derived Adipocytes, Medullary Adipocytes, and White Adipocytes. D. L. MacKay*, P. J. Tesar*, L. Liang*, S. E. Haynesworth. Biology, Case Western Reserve University, Cleveland, OH, USA.

The origin and phenotype of medullary adipocytes remain uncharacterized; however, human Mesenchymal Stem Cells (hMSCs) are a putative progenitor cell type. To investigate the above issues, hMSC-derived adipocytes and medullary adipocytes were analyzed for their expression of gene products that characterize white adipocytes. All three adipocyte phenotypes were cultured under identical conditions. hMSC adipogenesis was induced by culturing superconfluent cells for 12 days in DMEM-HG, supplemented with 10% FBS, 1 μM dexamethasone, 100 μM indomethacin, 0.5 mM 3-isobutyl-1-methylxanthine, and 10 μg/mL insulin. The hMSC-derived adipocytes were then cultured for an additional 18 days in Adipocyte Maintenance Medium, consisting of DMEM-HG, supplemented with 10% FBS and 10 μg/mL insulin. hMSC differentiation along the adipogenic lineage was monitored by measuring lipid accumulation. Cells fixed on sequential timepoints were stained with Nile Red and assayed by flow cytometry. Analysis indicated that lipid accumulation begins on day 2-3 of culture. The number of cells accumulating lipid increases steadily to around 50% by day 12-14, after which the percentage of adipogenic cells remains relatively constant. The volume of lipid per cell increases through day 30, and cells begin to acquire a unilocular morphology during week 4. Medullary adipocytes and white adipocytes were cultured in only the maintenance medium. Cell lysate was collected from each adipocyte phenotype, and semiquantitative PCR was performed using GAPDH for normalization. A gene expression profile was generated based on markers that have been reported either as specific to a particular fat phenotype, such as uncoupling protein 1, or highly upregulated as a function of adipogenesis. Markers in the latter category include key transcription factors, adiponectin, leptin, adipsin, lipoprotein lipase, and others. Undifferentiated hMSCs expressed no adipocyte-specific markers, with marker expression in hMSC-derived adipocytes beginning on day 1-2 of culture. Each of the markers investigated was expressed in all three adipocyte phenotypes, with the exception of uncoupling protein 1, which presents a crucial distinction between medullary adipocytes and brown adipocytes. The similarity of profiles between hMSC-derived and medullary adipocytes supports the hypothesis that hMSCs are precursors to medullary adipocytes; however, confirmation of this role will require further analysis. These PCR data serve as a preliminary, but fundamental, basis for the classification of medullary adipocytes, which, to the level analyzed, are not distinguishable from white adipocytes.

Disclosures: ***D.L. MacKay****, None.*

M046

The Degree of Mineralization is a Determinant of Bone Strength. A Study on Human Calcaneus. H. Follet*[1], G. Boivin[2], C. Rumelhart*[1], P. Meunier[2]. [1]Laboratoire de Mécanique des Solides (LMSo), INSA, Lyon, France, [2]Faculté de Médecine R. Laennec, INSERM Unité 403, 69372 LYON Cédex 08, France.

Strength of bones depends on bone matrix volume (BMV), bone microarchitecture but also on the degree of mineralization of bone (DMB). We have recently shown in osteoporotic patients treated with alendronate that fracture risk decreased and bone mineral density increased with a parallel increase of the DMB, due to a prolonged secondary mineralization but without modifications of BMV or bone microarchitecture. In the present study, DMB and strength were both measured at the tissue level in calcaneus bone samples taken at necropsy from 20 subjects (aged 78 ± 8 years, 8 women, 12 men) who died suddenly without apparent bone disease. DMB parameters measured on microradiographs

(mean DMB, distribution of DMB, more frequent maximum DMB value and width at half-maximum, an index reflecting the homogeneity of DMB) were compared with those reported in iliac cancellous bone samples of 43 human controls. Compression tests were performed on contiguous samples of the same calcaneus on a universal screw-driven machine (Schenck RSA 250). A 5000 N load cell (TME, F 501 TC) measured the compressive load and the displacement was measured directly on the sample, using a specific displacement transducer developed by the LMSo. The apparent Young modulus (E) and the maximal strength (sigmamax) until failure were measured. In human cancellous bone tissue, mean DMB (± SD) was higher in calcaneus (1.226 ± 0.125 g/cm3) than in iliac crest (1.099 ± 0.104 g/cm3). The more frequent maximum DMB values (DMB freq max) were 1.25 g/cm3 in calcaneus and 1.10 g/cm3 in iliac samples, and DMB was almost 2-fold more heterogeneous in calcaneus than in iliac samples (width at half maximum were 0.52 versus 0.28 g/cm3, respectively). Compression tests revealed significant positive linear correlations between DMB and both elastic modulus (r2 = 0.69) and maximal strength (r2=0.69). Correlations persisted (p<0.003) even after adjustment for cancellous bone volume for the Young modulus (E) and the maximal strength (sigmamax) (r2 = 0.44 and 0.41, respectively). We conclude that the greater the cancellous tissue was mineralized the higher was its stiffness and compressive strength. This may explain the increase in bone strength when DMB is modified without necessary changes of BMV and bone microarchitecture. The impact of such modifications on fracture risk and the therapeutic implications of these data remain to be evaluated.

Disclosures: ***H. Follet****, None.*

M047

Decreased Whole-Bone Strength in the SAMP6 Mouse Is Explained by Decreased Material Strength of Demineralized Bone Matrix. M. J. Silva, M. D. Brodt*, M. Ko*, N. Kusano*, R. P. Mecham*, B. Wopenka*. Orthopaedic Surgery, Washington University, St. Louis, MO, USA.

The senescence accelerated mouse, strain P6 (SAMP6) has been described as a model of senile osteoporosis. Long bones from SAMP6 mice are weak and brittle, with a 25% lower failure load and 60% lower energy-to-fracture compared to SAMR1 controls. These inferior mechanical properties exist despite increased cross-sectional moment of inertia (ie, geometric resistance to bending). Our objective was to identify the material basis for the inferior bone properties in SAMP6 mice. We analyzed femurs from 4- and 12-month SAMP6 and SAMR1 (control) mice. We evaluated tensile properties of demineralized bone using a novel method: femora were scanned by μCT (to assess cortical thickness) and then demineralized; waisted specimens were cut and then loaded to failure in tension. Demineralized bone specimens from SAMP6 were significantly weaker than SAMR1, with 45% lower ultimate force and 30% lower ultimate stress (Table); similar reductions in stiffness and energy-to-fracture were noted. Additional bones were analyzed for collagen content (by amino acid analysis) and mineral structure-composition (by Raman spectroscopy). Bones from SAMP6 mice had normal protein content but a 3% decrease in the fraction of hydroxyproline per total protein, resulting in a slight decrease in collagen per dry wt. (mg/mg): SAMR1, 10.5%; SAMP6, 9.7%. These data are consistent with an increase in ash fraction in SAMP6 bones previously reported. Raman spectroscopy revealed no inter-strain differences in the peak positions or bandwidths for the main bioapatite peaks (e.g., PO_4 mode, 960 cm^{-1}). In summary, the demineralized bone matrix of the SAMP6 mouse has inferior material properties compared to SAMR1. This finding, combined with the apparently normal mineral structure-composition, indicates that the weakness and brittleness previously noted in SAMP6 whole bones is explained by a defect in the bone matrix. Because there is only a slight decrease in collagen content, we hypothesize that other characteristics of the bone matrix (e.g., collagen fiber orientation, cross-links) are the source of the inferior material properties. We conclude that reduced bone matrix strength is an additional feature of the SAMP6 mouse that underscores its relevance as a model of skeletal fragility.

Demineralized bone properties (n=5-6/group)

	SAMR1 (Control) 4 mo.	SAMP6 4 mo.	SAMR1 (Control) 12 mo.	SAMP6 12 mo.
Ult. Force (N)	4.4 ± 0.7	2.5 * ± 0.6	3.2 + ± 0.3	1.7 *+ ± 0.6
Ult. Stress (MPa)	9.9 ± 1.6	7.1 * ± 1.8	8.3 + ± 0.7	5.2 *+ ± 1.9

* different from SAMR1; + different from 4-month

Disclosures: ***M.J. Silva****, None.*

M048

Osteopontin Promoter which Directs the Expression in Osteocytes in Response to Mechanical Stress Loading or Sex Hormone Depletion: An Analysis of Transgenic Mice. S. Fujihara*[1], M. Yokozeki*[1], Y. Higashibata*[2], F. Akiyama*[2], S. Nomura[2], K. Moriyama[1]. [1]Department of Orthodontics, School of Dentistry, University of Tokushima, Tokushima, Japan, [2]Department of Pathology, Osaka University Graduate School of Medicine, Osaka, Japan.

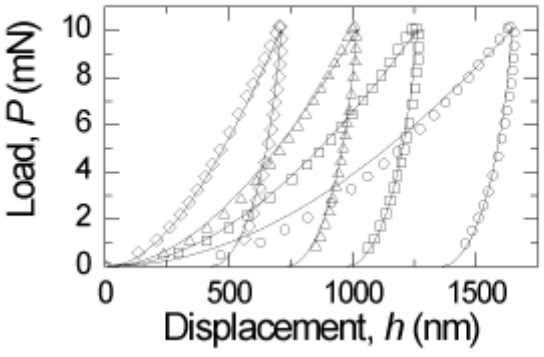
Recent investigations have revealed that osteocytes in the bone matrix act as sensory cells to detect environmental changes, such as mechanical stress loading or sex hormone depletion. We previously found the enhancement of osteopontin gene expression in osteocytes by mechanical stress loading during tooth movement. Other investigators reported the expression of osteopontin in osteocytes in the ovariectomized mice prior to bone resorption. The indispensable roles of osteopontin in response to such treatments were demonstrated by the analysis of phenotypes of osteopontin-deficient mice. To understand molecular mechanisms of osteopontin expression in response to mechanical stress loading or sex hormone depletion, we produce transgenic mice lines carrying GFP (green fluorescence protein) under the control of various lengths of osteopontin promoter. These transgenic mice and osteopontin knockout mice were treated as follows. 1) Orthodontic closed coil springs were bonded to maxillary first molars and incisors for the experimental tooth movement and maxillary first molars were mesially moved by continuous force (approximately 10g). 2) The mice were surgically ovariectomized or injected with leuplin, an antagonist of LH-RH receptor. Expressions of endogenous osteopontin and GFP were examined by in situ hybridization and immunohistochemistry and compared with each other. OPN mRNA-expressing cells were detected in a population of osteocytes, osteoclasts and osteoblasts on the alveolar bone surface after 72 hours of treatment in wild-type (non-transgenic) mice. The localization of GFP in Op5.5GFP mice was consistent with that of endogenous osteopontin during tooth movement or sex hormone depletion. On the contrary, the localizations of GFP-expressing cells in Op3.1GFP and Op1.5GFP were different from that of Op5.5GFP mice. Moreover, expression levels of GFP were decreased in Op3.1GFP and Op1.5GFP transgenic mice and were undetectable in Op0.9GFP transgenic mice. Taken together with the results obtained by the analysis of OPN-knockout mice, our experimental system and result may provide us with a clue for the elucidation of the molecular mechanism of bone remodeling caused by mechanical stress loading or sex hormone depletion.

Disclosures: ***S. Fujihara****, None.*

M049

Local Variations in Viscous-Elastic-Plastic Nanoindentation Responses of Healing Bone. M. L. Oyen*[1], C. Ko*[2]. [1]Biophysical Sciences, University of Minnesota, Minneapolis, MN, USA, [2]Oral Sciences, University of Minnesota, Minneapolis, MN, USA.

Information is scarce about mechanical properties of healing bone. In this study, viscoelastic and plastic properties of healing bone (adjacent to a dental implant) were assessed by nanoindentation experiments. The 4th premolar of a two-year-old Sinclair miniswine was surgically removed unilaterally (Animal Protocol #9910A2261). A smooth titanium threaded dental implant was inserted in the alveolar ridge after the extraction wound had healed for 7 months. The implant was protected from bite forces for 1 month, then the animal was sacrificed. Bone samples were harvested and embedded in polymer resin, sectioned, and polished to 0.5 μm for testing. Mechanical tests were performed in air with an MTS Nanoindenter XP using a Berkovich indenter, to a peak load of 10 mN at fixed loading and unloading rate of 0.33 mN/s. Spatial position was recorded for each test and used to determine the distance from the bone-implant interface. The load-displacement traces were analyzed using a new technique called the VEP model, which estimates viscous, elastic, and plastic coefficients for the material [J Mater Res 2003; 18(1):139-50]. Nanoindentation tests performed near the implant interface (<0.25 mm) showed substantial variations from point to point, as shown by four sample traces in the Figure (hollow symbols). It was found that the VEP indentation model could well-approximate load-displacement traces for bone, as shown by the solid lines in the Figure. Surprisingly, elastic modulus and viscosity terms were unchanged for the different locations (E = 16 GPa, time constant τ = 101 s), and all variations in the responses could be attributed to the Tabor hardness term, which measures resistance to permanent deformation. The Tabor hardness values varied from 0.28 to 5.15 GPa near the interface, and converged to 2.0 ± 0.3 GPa for tests > 2.5 mm from the interface. Nanoindentation testing revealed substantial variations in mechanical response near a bone-implant interface, which undergoes *de novo* mineralization (1-month healing). Application of a new technique for indentation analysis of viscoelastic-plastic materials showed that the viscoelastic properties remained unchanged, and that the variations could be all attributed to variations in the local Tabor hardness of the mineralized tissues.



Disclosures: ***M.L. Oyen****, None.*

M050

Does Laminarity Mediate Site-Specific Differences in Collagen Fiber Orientation in Primary Bone? An Evaluation in the Turkey Ulna. J. G Skedros, K. J. Hunt. Dept Orthopaedics, University of Utah, Salt Lake City, UT, USA.

It has been hypothesized that preferred orientations of primary vascular canals in primary cortical bone mediate important mechanical adaptations [de Margerie 2002, J Anat]. Specifically, bones that receive habitual compression, tension, or bending stresses typically have cortices with a low laminarity index (LI) (i.e., relatively low circularly (C) oriented

primary vascular canal area, and relative higher areas attributed to canals with radial (R), oblique (O), or longitudinal (L) orientations). In contrast, bones subject to habitual torsion have high LIs (i.e., relatively greater areas attributed to C-oriented primary vascular canals). Bone ultrastructure, characterized by regional variations in predominant collagen fiber orientation (CFO), may be the adaptive characteristic mediated by the degree of laminarity. We tested the hypothesis that site-specific variations in predominant CFO in the turkey ulna diaphysis strongly correlate with the degree of laminarity. Thin cross-sections (100+/-5 mm) from mid-diaphyses of 11 sub-adult and 11 adult domestic turkey ulnae were evaluated for predominant CFO and LIs using circularly polarized light images [Laminarity index (LI; based on %vascular canal area) = C/(C+R+O+L)]. Results showed that the mean LI of sub-adult bones (40 +/- 11) is significantly less than adult bones (51 +/ - 10) ($p<0.01$). This suggests that adult bones experience relatively more prevalent torsion compared to sub-adults. Moderate-to-high positive correlations were shown between LI and predominant CFO (sub-adults: $r = 0.735$; adults: $r = 0.866$; $p<0.001$). These data support the hypothesis that a causal relationship may exist between these variables. Furthermore, these data indicate that primary bone can exhibit site-specific material adaptations independent of secondary osteon formation. These results corroborate the concept that the sub-adult turkey ulna is subject to relatively more prevalent bending (relatively lower mean LI) compared to the torsionally loaded adult ulna (relatively higher mean LI). If predominant CFO and LI are sufficiently sensitive and specific parameters for inferring a bone's loading history, then these characteristics may be a reliable way of discerning regional strain environments when the application of strain gauges is difficult or impossible. We speculate that 'non-traditional' parameters such as energy absorption and fatigue resistance may be the mechanical enhancement mediated by these important material characteristics. Regional heterogeneities of primary bone organization may also be an important issue for investigating bone fluid-flow dynamics.

Disclosures: ***J.G. Skedros****, None.*

M051

Collagen Status and Brittleness of Human Cortical Bone in the Elderly. T. M. Keaveny[1], G. E. Morris*[1], E. K. Wong*[1], M. Yu*[1], A. N. Sakee*[2], N. Verzijl*[2], R. A. Bank*[2]. [1]Orthopaedic Biomechanics Laboratory, Dept. of Mechanical Engineering, UC Berkeley, Berkeley, CA, USA, [2]TNO Prevention and Health, Gaubius Laboratory, Leiden, Netherlands.

It is widely believed that factors other than bone density play a role in bone strength, and ultimately in fracture risk assessment for osteoporosis. Highly brittle bone is more susceptible to fracture during impact events such as a fall. The goal of this study was to determine if collagen status affects cortical bone brittleness among the elderly since this may provide the basis for development of new strategies for bone quality assessment. Using 88 cadaver bone specimens obtained from 42 elderly cadavers (23 males age=73.6±10.7 years, 19 females age=75.7±11.4 years), we measured the correlation between the tensile energy to failure of machined specimens of human cortical bone and a variety of parameters of collagen biochemical status as measured by HPLC. Low values of energy to failure indicate that the bone is relatively brittle. Results indicated that energy to failure was positively correlated ($p<0.05$ in all cases) with percent collagen content ($r=0.26$), Hyl ($r=0.25$), LP ($r=0.21$), and HP ($r=0.30$), and was negatively correlated with pentosidine ($r=-0.22$). As expected, energy to failure was also negatively correlated with cortical bone porosity ($r=-0.47$). These findings indicate that the status of the collagen plays a small but significant role in brittleness of elderly human cortical bone. Specifically, elderly individuals having high levels of non enzymatic crosslinks and low levels of enzymatic crosslinks and collagen content tend to have more brittle bone, and thus may be at elevated risk of fracture in the event of a fall.

Disclosures: ***T.M. Keaveny****, None.*

M052

Age-dependent Effect of Hrk Deficiency on Bone Quality in Mice. L. M. Wise*[1], A. Jurisicova*[1], A. Benito*[2], G. Nunez*[2], M. D. Grynpas[1]. [1]Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada, [2]Department of Pathology & Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI, USA.

Hrk and Bax are pro-apoptotic members of the Bcl-2 gene family. Aged Bax-deficient mice express a phenotype in both ovarian cell function and bone mineral density. Hrk works upstream from Bax in some cell types and is dependent on the presence of Bax to activate cell death. Given that Hrk has been implicated in regulation of oocyte survival, this gene may have a similar effect as Bax on bone quality. Accordingly, Hrk-deficient (knockout, KO) female mice and wild-type (WT) littermates were generated and euthanized after 3 months, 6 months and 16 months of age. Dual energy x-ray absorptiometry (DEXA) using a PIXImus™ mouse densitometer was performed on all mice to determine bone mineral content (BMC), bone mineral density (BMD), and overall tissue lean and fat mass. To evaluate mechanical properties of cortical bone and trabecular bone, respectively, 3-point bending was performed on the femurs and unilateral compression was performed on the 5th lumbar vertebrae. The results were normalized to determine bone material properties, such as elastic modulus, yielding behavior, maximum strength, and toughness. Bone architecture will be evaluated using micro Computed Tomography (μCT) and histomorphometry, while mineralization will be analysed using backscattered electron (BSE) imaging, microhardness testing, chemistry and x-ray diffraction. In order to evaluate the connection between ovarian cell function and bone quality, follicle numbers and hormone levels will be correlated with these results.

At 3 months of age, the KO mice were found to have significantly ($p<0.04$) higher BMD (whole mouse, excised femur, and excised spine) when compared to age-matched WT females. The difference in BMD between the KO and WT mice was reduced with increasing age. Mechanical testing on the 3-month old femurs resulted in a higher modulus in the WT mice compared to the KO mice, suggesting that the more highly dense bone (KO) does not necessarily have superior mechanical stiffness. Moreover, the 6-month old KO femurs yielded earlier and at a reduced stress compared to the WT mice. By contrast, compression testing on the 3-month old KO vertebrae resulted in delayed yield behavior and a higher yield stress compared to the WT mice. As well, 6-month old KO vertebrae failed at a higher overall stress than the WT. These findings indicate that Hrk-deficiency may have different effects on cortical and trabecular bone. Furthermore, differences in BMD and mechanical properties between the KO and WT mice seem to diminish with age.

Disclosures: ***L.M. Wise****, None.*

M053

The Effects of Myostatin Deficiency on Mouse Sagittal Suture Morphology and Mechanics. C. D. Byron*[1], J. Borke*[2], J. Yu*[3], D. Pashley*[2], N. Do*[2], M. W. Hamrick[1]. [1]Cell Biology and Anatomy, Medical College of Georgia, Augusta, GA, USA, [2]Oral Biology, Medical College of Georgia, Augusta, GA, USA, [3]Surgery, Medical College of Georgia, Augusta, GA, USA.

The purpose of this study is to test the hypothesis that increased muscle mass will increase sutural complexity and increase sutural tensile strength. Myostatin (GDF8) is known to act as a negative regulator for skeletal muscle cell development. Therefore, myostatin knockout mice show consistent, systemic doubling of skeletal muscle mass. Differences in suture morphology between knockout and wild type groups were measured using fractal dimension (FD) analysis software (Benoit 1.3). FD was used because it is a measure of a line's self-similarity and thus complexity (sutures with greater complexity have greater fractal dimensions). The mechanical properties of sagittal sutures were also compared using a Vitrodyne V1000 Universal Tester. Load and displacement were recorded for strips cut from sagittal sutures pulled at 10 microns per second. Differences between means were calculated using Student's t-test. Masseter muscle mass was significantly enlarged in the GDF8 knockout group suggesting an overall increase in cranial musculature ($P<0.001$). The FD analysis revealed a larger value for the GDF8 knockouts meaning they have a more complex and interdigitating sagittal suture ($P=0.026$). Mechanical properties analysis revealed high variance in ultimate tensile strength among individuals within each group. In addition, the stiffness of sagittal sutures was significantly different with the GDF8 deficient mice having a lower stiffness value ($P=0.016$). The larger FD value may relate to local tissue adaptation within the sagittal suture in response to greater tensional forces generated by a larger temporalis. This hypothesis suggests a complex and interdigitating pattern better withstands tensional forces. The sagittal suture mechanical properties do not support this. In fact, stiffness was actually decreased with larger muscle mass. This difference may relate to the arrangement of bridging fibers in a convoluted suture requiring more displacement before becoming loaded whereas in a straighter suture environment, bridging fibers are initially oriented relatively perpendicular to the suture. Thus, an equal load generates a smaller displacement. Increased muscle mass appears to directly affect FD and indirectly affect sutural mechanics by way of sutural complexity. Further tests to investigate biochemical differences between sutures are currently under investigation.

Disclosures: ***C.D. Byron****, None.*

M054

A Dominant-Negative Mutant of the E3 Ubiquitin Ligase, FWD1 Markedly Reduces Myeloma Burden in Bone in a Murine Model of Multiple Myeloma. B. Oyajobi[1], P. Williams*[1], A. Gupta*[1], S. Munoz*[1], B. Grubbs*[1], M. Zhao[1], D. Chen[1], K. Nakayama*[2], G. Mundy[1]. [1]Cellular & Structural Biology, Univ of Texas Hlth Sci Ctr, San Antonio, TX, USA, [2]Molecular & Cellular Biology, Kyushu University, Fukuoka, Japan.

Recent clinical studies with the proteasome inhibitor Bortezomib show that the ubiquitin/proteasome (Ub-prot) pathway controls behavior of multiple myeloma (MM) cells in patients. Independently, it has been shown that inhibition of this pathway also stimulates osteoblast differentiation. Since myeloma bone disease is characterized by a marked decrease in osteoblast activity which accompanies the increased osteoclast activity, we are exploring the potential for the regulation of Ub-prot pathway to beneficially influence MM bone disease. Here, we focused on the role of NF-κB and examined the effect of direct blockade of NF-κB on MM bone disease in vivo. Murine myeloma 5TGM1 cells inoculated into C57BL/KaLwRij mice via tail veins reproducibly home to medullary cavities and spleens resulting in elevated serum titers of the paraprotein, splenomegaly, and lytic lesions in bones. As NF-κB transcriptional activity is dependent on proteasomal function and specifically the E3 Ub ligase β-TrCP, 5TGM1 cells were transduced with plasmid encoding a dominant-negative (ΔF) mutant of FWD1, the murine homolog of β-TrCP or empty vector and stable G418-resistant clones generated (ΔF and EV cells respectively) which were then inoculated in mice. Five weeks post tumor inoculation, serum IgG2bκ titer, a marker of overall myeloma burden, was dramatically reduced in ΔF-bearing mice compared to EV-bearing mice. In addition, splenic wet weights in ΔF-bearing mice were significantly reduced compared to EV-bearing mice. Consistent with these findings, there was a significant reduction in radiographically-detectable lytic lesions in ΔF mice compared to EV mice. This was confirmed by histomorphometric analysis of long bones and spines of ΔF mice which revealed markedly reduced tumor volumes compared to EV mice. In addition, there was significantly reduced myeloma infiltration in spleens of ΔF mice compared to EV mice. To further elucidate mechanism(s) mediating this anti-myeloma effect, we assessed levels of NF-κB1 (p50) and NF-κB2 (p52) and their precursor proteins in ΔF and EV cells by western blotting using antibodies raised to the N-termini. p100, but not p105, was elevated in ΔF cells compared to EV cells. Together, these data strongly suggest that MM growth and behaviour in bone is dependent on NF-κB activity, and in particular the p100/p52 pathway. It is likely that Bortezomib and other proteasome inhibitors exert their potent anti-myeloma effects by inhibiting the Ub-prot pathway through this mechanism.

Disclosures: ***B. Oyajobi****, None.*

M055

Therapy of Renal Cell Carcinoma in Bone Using Zometa and the EGF-R Inhibitor, PKI166. K. Weber[1], M. Doucet*[2], J. Price*[2]. [1]Surgical Oncology and Cancer Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA, [2]Cancer Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA.

The purpose of our study was to determine the effect of Zometa, a potent bisphosphonate, alone and in combination with PKI166, an EGF-R tyrosine kinase inhibitor, in a nude mouse model of renal cell carcinoma (RCC) bone metastasis.

Proliferation studies, Western blots, and intratibial injection experiments using a nude mouse experimental model were utilized. 4 x 10^5 RBM1-IT4 cells, derived from a human RCC bone metastasis, were directly injected into the tibia of 50 nude mice that were randomized into 5 groups. Treatment consisted of PKI166 (100 mg/kg p.o. 3x/wk), Zometa (25 ug/kg SQ BID 5x/wk), PKI166 + Zometa, or PKI166, Zometa, and Taxol (200 ug/mouse i.p. weekly). Control mice received saline. Radiographic, histologic and immunohistochemical methods were used to analyze the results. A dose-response experiment was also conducted *in vivo* using Zometa .

RBM1-IT4 cells express high levels of EGF-R, and cell proliferation is stimulated 2.0-2.3-fold with addition of TGF-alpha or EGF. PKI166, which inhibits EGF-R signaling, significantly inhibited cell proliferation ($p<0.05$) and receptor autophosphorylation (87% decrease). By western blotting, RBM1-IT4 cells express RANKL, necessary for the stimulation of osteoclasts. The tumor cells also express IL-6, PTHrP, TGF-β, MMP-2, and MMP-9 which contribute to the bone destruction noted in osteolytic bone metastasis. A marked decrease in radiographic bone destruction was noted in all treatment groups, most prominent in the mice treated with Zometa ($p<0.05$). The tumor incidence was 90% in the control group and significantly decreased in the group treated with PKI166 and Zometa ($p<0.05$). A decrease in tumor weight was found in the 3 groups treated with PKI166 ($p<0.05$). Zometa alone had no effect on tumor weight *in vivo* or on tumor cell proliferation *in vitro*. Immunohistochemical analysis revealed a marked decrease in activated EGF-R, VEGF, and PCNA in tumors treated with PKI166. Factors in the EGF-R signaling cascade were not affected by Zometa, but the number of activated osteoclasts was decreased as documented by TRAP staining ($p<0.05$). In addition, growth plates in the mice treated with Zometa were widened and showed histologic evidence of decreased remodeling. The dose-response experiment identified these changes in the bone when using 5 ug/kg SQ 5 days/week.

These data show that inhibition of osteoclast-mediated bone resorption by Zometa allows maintenance of bone structure in a RCC bone metastasis model. It is most effective when combined with EGF-R blockade to decrease tumor growth.

Disclosures: ***K. Weber****, None.*

M056

Radio-sensitivity and the Effects of the Radioprotectant Amifostine on Ewing's Sarcoma and Rhabdomyosarcoma Relative to Human Endothelial Cells. B. S. Margulies*, M. J. Allen*, T. A. Damron*. Orthopedic Surgery, S.U.N.Y. Upstate Medical University, Syracuse, NY, USA.

Introduction: Radiation therapy is a well-established adjunct to surgical excision for soft tissue sarcomas and an alternative to surgery for Ewing's sarcoma of bone in pediatric patients. Radiation negatively impacts the growth plate in a growing child, leading to long-term complications. Radioprotectant drugs, such as the free radical scavenger amifostine (WR-2721), that protect from negative irradiation effects may be of value in these pediatric patients. In order to be clinically acceptable radioprotectants must be selective in their protection of normal tissues at the expense of the irradiated tumor. This selectivity has not been demonstrated for musculoskeletal sarcomas. The specific aim of this study was to use a cell culture model to compare the individual effects of radiation and amifostine on two pediatric sarcoma cell lines versus human endothelial cells. **Methods:** The TC71 Ewing's sarcoma, the RD rhabdomyosarcoma, and the HUVEC endothelial cell-lines were maintained under standard conditions. Amifostine (0-10 mM) was administered to 24-well plates containing $1x10^4$ cells/well. For the radiation experiments, $5x10^4$ cells/well were exposed to 0, 1, 2, 5, 10, and 20 Gy x-irradiation. The MTT assay was used to quantify the effects of treatment on cellular proliferation. The lactate dehydrogenase assay was used to quantify cytotoxicity. **Results:** Clinically relevant doses of amifostine (1-2 mM) caused a profound (>80%) decrease in viable cell numbers in both tumor cell lines ($p<0.001$ *vs*. controls for all doses). This decrease in viable cell numbers was associated with statistically significant increases in LDH activity (indicative of overt cytotoxicity) in both the TC71 and RD cell lines ($p<0.0.001$ for all doses). HUVEC viability was decreased to a lesser extent for all doses. Data from the irradiation experiments confirmed the extreme radiosensitivity of HUVEC cells (75% inhibition at doses of 2 Gy or greater; $p<0.001$). The two human sarcoma cell lines displayed intermediate sensitivity, with approximately 50-60% inhibition at all doses ($p<0.001$). Cytotoxicity appeared to be the predominant mechanism by which radiation modulated cell survival (data not shown). **Discussion:** Two key findings have been identified. First, amifostine has differential effects on tumor cells versus endothelial cells. Amifostine has a clear and consistent toxic effect for both TC71 and RD cells, in contrast to the low cytotoxicity observed in endothelial cells. The second important finding is confirmation of the radio-sensitivity of two pediatric tumor cell lines that are generally considered to respond well to radiation therapy.

Disclosures: ***B.S. Margulies****, None.*

M057

Effects of PTHrP from Human Mammary Cancer Cells on Human Osteoblasts in Co-Culture. J. J. B. Anderson, N. Doshi*, E. Klem*, C. Albright*. Nutrition, Univ. of North Carolina, Chapel Hill, NC, USA.

The stimulation of bone cells, especially osteoblasts, by secretory products of mammary tumor cells is considered to have an important role in mammary tumor metastasis to bone and subsequent bone degradation. We postulate that parathyroid hormone-related peptide (PTHrP), a product of human mammary cancer cells, acts directly on osteoblasts (OBs) to increase osteoclastic activity, including recruitment from precursor cells, differentiation of theses cells, and bone resorption. In our two-cell co-culture system, human fetal osteoblast-like cells (hFOB) are grown in a chamber separated from human mammary cancer cells (MCF-7), so that the cells are not in physical contact. In addition, non-tumorigenic human mammary epithelial cells (MCF-10A) were co-cultured with hFOB cells; in contrast to co-cultures of hFOB cells and mammary cancer cells, no changes were observed. We used an unbiased image analysis method and a monoclonal antibody to localize PTHrP protein in cultured human bone and mammary cells. Compared to normal MCF-10A human mammary epithelial cells and hFOB human osteoblast cells, MCF-7 human mammary tumor cells contained higher levels of PTHrP in their cytoplasm. When MCF-7 and hFOB cells were placed in co-culture, higher levels of PTHrP protein were found in hFOB cells compared to the same cells cultured in the absence of MCF-7 tumor cells. Also, these co-culture experiments using MCF-7 mammary tumor cells, but not MCF-10A normal mammary cells, resulted in decreased incorporation of bromodeoxyuridine (BrdU) by hFOB cells (6% vs. 10%). Using human mammary cancer cells suggest that they produce PTHrP, and possibly other products, that stimulate OB cells to produce cytokines that increase the full potential of osteoclasts to degrade bone. (Supported in part by a grant from the UNC Research Council to N. Doshi, and by a grant from the Association for International Cancer Research to C. Albright)

Disclosures: ***J.J.B. Anderson****, None.*

M058

Myeloma Cells Suppress BMP-2-mediated Osteoblast Differentiation: Impaired Bone Formation in Multiple Myeloma. T. Oshima*, M. Abe, T. Hara*, K. Kitazoe*, E. Sekimoto*, Y. Tanaka*, H. Shibata*, T. Hashimoto*, S. Ozaki*, S. Kido, D. Inoue, T. Matsumoto. Department of Medicine and Bioregulatory Sciences, University of Tokushima Graduate School of Medicine, Tokushima, Japan.

Multiple myeloma (MM) develops and expands in the bone marrow, and generates devastating bone destruction. Besides enhanced osteoclastic bone resorption, clinical evidence has also suggested suppression of bone formation as a contributing factor to the bone loss in MM. Consistently, we have demonstrated that serum levels of a bone formation marker osteocalcin were relatively suppressed compared to apparently increased levels of a bone resorption marker urinary deoxypyridinoline excretion in MM patients. In the present study, we therefore tested a hypothesis that MM cells produce a soluble inhibitor of osteoblast differentiation and function. Murine osteoblastic MC-3T3-E1 cells were cultured in medium containing beta-glycerophosphate and ascorbic acid. Alkaline phosphatase (ALP) activity in the cells was increased 5-fold at day 10 in the presence of BMP-2 at 50 ng/ml. Consistent with our hypothesis, we found that addition of 10% conditioned media from MM cell lines, ARH77, U266, and RPMI8226 suppressed approximately 90, 70, and 60% of ALP activities enhanced by BMP-2, respectively. These MM cell conditioned media also delayed osteocalcin mRNA induction, and almost completely suppressed mineralized nodule formation in MC-3T3-E1 cells stimulated by BMP-2. Although primary MM cells as well as ARH77 cells have been reported to secrete IGF binding protein-4, an inhibitor of IGF-I, a an excess amount of IGF-I did not rescue osteoblast differentiation suppressed by ARH77 conditioned media, suggesting that MM cells release potent factor(s) other than IGF binding protein-4 to suppress osteoblast differentiation. None of MM-derived bone resorptive factors which we tested so far including MIP-1alpha, MIP-1beta, IL-1beta and IL-6 were able to mimic such inhibitory effects. Interestingly, we found that ARH77 conditioned media suppressed Smad1 phosphorylation in MC-3T3-E1 cells induced by BMP-2. These results suggest that MM cell-derived unknown soluble factor(s) suppress osteoblast differentiation, through blockade of BMP-2 action. In conclusion, cellular interplays between MM cells and bone cells result in defective bone formation as well as efficient activation of osteoclastic bone resorption, leading to an uncoupling state of bone turnover which ultimately causes rapid loss and destruction of bone.

Disclosures: ***T. Oshima****, None.*

M059

The Extracellular Matrix Differentially Regulates the Expression of the PTH/PTHrP Receptor in Pancreatic Cancer. J. J. Grzesiak*[1], C. Chalberg*[2], K. Smith*[2], D. W. Burton[2], S. A. Silletti*[3], A. R. Moossa*[1], L. J. Deftos[2], M. Bouvet[1]. [1]Department of Surgery, Veteran's Affairs Medical Center, San Diego, San Diego, CA, USA, [2]Department of Medicine, Veteran's Affairs Medical Center, San Diego, San Diego, CA, USA, [3]Cancer Center, University of California, San Diego, San Diego, CA, USA.

Previous studies by our laboratory have demonstrated that parathyroid hormone-related protein (PTHrP) and its receptor are commonly expressed in pancreatic cancer and indicate a role for PTHrP in the regulation of this devastating disease. It has also been demonstrated recently that pancreatic adenocarcinoma is associated with increased production of the extracellular matrix (ECM), an important regulator of diverse cellular processes such as differentiation, proliferation and angiogenesis. The present study focused on the interactions among PTHrP, the PTH/PTHrP receptor and the ECM in the pathobiology of pancre-

atic cancer. Using the fast growing (FG) variant of the COLO 357 metastatic pancreactic adenocarcinoma cell line along with RT-PCR and radioimmunoassay methodologies, we demonstrate that FG cells express PTHrP and the PTH/PTHrP receptor when cultured on tissue culture plastic. With radioimmunoassays using polyclonal antibodies raised against the 1-34, 38-64 and 109-141 amino acid peptides of PTHrP, we found that FG cells secrete 9.36, 0.80 and 1.46 pg/μg cellular protein respectively, of PTHrP after 96 hours in culture; the intracellular PTHrP expression levels were 1.4, 0.11 and 0.57 pg/μg cellular protein, respectively. RT-PCR with primers specific for the PTH/PTHrP receptor indicate that FG cells express the receptor, albeit at very low levels. In cell culture experiments using FG cells on either type I collagen or fibronectin, however, we found that type I collagen increased the expression of the PTHrP receptor about 6-fold compared to fibronectin or tissue culture plastic. These observations suggest unique functional interactions among ECM proteins, PTHrP and its receptor and indicate important implications for our understanding of the complex mechanisms responsible for the progression of pancreatic cancer and its metastases. Further studies will be necessary to fully understand the relationship between the ECM and the PTHrP axis in pancreatic cancer.

Disclosures: ***J.J. Grzesiak****, None.*

M060

Expression of Alternatively Spliced Runx2 in Multiple Myeloma Patients and a Shift in Expression in Response to Thalidomide Treatment. N. Bose*[1], H. Bergemann*[1], J. J. Westendorf[2], S. V. Rajkumar*[3], A. M. Masellis[1]. [1]Leukemia Research, Virginia Piper Cancer Institute, Minneapolis, MN, USA, [2]Orthopaedic Surgery and Cancer Center, University of Minnesota, Minneapolis, MN, USA, [3]Mayo Clinic, Minneapolis, MN, USA.

In individuals with the hematopoietic malignancy multiple myeloma, there is evidence that bone formation rates are reduced and that increased osteoclastic bone resorption is associated with impaired osteoblast function. Our previous studies indicate that mesenchymal progenitor cells (MPCs) from newly diagnosed previously untreated myeloma patients express Runx2, but that the DNA binding ability of Runx2 is severely reduced compared to age-matched healthy donors. MPC cultures established from these same patients show decreased differentiation to the osteogenic lineage (*in vitro)* as determined by mineralization, bone alkaline phosphatase levels and expression of bone specific markers (osteonectin, osteopontin, ALP). Currently thalidomide and thalidomide analogues (IMiDs) are promising novel active therapeutic agents for myeloma patients, targeting the tumor cells and the bone marrow environment. The mechanism of action for thalidomide and IMiDs are unknown, although recent studies suggest they may regulate activity of transcription factors. Based on these observations, we examined the expression (by DNA sequencing) and function (by DNA binding) of Runx2 in myeloma MPCs treated *in vivo* or *in vitro* with thalidomide. Runx2 in myeloma patients did not contain any sequence mutations in the DNA-binding region, however a variant lacking 60 residues of exon 8 was found in 5 of 8 myeloma patients. The variant was present in only 2 of 5 healthy age-matched donors. Upon *in vivo* treatment of two patients with thalidomide, this Runx2 isoform was no longer detectable. We examined the DNA-binding activity of Runx2 upon *in vivo* treatment of these two patients with thalidomide. Although MPCs derived from previously treated myeloma patients had reduced Runx2 DNA-binding activity, MPCs derived from myeloma patients treated with thalidomide recovered Runx2 DNA binding activity. A correlation between reduced expression of the alternatively spliced Runx2 isoform and the enhancement in Runx2 DNA binding activity upon thalidomide treatment is being examined. Clinically, of 12 newly diagnosed myeloma patients treated with thalidomide (200 mg/day dose) and/or dexamethasone, 4 patients showed an increase in serum alkaline phosphatase (ALP) levels compared to levels prior to treatment. The increase in ALP indicates possible new bone formation, a process that is dependent upon Runx2 activity. Put together, our *in vitro* and *in vivo* data suggest that thalidomide may enhance Runx2 activity.

Disclosures: ***N. Bose****, None.*

M061

Preliminary Characterization of Serum Biomarkers for Multiple Myeloma with Bone Metastasis Using SELDI-TOF Mass Spectrometry. S. Bhattacharyya[1], J. Epstein[2], L. J. Suva[1]. [1]Orthopaedic Surgery, UAMS, Little Rock, AR, USA, [2]Myeloma Institute, UAMS, Little Rock, AR, USA.

The cause of most human disease lies in the functional dysregulation of protein interactions. These protein biomarkers, expressed as single or multiple markers in body fluids, with patterns of up and down regulation, have been shown to be indicators of disease susceptibility, onset, progression, drug response, toxicity etc. In this study the unique biomarker pattern from human serum samples for the early detection of Multiple Myeloma (MM) metastasis to bone was examined. MM is a severely debilitating neoplastic disease of B cell origin with the primary source of morbidity and mortality being bone metastasis. We have used surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF) to screen for potential biomarkers. Serum samples were collected from 48 MM patients, 24 with more than three bone lesions and 24 with no evidence of bone lesions. Serum samples were fractionated into 6 different fractions based on the charge of the serum proteins. Small aliquots from each fraction (15 ul) were applied in duplicate to IMAC3 ProteinChip arrays and the bound proteins detected using the ProteinChip Reader (Ciphergen PBS IIC) after laser desorption/ionization. The resulting spectra were compiled, normalized to total ion current and mass peaks with mass-to-charge ratios (*m/z*) between 2000 and 20000 were identified using ProteinChip Reader Software (Ciphergen Biosystems). Peaks with a signal-to-noise ratio >2 that were present in at least 10% of the spectra were identified from each fraction. No single peak was identified that could precisely distinguish between the two sample groups. Peak information from each fraction was separately analyzed using Biopattern software (Ciphergen); a statistical tool for the classification of SELDI generated datasets. An optimal decision-tree model from all the fractions was combined to generate a final decision-tree model that following blinded cross-validation on the same dataset produced a prediction sensitivity (accuracy of assigning the disease class) of 80% and a prediction specificity (accuracy of assigning the controls) of 82.5%. Additional studies with expanded sample sets are currently ongoing. These data suggest that SELDI profiling may provide critical insights into the rapid diagnosis of the skeletal involvement of MM and other skeletal diseases.

Disclosures: ***S. Bhattacharyya****, None.*

M062

Endothelin A Receptor Blockade Alters Bone Remodeling: Gender-Specific and Sex Steroid-Dependent Changes. K. S. Mohammad, T. A. Guise. Internal Medicine, University of Virginia, Charlottesville, VA, USA.

Osteoblastic metastases commonly affect patients with breast and prostate cancer and are incurable. In both clinical trials and preclinical animal models, treatment with a selective endothelin A receptor (ETAR) antagonist inhibits the progression of bone metastases. However, the effects of such antagonists on normal bone remodeling are unknown. Since many cancer patients are hypogonadal consequent to chemotherapy or adjuvant therapy, we evaluated bone remodeling during chronic ETAR blockade in both eugonadal and hypogonadal male and female mice. Orchiectomy, ovariectomy, or sham-surgery was performed on 4-week old C57BL6 mice, and ETAR blockade (ABT-627) or vehicle was started. Bone mineral density (BMD) was measured (at total body, femur, tibia and spine) over 36 weeks of treatment, and bone histomorphometry was performed.

In male mice, hypogonadism reduced BMD and increased histomorphometric indices of bone turnover. ABT-627 treatment increased BMD in hypogonadal males and marginally reduced BMD in intact mice. Histomorphometry of hypogonadal male bones showed that ABT-627 increased trabecular bone volume (TBV), number of osteoblasts (N.Ob), number of osteocytes (N.Ot) and mineral apposition rate (MAR) but had no effect on bone formation rate (BFR) or number or osteoclasts (N.Oc). In intact males, ABT-627 had no effect on TBV, N.Ob, N.Ot, N.Oc or BFR but caused a slight increase in MAR.

In female mice, hypogonadism caused marginal bone loss as measured by BMD and histomorphometry. However, ABT-627 treatment significantly reduced BMD in hypogonadal female mice but significantly increased it in intact mice. Hypogonadal female mice treated with ABT-627 had significantly reduced TBV, increased BFR and no change in N.Ob, N.Ot or MAR. In contrast, ABT-627 increased TBV, N.Ob and N.Ot but had no effect on MAR or BFR. ABT-627 reduced N.Oc in both intact and hypogonadal females.

Our data indicate that ETAR blockade has gender-specific effects on bone remodeling, which depend on the presence or absence of sex steroids. In males, the effects are on the osteoblasts. In females, both osteoblasts and osteoclasts are involved. ET_A receptor blockade may benefit patients with osteoblastic bone metastases and should not result in bone loss when used for prevention in hypogonadal men. However, in hypogonadal females, ETAR blockade may cause accelerated bone loss. In sex steroid-sufficient females, it substantially increases bone mass and may represent a new anabolic treatment for osteoporosis in this patient group.

Disclosures: ***K.S. Mohammad****, None.*

M063

Osteoprotegerin Expression Correlates with ER/PR Status in Human Breast Tumors. C. H. Van Poznak[1], S. S. Cross*[2], C. Hudis*[1], K. S. Panageas*[3], R. E. Coleman*[4], I. Holen*[4]. [1]Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [2]Pathology, University of Sheffield, Sheffield, United Kingdom, [3]Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [4]Clinical Oncology, University of Sheffield, Sheffield, United Kingdom.

In addition to its role in bone turnover, osteoprotegerin (OPG) has been reported to bind to and inhibit Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL). TRAIL is produced in tumors by invading monocytes where it induces apoptosis in neoplastic cells that are sensitive to this cytokine. OPG production by tumor cells would therefore be a novel mechanism whereby cancer cells evade host defences and gain a growth advantage. Preclinical data suggests that breast and prostate tumors may use osteoprotegerin (OPG) signaling as a survival mechanism though inhibition of TRAIL-induced apoptosis. Our preclinical data suggested that hormone independent breast cancer cells (MDA-MB-436) release OPG while hormone sensitive breast cancer cells (MCF-7) do not. To test this finding in the clinical setting, we examined human breast cancer for evidence of OPG staining. Specimens analyzed included estrogen/progesterone receptor (ER/PR) positive/positive (+) and ER/PR negative/negative (-) primary breast cancer and non-malignant breast tissue. With 20 specimens per group, we can detect a 40% difference in the frequency of OPG positive patients in the ER/PR(+) and ER/PR(-) groups with 80% power and 5% significance level (two-sided test).

Anonymized paraffin embedded specimens from the Memorial Sloan-Kettering Department of Pathology's archived tissue bank were stained using an immunohistochemistry (IHC) mouse monoclonal antibody that recognizes human OPG (R&D Systems, UK). 45 breast cases [20 ER/PR (-) tumor, 20 ER/PR (+) tumor and 5 non-malignant] were analyzed. Scoring examined both the intensity of staining (negative, weakly positive, strongly positive) and the percentage area of the tumor that each pattern of staining occupied. The non-malignant breast tissues all scored zero (5/5); tumor specimen results are outlined in the table below.

	OPG Negative (%)	OPG Positive (%)	
ER / PR (-)	9 (45%)	11 (55%)	
ER / PR (+)	3 (15%)	17 (85%)	p=0.0384

Our results demonstrate that OPG is expressed in primary human breast cancer tissue. OPG

expression correlates with ER/PR status. Further studies of the relationship between OPG, TRAIL, ER/PR and clinical outcome are in progress.

Disclosures: ***C.H. Van Poznak****, None.*

M064

Identification of Plasmin and MMP Cleavage Sites that Result in Release of Bone Matrix-Bound TGFβ by Breast Cancer Cells. S. L. Dallas[1], L. F. Bonewald[1], C. Barley*[2], Q. Chen*[1], J. Guthrie*[3]. [1]Univ. Missouri, Kansas City, MO, USA, [2]Univ. Manchester, Manchester, United Kingdom, [3]Midwest Research Institute, Kansas City, MO, USA.

Transforming growth factor beta (TGFβ) in the bone microenvironment is an important regulator of parathyroid hormone related protein production by breast cancer cells, which in turn regulates breast cancer-associated bone destruction. We have previously shown that breast cancer cells can release TGFβ from bone extracellular matrix (ECM) by proteolytic cleavage of latent TGFß binding protein-1 (LTBP1), an ECM glycoprotein that binds TGFß and regulates its activity. Cleavage of LTBP1 is associated with the formation of osteolytic lesions by breast cancer cell lines and with high expression of matrix metalloproteinases (MMPs) 2 and 9 and plasminogen activators.

To identify serine and MMP cleavage sites in LTBP1, recombinant LTBP1 fragments were expressed and purified using a mammalian expression system. Recombinant proteins were digested with MMPs and plasmin and analyzed by peptide mass mapping using MALDI-TOF mass spectrometry. An N-terminal LTBP1 fragment (a.a. 21-487) was cleaved by MMP9, but this cleavage site is not yet identified. Fragment 413 to 545, termed the "hinge domain" due to predicted flexibility, was cleaved by plasmin and MMP2 but not MMP9. Cleavage in this region is predicted to release a C-terminal LTBP1 fragment that includes the site for covalent linkage with TGFβ. The MMP2 cleavage site was determined to be between amino acids 488-545. Fragment 526 to 1014, which consists of a central stretch of 11 tandem EGF-like repeats, was resistant to cleavage with plasmin, MMP2 and MMP9. The C-terminal fragment, (a.a. 1008 to 1394), was cleaved by plasmin but not MMP2 or 9. The major plasmin cleaved fragment was analyzed by peptide mass mapping, revealing that all predicted masses after residue 1285 were absent. Edman sequencing confirmed plasmin cleavage on the carboxy side of Arg1285. To further investigate the role of MMPs in release of LTBP1 from bone ECM, fluorescence *in situ* zymography techniques were developed. MDA-231 breast cancer cells were cultured on bone ECM in which LTBP1 was labeled using fluorescent antibodies. Using a fluorescent gelatin substrate, MMP activity was observed in cells that contacted with LTBP1-positve fibrils. This system is currently being used for time lapse fluorescence imaging studies to monitor the dynamics of turnover of bone ECM proteins by breast cancer cells.

These studies have identified cleavage sites in LTBP1 for plasmin and MMPs, which may result in release of latent TGFβ from bone ECM. Targeting of these cleavage sites may provide a novel therapeutic approach to regulate breast cancer-associated bone destruction.

Disclosures: ***S.L. Dallas****, None.*

M065

Characterization of LuCap 23.1 and PC-3 Prostate Cancer Intra-Tibial Developmental Metastasis Models. J. S. Hahn*[1], M. P. Roudier[1], J. E. Quinn*[1], S. M. Ott[2], R. L. Vessella*[1]. [1]Urology, University of Washington, Seattle, WA, USA, [2]Endocrinology and Metabolism, University of Washington, Seattle, WA, USA.

New well-characterized pre-clinical models of prostate cancer (CaP) bone metastases are needed to further understanding of the development of CaP-related bone disease in patients. We characterized quantitatively and qualitatively our osteolytic PC-3 and osteoblastic LuCaP 23.1 intra-tibial developmental metastasis models. Using Faxitron X-rays, we defined early, intermediate and late stages of disease progression in each model. We studied 6 early, 5 intermediate, and 3 late samples of PC-3 tumor development and 3 early, 4 intermediate, and 5 late samples of LuCaP 23.1 tumor development and their corresponding controls. The take rates of PC-3 and LuCap 23.1 cells after tibial injection were 100% and 94%, respectively. BMD by DEXA of osteolytic and osteoblastic model tibiae showed significant decrease (from 0.04 to 0.02 g/cm^2; p = 0.003) and increase (from 0.04 to 0.14 g/cm^2; p = 0.00002) with time compared to controls respectively. Bone volume of osteolytic and osteoblastic models showed significant decrease (from 24.6% to 4.0%; p = 0.023) and increase (from 39.6% to 51.2%; p = 0.00006) with time compared to controls respectively. Qualitative analysis of LuCap 23.1 samples showed that tumor-induced bone formation was observed arising from the cancer stroma more than along the native bone trabecular surface; that bone-forming cells were spindle cells more than well-differentiated osteoblasts; that these cells were producing woven osteoid that was subsequently mineralized to woven bone. An alkaline phosphatase activity was demonstrated in these spindle cells, suggesting that they were pre-osteoblasts. Moreover when compared to clinical samples, the LuCaP 23.1 model qualitatively mimics the clinical CaP osteoblastic bone metastasis. Interestingly, in PC-3 numerous pre-osteoblasts were also observed associated with osteoclasts at each stage of the disease progression. The CaP cells could produce factors that arrest the maturation of osteoblast progenitors into mature osteoblasts. TRAP osteoclasts/mm of bone surface significantly increased (from 2.9 to 4.3; p = 0.00006) in osteolytic model and decreased without significance (from 1.6 to 0.86; p = 0.32) in osteoblastic models at early, mid and late stages compared to controls. Eroded surface increased (from 6.5% to 10.8%, p = 0.00008) in PC-3 and decreased in LuCaP 23.1 (from 4.6% to 2.8% p = 0.0003). In conclusion, PC-3 and LuCap 23.1 intra-tibial developmental metastasis models are suitable models of osteolytic and osteoblastic CaP bone metastasis for study of interactions of tumor and bone cells and new therapeutic modalities.

Disclosures: ***J.S. Hahn****, None.*

M066

Prostate Cancer Osteoblastic Metastases Show a Continuum of Bone Volumes from Osteopenic to Osteodense. M. P. Roudier[1], J. H. Hahn*[1], R. L. Vessella*[1], S. M. Ott[2]. [1]Urology, University of Washington, Seattle, WA, USA, [2]Endocrinology and Metabolism, University of Washington, Seattle, WA, USA.

Development of prostate cancer (CaP) osteoblastic bone metastasis is still not well understood. In order to characterize osteoblastic CaP bone metastasis, we studied a set of 221 bone biopsies from 12 androgen-deprived autopsy patients. All patients had been treated with androgen ablation therapy and subsequent chemotherapy when androgen therapy failed. Twenty bone sites were systematically obtained from pre-specified anatomic sites in each patient. The median age at death was 65 years (range: 45 to 83 years). The median time of androgen ablation was 2.5 years (range from 1.5 to 11 years). At initial microscopic assessment of the 221 specimens, 130 biopsies were either showing necrosis of tumor and bone (n=93) or tumor-free (n=37) and were excluded from the study. In the 91 remaining metastatic samples, quantitative histomorphometric measurements were made. At lower magnification, 4 major bone patterns were observed throughout the biopsies: osteodense, osteopenic, normal with minor change, and no bone change in response to the tumor. Except in one patient where no bone change was observed, the type of bone lesion varied among sites in each patient and could not be predicted by anatomic site or duration of metastasis. Bone volume (BV/TV) ranged from 3.72% to 73.6%: 38 were osteopenic (<20%); 43 were osteodense (>30%) and 10 were normal (20-30%). When normal eroded surface was defined as less than 7%, biopsies in the osteodense group could be further classified as being in a quiescent state (n=24) or an osteoresorptive state (n=19). The volume of woven bone ranged from 0% to 70.3% (median 20%). In this study, we observed a continuum of BV/TV from osteopenic to osteodense in the osteoblastic CaP metastatic process. The osteopenia might be related to androgen ablation that initially and significantly reduced the amount of lamellar bone, prior to any bone metastasis. Since the vast majority of prostate cancer bone metastases result in production of woven bone, the BV/TV will increase when metastasis occurs. If it increases only slightly, then the lesion will still exhibit an osteopenic character, even if of osteoblastic nature. The BV/TV may increase to a level where it is approximately equivalent to that of normal bone, or to a higher level, where it is classified as osteodense. When osteodense, the BV/TV bone values are even more noteworthy given that the starting point was most likely below that of normal bone. In this study, the histomorphometric results of CaP bone metastasis suggest that the osteoblastic bone pattern is confounded by androgen ablation, which precedes sometimes by years, evidence of bone metastasis.

Disclosures: ***M.P. Roudier****, None.*

M067

Activation of Rac and p38 Is Not Related to Resistance of Tumour Cells to Bisphosphonate-Induced Apoptosis. J. E. Dunford[1], S. Gordon*[1], F. P. Coxon*[1], R. J. Phipps[2], M. J. Rogers[1]. [1]Medicine and Therapeutics, University of Aberdeen, Aberdeen, United Kingdom, [2]Procter & Gamble Pharmaceuticals, Cincinnati, OH, USA.

Like osteoclasts, J774 macrophages and JJN3 myeloma cells undergo apoptosis following treatment with nitrogen-containing bisphosphonates (N-BPs) such as risedronate (RIS) and zoledronic acid (ZOL), due to inhibition of FPP synthase and loss of prenylated small GTPases. However, some tumour cells such as PC3 prostate cells, MDA-MB-231 and MCF7 breast cancer cells and U266 myeloma cells are relatively resistant to N-BP-induced apoptosis. We have recently shown that apoptosis of J774 cells induced by N-BPs is associated with activation of Rac and its downstream effectors PAK and p38 MAPK in J774 macrophages. The increase in the level of active, GTP-Rac and subsequent PAK/p38 activation is due to the accumulation of the unprenylated form of Rac, detected using a pull-down assay utilising PAK-conjugated agarose beads. Since activation of a p38-dependent stress response may prevent apoptosis, we examined whether the resistance of some tumour cell types to N-BPs is related to Rac/p38 activation.

In J774 cells, the timecourse of p38 activation following treatment with 100uM RIS closely resembled the accumulation of unprenylated Rap1A GTPase and the activation of Rac i.e. sustained activation from 24 hours onwards. In PC3, MDA-MB-231, MCF7, U266 and JJN3 cells, an increase in unprenylated Rap1A and phospho-p38 was also detectable from approximately 24 hours onwards. Hence, resistance of these cells to N-BP-induced apoptosis is not due to lack of cellular uptake.

Inhibition of p38 with 20uM SB203580 caused a consistent increase in RIS-induced apoptosis of J774 cells (measured by nuclear degeneration and caspase-3/7 activity) compared to treatment with 100uM RIS alone, suggesting that activation of p38 has an anti-apoptotic effect in these cells. However, little or no caspase-3/7 activity was observed in PC3, MDA-MB-231, MCF7 and U266 cells following treatment with 100uM N-BPs for 24-120 hours, and this was not increased by the presence of SB203580. Inhibition of p38 also had no effect on inhibition of growth of PC3, MDA-MB-231, MCF7, JJN3 or U266 cells by ZOL. In contrast to J774 cells, SB203580 delayed the onset of N-BP-induced caspase-3/7 activity in JJN3 cells, suggesting that p38 activation promotes apoptosis of JJN-3 cells.

These studies demonstrate that inhibition of protein prenylation by N-BPs such as RIS and ZOL causes activation of Rac and p38 in a wide variety of cell types *in vitro*. Although p38 activation may influence the ability of N-BPs to cause apoptosis in some cell types, it does not appear to account for the resistance of certain tumour cells to N-BP-induced apoptosis.

Disclosures: ***M.J. Rogers****, Procter and Gamble 2; Novartis 2.*

M068

The Effects of a Soluble Receptor Activator of NF-κB:Fc Fusion Protein on Osteolytic and Osteoblastic Human Prostate Cancer Cell Metastases. P. G. Whang*[1], E. Schwarz*[2], W. Dougall*[3], S. Gamradt*[1], J. R. Lieberman[1]. [1]Orthopaedic Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA, [2]Orthopaedic Surgery, University of Rochester School of Medicine, Rochester, NY, USA, [3]Immunex Corp., Seattle, WA, USA.

In this study we evaluated the efficacy of a soluble receptor activator of NF-κB:Fc (RANK:Fc) fusion protein in limiting the growth of osteolytic and osteoblastic prostate cancer (CaP) bone metastases and examined whether osteoclast activity is necessary for the formation of osteoblastic lesions.

Human CaP cells that generate osteolytic (PC-3) or osteoblastic (LAPC-9) lesions were injected into the tibias of SCID mice. Animals implanted with either PC-3 or LAPC-9 cells were divided into the following groups: Treatment (4 weeks of treatment after tibial injection), Delayed Treatment (4 weeks of treatment starting 4 weeks after tibial injection), and Controls (4 weeks of saline injections). The experimental groups received RANK:Fc protein (15 mg/kg) subcutaneously twice a week. All animals were sacrificed 8 weeks after tibial injection, at which time hindlimb tumor size was measured and radiographic analysis was performed. Tissues were also prepared for histomorphometric analysis.

In this study, RANK:Fc protein blocked osteoclast activity; histomorphometric analysis demonstrated significantly fewer osteoclasts in the RANK:Fc-treated groups ($p<.001$). In the PC-3 groups, the mean hindlimb tumor size was significantly smaller in the Treatment group than the Controls and Delayed Treatment groups ($P<.001$). All of the animals in the Control group formed osteolytic lesions (7/7), whereas none of the Treatment animals formed osteolytic lesions (0/7, $P<.001$); in the Delayed Treatment group, 4/7 animals formed lesions. The area of bone present in the Treatment group was significantly greater than that of Controls ($P<.001$). In the LAPC-9 groups, the mean hindlimb tumor diameter in the Treatment group was significantly less than that of the Controls ($P<.001$). All of the animals in the Control and Delayed Treatment groups formed osteoblastic lesions (8/8), while 7/8 animals formed blastic lesions in the Treatment group; all of the groups also had similar areas of bone formation.

We found that RANK:Fc protein prevented the formation of osteolytic PC-3 bone lesions, indicating that osteoclasts are critical for the development of osteolytic CaP metastases. However, osteoblastic lesions were observed in all LAPC-9 groups, suggesting that osteoclasts are not required for the formation of osteoblastic CaP lesions but may be involved in enhancing the growth of established blastic lesions. Thus, we believe that RANK:Fc protein may represent a novel therapy for metastatic bone lesions.

Disclosures: ***P.G. Whang****, None.*

M069

Pharmacokinetics and Pharmacodynamics (PK/PD) of Intravenous Pamidronate in Patients with Breast Cancer and Bone Metastases. S. C. L. Cremers*[1], S. E. P. Papapoulos[2], H. Gelderblom*[3], C. Seynave*[4], J. den Hartigh*[1], H. Pols[5], P. Vermeij*[1], C. van der Rijt*[4], L. van Zuylen*[4]. [1]Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, Netherlands, [2]Endocrinology, Leiden University Medical Center, Leiden, Netherlands, [3]Clinical Oncology, Leiden University Medical Center, Leiden, Netherlands, [4]Medical Oncology, Erasmus MC - Daniel den Hoed Cancer Center, Rotterdam, Netherlands, [5]Endocrinology, Erasmus Medical Center, Rotterdam, Netherlands.

Introduction: Bisphosphonates (BPs) given i.v. every 3 to 4 weeks are effective in the management of metastatic bone disease from breast cancer but responses among patients vary and it is not known whether current dose and dose intervals are appropriate for an individual patient. An influence of PK of BP on its antiresorptive action may contribute to this variation. To test this hypothesis we determined the skeletal retention of intravenous pamidronate and its association to the rate of bone resorption in patients with bone metastases from breast cancer

Methods: In a cross-sectional study, 24 hr urinary excretion of pamidronate and NTx were measured in 28 patients with bone metastases from breast cancer at the beginning, after 3-6 months or after 1 year of treatment with iv pamidronate 90 mg every 3-4 weeks

Results: Skeletal retention (dose – amount excreted into urine) varied between 12-98% (mean 59%) of the administered dose but there were no differences in retention between patients receiving pamidronate for the first time or after one year of treatment. Retention of pamidronate was related to the prevalent rate of bone resorption in previously untreated patients whereas no such relationship was found in previously treated patients. The rate of bone resorption normalized in all patients, but mean NTx levels started to increase again before the next dose. Retention of pamidronate and the specific pattern of bone resorption were described adequately by a physiology-based 3-compartment PK/PD model, in which the rate of bone resorption depends on the amount of BP attached to bone. This model enables now the prediction of the rate of bone resorption induced by different treatment regimens.

Conclusions: During 1 year of treatment with i.v. pamidronate, skeletal retention of the BP does not change, indicating no saturation of skeletal binding sites with treatment. The variability in retention among patients can be attributed to the number of potential binding sites. This is related, however, to bone resorption only before start of pamidronate treatment. The PK/PD model described can help to optimize BP treatment of both a population and individual patients with bone metastases from breast cancer.

Disclosures: ***S.C.L. Cremers****, None.*

M070

Effect of Anastrozole on Bone Mineral Density: 2-year Results of the 'Arimidex' (anastrozole), Tamoxifen, Alone or in Combination (ATAC) Trial. R. Eastell*. Clinical Sciences Centre, University of Sheffield, Sheffield, United Kingdom.

The ATAC trial is a randomized, double-blind trial of anastrozole (1 mg/day, A), tamoxifen (20 mg/day, T) or the combination (C) in 9366 postmenopausal women with early breast cancer (adjuvant therapy). At the first analysis (median follow-up 33 months for disease-free survival) A was superior to T for all major efficacy endpoints (ATAC Trialists' Group. *Lancet* 2002, **359**: 2131-2139). Here we report the updated bone mineral density (BMD) results of the ATAC bone sub-protocol in a subset of 308 women from the ATAC trial and 46 unrandomized control patients with breast cancer receiving no hormone therapy. The control group was included for observational reasons. We previously reported the 1-year BMD data (as measured by DXA at the lumbar spine [LS] and total hip [TH]); A was associated with bone loss at the spine and hip and T with an increase in BMD, the differences between A and T being statistically significant. We now present the estimated % changes (95% confidence interval) from baseline in LS- and TH-BMD, following ANOVA on log-transformed data, after 2 years of therapy, alongside the results at 1 year.

	LS-BMD		TH-BMD	
	Year 1	Year 2	Year 1	Year 2
A	–2.6 (–3.3 to –1.8) [n=71]	–4.0 (–5.0 to –3.0) [n=58]	–1.7 (–2.3 to –1.0) [n=71]	–3.2 (-4.1 to -2.4) [n=58]
T	1.2 (0.4 to 2.0) [n=69]	1.9 (0.9 to 2.9) [n=64]	0.8 (0.1 to 1.6) [n=68]	1.2 (0.3 to 2.0) [n=63]
C	0.1 (–0.7 to 1.0) [n=64]	0.8 (–0.3 to 1.9) [n=51]	0.8 (0.0 to 1.5) [n=62]	1.1 (0.1 to 2.1) [n=48]
Controls	–0.2 (–1.3 to 1.0) [n=38]	0.3 (–1.1 to 1.7) [n=31]	0.0 (–0.9 to 0.9) [n=39]	-0.1 (–1.3 to 1.1) [n=32]

Therapy with A continues to be associated with bone loss while T is associated with an increase in BMD at 2 years. The rate of bone loss with A is approximately constant over both 1 and 2 years of therapy. Between year 1 and year 2 there were 13 fewer BMD assessments available for A and five fewer for T. There were various reasons for the fewer assessments, most of which were not related to bone effects, and only three patients on A and two on T were withdrawn for adverse events related to osteoporosis / osteopenia. When the BMD analyses were repeated with the exclusion of those patients where both year 1 and 2 data were not available, the overall results were similar. Further follow-up for BMD assessments will provide information on whether BMD loss shows stabilization over time; a 5-year assessment of BMD is planned. The impact of A on bone needs to be balanced against the overall efficacy and tolerability benefits observed in the main ATAC trial.

Disclosures: ***R. Eastell****, AstraZeneca Pharmaceuticals 2.*

M071

Bone Morphogenetic Proteins (BMPs) and Parathyroid Hormone-related Protein (PTHrP) Are Bone Microenvironment Factors that Modulate Prostate Cancer (CaP)-induced Bone Remodeling through Regulation of Vascular Endothelial Growth Factor (VEGF). J. Dai[1], J. Zhang[1], A. Koh*[2], L. McCauley[2], E. T. Keller[1]. [1]Ulam, University of Michigan, Ann Arbor, MI, USA, [2]Dept. of Periodontics, University of Michigan, Ann Arbor, MI, USA.

Advanced CaP induces a heterogeneous mixture of osteolytic and osteoblastic bone metastases. Many factors appear to be involved in CaP-induced bone remodeling, including BMPs and PTHrP. One factor that appears to be modulated by both BMP and PTHrP is VEGF which has recently been demonstrated to modulate bone remodeling. The goal of this study was to determine if prostate cancer-produced VEGF's modulate bone remodeling in CaP-induced skeletal lesions. We have previously shown that BMPs are expressed in CaP cells and promote VEGF expression. To determine if PTHrP modulates VEGF expression, we incubated MC3T3 pre-osteoblast cells with PTHrP. At 6 hours post-treatment, PTHrP induced a fivefold increase of VEGF levels. Thus, both BMPs and PTHrP can induce VEGF expression. To determine if VEGF contributes to BMP's ability to mediate prostate cancer-induced osteoblastogenesis, primary human osteoblast-like cells (HOB) were treated with C4-2B conditioned media (CM) plus noggin or neutralizing anti-VEGF antibody. Alkaline phosphatase (ALPase) activity and osteocalcin (OCN) level in the supernatants and in vitro mineralization ability was measured as indices of osteoblastogenesis. CM increased ALPase and OCN production and mineralization. Noggin decreased CM-induced ALPase levels, OCN levels, and mineralization by approximately 50-65%, 40-45% and 32-37%, respectively. Anti-VEGF antibody decreased CM-induced ALPase levels, OCN levels, and mineralization by approximately 27-32%, 26-39%, 22-25%, respectively. Combining noggin and anti-VEGF antibody had no greater effect than noggin alone. Finally, we overexpressed VEGF in C4-2B and treated cells with noggin. VEGF overexpression abrogated the noggin-induced reduction by approximately 40-50% for CM-induced ALPase levels, OCN levels and mineralization. This latter result indicates that VEGF induces osteoblast activity independent of noggin-sensitive BMPs. Taken together, these findings indicate CaP cells ability to express BMPs and PTHrP results in modulation of VEGF expression which, in turn, can promote osteoblastic activity. We conclude that in addition to its well recognized pro-angiogenic activity, VEGF may play an important rule in the development of CaP-induced osteoblastic metastases.

Disclosures: ***J. Dai****, None.*

M072

Estrogen Activates PTHrP Gene Expression in a Subset of Breast Cancer Cell Lines. J. Gordon*[1], H. Nakshatri*[2], Z. Bouizar[3], J. Foley[1]. [1]Medical Sciences, Indiana University School of Medicine, Bloomington, IN, USA, [2]Department Surgery, Indiana University School of Medicine, Inidianapolis, IN, USA, [3]INSERM, Paris, France.

Estrogen receptor (ER) positive primary breast cancers are more likely to metastasize to bone as compared to visceral sites. Activation of PTHrP gene expression appears to be crucial to the proliferation and survival of breast cancer cells that have metastasized to bone. It is unclear whether there is a relationship between estrogen signaling and PTHrP gene expression in breast cancer cells. MCF-7 cells are ER alpha positive and express low levels of PTHrP mRNA. As assayed by quantitative RT-PCR, 10nM 17 beta-estradiol (E2) treatment modestly increased PTHrP mRNA 1.6-fold at 2h and levels returned to baseline by 6h. MDA-MB-231 cells lack ER alpha and express high levels of PTHrP relative to other breast cancer cell lines. Using a novel MDA-MB-231 line, permanently transfected with the ER alpha, we found that 10nM E2 treatment increased the PTHrP transcripts 3-fold within 2h and the higher levels persisted for 24h. Employing a reporter construct containing all three PTHrP promoters in 4.3 kb of upstream sequence, we found that cotransfection with ER alpha in the presence of E2 increased luciferase activity 10-fold in breast cancer cell lines that express high levels of endogenous PTHrP. Activity from this construct was repressed by E2 in cells that expressed low levels of endogenous PTHrP. Other reporter constructs containing the PTHrP promoters in isolation or lacking sequences upstream of P1 were repressed or unaffected in these assays. Future experiments will determine which of the enogenous PTHrP promoters respond to E2, as well as identify ER alpha binding sites within the 5' region of the PTHrP gene and the factor/s in the high PTHrP-producing cell lines that cooperate with ER to activate gene expression. These findings suggest E2 signaling mediated by ER alpha has the potential to activate PTHrP gene expression in a subset of breast cancer cell lines.

Disclosures: ***J. Gordon****, None.*

M073

In vivo **Visualization and Quantitation of Metastatic Bone Disease Progression in SCID Mice.** L. M. Kalikin*[1], A. Schneider[2], M. A. Thakur*[3], L. B. Griffin*[4], A. Rehemtulla*[4], L. K. McCauley[2], K. J. Pienta*[1]. [1]Urology, The University of Michigan, Ann Arbor, MI, USA, [2]Periodontics, Prevention and Geriatrics, The University of Michigan, Ann Arbor, MI, USA, [3]Internal Medicine, The University of Michigan, Ann Arbor, MI, USA, [4]Radiation Oncology, The University of Michigan, Ann Arbor, MI, USA.

Metastatic bone disease develops when cells from primary tumors, especially prostate and breast, enter into circulation and colonize within the skeletal system. These bony tumors have a significant impact on patient quality of life as they generally cause significant pain, reduced function, and poor sleep. Some patients may also experience pathological fractures, hypercalcemia, and spinal cord compressions. As well, treatment side effects may include nausea, constipation, confusion, and fatigue. In 2003, 90% of the almost 29,000 men in the U. S. predicted to die from hormone refractory prostate cancer will also have disseminated bone disease. While disease-free survival periods have steadily increased, there is still no curative therapy for advanced prostate cancer and complications related to distant organ metastases. To gain insight into the pathogenesis of this disease, we developed a murine metastatic prostate cancer model utilizing the bioluminescence chemistry of the firefly (*Photuris pyralis*) luciferase enzyme. Luciferase-tagged PC-3, a human prostate cancer cell line derived from a bone metastasis, was inoculated intracardiac into male SCID mice, and anesthetized animals were imaged weekly in conjunction with intraperitoneal luciferin injections using a CCD camera. Animals were sacrificed after 7 weeks, and 80% presented with histologically confirmed tumors of the tibia, femur, and mandible, all of which colocalized with distinct biophoton imaging signals. Similarly, soft tissue tumors of the adrenals, lymph nodes, liver, and lungs were histologically verified at sites predicted by weekly imaging. Using imaging software, tumor growth rates were calculated by quantifying photon emissions within defined regions of interest, and similar kinetics were demonstrated between bone tumors. Thus, we have generated a non-invasive murine model that allows spatial-temporal visualization and quantitative monitoring of metastatic progression. As mice are reimaged weekly, fewer animals are required, and experimental variability is reduced compared to other models. This model is sensitive enough to detect bone micrometastases as early as one week after intracardiac injection and therefore will be a valuable tool to study the mechanisms of skeletal tumorigenesis and the effectiveness of pharmacological regimens against skeletal colonization by circulating prostate cancer cells.

Disclosures: ***L.M. Kalikin****, None.*

M074

Metastatic Breast Cancer Cells Reduce Osteoprotegerin Secretion by Human Osteoblastic Cells. P. Kapoor*[1], Z. Li*[1], Z. Zhou*[1], H. Donahue[1], D. Welch*[2]. [1]Orthopedics, Milton S Hershey Medical Center, Hershey, PA, USA, [2]Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Breast cancer metastasis, to a large extent, preferentially occurs to bone and leads to the formation of osteolytic lesions within the bone microenvironment. While the mechanism by which this occurs is unclear, it likely involves localized increases in osteoclastogenesis and osteoclast activity. Previous studies suggest that human breast cancer cell lines inhibit murine bone osteoprotegerin (OPG) expression and increase RANKL expression; thereby leading to increased osteoclastogenisis. Furthermore, in vivo studies suggest that increased breast cancer cell metastasis is correlated with increased osteolysis. In the current study we examined whether breast cancer cells can also inhibit OPG release from human osteoblastic cells. We also examined whether breast cancer cell metastatic potential was related to inhibition of osteoblastic OPG secretion. We utilized a highly metastatic and tumorigenic breast cancer cell line MDA-MB-435 (435), which does not secrete detectable levels of OPG, and 435 cells expressing the metastasis suppressor gene BRMS1 (4235/BRMS1), which are equally tumorigenic but much less metastatic than 435 cells. 435 and 435/BRMS1 cells were co-cultured for 3 days with the human fetal osteoblastic cell line hFOB1.19 (hFOB) at a ratio of 1:1. OPG released into the media was quantified by ELISA. Neither 435 nor 435/BRMS1 cells secreted detectable OPG, whereas hFOB cells secreted abundant OPG. However, OPG levels in hFOB cells cocultured with 435 or 435/BRMS1 cells were reduced by 2 fold and 1.9 fold, respectively (P<0.05 vs. hFOB in monoculture). 435 and 435/BRMS1 conditioned media reduced secretion of OPG levels by hFOB cells to similar degrees. These studies suggest that human breast cancer cells reduce human bone cell OPG levels, through release of a soluble factor, as suggested by studies with murine models. Interestingly, the expression of the metastasis suppressor gene, BRMS1, did not affect the ability of 435 cells to decrease hFOB OPG secretion. Thus BRMS1 mediates its metastasis suppressor effects at the bone level by a mechanism independent of osteoblast OPG regulation.

Disclosures: ***P. Kapoor****, None.*

M075

In-Vivo Cross-Calibration of a New Peripheral Densitometer: The Osteometer Dexacare G4. R. Patel*, G. M. Blake, I. Fogelman. Osteoporosis Research Unit, Guy's, King's & St. Thomas' School of Medicine, London, United Kingdom.

The purpose of this study was to compare forearm bone mineral measurements made with a new generation of peripheral DXA system, the Osteometer Dexacare G4, 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 101 subjects (mean age: 58 years, range 19 to 86 years) referred by their primary care physician for a densitometry investigation. Fifty three of these subjects (mean age: 56 years, range 25 to 81 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 between 0.93 and 0.95. The results also showed that for all variables, the intercepts were not statistically significantly different from zero ($p < 0.001$). Regression analysis was therefore repeated with the regression line forced through the origin for each variable. The precision errors for the Dexacare G4 were not statistically significant different from the DTX-200 for any of the sites. The RMSE errors from the regression analysis were consistent with the precision errors. Results of this preliminary comparison show that the Dexacare G4 system satisfactorily reproduced the performance of the earlier DTX-200 model.

Variable	Regression Analysis (n = 101)			CV% (n = 53)	
	Slope (SE)	r	RMSE	DTX-200	Dexacare G4
BMD (g/cm^2)	0.921 (0.005)	0.954	0.022	1.6%	1.5%
BMC (g)	0.904 (0.005)	0.946	0.155	1.6%	1.7%
Area (cm^2)	0.986 (0.003)	0.930	0.213	1.2%	1.2%

Disclosures: ***R. Patel****, None.*

M076

Does Femur Positioning Affect Femur Bone Mineral Density Measurement? N. Kaech*, D. Krueger, J. Harke, N. Binkley. Osteoporosis Clinical Center and Research Program, University of Wisconsin, Madison, WI, USA.

It is often recommended that the femoral shaft be parallel to scan direction when obtaining proximal femur bone mineral density (BMD) measurements using dual-energy x-ray absorptiometry. However, use of a positioner that allows measurement of both femurs (DualFemur GE-Lunar, Madison WI) has recently been suggested. In our experience, use of this positioner often results in abduction or adduction of the femoral shaft. Whether this impacts BMD measurement or diagnostic classification has received limited evaluation. We hypothesized that femoral abduction or adduction would alter BMD measurement and therefore diagnostic categorization.

To determine the effect of bilateral femur positioner use, proximal femur BMD measurements were obtained on 54 subjects (32 men/22 women). All scans were obtained using a GE Lunar Prodigy densitometer by one technologist. A spine and left hip measurement was obtained using a single femur positioner. Subsequently, a bilateral femur scan was performed without subject repositioning using a standard GE Lunar DualFemur positioner.

Study participant mean (SEM) age was 69.7 (1.4), range 50-86 years. Their weight was 170.3 (2.8) pounds. Based upon L1-L4 BMD and single left hip measurement, 54%, 37% and 9% were normal, osteopenic and osteoporotic respectively. Left proximal femur BMD measurements were extremely highly correlated ($r > .995$) between the single and bilateral positioner. Similarly, mean BMD of the total femur, femoral neck or trochanter did not differ between positioners. Finally, diagnostic categorization using the WHO criteria was rarely affected by use of the bilateral positioner. Specifically, individual site diagnostic categorization changed in 2, 1 and 4 individuals at the total femur, neck and trochanteric regions. However, the overall diagnostic categorization of only one individual was changed (from osteopenia to normal) by use of the bilateral positioner.

In conclusion, though use of the bilateral positioner may lead to abduction or adduction of the femoral shaft, BMD measurement and diagnostic categorization are unaffected. Use of this bilateral femur positioning device does not compromise BMD diagnostic accuracy.

Disclosures: ***N. Kaech****, None.*

M077

Bone Mineral Density and Body Composition of Osteoporotic SAMP6 and SAMR1 Mice. I. Schoenmakers*[1], R. Bakker*[2], M. Gros-van Hest[1], S. H. M. Arts*[2], K. E. de Rooij[3], M. C. de Wilde*[1], A. L. B. van Helvoort*[1]. [1]Biomedical Research Department, Numico Research B.V., Wageningen, Netherlands, [2]Agrotechnology and Food Sciences, Wageningen University, Wageningen, Netherlands, [3]Endocrinology, Leiden University Medical Center, Leiden, Netherlands.

SAMP6 mice are a model for senile osteoporosis, exhibiting a low peak bone mass and an early onset of bone loss when compared to the normally ageing strain SAMR1. Differences in body weight and size were however observed between the SAMP6 and SAMR1 strains, possibly concealing differences in bone mineral density (BMD) as measured *in vivo* with dual-energy X-ray absorptiometry (DXA).
To investigate differences in body composition and BMD at different skeletal sites, 24 SAMP6 and 16 SAMR1 were examined with whole body DXA (Piximus) from 22 to 32 weeks of age, i.e., during the phase of linear bone loss. To test sensitivity of the detection of the anabolic effects 17ß-oestradiol in SAMP6 mice, a subcutaneous slow release pad was placed in 8 of the SAMP6 (SAMPBE). All animals were fed a diet meeting the dietary requirements of mice. Total bone mineral density, lean body mass and fat percentage as well as density of the lumbar spine and femur were determined. The skull and tail were excluded from the analysis.
SAMP6 had a significantly higher fat percentage and lower BMD at the lumbar spine compared to SAMR1 at all ages. The BMD of the total body and the femur revealed smaller differences between SAMP6 and SAMR1 mice, not reaching significance at all ages. Treatment of SAMP6 mice with 17ß-oestradiol significantly increased BMD at both the lumbar spine and the femur.
Although the expected differences in BMD between SAMR1, SAMP6 and SAMPBE could be detected, size differences between strains might have caused a relative insensitivity in the detection of differences in the BMD of the femur. Correction for the possibly confounding lower femur size in SAMR1 may prove to increase sensitivity of the detection of differences between these two strains of mice.

Disclosures: ***I. Schoenmakers****, None.*

M078

Choice of Cross-Calibration Phantom forDXA of the Lumbar Spine and Total Hip. S. A. Jackson*, C. G. Miller. Bio-Imaging Technologies Inc., Newtown, PA, USA.

DXA technology has been in existence since 1987 and some of the original equipment is still in operation today. Since patients undergoing BMD examinations tend to be followed for many years, it has always been recommended that they are measured on the same machine. This is to avoid the inherent errors associated with calibration and scan mode differences between various models of DXA scanner. However, as the first generation of machines are currently being discontinued and are no longer supported by the manufacturer, the prospect of a patient switching to a different machine and even a different manufacturer is becoming more frequent. Consequently, the need to cross-calibrate old and new DXA machines in now a common problem.
The ideal way to perform a cross-calibration is to scan a large number of subjects, with a range of BMD values, on both machines, and then calculate a regression equation. However, this approach may not always be appropriate for a variety of reasons, including cost, logistics and ethics. The use of static test objects (Phantoms) is another way to perform cross-calibration, but there are several issues to consider in determining the most appropriate phantom to use.
This work compares human data to that of the only three phantoms which provide a suitable range of BMD values. The human data for spine (L1-L4) and Total hip were acquired on Hologic QDR 1000 and GE Lunar Prodigy scanners from the CaMos study. The subjects aged between 22 and 72 years with BMD values covering the clinical range from normal to osteoporotic. The phantoms compared were the European Spine Phantom (ESP), the GE Lunar Aluminum step wedge (AL) and the Bona Fide Spine Phantom (BFP). All phantoms were measured ten times on each machine and the means of individual vertebral sections used to calculate a regression relationship. To be useful for cross-calibration, the ideal phantom will have the same regression equation as the human data. For the spine, both the ESP and the AL regressions exhibit different slopes to the human data which translates to errors of between -4.4 and 19.4% over the 0.5 to 1.5 g/cm^2 BMD range (GE Lunar). The BFP data has virtually identical slope to the human data with a minimal offset of approximately 0.015 g/cm^2. For the total hip a similar relationship occurs with the BFP and human data regressions exhibiting the same slope with a constant offset of 0.05 g/cm^2.
Of the three commercially available phantoms studied, the BFP exhibits the closest regression to human data for both the spine and total hip. Allowing for the small constant offset, the BFP phantom data provides the most utility and least error for cross-calibrations in clinical trials and DXA machine changes.

Disclosures: ***S.A. Jackson****, Bio-Imaging Technologies Inc 3.*

M079

Diagnosis of Osteoporosis Using the Ultradistal Radius Region of Interest. G. M. Kiebzak[1], E. M. Lewiecki[2], S. M. Petak[3]. [1]St. Luke's Episcopal Hospital, Houston, TX, USA, [2]New Mexico Clinical Research & Osteoporosis Center, Albuquerque, NM, USA, [3]Texas Institute for Reproductive Medicine and Endocrinology, Houston, TX, USA.

Aim. To determine if more patients are diagnosed as osteoporotic when using the ultradistal (UD) radius region of interest (ROI) in addition to PA lumbar spine, proximal femur (hip), and radius "midshaft."
Background. WHO criteria for classifying patients as normal, osteopenic, or osteoporotic are based on bone mineral density (BMD) of the lumbar spine, hip, and forearm. There is no consensus regarding which forearm ROIs should be used with the WHO criteria. Since UD radius has a greater ratio of trabecular to cortical bone than midshaft portions of the radius, it is likely more patients would be classified as osteoporotic if the UD radius is measured.
Methods. Data were obtained from three centers with differing patient demographics, thus reducing bias due to patient characteristics. Data were used only from patients that had a spine, hip, and forearm scan on the same day. Central DXA machines included a GE-Lunar DPX-L, DPX IQ, and Prodigy, and a Hologic Delphi. Hologic data were for UD radius+ulna. Diagnoses (WHO criteria) were made with and without the UD radius ROI T-scores, in addition to lumbar spine (L2-L4 or L1-L4), hip (lowest T score from femoral neck, greater trochanter, or total), and radius 33% ROI. Only pooled results are shown.
Results. For all GE-Lunar patients (n = 511, mostly women, age range 20-95 yrs) the distribution of normal, osteopenic, osteoporotic *not* using the UD radius ROI was 112, 211, 188 respectively. The distribution when *using* the UD radius site was 83, 169, 259. The ratio of normal/osteopenic vs. osteoporotic was significant (P<0.0001, one-tailed Fisher's Exact Test). For all Hologic patients (n = 196, age range 44-93), the distributions were 36, 91, 69 not using and 35, 89, 72 using the UD radius+ulna ROI (not significantly different). Distributions were similar after accounting for sex, age, use of steroid medications, and hyperparathyroidism. The group mean T-scores were lowest for the UD radius region compared to spine and hip with GE-Lunar but not Hologic patients.
Conclusions. More patients (38% in our results) are diagnosed as osteoporotic when using the UD radius ROI with GE-Lunar machines. Using the Hologic UD radius+ulna ROI did not increase the number diagnosed as osteoporotic. These data suggest that the prevalence of osteoporosis is greater when BMD is measured at the UD radius on GE-Lunar machines, and that these patients may be more likely to receive pharmacologic intervention. Further study is necessary to determine the applicability of WHO criteria to T-scores from the UD radius ROI and the origin of manufacturer-specific discrepancies.

Disclosures: ***G.M. Kiebzak****, None.*

M080

Radiation Dose Associated with Peripheral Quantitative Computed Tomography Scanning in Children. C. L. Gordon, C. E. Webber, M. Cottreau*. Radiology, McMaster University, Hamilton, ON, Canada.

Peripheral Quantitative Computed Tomography (pQCT) is valuable for longitudinal studies in children because it is not confounded by changes in bone size and can simultaneously assess bone and muscle cross-sectional area in the limbs. Despite these benefits there is still the perception that this CT procedure entails a significant dose burden for children. In this study we prepared a cylindrical limb phantom to evaluate the dose received by arm and leg tissue during scanning on the Stratec XCT 2000 pQCT system. The phantom was made of polyethylene to simulate soft tissue and had an outer diameter of 80 mm. At the center of the phantom there was a 25 mm bore into which an aluminum tube with 5 mm thick walls was inserted. This aluminum insert was used to simulate cortical bone. The absorbed dose was measured using a CT ion chamber (Radcal Corporation). The probe of the ion chamber was placed at the center of the aluminum tube insert to record the marrow dose. It was also placed in a bore centered 10 mm below the surface of the phantom to record the soft tissue dose. Doses were recorded with the phantom scanned on an XCT 2000 with typical scanning parameters for pediatric forearm and tibia assessment. The scout view parameters were: 30 mm/sec, 1.0 mm between lines, 40 scan lines. The CT scan parameters were: 90 mm field of view, 0.4 mm voxel, 15 mm/sec scan speed. Two sets of readings were taken two weeks apart. The average values are listed below.

	Scout scan	CT Scan
Tissue dose (mRem)	3.8	2.4
Marrow dose (mRem)	Nil	0.7

This dose data shows that a typical pQCT examination consisting of a scout scan and two CT scans entails a dose burden of approximately 9 mRem. This is the local absorbed dose received by the radius or tibia. Since there is no scatter to the trunk during scanning this local dose translates into an effective dose equivalent of approximately 1 µSv. We conclude that the doses received from pQCT exams are safe for children and is negligible compared to the doses received from an axial QCT exam (30-60 µSv) or annually from background radiation (2500 µSv).

Disclosures: ***C.L. Gordon****, Orthometrix Inc 5.*

M081

In-vivo Bone Mineral Measurement of the Hip using the Lexxos Cone Beam Densitometer: Precision and Cross-calibration with Hologic Densitometers. K. M. Knapp[1], R. M. Koa-Sales*[1], V. Boudousq[2], I. Ruiz[2], P. O. Kotzki[2], D. Chappard[3], M. Audran[3]. [1]Imaging Sciences, Guy's, Kings and St Thomas' School of Medicine, London, United Kingdom, [2]Service de Médecine Nucléaire, CHU de Nîmes, Nîmes, France, [3]INSERM EMI 0335 - Service de Rhumathologie, CHU d'Angers, Angers, France.

The Lexxos cone beam densitometer (DMS, France) utilises digital radiological flat panels and conically collimated X-rays. This enables short acquisition times of 3s using two X-ray exposures, avoiding the need for scanning. The aim of this study was to evaluate the short-term in-vivo precision and cross-calibration of the Lexxos (DMS, France) with Hologic (Bedford, MA) fan-beam densitometers. Three centres from two countries (France and UK) participated in the study. The short-term in-vivo precision was evaluated using two groups of females; group 1 consisted of 71 young women aged 20-40 (mean 30, SD ± 6), and, group 2 consisted of 81 women between the ages of 41 and 80 (mean 57, SD ± 9).

Each woman had repeated hip examinations with repositioning between the acquisitions. The short-term precision of these two groups and the combined groups was calculated using the root mean square coefficient of variation (RMS CV%). Correlations between the Lexxos and Hologic QDR2000 and QDR4500 densitometers were evaluated using a third group of subjects (QDR2000 n=60, QDR4500 n=219) and using linear regression to calculate the agreement between densitometers

	Femoral neck	Trochanter	Intertrochanter	Total Hip
RMS CV% Group 1	1.60	1.53	1.18	1.46
RMS CV% Group 2	1.72	1.87	1.10	1.10
RMS CV% Combined	1.67	1.72	1.14	1.28
r QDR2000	0.89	0.80	0.92	0.89
RMSE g/cm^2	0.063	0.084	0.063	0.063
Slope (SE) QDR2000	0.973 (0.052)	0.898 (0.073)	0.932 (0.042)	0.928 (0.050)
Intercept (SE) QDR2000	0.065 (0.038)	0.178 (0.047)	0.174 (0.042)	0.169 (0.042)
r QDR4500	0.97	0.92	0.92	0.94
RMSE g/cm^2	0.032	0.055	0.071	0.055
Slope (SE) QDR 4500	0.948 (0.016)	0.985 (0.029)	0.879 (0.024)	0.922 (0.023)
Intercept (SE) QDR4500	0.026 (0.012)	0.076 (0.019)	0.122 (0.025)	0.088 (0.020)

SE= Standard Error

The RMS CV% ranged from 1.10 to 1.87%, which is similar to the values expected for hip dual X-ray absorptiometry (DXA). The correlation results demonstrated good agreement between the Lexxos and QDR densitometers, with the best agreement being with the QDR4500. In conclusion, these results demonstrate that cone-beam DXA performance is equivalent to fan beam DXA.

Disclosures: ***K.M. Knapp****, None.*

M082

Changes in Appropriate Use of Bone Densitometry From 1997 to 2001 in a Large Multispecialty Clinic. J. T. Schousboe[1], J. Fowles*[2], E. A. Kind*[2], C. Craft*[2], S. A. Adlis*[2], C. R. DeBold*[1]. [1]Park Nicollet Clinic, Minneapolis, MN, USA, [2]Park Nicollet Institute, Minneapolis, MN, USA.

Underuse of bone densitometry (BD) for those who have suffered a fracture has been documented, but studies regarding appropriate use among the wider postmenopausal female population have not been reported.

We randomly selected 3 separate cohorts of 175 women age 55 to 80 among those who had received care at Park Nicollet Clinic for five or more years previous to an index visit during August of 1997, 1999, or 2001 (525 total patients). Patients were classified as meeting appropriate criteria for BD according to variations of 3 decision rules: the Osteoporosis Risk Assessment Instrument (Modified ORAI), the Simple Calculated Osteoporosis Risk Estimation (SCORE), and the European Society for Osteoporosis and Bone Diseases guidelines (Modified Euro).

From each medical record we extracted the patient's age, most recent weight, fracture since age 45, radiographic osteopenia, use of hormone replacement therapy (HRT) or other drugs to prevent osteoporosis, use of oral glucocorticoid therapy for three months or longer, current cigarette smoking, and race. The 3 decision rules for appropriate use of BD were:

Modified Euro: Age $\geq$ 65, fracture since age 45, radiographic osteopenia, weight < 127 lbs, use of oral glucocorticoid, not on HRT.

Modified ORAI: ORAI $\geq$ 9 (see JAMA 2001; 286: 57-63), fracture since age 45, or use of oral glucocorticoid.

SCORE $\geq$ 6 (see JAMA 2001; 286: 57-63).

The percentages of women meeting criteria for BD for all three years was 83.1% (Modified Euro), 56.51% (Modified ORAI), and 73.76% (SCORE). The table shows the percentage among women who met criteria for a bone densitometry test and who had one before the index visit.

Criteria	1997	1999	2001
Mod Euro	7.5	23.9	47.3
Mod ORAI	7.8	24.3	56.2
SCORE	7.5	23.4	48.0

P <0.0001 for trend with all three criteria

In 2001, among those not meeting criteria for BD, 29% to 32% had the test, indicating some inappropriate use may now be occurring. Significant improvement in use of BD in those who meet various criteria sets has occurred between 1997 and 2001, but, for postmenopausal women age 55-80, underuse of bone densitometry when indicated remains the dominant problem.

Disclosures: ***J.T. Schousboe****, None.*

M083

3 Dimensional X-Ray Absorptiometry (3D-XA): Validation of 3D Reconstruction of Human Proximal Femurs using a DXA Device. S. Kolta[1], A. Le Bras*[2], V. Bousson*[3], D. Mitton*[2], J. A. De Guise*[4]. [1]Rheumatology, Cochin hospital, Paris, France, [2]LBM-ENSAM-CNRS, Paris, France, [3]Lariboisière Hospital, Paris, France, [4]LIO, Montréal, PQ, Canada.

Bone Mineral Density measured by Dual energy X-ray Absorptiometry (DXA) has proven to be an important risk factor for fracture. However, being an areal density, the results are highly dependent on patient positioning and inter individual anatomical differences. Recent advances in stereoradiographic reconstruction techniques (Non Stereo-Corresponding Contour, NSCC) yield 3D bone geometry from 2D contours identified on biplanar radiographs with an accuracy close to that of 3D CT-scan reconstruction. This algorithm performs an elastic deformation of a generic object to fit its 3D retro-projected contours with real contours on X-rays. The aim of our study was to evaluate the feasibility and accuracy of 3D reconstruction of human proximal femurs using 2 perpendicular DXA scans. Twenty eight excised proximal femurs, 23 F and 5 M aged 84±13 years provided by Institut d'Anatomie René Descartes (Paris) were scanned using a multislice CT-scan device (Somatom Plus 4, Siemens) with a high spatial resolution acquisition protocol. The 3D reconstruction containing up to approximately 10000 points were obtained (accuracy ± 1mm) using the SliceOmatic® software. Another specimen (female, 89 y) was reconstructed with a similar protocol and represented the generic shape used in NSCC. Nine anatomic areas allowing to generate 3D contours were defined from this generic object. DXA scans of each femur were performed using a Delphi-W device (Hologic) in a 14 cm depth water in PA and lateral incidences. Beforehand, calibration of the DXA environment was performed allowing geometrical localisation of X-ray source and detectors in a global referentiel. From both DXA images, contours of the areas defined on the generic object were identified. The accuracy of 3D-XA reconstructions was evaluated in comparison with the CT-scan ones by superimposing the two models using geometrical transformations and a least square matching method. Results were expressed as point to surface distances. The NSCC method succeeded for 25 of the 28 proximal femurs. Mean difference between the CT-scan and the 3D-XA reconstructions was 0.06±1.02 mm. Mean absolute value was 0.8 mm and 95% of errors were less than 2.1 mm. Maximal error reached 7.8 mm on areas as the greater or lesser trochanter. This pilot study shows that 3D-XA of proximal femur is feasible with accuracy close to that of CT-scan. Association of 3D-XA structural parameters with classical DXA bone density results could provide a new tool to predict in a better way the osteoporotic hip fracture risk

This work was supported by Biospace Instruments, France

Disclosures: ***S. Kolta****, None.*

M084

3 Dimensional X-Ray Absorptiometry (3D-XA): Validation of 3D Reconstruction of Human Vertebrae using a DXA Device. S. Kolta[1], A. Le Bras*[2], W. Skalli*[2], D. Mitton*[2], J. A. De Guise*[3], J. Fechtenbaum[1], C. Roux[1]. [1]Rheumatology, Cochin Hospital, Paris, France, [2]LBM-ENSAM-CNRS, Paris, France, [3]LIO, Montréal, PQ, Canada.

Lumbar spine bone mineral density (BMD g/cm²) measured by Dual energy X-ray Absorptiometry (DXA) has proven to be an important risk factor for vertebral fracture. Being an areal density, it is highly dependent on patient positioning and inter individual variations. Other parameters such as vertebral body size and shape independently affect its strength. Recent advances in stereoradiographic reconstruction techniques allow obtaining 3D bone structure geometry from landmarks identified on two radiographs and a priori knowledge (1). This technique was named NSCP (Non Stereo Corresponding Points). The aim of our study was to evaluate the feasibility and accuracy of 3D reconstruction of human vertebrae using 2 perpendicular DXA scans. Sixteen human dried lumbar vertebrae from the Laboratoire d'anatomie des St Pères (Paris, France) were included in this study. They were scanned using a helical CT-scan device. The 3D reconstruction containing up to approximately 4000 points, were obtained using the SliceOmatic® software (accuracy ± 1 mm). DXA acquisitions of the vertebrae were performed using a Delphi-W device (Hologic). First, DXA spatial calibration was performed as described elsewhere (2). Secondly the vertebrae were embedded in foam and put inside the calibrating box which was put on a 36 mm thick plexiglas plate in order to simulate soft tissues. The DXA scans were acquired in frontal and lateral incidences. From both DXA images, approximately 25 anatomical landmarks were identified and reconstructed using NSCP algorithm (1). A visual control was then done to compare the retro-projection of the 3D model on the real vertebral contours seen on both DXA images. The accuracy of 3D-XA reconstructions was evaluated in comparison with the CT-scan ones by superimposing the two models using geometrical transformations and a least square matching method. Results were expressed as point to surface distances. Quantitative comparisons between the CT-scan reference reconstructions and the 3D-XA yielded mean difference of 0.05±1.24 mm. Mean absolute value was 1.0 mm and 95% of errors were less than 2.5 mm. Maximal error reached 6.5 mm on areas as the transverse processes. This pilot study shows that 3D-XA of human vertebrae is feasible with an accuracy close to that of the CT-scan reconstruction.

This work was supported by Biospace Instruments, France.

(1) Mitton et al, Med Biol Eng Comput ; 2000

(2) 3 Dimensional X-Ray Absorptiometry (3D-XA) : Validation of 3D reconstruction of human proximal femurs using a DXA device. S Kolta, A Le Bras, V Bousson et al (ASBMR 2003)

Disclosures: ***C. Roux****, None.*

M085

A Comparison of Lumbar Spine Quantitative Computed Tomography and Dual Energy X-Ray Absorbtiometry Measurements of Femur Neck and Spine. M. Calguneri*[1], M. Ariyurek*[2], D. Gunes*[3], I. Ertenli*[1], S. Kiraz*[1], S. A. Bilgen*[1], M. A. Ozturk*[1]. [1]Department of Internal Medicine Section of Rheumatology, Hacettepe University Faculty of Medicine, Ankara, Turkey, [2]Department of Radiology, Hacettepe University Faculty of Medicine, Ankara, Turkey, [3]Department of Internal Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey.

Dual energy x ray absorptiometry (DEXA) measurements of femur neck and lumbar spine is compared with quantitative computed tomography (QCT) assessments of the lumbar spine in patients with and without osteoarthritis (OA)in this study.30 women with gen-

eralized OA, mean age 49.53±10.59, and 20 healthy women,mean age 46.80±8.66, were included . Bone mineral density (BMD)of trabecular and cortical bone were measured at the midvertebral bodies of the lumbar spine by QCT. And BMD of the lumbar spine and femur neck was assessed by DEXA.There was no significant difference between DEXA and QCT measurements of lumbar spine in OA and healthy groups. But DEXA results of femur neck between groups (0.830 in OA vs 0.755 in healthy group)was different(p=0.05).QCT measurements of the trabecular bone was significantly lower in subjects older than 45 years old. We also compared the QCT results according to lumbal t score lower and higher than -2.5. Trabecular and cortical BMD was lower in the osteoporotic group (118.3 vs 129.85, 301.71 vs 319.49 respectively) but the difference was not significant.When lumbal t score lower and higher than -1 was evaluated, we found significant differences in the QCT measurements between groups.(table1)

Table 1:

Lumbal t-1(26) p

Trabecular BMD 112.82±24.28 144.45±25.22 0.001

Cortical BMD 294.16±41.94 341.00±50.64 0.001

We believe that OA changes of the lumbar spine elaborate the results of DEXA and more precise measurements of the BMD are needed.We thought it was because of our small sample size that there was no significant difference in measurements of DEXA and QCT among OA and healthy groups. But in the group with osteopenia according to DEXA, the decrease in the cortical and trabecular BMD in QCT was more pronounced. There was no difference in QCT measurements between subjects diagnosed as osteoporotic and not osteoporotic according to DEXA, which may be caused by the small number of subjects with osteoporosis (only 8 subjects). According to age groups above and below 45, we found significant differences in QCT measurements of trabecular bone and none of the others, which may be a clue for the value of QCT showing the aging process in bones earlier. We will continue the measurements with both methods and compare the results with plain x rays of subjects. We believe that as the sample size increases the diagnostic utility of QCT in OA will be evident.

Disclosures: ***M. Calguneri****, None.*

M086

Differences in Heel BMD, Stiffness Index, and QUI Compared to Total Hip BMD in Old Men. Results from the Swedish Part of the Mr OS Study. H. Mallmin[1], E. Ribom*[1], E. Orwoll[2], O. Johnell[3], Ö. Ljunggren[4]. [1]University Hospital, Dept of Orthopedics, Uppsala, Sweden, [2]Oregon Health and Sciences University, Clin Research Center, Portland, OR, USA, [3]Malmö General Hospital, Dept of Orthopedics, Malmö, Sweden, [4]University Hospital, Dept of Medicine, Uppsala, Sweden.

We have investigated the associations between DXA total hip BMD and in DXA heel BMD/QUS heel in elderly Swedish men. The participants were a subset of the Swedish part of the MrOS study, an ongoing intercontinental (USA, Hong Kong and Sweden) prospective study in men for evaluation of fracture risk. The study cohort was population-based, participant rate of 50%. 295 men, age 76.4 +-3.5 range 70.3-80.5, were measured bilaterally DXA, Prodigy; QUS-heel measurements with the Sahara QUS-equipment and Achilles express, and DXA-heel. The average for left and right side was calculated and the manufacturers recommended white male reference-populations T-scores (all four have different reference populations) was used for calculations. In addition, we compared these values with T-scores derived from a population-based (participant rate 50%) sample of 340 Swedish women, 20-40 yrs, measured with the same techniques. Results: Best correlations were found for heel QUS's, Table 1. The proportion of individuals with manufacturer derived T-score cut offs for "osteoporosis" were; 9%, 5%, 8% and 2% for DXATot hip, DXA Heel, QUS Ach, and QUS Sah respectively and for "osteopenia"; the proportions were correspondingly; 35%, 30%, 32%, 35%. The corresponding proportions for a homogenous female derived T-scores were for "osteoporosis"; 8%, 1%, 2%, 8% and "osteopenia"; 32%, 11%, 30% and 28% respectively. The correlations between mean values of the total hip BMD and different measures of the heel in elderly men show r-values between 0.7 and 0.59. When applying a T-score based on young Swedish women the proportion of men diagnosed as having osteoporosis did not differ in total hip, but was substantially lower in the heel measured by DXA or QUS.

Table 1.

	DXA BMD Total Hip	DXA BMD Heel	Ach Stiffness Heel	Sahara QUI Heel
DXA BMD Total Hip	1.00	0.7	0.61	0.59
DXA BMD Heel	0.7	1.0	0.78	0.75
Ach Stiffness Heel	0.61	0.78	1.0	0.9
Sahara QUI Heel	0.59	0.75	0.9	1.0

Disclosures: ***H. Mallmin****, None.*

M087

The Difference of Bone Mineral Density Between Both Hips Influences the WHO Classification. J. L. Mansur, M. C. Cianciosi*, A. Martella*. Center of Endoc and Osteoporosis La Plata , Argentina, La Plata, Argentina.

Some articles have shown a good correlation between the Bone Mineral Density (BMD) of both hips. However, last year in ASBMR it was shown that a significant number of women have difference between sides: in Femoral Neck (FN) 23.3 % had a difference > 5 % and 18.7 % had a difference > 0.5 St D, and in Total Hip (TH) 12.7 % had a difference > 5 % and 6.7 % had a difference > 0.5 St D (1). We wanted to know if this difference could alterar the WHO classification.

Patients and methods: We prospectively study the BMD of both hips (Lunar Prodigy) of 100 postmenopausal women. The presence of scoliosis in the image of spine scans, and previous treatments with drugs that affect bone metabolism were exclusion criteria. They were classified in Normal (n), osteopenia (op) or Osteoporosis (OP) in both sides. We report the difference between both sides in 1) % and 2) T-Score, in four regions: A) Femoral neck (FN), B) Ward area (W), C) Trochanter (Troch), and D) Total Hip (TH). We study the group as a whole (multivariate regression) and divided in tertils of age and weight.

Results:

Age: 60,0 (SD: 9,9) years. Menop: 48,3 (4,4) years. Weight: 68,1 (13) Kg. Height: 156 (6) cm

- Difference mean: FN: 4,4 % (4.3 SD), W: 4,6 % (4.4), Troch: 4,8 % (4.0), TH: 3,1 % (2.9)
- Correlation between sides: FN: 0.87, W: 0,90, Troch: 0,92, Shaft: 0,95, TH: 0,95
- % of patients with difference between sides:

in FN > 5 % in 25 % of the women and > 0.5 SD in 17 %

in TH > 5 % in 18 % of the women and > 0.5 SD in 10 %

In FN 81 % of the women have concordance between both sides (n-n, op-op or OP-OP) and 19 % have discordance (n-op in 14 % and op-OP in 5 %)

In TH 84 % of the women have concordance between both sides (n-n, op-op or OP-OP) and 16 % have discordance (n-op in 11 % and op-OP in 5 %)

In older women (>60 years) discordance were present in 32.5 % of 40 women in FN, and in 17.5 % in TH.

Dividing them into tertiles, heavier (>71 Kg) and older (>60 years) women have more difference between both sides in FN, Troch and TH, as was shown in ASBMR 2002 (1).

In multivariate analysis the difference between both Troch and between both TH increases when weight and age increases.

Conclusion:

Although the correlation between both hips is high, a significant number of patients without scoliosis have difference between sides and nearly 20 % is classified in a different way if we choose only one side. Bilateral DXA measurements are recommended, especially in older and heavier patients.

(1) J Bone Min Res 17 (suppl 1) S152

Disclosures: ***J.L. Mansur****, None.*

M088

Performance Evaluation of a New DXA System: The Lunar Bravo. S. B. Broy, L. G. Jankowski*. Illinois Bone and Joint Institute, Chicago, IL, USA.

The Lunar Bravo (GE Medical Systems) is a small footprint spine/hip DXA scanner designed for offices with limited space. The Bravo scanner arm rotates to the side for patient access and positioning. It uses established pencil beam technology with several features previously found only in fan-beam systems. These include a spine/bilateral femur mode (OneScan) that eliminates the need to reposition between spine and femur scans, automated software to help identify problem scans, and integrated physician reporting software. We evaluated Bravo precision and accuracy compared to an existing DXA system.

Fifteen female volunteers aged 44 to 82 years (mean age 62 ± 12 years) were measured three times each on the Bravo using the OneScan mode. The subject is placed supine on the scanner table with legs flat and feet in the standard bilateral femur positioner. After the spine scan is completed, the scanner moves automatically to each femur, the operator confirms the start point, and the scan is completed without moving the patient. Precision was determined for the spine, femoral neck and total femur as the RMS standard deviation of the repeat measurements, expressed as a percent (%CV). Each subject was also scanned on a Delphi (Hologic) DXA system using the standard spine (legs elevated) and bilateral femur scan protocols. A two-tailed, paired t-test was used to determine the relationship between the Bravo and Delphi BMD and T-score values at the L1-L4 spine, femoral neck and total femur using the first measurement result from the Bravo for the comparison.

Bravo precision error was 1.2% at the spine, 1.8% at the femoral neck, and 0.9% for the total femur. There were significant differences in BMD values between devices due to calibration differences, but the values were highly correlated (r = 0.94-0.97). There was no significant difference in T-scores at the spine or total femur; a small (0.3 SD) difference was seen at the femur neck. The reasons for the higher femoral neck T-scores are unclear. They may be related to differences in the normative databases, variations in abduction or rotation due to the bilateral femur positioner, neck-box placement and edge detection, or differences between fan-beam and pencil-beam systems. The small difference in femoral neck T-scores may not be clinically significant.

We conclude that the Bravo provides accurate and precise spine and hip DXA measurements, consistent with results from other DXA systems.

	Bravo	**Delphi**	P-value for difference
L1-L4 BMD (g/cm^2)	1.16	0.98	< 0.001
L1-L4 T-score	-0.4	-0.6	0.23 (NS)
Femur Neck BMD (g/cm^2)	0.93	0.78	< 0.001
Femur Neck T-score	-0.4	-0.7	< 0.01
Total Femur BMD (g/cm^2)	0.96	0.91	< 0.001
Total Femur T-score	-0.3	-0.3	0.58 (NS)

Disclosures: ***S.B. Broy****, GE Medical Systems Lunar 2.*

M089

Precision Comparison of Hologic and Lunar DXA Densitometers using the European Spine Phantom. C. Horvath, S. Meszaros*, V. Ferencz*. Department of Internal Medicine, Semmelweis University, Budapest, Hungary.

Dual-energy X-ray absorptiometry (DXA) scans are widely used to assist in the diagnosis of osteoporosis and to monitor bone mineral density (BMD) change following treatment. In order to assess the significance of any observed difference in BMD, it is necessary to determine the precision error of the DXA system. A large precision error limits the ability to detect small changes, while a smaller precision error allows for a more rapid assessment of a differ-

ence in BMD. It is therefore imperative that DXA instruments perform according to the highest precision and accuracy standards.
In this study, we compared the precision error in a group of Hologic and GE Lunar DXA systems participating in a nation-wide investigation of osteoporosis. The European Spine Phantom (ESP) was used as a calibration standard. The ESP is a semi-anthropomorphic phantom recognized as a calibration standard for DXA [1]. A single European Spine Phantom (ESP-03-202) was circulated to 29 different DXA densitometers in Hungary: 17 manufactured by GE Medical Systems Lunar and 12 manufactured by Hologic. The ESP was scanned 11 times on each system using the standard AP-spine acquisition mode. Each scan was analyzed according to manufacturer recommended procedures by a trained technologist. The average BMD value, standard deviation, and root mean square precision (%CV) were calculated for each system. Precision error (variances) for GE Lunar and Hologic were compared using an F-test to evaluate the significance of any observed differences.

	Mean BMD (g/cm^2)	**SD** (g/cm^2)	**% CV**
GE Lunar	1.144	0.0057	0.49 %
Hologic	1.011	0.0061	0.60 %

Expected differences in mean BMD between GE Lunar and Hologic values (13%) were seen, consistent with known calibration differences. Precision error for the GE Lunar systems ranged from 0.32% to 0.73%, with an average of 0.49%. Hologic precision errors ranged from 0.39% to 0.99%, with an average of 0.60%. The average GE Lunar precision error was significantly less than the average Hologic precision error ($p = 0.003$). In conclusion, interscanner variability of the ESP BMD measurements for instruments of the same manufacturer is very low, and the precision of all the tested instruments is extremely good. Small but significant differences in precision exist between individual instruments and between manufacturers. Precision error in patients is expected to be larger than that reported with the ESP.
1. Kalender W.A. et al. *Eur J Radiol* 1995; 20:83-92.

Disclosures: ***C. Horvath****, None.*

M090

CTXA Hip - An Extension of Classical DXA Measurements Using QCT. C. E. Cann[1], J. E. Adams*[2], G. Wood*[3], J. K. Brown*[1]. [1]Mindways Software, Inc., San Francisco, CA, USA, [2]University of Manchester, Manchester, United Kingdom, [3]Schnectady Radiology, Schnectady, NY, USA.

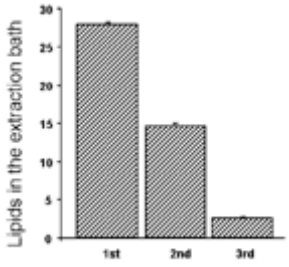
Bone mineral density (BMD) estimates for the proximal femur using DXA are currently considered the standard for making a diagnosis of osteoporosis in an individual patient using BMD alone. Quantitative computed tomography (QCT) BMD estimates have generally been restricted to the spine or peripheral sites, and little prospective clinical data are available to form a context for interpretation of these measurements in terms of fracture risk, even though they have been shown to be very sensitive indicators of change in BMD. We have developed a 3D QCT BMD measurement for the proximal femur which provides DXA-equivalent clinical data, and in addition provides separate estimates for cortical and trabecular bone in standard DXA hip ROIs.
69 women were scanned using 3D QCT and using DXA (Hologic QDR4500). QCT data were projected to a DXA-like 2D image using commercial software (CTXA Hip, Mindways Software, Inc, San Francisco, CA), and the area BMD measurements for Total Hip and Femoral Neck compared to DXA results. In addition, 22 women were scanned using 3D QCT at 0,1,2 years as part of a placebo group in a clinical trial, and those data were analyzed using CTXA Hip for long term reproducibility.
Long term reproducibility was 0.011 g/cm2 for Total Hip and 0.012 g/cm2 for Femoral Neck. The correlation of Total Hip BMD CTXA vs. DXA was R=0.966, and for Femoral Neck was R=0.946 (SEE 0.044 g/cm2 in both cases). In older osteoporotic women, cortical bone comprised 62+/-5% (mean+/-SD) of total hip bone mass.
A new method for QCT BMD estimates in the proximal femur, CTXA Hip, has been shown to correlate highly with current DXA hip measurements, with high reproducibility in vivo. In addition, this method has the ability to evaluate separately changes in the cortical and trabecular bone in the standard DXA ROIs in the hip, and to determine the distribution of bone within these compartments in osteoporosis or other disease states.

Disclosures: ***C.E. Cann****, None.*

M091

Ex-vivo **Bone Mineral Measurement of the Wrist Using the Lexxos Cone Beam Densitometer: Influence of Medullar Lipids and Comparison with Hologic 4500.** D. Chappard, M. Moquereau*, P. Mercier*, Y. Gallois*, S. Sourice*, M. F. Basle*, M. Audran. LHEA Faculty of Medecine, INSERM 0335, Angers, France.

The development of new DXA systems is required to shorten the time of analysis while preserving the same level of precision and reproducibility. The cone beam technology was recently proposed in a 3rd generation of densitometers. The Lexxos (DMS-France) uses a new type of large (20*20 cm) CCD detectors that can provide the image of a hip in a single shot. The system is now available for lumbar spine, hip and wrist measurements. The influence of bone marrow lipids is a well known problem in DXA and QCT analyses. We have designed a cadaver study to compare the influence of medullary fat on the measures performed with the Hologic QDR4500 and the Lexxos. Twelve human distal radius were obtained from the Anatomical laboratory and defleshed. Bones were immersed in a polyethylene tank containing a constant height of water. They were analyzed in parallel with the QDR4500 and the Lexxos; BMD was measured at the ultradistal radius with standard softwares and on a standardized square ROI centered under the articular surface. Bones were then extensively defatted in several bath of chloroform/acetone (in 3 baths) and complete delipidation was controlled by biochemical dosages. They were rehydrated and a new series of measurement was performed on both densitometers. Bone specimens were then thoroughly dried for several days and were sectioned using a banding saw. A cube was prepared at the ultradistal radius, similar in size with the ROI measured by the densitometers. Trabecular bone volume and other histomorphometric descriptors of 3D bone architecture were measured on a microcomputerized tomograph (Skyscan 1072). Then, specimens were calcinated in a muffle furnace at 800°C for 24 hours and ashes were weighted on a precision scale. The image quality was better with the Lexxos but delipidation did not improve quality. The automatic algorithms used by both densitometers for contour detection often needed an interactive re-drawing of boundaries. Delipidation had a significant effect on measurements.



Disclosures: ***D. Chappard****, None.*

M092

Measuring Bone Density in Children. T. L. Kelly, K. E. Wilson. Hologic, Inc., Bedford, MA, USA.

Densitometry referrals for children, some as young as 3 years old, are increasing in hospitals and clinics across the country. This paper addresses the key technical components of measuring bone density in children, e.g. selecting the most appropriate scan site, and interpreting densitometry results in pediatric subjects.
AP Spine examinations should be the first choice for pediatric skeletal assessment for a variety of practical and technical reasons. AP Spine exams present a well defined anatomical region which greatly simplifies ROI placement, an important consideration in growing children. Subject positioning and analysis are straightforward, precision is typically better than 1%, and short exam times improve compliance. The AP Spine site also provides reliable diagnostic information and responds well to therapy. In contrast, the forearm site is not recommended because long bones undergo both appositional and linear growth. As a consequence, the same physical region of bone cannot be measured during serial evaluation. Hip exams are challenging because the anatomy, and in particular the greater trochanter, is not well developed in young children. The developing anatomy complicates initial ROI placement and creates problems during serial evaluation. Whole body studies provide global assessment of skeletal health and body composition and may be indicated in some subjects (e.g. to evaluate the effects of growth hormone deficiency or wasting disease). However, whole body examinations are not widely available, involve considerably longer exam times, and may be less sensitive to changes in bone density. For these reasons, whole body studies are employed principally in medical research.
It is widely recognized that T-scores, WHO definitions, and other adult fracture risk concepts have no practical value in children. A child's skeletal status should be assessed relative to overall growth and development. Z-scores provide relevant age-related diagnostic information, but must be used appropriately. For example, a child who is small for his age may have perfectly normal bones for his size. We propose to report pediatric BMD results as age and gender matched percentiles, along with height and weight percentiles. This approach may help determine whether skeletal deficiencies are due to primary causes or are related to secondary causes, such as small stature or delayed maturation. Further, as with the use of growth charts, ethnicity should be ignored. Ethnicity is self reported and often unreliable. There is also the problem of mixed ethnicities, and the effects of ethnicity on BMD largely disappear after adjusting for height and weight.

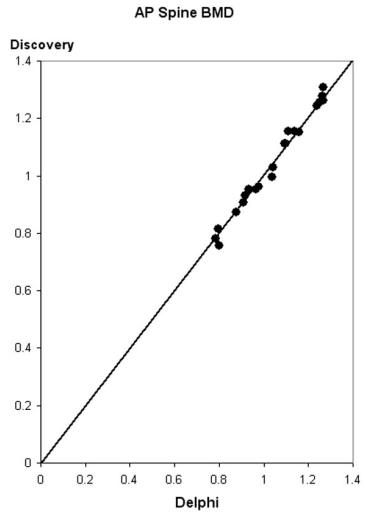
Disclosures: ***T.L. Kelly****, None.*

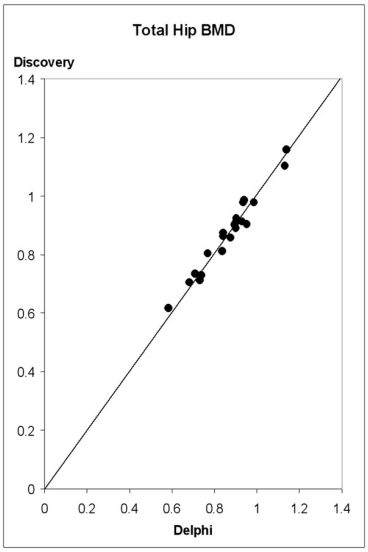
M093

Discovery BMD Correlation Study. A. J. Laster*[1], C. Smith*[1], T. L. Kelly[2], L. A. Wierbowski*[2], K. E. Wilson[2]. [1]Arthritis and Osteoporosis Consultants of the Carolinas, Charlotte, NC, USA, [2]Hologic, Inc., Bedford, MA, USA.

A bone mineral density (BMD) correlation study at the AP Spine and Hip was performed to examine the relationship between the Hologic Discovery and Delphi models. The Discovery is a new densitometer introduced by Hologic which supports a reduced scan time (66%) with reduced radiation dose (30%) to the patient.
BMD at the AP spine and hip of 21 women, age range 28 to 88 years, was measured using the "Express" 10 second scanning mode on a Discovery and using the "Fast" 30 second scanning mode on a Delphi. Linear regression of the BMD's was performed with the BMD measured with Discovery as the dependent variable. If the intercept was not significantly different than zero at the 95% confidence level, the intercept was restricted to zero.
The two models were highly linearly correlated (see figure), with $r = 0.99$ for the spine and $r = 0.98$ for the total hip and femoral neck regions. None of the offsets for the AP spine (L1-L4), total hip, and femoral neck regions were significantly different from zero. The slope for the AP Spine was 1.004 ± 0.004 when the offset was restricted to zero. The slopes were 1.005 ± 0.006 for the total hip and 1.017 ± 0.009 for the femoral neck when the offsets were restricted zero.
The Discovery's 10 second Express scan mode and the Delphi's 30 second Fast scan mode were found to be highly linearly correlated, without significant offsets, and slopes which were not significantly different from unity. Other studies have found similar results when

comparing the 30 second Fast scan mode and the 60 second Array scan modes, and when comparing the QDR Delphi to the QDR-4500. Therefore, all of these models and scan modes provide equivalent diagnostic and BMD information, however, the scanning time and radiation dose has been significantly decreased on the Discovery.





Disclosures: ***A.J. Laster****, Hologic, Inc. 2.*

M094

Use of a Combination DXA System and Exam Table (DEXAM) for Assessing Spine and Hip Density: The GE Lunar Duo. L. S. Blumenthal*. Women's Health Consulting, Illinois, Chicago, IL, USA.

While primary care physicians see many patients at risk for osteoporosis, they frequently do not offer central bone densitometry due to lack of space for a full table densitometer. Many physicians also have concern over the degree of expertise needed to operate a DXA system. Recently, the Lunar Duo (GE Medical Systems), a combined DXA/exam table (DEXAM), was introduced to bring central densitometry to the primary care office. The Duo consists of a spine/hip DXA scanner embedded in an exam table, complete with drawers, procedure tray, retractable leg supports, paper roll, and washable table pad. The scanner arm swings to the side for easy exam table access. The Duo offers simplified acquisition and analysis with OneScan: spine and dual femur measurement in a single exam without repositioning the patient. Computer assisted densitometry (CAD) software automatically identifies scans with nonstandard positioning, artifacts, or unusual BMD values for operator review. The performance of the Duo was compared to a dedicated full table bone densitometer.

Fifteen female volunteers were measured on the Duo and on the Lunar Prodigy (GE Medical Systems) using OneScan. Volunteers were 45 to 78 years of age (average age 57 ± 11 years). Each subject was measured 3 times on the Duo with interim repositioning to determine precision error. BMD results were determined using manufacturer recommended analysis protocols. Precision error was calculated as the RMS standard deviation for the repeat measurements, expressed as a percent (%CV). Duo spine and hip BMD values were compared to the Prodigy values using a paired Student's t-test based on the first measurement obtained from the Duo.

Scan times for the Duo were slightly longer than for the Prodigy, but both were greatly reduced with OneScan. Precision error was consistent with published values for existing fan beam DXA systems, even using the simplified OneScan protocol. The Duo spine and femur BMD results were not significantly different from Prodigy values, except for a small (1.6%) difference seen at the total femur that was not considered clinically significant. The Duo was determined to be completely functional for patient and physician use as an exam table.

We conclude that the Lunar Duo can provide accurate and precise bone density measurements of the spine and hip in a system that can also function as an examination table.

	L1-L4 Spine	Femur Neck	Total Femur
Duo Precision (%CV)	0.8 %	2.1 %	1.2 %
Duo BMD (g/cm^2)	1.178	0.953	0.995
Prodigy BMD (g/cm^2)	1.188	0.967	1.011
p-value for difference	0.37 (NS)	0.11 (NS)	0.01

Disclosures: ***L.S. Blumenthal****, GE Medical Systems Lunar 2.*

M095

The Influence of Vertebral Fractures and Fracture Severity on Lumbar Spine Bone Mineral Density in Postmenopausal Women. C. Wu*, G. von Ingersleben, S. Zaim*, E. Griffith*, T. Fuerst*. Synarc Inc, San Francisco, CA, USA.

Lumbar bone mineral density can be influenced by various degenerative changes, surgical procedures and artifacts that can prevent an accurate assessment of spine T-score and fracture risk. We investigated the relationship of vertebral fracture lumbar BMD assessed in the anterior-posterior projection.

We measured lumbar BMD (L1-L4) in 422 postmenopausal women with fractures in the lumbar spine, aged 50-85 years. Lateral lumbar spine radiographs were obtained in all subjects and graded for vertebral fractures according to the Genant semiquantitative method (mild = 20-25% height reduction, moderate = 25-40% height reduction, severe = >40% height reduction). In each woman found to have a single vertebral fracture in the lumbar spine, the T-score of each individual vertebra was calculated and the T-score of fractured vertebra was compared to the T-score of non-fractured vertebrae. The difference in T-score was evaluated for any fracture and by fracture grade.

The T-score differential was found to increase with severity of vertebral fracture. On average, mild fractures had a negligible effect on T-score in L1 and L2 but a large effect in L3 and L4.

Vertebral Fracture Grade	Mean T-score Differential (Fracture - No Fracture)			
	L1 (n)	L2 (n)	L3 (n)	L4 (n)
Grade = 1	0.12±0.72 (93)	0.02±0.55 (28)	0.64±0.84 (28)	0.74±1.01 (33)
Grade = 2	0.54±1.01 (66)	0.43±0.65 (33)	0.60±0.35 (18)	0.06±0.76 (4)
Grade = 3	1.26±0.94 (22)	0.70±0.78 (3)	2.14 (1)	NA (0)
Grade ≥ 1	0.37±0.91 (181)	0.26±0.64 (64)	0.21±0.99 (47)	0.67±1.00 (37)

The influence of vertebral fractures on lumbar BMD and T-score in postmenopausal women increases with the severity of the fracture. The T-score of a fractured vertebra was higher than vertebrae without fracture, ranging from about 0.5 to 0.75 T-score units. While the impact on total spine BMD and T-score is smaller, it remains valuable to exclude fractured vertebrae during the scan analysis.

Disclosures: ***C. Wu****, None.*

M096

Comparison of Lunar and Hologic BMD and Hip Structural Analysis Measurements. M. A. Laskey*[1], B. C. C. Khoo*[2], R. I. Price[2], A. Prentice[1], T. J. Beck[3]. [1]MRC Human Nutrition Research, Cambridge, United Kingdom, [2]Sir Charles Gairdner Hospital, Perth, Australia, [3]Johns Hopkins University, Baltimore, MD, USA.

Bone fragility depends on the quantity, distribution and material properties of bone. Bone mineral density (BMD), from conventional DXA analyses, is influenced by these properties, it does not measure them. HSA attempts to express data recorded in a DXA scan in a mechanically relevant way so that strength properties can be addressed directly. This study compares femoral neck BMD and HSA results from 2 DXA scanners: Lunar MD and Hologic QDR-1000W. Eight female and 3 male volunteers aged 22-60 years had duplicate hip scans, without repositioning, on both DXA systems within one week. Hip scans were analysed using conventional DXA analyses (BMD) and HSA software (version 1). HSA measurements at the narrowest point on the femur neck (NN) included: BMD, cross-sectional area (CSA), section modulus (Z, indicator of bending strength), neck width (NW), cortical thickness (CT) and buckling ratio (BR, index of cortical stability).

Precision (CV) for repeat measurements were: conventional neck BMD 1-2%, NNBMD 2-3%, CSA 3-4%, Z 2-5%, NW 2-5%, CT3%, BR 6-10%. HSA precision appeared to be independent of DXA system, inferior to BMD measurements, but prone to outlying results. When the difference between the 2 DXA system measurements was plotted against the mean for each index (Bland and Altman plot), gradients were significantly different from zero. After conversion into natural logs, gradients for all measurements became nonsignificant. The constant remained significant for all measurements indicating that calibration did not remove systematic differences between scanners except for NW. For NNBMD, CSA, Z and CT, Hologic results were greater than Lunar values (difference about 50%) but lower for BMD and BR (differences of –17% and -50%). These results suggest that differences in pixel value but not spacing are affected. Regression analyses of BMD against HSA measurements demonstrated that BMD, for both DXA systems, was a good predictor of NNBMD, CSA and CT (>80% of variance), reasonable predictor of Z (>60%) but poor predictor of NW and BR (<60%).

In summary, there are large systematic differences in BMD and HSA measurements between the Lunar and Hologic DXAs, making comparison of results from different DXAs difficult. In contrast to BMD, HSA measurements are determining different properties of a hip and provide mechanically relevant information, but do so with inferior precision.

Disclosures: ***M.A. Laskey****, None.*

M097

A Novel Method for the Measurement of Degree of Mineralization in Bone using Bench-top μCT. T. Dufresne*, P. A. Chmielewski*, B. Borah*. Procter & Gamble Pharmaceuticals, Mason, OH, USA.

High resolution 3-D micro computed tomography has been extensively used in recent years to accurately measure the complex trabecular architecture of human and animal samples. We describe the viability of using a bench-top μCT scanner (Scanco μCT20) to measure the degree of mineralization in bone biopsies. The technique relies on the physical property of CT that the grey level intensity (x-ray attenuation coefficient) is proportional to radio opacity (density) of material. However, the effect of beam hardening due to the polychromatic radiation of the scanner and partial volume averaging due to limited resolution (typically 20 - 30 μm) need to be considered. A calibration phantom of multiple concentrations of hydroxyapatite was used to calibrate the CT grey scale to density values. A linear relationship was observed between equivalent % calcium content of the phantom and x-ray attenuation. After correction for beam hardening, the peak values obtained in the histograms of human iliac crest samples seem to correspond closely with those predicted by other methods, such as micro-radiography. The resulting histogram reveals that the tail on the low grey level side is very large, due to the partial volume averaging (Figure 2). Since only the surface voxels should be affected by the partial volume averaging effect, all surface voxels were peeled off from the segmented bone image, resulting in a histogram which is more gaussian (Figure 3). We will present data from pre-clinical (ovariectomized minipig) and human (PMO) iliac crest biopsy studies, which provide validation that the increase in mineralization induced by an anti-resorptive such as risedronate, can be detected by this new method.

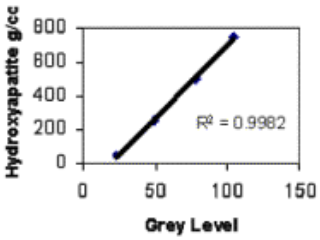


Figure 1) Calibration curve between the Hydroxyapatite % calcium equivalent and

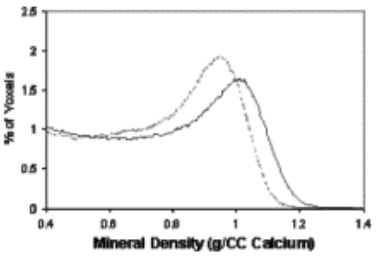


Figure 2) Histogram of baseline (dotted line) and 3 yr risedronate treatment without partial volume averaging correction.

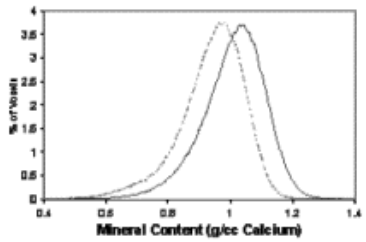


Figure 3) Histogram of baseline (dotted line) and 3 yr risedronate treatment in a patient with partial volume

Disclosures: ***T. Dufresne****, None.*

M098

Simple Indices on Plain X-rays Strongly Predict Risk of Hip Fractures in Mexican Men and Women. P. Clark[1], J. Talavera*[2], L. Palermo*[3], S. R. Cummings[3]. [1]Clinical Epidemiology Unit, Centro Medico Nacional, IMSS-Faculty of Medicine UNAM, Mexico City, Mexico, [2]Clinical Epidemiology Unit, Centro Medico Nacional, IMSS, Mexico City, Mexico, [3]SF Coordinating Center, San Francisco, CA, USA.

Hip x-rays are universally available, even where densitometry is not affordable. We postulated that simple measurements on hip x-rays may be powerful predictors of hip fracture in men and women. We conducted a study to test the hypothesis that simple measurements on hip x-rays could predict hip fractures and identify women and men at high risk of fracture. A total of 254 pelvic radiographs from a case control study on hip fractures and risk factors in individuals over 45 years old were included for this study; 184 women (78 cases and 106 controls) and 70 men (33 cases and 37 controls) Two measurements were performed by two trained observers in all studies: Singh index, a measure of the quality and connectivity of bundles of trabecular bone of the proximal end of the femur were the change in trabecular patterns is graded in six categories from 6 normal pattern to 1 the worst, and the Cortical Index obtained by dividing the sum of the thickness of the internal and external cortices by the total diameter of the shaft at the distal limit of the sub trochanteric region, measurements of 0.20 to 0.49 are associated with osteoporotic changes. In age-adjusted generalized logit models, for every 1 unit decrease in Singh Index, men and women had a 45.5 (7.6-273.2) and 6.8 (3.7-12.5) increased risk of trochanteric fracture. For cervical fracture a risk of 23.8(4.0-143.2) for men and 21.0(7.8-56.3) for women was found. After adjusting for Cortical Index, Singh index remained predictive of trochanteric fractures in men 52.5(7.4-370.0) and women 5.3(2.6-10.9) and cervical fractures in men 13.1(1.9-90.9) and women 25.8 (7.8-86.1). For Cortical Index alone, a 0.1 decrease was also associated with increased risk of trochanteric [3.1 (1.6-6.3) men, 3.2 (2.1,4.9) women] and cervical fractures [4.1 (1.7-9.7) men, 3.2 (1.8,5.8) women], but not when Singh Index was included in the model.
This is the first study to show that measurements of hip structure predict hip fractures in both men and women. These simple structural measurements are powerful measurements of risk of hip fracture and particularly valuable where densitometry is unavailable.

Disclosures: ***P. Clark****, None.*

M099

Patients with Cystic Fibrosis Have Normal Bone Geometry but Reduced Bone Mineral. J. H. Cole*[1], K. Kent*[2], G. S. Bhudhikanok*[3], L. K. Bachrach[3], M. C. H. van der Meulen[1]. [1]Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA, [2]Department of Medicine, Stanford University, Stanford, CA, USA, [3]Department of Pediatrics, Stanford University, Stanford, CA, USA.

Low bone density and fractures occur more commonly in patients with cystic fibrosis (CF). Large deficits have been observed in bone mineral content (BMC), bone mineral density (BMD), and bone mineral apparent density (BMAD) at various anatomic sites (Bhudhikanok *et al*. 1996). The purpose of this study was to assess the correlation between measures of bone mineral and calculated cross-sectional bone strength indices in patients with CF. The bone mineral of 41 CF patients (15 male, 26 female, 8-48 years) was assessed at baseline and at an average follow-up time of 1.5 years for the femoral neck and whole body using dual energy X-ray absorptiometry in pencil beam mode (QDR 1000W, Hologic Corp, Waltham, MA). BMC (g) and areal BMD (g/cm^2) were recorded directly from the scans, and BMAD (g/cm^3), an estimate of volumetric mineral density (Katzman *et al*. 1991), was calculated for the femoral neck and mid-diaphysis. Strength at the left femoral mid-diaphysis was assessed with a series of subregion analyses on each whole body scan (van der Meulen *et al*. 1996). The diameter and cross-sectional area of the mid-shaft were estimated and used to compute two geometry-based structural measures, the whole bone strength index (S_B) and cross-sectional strength index (Z). Relationships between strength measures (S_B, Z) and bone mineral measures (BMC, BMD, BMAD) were examined using simple linear regressions. S_B and Z showed positive relationships with BMC (r^2=0.58-0.90) and BMD (r^2=0.39-0.86) at all sites at baseline and follow-up ($p<0.0001$). Changes in S_B and Z from baseline to follow-up were linearly related to changes in BMC and BMD ($p\leq0.025$). Both S_B and Z displayed a strong positive linear relationship with body mass at baseline and follow-up (r^2=0.70-0.78, $p<0.0001$). The relationship of Z with body mass was compared to that of normal Caucasian subjects (48 male, 53 female, 9-26 years) and was statistically identical, indicating similar mid-diaphyseal geometry for body size. However, this geometric analysis assumes the same mineral distribution for CF and normal subjects and does not take into account the lower BMAD in CF subjects. Taken together, the similar geometry but reduced BMAD in CF patients indicate that the deficits in bone mineral density with CF produce reduced bone strength. The femoral diaphysis of CF patients would need to be larger to compensate for the lower mineralization. These findings suggest a potential explanation for increased bone fragility observed in CF patients.

Disclosures: ***J.H. Cole****, None.*

M100

Relationship Between the Regularity of 3D Trabecular Bone Samples and of their 2D Projections. S. Bretteil*[1], R. Jennane*[1], G. Lemineur*[1], A. Estrade*[2], B. Brunet-Imbault*[3], C. L. Benhamou[3], E. Lespessailles[3], R. Harba*[1]. [1]University of Orleans, Laboratory of Electronics, Signals, Images, Orleans, France, [2]University of Orleans, MAPMO, Orleans, France, [3]Inserm ERIT-M 0101, Orleans, France.

Fractal analysis applied to bone radiographs is often used to characterize the microarchitecture changes in osteoporosis. However the theoretical background of this analysis is

not perfectly mastered. Indeed the relationship between 2D and 3D parameters remains unclear even if a high correlation has been experimentally found between those two parameters. Recently, a theoretical study has shown that 3D regularity (R_{3D}) of a 3D isotropic fractal is equal to the regularity of its projection (R_{2D}) increased of 0.5 ($R_{2D} = R_{3D} + 0.5$). The object of this contribution is to study the 3D regularity of 22 femoral head samples and the 2D regularity of their projections to assess if this relation can be applied in this case. In addition, the correlation between morphological parameters and the 2D and 3D regularities will be also calculated.
3D X-Ray microtomographic images were acquired using a microQCT device (Skyscan 1072). The projections were numerically simulated. Each 3D image had a size of 4.8×4.8×4.8 mm^3 with an isotropic voxel size of 12.02 µm. 3D regularity was calculated with the box counting method and that of the projection with the variance method. Moreover 3D morphological parameters were calculated by the Mean Intercept Length method : bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), trabecular number (Tb.N) and bone surface fraction (BS/TV).
We found a difference of 0.53 ± 0.046 between the 2D and the 3D regularity. This shows that the theoretical result found on certain fractal objects about their regularity ($R_{2D} = R_{3D} + 0.5$) can be applied to this kind of binary object (trabecular bone tissue). The following table shows the correlation coefficient between 3D morphological parameters and regularities.

Correlation coefficient	2D regularity	3D regularity
BV/TV	-0.769	-0.955
Tb.N	-0.687	-0.784
Tb.Th	-0.656	-0.872
Tb.Sp	0.668	0.784
BS/TV	0.693	0.879
3D regularity	0.845	1

We observe a good correlation between morphological parameters and 2D as well as 3D regularity. This study suggests that a simple 2D measurement allows to quantify accurately the 3D trabecular structure and can be helpful for the early diagnosis of osteoporosis.

Disclosures: ***C.L. Benhamou****, None.*

M101

An In-Vivo Cone Beam Micro-CT Scanner for Whole Body Investigations of Mice. K. Engelke[1], M. Karolczak*[1], S. Ulzheimer*[1], H. Fuchs*[2], M. H. de Angelis*[2], W. Kalender*[1]. [1]Inst Med Phys, Univ of Erlangen, Erlangen, Germany, [2]Inst Exp Gen, GSF Nat Res Cen f Environ and Health, Oberschleissheim, Germany.

In-vivo micro CT investigations of genetically engineered laboratory animals such as mice provide exiting opportunities for the study of osteoporosis and arthritis. The possibility of longitudinal studies for monitoring disease progression and effectiveness of pharmaceutical interventions in the same animal will greatly reduce the number of animals required to achieve a given study power. Here we report on the development of a micro-CT scanner enabling 3D in-vivo scanning of mice.
The cone-beam microtomography imaging system consists of a microfocus X-ray tube (20-90 kV, focus: Ø<15 µm, power: 80 W), 2D CCD detector (1024 x 2048 elements, magnifying fiber-optic taper, effective pixel pitch at input window: 66 x 66 $µm^2$, input window size: 60 x 120 mm^2, $Gd2O_2S$:Tb phosphor, dynamic range: 14 bit, readout time: 1.2 s, Peltier cooling) and a high accuracy positioning system. The anesthetized animals are mounted in a vertical position. X-ray tube and detector are fixed and located 500 mm apart. The mice can be translated between them so that the magnification can be optimally adapted its size. The maximum sample diameter is 60 mm. The temperature in the vicinity of the animal is controlled throughout the scan. For tomographic reconstruction an approximate Feldkamp algorithm with integrated scanner misalignment correction is used.
In the scanner so far acquisition times of 20 min per scan were achieved for full body scans of living mice. Anesthesia times were approx. 30 min and were well tolerated by the animals. Dose values of typically 100 to 300 mGy were determined. Tomographic reconstruction times (data volume: 512^2x1024 voxels) were 15 minutes on a dual Pentium IV 2GHz computer with 2 GB memory. The sampling distance for whole body scans was 65-100 µm. Resolution can be increased in smaller objects. For examples, in the extremities or the tail the sampling distance can be reduced to 10 – 20 µm. Motion artifacts remain a problem in scanning abdominal regions.
An in-vivo µCT scanner for mice was successfully developed and tested. Due to the variable magnification scan parameters can be optimally adapted to the size of the mouse. Scan times of 20 min allow for high throughput in-vivo scanning of mice and provide the possibility of longitudinal studies in one animal.

Disclosures: ***K. Engelke****, None.*

M102

Development, Optimization and Validation of Automated/Interactive Strut Analysis Software: a New Quality Assessment Approach. S. Picard, D. Brown*, J. P. Brown. Centre de recherche du CHUQ - Pavillon CHUL, Groupe de recherche en maladies osseuses, Ste-Foy, PQ, Canada.

Histomorphometry is both a theoretical and practical assessment tool in bone science. Some histomorphometric parameters have been developed and improved but none have been evaluated or validated as it is the case for several chemistry and biochemistry methods and as proposed by the ICH Guidelines or other regulatory agencies. The aim of this study was to evaluate and validate new automated histomorphometric strut analysis software in conformities with ICH Guidelines for development, optimization and validation approach. Iliac biopsies were obtained from 12 women aged from 33 to72 years old; mean 59. Strut analyses were performed with new strut analysis Nova Prime Bioquant's (R&M biometrics, Nashville) image-analysis software. Calibration of the system was done before histomophometric data collection. Saved JPEG and TIFF images from twelve trans-iliac biopsy section have been used for this study. No significant difference has been found between the two saved image types. Correlation between manual and the interactive automated method was evaluated Repeatability and intermediate precision study have also been performed. Very good results were obtained when comparing interactive and manual strut analyses. Coefficient of variation from intermediate and repeatability assessment was satisfactory for all parameters as well as when comparing manual and interactive measurements. In conclusion, our data presents the first validation procedure for histomorphometric struts analysis. The results demonstrate that the automated/interactive strut analysis approach is a valuable and reproducible for the assessment of mechanical resistance of bone tissue.

Intermediate precision — Coefficient of variation (%)

Free/TV	Free/BV	Node/TV	Node/BV	Total Strut Length	NF Strut %	NN Strut %	FF Strut %
3,7%	3,9%	1,7%	5,1%	2,1%	4,3%	15,2%	3,0%

Repeatability — Coefficient of variation (%)

Free/TV	Free/BV	Node/TV	Node/BV	Total Strut Length	NF Strut %	NN Strut %	FF Strut %
3,20	7,04	8,53	4,15	1,93	8,58	7,04	12,40

Disclosures: ***S. Picard****, None.*

M103

Biomechanical Impact of Aluminum Accumulation on the Pre- and Post-Yield Behavior of Rat Cortical Bone. G. R. Cointry*[1], D. Salica[1], A. L. Negri*[2], J. L. Ferretti[1]. [1]Centro de Estudios de Metabolismo Fosfocálcico, Faculty of Medicine, UNR, Rosario, Argentina, [2]Research Department, Del Salvador University, Buenos Aires, Argentina.

In order to analyze the biomechanical impact of a chronic aluminum accumulation in bone tissue on the whole-bone behavior, 14 rats aged 90 days received ip doses of 27 mg/d of elemental Al as Al(OH)3 during 26 weeks while other 14 remained as controls. Their femur diaphyses were studied tomographically (pQCT) and mechanically tested in bending. The load/deformation curves obtained allowed distinction between effects observed during the linearly elastic (Hookean) and nonlinear (non-Hookean) behaviors of bones before and after the *yield point*, respectively.
No effects on body weight were observed. Aluminemia and bone histological data confirmed the Al accumulation. Treatment reduced the cortical bone mineralization (volumetric cortical BMD, $p<0.01$) with a negative impact on the intrinsic bending stiffness of the cortical tissue (calculated Young's elastic modulus, $p<0.05$). Despite of the absence of any cortical mass increase (cortical cross-sectional area), an improvement of the spatial distribution of the available cortical tissue (cortical cross-sectional moment of inertia for A-P bending, $p<0.05$) occurred through a directional modulation of the *modeling drifts* during growth.
This presumably adaptive response should have resulted adequate for maintaining a normal diaphyseal *stiffness* (load / deformation ratio) according to the bone "mechanostat" theory, but not so to provide a complete neutralization of the impaired diaphyseal *strength* (ultimate load reduction, $p<0.05$). Although a relative inhibition of bone formation could not be discarded, an Al-induced impairment of the bone ability to resist loads beyond the yield point (difference between the ultimate and yield loads, $p<0.01$) should have caused the striking disruption observed between effects on bone stiffness and strength. In addition to describe an unusual finding, these results suggest that the little-known microstructural factors affecting the *post-yield* behavior of cortical bone in these and other conditions ought to be further investigated in specifically designed studies as a promising field in skeletal research.

Disclosures: ***J.L. Ferretti****, None.*

M104

Effects of Hypophysectomy (Hx) and Recombinant Human Growth Hormone (rhGH) on Material Properties and Pre- and Post-yield Strength of Rat Remur Diaphyses. S. Feldman*, G. R. Cointry*, M. R. Ulla, R. Capozza*, L. Sarrió*, J. L. Ferretti*. Centro de Estudios de Metabolismo Fosfocálcico, Faculty of Medicine, UNR, Rosario, Argentina.

Aiming to analyze biomechanically the musculoskeletal effects of Hx and a partial replacement with rhGH, we determined the intrinsic stiffness (elastic modulus, E) and volumetric BMD (vBMD) of the cortical bone; the area and moment of inertia (CSMI) of the cross-sections, and the structural stiffness and pre- and post-yield strength of the femur diaphyses (by pQCT and mechanical tests), and the gastrocnemius weight of rats, either intact (n = 9) or Hx at 15 days of age (20), otherwise untreated (Hx controls, 4) or given 30 (8) or 150 (8) mIU/d sc of rhGH since 15 days after surgery during 45 days.
The Hx delayed the osteomuscular development (gastrocnemius weight, bone geometric properties) with no complete catch-up, thus affecting the diaphyseal stiffness and strength. It also reduced the cortical vBMD through an undefined mechanism, paradoxically increasing the elastic modulus of cortical bone. The Hx also affected the correlation between bone geometric and material properties (CSMI *vs* E), suggesting an anti-anabolic shift of the bone *mechanostat setpoint* for triggering the bone modeling response to strains provoked by mechanical usage, that was partially prevented by rhGH. As an integrated result, Hx reduced the stiffness and the post-yield and ultimate strength of the diaphyses. The rhGH treatment tended significantly to prevent Hx effects on bone and muscle development correlatively; but it failed to prevent the bone material stiffening (E) and the impairment in structural stiffness / strength of the diaphyses.

The Hx/rhGH effects on bone/muscle development (geometry) were closely parallel as expected. Not so the curious, demineralizing / stiffnening effect of Hx on bone tissue and the unusual effects observed on the post-yield strength (less clearly related than the former to muscle development and unaffected by rhGH at the assayed doses). These should reflect changes in bone tissue microstructure associated with crack generation and progress, unrelated to bone mineral mass (delay in collagen turnover with associated changes in crystal size and arrangement), resulting from the suppression of some other hormones, presumably thyroid. The effects of larger rhGH doses and the interaction of other hormones with the described effects remain to be investigated.

Disclosures: ***J.L. Ferretti****, None.*

M105

A DXA Study of Muscle-Bone Relationships in the Whole Body and Limbs of 1,900 Normal Men and Pre- and Post-Menopausal Women. C. Cure-Cure*[1], S. García[2], P. Cure-Rodríguez*[1], G. Cointry*[2], R. Capozza*[2], J. L. Ferretti[2]. [1]Universidad Metropolitana de Barranquilla, Barranquilla, Colombia, [2]Centro de Estudios de Metabolismo Fosfocálcico, Faculty of Medicine, UNR, Rosario, Argentina.

In whole-body studies with DXA *[Ferretti et al; Bone 22:683,1998*, n = 1,450*]* we had shown that the densitometric mineral mass, either crude (BMC) or statistically adjusted to fat mass (FA-BMC) in order to avoid any fat interference with its determination, correlated linearly with the lean mass (LM) showing similar slopes but decreasing intercepts in the order: pre-MP women > men > post-MP women > children. This evidenced 1. the homogeneous control of bone status by muscle strength in the species through the bone "mechanostat", and 2. the interaction of sex hormones with that regulation. Now we aim to expand that evidence by studying 1,900 normal Hispanic adults (60men, 600 pre-menopausal women, 1,240 post-menopausal women), including also the same determinations in the upper and lower limbs.

In all the studied regions the slopes of the BMC or FA-BMC vs LM relationships were always parallel. However, interesting region-related differences were found between the intercepts of the curves. In the whole body, the crude-BMC/LM relationships showed the same intercept differences as previously observed (pre-MP women > men > post-MP women). In the lower limbs the variance of the data was substantially reduced, and those differences were highly significant but lesser in magnitude, showing the order: pre-MP women > men = post-MP women. In the upper limbs the decreasing intercept order was: men > pre-MP women > post-MP women. After fat-adjustment of the BMC, the intercept order in both limbs was men > pre-MP women > post-MP women. Parallelism of the curves was maintained in all cases.

The parallelism of the curves suggests a common biomechanical control of bones by muscles in the species. Assuming that LM is proportional to muscle mass, results suggest that the sex-hormone-induced differences in the DXA-assessed muscle-bone proportionality in humans would vary according to the region studied. Assuming also that BMC adjustment reduced the influence of fat on body weight, this could be related to the different weight-bearing nature of the musculoskeletal structures studied. The study design did not account for some gender-related aspects of hand/foot skeletal morphometry which could also help to explain those differences.

Disclosures: ***J.L. Ferretti****, None.*

M106

Short Term Stability of Bone Formation in the Human Iliac Crest Evaluated using a Novel Quadruple Tetracycline Labeling Technique. H. Zhou[1], M. Bostrom[2], D. W. Dempster[1], V. Shen[3], F. Cosman[1], R. Lindsay[1]. [1]Regional Bone Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Medicine, Hospital for Special Surgery, New York, NY, USA, [3]Skeletech, Inc., Bothell, WA, USA.

Classically, bone remodeling has been evaluated in iliac crest biopsies following double tetracycline labeling. This technique allows a single time point evaluation of bone formation based upon the extent of, and distance between the two tetracycline labels. Repeat biopsies are required to determine changes over time and are generally not feasible in short time frame (< 9 months). To overcome this problem, and allow more short term (6-8 weeks) evaluation of changes in bone formation we investigated a quadruple labeling system. Standard tetracycline labels were administered to healthy postmenopausal women following the 3-10-3 schedule. Six weeks later, labeling was repeated following the same schedule. Iliac crest biopsies were performed 1 week following completion of the final tetracycline label. In 3 subjects labeling was performed with tetracycline followed by oxytetracycline and the labels reversed for the second course. In 3 more subjects the same tetracycline was given twice for the initial labeling, followed 6 weeks later by the other tetracycline. Standard procedures were used to prepare the biopsies for histomorphometry. Double and quadruple labels were identified and dynamic parameters of bone formation calculated for each set of double labels. When only double labels were used all dynamic measures of mineralization were similar using either the first or second set of labels with both tetracycline schedules (MAR 0.46+/-0.15 and 0.0.43+/-0.13 mc/day; BFR 0.027+/-0.01 and 0.019+/-0.01 mc.mc2/day in cancellous bone). This conclusion held true in cancellous bone, the endocortical junction, and within the cortex. Periosteal labels were insufficient to allow conclusions to be drawn. In general, label identification was easier when one tetracycline was given for the first pair of labels and a different tetracycline used for the second pair. For short term evaluation of bone formation in cancellous and cortical bone, as well as at the endocortical junction, quadruple labeling allows two measures of bone formation to be made within a short period in a single biopsy.

Disclosures: ***H. Zhou****, None.*

M107

Fracture Load of Fresh-Frozen Cadaveric Proximal Femora Correlates Much Better with Non-Invasive, 2D Radiographically Derived Micro-Structural and Macro-Anatomic Indices Than with BMD. C. D. Arnaud[1], D. Steines*[1], S. Liew*[1], A. Nazarian*[2], B. Snyder*[2], B. J. Linder*[1], P. Lang*[1]. [1]Imaging Therapeutics, Inc., San Mateo, CA, USA, [2]Orthopedic Biomechanics Laboratory, Harvard Medical School, Boston, MA, USA.

We showed that 2D measurements of trabecular structure in ordinary radiographs of cores of proximal cadaveric femora correlate with similar measurements made with 3D μCT. Those 2D measurements also correlate with biomechanical failure loads applied to cores and with bone stiffness. The present study was done to determine if 2D measurements of bone structure could be measured in radiographs of whole proximal cadaveric femora and to compare the correlation between fracture load and BMD and fracture load and radiographically derived micro-structural and macro-anatomic indices. DXA-BMD and standard radiographs were obtained from the proximal region of fifteen intact cadaveric femora. Using an Instron mechanical testing device, the femora were tested biomechanically at 15-55 degrees of tilt and 8 degrees of external rotation to generate a 2 dimensional load vector at the femoral head. Radiographs were analyzed in several regions of interest at the femoral head, neck and proximal shaft to yield indices of trabecular micro-structure and macro-anatomic indices (such as cortical thickness). There was a weak positive correlation of femoral neck BMD with femoral neck fracture load ($r=0.34$, $p=0.11$). Femoral neck fracture load was more highly correlated with 2D indices of proximal femoral trabecular structure such as mean node-to-free-end segment thickness ($r=0.50$, $p<0.03$) and local maximum of inter-trabecular spacing ($r=-0.59$, $p=0.01$) and macro-anatomic measurements such as mean cortical thickness ($r=0.66$, $p<0.005$). Multivariate linear regression analyses of these combined structural and macro-anatomic parameters provided estimates of predicted fracture load that correlated highly ($r=0.78$, $p<0.02$) with actual fracture load.

We conclude that 2D measurements of bone structure on hip radiographs correlate highly with biomechanical fracture loads at the femoral neck. These results suggest that inexpensive and noninvasive proximal femoral trabecular micro-structure and macro-anatomic analysis from hip radiographs may yield vastly improved diagnostic assessment of osteoporosis and estimation of fracture risk as compared with BMD.

Disclosures: ***C.D. Arnaud****, None.*

M108

Community Osteoporosis Ultrasound Screening: Reported Outcomes. J. A. Robbins[1], G. Voelm*[2]. [1]Internal Medicine, UC Davis, Sacramento, CA, USA, [2]Personal Health Institute, Carmichael, CA, USA.

Many individuals are now self-referring for measurements of BMD in drug stores and at health clubs. There is a long history of community screening for conditions such as hypertension, anemia, and glaucoma, frequently with relatively poor follow-up and treatment. Now individuals are ordering BMD, carotid ultrasounds and even MRIs for themselves. It is not clear what becomes of the results of screening for which individuals have self-referred and paid, nor how it affects future diagnostic studies and/or treatment. This study investigates what happened to a sample of individuals who had paid an average of $25 to have a heel ultrasound preformed in northern California or neighboring Nevada. All of those tested had been given a printout of their results and instructed to take it to their physicians. Telephone questionnaire interviewers called to a stratified sample of 287 individuals with the ultrasound defined osteoporosis, 235 osteopenia and 287 normals, approximately 1 year after testing in order to see what they had done with their results, if they had taken them to their MDs, and how the MDs had responded. The response rate to the telephone survey was just under 50%> Non-response was mostly due to our inability to establish phone contact in a short period. The average age was 66 (std =11) and was almost entirely composed of women. More than 70% of those responding correctly recalled their results (osteoporotic, osteopenic or normal), although a third of individuals with results in the osteoporotic range remembered them as less severe. 71% of all tested individuals who responded reported taking the results to their physicians, 83% of those in the osteoporotic range. Most physicians did not order more testing in normal or osteopenic individuals but did in 50% of those reported by ultrasound to be osteoporotic. A full two thirds of heel ultrasound osteoporotic individuals reported being treated, 28% of these without further testing. An average of 20% of the sample reported improving their diet and exercise behaviour.

Community based BMD screening appears to be having a bigger impact than was seen with previous community screening projects. Most individuals reported taking the results to their physicians. If the results did not suggest osteoporosis, even in this relatively old population, most physicians accepted the values reported, from a non-"gold standard" technique, as sufficient testing. A small but significant number of MDs are prescribing treatment without further studies. These results suggest that public osteoporosis screening substantially increases both diagnosis and treatment. What this means medically and financially deserves further study.

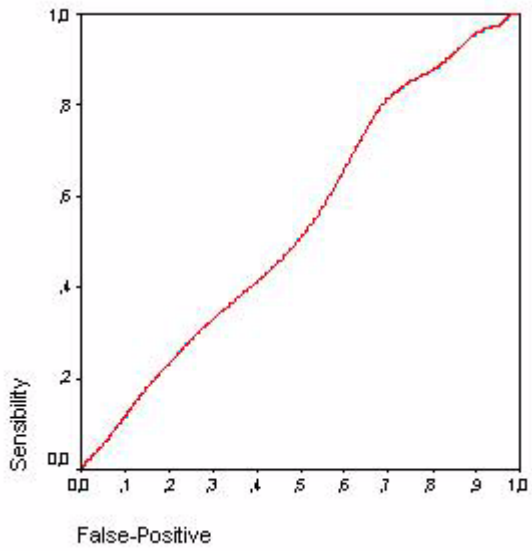
Disclosures: ***J.A. Robbins****, None.*

M109

Quantitative Ultrasound Measurements are not Useful to Discriminate Postmenopausal Women with Colles Fracture from Controls. M. Sosa[1], P. Saavedra*[1], The GIUMO Study Group*[2]. [1]Bone Metabolic Unit, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria. Canary Islands, Spain, [2]Cooperative Working Group, SEIOMM Italfarmaco, Spain.

Background. There are some controversial findings when studying whether or not Colles´ fracture is associated to low values of bone mineral density (BMD). Indeed the controversial is even greater when BMD is measured by Quantitative Ultrasounds (QUS).The objective of our study was to investigate if QUS are valid to discriminate both healthy and fractured women.
Methods. Case-control study performed on 698 postmenopausal caucasian women. 213 women had suffered a Colles´fracture (CFx) in the three previous months to the study. 485 women were controls. QUS measurements were performed in the calcaneus using a Sahara, Hologic, Clinical Ultrasonometer. Tscores were calculated based upon Spanish normal values. ROC curves were also calculated.
Results.To obtain 5% of false-negative Tscore cut-off had to be establish in -2.60 and to obtain 10% of false positive Tscore cut-off had to be establish in -0.105.
Conclusion. Tscores only permit to identify 11% of high CFx risk patients and 9.2% of low risk CFx patients. These results show that QUS are not useful for risk assessment of Colles´fracture.

	QUI		
Risk	Cases	Controls	Total
High	23	41	64
	12.7%	10.2%	11.0%
Undefined	149	317	466
	82.3%	78.7%	79.8%
Low	9	45	54
	5.0%	11.2%	9.2%
Total	181	403	584
	100%	100%	100%



Disclosures: ***M. Sosa****, None.*

M110

Measurement of Ultrasound Transmission Velocity (UTV) Identifies Critical Bone Quality in an Animal Model before Implantation. P. H. Kann[1], K. A. Grötz*[2], R. Brahm*[2], B. Al-Nawas*[2]. [1]Endocrinology & Diabetology, Philipps University Hospital, Marburg, Germany, [2]Oral and Maxillofacial Surgery, Johannes Gutenberg University Hospital, Mainz, Germany.

INTRODUCTION: UTV measurement may provide information about bone quality in systemic bone diseases as well as in localised diseases or conditions. Information about local bone situation obtained by UTV measurement might be useful in planning therapeutic strategies in tooth implantation in maxillofacial surgery.
AIM: The aim of this study was to investigate in an animal model in vivo whether UTV measurement might be prognostically relevant in considering the quality of the jaw bone before implantation.
MATERIALS AND METHODS: In 14 beagle dogs, following the extraction of the premolars 3 months earlier, UTV in both the upper and lower jaw was measured in intubation anaesthesia. Each site was measured 4 times using a miniaturised device (IGEA, Italy; 2 MHz). Subsequently, two self-cutting tooth implants each were inserted into this area, and the maximum torque during implantation as a surrogate for the implant stability was measured (Branemark DEC 600 motor).
RESULTS: UTV in the upper jaw (1691 ± 119 m/s) was significantly lower than UTV in the lower jaw (2161 ± 173 m/s; $p < 0.001$). Correspondingly, torque tended to be lower in the upper jaw (40.2 ± 9.1 Ncm) than in the lower jaw (45.3 ± 3.95 Ncm; $p = 0.15$). In 2/28 cases, torque was below a critical value of 30 Ncm: Both cases showed significantly lowered UTV values < 1600 m/s ($p = 0.003$).
CONCLUSION: The jaw bone of the beagle dog showed high mechanical quality, characterised by both high torque and UTV values. Two critical cases did not provide adequate stability of the implants (torque < 30 Ncm). Both these situations could be identified before implantation by UTV values below a critical range of 1600 m/s. Therefore, measurement of UTV of the jaw bone in vivo may be used as a non invasive diagnostic approach before implantation to identify critical bone quality with an impact for therapeutic strategy.

Disclosures: ***P.H. Kann****, None.*

M111

Use of Quantitative Ultrasound (QUS) at the Phalanges for a First Level Screening Program for Recently Menopausal Women without Clinical Risk Factors for Osteoporosis for Referral BMD Assessment by DXA. C. Cormier[1], L. Boubli*[2], J. Boulanger*[3], E. Drapier-Faure*[4], P. Fardellone*[5], N. Genin*[6], F. Lavieille*[7], P. Lopes*[8], C. Nahmanovici*[9], D. Elia*[10]. [1]Rheumatology a, Cochin Hospital APHP, Paris, France, [2]Hopital Nord, Marseille, France, [3]Centre de Gynécologie-Obstétrique, CHU, Amiens, France, [4]Hopital E. Herriot, Lyon, France, [5]Rheumatology, Teaching Hospital, Amiens, France, [6]Gynécologue, Monaco, France, [7]Gynécologue, Bordeaux, France, [8]CHU, Nantes, France, [9]Gynécologue-obstétricien, Nice, France, [10]Centres de menopause MG et FMP, Paris, France.

According to French guidelines, for women who don't present any clinical risk factor for osteoporosis at menopause, Dual-energy X-ray Absorptiometry (DXA) is not routinely recommended in their postmenopausal life. This multicenter study conducted in France on young postmenopausal women aims to identify women by phalangeal QUS who, even if they do not present any clinical risk factors for osteoporosis at menopause, are nevertheless osteoporotic after 5 to 10 years. The study data collected in 7 cities of France involve 435 postmenopausal women in the age range 55-65 yrs, postmenopause for 5 to 10 years and furthermore a group of 373 women (age range 45-55 yrs) were also evaluated by phalangeal QUS as a menopause reference. The QUS measurements were collected with the DBM Sonic Bone Profiler (IGEA, Italy). Two parameters were analysed: AD-SoS (Amplitude Dependent Speed of Sound) and UBPI (Ultrasound Bone Profile Index). In a subgroup of 150 women randomly selected in the postmenopausal group, a DXA evaluation (lumbar spine and femoral neck) was carried out in order to identify osteoporotic, osteopenic and normal subjects. Subjects in postmenopause show significantly lower values of AD-SoS and UBPI compared to women at the climacteric ($p<0.0001$). A strict correlation between AD-SoS and age is observed in postmenopausal women ($r=-0.44$, $p<0.0001$), whilst at the climacteric the correlation is lower ($r=-0.13$, $p<0.05$).
When the group of subjects with QUS and DXA measurements were investigated, best performances were obtained when the selection of high risk patients was based on the presence of at least one of the 2 QUS parameters below the T-score threshold of -2.25SD. In this case 95.2% (20/21) of osteoporotic subjects were revealed and the overall percentage of missed cases is 0.7 %. This QUS threshold refers 45.3% (N=68) of patients to a further DXA examination. Among these 68 subjects, 20 are osteoporotic, 37 osteopenic and 11 normal. These results show a good performance of QUS at the phalanges as a screening tool to identify osteoporotic women in recent menopause, who otherwise would not have been identified as at risk.

Disclosures: ***C. Cormier****, None.*

M112

Calcaneal Ultrasound from Age 6 to Age 19. K. M. Davies, J. M. Lappe, G. Lypaczewski*. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

A cohort of healthy, white children and adolescents aged 6 to 19, 208 girls and 202 boys, was recruited for a cross-sectional study of bone ultrasound characteristics. The Osteometer DTU-One was used to measure speed of sound (SOS) and attenuation (BUA) in the calcaneus. When averaged by age group over each sex, SOS seems to change little if any, varying within a band 10 m/s wide about 1552 m/s. The BUA shows a rise from the age of 7 to about 17, increasing from about 35 dB/MHz to about 50, or about 43%. In the figure, error bars are standard error. The curves for boys and girls are similar. In this study, Tanner stage was self-assigned, and not useful for this analysis. In regression on the individuals, SOS depends on age, R^2 of 4% ($P < 0.0001$) and a change of 6.7 m/s on a base of 1551 m/s over 13 years. BUA depends on age and weight, R^2 is 47.5% ($P < 0.0001$), and the change of 16.7 dB/MHz on a base of 33.6 dB/MHz is 60% due to weight change and 40% due to age change. Conclusion: Change in calcaneal SOS is very slight and in BUA is substantial from age 6 to age 19, independent of sex.

Disclosures: ***K.M. Davies****, None.*

M113

Quantitative Ultrasound of the Calcaneous in Children from Cameroon Central Africa, Jamaica, U.S.A., and Honduras: Effects of Ethnicity, Age, Sex and BMI. W. D. Bronson[1], E. Brooks*[2], T. Alderson*[1], S. Dongmo*[3], R. Zebaze*[1]. [1]Arthritis and Osteoporosis Center, Heartland Health System, St. Joseph, MO, USA, [2]Missouri Western State College, St. Joseph, MO, USA, [3]Bafut District Hospital, Bafut, Cameroon.

The calcaneal stiffness index (SI) and BMI of a convenience sample of 739 healthy children ages 5-12 years old were measured. The children were from very different socio-economic, cultural and geographic environments: Cameroon, Central Africa n=100, Caucasian U.S.A. n=214, African American U.S.A. n=49, Jamaican N=223, rural Honduran n=153. All measurements were obtained using the same Achilles ultrasonometer manufactured by Lunar Corp. Shims were used to properly position the child's foot. A between subjects factorial MANOVA was calculated comparing the BMI and SI scores for subjects in one of four age categories and in one of five ethnic racial groups. Both BMI and SI scores were significantly influenced by age and ethnic racial group. A MANOVA was calculated examining the effect of age (5&6; 7&8; 9&10; 11&12) on BMI and SI. A significant interaction effect was found (Lambda(6,1436) = 33.14, p<.001). The SI was significantly different by age categories (F(3,719) = 65.30, p<.001). The SI was significantly different between all age groups except the 5 and 6 and 7 and 8 year olds. A MANOVA was calculated examining the effect of ethnic/racial on BMI and SI scores. A significant interaction effect was found (Lambda(8, 1436) = 25.43, p<.001). To understand where the differences were between the ethnic racial groups post hoc Scheffe were performed which revealed significant differences between all groups except the Caucasian and Honduran populations. The 3 groups of African ancestry had significantly higher SI than the Caucasian and Honduran children. The SI between the Cameroon, Jamaican and African American children were also statistically significantly different. Multiple regression was calculated to predict subject's SI based on their racial/ethnic group (Black, Caucasian, Honduran), age, and BMI. A significant regression was found (F(3, 735)=130.81,p<..001)with an R2 of .348. While ethnicity, age and BMI do impact SI, a genetic/geographic/environmental influence may be important on bone acquisition and SI.

	S.I.			
	5&6	7&8	9&10	11&12
Cameroon-African	70.7	72.1	84.7	87.7
African-American	78.2	78.2	90	104
Caucasian-U.S.A.	67	69.6	74.3	85.7
Jamaican	77	81	86.7	98.7
Honduran	71.7	72.2	76.5	80.8

Disclosures: ***W.D. Bronson****, None.*

M114

Bi-Directional Axial Transmission: An In Vitro Investigation of the Relationships between Cortical Bone Properties and SOS. E. Bossy*[1], L. Akrout*[1], V. Bousson*[2], C. Latremouille*[3], J. Laredo*[2], M. Talmant*[1], P. Laugier[1]. [1]Lab. Imagerie Parametrique, CNRS, Paris, France, [2]Service de Radiologie, Hopital Lariboisiere, Universite Paris 7, Paris, France, [3]Hopital Broussais, Universite Paris 6, Paris, France.

The so-called axial transmission technique provides a measurement of the velocity (SOS) of elastic waves propagating along the surface of cortical bone and radiating into soft tissue. A major source of measurement error comes from the variations of soft tissue thickness in the region of measurement resulting in a bias on the estimate of the SOS. We have developed a new technique, using a bi-directional transmission mode, which eliminates preliminary measurement of the thickness and velocity of soft tissue, based on a proprietary new probe design. The probes with central frequencies of 1 and 2 MHz consist in 1D linear arrays of small elements. The residual relative error on SOS values after correction for inclination between the probe and Aluminum or Perspex samples was reduced to 0.2%-0.3%.

Our goal was to investigate the relationship between SOS, cortical thickness (CT) and BMD, using this new device. SOS measurements were performed in vitro on 50 excised human radius with soft tissue removed, at the distal third in the posterior, median and anterior quadrant. Mean SOS values were 3950 m.s-1 (range 3780 - 4120 m.s-1) and 4033 m.s-1 (range 3850 - 4190 m.s-1) at each side for the 1 MHz and 2 MHz probes respectively. At the posterior quadrant, precision measurement expressed as variation coefficient (CV) was 0.4%.

3D quantitative computed tomography (QCT) measurements (Siemens Somaton 4 Plus) were performed on the same samples. Relationships between SOS and QCT data measured at several matched sites were assessed using linear regression.

No correlation between cortical thickness and SOS was observed for both frequencies, expect at 1 MHz for thin bones (CT < 0.2 mm). BMD is found to correlate weakly but significantly with SOS for both 1 and 2 MHz probes (r2=0.2 ; N=100). These results are consistent with the data reported by Sievänen et al. (Osteoporosis Int., 2001) and Njeh et al. (Ultrasound Med Biol, 2001) and are strongly supported by predictions obtained using 3D numerical computation based on a finite difference method.

These results tend to show that SOS values poorly correlate with density or thickness but are likely to depend on other bone material properties (elasticity) .

Disclosures: ***E. Bossy****, None.*

M115

Combining Sites for Fracture Discrimination: Results for Axial Transmission Ultrasound Compared with a Theoretical Model. K. M. Knapp, G. M. Blake, T. D. Spector, I. Fogelman. Imaging Sciences, Guy's, King's and St Thomas' School of Medicine, London, United Kingdom.

Clinicians frequently use spine and hip dual x-ray absorptiometry (DXA) for the assessment of fracture risk. However, the information added by a second site using DXA is limited because the measurements are too well correlated [1]. However, measurements of peripheral sites using axial transmission ultrasound are not so well correlated and there may be a benefit in using a combination of sites in this case. The aim of this study was to establish the benefit of combining sites in vertebral and Colles' fracture patients. Secondly, the findings were used to evaluate the predictions of a theoretical model for combining sites. The Sunlight Omnisense was used to measure the speed of sound (SOS) at the radius, phalanx and metatarsal. The study population consisted of 191 healthy postmenopausal women (mean age 59y ± 7), 115 women with atraumatic vertebral fractures (72y ± 8) and 63 untreated women with low trauma wrist fractures (69y ± 8). Logistic regression analysis was used to calculate the age adjusted odds ratios (OR) for fracture discrimination. OR for the combinations of sites were calculated by taking the mean Z-score for the two measurements and renormalizing the population standard deviation [2]. The theoretical OR values were calculated using the single site OR's, combined based on a mean Z-score approach, assuming a Gaussian distribution and utilising the correlation coefficient between the measurements [1]. SOS measurements were able to discriminate fracture cases from controls in all cases. Additionally, there were limited improvements in fracture discrimination obtained by combining measurement sites. When comparing the results from the patient data with the results of theoretical modelling, there was good agreement, demonstrating that this model was an appropriate way of predicting the effect of combining sites.

[1] Patel, J Bone Miner Res 2002 ;17 :S312
[2] Frost, Osteoporosis Int 2001;12 :471-447

SOS Site	Vertebral Fracture OR (95% CI		Wrist Fracture OR (95% CI	
	Actual OR	Theoretical OR	Actual OR	Theoretical OR
Rad	1.32 (1.00-1.74)	-	1.50 (1.07-2.10)	-
Plx	1.59 (1.05-2.41)	-	1.85 (1.06-3.23)	-
Met	1.50 (1.04-2.16)	-	1.74 (1.12-2.71)	-
Rad & Plx	1.57 (1.09-2.26)	1.54	2.00 (1.21-3.33)	1.81
Rad & Met	1.69 (1.15-2.49)	1.51	1.67 (1.05-2.67)	1.79
Plx & Met	1.78 (1.18-2.70)	1.69	1.86 (1.11-3.10)	2.02

Disclosures: ***K.M. Knapp****, None.*

M116

Female Premenopausal Bone Fracture Risk Depends on Gc Phenotype. A. L. Lauridsen*[1], P. Vestergaard[2], A. P. Hermann*[2], H. J. Moller*[1], L. Mosekilde[2], E. Nexo*[1]. [1]Department of Clinical Biochemistry, Kommunehospitalet, Aarhus University Hospital, Aarhus, Denmark, [2]Department of Endocrinology and Metabolism, Amtssygehuset, Aarhus University Hospital, Aarhus, Denmark.

The aim of our study was to examine the relation between Gc phenotype and bone fragility. The multifunctional plasma protein Gc, also known as Vitamin D binding protein, DBP, or Gc globulin, has two functions with relation to bone tissue: it is the major carrier protein of vitamin D in the circulation and deglycosylation converts it into a very potent macrophage- and osteoclast-activating factor, Gc-MAF. Gc occurs in several isotypes.

By isoelectric focusing we identified the Gc phenotype of 595 Caucasian recent postmenopausal women enrolled into the Danish Osteoporosis Prevention Study (DOPS) and identified three groups: Gc1-1, n=323, Gc1-2, n=230, and Gc2-2, n=42. Differences among the three groups were examined with respect to number of fractures before enrollment, bone mineral content and density, and various clinical and biochemical variables, including the concentration of Gc measured by immunonephelometry and the concentration of the macrophage marker soluble CD163 measured by ELISA.

The risk of having at least one premenopausal bone fracture (total number of women with fracture=179) differed significantly (p=0.017) among women with phenotype Gc1-1 (110/323=0.34), Gc1-2 (63/230=0.27), and Gc2-2 (6/42=0.14). The difference was even more striking (p=0.005) for fractures caused by low-energy traumas. Using logistic regression we found the relative risk of premenopausal fracture to be 3.1 in Gc1-1 compared to Gc2-2. We propose the mechanism to be differences in osteoclast activity mediated by Gc-MAF, a theory supported by our finding of higher level of Gc and of the macrophage marker soluble CD163 in women with Gc1-1 compared to Gc2-2.

In conclusion female fracture risk before menopause is related to Gc phenotype.

Disclosures: ***A.L. Lauridsen****, None.*

M117

Meta-analysis of Molecular Association Studies: The Vitamin D Receptor Gene Polymorphisms and Bone Mineral Density as a Case Study. A. Thakkinstian*[1], C. D'Este*[2], J. Eisman*[3], T. Nguyen*[3], J. Attia*[2]. [1]Clinical Epidemiology Unit, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rachatevi, Rama 6 Rd., Bangkok, Thailand, [2]Centre for Clinical Epidemiology and Biostatistics, Faculty of Medicine and Health Science, Newcastle University, NSW, Australia, [3]The Garvan Institute of Medical Research, Darlinghurst, NSW, Australia.

Introduction With the rise of molecular and genetic epidemiology, molecular association studies are increasingly common; however meta-analysis of these studies has been a neglected area. This study has performed a meta-analysis of the association of the vitamin D receptor (VDR) gene polymorphisms and bone mineral density/osteoporosis and highlight methodological issues.
Methods Studies published from 1994 to 2001 were identified via Medline using PubMed software. The reference lists of the articles retrieved were also reviewed. Where eligible papers had insufficient information, we contacted authors by mail (up to 3 mailings) for additional information. Any observational study, which tested the association between VDR BsmI genotypes and either bone mineral density (BMD), or osteoporosis, at the femoral neck or spine in adult women, were included in review. Data was extracted independently by two reviewers (AT & JA) using a standardised data extraction form.
Results The B allele was significantly associated with BMD at the spine; it appeared to follow a recessive model with the BB genotype having lower BMD than Bb/bb genotypes at baseline, and leading to greater bone mineral loss over time. Methodological lessons highlighted include the need to check Hardy-Weinberg equilibrium, the importance of exploring heterogeneity, pooling the data in a manner that is sensitive to genetic models, and avoiding multiple comparisons.
Conclusion With the proliferation of molecular association studies, there will be an increased need to quantify the magnitude of the risk associated with genetic polymorphisms. This will likely entail meta-analytic methods, and this meta-analysis highlights some of the methodological issues that will need to be resolved.

Disclosures: ***A. Thakkinstian****, None.*

M118

Cytochrome P450c17α (*CYP17*) Gene Polymorphism Indirectly Influences on Bone Density through their Effects on Endogenous Androgen in Postmenopausal Japanese Women. I. Gorai[1], M. Inada*[2], H. Morinaga*[2], Y. Uchiyama*[2], H. Yamauchi*[2], O. Chaki[2], F. Hirahara*[2]. [1]Obstetrics and Gynecology, International University of Health and Welfare Atami Hospital, Atami, Japan, [2]Obstetrics and Gynecology, Yokohama City University School of Medicine, Yokohama, Japan.

Bone density and bone loss have strong genetic inclination. We aimed to assess whether circulating sex steroid hormones influence bone measured as bone density and biochemical markers, and whether some part of this influence can be explained by genetic variation measured as polymorphisms in candidate genes influencing circulating hormone levels in 250 postmenopausal Japanese women aged 46 yr and over who had been followed for eight years. We investigated the polymorphisms of estrogen metabolizing enzyme genes, *CYP17*; estrogen biosynthesis (high activity, A2/A2), *CYP1A1*; hydroxylation (high inducibility, vt/vt) and *COMT*; inactivation (low activity, L/L). The *CYP17* has both 17α-hydroxylase and 17, 20-lyase activities and catalyzes two distinct steps in steroid hormone production. Genomic DNA was extracted from peripheral leukocytes and PCR-based restriction fragment length polymorphism (RFLP) assays were used to determine *CYP17*, *CYP1A1* and *COMT* gene polymorphism. There were significant correlations between the levels of androstenedione (AND) or dehydroepiandrosterone (DHEA), and bone densities at all sites examined. Women with A2/A2 genotype had significantly higher level of DHEA than that with A1/A1 or A1/A2 genotypes (DHEA P=0.0021 and P=0.0013, respectively). There was no significant difference between estrogen levels and *CYP17* genotype. We could not find any significant correlations between sex steroid hormone levels and *CYP1A1* or *COMT* gene polymorphism. There were no significant differences in bone density and annual changes of BMD at each site examined among each *CYP17*, *CYP1A1* or *COMT* genotypes except in bone loss at distal radius. The annual loss of distal radius BMD in women with L/L genotype was significantly higher than in those with H/H or H/L genotypes (P=0.0127 and P=0.0154, respectively). The results suggest *CYP17* gene polymorphisms have some indirect influences on bone density through their effects on endogenous androgen.

Disclosures: ***I. Gorai****, None.*

M119

Association of Aromatase Gene Polymorphisms with Bone Mineral Density in Men and Women: The Framingham Offspring Study. D. Karasik[1], A. M. Shearman[2], L. A. Cupples*[3], S. Demissie*[3], K. Gruenthal*[2], D. E. Housman*[2], D. P. Kiel[1]. [1]Hebrew Rehab Ctr for Aged & Harvard Med Sch, Boston, MA, USA, [2]Ctr for Cancer Research, MIT, Cambridge, MA, USA, [3]Biostatistics, BU Sch of Public Health, Boston, MA, USA.

Aromatase catalyses the conversion of androgens into estrogens, and the aromatase gene (CYP19) has been suggested to confer susceptibility to osteoporosis. We studied the association between CYP19 polymorphisms and bone mineral density (BMD) in 732 men and 795 women (mean age 60 ys, range 29-86) from the Offspring Cohort of the Framingham Study. BMD (g/cm^2) was measured at the hip (femoral neck, trochanter, Ward's area) and L2-L4 lumbar spine (LS BMD) with Lunar DPX-L densitometer. DNA samples were genotyped for four polymorphisms, one previously reported tetranucleotide (TTTA)n repeat, and three novel SNPs: rs700518, rs4646, and rs726547. Information on age, height, body mass index (BMI), menopausal status (y/n) and current estrogen use (y/n) in women, was obtained at the time of BMD examination.
Sex-specific linear regression and ANCOVA were used to compare BMDs adjusted for age, height, BMI, menopause, and estrogen use, according to CYP19 polymorphism. Probability of each haplotype was computed using the expectation-maximization algorithm.
Frequencies of the minor allele were 47.8% (G), 26.1% (A), and 10.9% (A), for rs700518, rs4646, and rs726547, respectively. Out of six (TTTA)n alleles, the most frequent were those with 7 (51.3%) and 11 (31.9%) repeats. All four CYP19 polymorphisms were in strong linkage disequilibrium.
For the (TTTA)n repeat, our analysis compared BMD measures across the three genotypes obtained when the alleles were defined as short (7 repeats) or long (8-12 repeats). In men the (TTTA)n repeat was significantly associated with BMD (p=0.004) at the spine only. Homozygotes for the short allele had 3.4% lower LS BMD than the other genotypes. In contrast, in women (TTTA)n was not related to BMD at hip and spine ($p > 0.6$).
LS BMD was marginally significantly associated with rs700518 in men and with rs726547 in women ($p \sim 0.05$). No association was observed between rs4646 and BMD values in either sex ($p > 0.06$). Haplotype analysis provided significant (p=0.03) association of a frequent (47%) combination of (TTTA)n shortest allele, rs700518(A) and rs726547(G) with lower LS BMD in men. Polymorphisms in the CYP19 gene, including common (TTTA)n repeat, are associated with BMD in older Caucasian men and women, although this effect is limited to lumbar vertebrae and is pronounced in men. Since the male skeleton is highly dependent on estradiol, these findings underscore the importance of genes such as aromatase, that influence the metabolism of testosterone to estradiol and may play a role in bone mass.

Disclosures: ***D. Karasik****, None.*

M120

C4887A Polymorphism of the CYP1A1 Gene Conditions Estrogen Metabolism and Bone Density. N. Napoli[1], S. Sheikh[1], S. Mumm[1], C. Baldus*[1], J. James*[1], C. Mueller*[1], J. Fryer*[1], T. Klug*[2], G. B. Rini[3], R. Civitelli[1], R. C. Armamento-Villareal[1]. [1]Bone and Mineral Diseases, Washington University, St. Louis, MO, USA, [2]Immunacare Corp., Bethlehem, PA, USA, [3]University of Palermo, Palermo, Italy.

The ratio of urinary 2-hydroxyestrogen (inactive) / 16α-hydroxyestrogen (agonist) products of estrogen metabolism is an important determinant of postmenopausal bone density. We tested the hypothesis that polymorphisms of CYP1A1, a gene that codes one of the estrogen metabolizing enzymes, affects the balance between inactive and active metabolites, and in turn bone density in postmenopausal women. In a cohort of 143 untreated women, we detected 3 restriction fragment length polymorphic (RFLP) variants. Of these, the C to A transversion at position 4887 revealed significant interracial differences in allele frequencies (table). Biochemical analysis of urine estrogen metabolites revealed that, relative to individuals with the most prevalent CC haplotype, subjects with the CA and AA variants combined have significantly higher urine levels (μg/gm creatinine) of 16α-hydroxyestrone ($16\alpha OHE_1$: 6.70±0.94 vs. 4.36±0.49, p=0.03) and urinary estriol (E_3) (9.20±1.35 vs. 5.26±0.75, p=0.02). The inactive 2-hydroxyestrone ($2OHE_1$) and 2-methoxyestrone ($2MeOE_1$) were not significantly different among the groups, resulting in significantly lower ratios of inactive to active metabolites in the variants compared to the wild type (1.61±0.25 vs 2.19±0.13, p=0.04, for $2OHE_1/16\alpha OHE_1$; and 1.03±0.09 vs 1.27±0.05, p=0.02, for $2OHE1+2MeOE1/16\alpha OHE_1+E_3$). Ratios were lower in the two variant haplotypes regardless of race. Surprisingly, proximal femur bone mineral density was lower in the CA and AA variants compared to the CC genotype (0.657±0.02 vs 0.710±0.01, p=<0.01 in the femoral neck; 0.592±0.02 vs 0.647±0.01, p=<0.01 in the trochanter; and 0.793±0.02 vs 0.849±0.01, p=0.03 in the total hip). The other 2 CYP1A1 polymorphsisms (A4889G, T6235C) were not associated with differences in either urinary metabolites or bone density. In conclusion, we identified 3 polymorphic variants of the CYP1A1 gene, whose prevalence varies among ethnic groups. Of these, only the C4887A variant (Thr461Asn) is associated with biologic differences in estrogen metabolism, which translate into differences in bone density.

Genotype	Total	Whites	Asians	Blacks
N	135	124	6	4
C / C (%)	83.7	84.7	83.3	50
C / A (%)	15.6	14.5	16.7	50
A / A (%)	0.7	0.8	0	0

Disclosures: ***R.C. Armamento-Villareal****, None.*

M121

Association of Endothelial Nitric Oxide Synthase (NOS3) Genotypes with Bone Mineral Density (BMD), Bone Loss and Risk of Fracture in Older Women. B. C. Taylor[1], P. J. Schreiner*[1], J. M. Zmuda[2], S. P. Moffett*[2], T. A. Hillier[3], S. R. Cummings[4], K. E. Ensrud[1]. [1]U of MN, Minneapolis, MN, USA, [2]U of Pitt, Pittsburgh, PA, USA, [3]KP Center for Health Research, Portland, OR, USA, [4]UCSF, San Francisco, CA, USA.

Nitric oxide (NO) is an important bone-signaling molecule. A G894T polymorphism in exon 7 of NOS3, which results in a Glu298Asp amino acid substitution, has been associated with differential NO production. We examined the associations between this G894T polymorphism and BMD, BMD loss and the incidence of fracture among 3489 women aged 65 years and older in the Study of Osteoporotic Fractures.

Calcaneal BMD was measured at an initial exam with SPA and after an average of 5.9 years with SXA (r=0.94 for SPA and SXA). Hip BMD was measured at an initial exam and after 3.7 years with DXA. Incident hip fractures (n=232) were confirmed by review of radiographic reports (mean follow-up 8.7 years); follow-up was >98% complete. Incident vertebral fractures (VF, n=86) were defined by morphometry using lateral spine radiography at baseline and an average of 3.7 years later. We performed multiple linear, logistic and Cox regression analyses for the effect of the Glu298Asp polymorphism on BMD, vertebral fracture and hip fracture, respectively.
The frequencies of the NOS3 G894T genotypes were G/G = 45.4%, G/T = 43.4%, and T/T = 11.3%. There were no significant associations between NOS3 genotypes and initial calcaneal BMD, hip BMD, or rate of change in hip BMD. There was a significant ($p<0.05$) association between genotype and rate of change in calcaneal BMD; women with the T/T genotype experienced a greater loss of calcaneal BMD (1.98% per year) than women with the G/G genotype (1.75% per year, p=0.02 G/G vs. T/T), whereas those with the G/T genotype experienced a more moderate reduction in BMD (1.81% per year, p=0.09 G/T vs. T/T). NOS3 genotype was not significantly associated with VF; the OR and 95% CI for G/T vs. G/G was 0.80 (0.50, 1.28) and for T/T vs. G/G was 1.43 (0.77, 2.66). However, women with the heterozygous G/T genotype had a statistically significant lower rate of hip fracture than either the G/G genotype (HR=0.67 95% CI: 0.50, 0.89) or the T/T genotype (HR=0.63 95% CI: 0.42, 0.96). Further, the association between NOS3 and hip fracture was not substantially altered by adjustment for age, BMD, BMI, or other factors known to be associated with hip fracture.
In conclusion, the Glu298Asp polymorphism does not contribute substantially or consistently to variations in BMD in older white women. Our findings require confirmation in other studies and additional analyses of other NOS3 polymorphisms and genotype-by-environment interactions, but suggest allelic variation at the NOS3 locus is associated with hip fracture risk among older women.

Disclosures: ***B.C. Taylor****, None.*

M122

Polymorphisms of Vitamin D Receptor, Estrogen Receptor α and Transforming Growth Factor-β1 Genes are Associated with Quantitative Calcaneal Ultrasound in Postmenopausal Women. J. Koh*[1], I. Nam-Goong*[1], E. Kim*[2], Y. Chung*[3], J. Hong*[4], S. Kim*[5], G. Kim[1]. [1]Division of Endocrinology and Metabolism, Asan Medical Center, Seoul, Republic of Korea, [2]Department of Internal Medicine, Ulsan University Hospital, Ulsan, Republic of Korea, [3]Department of Internal Medicine, Seoul Veterans Hospital, Seoul, Republic of Korea, [4]Asan Life Sciences, Seoul, Republic of Korea, [5]Department of Orthopedic Surgery, Kyungpook National University, Taegu, Republic of Korea.

Objective: Quantitative ultrasound (QUS) of bone is a new radiation-free, low cost method that measures both bone mass and bone quality. We investigated associations between QUS parameters and polymorphisms of vitamin D receptor (VDR), estrogen receptor α (ERα) and transforming growth factor-β1 (TGF-β1) gene in postmenopausal women who resided in a community.
Methods: From 206 postmenopausal women, aged 60 to 69 years, broadband ultrasound attenuation (BUA) and speed of sound (SOS) were measured at the left calcaneus using QUS measurement of bone, and stiffeness index (SI) was calculated. We determined the *Bsm*I and *Fok*I polymorphisms of VDR gene and the *Xba*I and *Pvu*II polymorphisms of ERα gene using polymerase chain reaction-restriction fragment length polymorphism method, and T[29]àC polymorphism of TGF-β1 gene using an allele-specific polymerase chain reaction assay.
Results: The subjects carrying the X allele of ERα gene *Xba*I polymorphism had significantly higher SI (T-score) compared with those without this allele (p=0.014). There were no significant differences in the QUS parameters among the VDR genotypes and TGF-β1 genotype. However, an association between the two VDR genotypes and SI (T-score) was noted in the subjects with the CC genotype of the TGF-β1 gene T[29]àC polymorphism (p=0.003 for the *Bsm*I polymorphism and p=0.016 for the *Fok*I polymorphism), but this was not significant in those without these genotypes.
Conclusion: This study indicates that the *Xba*I polymorphism of ERα gene and the interactions between the VDR and TGF-β1 genes influence the QUS parameters in postmenopausal women.

Disclosures: ***J. Koh****, the Korea Health 21 R&D Project 2.*

M123

Estrogen Receptor α Genotype is Associated with Bone Response to Exercise in Early Pubertal Finnish Girls. M. Suuriniemi[1], A. Mahonen[2], Q. Wang*[3], A. Lyytikäinen[3], M. Alen*[4], S. Cheng[3]. [1]Department of Cell Biology, University of Jyväskylä, Jyväskylä, Finland, [2]Department of Medical Biochemistry, University of Kuopio, Kuopio, Finland, [3]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [4]PEURUNKA-Medical Rehabilitation Center, Jyväskylä, Finland.

Estrogen receptor α (ERα) mediates the effects of estradiol on the skeleton. Recently, ERα has also been found to be involved in the responses of bone cells to mechanical strain. The purpose of this study was to evaluate whether girls with different ERα genotype differ in bone mass and geometry with respect to different levels of physical activity (PA). The subjects were healthy 10-12 year-old Finnish girls (n=217) with Tanner stage I-II who enrolled in an intervention study (the CALEX-study). Level of PA was assessed using a questionnaire designed to evaluate the frequency, intensity, duration, and type of the first three favourite PA during the past 6 months. Genotyping of the ERα locus at the PvuII polymorphic site was performed. Bone properties of the total body (WB), total femur (TF), femoral neck (FN), and lumbar spine (L2-L4) were measured by DXA (Prodigy, GE Lunar), and the distal radius and tibia shaft were measured using pQCT (XCT 2000, Stratec). Our results showed that girls who were physically more active with heterozygote ERα genotype (Pp) had significantly higher values of bone mineral density (BMD) in WB, TF, FN, and L2-L4 than their less active counterparts (p=0.001-0.017). They also had thicker cortex (p=0.001) and higher volumetric BMD of the tibia (p=0.017). No differences were found in bone mass and geometry of the distal radius which was not a weight-bearing bone, nor in Tanner stage, height, weight, body mass index, and energy intake among girls with different PA or genotype. Bone properties did not differ in either homozygote groups (PP and pp) regardless of the PA level. Our results suggest that the heterozygote group of the PvuII polymorphism in the ERα gene may benefit most from the effect of exercise at loaded bone sites. This raises the question: Is the P/p heterodimeric form of the ERα different in its function from the two homodimeric forms?

Disclosures: ***M. Suuriniemi****, None.*

M124

Common Variations in Intron 4 of Aromatase Gene Influence Bone Mineral Density in Men. J. A. Riancho[1], A. L. Zarrabeitia*[2], C. Valero*[2], M. T. Zarrabeitia*[3], J. L. Hernandez*[2], J. Gonzalez-Macias[1]. [1]Internal Medicine, Hosp. U.M. Valdecilla, University of Cantabria, Santander, Spain, [2]Internal Medicine, Hosp. U.M. Valdecilla, Santander, Spain, [3]Legal Medicine, University of Cantabria, Santander, Spain.

Estrogens play an important role in bone homeostasis in both sexes. In men and in postmenopausal women most estrogens are synthesized in peripheral tissues from androgen precursors, in reactions catalyzed by aromatase, the product of CYP19 gene. Rare null mutations of CYP19-aromatase gene are associated with abnormal skeletal maturation.
The purpose of this study was to determine if some common polymorphisms of CYP19 gene also influence bone mass in men.
The study group comprised 200 men without diseases known to affect bone homeostasis. DNA was isolated from peripheral blood and intron 4 of CYP19 was amplified by PCR using FAM-labelled primers. The region contains a tetranucleotide repeat. Allele size was determined by capillary electrophoresis and consecutive numbers (1-8) were given to alleles of different length. Genotypes were classified into three groups according to the sum of both alleles (group 1 [35%], allele sum <5; group 2 [29%], allele sum 5-7; group 3 [36%], allele sum >7). Bone mineral density was measured by DEXA (Hologic).
In young individuals (aged 45 or less, n=128), no association was found between BMD and CYP19 polymorphisms. However, among those aged 46-75 years (n=72), there was a significant association between CYP19 genotype and BMD at the lumbar spine (Z scores of the three genotypic groups: –1.2, -0.6 and –0.06, respectively; p=0.02), femoral neck (Z scores:-0.1, -0.3 and 0.4; p=0.03), total hip (Z scores: -0.1, 0, 0.6; p=0.03) or Ward's triangle (Z scores:-0.1, -0.1, 0.7; p=0.02). There were no significant differences in body weight, height or calcium intake.
In conclusion, a common polymorphism in CYP19-aromatase gene is associated with marked differences in BMD, both at the hip and the spine, in middle-aged and elderly males. Since no such association is found in young men, it seems likely that the polymorphism influences the maintenance of bone density after the peak bone mass is attained. On the other hand, these results give further support to the hypothesis of an important role of estrogens in skeletal homeostasis in men.

Disclosures: ***J.A. Riancho****, None.*

M125

Polymorphisms in the Interleukin-One Gene Cluster and the Risk of Aseptic Loosening after Total Hip Arthroplasty. A. Gordon*[1], J. M. Wilkinson[1], A. G. Wilson*[2], I. Stockley*[3], D. MacDonald*[4], R. Eastell[1]. [1]Bone Metabolism Unit, University of Sheffield, Sheffield, United Kingdom, [2]Division of Genomic Medicine, University of Sheffield, Sheffield, United Kingdom, [3]Department of Orthopaedics, The Northern General Hospital, Sheffield, United Kingdom, [4]Department of Orthopaedics, St James's University Hospital, Leeds, United Kingdom.

Aseptic loosening due to periprosthetic bone loss is a major cause of implant failure after total hip arthroplasty (THA). Interleukin 1α (IL-1α) and interleukin 1β (IL-1β) are thought to play a role in aseptic loosening by stimulating osteoclast activity. Interleukin 1 receptor antagonist (IL-1RA) is an anti-inflammatory soluble peptide that can block the activity of IL-1α and IL-1β. Polymorphism within the genes coding for these cytokines has been associated with differences in susceptibility to conditions associated with bone loss, including juvenile chronic arthritis, chronic periodontitis and postmenopausal bone loss. In this study we tested whether polymorphisms in the IL-1 gene cluster resulted in a differential risk of aseptic loosening in 481 white Caucasians (214 failed versus 267 radiologically intact implants) at 11.7± 4.1 years following primary cemented THA for osteoarthritis.
Genomic DNA extracted from peripheral blood was genotyped using the Taqman 5' nuclease method. Carriage rates were calculated and analysed using the χ^2 test.
Association of single nucleotide polymorphisms within the IL-1 gene cluster and osteolysis: Allele 2 carriage rate.

Population	IL-1B+3954	IL-1B-511	IL-1A+4845	IL1-RN+2018
THA Control (%) (n=267)	44.9	52.8	51.7	49.1*
THA Osteolysis (%) (n=214)	42.1	55.6	51.9	39.3

*χ^2 P=0.045
The genotype frequencies were in Hardy-Weinberg equilibrium for both intact and loose implant populations (Chi-squared P>0.05)
Using multivariate Cox proportional hazards model, carriage of the IL-1RN+2018 allele

was a significant independent risk factor for loosening (hazards ratio 0.71, 95%CI 0.54-0.94, P=0.02). Other significant risk factors for aseptic loosening included gender and age at THA (P<0.05). Our data suggests that the IL-1RN single nucleotide polymorphism at +2018 may influence the risk of aseptic loosening after THA, however the exact mechanism remains unclear.

Disclosures: ***A. Gordon****, None.*

M126

Genetic and Environmental Determinants of Bone Mineral Density in Chinese Women. H. Lau*[1], A. Ho*[1], K. Luk*[2], A. Kung*[1]. [1]Medicine, The University of Hong Kong, Hong Kong, China, [2]Orthopaedics & Traumatology, The University of Hong Kong, Hong Kong, China.

Bone mineral density (BMD) is a major determinant of osteoporosis and fractures. Several hereditary and environment factors have been implicated in the pathogenesis of osteoporosis, but the ways they interact remain poorly understood. The relative importance of genetic and environmental determinants of BMD was assessed in 282 southern Chinese women. The candidate genes studied include estrogen receptor (ER) alpha and beta, calcium sensing receptor, transforming growth factor beta1 and vitamin D receptor. We sequenced the 5' region of the COL1A1 gene and all the exonal regions of the LRP5 gene to determine the polymorphisms and sequence variations in Chinese. The polymorphisms at Sp1 binding site, –1339 C/G and –1997G/T of COL1A1, and Q89R, V740V and V1330A of LRP5 were studied. Social, medical, and reproductive history, dietary habits and lifestyle factors were determined using a structured questionnaire. Stepwise multiple regression analysis revealed that body weight was the strongest predictor of BMD in premenopausal women, accounting for 18% of the variance at the spine, 17.2% at femoral neck, 27.2% at total hip and 8.7% at the Ward's triangle. Other significant predictors were the dinucleotide CA repeats polymorphisms of ER beta, accounting for 3.1% of the variance at spine and total hip; the ER beta AluI genotype at spine (3.3%); and the Q89R genotype of LRP5 gene accounting for 3.3% at Ward's triangle. As for postmenopausal women, body weight was the strongest predictor of BMD, accounting for 28.6% of the variance at the spine, 29.6% at femoral neck and 31% at total hip. Age accounted for 14.5% of the variance at total hip and femoral neck. Other significant predictors were VDR Fok I genotype, accounting for 1.5% at the spine and 1.1% at trochanter; weight bearing physical activity at spine (1.1%), total hip (2.9%) and Ward's triangle (1.7%); calcium intake at the spine (1.8%) and total hip (1.7%). We concluded that body weight, polymorphisms of ERbeta and LRP5 gene together with age explained approximately 30% of the variance of bone mass in premenopausal women, whereas age, weight, VDR Fok I genotype and environmental factors explained about 50% of the variance of bone mass in postmenopausal women. Understanding the factors determining BMD at different stages of the woman's life enable better strategies for prevention against bone loss.

Disclosures: ***H. Lau****, None.*

M127

The Vitamin D Receptor FokI Start Codon Polymorphism, Bone Mineral Density and Bone Turnover in Postmenopausal Women. V. A. Myakotkin*, M. Y. Krylov*, L. I. Benevolenskaya. Genetics, Institute of Rheumatology Russian Academy of Medical Sciences, Moscow, Russian Federation.

Vitamin D receptor (VDR) gene polymorphisms have been reported to account for most of the well established genetic influence on bone mineral density (BMD). The associations BMD and biochemical markers of bone remodelling and a T/C polymorphism in the first of the two initiation codons in the vitamin D receptor VDR gene were studied in our reseach. This polymorphism was detected by restriction fragment length polymorphism analysis, using polymerase chain reaction (PCR) and the restriction endonuclease FokI. The presence of the restriction site, designated as f, allows protein translation to initiate from the first ATG, while the allele lacking the site, indicated as F, initiates translation at a second ATG. In this study we investigated the role of FokI polymorphism in a group of 136 postmenopausal women of Russian descent, aged 55-83 years, stratified for BMD (DEXA,g/cm2) into osteoporotic (n=76) and normal (n=60)groups. The serum osteocalcin and breakdown products of C-telopeptides alpha 1 chains of type I collagen were studied by means of commercial kits. The distribution of FokI genotypes in osteopototic women(FF=21.1%,Ff=65.8%,ff=13.1%) was different significantly (p=0.026) from control group (FF=30.0%,Ff=43.3%,ff=26.7%). A significantly higher prevalence of Ff genotype in osteoporotic than normal women was observed (p=0.009). Patients with the ff genotype had at average lower BMD (0.572 plus/minus 0.121 g/cm2) at the hip than those with the Ff genotype (0.626 plus/minus 0.064 g/cm2, p=0.044). FF patients had an intermediate mean value BMD at the hip. A similar pattern of lower lumbar spine BMD was found in ff osteoporotic patients too, but it did not reach statistical significance (p=0.19). We found the certain relationship between FokI genotypes and the means levels of the serum osteocalcin (FF=53.9 plus/minus 25.2 ng/ml,Ff=39.8 plus/minus 17.2 ng/ml,ff=34.2 plus/minus 6.3 ng/ml), but its did not reach statistical significance (p=0.06). We conclude, that the FokI genotypes of the vitamin D receptor gene is related to bone mass at the hip in Russian postmenopausal women with osteoporosis.

Disclosures: ***V.A. Myakotkin****, None.*

M128

Interleukin-7 Influences Osteoclast and Osteoblast Function *in vivo* but Is not a Critical Factor in Ovariectomy-Induced Bone Loss. S. Lee[1], J. F. Kalinowski*[1], A. Cabrera-Hernandez*[1], D. Adams[2], G. Gronowicz[2], J. A. Lorenzo[1]. [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT, USA.

Interleukin-7 (IL-7) is produced by stromal cells in bone marrow and is a major regulator of B and T-lymphopoiesis. We previously reported that IL-7 directly inhibits osteoclastogenesis *in vitro* and that bone marrow cell cultures from IL-7 deficient (IL-7KO) mice produced significantly more TRAP(+) OCL. However, others have found that a neutralizing antibody to IL-7 blocked ovariectomy (OVX)-induced bone loss in mice. We have now examined if differences exist between the bones of wild type (WT) and IL-7KO mice and if OVX altered bone mass in IL-7KO mice. Studies were in 2 month old sham-operated (SHAM) and OVX female mice that were sacrificed 3 weeks after surgery. IL-7KO mice and WT controls were in a C57BL/6 background. Vertebrae were evaluated by micro-computed tomography (μ-CT) while femurs were examined by histomorphometry. IL-7 KO mice were confirmed as IL-7 deficient by their almost total lack of mature B cells in bone marrow.

In vertebrae there was no significant difference between the bone volume of WT and IL-7KO SHAM mice. However, IL-7KO mice had significantly decreased (p<.05) connectivity and trabecular numbers (30% and 10%, respectively). Ovariectomy decreased vertebral trabecular bone volume (TBV) by 21% (p<.05) in WT mice and by 17% (p<.05) in IL-7 KO mice compared to SHAM. In femurs there was a trend for IL-7 KO SHAM mice to have decreased (30%) TBV compared to WT SHAM mice but this effect was not significant. Femurs from IL-7 KO SHAM mice had significantly increased (p<.05) cortical width (16%) and percent osteoclast (OC) surface (23%) and significantly decreased (p<.05) osteoblast (OB) surface (30%) compared to WT SHAM. As in the vertebrae, OVX significantly decreased femoral TBV in WT and IL-7 KO mice (43% and 73%, respectively, p<.05 for both) compared to SHAM. These findings demonstrate that IL-7 KO mice have increased OC and decreased OB compared to WT mice but mimic WT mice in the amount of bone lost after OVX. We conclude that IL-7 influences OC and OB function *in vivo* but may not to be a critical factor in OVX-induced bone loss.

Disclosures: ***S. Lee****, None.*

M129

Glucose-dependent Insulinotropic Peptide: Differential Effects on Hepatic Artery versus Portral Vein Endothelial Cells. K. Ding[1], Q. Zhong[1], D. Xie[1], A. L. Mulloy[2], C. M. Isales[2]. [1]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA, USA, [2]Medicine, Medical College of Georgia and the Augusta VA Hospital, Augusta, GA, USA.

We have previously reported that glucose-dependent insulinotropic peptide (GIP), produced by the endocrine cells in the small intestine, is an anabolic hormone with effects on both bone and vascular endothelium (1). A study by Kogire et al. (2) demonstrated contrasting effects of glucose-dependent insulinotropic peptide. GIP infusion in dogs resulted in an increase in portal vein circulation but a drop in hepatic artery blood flow. In an effort to evaluate whether these different responses were related to intrinsic differences in GIP effects in these two tissues we isolated canine hepatic artery (HAEC)and portal vein (PVEC) endothelial cells. Initial experiments examined signal transduction pathways through which GIP is known to act. Changes in intracellular calcium were measured using the calcium sensitive probe Fura-2. HAEC demonstrated a concentration dependent increase in intracellular calcium in response to GIP (GIP: 10-11M: 0.31; 10-10M: 0.38; 10-9M: 0.86; 10-8M: 1.22; 10-7M: 1.0; increase in 340/380 ratio over baseline). GIP did not induce any changes in intracellular calcium in PVEC. Other second messengers including cAMP and cGMP (by radioimmunoassay) were measured in HAEC and PVEC in response to GIP but neither second messenger increased. Effects of GIP on proliferation were measured next using 3H-thymdine incorporation as an index of proliferation. We found that GIP increased 3H-thymidine incorporation in both cell types but GIP effects were small in HAEC (HAEC: GIP: 10-11M: 99+0.6; 10-10M: 108+7.9; 10-9M: 125+3.1; 10-8M: 126+8.9; 10-7M: 128+10.3 % control+SEM). PVEC: GIP: 10-11M: 116+7.8; 10-10M: 142+4.2; 10-9M: 143+1.2; 10-8M: 153+1.9; 10-7M: 150+2.0 control+SEM. Endothelin-1 (ET-1) is a potent vasoconstrictor produced by endothelial cells so we next examined GIP effects on ET-1 secretion measured by ELISA. HAEC: Control: 1.56+0.25; GIP: 10-10M: 2.03+0.21; 10-9M: 2.6+0.18; 10-8M: 2.99+0.17; 10-7M: 3.08+0.23, ET-1 ng/ml Means+SEM. GIP had no effect on ET-1 secretion in PVEC. Effects of GIP on the vasodilator PGF 1alpha were also measured and no differences in PGF 1alpha concentrations were observed (HAEC Control: 2.07+.208 vs. PVEC: 3.04+.667, PGF 1alpha ng/well Means+SEM). Taken together our data demonstrate distinct differences in GIP effects on HAEC vs. PVEC. We conclude that GIP stimulation of ET-1 from HAEC may explain GIP's vasoconstrictive effect on this vascular bed.

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Disclosures: ***K. Ding****, None.*

M130

Pituitary Adenylate Cyclase Activating Peptide Receptors are Present on Osteoblastic-like Cells. B. Kang[1], Q. Zhong[1], K. Ding[1], J. Xu*[1], C. M. Isales[2]. [1]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA, USA, [2]Medicine, Medical College of Georgia and the Augusta VA Hospital, Augusta, GA, USA.

Pituitary adenylate cyclase activating peptide (PACAP) is a neuropeptide widely distributed in the central nervous system and peripheral tissues. The PACAP receptor belongs to the seven transmembrane G-protein coupled family of receptors which also includes PTH/PTHrP, calcitonin, GIP, VIP and GLP-1. The presence of PACAP receptors in bone cells has been controversial with some reports not finding PACAP receptors in osteoblast(1, 2), though others reporting effects of PACAP on osteoblastic-like cells(3). PACAP receptors have been reported on osteoclasts(4) and addition of PACAP to isolated osteoclasts inhibits bone breakdown(5). In an effort to reevaluate the presence or absence of PACAP receptors on osteoblasts we utilized an osteoblastic-like cell line, MG63, using RT-PCR with PACAP specific primers. A single PCR product was identified demonstrating the presence of PACAP receptors in MG63 cells. To further evaluate whether differences in our results and those of previous investigators related to the presence of subpopulations of cells variably expressing the PACAP receptor we performed in situ hybridization experiments with PACAP receptor specific probes. The PACAP receptor was found to be equally distributed between the whole population without any variation in expression. In other cell types PACAP appears to play a role in cell proliferation and differentiation. To evaluate this possibility we next performed experiments using 3H-thymdine incorporation as an index of proliferation. We found that PACAP increased 3H-thymidine incorporation in a biphasic manner: PACAP 10 nM: 5506+290; 50 nM: 5750+418; 100nM: 4567+149; 500 nM: 3961+66; 1 uM: 3794+270; 2 uM: 3218+239 cpm+SEM). Taken together our data demonstrate the presence of functional PACAP receptors in an osteoblastic-like cell line and suggests that this peptide may be playing a role in normal bone turnover.

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Disclosures: ***B. Kang****, None.*

M131

Analysis of CTGF and Collagen Type I Expression in Muscles of a Rat Model of Work-related Musculoskeletal Disorders. M. C. Rico[1], M. Amin*[2], U. Chizea-Abuah*[1], A. E. Barr*[2], S. N. Popoff[1], F. F. Safadi[1], M. F. Barbe*[2]. [1]Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA, [2]Physical Therapy, Temple University School of Medicine, Philadelphia, PA, USA.

Work-related musculoskeletal disorders (MSD) are the result of prolonged repetitive, forceful, or awkward movements. In recent studies, we have shown that performance of highly repetitive tasks is associated with an increase in serum levels of IL1α and widespread increases in activated macrophages in musculoskeletal tissues. We have shown previously that neural tissue fibrosis is associated with increased expression of connective tissue growth factor (CTGF) and collagen type I in the forelimb with performance of repetitive tasks. Studies have shown that CTGF is implicated in the pathogenesis of fibrosis by inducing fibroblasts to produce collagen. The purpose of this study was to examine expression of CTGF and collagen type I in forelimb muscle and tendons following performance of a highly repetitive reaching task for up to 9 weeks. Rats reached for food at a rate of 4 reaches/min, 2hrs/day, and 3 days/week for either 0 (control), 5 or 9 weeks. Expression of CTGF and collagen type I using RT- PCR analyses showed significant increases in muscles of reach limbs of trained animals at 5 and 9 weeks compared to the non-reach limbs and 0 week controls. Immuno-histochemical expression of CTGF and collagen type I was increased in fibroblasts of tendons, perimysium, and epimysium by 9 weeks. CTGF immuno-expression also increased in cytoplasm of muscle cells and in mast cells. Our results show that performance of highly repetitive tasks is associated with fibrosis mediated by CTGF. Further studies will lead us to understand the mechanism of CTGF in the pathogenesis of work-related MSD.

Disclosures: ***M.C. Rico****, None.*

M132

A Comparison of In Vitro Osteogenic Response of Cells from Ovariectomized and Normal Rats to bFGF and BMP-2. T. Haque*, H. Uludag. University of Alberta, Edmonton, AB, Canada.

Ovariectomized rats serve as a routine model for post-menopausal osteoporosis. Removal of systemic estrogen causes significant deterioration of bone structure, coupled with changes in the cellular population of bone marrow environment. Protein growth factors have been evaluated in ovariectomized rats because of their potential to modulate cellular population in the bone marrow. To better understanding of the responsiveness of osteogenic cells in bone marrow to protein growth factors, we investigated the osteogenic population of bone marrow aspirates from ovariectomized and age-matched normal Sprague-Dowley rats. The rats underwent bi-lateral ovariectamy at 3 months and 3-4 months were allowed for development of osteopenia. Bone marrow aspirates from femurs were characterized for colony formation on tissue culture plates, alkaline phosphatase activity (ALP) as well as calcification. The responsiveness of the cells to the anabolic agents basic Fibroblast Growth Factor (FGF-2) and Bone Morphogenetic Protein-2 (BMP-2) was investigated. A significant increase (>5-fold) in colony formation was observed as a result of ovariectomy, but the proportion of ALP-expressing colonies (~40%) was not influenced by the ovariectomy. Similarly, calcification (both in absolute amounts and in number of calcified nodules) was enhanced in cells from OVX rats. bFGF (50 ng/mL) was found to decrease the proportion of ALP-positive colonies (after 10 days), as well as to reduce calcification (after 21 days) in both types of cells. BMP-2 (200 ng/mL) did not influence the investigated parameters when the cells were treated in the early time points (<10 days), but appeared to increase calcification when the cells were treated with the protein at later time points (>7 days). We conclude that the ovariectomized rats have a more abundant osteogenic cell population, but the responsiveness of the cells to bFGF and BMP-2 treatment was similar in both normal and ovariectomized rats.

Disclosures: ***H. Uludag****, None.*

M133

Modifying Glycoproteins for Bone Affinity. H. Uludag[1], S. Gittens*[2], R. F. Zernicke*[3], J. R. Matyas*[4]. [1]Chemical and Materials Engineering, University of Alberta, Edmonton, AB, Canada, [2]Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada, [3]Faculty of Kinesiology, University of Calgary, Calgary, AB, Canada, [4]Cell Biology and Anatomy, University of Calgary, Calgary, AB, Canada.

Protein Growth Factors (such as BMPs and FGFs) with high affinity to bone matrix could serve as novel therapeutic agents capable of seeking bone after systemic administration. Growth Factors, capable of stimulating new bone formation, are typically secreted as glycoproteins, where numerous carbohydrate moieties are attached to the polypeptide core. The carbohydrate moieties offer a possibility for direct chemical modification of the proteins to attach bone-targeting molecules with a high bone affinity. Towards this goal, carbohydrate moieties of a model protein, fetuin, have been modified with bisphosphonates. Oxidization of the carbohydrate groups by periodate (4 mM) resulted in reactive aldehydes moieties (<30 per protein), which reacted with hydrazide-based linkers to couple 1-aminomethane-1,1-diphosphonate. The degree of modification (i.e., number of bisphosphonates conjugated per protein) was controlled by the reaction concentrations and varied between 1 to 8 bisphosphonates per protein. The modified proteins exhibited a high mineral affinity, as assessed by in vitro binding studies to synthetic hydroxyapatite and to various bone matrices freshly obtained from rats. Compared to bisphosphonates attached directly to the polypeptide core, carbohydrate modified proteins were observed to display a superior affinity to bone matrices, possibly due to increased flexibility of the carbohydrate moieties. The observed bone affinity was directly proportional to the number of bisphosphonates on the protein. The stability of the conjugate affinity to mineral was tested in serum-containing medium and the results indicated that the conjugates retained their bone affinity up 7 days in vitro. The protein bioactivity was not evaluated in this study (since fetuin served only as a model protein), but, based on the non-essential role of carbohydrates in protein activity, the proposed approach is expected to yield proteins with (i) high affinity to bone tissue and (ii) full retention of activity. Current studies are evaluating the ability of bisphosphonate-modified fetuin to target bone tissue in osteoporotic animals.

Disclosures: ***H. Uludag****, None.*

M134

Delivery of Novel Peptide Bone Growth Factor in Injectable Fibrin Sealant for Fracture Repair. D. R. Sindrey, E. Plawinski*. Millenium Biologix Inc., Mississauga, ON, Canada.

Current technologies for the treatment of local bone repair utilize various biologically compatible carriers to maintain exogenous bone growth factors at the trauma site. These include collagen sponges or paste, demineralized bone or synthetic polymers. Each has inherent disadvantages with respect to disease transmission or adverse events. Fibrin sealant has also been used in similar limited applications and has many advantages over the latter carriers. We have tested the feasibility of combining a novel small molecular weight bone growth factor, BCSP™ (Bone and Cartilage Stimulating Peptide), with Tisseel™ fibrin sealant, as an injectable carrier for fracture repair. Injected alone, the small Mol. Wt. 9mer peptide is quickly removed from the trauma site. Mixed with the fibrin sealant, the peptide will be immobilized at the trauma site when injected, where the peptide will be slowly delivered to stimulate bone repair. This study investigated the bone forming ability of the synthetic BCSP peptide delivered in fibrin sealant in a non-trauma rat tibia assay as a preclinical development candidate for fracture repair. Thrombin and Tisseel solutions from several kits were prepared according to kit protocols. BCSP peptide dissolved in phosphate buffer was added to the thrombin component and adjusted to pH 7.4. Dilutions of peptide and thrombin stock were made to yield doses of 0.5, 2.0 and 5.0 mgs per 200 uL when combined with fibrin upon injection. Male Wistar rats (n=8 per group) weighing 125-200g, were randomized and then anaesthetized using Isoflurane. Delivery of the fibrin sealant/peptide mix was made using a dual syringe injector fitted with spiral mixing chamber and 25G needle. Test material was deposited onto the periosteal surface of the right medial tibia surface proximal to the knee under the Tibialis cranialis muscle group. The control groups were injected with phosphate buffer plus Tisseel or BCSP in PBS only. Animals received one injection. Tibia were dissected out after 7 days and submitted to DEXA scan, micro CT and demineralized histology sectioning. After 7 days, one injection of BCSP™ peptide increased local bone formation in the tibia in a dose dependent manner proportional to dose size. Increases in local BMD compared to contralateral limb were 10%, 6.5% and 3% for the 5mg, 2.5mg and 0.5mg dose respectively. The fibrin control group receiving only fibrin sealant increased local BMD values by 3% whereas animals receiving peptide dissolve in phosphate buffer showed no significant increases in bone

mineral density. Fibrin sealant provides a convenient and quality controlled human product for controlled release delivery of small peptide or protein bone growth factors to trauma or depot sites for bone stimulation.

Disclosures: ***D.R. Sindrey****, Millenium Biologix Inc. 1, 3.*

M135

Stimulation of Bone Formation by Inhibitors of the Hedgehog Signaling Pathway is BMP Dependent. G. R. Rossini*, G. Gutierrez, A. Escobedo*, D. Horn*, G. R. Mundy, I. R. Garrett. OsteoScreen, San Antonio, TX, USA.

Bone morphogenetic proteins (BMPs) are centrally involved in embryonic morphogenesis and post-natal bone formation. Hedgehog proteins(HH) are also important in skeletal morphogenesis, but their role in post-natal bone formation and interactions with BMP pathways are not well understood. To examine the role of hedgehog proteins in post-natal bone formation and their dependence on BMP pathways, we treated calvaria isolated from neonatal mice with sonic hedgehog (Shh) in concentrations of 0.05-5ug/ml, but found no effect on osteoblast differentiation or bone formation. However, when we used structurally unrelated inhibitors of the hedgehog signaling pathway such as cyclopamine, which binds specifically to the HH signaling receptor Smo, filipin and cyclodextrin which both modify HH signaling by complexing with cholesterol and other sterols, we found they stimulated bone formation by 23-31% which is similar to effects seen with growth factors such as BMP-2 or FGF-1. To determine if this effect was dependent on BMPs, we added noggin, the naturally occurring BMP inhibitor at concentrations of 2ug/ml to these bone cultures incubated with these HH inhibitors. Noggin inhibited cyclopamine-stimulated bone formation. These results suggest that the effects of hedgehog inhibitors are mediated by extracellular BMPs. Next, we determined if the addition of BMP in small amounts would enhance the effects of hedgehog inhibitors on bone formation. We found the addition of rhBMP-2 at 2-50ng/ml enhanced bone formation stimulated by cyclopamine by 42%. We next investigated the effects of hedgehog signaling inhibitors on bone formation in vivo. Cyclopamine markedly stimulated bone formation when injected directly over the calvaria of mice at a dose of 10mg/kg/day 3 times day for only 5 days by 32%, indicating that the hedgehog pathway regulates osteoblast differentiation and postnatal bone formation in vivo. Our data suggest that important interactions occur between the hedgehog pathway and the BMP2 pathway in post-natal bone formation, and suggest new approaches and potential targets for the stimulation of bone formation

Disclosures: ***G.R. Rossini****, OsteoScreen 1, 3.*

M136

A Novel Induction Pathway of Sprouty1 by PLCγ. M. Abe, M. C. Naski. Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Genetic studies of receptor tyrosine kinase (RTK) signaling have identified sprouty is an essential antagonist of RTK signaling. Sprouty gene expression is dramatically and rapidly induced following RTK activation. The induction of sprouty expression requires action of the ERK MAP kinases. However, the contributions of other signaling events to sprouty induction is uncharacterized. Here we report that the induction of sprouty1 gene expression in response to basic FGF requires elevations of intracellular calcium. Sprouty1 was rapidly induced by bFGF and this induction was inhibited by chelation of either intracellular or extracellular calcium. Additionally, blockade of calcium channels with $CdCl_2$ abolished sprouty1 induction by bFGF treatment. Interestingly, calcium ionophores did not induce sprouty1 indicating an essential requirement for activation of the FGF receptor signaling pathways. Accordingly, constitutively active forms of fibroblast growth factor receptor 3, but not receptor variants unable to activation of phospholipase C gamma, induced sprouty 1 gene expression. These finding demonstrate the essential contribution of PLC and $Ca2^+$to sprouty gene induction and implicate PLC as a critical regulator of FGF receptor signaling.

Disclosures: ***M. Abe****, None.*

M137

Identifying a Novel Potential Bone Anabolic Role for Fibroblast Growth Factor 9. A. Houghton, R. S. Cooper*, M. Jankowsky*, D. Ebert*, A. Dickason*, M. Petrey*, D. Ji, M. W. Lundy, N. Jaiswal*, Y. O. Taiwo. Skeletal Research, P&G Pharmaceuticals, Mason, OH, USA.

In order to identify potential therapeutic targets which modulate the process of bone formation we have performed numerous genomic studies on a variety of in vitro models of osteoblast differentiation, including primary human mesenchymal stromal cells (MSCs). In these experiments we have repeatedly observed that the expression of Fibroblast Growth Factor Receptor 3 (FGFR3) is significantly increased when MSCs are induced to differentiate by BMP-2 (100ng/ml). In order to further investigate the role of FGFR3 in osteoblasts and bone formation we investigated the effects of a relatively selective FGFR3 ligand, FGF9, on osteoblast differentiation and bone formation both in vitro and in vivo. In cultures of MSCs FGF9 dose dependently increased osteoblast differentiation, as measured by alkaline phosphatase, with doses as low as 1ng/ml inducing statistically significant increases. To determine the effects of FGF9 on bone formation in vitro, FGF9 was tested in the mouse calvarial organ culture assay. Briefly, calvarial bones were dissected, weighed and cultured continuously in media containing various doses of FGF9 for 7 days. Analysis of the calvaria established that FGF9 exhibited a biphasic response, and significantly increased calvarial weight at doses of 10 and 30ng/ml. Histological analysis revealed that FGF9 caused significant increases in osteoid formation at doses as low as 1ng/ml, osteoclastic activity, and at higher doses of FGF9 an increase in cellularity which was indicative of a proliferative response not seen in MSCs.

As a preliminary investigation of the in vivo anabolic effects, FGF9 was tested in the calvarial local injection model. 30 or 300µg/Kg/day doses of FGF9 were administered directly onto mouse calvarial bones by subcutaneous injection, once daily for 5 days and then mice were maintained for a further 14 days. µCT measurements clearly demonstrated that the high dose of FGF9 caused a highly significant 40% increase in calvarial bone thickness and a 360% increase in exocranial mineral appositional rates. However the low dose of FGF9 had no effect on either parameter, suggesting that doses above 30µg/kg are required for a bone anabolic response.
In conclusion, in vitro and preliminary in vivo data suggest that FGF9 is a potent inducer of bone formation with a mechanism of action dissimilar to FGF2. However it remains to be determined whether systemic administration of FGF9 will result in comparable bone anabolic effects.

Disclosures: ***A. Houghton****, Procter & Gamble 3.*

M138

FGF-23 Regulates the Renal Sodium Phosphate Co-Transporter in Opossum Kidney Cells. M. D. Ruppe*, J. Y. Cho*, S. M. Jan de Beur. Medicine, Division of Endocrinology and Metabolism, Johns Hopkins, Baltimore, MD, USA.

Phosphorus plays a critical role in skeletal and cellular functions involving intermediary metabolism and energy-transfer mechanisms. Phosphate homeostasis is regulated primarily via modulation of the renal type IIa sodium-phosphate transporter (NPT-2a). Fibroblast Growth Factor-23 (FGF-23) is a potent inhibitor of phosphate transport *in vitro* and its excess leads to phosphaturia, hypophosphatemia, and osteomalacia *in vivo*. Excess circulating full-length FGF-23 is central in the pathophysiology of several renal phosphate-wating syndromes including oncogenic osteomalacia, X-linked hypophosphatemic rickets and autosomal dominant hypophosphatemic rickets. The mechanism by which excess FGF-23 leads to hypophosphatemia is poorly understood. We hypothesized that FGF-23 inhibits phosphate reabsorption via regulation of NPT-2a. To test whether FGF-23 down-regulates the endogenous type IIa sodium phosphate co-transporter (NaPi-4) RNA expression, opossum kidney (OK) cells were incubated with one of three different serum-free media preparations: full length FGF-23 (20 ng/ml); dexamethasone (10^{-6} M), an NPT-2 transcriptional down-regulator; or untreated media. Total RNA was extracted, fractionated by electrophoresis, transferred to a nylon membrane and hybridized with a radiolabeled fragment of NaPi-4. Signal was quantitated by phosphoimager and normalized to GAPDH expression. FGF-23 exposure reduced NaPi-4 RNA expression, as did dexamethasone treatment when compared to untreated media. To determine if FGF-23 regulation of the type IIa sodium phosphate co-transporter is solely transcriptional or also involves endocytic retrieval from the membrane by a mechanism similar to PTH, we generated a Npt-2a-green fluorescent protein fusion protein (GFP-Npt-2a) and visualized the cellular distribution in response to stimuli. GFP-Npt-2a (5 mcg) or control vector (5 mcg) was transfected into sub-confluent OK cells. To prevent intracellular degradation of the expressed fusion protein, cells were treat with leuptin (100 mcg/ml). Fluorescent imaging demonstrated patches of Npt-2a co-transporters localized to the membrane. After a 3 hour incubation with PTH (10^{-7}M) or FGF-23 (20 ng/ml), treated cells exhibited a diffuse cytosolic and perinuclear distribution compared with untreated cells that exhibited a persistent patchy membrane distribution. These studies suggest that FGF-23 mediates reduced renal phosphate transport through reduction in the type IIa sodium phosphate co-transporter RNA expression and enhanced endocytic retrieval from the renal epithelial membrane.

Disclosures: ***M.D. Ruppe****, None.*

M139

Heat Shock Factor-2 is a Down Stream Target of b-FGF to Induce RANKL Expression in Stromal/Osteoblast Cells. J. L. Roccisana*[1], N. Kawanabe[1], G. D. Roodman[2], S. V. Reddy[1]. [1]Medicine-Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Medicine-Hematology/Oncology, University of Pittsburgh and Department of Veterans Affairs Medical Center, Pittsburgh, PA, USA.

Tumor necrosis factor family member, RANK Ligand (RANKL) is a critical osteoclastogenic factor that is expressed on marrow stromal/osteoblast cells. Most resorption stimuli including b-FGF induce osteoclast formation by modulating RANKL gene expression in these cells. To characterize the transcriptional control of human RANKL gene expression in stromal/osteoblast cells, we previously cloned a 2 Kb hRANKL gene promoter region and identified the presence of Heat Shock Factor (HSF) responsive elements (HSE). We also demonstrated that the expression of HSF-2 but not HSF-1 was expressed in stromal/osteoblast cells and found that HSFs transactivate hRANKL gene promoter activity. We have further examined the participation of HSF-2 in b-FGF stimulated RANKL expression in human bone marrow derived stromal cells (SAKA-T). b-FGF treatment (24 hr) of SAKA-T cells transfected with the luciferase reporter plasmid containing the hRANKL HSE region (-2Kb to -1275 bp) upstream to a heterologous (SV40) promoter showed increased levels of transactivation. Western blot analysis demonstrated a 2.5 fold enhanced level of RANKL expression in SAKA-T cells stimulated (48 hr) with b-FGF. Furthermore, b-FGF treatment to these cells resulted in a 3 fold increase in Heat Shock Protein (HSP)-27 phosphorylation compared to untreated control cells. HSF-2 levels were increased moderately (1.5-2 fold) in SAKA-T cells stimulated with b-FGF. However, b-FGF has no significant effect on the status of HSP-27, HSP-70 and HSP-90 expression in these cells. In addition, over-expression of HSF-2 in SAKA-T cells resulted in a 5 fold increase in the levels of RANKL expression in these cells, further confirming a functional role of HSF-2 in RANKL gene expression. These data suggest that HSF-2 is a downstream target of b-FGF to induce RANKL expression in stromal/osteoblast cells, and that HSF may play an important role in modulating RANKL gene expression in the bone microenvironment.

Disclosures: ***J.L. Roccisana****, None.*

M140

Disruption of the FGF-2 in Mice Reduces the Expression of Key Regulators of Osteoblast Precursor Proliferation and Differentiation. T. Naganawa*[1], L. Xiao[1], E. Abogunde*[1], T. Sobue[1], J. D. Coffin[2], M. M. Hurley[1]. [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Pharmaceutical Sciences, University of Montana, Missoula, MT, USA.

FGF2, an important modulator of cartilage and bone is expressed and regulated in osteoblastic cells. Mice with disruption of the Fgf2 gene developed osteopenia with aging that is manifested by significantly decreased trabecular bone volume, mineral apposition and bone formation rates. In this study, we utilized tibial bones from 9-10 month old male Fgf2 wild type (+/+) and Fgf2 knockout (-/-) mice to assess basal expression of mRNA for the osteoblastic markers, type 1 collagen (Col1A1) and osteocalcin (OC), as well as the expression of bone morphogenetic protein 2 (BMP2) and Runx2. The mRNA levels for Col1A1 and OC were reduced by 68 and 44%, respectively, in tibiae from Fgf2-/- mice compared with Fgf2+/+ mice. In addition, Runx2 mRNA was reduced by 40% and BMP2 mRNA was decreased by 78 % in tibiae from Fgf2-/- mice. Because of the striking decrease in BMP2 in whole tibiae, we further examined its expression in marrow stromal cells from 12 month old male Fgf2+/+, Fgf2+/- and Fgf2-/- mice. Cells were cultured for 7 days and total RNA was obtained for Northern analysis. There was a 68 and 84% reduction in BMP2 mRNA in stromal cells from Fgf2+/- and Fgf2-/- mice, respectively, compared to Fgf2+/+ mice. We next examined colony formation and gene expression in marrow stromal cells from 24 month old male Fgf2 +/+ and Fgf2-/- mice. Cells were cultured in the absence or presence FGF2 (0.1nM) that was added only for the first 3 days of culture. Starting on day 3 of culture, cells were fed with differentiation medium containing beta-glycerophosphate (8 mM) and ascorbic acid (50 µg/ml). Cultures were harvested at 8 and 16 days and stained for alkaline phosphate (ALP) colonies. The number of ALP+ colonies was reduced and colony area was significantly decreased in Fgf2-/- cultures compared with Fgf2+/+ cultures (32.4 ± 5.9 vs 91.9 ± 5.9, $p<0.01$). Exogenous FGF2 markedly increased colony area in cultures from Fgf2-/- and Fgf2+/+ mice (70.9 ± 4.2 vs 160.3 ± 12.7, $p<0.01$). On day 16 of culture, the number of mineralized colonies was significantly decreased in vehicle treated cultures from Fgf2-/- mice compared with Fgf2+/+. Exogenous FGF2 markedly increased mineralized nodule formation in cultures from both Fgf2-/- and Fgf2+/+ mice. Similar to our in vivo data, Northern analysis revealed marked decrease in Col1A1, OC, Runx2 and BMP2 mRNA levels in cultures from Fgf2-/- compared with Fgf2+/+ mice. We conclude that FGF2 plays an important role in bone formation and propose that the reduction of bone formation in Fgf2-/- mice may correlate with suppression of BMP2 and Runx2 gene expression.

Disclosures: ***T. Naganawa****, None.*

M141

The Effects of rhFGF2/sTNF-R1 on Bone Formation during Distraction Osteogenesis in ZDF Rats. J. Aronson*, Z. Liu*, E. C. Wahl*, L. Liu*, D. S. Perrien, P. A. Kern*, J. L. Fowlkes, K. M. Thrailkill, R. C. Bunn*, L. J. Suva, R. A. Skinner*, C. K. Lumpkin. Pediatrics, Orthopedics and Physiology, University of Arkansas for Medical Sciences & Lab Limb Regeneration Research, Arkansas Children's Hospital Research Institute, Little Rock, AR, USA.

A previous study has shown that bone formation is impaired during distraction osteogenesis (DO) in Zucker Diabetic Fatty (ZDF) rats, a model of Type 2 Diabetes mellitus (T2DM). Several studies have demonstrated that FGF2 has a strong mitogenic activity and local delivery of recombinant human FGF2 (rhFGF2) at a fracture site resulted in enhancement of fracture callus formation in normal and Type 1 Diabetic rats. Moreover, increased expression of TNFa has been documented in many tissues in T2DM. TNFa may inhibit bone formation through suppression of osteoblast differentiation. We hypothesize that exogenous application of rhFGF2 combined with an antagonist of TNFa (sTNF-R1) could restore normal bone formation during DO in ZDF rats. 22 male ZDF rats (ZDF/Gemi-fa/fa), 10-11 weeks of age, were divided into treated and control groups (n=11 per group). All rats underwent the standard DO protocol, including placement of the external fixators and osteotomies to the left tibia. Distraction was initiated the following day (1 day latency) at 0.2 mm bid and continued for 14 days. Treated rats received an injection of rhFGF2/ hyaluronic acid gel (25mg/25ml/gap) into the hematoma of the osteotomic gap, while control rats received an injection of hyaluronic acid gel (25ml/gap). sTNF-R1 (8mg/kg) or the same amount of saline was subcutaneously injected into treated and control rats respectively every 2 days for 14 days. After sacrifice, the lengthened tibiae were harvested and the distraction gaps were radiographically and histologically analyzed. Blood glucose levels of rats were significantly increased in both treated and control groups after 14 days ($P<0.001$). Body weight changes were identical in both groups. The radiographic analysis showed a significant increase in the area (51.4±4.2% vs 32.7±5.2%) ($P<0.01$) and the density (28.2±1.8% vs 23.2±1%) ($P<0.05$) of mineralization in the distraction gaps of treated rats when compared to control rats. The histological analysis demonstrated that a significant increase of bone formation was found in endosteal bone formation in treated rats versus control rats (25.5±8% vs 1.2±1.2%) ($P<0.005$). Similarly, enhanced periosteal bone formation was found in treated rats compared with control rats (20.5±7.8% vs 7.8±3.3%) but did not reach significance. The results demonstrate that local application of FGF2 combined with systemic sTNF-R1can enhance bone formation by increasing endosteal and periosteal bone formation during DO in ZDF rats.

Disclosures: ***J. Aronson****, None.*

M142

FGF23 and FRP4 Internalize Sodium-Phosphate Co-Transporter (NaPi2a) in Opossum Kidney Proximal Tubule Cells. S. P. O'Brien*[1], A. E. Bowe*[1], A. Byrne*[1], W. Weber*[1], M. Pragnell*[1], R. Kumar[2], S. C. Schiavi[1]. [1]Receptor Ligand Therapeutics, Genzyme Corporation, Framingham, MA, USA, [2]Dept. of Medicine, Nephrology Research Unit & Mayo Proteomics Center & Biochemistry and Mol. Biology, Mayo Clinic and Foundation, Rochester, MN, USA.

Fibroblast Growth Factor 23 (FGF23) and Frizzled Related Protein 4 (FRP4) are over-expressed in tumors associated with oncogenic osteomalacia, a syndrome characterized by hypophosphatemia, hyperphosphaturia, and defects in bone mineralization. Multiple lines of evidence from several independent research groups have shown that over-expression of FGF23 *in vivo* leads to hypophosphatemia and hyperphosphaturia. Infusion of recombinant FRP4 protein in mice or rats also reduces serum phosphorus levels and increases urinary phosphorus excretion without altering serum calcium, parathyroid hormone (PTH) or 1,25 dihydroxyvitamin D levels. We have previously shown that similar to the well characterized actions of PTH, FGF23 and FRP4 proteins inhibit phosphate uptake in renal proximal tubule cells *in vitro*, suggesting these proteins have a direct effect on control of renal phosphate reabsorption. Regulation of phosphate transport by PTH has been well characterized and is predominantly due to surface retrieval and lysosomal degradation of NaPi2a in the renal proximal tubule. In the present study, we asked whether FGF23 and FRP4 can also alter the surface expression of the renal sodium-phosphate co-transporter, NaPi2a. We generated an opossum kidney (OK) cell line stably expressing a chimeric NaPi2a protein that contains a V5 epitope tag within the second extracellular loop. PTH-induced, dose-dependent internalization of NaPi2a-V5 protein was confirmed by anti-V5 immunostaining of non-permeabilized cells. NaPi2a-V5 was also internalized after a three hour incubation of these cells with FGF23 or FRP4. Internalization was not induced by FGF1 or an inactive fragment of PTH (13-34) confirming that this response is specific to proteins that inhibit phosphate transport. These data suggest that FGF23 and FRP4 inhibit phosphate transport by inducing internalization of the NaPi2a transporter.

Disclosures: ***S.P. O'Brien****, None.*

M143

Overexpression of Cox-2 Suppresses the High Constitutive Expression of IGFBP-6, a Strong Inhibitor of Bone Cell Differentiation. S. Hall*, R. Merid*, J. C. Felt*, T. A. Linkhart, D. J. Baylink, D. D. Strong. Musculoskeletal Disease Center, J.L. Pettis VAMC, Loma Linda, CA, USA.

The insulin-like growth factor (IGF) and prostaglandin (PG) systems are modulators of osteoblast differentiation and proliferation. In previous studies, we found that IGF binding protein 6 (IGFBP-6) completely halts osteoblast differentiation and is constitutively expressed at high levels. In contrast, PGs stimulate osteoblast differentiation and proliferation and stimulate bone formation, *in vivo*. The present study was undertaken to look at interactions between these two systems. We hypothesized that some PGs may act to stimulate bone formation by suppressing the expression IGFBP-6. Because there are numerous PGs that could be responsible for this stimulatory effect, we focused our studies on cyclooxygenase-2 (Cox-2), a rate-limiting enzyme for the production of several PGs. Cox-2 has been shown to increase bone formation and fracture repair, and may mediate increased bone formation in response to mechanical loading. To test our hypothesis, we assessed if overexpression of Cox-2 from a plasmid expression vector would decrease IGFBP-6 promoter activity. To overexpress Cox-2, we cloned full-length human Cox-2 cDNA into the VR1012 enhanced plasmid expression vector. We inserted 158 base pairs of the IGFBP-6 proximal promoter into the $pGL3_{Basic}$ luciferase reporter vector to assess promoter activity. This vector (p158Luc) supports basal IGFBP-6 transcription in osteoblasts. We co-transfected the vectors into two human osteoblast-like osteosarcoma cell lines (U-2 and SAOS-2) and normal mandibular cells. The empty VR1012 and $pGL3_{Basic}$ vectors were used as controls. Luciferase and protein content were determined 24 hours after transfection as a measure of promoter activity. In U2 cells cotransfected with the p158Luc promoter vector and the empty VR1012, promoter activity was several hundred-fold above the promoterless pGL3 basic control ($p<0.0001$). IGFBP-6 promoter activity was reduced 7 to 8-fold when cells were co-transfected with the hCox-2-VR1012 vector compared to co-transfection with the empty VR1012 vector ($p<0.001$). Overexpression of Cox-2 also inhibited IGFBP-6 promoter activity in SAOS-2 and normal mandibular osteoblast cultures, although to a lesser extent (2 to 3-fold, $p<0.01$, for both). In conclusion, 1) Cox-2 inhibits IGFBP-6 promoter activity 7 to 8-fold in U2 cells, and 2 to 3-fold in SAOS-2 and normal mandibular cells; 2) Since IGFBP-6 is a potent and constitutively expressed inhibitor of osteoblast differentiation, it follows that the action of Cox-2 to inhibit IGFBP-6 expression may be a novel mechanism by which Cox-2 mediates a marked stimulation of bone formation.

Disclosures: ***S. Hall****, None.*

M144

Identification of a Novel Mechanism by Which Activation of the PKC Suppresses the Catalytic Activity of the IGFBP-4 protease/Pregnancy-associated Plasma Protein (PAPP)-A. A. S. Sivanandam*[1], S. Mohan[1], H. Kita*[2], S. Kapur*[1], S. T. Chen*[1], T. A. Linkhart[1], G. Bagi*[1], D. J. Baylink[1], X. Qin[1]. [1]Musculoskeletal Disease Center, J.L. Pettis VAMC, Loma Linda, CA, USA, [2]Mayo Medical And Graduate School, Rochester, NY, USA.

IGFs play a pivotal role in regulating osteoblast proliferation, differentiation, and apoptosis *in vitro* and bone metabolism *in vivo*. IGFBP-4, the most abundant IGFBP produced by human osteoblasts (hOBs), counter-regulates the activity of IGFs. Importantly, the effective concentration of the IGFBP-4 in hOB culture is subject to regulation by an IGF-

dependent IGFBP-4 protease, the pregnancy-associated plasma protein-A (PAPP-A), which is in turn regulated by a number of osteoregulatory reagents. Among these regulators of IGFBP-4 proteolysis, PKC activators exert the most dramatic effect in that IGFBP-4 proteolysis in the conditioned medium (CM) of treated cells is essentially blocked (Chen et al. 2002. Endocrinology 143:1199-1205). This study was undertaken to determine the mechanism by which activation of PKC suppresses IGFBP-4 proteolysis. Treatment of hSFs/hOBs with TPA (100 nM) reduced IGFBP-4 proteolysis without significantly decreasing the PAPP-A level in the CM. Immunodepletion of the proform of eosinophil major basic protein (proMBP), a known PAPP-A inhibitor, from CM of TPA-treated cells (TPA CM) failed to increase IGFBP-4 proteolytic activity. Transduction of hSFs with proMBP retrovirus increased the concentration of proMBP in the CM up to 30 ng/mL and led to a moderate reduction in IGFBP-4 proteolysis. In contrast, TPA treatment blocked IGFBP-4 proteolysis but failed to induce a detectable amount of proMBP in the CM. While proMBP overexpression led to formation of covalent proMBP-PAPP-A complex and reduced migration of PAPP-A on SDS-PAGE, TPA treatment dose- and time-dependently increased the conversion of a slow-migrating PAPP-A species (SM-PAPP-A) to a fast-migrating PAPP-A species (FM-PAPP-A). Since PAPP-A monomers derived from reduced SM-PAPP-A and FM-PAPP-A co-migrated on SDS-PAGE, conversion of SM-PAPP-A to FM-PAPP-A is unlikely to be caused by proteolytic cleavage of PAPP-A itself. Importantly, the increase in the ratio of FM-PAPP-A:SM-PAPP-A forms is correlated with the extent of reduction in IGFBP-4 proteolysis. Conclusion: Based on our findings, we conclude that activation of PKC suppresses PAPP-A activity through a mechanism in addition to regulation of proMBP, namely by a novel post-translational modification of PAPP-A.

Disclosures: ***X. Qin****, None.*

M145

Lack of IGF-I Exerts Different Effects Compared to IGF-II on the Mechanical Properties of Bone in Mice during Postnatal Growth. S. Mohan, J. E. Wergedal, D. J. Baylink. Musculoskeletal Disease Center, J.L. Pettis VAMC, Loma Linda, CA, USA.

A major function of bone is to provide mechanical support to the body. The strength of the bone is dependent upon a number of variables of which bone size and bone density represent two major determinants. Our recent findings using transgenic mice lacking IGF-I, IGF-II or growth hormone (GH) reveal that some aspects of GH/IGF system are involved in regulating both bone size and bone density throughout from prepubertal, pubertal and postpubertal growth phases. To evaluate the relative contribution of IGF-I, and IGF-II on bone mechanical properties, we measured bone strength parameters in mice lacking IGF-I or IGF-II and corresponding control mice at day 23 (prepubertal), 31 (pubertal) and 56 (postpubertal) in the femur by 3-point bending analysis using an Instron Mechanical Tester. In control mice, maximal load and area of moment of inertia increased by similar magnitude (50-60%, P<0.01) during pubertal growth period. However, during postpubertal growth period, maximum load increased to a much greater extent (100%) compared to moment of inertia (50%) in control mice. Values for IGF-I and IGF-II knockout mice are shown as % of corresponding control mice (*=P<0.01, n=8-20 per group)

Table 1

Parameter	Day 23		Day 31		Day 56	
	IGF I	IGF-II	IGF-I	IGF-II	IGF-I	IGF-II
Maximum Load (N)	38*	78*	26*	67*	20*	81*
Area of Moment of Inertia (mm4)	14*	53*	10*	50*	11*	65*
Elastic Modulus (GPa)	413*	129	192*	102	141*	114
Cort. BMD (mg/cm^3)	72*	98	63*	93	68*	97

The magnitude of difference in the maximum load and area of moment of inertia between IGF-II knockout mice and control mice was less at day 56 compared to day 31. However, that was not the case for IGF-I knockout mice. Elastic modulus was significantly higher in IGF-I knockout mice compared to control mice but not in IGF-II knockout mice. Based on these data, we conclude that: 1) bone size (area of moment of inertia) contributes more to bone strength during pubertal growth period than during postpubertal growth period; 2) increased elastic modulus may compensate for the decreased cortical bone density in IGF-I knockout mice, 3) IGF-I but not IGF-II influences mechanical properties of bone throughout postnatal growth period; and 4) IGF-I and IGF-II genes may exert their effects via different mechanisms on bone mechanical properties in mice. (Supported by the Department of the Army [DAMD17-97-2-7016]. The content does not reflect the position/policy of the government or NMTB).

Disclosures: ***S. Mohan****, None.*

M146

Growth Hormone Expressed in Erythroid Marrow is Osteogenic in Transgenic Mice in the Absence of Local IGF-I Induction. J. R. Kunes*, S. E. Sparks*, D. King. Biochemistry and Molecular Pathology, Northeastern Ohio Universities College of Medicine, Rootstown, OH, USA.

It is widely acknowledged that growth hormone (GH) acts through insulin-like growth factors (primarily IGF-I, but also IGF-II) to produce its anabolic effects. We have previously shown that transgenic mice expressing human GH in their erythroid marrow deposit more bone than normal controls. The current study was designed to test the hypothesis that the osteogenic effect of growth hormone is dependent on elevated local levels of IGF-I. Skeletally mature (12 week) mice from two GH transgenic lines and nontransgenic control littermates were used to test the hypothesis. Protein was prepared from crushed long bone shafts, acid extracted, and subjected to column chromatography to quantitatively separate the IGFs from their binding proteins. Additional long bone shafts were used for total RNA purification. Quantitative reverse-transcriptase PCR reactions were analyzed in real time to determine relative abundance of mRNAs coding for IGF-I, IGF-II, and the IGF binding proteins BP-3 and BP-5. While there were trends toward increased expression of all four genes in the bones of transgenics, only the BP-3 RNA levels were elevated (2-fold) over those of control mice with statistical significance. At the protein level, bone-localized concentrations of IGF-I mirrored serum levels. This suggested that the relatively low concentrations of human GH present in the serum may have induced IGF-I at low levels in the liver for general systemic distribution. The disproportionate effect of GH expression on the bones of the transgenics points to a likely direct local effect of GH on osteoblasts, with additional osteogenic contributions from up-regulated expression of BP-3. Evidence in the literature indicates that GH does not require elevated IGF-I in order to induce BP-3. BP-3 is normally soluble, but can also bind matrix and can be osteogenic. BP-3 may act directly and locally to induce osteoblasts in the GH transgenics, or it may act by altering the local availability of relatively normal levels of IGF-I to produce an osteogenic effect. Additionally, BP-5, also known to have independent osteoinductive effects, is not likely part of the induction cascade in the transgenic bones, as indicated by statistically insignificant modulations in its level of expression. An important distinction between our new findings and the literature is that the major source of GH in the bones of our transgenic mice is local, as opposed to more typical systemic exposure from pituitary GH or recombinant GH injections. The molecular evidence indicates that GH expression in the bone/marrow microenvironment produces an osteogenic effect without significant induction of IGF-I.

Disclosures: ***J.R. Kunes****, None.*

M147

Locally Delivered TGF-β2 Enhances Implant Fixation and Bone Regeneration in Rat Model. A. De Ranieri*, S. Kuroda, A. S. Virdi, D. R. Sumner. Anatomy and Cell Biology, Rush University, Chicago, IL, USA.

Currently, we and others are using a rat model to investigate methods of enhancing implant fixation. The present experiment evaluated the effect of three concentrations of TGF-β2 on bone regeneration and mechanical fixation. Five experimental groups (10 animals per group, 50 animals total) received unilateral femoral implantation of titanium rods in an IACUC-approved study. The five groups consisted of rats implanted with (1) uncoated titanium (Ti), (2) hydroxyapatite/tricalcium phosphate coated titanium (Ti/HATCP), and (3-5) 1 of 3 doses of TGF-β2 (0.1μ g, 1μ g, 10μ g) loaded upon Ti/HATCP implants. Animals were killed four weeks post-surgery. For each group, 7 implanted femurs were first analyzed by micro computed tomography (μCT), followed by a mechanical pull-out test to measure the strength of fixation of the implant. The remaining three specimen were embedded in plastic and sectioned for backscatter scanning electron microscopy and routine histology. From the μCT data, five comparable regions were selected along the length of the implant. The scanned sections were contoured to exclude the implant and the bone volume per total volume (BV/TV) was calculated for the region between the endocortical perimeter and the implant (diaphyseal sites) or between the margins of the surgical defect and the implant (distal metaphyseal site). The mechanical pullout tests showed an increase in strength in the TGF-β treated groups and this difference was significant between the 1 μg TGF-β2-treated group and the Ti group ($p < 0.05$). In four of the five μCT sections, the 10 μg TGF-β2-treated group had significantly higher BV/TV than the Ti group ($p < 0.05$). These findings are consistent with previous studies in a canine model which demonstrated enhanced bone ingrowth into porous coated implants with TGF-β2. There are two probable explanations for why the dosage for the greatest effect was different between the findings from mechanical testing (1 μg treatment) and μCT (10 μg treatment). First, the rather small sample size may have obscured treatment effects at all doses. Secondly, some of the mechanical strength may be from unmineralized matrix and microarchitectural differences (such as direct bone-to-implant contact) not evaluated by μCT.

Disclosures: ***A. De Ranieri****, NIH AR 42862 2; NIH RR 16631 2.*

M148

Analysis of Protein Secretion Profiles in Undifferentiated and ATRA-Differentiated C3H10T1/2 Cells upon TGFB Stimulation using the Ciphergen ProteinChip System. D. S. Bischoff*[1], S. Jia*[2], D. T. Yamaguchi[1]. [1]VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA, [2]Ciphergen Biosystems, Inc., Freemont, CA, USA.

Fracture healing is a complex process involving several coordinated steps including hemostasis, inflammatory reaction at the fracture site, proliferation and recruitment of mesenchymal cells, cartilage formation with subsequent replacement of cartilage with bone, and finally remodeling of the healed bone. Healing events are mediated by a variety of growth factors and cytokines that attract mesenchymal cells to the fracture site and then stimulate proliferation and differentiation to form bone. The role of TGFB on the expression of these cytokines was explored in C3H10T1/2 cells, a murine pluripotent mesenchymal precursor cell line, that is used as a mesenchymal stem cell model as well as a model for osteogenic and chondrocytic differentiation. Undifferentiated cells and those induced to differentiate towards the osteoblastic lineage by all-trans retinoic acid (ATRA) treatment were stimulated with TGFB. Supernatants were collected at 0, 24, 48, and 72 hr post-stimulation. After concentration, protein biomarkers were identified with the Ciphergen ProteinChip Biomarker System. This novel system allows for rapid analysis of hundreds of proteins simultaneously by Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF- MS). Three ProteinChips were used which selectively bind different biochemical classes of proteins – positively charged, negatively charged, or metal binding. Initial experiments have identified several protein biomarkers with molecular weights ranging from 6.9 to 28 kDa that are up-regulated in ATRA-differentiated cells but are not expressed in undifferentiated cells. Other biomarkers were down-regulated in ATRA-treated cells and may reflect differences between differentiated and undifferentiated cells. The molecular weight and isoelectric point of these biomarkers was used to search the Swiss-Prot database to identify potential candidate cytokines and growth factors that have similar protein characteristics. Purification and immunoprecipitation experiments are

underway to confirm the identity of these biomarkers. This study illustrates the usefulness of ProteinChip assays for both identification of novel proteins involved in bone formation, repair, or differentiation and as a complementary approach to mRNA gene array analysis to confirm changes in protein regulation suggested by gene chips.

Disclosures: ***D.S. Bischoff****, None.*

M149

Inhibin A Prevents Orchidectomy-Induced Bone Loss in Mice. D. Gaddy[1], D. S. Perrien[1], N. S. Akel*[1], D. C. Montague[1], R. A. Skinner*[2], F. L. Swain*[2], L. J. Suva[2]. [1]Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Orthopaedic Surgery, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

We have previously demonstrated that Inhibin-A suppresses and Activin-A stimulates osteoblast and osteoclast differentiation in primary Swiss-Webster murine bone marrow cultures, as well as osteoblastogenesis in cultures of primary human bone marrow cells. These data led to our hypothesis that Inhibins act to suppress bone turnover and maintain bone mass through direct inhibitory effects on osteoblast and osteoclast development. To test this hypothesis in vivo in male mice, we utilized the GeneSwitch system which uses transgenic transactivator mice with liver-specific expression of a mifepristone-activated chimeric nuclear receptor (GLVP), crossed with transgenic target mice containing a GVLP-responsive promoter upstream of polio-virus IRES (internal ribosome entry site)-linked sequences coding for the alpha- and beta-subunits of inhibin A. This intercross produced "bigenic" mice capable of regulable expression of inhibin A from the liver, which when induced was associated with suppressed levels of FSH (Mol Endo, 2000 Jul;14(7):1075-85). We determined that both the GLVP only (monogenic) and the bigenic crossed mouse strains obtained peak bone mass at 5-6 months of age, as determined by bone densitometry using the Piximus (Lunar). At peak bone mass, baseline BMD measurements were performed prior to sham or orchidectomy (ORCH) of male mice, and the subcutaneous placement of mefipristone or vehicle-containing pellets (Innovative Research). Animals were followed for 4 weeks prior to obtaining femoral bone marrow for osteogenic culture, and tibial analyses of bone volume by microCT. Marrow cultures were initiated from both monogenic and bigenic mice, and cultured in the presence of ascorbic acid and betaglycerolphosphate, and in the presence or absence of Inhibin A, noggin or BMP2. As we previously reported (Endocrinology 2002 Jan;143(1):74-83), both Inhibin A and noggin suppressed recruitment of cells into the osteoblastic lineage (number of AP+ CFU-F), as well as mineralization (number of CFU-OB, and alizarin red normalized to protein). Induction of monogenic mice with mefipristone had no effect on either intact or ORCH bone volume or architecture in the proximal tibia. However, inhibin expression induced by mefipristone in bigenic mice protected against ORCH-induced volumetric bone loss (BV/TV) ($p<0.05$), which appeared to be due to a decrease in trabecular number. Together, these data provide direct in vivo evidence that Inhibin A regulates bone turnover and bone mass through bone marrow cell differentiation.

Disclosures: ***D. Gaddy****, Diagnostics Systems Laboratories 5.*

M150

Distinct Regulation of Bone and Muscle Maintenance During Hindlimb Suspension by Concentric and Eccentric Exercise. D. S. Perrien[1], N. S. Akel*[1], D. C. Montague[1], M. Knox*[2], J. D. Fluckey*[2], E. E. Dupont-Versteegden*[2], C. A. Peterson*[2], L. J. Suva[3], D. Gaddy[1]. [1]Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Geriatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [3]Center for Orthopaedic Research in Orthopaedic Surgery, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Exposure to microgravity causes dramatic loss of both muscle and bone mass. In normal gravity, resistance exercise is commonly used to increase both muscle and bone. We tested a novel form of resistance exercise (RT) in rats using either concentric only (Conc) or concentric plus eccentric (C+E) force production to offset the loss of musculoskeletal mass during 2 weeks of hindlimb suspension (HS). In two separate studies, male, Sprague-Dawley rats (6-months old; 4 or 5 per group) were operantly conditioned, and then randomly assigned to groups of sedentary control (CON) or hindlimb suspension (HS), +/- the training (CONRT or HSRT, respectively). Resistance exercise consisted of 2 sets of ~21 repetitions of 100-300 Newtons of either Conc or C+E force, 3 days/week for 2 weeks during suspension. Volumetric bone density (BMD) and architecture of the proximal tibia were analyzed by pQCT and microCT.

In Study 1, using Conc RT, HS significantly ($p<0.05$) reduced volumetric BMD of trabecular bone, which was restored to CON levels in Conc HSRT rats. Similarly, 2 weeks of HS significantly ($p<0.05$) increased trabecular spacing, and decreased trabecular number, thickness (TbTh), and bone volume (BV/TV) in HS rats compared to CON ($p<0.05$). Trabecular thickness of HSRT rats was significantly greater than HS ($p<0.05$), whereas spacing, number, and BV/TV were not significantly different from CON or HS rats. In contrast, the loss of muscle mass in the soleus and gastrocnemius in HS compared to CON were not prevented by the Conc HSRT regimen ($p<0.05$).

In the second study, using C+E RT, significant decreases in trabecular density as well as TbTh were observed in HS ($p<0.05$), and these decreases were prevented by C+E HSRT. In addition, loss of soleus muscle mass in HS compared to CON ($p<0.01$) was now prevented by the C+E HSRT regimen ($p<0.01$). These data indicate that resistance training using only concentric force is able to prevent bone, but not muscle loss during 2 weeks of HS, whereas the addition of the eccentric component (resistance exercise) prevents both bone and muscle loss. Together, these data indicate that inclusion of eccentric exercise provides superior protection against the loss of musculoskeletal mass that is induced by HS.

Disclosures: ***D.S. Perrien****, None.*

M151

Limitations of Long Term Exercise Interventions Aimed at Improving Bone Health in Normally Active Boys. S. L. Bass[1], L. Saxon*[2], S. Iuliano-Burns*[3], G. Naughton*[4], R. Daly[1], C. Nowson*[1], E. Briganti*[5], S. Austen*[1]. [1]Centre for Physical Activity and Nutrition, Deakin University, Victoria, Australia, [2]Department of Orthopaedic Surgery, Indiana University, Indianapolis, IL, USA, [3]Department of Medicine, Melbourne University, Victoria, Australia, [4]The Childrens Hospital, Sydney, Australia, [5]Department of Epi & Preventative Medicine, Monash University, Victoria, Australia.

In both boys and girls twenty minutes of moderate impact exercise two to three times a week combined with 400 mg/d of increased dietary calcium resulted in a greater osteogenic effect at the loaded sites than either intervention alone.1,2 Greatest effects were evident in those children with the lowest levels of participation in organised sport. The questions asked were: i) Did an additional 8 months of exercise increase bone mass? ii) Did calcium supplementation in phase 1 influence the effect of exercise in phase 2? In phase 1, boys aged 9.0 (1.1) yrs (mean (SD)) of Tanner stages 1 or 2 were randomly assigned to either moderate impact exercise (with or without calcium) or control (with or without calcium). In phase 2, 71 of the original 89 boys continued with their randomised assigned exercise allocation in the following school year without the additional calcium: 29 exercisers (14 calcium and 15 placebo in phase 1) and 38 controls (14 calcium and 24 placebo in phase 1) completed at least 1 session per week (mean 1.7 and 1.4 sessions per week respectively). Regional BMC, anthropometry, sexual maturity, dietary calcium intake using a 24 hour recall and FFQ and physical activity levels were assessed at the beginning and end of the first year and the end of the second year. ANOVA was used to determine the effect of exercise. Effect modification by calcium supplementation in phase 1 on the effect of exercise in phase 2 was examined by the inclusion of an interaction term in the ANOVA. During phase 2 a greater proportion of the controls participated in high impact sports than the exercise group (67% vs 40%). In phase 2, there were no differences in BMC accrual at the weight bearing sites between the exercise groups, nor was there any effect modification by previous calcium supplementation. In summary, there are several factors that appear to have affected the success of this program, including reduced compliance in the second year of the intervention, high rates of participation in organised sport in the controls and difficulties associated with conducting long-term exercise interventions. In conclusion, these results have implications for the delivery of public health programs aimed at improving bone health in children through exercise.

1.Iuliano et al JBMR 2003;18:1,156-62 2. Bass et al JBMR 2002; 17:1,S294

Disclosures: ***S.L. Bass****, None.*

M152

Moderate Physical Activity Is not Associated with Increased Bone Mineral Increment in Girls During Late Adolescence – a 5 Years Prospective Study. Ö. Valdimarsson*[1], G. Sigurdsson[2], L. Franzson*[2], L. Steingrímsdóttir*[3], M. K. Karlsson[1]. [1]Dept of Orthopaedics, Inst of Orthopaedics, Malmo, Sweden, [2]Department of Medicine and Clinical Chemistry, Reykjavik, Iceland, [3]Icelandic Nutritional Council, Reykjavik, Iceland.

The purpose of this study was to evaluate the association between moderate physical activity and accrual of bone mineral density (BMD, g/cm2) and bone size in girls during adolescence.

A population based observational cohort including 78 Caucasian girls from Reykjavik, Island, with mean age 13.4 ± 1.0 years was prospectively followed for 5 years. Bone mineral density (BMD; g/cm2) and bone size was measured at the distal forearm by single photon absorptiometry (SPA) at baseline. After 3 and 5 years, BMD and bone size were re-measured with dual X ray absorbtiometry (DXA) at distal forearm, lumbar spine, femoral neck and total skeleton. The Z - scores (the number of standard deviations above or below age predicted mean) was calculated for the forearm measurements as to be able to compare the changes in the forearm during the study period. A questionnaire evaluated physical activity during the study period. Changes in bone mass and bone size were compared between the half of girls with the highest ("high") and the half of girls with the lowest ("low") level of physical activity. Data is presented as mean ± SD.

BMD (g/cm2), adjusted for differences in age-, height-, weight- and menarcheal age, was at baseline higher at the forearm (0.36 ± 0.05 versus 0.33 ± 0.04, p=0.001) and at 5 years higher in the forearm (0.57 ± 0.04 vs. 0.55 ± 0.04, p=0.04), the total body (1.06 ± 0.08 vs. 1.01 ± 0.06, p=0.001), the lumbar spine (1.09 ± 0.11 vs. 1.04 ± 0.09, p= 0.018) and the femoral neck (0.95 ± 0.12 vs. 0.87 ± 0.10, p=0.002) in the "high" physical active girls in comparison with the "low" active girls. No differences were found in bone size. In contrast, the accrual of forearm BMD and bone size from age 13 to 18 years, did not differ when comparing the "high" and the "low" active groups.

Moderate physical activity in girls is associated with high BMD at age 13 but does not seem to influence the accrual of forearm BMD or bone size during the later parts of the adolescence.

Disclosures: ***Ö. Valdimarsson****, None.*

M153

Absence of Mechanical Load Results in an Attenuation of Marrow Ablation-Induced Increase in Cancellous Bone Formation Rate. M. R. Allen, S. A. Bloomfield. Health & Kinesiology, Texas A&M Univeristy, College Station, TX, USA.

Prolonged periods of disuse result in significant loss of bone mass due to decrements in osteoblast number and function. Rodent hindlimb unloading studies have documented significant decrements in cancellous bone volume, osteoblast surface and bone formation rate yet little data exists on the mechanisms responsible for these disuse-induced adaptations.

The purpose of this study was to utilize marrow ablation, a potent bone formation stimulus, to determine if osteoblast function could be stimulated in unloaded bones. Four-month-old C3H male mice were divided into four groups: baseline control (BC), weight bearing control + ablation (CAB), hindlimb unloaded (HU) and HU + ablation (HUAB). Groups of animals (n=6-8) were sacrificed at both 10 and 14 days post-ablation. Right femoral metaphyseal bones were assessed using peripheral quantitative computed tomography and histomorphometry. There was no significant effect of sacrifice day on any measured parameter, thus data from animals sacrificed at 10 and 14 days post-ablation were pooled. Ablation-induced increases in cancellous bone mineral density (BMD), bone volume (BV/TV) and osteoblast surface (Ob.S) were similar in CAB and HUAB groups with respect to non-ablated controls. There was no effect of ablation on mineralizing surface (MB/BS), yet HU animals had significantly lower (-40%) values compared to weight bearing animals. The effect of ablation on bone formation rate (BFR) was load-dependent. Bone formation rate of CAB animals (102 ± 12 $\mu m^3/\mu m^2$/day) was significantly higher compared to both BC (55 ± 7 $\mu m^3/\mu m^2$/day) and HUAB (47 ± 8 $\mu m^3/\mu m^2$/day); there was no difference between HUAB and HU groups. These data suggest that while osteoblasts retain the capacity proliferate (increased Ob.S) and function (increased BV/TV and MAR) in response to a potent bone formation stimulus during disuse, there is a significant deficit in mineralization, likely producing tissue that is compromised with respect to mechanical strength.

Disclosures: ***M.R. Allen****, None.*

M154

Does Sex Hormones Override the Effect of the Physical Activity on Bone and Muscle in Early Pubertal Girls? S. Cheng[1], Q. Wang*[1], M. Suuriniemi[2], A. Lyytikäinen[1], P. Nicholson*[1], E. Helkala*[1], H. Kröger*[3], M. Alen*[4]. [1]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [2]Department of Cell Biology, University of Jyväskylä, Jyväskylä, Finland, [3]University of Kuopio, Jyväskylä, Finland, [4]PEURUNKA-Medical Rehabilitation Center, Jyväskylä, Finland.

The purpose of this study was to evaluate the influence of physical activity (PA) and sex hormones and its interaction on bone and muscle mass in 10-12 year-old Finnish girls (n=237) at maturation stage I-II (Tanner stage). Serum estradiol (E2) and testosterone (Ts) were analysed using immunofluorometry (DelfiaT, Wallac Oy, Turku). Bone mineral content (BMC) and density (BMD), and cross-sectional area (CSA) of the bone and muscle were measured at the tibia shaft by pQCT (XCT 2000, Stratec) and analysed with BonAlyse software (BonAlyse, Finland). Scores of PA were obtained by a questionnaire designed to evaluate the frequency, intensity, duration, and type of PA. Based on the levels of E2 and Ts, and PA scores, subjects were divided into low and high groups using the median values. Two-way ANOVA was applied to test the interaction of PA with E2, and PA with Ts on bone and muscle mass after adjusting for body mass index. At the Tanner stage I, PA and E2 had positive effects on bone CSA (p=0.049 & p<0.001, respectively) and BMC (p=0.006 & p<0.001, respectively). Girls with a higher level of E2 also had a bigger muscle CSA (p=0.021). On the other hand, PA tended to have a positive effect on BMD (p=0.051). No significant effect of Ts on bone and muscle variables was found in Tanner stage I. However, there was an interaction of E2 with PA on BMD (p=0.008) and a tendency towards an interaction of Ts with PA (p=0.06) on BMD. For girls at Tanner stage II, both E2 and Ts showed significant positive effects on the bone BMC (p=0.028 & p=0.01, respectively), and muscle CSA (p=0.032 & p=0.011, respectively). Girls with high level of Ts also showed higher BMD compared to their counterparts (p=0.016). For girls at Tanner Stage II there was no significant effect of PA on any of the measured variables, nor was there any interactions between sex hormones and PA. The results suggest that E2, Ts and PA are independent predictors for bone and muscle size and mass and have a combined effect on bone mineral density in prepubertal girls. With advanced maturation status the influence of sex hormones on bone and muscle mass might override the effect of physical activity.

Disclosures: ***S. Cheng****, None.*

M155

Comparison of Serum Leptin Concentration Under Simulated Microgravity Condition or Food Restriction or Combination of Two. K. Baek*, A. Currado*, M. R. Allen, S. A. Bloomfield. Texas A&M, College Station, TX, USA.

Leptin is a potential candidate responsible for linking energy metabolism to bone mass. Because astronauts are commonly in negative energy balance during spaceflight, this study was designed to assess independent effects of simulated microgravity and food restriction on bone mass and serum leptin. Male Sprague-Dawley rats (6-mo-old, skeletally mature) were randomly assigned to 4 groups (n=12 each). One group was subjected to hindlimb unloading (HU) and fed 100% of their food requirement (HU100), while another HU group was fed 70% of their food requirement (HU 70). Two cage-activity control groups were fed 100% (Con100) and 70% (Con70) of their requirement. On day 0 and day 28, peripheral computed tomography (pQCT, Stratec XCT Research M;Norland)) was used to assess bone mineral density (BMD) and serum was collected to measure leptin levels by ELISA (Crystal Chem. Chicago, IL). A decrease in serum leptin was observed after 28days in Con70, HU 100 and HU70, but not in Con100. The decrease in serum leptin was greater in HU100 group than Con70 group (-60% vs -27%, respectively), even though body weight decreased similarly and final body weights were similar in both group. Furthermore, serum leptin was non-detectable in HU70 group after 28 days. Total BMD decreased over 28d in all groups but the decline in HU groups was greater than that in Con groups (-8% vs –4%, respectively). The change in total BMD at the proximal tibia was moderately correlated with the change in serum leptin values over 28d (R=.48, p<0.01).We conclude that other factors besides the decrease of body weight or food restriction is associated with a decrease of serum leptin concentration during HU .

Disclosures: ***K. Baek****, None.*

M156

Accuracy and Precision Error of Muscle Cross-sectional Area Measured Using Peripheral Quantitative Computed Tomography in Adults. C. L. Gordon, C. E. Webber, L. F. Beaumont*. Radiology, McMaster University, Hamilton, ON, Canada.

Purpose: Using muscle area from standard spiral Computed Tomography (CT) as the gold standard this study assessed the accuracy of muscle cross-sectional area derived from pQCT images acquired at 0.4 mm and 0.8 mm voxels. The precision error for muscle area derived at these two voxels was also assessed.

Method: Twenty subjects participated in this study. Two pQCT images of the leg were obtained at 66% of the distance from the medial maleolus to the medial condyle of the tibia using a STRATEC XCT2000. Image #1 was acquired with standard scan parameters (voxel=0.8 mm, scan speed=30 mm/sec). For image #2 the voxel and speed were lowered to 0.4 mm and 20 mm/sec. The spiral CT images were acquired on a General Electric scanner with 0.4 mm voxels and 2 mm thick slices. pQCT muscle cross-sectional area was derived using standard analysis parameters. To reduce noise, median filtering was applied to the 0.4 mm voxel image before analysis. Custom software was used to extract muscle area from the spiral CT images. To assess precision , 10 subjects had the 0.8 mm and 0.4 mm voxel pQCT images acquired on three different days within three weeks. Subjects were not repositioned between the scans. Least squares analysis was used to compare pQCT and spiral CT muscle area. Precision error was assessed as percent coefficient of variation.

Results: There was good agreement between pQCT and spiral CT muscle cross-sectional area (R^2 >0.9, p<0.001). As well, good agreement was found between muscle area derived from the 0.8 mm voxel pQCT image and the filtered 0.4 mm voxel image. The precision error for muscle cross-sectional area derived at either voxel setting was small (1-2%).

Conclusion: Muscle cross-sectional area derived from pQCT images is accurate when compared to muscle area from spiral CT. Second, filtering 0.4 mm voxel pQCT images to remove noise does not reduce the accuracy or precision of muscle measurements.

Disclosures: ***C.L. Gordon****, Orthometrix Inc 5.*

M157

Bone Adaptation Response to Mechanical Loading In Mice Ulnae. M. P. Akhter, S. J. Short*, D. J. Wells*, D. M. Cullen. Medicine, Creighton University, Omaha, NE, USA.

Bone adaptation response to mechanical loading is influenced by factors that include genetics and the level of induced strain on the periosteal surface. This project investigated the genetic influences on bone adaptation response to varying strain levels within ulnae of three mouse (C57BL/6J[**B6**], DBA/2J[**D2**], C3H/HeJ[**C3**]) breeds. Adult female mice (n=24/breed, 4 mo. old) were subjected to *in vivo* ulna loading with a schedule of 36 cycles (@2Hz) per loading session, 3d/wk (Monday, Wednesday, and Friday) for 3 weeks. Each mouse breed received three levels of compressive force (N) to induce periosteal surface strains ranging from 800 to 3400μ in the mid-shaft of right ulnae (left ulnae non-loaded). Calcein injections were given on 3 and 10d before killing and all ulnae were collected and placed in 70% alcohol for standard histomorphometric analyses to quantify the parameters of bone formation. The periosteal mineralizing surface [**PMS**=(0.5sL+dL)/BS] was measured within each ulna (left and right). Mid-shaft (medial) periosteal surface strains were measured using strain gages (EA-09-032-SG-120 Micro Measurements, NC) in separate groups of adult female mice within each breed (5mice/breed). The differences in **PMS** due to: a) loading (left vs. right), and b) force magnitude, were compared using ANOVA at a significance level of P<0.05. At only the higher strain levels (>2000μ) the periosteal mineralizing surface (**PMS**) was different between the loaded and the nonloaded ulnae. The net periosteal mineralizing surface (**nPMS)** per unit-applied force ([Loaded-nonloaded ulna]/force, at the highest force level only) tended to be greater in **DBA** (12.7/N) as compared to both **C3** (9.2/N) and **B6** (10.3/N). In addition, these results suggest that with 36 loading cycles per session, bone in ulnae is osteogenic at strain levels greater 2000μ. This compares to reports in rat tibia and turkey ulna that show responses in the range of 1000-1500 μ. Therefore, the osteogenic threshold for ulnae bone adaptation is greater than what has been reported for mice tibiae.

Table: Ulna histomorphometric measurements

Mean ± SD	Force (N)	Microstrain (μ)	PMS [non-loaded] (%)	PMS [loaded] (%)	nPMS / Force (% / N)
C57BL / 6J	0.8	902±126	2.04 ± 2.27	1.82± 1.96[a]	-
	1.5	1691±239	3.28 ± 4.60	2.22 ± 4.34[a]	-
	3.0	3381±477	2.89 ± 4.51	33.72 ± 11.79[b]	11.24±3.9
DBA / 2J	0.5	727±291	0.67 ± 0.91	3.76 ± 5.89[a]	-
	1.0	1454±582	2.68 ± 2.76	2.71 ± 3.84[a]	-
	2.0	2908±989	0.48 ± 0.80	25.80 ± 6.62[b]	12.90±3.3
C3H / HeJ	0.8	657±214	6.01 ± 8.0	1.17 ± 2.1[a]	-
	1.5	1232±401	7.51 ± 8.22	9.23 ± 12.24[a]	-
	3.0	2463±801	2.23 ± 2.66	29.73 ± 14.93[b]	9.9±5.0

[a] Different than the highest force loading group *within* each breed (P < 0.05)
[b] Differences due to loading (P < 0.05)

Disclosures: ***M.P. Akhter****, None.*

M158

Anabolic Cyclic Fluid Flow Stimulation is Responsible for Trabecular Bone Adaptation. Y. Qin, T. Kaplan*, W. Lin*. Biomedical Engineering, SUNY Stony Brook, Stony Brook, NY, USA.

It has been demonstrated that anabolic hydraulic fluid flow significantly mediates bone mass and morphology in the cortical region, e.g., apply cyclic fluid pressure in the medullary canal resulted in surface and intracortical remodeling. While the fluid stimulus can be controlled quantitatively and potentially applied for therapeutic in promoting turnover and remodeling, it is hypothesized that low magnitude, cycle oscillatory fluid flow strongly influence bone's adaptive response in a manner of sensitive to flow rate and repetitive flow duration. The rate of bone's remodeling response to fluid flow stimuli was evaluated in trabecular bone region using an avian in vivo model. Total of 12 one-year old male turkeys was used in this study. A special designed fluid loading device was firmly attached on bone allowing oscillatory intramedullary pressure (ImP) stimuli. A sinusoidal fluid pressure was applied to the experimental ulna 10 min/day for 4 weeks. Three experimental groups of loading were performed: (A) 30 Hz, 76 mmHg (p-p); (B) 1 Hz, 76 mmHg (p-p); and (C) 30 Hz, 10 mmHg (p-p). All animals were labeled weekly using fluorochrome solutions, e.g., altered by tetracycline and calcein (15mg-Kg^{-1}) through IV. The trabecular regions were analyzed using histomorphometry measurement. The results reveal an increase of 22.7%±7.2 in trabecular volume fraction ($p<0.05$) for group A. New bone volume in Group B (1Hz, 76 mmHg) had only 0.5 % increase between loaded and contralateral control bones with no significance. Low magnitude, high frequency fluid stimulation (Group C) increases new bone (15.7%±7.4, $p<0.05$). This result indicates when applying physiologic fluid pressure (i.e., 76 mmHg), a high daily cycle number (18 k) of stimuli with high flow rate (30 Hz) generates much higher remodeling response (23% increase) than a low cycle number stimuli (0.6 k) (0.5% increase). Interestingly, those bones loaded at the high cycle numbers, but much smaller fluid magnitude perturbation (i.e., 18 k cycles with 10 mmHg) has shown significant bone turnover in the trabecular region (i.e., 16% increase). This implies that bone turnover may be more sensitive to the flow cycle number (elevated by frequency) than the pressure intensity. These data suggest that repetitive fluid stimuli elevated with high flow rate has strong influence on bone remodeling, which may influence fluid perfusion, convection, and surface fluid shear stress.

Disclosures: ***Y. Qin****, None.*

M159

Female Collegiate Gymnasts Have Greater Total and Cortical Bone Size in the Mid-Femur than Non-gymnasts. C. M. Modlesky*[1], M. L. Cavaiola*[1], J. M. Slade*[2], R. D. Lewis[3], G. A. Dudley*[2]. [1]Department of Health, Nutrition and Exercise Sciences, University of Delaware, Newark, DE, USA, [2]Department of Exercise Science, The University of Georgia, Athens, GA, USA, [3]Department of Foods and Nutrition, The University of Georgia, Athens, GA, USA.

Artistic female gymnasts are noted for their highly mineralized skeleton, as evidenced by areal bone mineral density (aBMD) values up to 30 % higher than age-, height- and weight-matched controls. However other features that contribute to bone strength, such as geometric structure, are not adequately described in this unique group. The purpose of this study was to compare the geometric structure of the mid-third of the femur (MTF) in female collegiate artistic gymnasts (n = 7) and controls (n = 7) of similar age, height and weight. aBMD, bone mineral content (BMC) and bone area of the MTF were determined using total body scans from dual-energy X-ray absorptiometry (Delphi A, Hologic Inc). Approximately 10 T1-weighted axial images representing the MTF were collected using a magnetic resonance imager. The volume and anterior, posterior, medial and lateral widths of the MTF and its cortical, endosteal and medullary compartments were assessed using a modified version of the X-vessel software program. Volumetric BMD of the total MTF (vBMDt) was determined by dividing BMC by total bone volume. Volumetric BMD of the endosteal and cortical compartments combined (vBMDc) was determined by dividing BMC by endosteal volume + cortical volume. aBMD, BMC and bone area were higher in GYM than CON (21, 29.6 and 6.8 %, respectively). These differences were accompanied by greater total and cortical volume of the MTF in GYM than CON (19.2 and 30.1 %, respectively). Moreover, total MTF was wider in the anteroposterior and mediolateral planes (10.0 and 14.0 %, respectively) and cortical wall MTF was wider in its anterior, posterior, medial and lateral regions (24, 35, 22, 29 %, respectively) in GYM than CON. There were no statistically significant differences in endosteal and medullary cavity volume or width; nor were there differences in vBMDt and vBMDc. The findings suggest that the higher aBMD observed in the MTF of artistic gymnasts is due to a larger diameter and volume of the total bone and its cortical compartment.

Disclosures: ***C.M. Modlesky****, None.*

M160

Increases in Bone Area and Periosteal Circumference are Greater with Gross Motor vs Fine Motor Exercise in Preschool Children: A Persistent Effect 12 Monts Post Intervention. T. L. Binkley*, J. Wermers*, B. L. Specker. EA Martin Program in Human Nutrition, South Dakota State University, Brookings, SD, USA.

We previously found in a 1-year randomized factorial trial among 178 preschool children that calcium intake (1g/d, 5d/wk) modified the leg BMC and tibia geometry response to exercise (30 min/d, 5d/wk of gross motor (GM) or fine motor (FM) exercise). The purpose of this study was to determine if changes persisted 12 months post intervention. Whole body and regional bone mineral content (BMC) by DXA and 20% distal tibia pQCT bone measurements were obtained at 0, 12 and 24 months. 90% of the children (N=161) were followed an additional 12 months after cessation of intervention (24 mo). Children in the GM group had greater average accelerometer counts and more time in vigorous activity at 18 months (6 mo post-intervention) than children in FM ($p=0.04$ & $p=0.05$ respectively, N=60). There were no differences in accelerometer readings at 24 months (N=58). Regression models controlling for time between scans, age, gender, childcare center, and change in weight, height and percent body fat, and for BMC outcomes change in bone area, indicate that increases from 12 to 24 months were greater in GM vs. FM group for whole body bone area (BA) ($p=0.08$), arm BMC and arm BA ($p= 0.03$ & $P=0.02$), leg BA ($p=0.05$). There were no differences in BMC or BA changes from 12-24 months by calcium supplementation. Differences in tibia periosteal circumference persisted at 24 months (GM 51.7 mm vs. FM 50.7 mm, $p=0.07$). There were no statistically significant differences in endosteal circumference, cortical area or cortical thickness by activity or supplement group at 24 month. In summary, preschool children randomized to GM exercise had greater vigorous activity 6 months post intervention than children randomized to FM exercise. A larger bone area and tibia periosteal circumference was observed in GM vs. FM 12 months post intervention. Whether differences 12 months post intervention are due to greater activity levels following the intervention or a delayed bone response to exercise is not known.

Disclosures: ***T.L. Binkley****, None.*

M161

Skeletal Response to Hind Limb Unloading in Mature Female Rats. J. A. Brittain*[1], A. Ethriedge*[1], E. A. Lucas[1], D. Bellmer*[2], B. H. Arjmandi[1], B. J. Smith[1]. [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Oklahoma Food and Agriculture Products Research and Technology Center, Oklahoma State University, Stillwater, OK, USA.

While most studies examining the effects of hind limb unloading (HLU) on the mature skeleton have focused on male animals, the intent of this study was to determine the skeletal response to unloading on the female skeleton. Six month old female virgin Sprague-Dawley rats were acclimated for five days and then either HLU (n=9) or maintained as ambulatory controls (AMB; n=9) for twenty-one days. Throughout the suspension period all animals received a standard semi-purified diet with the food intake of the AMB group matched to that of HLU animals. At the end of the unloading period, animals were sacrificed and tissues harvested. Tibial bone mineral content (BMC) and density (BMD) were decreased in response to unloading by 7% and 4% respectively, but bone mineral area (BMA) was unaltered. Assessment of distal femoral microarchitecture by micro-computed tomography (Scanco Medical μCT 40), revealed that the bone volume fraction (BV/TV) and trabecular thickness (TbTh) were significantly reduced by unloading, while trabecular number (TbN) only tended to be reduced. Additionally, HLU resulted in a significant increase in the structural model index (SMI) translating to a change toward a more rod-like structure. Analysis of cortical bone strength of the femur middiaphyses showed structural properties of the femur (i.e. ultimate and yield load) were reduced in the HLU group, but when the size of the bone was considered, material properties were unaffected. These alterations in bone associated HLU seemed to result from an increase in bone resorption as indicated by an elevation ($P<0.05$) in total urinary deoxypyridinoline excretion, and no change in markers of bone formation, serum osteocalcin and alkaline phosphatase. We conclude that the mature virgin female rat experiences significant reduction of trabecular bone due to skeletal unloading, similar to our studies with male rats. (OCAST HR01-133)

Disclosures: ***J.A. Brittain****, None.*

M162

Changes in Cortical Bone Formation of C3H/HeJ Mice Induced by Altered Mechanical Loading. R. A. Garman[1], L. Q. Xie*[1], L. R. Donahue[2], C. T. Rubin[1], S. Judex[1]. [1]Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, NY, USA, [2]The Jackson Laboratory, Bar Harbor, ME, USA.

The adaptive response of bone to changes in its mechanical loading environment is influenced by an individual's genotype. We have recently shown that trabecular bone of the distal femur of C3H/HeJ (C3H) mice was unresponsive to both anabolic and catabolic signals while catabolic pressure was capable of changing cortical bone morphology in the absence of altered bone mass. The effect of these signals on the formative processes in cortical bone, however, has not been established. Here, we evaluated changes in indices of bone formation in the cortical tibial diaphysis and metaphysis of C3H mice in response to mechanical vibration and disuse. Adult female C3H mice were subjected to low-magnitude (0.25g), high frequency (45Hz) mechanical vibrations applied for 10min/d (n=10), hindlimb suspension (n=12), or free cage activity (n=10). After 2 wk of mechanical loading, no changes in mineral apposition rate (MAR) or bone formation rate (BFR) were observed in the metaphysis, although a greater percentage (+153%, $p<0.001$) of the diaphyseal periosteal surface was single-labeled in mechanically loaded animals than in controls. Unloading caused higher periosteal BFR (+287%) and percentage of double labeled surface (+507%) in the metaphysis of those animals that displayed double labels (n=4 for disuse and n=2 for control). In the mid-diaphysis, endocortical (-51%, $p<0.03$) and periosteal (-86%, $p<0.05$) mineralizing surfaces were both smaller in disuse mice as compared to controls. Periosteal BFR was 79% less in unloaded mice than in control mice ($p<0.053$). Failure of the mechanical signal to increase cortical bone formation is consistent with our previous trabecular bone data, which indicated that the skeleton of C3H mice is unresponsive to a mechanical signal capable of increasing bone formation in both BALB/cByJ and C57BL/6J mice; this further demonstrates the strong influence of genetic variations on bone's mechano-sensitivity. The greater periosteal BFR in the metaphysis with hindlimb unloading may, at least in part, explain a previously observed compensatory mechanism in the femoral metaphysis of C3H mice, where disuse caused larger periosteal and endocortical surface envelopes in the absence of altered bone mass. The smaller bone formation rates in the diaphysis support previous data from the femoral mid-diaphysis in which disuse enlarged the endocortical surface envelope while bone quantity was maintained. Taken

together, our results suggest that the skeleton of C3H mice is responsive only to mechanical unloading, which may both decrease or increase bone formation dependent on the specific anatomical site.

Disclosures: ***R.A. Garman****, None.*

M163

Interleukin-6 Alters the Gene Expression of OPG in Osteoblasts in Response to Orthopedic Particulate Debris. T. H. Lamirand*, J. A. Struve, J. T. Ninomiya. Department of Orthopaedic Surgery, Medical College of Wisconsin, Milwaukee, WI, USA.

Aseptic loosening remains the primary cause of failure following total joint replacement. Despite advances in materials and engineering, the generation of particulate wear debris leads to bone resorption and implant failure. Recently, two proteins that play a critical role in osteoclast maturation, receptor activator of NFkB (RANK) and its ligand, (RANKL) have been described. These two proteins, along with the soluble decoy receptor, osteoprotegerin (OPG), work in concert to modulate osteoclast activity and bone resorption. The inflammatory cytokine IL-6 also alters osteoclast formation, and is secreted by osteoblasts in response to particulate debris. The addition of wear debris to osteoblasts increases expression and secretion of RANKL and IL-6, resulting in the process of osteolysis and implant loosening. However, the relationships between IL-6 and the gene expression and secretion of RANKL are not well described. Therefore, we hypothesized that IL-6 secretion modulates the production of RANKL and OPG, resulting in increased osteoclast maturation.
The roles of IL-6 and titanium particles on the gene expression and secretion of RANKL and OPG were investigated in murine osteoblastic MC3T3-E1 cells. Conditioned media were collected after the addition of titanium particles, and IL-6 ELISA was performed. Alterations in the gene expression of IL-6, RANKL, and OPG were determined by RT-PCR. Exogenous murine IL-6 was added to the cells and particles in culture, and further experiments also included the addition of IL-6 neutralizing antibodies. The effects of particles and IL-6 on osteoclast maturation were determined using a murine marrow cell assay.
Compared to unstimulated controls, titanium particles increased the secretion of IL-6 into the conditioned media. Particles also increased the gene expression of both RANKL and OPG.The addition of rmIL-6 did not increase RANKL, but produced a dose-dependent decline in OPG, increasing the ratio of RANKL to OPG. Anti-IL-6 IgG did not alter RANKL transcription, but increased OPG 2.8-fold, resulting in a reduction in the RANKL to OPG ratio.
Our results demonstrate that osteoblasts secrete IL-6 in response to particulate debris, and that IL-6 increased osteoclast maturation by decreasing the gene expression of OPG. These findings suggest an important autocrine role for IL-6 in the regulation of bone resorption through modulation of OPG secretion.

Disclosures: ***J.T. Ninomiya****, None.*

M164

RANKL and TNF-α Contribute to Acid-Induced Bone Calcium Efflux. K. K. Frick, S. B. Smith*, D. A. Bushinsky. Medicine/Nephrology Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA.

Chronic metabolic acidosis stimulates net calcium efflux from bone due to increased osteoclastic bone resorption and decreased osteoblastic collagen synthesis. We have previously shown that incubation of neonatal mouse calvariae in medium simulating physiologic metabolic acidosis leads to a significant, cyclooxygenase-dependent, increase in bone cell receptor activator of NFκB ligand (RANKL) RNA expression as compared to incubation in neutral medium. To test the hypothesis that acid-mediated increase in RANKL expression is a primary mechanism for this stimulated osteoclastic resorption, we incubated calvariae with, or without, the RANKL decoy receptor osteoprotegerin (OPG). Concentrations of OPG up to 25 ng/ml, far greater than physiologic, did not significantly decrease the robust acid-induced calcium efflux from bone. To confirm this observation, we utilized a soluble protein in which RANK is fused to an immunoglobulin Fc moiety (RANK / Fc); this protein binds to, and inactivates, RANKL. Incubation of calvariae with RANK / Fc (up to 50 ng/ml) also did not significantly diminish acid-induced net calcium efflux. Thus acid-induced net calcium efflux appears to involve mechanisms in addition to the RANK / RANKL pathway. Osteoblasts produce tumor necrosis factor-α (TNF-α), which also stimulates the maturation and activity of osteoclasts. A monoclonal antibody (Mab) to TNF-α will bind to, and inactivate, TNF-α. Incubation of calvariae with TNF-α / Mab (up to 250 ng/ml) did not significantly decrease acid-induced net calcium efflux. However, the combination of RANK / Fc plus TNF-α / Mab caused a significant (30%, $p< 0.02$) reduction in acid-induced calcium efflux while the combination of RANK / Fc plus an isotype-matched control for the Mab had no effect on calcium release. Thus inhibition of both RANKL and TNF-α is necessary to reduce acid-induced, cell-mediated net calcium efflux from bone; however, other factors must also be involved in mediating acid-induced bone resorption.

Disclosures: ***K.K. Frick****, None.*

M165

Oxytocin: a New Activator of Bone Cell Activity. S. Colucci*, G. Colaianni*, R. Tamma*, G. Mori*, C. Camerino*, L. Mancini*, A. M. Zallone. Human Anatomy and Histology, University of Bari, Bari, Italy.

Oxytocin (OT) is a well known hypothalamic hormone, whose effects in recent years have been demonstrated to be more widespread than previously believed. It was first known for his effects on lactation and parturition, but recently its receptors (OTR) have also been identified on a variety of normal and transformed cells. We found their expression on osteoblasts, and both on osteoclast precursors and mature cells. In this research we studied the signal transduction pathways activated by OT and searched for its biological effects. OTR belongs to the class I G protein-coupled receptor (GPCR) family and has 7 transmembrane domains. Its stimulation induced in both cell kinds an increase of intracellular calcium concentration, but with a different pattern: a single spike, returning immediately back to the baseline in osteoblasts and a slower, but long lasting, increase in osteoclasts. In both cell kinds ERK was phosphorylated after 5' of OT treatment and was back to basal levels after 20'. The ERK activation was inhibited by inhibitors of G proteins. Treatment with OT caused receptor endocytosis that, however, were again exposed on the membrane after 24-48 hours, as seen by immunofluorescence and confirmed at the mRNA level by PCR.
Human osteoblasts treated with OT displayed an increased proliferation rate, well evident after 48-72 hours. Moreover, in differentiated osteoblasts OT reduced the expression of osteoprotegerin and induced an increase in RANK-L.
OT given to human blood monocytes in the presence of MCSF induced in three days a significant increase in cell proliferation and increased expression of RANK. At day 12 of culture the number of TRAP-positive cells was higher than in untreated controls. In mature osteoclasts the anti-apoptotic pathway AKT was also phosphorylated within 5'. Bone resorption activity of mature osteoclasts resulted, however decreased by 30% in a 48 hour period.
All together these results indicate that the hormone, stimulating the proliferation of both cell kind, maintain a high rate of cell activity in bone. The increased osteoclastogenesis, coupled to the decreased bone resorption, can be justified in the logic of the time lapse between the stimulus and the end effect: when oxytocin is released (and/or receptor expression is up-regulated) osteoblasts proliferate, thus increasing bone formation, and resorption is decreased, but again, in a longer time lapse, a large number of osteoclasts will differentiate from the increased precursor pool, maintaining bone turnover at a high level.

Disclosures: ***A.M. Zallone****, None.*

M166

OPG Regulates Bone Formation Through the Coupling Mechanism with Bone Resorption. M. Nakamura*[1], N. Udagawa[2], Y. Kobayashi[3], K. Takaoka[4], H. Ozawa[3], H. Miyazawa*[1], N. Takahashi[3]. [1]Pediatric Dentistry, Matsumoto Dental University, Shiojiri, Japan, [2]Biochemistry, Matsumoto Dental University, Shiojiri, Japan, [3]Institute for Oral Science, Matsumoto Dental University, Shiojiri, Japan, [4]Department of Orthopaedic, Osaka City University School of Medicine, Osaka, Japan.

Deficiency of OPG in mice induces severe osteoporosis caused by enhanced bone resorption. Interestingly, bone formation was also accelerated in the deficient mice. We examined whether bone formation is coupled with bone resorption in OPG deficient (-/-) mice using risedronate, a bisphosphonate. Histomorphometric analysis showed that bone formation-related parameters as well as bone resorption-related parameters were strikingly elevated in vertebrae of OPG (-/-) mice (Table). When risedronate was daily injected into OPG (-/-) and wild-type mice for 30 days, bone formation-related parameters in OPG (-/-) mice were sharply decreased with the suppression of bone resorption. OPG (-/-) mice also exhibited high serum alkaline phosphatase (ALP) activity and osteocalcin concentrations, both of which were decreased to the levels of wild-type mice by the risedronate injection. The serum level of RANKL was markedly elevated in OPG (-/-) mice, but the treatment of OPG (-/-) with risedronate showed no effect on the circulating levels of RANKL. When collagen sponge disks containing recombinant human bone morphogenetic protein-2 (rhBMP-2) were implanted into OPG (-/-) and wild-type mice, rhBMP-2-induced ectopic bone formation at 3 weeks was not accelerated in OPG (-/-) mice even in a high turnover state of bone. However, attenuation of mineral density from the ectopic bone after 6 weeks of the implantation was accelerated in OPG (-/-) mice. These results suggest that bone formation is accurately coupled with bone resorption at the local sites in OPG (-/-) mice, and serum RANKL levels do not reflect this coupling.

Means ±SEM of 9 mice. *significantly different from wild-type mice, $p<0.0001$

	Wild-type mice	OPG (-/-) mice	OPG (-/-) mice
Risedronate injection	-	-	+
Osteoclast number (number/mm)	0.88 ± 0.13	3.36 ± 0.31*	0.81 ± 0.14
Bone formation rate (mm^2/mm^2/year)	0.11 ± 0.01	0.37 ± 0.02*	0.08 ± 0.01
Serum ALP activity (mU/ml)	37.1 ± 5.8	171.4 ± 16.0*	13.2 ± 0.7
Serum RANKL conc. (ng/ml)	0.05 ± 0.02	1.59 ± 0.37*	1.73 ± 0.24*

Disclosures: ***M. Nakamura****, None.*

M167

Retinoic Acid Downregulates Osteoprotegerin in Human Osteosarcoma Cells. A. C. Jacobson*, S. Johansson*, H. Brandstrom*, H. Melhus. Medical Sciences, Uppsala, Sweden.

Retinoic acid (RA) is known to stimulate osteoclast formation and activity in vitro. A high intake of vitamin A increases bone resorption in animals and is associated with increased risk for osteoporosis in humans. Osteoprotegerin (OPG) is a potent inhibitor of osteoclast differentiation and activation. We have studied the effect of RA on OPG expression in human osteosarcoma MG-63 and SaOs cells. RA in serum-free medium dose- and time-dependently decreased protein levels of OPG, as measured by ELISA. The maximum effect (-56.3%, $p<0.01$) was seen after 24 hours at a dose of 10^{-6} M. The effect on OPG

mRNA was studied with Northern blot. The OPG mRNA was decreased with a maximum effect after 4 hours in MG-63 cells. The less differentiated osteosarcoma, SaOs, expressed low levels of OPG. Treating SaOs with 10^{-5} -10^{-6} M RA in cultures decreased cell growth, whereas MG-63 cell growth was unaffected.
In conclusion we demonstrate that RA down-regulates OPG in MG-63 cells and depress the growth of SaOs cells, indicating that regulation of OPG is a mechanism by which RA can stimulate bone resorption.

Disclosures: ***A.C. Jacobson****, None.*

M168

OPG and RANKL Levels during Osteogenesis Depend on Substrate Microarchitecture. B. D. Boyan[1], S. Lossdoerfer*[2], D. M. Ranly*[1], Z. Schwartz[1]. [1]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [2]Orthodontics, University of Bonn, Bonn, Germany.

Recent studies have shown that osteogenesis on osteoclast-resorbed bone surfaces is sensitive to resulting microarchitectural features of the substrate. When osteoblasts are cultured on model surfaces with similar microrough architecture to osteoclast resorbed bone, they exhibit decreased proliferation and enhanced differentiation, as well as greater responsiveness to osteotropic factors including 1,25(OH2D3, estrogen, and BMP-2. In vivo studies using these model surfaces also show that bone formation is enhanced, suggesting that bone resorption may be modulated as well. PGE2 and TGF-beta-1 are increased in osteoblast cultures grown on rough microtopographies and there is a synergistic effect with 1,25(OH2D3-treatment. These factors are at levels that stimulate osteogenesis and TGF-beta-1 is at levels that inhibit osteoclast activity, susupporting the hypothesis that surface microtopography modulates release of factors from osteoblasts that modulate osteoclasts. To test this, we assessed the effect of surface microtopography on production of the osteoblast-generated decoy receptor osteoprotegerin (OPG), which modulates the interaction betweeen osteoblasts expressing RANK ligand (RANKL) and osteoclasts expressing RANK. MG63 human osteoblast-like osteosarcoma cells were grown on tissue culture plastic (Ra<0.2 um), smooth Ti disks (Ra<0.2 um), Ti disks that were grit blasted and acid etched (Ra=4um), and Ti plasma sprayed disks (Ra=5um). At confluence, cultures were treated for 24h with 0, 10-8 or 10-7M 1,25(OH2D3. Cell number was determined. Soluble RANKL (decoy ligand for OPG) and OPG in the conditioned media were measured using immunoassay kits. Cell number was reduced on the microrough surfaces; 1,25(OH)2D3 caused dose-dependent decreases on Ti surfaces but not plastic. Soluble RANKL was low on all surfaces. OPG levels were higher on the rough Ti surfaces; 1,25(OH2D3 increased OPG by 50% on the smooth Ti surface but on the microrough Ti surface (Ra=4 um), 10-9 M elicited a 100% increase and 10-8 M increased OPG by 200%. This effect was greater on the Ti plasma sprayed surface, where 10-8 M increased OPG by 350%. Real time PCR confirmed these results. Thus, on rougher microtopographies, osteoblasts secrete factors that enhance osteoblast differentiation while decreasing osteoclast formation and activity. Moreover, this surface-dependent effect is increased in a synergistic manner by 1,25(OH)2D3, which also regulates osteoclastic activity.

Disclosures: ***B.D. Boyan****, Institut Straumann 2, 5.*

M169

The Establishment of 6xOSE2-Luc Stable Cell Line that Efficiently Evaluates Cellular Runx2 Activity. H. J. Kim[1], J. H. Kim*[1], J. Y. Kim*[1], J. K. Kim*[2], K. O. Oh*[2], H. J. Kim*[3], H. M. Ryoo[1]. [1]Biochemistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [2]OCT inc., Chonan, Republic of Korea, [3]Pedodontics, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea.

Runx2 is an essential transcription factor for osteoblast differentiation from early commitment step to final differentiation. Depending upon its crucial role in osteoblast differentiation, the transcriptional activity of Runx2 protein implicates more valuable information for osteoblast differentiation than any other parameters, such as Runx2 mRNA level or protein level. Thus, a sensitive, specific and consistent method for the determination of the activity of Runx2 transcription factor has long been required. Here we established a stable cell line that carries 6xOSE2-Luciferase reporter vector. The characters of cloned cell lines were little different from the mother cells, premyoblastic C2C12 cells. The cells specifically responded with Runx2 modulating agents, such as FGF2, TGFb1 and BMP2, and the reporter activity was strongly enhanced by forced expression of Runx2 isoforms. The stable cell line responded 5-6 folds more sensitively than the transiently transfected cells and the activity was consistently maintained under the pressure of continuous G418 treatment. Together, the 6xOSE2-Luc stable cells could be a good biological evaluation system to assess the Runx2 modulating environmental stimulation.

Disclosures: ***H.J. Kim****, None.*

M170

Possible Role of (Pre-) Osteoclasts in Modulating TRAP Activity of Osteoblasts. S. G. Perez[1], I. Vogels*[2], W. Beertsen[3], V. Everts[1], S. G. Perez[4]. [1]Periodontology (ACTA) and Cell Biology and Histology (AMC), ACTA and AMC, University of Amsterdam, Amsterdam, Netherlands, [2]Cell Biology and Histology (AMC), AMC, University of Amsterdam, Amsterdam, Netherlands, [3]Periodontology (ACTA), ACTA , University of Amsterdam, Amsterdam, Netherlands, [4]Cell Biology, Academic Medical Center, Amsterdam, Netherlands.

Tartrate resistant acid phosphatase (TRAP) is considered to be an enzyme primarily expressed by osteoclasts. However, several observations suggest its presence also in other cells such as osteocytes, chondroblasts and osteoblasts. Since TRAP-activity in the latter cell type is mainly seen at sites close to osteoclasts, we wondered whether osteoclasts play a role in the modulation of TRAP-activity of osteoblasts. This question was addressed by co-culturing primary human osteoblasts with peripheral blood monocytes or osteoclasts or with conditioned medium (CM) obtained from cultured monocytes. A semi-quantitative RT-PCR method was used to compare the mRNA levels of TRAP between CM-treated osteoblasts and control cultures. The activity of TRAP was assessed by histochemical and biochemical assays.
Our data showed that in the co-cultures the osteoblasts in the direct vicinity of monocytes or osteoclasts expressed high levels of TRAP activity. When osteoblasts were cultured in the presence of CM, high TRAP activity was detected in all osteoblasts. In control cultures no TRAP activity was found. The expression of TRAP mRNA was found in osteoblast cultures either with or without CM. No difference in level of expression between these cultures was noted. CM that was heated prior to addition to osteoblasts did not induce TRAP-activity.
The presented data show that osteoblasts express TRAP activity if stimulated by monocytes/(pre-)osteoclasts. We propose that the enzyme is present in (non-stimulated) osteoblasts in a latent form and that activation is induced by, yet unknown, cytokines and/or growth factors released by monocytes/(pre)-osteoclasts.

Disclosures: ***S.G. Perez****, None.*

M171

Antioxidant Enzyme Paraoxonase-2 is Expressed in Human and Mouse Osteoblastic Cells. H. T. Kha*, J. A. Richardson*, C. J. Ng*, S. T. Reddy*, F. Parhami. Department of Medicine, UCLA, Los Angeles, CA, USA.

Osteoporosis and atherosclerosis often develop in parallel with aging. The oxidation of low-density lipoproteins (LDL) is a key process in atherogenesis. We previously showed that oxidized lipids and lipoproteins inhibit osteogenic differentiation of osteoprogenitor cells, and therefore may contribute to the development of osteoporosis as well as atherosclerosis. Paraoxanase (PON) is a family of enzymes that can inhibit LDL oxidation and/or destroy the biologic activity of LDL oxidation products. PON-1 and PON-3 are high-density lipoprotein (HDL)-associated enzymes and their expression is restricted primarily to the liver. Conversely, PON-2 is not HDL-associated and exerts its antioxidant functions at the cellular level. PON-2 is widely expressed and is found in a number of tissues including the brain, liver, kidney and testis.
In this study, we examined the expression of PON-1 and PON-2 in osteoblastic cells. Northern blot analysis showed the expression of PON-2 mRNA in several osteoblastic cell lines including: osteoblast-like human osteosarcoma cells, SAOS-2 and U2OS, osteoblast-like rat osteosarcoma UMR106 cells, mouse preosteoblastic MC3T3-E1 cells, and pluripotent mouse marrow stromal cells, M2-10B4 (M2). Furthermore, high levels of PON-2 mRNA expression were found in adherent bone marrow cells, which include marrow stromal cells and macrophages, isolated from C57BL/6 mice. No expression of PON-1 mRNA was found in any of these cell types.
Next, we examined the expression of PON-2 mRNA during the osteogenic differentiation of M2 and MC3T3-E1 cells *in vitro*. RNA was extracted after incubation for 2-12 days in an osteogenic medium consisting of 10% fetal bovine serum, 50 µg/ml ascorbate and 3 mM beta-glycerophosphate. Results showed that PON-2 mRNA was expressed throughout the differentiation time course in both M2 and MC3T3-E1 cells.
Treatment of M2 cells for up to 8 days with minimally oxidized LDL (MM-LDL) in osteogenic medium inhibited their osteogenic differentiation as assessed by the inhibition of alkaline phosphatase activity and mineralization. In parallel, PON-2 mRNA expression was increased 23% in response to MM-LDL compared to vehicle-treated control cells. This type of response is suggestive of a defensive reaction against the oxidant stress inflicted by MM-LDL. In conclusion, the previously unrecognized expression of PON-2 in osteoblastic cells may provide an internal mechanism of protection from inhibitory oxidized lipids and other inducers of oxidant stress that may have adverse effects on the osteogenic differentiation and activity of osteoblasts.

Disclosures: ***H.T. Kha****, None.*

M172

The Cis-regulatory Region, -9624 to +1996 for Dentin Matrix Protein Contains Modules that Increase Expression in Early Pre-Osteocytes in the Context of a Mineralized Matrix. W. Yang*[1], Y. Lu*[1], M. A. Harris[1], D. Guo*[1], J. Gluhak-Heinrich*[2], J. Zhang*[1], A. Krisnaswamy*[1], L. F. Bonewald[1], J. Q. Feng[1], A. Lichtler*[3], D. Rowe*[3], S. E. Harris[1]. [1]Dept. of Oral Biology, U. of Missouri at Kansas City, Kansas city, MO, USA, [2]Dept. of Orthodontics, UT Health Science Center, San Antonio, TX, USA, [3]UConn Health Center, Farmington, CT, USA.

Dentin Matrix Protein I (DMP1) is selectively expressed in osteocytes in mature bone. The DMP1 gene is also activated in response to mechanical loading in osteocytes, but not osteoblasts, in both the tooth movement model and the rat ulnae fatigue loading model. Understanding the *cis* regulation of DMP1 gene in response to the osteoblast differentiation program will lead to deeper mechanistic insight into osteocyte specific gene expression and the response of DMP1 gene to mechanical strain. Our first approach was to analyze the mouse and human DMP1 genes for islands of sequence conservation using *Vista* and *Family Relations* computer programs. Islands of conservation were detected 10kb 5' from the transcription start site, within all of the introns, and in 3 kb of the 3' flanking region. Northern analysis of DMP1 expression in 2T3 osteoblasts demonstrated that in BMP2 treated cultures, there was a 5 to 20 fold increase in DMP1 expression between 7 and 15 days, a time of active mineralized matrix production. DMP1 expression was most intense within the mineralized nodules, as determined by in situ hybridization. We prepared a DMP1 construct in which the

-9624 of the 5'flanking region, exon1 and approximately 1900 bp of the first intron was ligated into a splice acceptor GFPtopaz construct. This construct was stably transfected into 2T3 osteoblasts from which 5 independent cell clones were derived. The patterns of GFP expression, derived from single cell clones, were then monitored for up to 22 days, with and without the addition of 100 ng/ml BMP2. At confluence, the GFP pattern was diffuse and of low magnitude. The BMP2 treated cultures begin forming well organized mineralized matrix at 7 to 12 days. During this period, there is a dramatic and localized increase in GFP expression in the mineralizing matrix. Many of the strong GFP positive cells were dendritic in morphology and most likely represent maturing osteocytes in the context of a mineralizing matrix. Thus high levels of expression of DMP1 is dependent on formation of a mineralized matrix and presumed osteocytes within that matrix. Experiments are now directed at determining if the cis-regulatory regions for the osteocyte specific and mechanical strain response regions reside in this -9624 to +1996 region.

Disclosures: ***W. Yang****, None.*

M173

Expression of Matrix Extracellular Phosphoglycoprotein (MEPE) is Dependent on Differentiation of Primary Human Osteoblasts and of the Osteosarcoma Cell Line HOS 58. E. Schmidt*, B. Hennies*, M. Huefner*, H. Siggelkow. Endocrinology and Gastroenterology, University of Goettingen, Goettingen, Germany.

MEPE (matrix extracellular phosphoglycoprotein) is an extracellular phosphoglycoprotein, characterized by a high expression in tumors causing oncogenetic hypophosphatemic osteomalacia (OHO). An increased expression of MEPE in mineralizing murine osteoblasts compared to non mineralizing conditions has been observed and a role in mineralisation was assumed. We analyzed the expression of MEPE, alkaline phosphatase (AP) and osteocalcin (OC) in a differentiation model of primary human osteoblasts (pHOB) and of osteosarcoma HOS 58 cells. Primary human osteoblasts in second passage isolated from spongiosa of the iliac crest and HOS-cells in passage 68 were investigated with respect to the mRNA expression of MEPE, AP and OC and the housekeeping gene L7 by semiquantitative PCR with an internal standard. In HOS 58 osteosarcoma cells MEPE expression did not change during time in culture under basal conditions. Inkubation with 5 mM beta-glyerolphosphate (b-GP) on day 14 led to a significant stimulation of MEPE mRNA ($p<0.01$), whereas 10 und 20 mM b-GP caused a significant decrease of MEPE expression ($p<0.01$) together with increasing differentiation according to AP and OC expression. In pHOB MEPE decreased to 25 % of maximal expression during differentiation in culture (day 4 to 28). MEPE was not inducible by 2.5 mM to 20 mM b-GP at this time point using b-GP alone. In contrast, ascorbate stimulated and transforming growth factor beta, bone morphogenetic protein 2 and dexamethasone decreased the expression level.
Our results confirm that MEPE is a differentiation marker also in human osteoblasts. In contrast to the murine system MEPE decreased with further differentiation induced by time in culture, differentiation media or b-GP.

Disclosures: ***H. Siggelkow****, None.*

M174

Availability of Promoter-GFP Transgenic Mice for Identifying Subpopulations of Cells within the Osteoprogenitor Lineage. I. Bilic*, M. S. Kronenberg, X. Jiang*, I. Kalajzic*, D. W. Rowe. Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA.

The stem cell gene anatomy project (SC-GAP, http://www.scgap.org/) sponsored by NIDDK is a consortium of investigators who are sharing methodologies and resources to identify subpopulations of cells within a lineage and to define the molecular profile of the cells as they progress through stages of differentiation. The ultimate goal of the initiative is to develop and provide these reagents and strategies to the entire cell biology community.
This poster will describe the various transgenic mice that are available from this project that are useful in studies of the osteoprogenitor lineage. All of the GFP marker mice are conceptual derivatives of the pOBCol3.6GFP and pOBCol2.3GFP transgenic mice which have been consistent markers of preosteoblast, early osteoblast and late osteoblast differentiation. These transgenes are in CD1 mice and are currently being breed into C57/Bl6 and C3H backgrounds. The Col3.6 construct is available as a GFPtpz, GFPcyan and GFPsaph while the Col2.3 is GFPemd.
Previously we reported mice transgenic for the rat 1.7 kb osteocalcin-GFP transgene that showed expression restricted to rare individual osteoblasts and osteocytes in contrast to the wide expression of the Col2.3 transgene in the culture and in intact bone. We have recently generated C57/Bl6 transgenic mice with the human 3.8kb osteocalcin promoter driving GFPtpz. Screening tail biopsies revealed the strong expression of the transgene similar to our pOBCol2.3GFP mice suggesting this will be a strong and widely expressed marker of late osteoblast differentiation.
Currently we are examining promoters that may identify cells prior to preosteoblast differentiation. Fibronectin was selected because of its strong expression in developing embryos and its known expression in primary osteoblast cultures. Recently, we have produced two transgenic lines, in which the rat 4.5kb fibronectin promoter drives strong GFPtpz expression in the intervertebral disc region of the tail clips. In marrow stromal cell cultures, this transgene does not activate before pOBCol3.6GFP, and is restricted to large cells at the periphery of the nodule. In the histological sections of adult mice tissues from two different transgenic lines, GFP expression is restricted to cells of nucleus pulposis of the tail vertebral disc without any expression within bone. The value of this transgene for studying bone cell lineage is still under investigation. The progress of this and other constructs will be available through the SC-GAP program will be listed on a web site, http://skeletalbiology.uchc.edu/30_ResearchProgram/304_gap/index.htm.

Disclosures: ***I. Bilic****, None.*

M175

Acute Response of Resident Osteoblasts to Total Bone Marrow Ablation. L. Wang*, Y. Liu*, X. Jiang*, P. Liu, D. Rowe. University of Connecticut, Farmington, CT, USA.

Total body irradiation (TBI) sufficient to ablate the hematopoietic elements has been used to perform experiments designed to show engraftment of osteoprogenitor cells. Although hematopoietic recovery after bone marrow transplantation is well documented, the temporal response and recovery of endogenous bone cells is not adequately described and needs to be understood to plan and interpret bone cell engraftment studies. Mice 6-10 months of age that were transgenic for pOBCol3.6GFP, a sensitive transgene for assessing the metabolic activity of resident preosteoblasts and early osteoblasts were subjected to 900 cGy. This was followed immediately by bone marrow transplantation (1.5x106 cells via the periorbital sinus) from a nontransgenic donor and an injection of xylenol orange and BrdU 2 days prior to sacrifice. In both cases the recipients and donors came from a CD1 background. All irradiated mice not receiving a transplant died 5-14 days later while all mice that received the transplant survived. After sacrifice, the decalcified and non-decalcified femurs were prepared for frozen sectioning using the CryoJane tape method. Serial images that encompassed the majority of the femur were recorded by a Zeiss Axioplan 200/Improvision workstation. From that series, a composite image of GFP expression throughout the bone was reconstructed. Day one post irradiation (D1PI), there was widespread autolysis of marrow elements which progressed to a major dropout of cellularity between D3-5PI. Expression of GFP was unchanged during this time interval. By D7-9PI when the hematopoietic elements had begun to fill the marrow space there was an expansion in the number of pOBCol3.6+ cells on the endosteal and trabecular surface that became maximal by D14PI and returned to normal levels by D21PI. The wave of new osteogenesis is accompanied by an increase in xylenol orange labeling along the endosteal and trabecular surfaces. TRAP staining showed a wave of osteoclastic activity that paralleled the GFP expression. BrdU labeling was strong in the marrow space but was not increased in the bone lining cells. Taken together the resident osteoblast population appears to tolerate TBI and show a brief wave of new bone formation that appears to emanate from lining (non-proliferative and GFP negative) cells. Because there was no evidence of graft rejection up to 28 days, this model of transplantation provides an immunological window to assess early engraftment of a nontransgenic recipient with marrow from mice bearing a visual marker gene. Initial experience has shown no evidence of bone cell engraftment by transplanted transgenic marrow and suggests that other strategies to disrupt resident osteoblasts may be necessary to promote bone cell engraftment.

Disclosures: ***L. Wang****, None.*

M176

Arachidonic Acid and its Metabolites Prostaglandin 1 and 2 Increase Intracellular Calcium in Cultured Human Osteoblast-like MG63 Cells. A. Terranegra*[1], G. Vezzoli*[2], B. Baggio*[3], G. Priante*[3], D. Adamo*[1], C. Bianchin*[2], T. Arcidiacono*[1], A. Rubinacci[4], L. P. E. Soldati*[1]. [1]Department of Sciences and Biomedical Technologies, University of Milan, Segrate (Mi), Italy, [2]Division of Nephrology, Dialysis and Hypertension, Vita e Salute University, Milan, Italy, [3]Department of Medical and Surgical Sciences, University of Padua, Padua, Italy, [4]Bone Metabolic Unit, San Raffaele Hospital, Milan, Italy.

Arachidonic acid (AA) affects the activity of most known ion channels. Its effect may be positive or negative with different mechanisms of activity according to different channel types. The aim of the present research was to study the effect of AA on intracellular Ca (Cai) and Ca transport in osteoblastic cells. The mechanism of action of AA was explored. Cai was measured by fluorescence method on cultured human osteoblastic cells MG63 using the dye fura-2.
Exposure of cells to 200-300 microM AA elicited a concentration-dependent increase in Cai, that was derived mainly from extracellular Ca. In fact, in the absence of extracellular Ca (1 mM EGTA in extracellular fluid), 250 microM AA was ineffective on Cai. However, if endoplasmic reticulum Ca pumps was blocked by thapsigargin, a transient increase of Cai was recorded, indicating that AA was also active on intracellular Ca store. The Cai response to AA was not decreased by nifedipine, suggesting that AA did not activate a voltage-dependent Ca2+ channel. The Cai response to AA was completely inhibited by indomethacin at micromolar concentrations (50% of inhibition was obtained with indomethacin 145 microM). This finding indicates that the Cai response could be mediated, at least partly, by cyclooxygenase products of AA. To confirm this hypothesis, we tested the effect of prostaglandin 1 and 2 (PGE1, PGE2), that produced an increase of Cai at concentrations between 1-250 μM, even though the AA-induced Cai response far exceeded that induced by prostaglandins (AA-induced Ca influx: 1.4 +/- 0.03 nM/s; PGE1 and PGE2-induced Ca influx: about 0.3 nM/s).
We conclude that AA could modulate Cai in osteoblast-like cells MG63, through a different influence on Ca transport across plasma membrane and endoplasmic reticulum membrane. Its intracellular activity may be exerted via two mechanisms: directly via membrane lipid-protein interaction and indirectly via cyclooxygenase pathway.

Disclosures: ***A. Terranegra****, None.*

M177

Peroxynitrite Produced by Nitric Oxide and Superoxide Suppresses the Osteoblastic Differentiation. H. Hikiji[1], T. Abe[1], T. Koizumi*[1], W. S. Shin*[2], S. Shima*[1], N. Koshikiya*[1], T. Susami*[1], T. Takato*[1], T. Toyo-oka*[2]. [1]Oral and Maxillofacial Surgery, University of Tokyo, Bunkyo-ku, Tokyo, Japan, [2]Organ Pathophysiology and Internal Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Nitric oxide (NO) synthesized by nitric oxide synthases (NOS) plays an important role in bone metabolism. Endotelial NOS (eNOS) produces a little amount of NO which is related to bone formation. On the other hand, inducible NOS produces a large amount of NO which is related to bone loss. NO plays a role in both bone formation and bone resorption. However, how NO controls bone formation and resorption has been unknown. We have found that NO and its metabolite, peroxynitrite, control inflammation-mediated bone metabolism. Tumor necrosis factor-alpha and interleukin-1beta expressed inducible NO synthase gene with little effect on constitutive NO synthase gene. We measured NO production, alkaline phosphatase (ALPase) activity, osteocalcin gene expression, superoxide synthesis, peroxynitrite metabolites (nitrotyrosine) in order to determine the roles of these free radicals on the osteoblastic activities. Osteoblastic MC3T3-E1 cell line was stimulated by NO donor (ethanamine, N-ethyl-,compound with 1,1-diethyl-2-hydroxy-2-nitrosohydrazine; ethanamineNO), superoxide donor (pyrogallol) and/or peroxynitrite scavenger (urate). EthanamineNO dose dependently elevated the ALPase activity. Combination of ethanamineNO and pyrogallol reduced both ALPase activity and the osteocalcin gene expression. Peroxynitrite have been found to inhibit the osteoblastic differentiation, since both ALPase activity and osteocalcin gene expression were recovered by urate. We have also found that combination of proinflammatory cytokines (tumor necrosis factor-alpha plus interleukin-1beta) yield NO, superoxide and peroxynitrite. Therefore, we conclude that peroxynitrite produced from NO in the co-existence of superoxide, but not NO per se, would hide the stimulatory effect of NO itself on the osteoblastic activity and inhibit the osteoblastic differentiation.

Disclosures: ***H. Hikiji****, None.*

M178

Cytokine-Induced Inhibition of Osteoblastic Activity Is Reduced by Targeting of iNOS with Antisense DNA Plasmid. T. Abe[1], H. Hikiji[1], T. Koizumi*[1], W. S. Shin*[2], N. Koshikiya*[1], S. Shima*[1], T. Susami*[1], T. Takato*[1], T. Toyo-oka*[2]. [1]Department of Oral and Maxillofacial Surgery, University of Tokyo, Tokyo, Japan, [2]Department of Organ Pathophysiology and Internal Medicine, University of Tokyo, Tokyo, Japan.

Tumor necrosis factor (TNF) –alpha and interleukin (IL)-1beta enhance bone resorption, and may lead to inflammatory diseases such as osteoporosis and rheumatoid arthritis under several pathological conditions. These cytokines are reported to cause inducible nitric oxide synthase (iNOS) gene expression and nitric oxide (NO) production. In contrast, NO itself enhances osteoblastic differentiation *in vitro*. Therefore, these contradictory results mean that the bone-resorbing effect of cytokines is not mediated *via* NO *per se*. In this time, we have another report that the cytokines generate both NO and superoxide in osteoblasts and that NO and superoxide produce an even more toxic product, peroxynitrite, inhibiting osteoblastic differentiation at the same time. The first purpose of the present study is to examine effects of the specific inhibition of iNOS expression on osteoblastic cells. The second is to inspect whether iNOS antisense plasmid prevents cytokine-induced reduction of osteoblastic activity. Antisense techniques are specific for inhibiting the biosynthesis of a single protein. Especially, antisense DNA plasmids have advantages over synthetic antisense oligonucleotides since oligonucleotides must be repeatedly added at high concentrations in culture medium, and are not suitable for the long-term experiment. Here, we established stable transformants derived from osteoblastic MC3T3-E1 cells in which transfected plasmids produced iNOS antisense RNA continuously. We identified transformed antisense cell lines by 1) the detection of antisense transcripts, 2) the attenuated expression of iNOS protein, 3) the reduction of NOS activity and 4) the level of NO production. With these cells, we analyzed growth and osteoblastic differentiation by the parameters of type I collagen, alkaline phosphatase (ALPase), osteocalcin and Core binding factor A1 mRNA levels, ALPase activity in the presence or absence of proinflammatory cytokines. These cell lines targeting iNOS, which showed decreased production of both NO and peroxynitrite, prevented the inhibition of osteoblastic with the cytokine-stimulation. These results show that the antisense DNA plasmid of iNOS is potent to reduce the cytokine-induced inhibition of osteoblastic activity.

Disclosures: ***T. Abe****, None.*

M179

Cyclo-Oxygenase-2 Produced by High Passage MC3T3-E1 Cells Mediates Down Regulation of Proliferation and Differentiation of Low Passage Cells During Osteogenesis In Vitro. W. J. Peterson[1], 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 691/11g, Greater Los Angeles Healthcare System and UCLA School of Medicine, Los Angeles, CA, USA.

Previous reports suggest that certain age-related pathologies may be associated with accumulation of replicative senescent bone cells in osseous tissues. Components of heterogeneous populations of cells may be capable of regulating themselves as well as other cell types by autocrine and paracrine processes, respectively. It is possible that as replicative senescent cells accumulate in normal populations of bone cells they may down regulate surrounding cells, thus compromising bone formation and facilitating development of osteoporosis. To better understand the role of replicative senescence in aging bone, we have been using MC3T3-E1 cells as an in vitro model of cellular aging. Although, this model does not perfectly match the human fibroblast model in terms of having a finite number of cumulative cell divisions, it does show passage-related decline in cell proliferation and differentiation. For example, high passage (HP > 65 passages) cells show: (a) altered cell morphology; (b) decreased ability to respond to TGF-b1 and BMP-2; (c) diminished gap junctional intercellular communication; (d) decline in their ability to produce alkaline phosphatase and osteocalcin; and (e) alteration in gene expression. To test the hypothesis that HP cells may down regulate low passage (LP < 40 passages) cells in their bone forming activity, co-cultures were established between LP and HP cells to determine the effect of their cellular interaction. In some studies, LP cells were cultured in culture fluid taken from HP cells to assess the presence of soluble mediators of inhibition. In other studies, Cox inhibitors were added to some co-cultures to determine whether soluble mediators of inhibition were prostaglandins. Data are expressed as the mean ± SE. Results show: HP cells inhibit growth of LP cells kinetically between days 4-12 of culture; (2) The optimal ratio for maximum inhibition of proliferation is 2 LP cells to 1 HP cell; (3) Culture fluid from HP cells also inhibit growth of LP cells; (4) ECM from HP cells is not responsible for growth inhibition of LP cells; (5) HP cells inhibit alkaline phosphatase production by LP cells; (6) NS-398 reduced the level of HP cell -induced growth inhibition of LP cells. These results suggest that cells undergoing replicative senescence may down regulate proliferation and differentiation of normal neighboring LP cells by releasing prostaglandins.

Disclosures: ***W.J. Peterson****, None.*

M180

Sustained Inhibition of COX-2 Decreases Fracture Healing Success. J. P. O'Connor, A. M. Simon*. Department of Orthopaedics, UMDNJ-New Jersey Medical School, Newark, NJ, USA.

Prostaglandins produced by cyclooxygenase-2 (COX-2) have profound effects on bone metabolism. We have shown previously that pharmacological inhibition of COX-2 dramatically impairs fracture healing in rats and that targeted mutation of the COX-2 gene in mice also leads to impaired fracture healing [Simon et al., JBMR 17:963-976,(2002)]. In both instances, little or no COX-2 activity was present throughout the course of fracture healing. This is unlike common clinical scenarios when non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit COX-2 activity are generally used within the first 10 days following the fracture. Also, we had shown previously that chondrocytes within the fracture callus of rats treated with COX-2 selective NSAIDs disappeared from the callus between 2 and 3 weeks post-fracture indicating that endochondral ossification had ceased leading to development of fracture non-unions. Chondrocyte disappearance occurs well after the initial inflammatory phase of fracture healing that is correlated with high COX-2 activity. Therefore, we wished to determine if there was a specific phase during fracture healing when inhibition of COX-2 activity would be most deleterious to fracture healing. To identify any COX-2 dependent phase of fracture healing, rats were treated with the COX-2 selective NSAID, celecoxib (4 mg/kg/day), for 5 days prior to fracture; 5, 10, 15, 21, or 28 days post-fracture; and between 7-28 and 14-28 days post-fracture (average sample size per time point of 20 rats). Fracture healing was assessed radiographically and by mechanical testing. We found that inhibition of COX-2 during the early phases of fracture healing significantly impaired fracture healing success though longer periods of COX-2 inhibition were more deleterious. Histological analysis indicated that fracture healing failed in the celecoxib treated animals due to pre-mature differentiation of chondrocytes within the fracture callus. Together our observations suggest that COX-2 function is important for multiple phases of fracture healing including the early inflammatory phase as well as the later endochondral ossification phase.

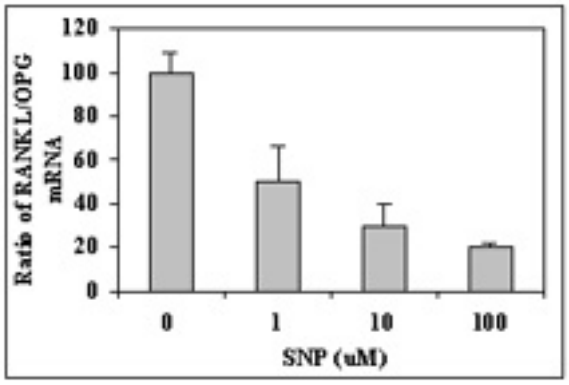
Disclosures: ***J.P. O'Connor****, None.*

M181

Nitric Oxide Regulates RANKL and OPG Expression in Bone Stromal Cells. X. Fan[1], E. Roy*[1], L. Zhu*[1], T. C. Murphy*[1], M. Hart*[1], C. J. Rosen[2], M. S. Nanes[1], J. Rubin[1]. [1]Emory University / VA Medical Center, Decatur, GA, USA, [2]Jackson Laboratory, Bar Harbor, ME, USA.

Bone remodeling reflects equilibrium between bone resorption and formation; the local expression of Receptor Activator of NFκB ligand (RANKL) and osteoprotegerin (OPG) in bone determines the entry of monoblastic precursors into the osteoclast lineage and subsequent bone resorption. Nitric oxide (NO), an intracellular messenger, has complex effects on bone cells, but many studies have shown that NO can slow bone remodeling and bone loss in animals and even humans. Evidence of an interaction of NO with RANKL and OPG has not been studied. Using real-time PCR, we show here that 24 h treatment of ST-2 murine stromal cells with 100 μM sodium nitroprusside (SNP), a NO donor, inhibited $1,25(OH)_2D_3$-induced RANKL mRNA to less than 33 ± 7% of control level while OPG mRNA increased to 160% of control. Therefore, the ratio of RANKL/OPG mRNA decreased in a dose-dependent fashion with respect to NO treatment (see Figure). Furthermore, western blot and ELISA confirmed real-time PCR findings, i.e., that RANKL protein decreased and OPG protein increased after treatment with NO. PTH-induced RANKL expression in primary stromal cells was also subject to inhibition by SNP, indicating that the NO effect was not due to inhibition of vitamin D action. NO donor did not change the stability of RANKL or OPG mRNA assessed by message half-life, suggesting that NO's predominant effect was on transcription of these osteoactive genes. Finally, cGMP, which can function as a second messenger for NO, did not reproduce the nitric oxide effect, nor did inhibition of endogenous guanylate cyclase prevent the NO effect on RANKL and OPG. Hence, NO effect on transcriptional regulation was independent of cGMP action. Our results suggest a significant distal effect of NO to decrease the ratio of RANKL/OPG, which should lead to decreased recruitment of osteoclasts and a increase in bone formation. Thus drugs and conditions which cause local increase in nitric oxide formation in bone

may have positive effects on bone remodeling.



Disclosures: ***X. Fan****, None.*

M182

Production of PGD_2 and Characterization of its Receptors in Human Osteoblasts in Culture. M. A. Gallant*, R. Samadfam*, J. A. Hackett*, A. J. de Brum-Fernandes. Rheumatic Diseases Unit, Université de Sherbrooke, Sherbrooke, PQ, Canada.

Prostaglandins, mainly PGE_2, have complex actions on bone metabolism and can regulate it either positively or negatively, depending on the interactions with different cells and types and subtypes of receptors. Little is known about the possible effects of prostaglandin D_2 (PGD_2) on bone metabolism. Our objective was thus to characterize the PGD_2 receptors present in primary cultures of human osteoblasts. Using human mature osteoblasts obtained from trabecular bone explants, we have recently shown that human osteoblasts in culture present DP, EP_4, FP, IP and TP receptors. Recently, a novel G protein-coupled receptor named CRTH2 (Chemoattractant Receptor-homologous molecule expressed on TH2 cells) was cloned. CRTH2, present mainly on T lymphocytes and eosinophils, binds PGD_2 and activates a $G\alpha_{i/0}$ protein. Our results with RT-PCR analysis revealed the presence of CRTH2 receptor mRNA in primary cultures of human osteoblasts and also in human osteosarcoma cell line MG-63, but not in Saos-2 cells. To verify the functionality of the two PGD_2 receptors (DP and CRTH2), we stimulated human osteoblasts with different agonists of these receptors and determined their effects on intracellular levels of cAMP and intracellular calcium. PGD_2 (EC_{50}: 5 nM), which stimulates both receptors and BW245C (EC_{50}: 1 nM), a DP specific agonist, dose-dependently increased the levels of intracellular cAMP. This effect was reversed by the DP receptor antagonist BW A868A with IC_{50} of 5 and 50 nM for 1 µM PGD_2 and 1 µM BW245C, respectively. Stimulation with 1µM DK-PGD_2, a specific CRTH2 agonist, increased the intracellular level of calcium in human osteoblasts by 3 to 6 times the basal level. Using chemotactic chambers, we demonstrated that human osteoblasts migrate towards a gradient of DK-PGD_2 but not of BW245C. The ability of prostaglandin D_2 receptors to modulate cell growth was assayed using incorporation of [^{3}H]-thymidine; neither BW245C nor DK-PGD_2 changed the proliferation rate of osteoblasts in these conditions. When stimulated by IL-1, primary human osteoblasts increase 10-fold their production of PGD_2 compared to control. In conclusion, these results show that human osteoblasts produce PGD_2, and that PGD_2 may act on two receptors, namely DP and CRTH2, both present in these cells. The DP receptor is coupled to an increase in icAMP while the CRTH2 is coupled to an increase in intracellular calcium and is probably a chemotactic agent for osteoblasts.

Disclosures: ***M.A. Gallant****, None.*

M183

Prostaglandin Receptors and the Expression of OPG in Human Osteoblasts. R. Samadfam*[1], J. Antoniou*[2], A. J. de Brum-Fernandes*[1]. [1]Rheumatic Diseases Unit, Université de Sherbrooke, Sherbrooke, PQ, Canada, [2]Orthopedics, Jewish General Hospital, Montreal, PQ, Canada.

RANKL and OPG are produced by osteoblasts and play a central role in the control of osteoclastogenesis, osteoclast activity and bone remodeling. Several studies have shown the implication of prostaglandins in the expression of RANKL and OPG, but the receptors and enzymes implicated in these effects of prostaglandins remain to be studied. In the present study, we investigated the roles of cyclooxygenases and prostaglandin receptors on the expression of OPG in human osteoblasts in culture. These cells were obtained from femoral heads of patients undergoing hip arthroplasty. The concentration of OPG in the supernatants was determined by sandwich ELISA with a detection limit of 0.03 ng/ml and intra- and interassay variabilities of 10 and 13%, respectively. In basal conditions the concentration of OPG detected in the supernatants was 7 ± 0.9 ng/ml, and it was not changed by treatment with inhibitors of COX-1 (valeryl salicylate 10^{-4} M), COX-2 (NS 10^{-5} M) or non-specific COX inhibitors (diclofenac 10^{-5} M, naproxen 10^{-5} M and indomethacin 10^{-5} M). These results are in accordance with our previous findings showing that non-stimulated human osteoblasts do not present any cyclooxygenase activity. Exogenous PGE_2 at 10^{-6} M decreased the concentration of OPG in the supernatant by 65%. Of all the EP receptor agonists tested (11-deoxy PGE_1, 17 phenyl PGE_2, sulprostone, Butaprost at 10^{-6} M), only 11-deoxy PGE_1, an EP4 and EP2 receptor subtype agonist, dose-dependently (10^{-8}-10^{-6} M) decreased the production of OPG. Butaprost, a specific EP2 receptor agonist, had no effect, suggesting that the response obtained with 11-deoxy PGE_1 was mediated only by the EP4 receptor. These results were further confirmed by the specific EP4 receptor antagonist, L-161982 (10^{-6} M) that completely blocked the response to 11-deoxy PGE_1. We also tested the effects of DP, FP, IP, and TP receptor agonists (respectively BW 245C, fluprostenol, carbaprostacyclin, and U46619) at concentration of 10^{-6} M on the expression of OPG. Among the agonists tested only BW 245C, a DP receptor agonist decreased the expression of OPG by 55%. The effect of BW 245C was dose dependent and was completely blocked by the DP antagonist BW A868C. Our results show that activation of the EP4 or DP receptors inhibits OPG expression by human osteoblasts in culture.

Disclosures: ***R. Samadfam****, None.*

M184

Activation of Mitogen-Activated Protein Kinase (MAPK) Pathway in Mesenchymal Stem Cells (MSC) Derived from Control and Osteoporotic Postmenopausal Women. J. P. Rodriguez, S. Rios*, M. Fernandez*, A. M. Pino*, R. Hess*, J. F. Santibañez*. Laboratorio de Biología Celular, INTA. Universidad de Chile, Santiago, Chile.

Several evidences indicate that the MAPK pathway is involved in the osteogenic differentiation of MSC. Results presented in the literature are still controversial. Thus, some authors show that the phosphorylation of components of the MAPK pathway stimulates the differentiation of MSC to the osteogenic lineage; however, other authors show that the continuos inhibition of the phosphorylation of MAPK pathway stimulate the osteogenic differentiation of MSC. On the other hand, we reported that MSC derived from control and osteoporotic donors share some functional characteristics, but differ importantly in others, mainly in their ability to differentiate to the osteogenic and adipogenic lineages. The aim of the present work is to analyze whether changes in the activation of the MAPK transduction pathway explain, at least in part, differences observed for us previously in the dynamic response of MSC derived from control and osteoporotic postmenopausal women.
For this, MSC were isolated from bone marrow obtained from control and osteoporotic postmenopausal women. MSC were isolated by fractionation on a 70% Percoll density gradient. The MSC-enriched fraction was cultured in Dulbecco's Minimal Essential Medium containing 10% fetal bovine serum, at 37º C in a humidified atmosphere of 5% CO_2.
The results obtained show that in basal conditions the ratio between phosphorylated extracellular signal regulated kinase (p-ERK) and total extracellular signal regulated kinase (ERK) is higher in osteoporotic than control cells. This ratio increases only in MSC derived from control donors when were cultured in osteogenic differentiation medium. Also, we found that inhibition of the MAP kinase pathway by PD98059, a specific MAPK/extracellular signal-regulated kinase (MEK) inhibitor, produced a different effect on the ability of control and osteoporotic cells to deposit calcium.
We conclude that the MAP kinase pathway is differently activated in MSC derived from control than osteoporotic postmenopausal women. The high pERK/ERK ratio observed in MSC derived from osteoporotic donors under basal conditions, could determinate the unresponsiveness of these cells to the osteogenic differentiation stimulus.

Disclosures: ***J.P. Rodriguez****, None.*

M185

Prostaglandin E_2 Increases the Osteogenic Capacity of Rat Bone Marrow by Promoting the Survival of Stromal Cells via Binding the EP_4 Receptor, Activation of Sphingosine Kinase and Inhibition of Caspase Activity. M. Weinreb, D. Shamir*, S. Keila*. Oral Biology, Tel-Aviv University Goldschleger School of Dental Medicine, Tel-Aviv, Israel.

Prostaglandin E_2 (PGE_2) stimulates bone formation and increases osteoblast number in vivo. We showed previously that PGE_2 increases the osteogenic capacity of bone marrow (i.e. number of osteoprogenitors) in vivo and in vitro as a major mechanism of this stimulation of bone formation. In this study we explored intracellular mechanisms of this effect. Bone marrow stromal cells were harvested from 5-7 week-old SD rats, cultured for 21 days with 12% serum and dex (dexamethasone, 10 nM) and mineralized nodules were counted. PGE_2 (100 nM) increased nodule formation whether present for the entire 21-day period or for the first 12 hours only. Therefore we incubated bone marrow cells with various compounds for 12 hours (without dex), washed the non-adherent cells and determined the % surviving stromal cells. PGE_2 (100 nM) increased stromal cell number 2-2.5 fold. This effect was mediated by cAMP since forskolin (an adenylate cyclase stimulator) mimicked the PGE_2 effect (~ 4-fold at 10 µM). Furthermore, the PGE_2 effect was mediated via binding of the EP_4 receptor since it was abrogated by L-161,982 (a selective EP_4 antagonist) and mimicked by 11-deoxy-PGE_1, an EP_4/EP_2 agonist. In agreement, daily injection of an anabolic dose of PGE_2 (5 mg/kg) for 2 weeks increased ex-vivo nodule formation, an effect blocked by pre-injection of L-161,982 (10 mg/kg). The effect of PGE_2 in vitro on stromal cell number involved activation of sphingosine kinase (SPK) since SPP (sphingosine-1-phosphate, the SPK product) mimicked (~4-fold increase at 10 µM) and DMS (N,N-dimethyl sphingosine, an SPK inhibitor) abrogated the PGE_2 effect. Addition of SPP overcame the inhibition of DMS. Nodule formation on day 21 was increased after an initial 12-hour incubation with SPP, confirming the correlation between increased number of surviving stromal cells to increased nodule formation. Stromal cell number was increased 4-7 fold by inhibitors of caspase 9, 8 and 3 (max. effect at 10-50 µM) and this concentration increased also nodule formation. When PGE_2 and caspase inhibitors were co-incubated, the PGE_2 effect was lost, suggesting that it shared pathways with the caspase system. Indeed, direct measurement of caspase activity of marrow cells revealed that PGE_2 treatment significantly inhibited the activity of caspases 3 and 8. Since SPP and caspase inhibitors are anti-apoptotic, we conclude that PGE_2 increases the osteogenic capacity of rat bone marrow by promoting the survival of stromal cells via binding the EP_4 receptor, activation of sphingosine kinase and inhibition of caspase activity.

Disclosures: ***M. Weinreb****, None.*

M186

Overexpression of Cyclooxygenase-2 Decreases Proliferation of Osteoblastic Cells. Z. Xu*, O. Voznesensky, S. Choudhary*, L. G. Raisz, C. C. Pilbeam. Medicine, University of Connecticut Health Center, Farmington, CT, USA.

Constitutive expression or overexpression of cyclooxygenase (COX)-2 promotes tumor progression in multiple tissues, secondary to decreased apoptosis and increased cell proliferation. We examined effects of endogenous COX-2 expression by comparing cultured primary osteoblasts obtained by sequential enzymatic digestion of calvariae from COX-2 knockout (KO) mice and their wild type (WT) littermates. COX-2 expression is transiently induced in WT cultures by addition of fresh serum. There was decreased proliferation in COX-2 WT cultures compared to KO cultures, as measured by ^{3}H-thymidine incorporation into replicating DNA over the last 2 h of culture normalized to cell count in parallel cultures. Apoptosis was also decreased in WT compared to KO cells, but the effect on proliferation was predominant since cell number was decreased in WT relative to KO cultures. Because the transient induction of COX-2 expression might have different effects on cell function than constitutively overexpressed COX-2, we developed a retroviral system to infect primary calvarial osteoblasts from COX-2 KO mice with a bicistronic construct co-expressing green fluorescent protein (GFP) and COX-2 (CMV-COX-2/GFP) or empty vector (CMV-GFP) under the control of the CMV promoter. Retroviruses were generated in 293 GPG packaging cells and the production of COX-2 confirmed by Northern and Western analyses. Primary osteoblasts were infected at 70% confluence with COX-2 expressing vector (V+C) or empty vector (V-C). The pattern of GFP expression was similar in both cultures. Because the conditioned medium carrying the COX-2 expressing retrovirus contained PGE_2, transduced osteoblasts were replated in fresh medium for experiments. After 4 days of culture, medium PGE_2 was increased 40% and cell number was decreased 20% in KO cells transduced by V+C compared with KO cultures transduced with V-C. Proliferation, as measured by a BrdU proliferation assay, was decreased 60% in V+C cells compared to V-C cells. An inhibitor of COX activity, indomethacin (1 μM) increased cell number and proliferation in KO cells transduced with V+C, while PGE_2 (1 μM) decreased cell number and proliferation in KO cells transduced with V-C. Similar to the effects of normally inducible COX-2 expression, transduction of KO cells with V+C increased alkaline phosphatase activity compared to KO cells transduced with V-C. We conclude that both the transient induction of endogenous COX-2 and the constitutive overexpression of COX-2 can decrease proliferation in osteoblastic cells and enhance osteoblastic differentiation. We speculate that overexpression of COX-2 will not promote tumor progression in osteoblastic cells.

Disclosures: ***Z. Xu****, None.*

M187

Metabolic Acidosis Increases Expression of Cyclooxygenase-2 mRNA in Bone. N. S. Krieger, D. A. Bushinsky. Medicine, University of Rochester, Rochester, NY, USA.

As humans age, the documented decline in renal function makes us less able to excrete our daily endogenous acid production, resulting in a mild chronic metabolic acidosis that is associated with an increased renal loss of body calcium. Chronic metabolic acidosis induces net calcium efflux from bone through inhibition of osteoblastic bone formation and stimulation of osteoclastic bone resorption. Acid-induced bone resorption is mediated by a stimulation of osteoblastic prostaglandin E_2 (PGE_2) synthesis, and this net calcium efflux from bone is correlated directly with PGE_2 production. Indomethacin, an inhibitor of both constitutive and inducible cyclooxygenase activity, completely inhibits acid-induced bone resorption. NS398, a specific inhibitor of cyclooxygenase-2 (COX-2), the inducible form of the enzyme which converts arachidonic acid to prostaglandins, also completely inhibits both acid-induced PGE_2 production and net calcium efflux. In this study we tested the hypothesis that chronic metabolic acidosis stimulates PGE_2 synthesis through increased expression of COX-2 mRNA. Primary cells were isolated from neonatal mouse calvariae by collagenase digestion and cultured until confluent. The cells were then incubated overnight in serum-free medium and subsequently switched to either physiological acidic (Acid, pH ~ 7.40, HCO_3^- ~24 mM) or neutral (Ntl, pH ~ 7.15, HCO_3^- ~14mM) pH medium containing 15% heat-inactivated horse serum. At 30 and 60 min, cells were collected and RNA isolated for Northern analysis using a COX-2 cDNA probe. Data were normalized by reprobing blots for GAPDH and results were expressed as percent of Ntl at each time point. While there was no difference in COX-2 mRNA levels between Acid and Ntl at 30 min, after 1h of incubation in Acid medium COX-2 mRNA increased approximately 2-fold compared to Ntl ($p<0.05$). Thus, the increase in osteoblastic production of PGE_2 observed in response to chronic metabolic acidosis and necessary for acid-induced bone resorption, is mediated by induction of COX-2 mRNA synthesis. This suggests the possibility that inhibition of COX-2 in humans may reduce age-related skeletal demineralization.

Disclosures: ***N.S. Krieger****, None.*

M188

Analysis of the Colony Formation, Expansion and Differentiation of Bone Marrow Stromal Cells Derived from COX-2-/- Mice. X. Zhang, E. Schwarz, R. O'Keefe. Orthopaedics, University of Rochester, Rochester, NY, USA.

To better understand the role of COX-2 in regulating mesenchymal stem cell differentiation into osteoblastic lineage, we combined colony formation assays with fluorescence activated cell sorting (FACS) analysis for alkaline phosphatase (ALP) and BRDU pulse labeling to examine the recruitment, proliferation and differentiation of bone marrow stromal cell culture derived from wild type or COX-2-/- mice. The lack of COX-2 expression had no apparent effect on the number of CFU-F, but lead to a slight reduction of the number of CFU-ALP and significant reduction of the area of CFU-ALP+ colonies. The addition of exogenous PGE2 (10-7M starting from day 1) significantly increased CFU-F, CFU-ALP and CFU-O in both wild type and COX-2-/- cultures, with a much larger induction in the COX-2 -/- cultures. Consistent with the colony assays, FACS analysis for intracellular alkaline phosphatase (ALP) demonstrated a significant reduction of ALP+ cells in COX-2-/- cultures in comparison with wild type controls. The addition of exogenous PGE2 significantly increased the percentage of ALP+ cells by 2 to 3 folds in wild type culture, 5 folds in the COX-2-/- culture. BRDU pulse labeling of the culture for 1.5 hour on day 7 demonstrated that PGE2 increased the percentage of ALP+ and BRDU+ (double positive) cell population, proportional to that of the ALP+ cells. This data suggests that exogenous PGE2 has no effect on the proliferation of the ALP+ cells. PGE2 could also induce the BRDU+ and ALP- cell population at the early culture, but this effect was variable among experiments. Averaging over several experiments, the induction of proliferation was not significant. Taken together, our current data supports that COX-2 is necessary for the differentiation of mesenchymal stem cells into osteoblasts, whereas exogenous PGE2 acts on recruitment, differentiation of mesenchymal stem cells and possibly the proliferation of osteoprogenitors.

Disclosures: ***X. Zhang****, None.*

M189

Role of c-src Inhibition in the Differentiation of Rat Osteoblasts *in vitro*. T. A. Owen[1], S. L. Smock*[1], Y. C. Clancy*[1], S. Prakash*[1], T. A. Castleberry[1], M. R. Moalli[2], L. C. Pan[1]. [1]Cardiovascular and Metabolic Diseases, Pfizer Global R&D, Groton, CT, USA, [2]Comparative Medicine and Physiology, Pfizer Global R&D, Groton, CT, USA.

C-src has been implicated in the function of both osteoclasts and osteoblasts. Although src has a ubiquitous tissue distribution, the only prominent phenotype reported for src-null mice is osteopetrosis. Osteoblasts derived from src-null mice or osteoblasts from normal mice in which src activity was inhibited by anti-sense oligonucleotides or compounds show augmented differentiation as measured by increases in alkaline phosphatase (AP) and nodule formation. To further elucidate the molecular mechanisms through which this enhanced differentiation occurs, we have undertaken a systematic investigation of the effects of c-src inhibition on parameters of primary rat osteoblast (ROB) differentiation using PP2, a pyrazolopyrimidine with an IC50 of ~500 nM for src kinase inhibition. Addition of PP2 with differentiation medium at each feeding beginning on day 4 for the duration of the culture resulted in stimulation of differentiation as reflected by increased nodule formation. Levels of total c-src protein and those of the related kinases c-yes and c-fyn were essentially unchanged throughout differentiation, even following chronic PP2 treatment. Focal adhesion kinase (FAK) protein, a src substrate involved in cell interaction with the extracellular environment, was readily detectable throughout the timecourse, but its expression was reduced approximately 50% in all PP2 treated cultures. We also found that c-cbl, another src substrate involved in adhesion, was detectable at days 2, 4, and 12 of culture and, although not detectable on intervening days, its expression was dramatically reduced in the PP2 treated cells at day 12. Total DNA and total soluble protein per well increased as expected through day 6 and then were stable through day 12, regardless of the presence of PP2. [^{3}H]-thymidine incorporation per cell was high early in the cultures and decreased with time, but was increased approximately 2-fold in the PP2 treated cells at day 6. After day 6, AP activity increased approximately 50% and by day 10, mineralized nodule area was over 10-fold higher in PP2 treated cultures. These changes were also reflected by increased AP staining, nodule number, and calcium deposition in the cell layer. [^{3}H]-proline incorporation into collagen also increased by 20-50% in the PP2 treated cultures. Based on these data, we suggest that inhibition of c-src activity in osteoblasts results in increased osteogenesis by prolonging the proliferation of osteoblastic precursors which form additional mineralized nodules upon differentiation of the cultures.

Disclosures: ***T.A. Owen****, Pfizer, Inc. 1, 3.*

M190

Shell Matrix Soluble Signal Molecules Induce Bone Cells Differentiation in Vitro Studies. D. Carlisi*[1], L. Pereira-Mouriès*[2], M. Almeida*[2], M. Lamghari*[2], M. Rousseau*[2], E. Lopez*[2], C. Milet*[2]. [1]SIERA SA, Paris, France, [2]Usm0401 (bome), Muséum National d'Histoire Naturelle, Paris, France.

We evaluated osteogenic activities of water soluble mother-of-pearl (nacre) organic matrix components extracted from the shell of bivalve mollusc Pinctada maxima on different mammalian cell types. The effect of nacre molecules on the osteogenic pathway was characterised from the step of cell recruitment to the outcome bone matrix mineralization.
We extracted the Water-Soluble Matrix (WSM) from P. maxima nacre powder without demineralization process to avoid denaturation of the biological activity of molecules. We used three mammalian osteoblastic cell types. Undifferentiated cells - human fibroblast MRC5 cell line and bone marrow stromal cells obtained from femurs of young rats - and the mouse pre-differentiated MC3T3-E1 cell line. Alkaline Phosphatase (ALP) activity was used as a marker of osteoblastic differentiation and/or stimulation. We evaluated ALP activity variation in undifferentiated cells. Using RT-PCR technique, we analysed Osteocalcin expression as a specific osteogenic markers for MRC5 differentiation. Pre-osteoblastic MC3T3-E1 cells were used as a model of mineralization in the osteogenic pathway. In vitro, these cells can differentiate in osteoblasts able to mineralize bone matrix when treated by b-glycerophosphate and ascorbic acid.
WSM stimulates ALP activity of human fibroblasts cells in culture and in bone marrow stromal cells. The effects were compared with those obtained with recombinant human Bone Morphogenic Protein-2 and Dexamethasone, which also stimulated ALP activity in MRC5 cells. We also demonstrated the stimulation of ALP mRNA expression in MRC5 cells treated by WSM using RT-PCR technique. In MRC5 WSM-treated cells, Osteocalcin mRNA expression was induced by 3-9 days. MC3T3-E1 cells were supplied with WSM extract and cultures were processed for bone mineralization detection. In pre-osteoblasts

MC3T3-E1 cell line, WSM shortens the period of time necessary to reach mineralization in vitro. The delay was 21 days long with b-glycerophosphate and ascorbic acid controls. Mineralization is occurred after only 6 days when WSM was added to the culture media.
Our data provides strong evidence for the presence in nacre WSM, of signal molecules, recognized by mammal cells and involved in cellular route assignment leading to cell differentiation in the osteogenic pathway. WSM accelerates mineralization process of pre-differentiating cells (MC3T3-E1), indicating that active molecules contained in nacre are able to act not only in recruitment and controlling osteoblastic precursors but also during the differentiation pathway and the final step of mineralization.

Disclosures: ***D. Carlisi****, None.*

M191

CCAAT/Enhancer Binding Protein Homologous Protein (CHOP) Induces Osteoblastic Cell Differentiation. R. C. Pereira, A. M. Delany, E. Canalis. Research, Saint Francis Hospital and Medical Center, Hartford, CT, USA.

CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), a member of the C/EBP family of transcription factors, plays a role in cell survival, differentiation and endothelial reticulum stress-induced apoptosis. CHOP binds to C/EBPs to form heterodimers that do not bind to classic consensus C/EBP sequences, often acting as a dominant negative inhibitor of classic C/EBPs. As a consequence, it prevents the differentiation of undifferentiated preadipocytes to mature adipocytes. The function of CHOP in bone is not known and we postulated it could induce osteoblastogenesis. We investigated the effects of constitutive CHOP overexpression in murine ST-2 stromal cells transduced with retroviral vectors. In the presence of β-glycerophosphate, ascorbic acid and BMP-2, ST-2 cells differentiated toward the osteoblastic phenotype. CHOP overexpression accelerated osteoblast differentiation as determined by the appearance of mineralized nodules, and the expression of osteocalcin and alkaline phosphatase mRNAs. The acceleration of osteoblastogenesis was more pronounced in the presence than in the absence of BMP-2. CHOP overexpression also opposed adipogenesis. In ST-2 cell cultures, CHOP overexpression did not cause substantial changes in cell number; however, as the cells entered terminal differentiation they underwent apoptosis and this process was accelerated by CHOP and BMP-2. CHOP overexpression did not modify C/EBP α or β mRNA levels, but decreased C/EBP δ mRNA after 24 days of culture. Electrophoretic mobility shift assays demonstrated that overexpression of CHOP decreased the binding of C/EBP α and β to a C/EBP consensus sequence, demonstrating the potential for a dominant negative role of CHOP in these cells. In conclusion, CHOP induces the differentiation of stromal cells toward the osteoblastic and away from adipogenic pathway, possibly through its interactions with C/EBP α and β.

Disclosures: ***R.C. Pereira****, None.*

M192

Effects of Fatty Acids on Lipid Accumulation, Differentiation and Cell Death in Primary Human Osteoblasts. J. E. Reseland*, M. Monjo*, J. O. Gordeladze*, C. A. Drevon*. Institute for Clinical Dentistry, University of Oslo, Oslo, Norway.

Lifestyle factors like dietary fat intake are known to affect biological functions of cells originated from the mesenchymal lineage (adipocytes and myoblasts), but the effects of fatty acids on osteoblasts have not been extensively examined. The aim was to evaluate the effects of different concentrations (0.05, 0.1 and 0.5 mM) of saturated (palmitic acid,16:0; PA) and polyunsaturated (eicosapentaenoic acid, 20:5 n-3; EPA) fatty acids on the lipid accumulation, differentiation and cell death in cultured primary human osteoblasts.
We observed less than 20 % cytotoxicity after incubation with 0.5 mM FA for 7 days as compared to control. PA induced death by necrosis, assessed by Hoechst 33342 and propidium iodide staining, whereas EPA induced mainly apoptosis through caspase-3 activation and altered Bcl-2/Bax mRNA ratio. Primary cultures of differentiated osteoblasts accumulated lipid droplets upon incubation with EPA, and cellular accumulation of EPA was 2.8 fold higher (p= 0.017) than the accumulation of PA after 6 hours.
mRNA expression of the osteoblast marker OSF-2 (Runx2) and leptin was reduced by EPA, as compared to PA and control osteoblasts incubated without fatty acids. There was no change in expression of alkaline phosphatase (ALP), whereas a moderate increase in collagen I mRNA was observed. Moreover, osteocalcin mRNA and the % of mineralized cell surface were enhanced upon incubation with EPA, indicating increased differentiation and biological activity of the primary osteoblasts.
In conclusion, EPA induced maturation of osteoblasts and apoptosis, being the main endpoint of differentiation for osteoblasts, whereas PA had no effect on differentiation, and promoted cell death by necrosis. Thus, dietary fatty acids may individually influence osteoblast differentiation and biological function, suggesting that dietary FA pattern may play a role in bone turnover and skeletal biology.

Disclosures: ***J.E. Reseland****, None.*

M193

Zinc Transporters in the Osteoblast: Gene Expression, Regulation, and Cellular Distribution. Z. Tang*, M. A. Khadeer*, A. Gupta. Oral & Craniofacial Biological Sciences, University of Maryland, Baltimore, Baltimore, MD, USA.

Zinc (Zn) is an essential trace element for normal bone growth and development. Zn has been suggested to affect bone mineralization, either directly as a divalent cation acting on nucleation and mineral growth, or indirectly as a cofactor for alkaline phosphatase and other metalloenzymes. Zn has also been suggested to act in collaboration with IGF-1 and TGF-β1 in stimulation of bone growth. Although the anabolic effect of Zn on osteoblasts has been well documented, not much is known about the uptake mechanisms for Zn in bone cells. There are at least two distinct families of Zn transporters that mediate cellular uptake and subcellular compartmentalization of Zn. First, we propose that the initial entry of Zn into osteoblasts is mediated by at least one family of Zn transporters. The gene expression of the Zrt-Irt-like protein (ZIP) family of Zn transporters was detected in the human (h) fetal osteoblast cell line hFOB1.19 by both RT-PCR and Northern blot analysis. In addition, the gene expression of Znt-5, a Zn transporter purported to mediate uptake of Zn into the Golgi apparatus, was also apparent in osteoblasts. Furthermore, the hZIP-1 protein could be detected as an ~35 kD protein by Western analysis of hFOB cell lysates using a chicken polyclonal antibody. Confocal microscopy revealed the cellular distribution of hZIP-1 as being partly diffuse throughout the cytosol, including expression at the osteoblast plasma membrane. Second, we have examined the regulation of hZIP-1, and alkaline phosphatase (ALP), which is a marker of osteoblast differentiation, in response to changes in *in vitro* ambient levels of Zn, or by the addition of IGF-1 (10^{-8} M) alone. Osteoblasts in culture were exposed to ~10 μM Zn (added as $ZnSO_4$ to the culture medium) for five days. We found that the gene expression of hZIP-1, Znt-5, and ALP were increased following exposure to external Zn, whereas IGF-1 alone induced the expression of hZIP-1. In a study designed to overexpress the hZIP-1 transporter in osteoblasts using a recombinant adenovirus, preliminary data suggests an increased gene expression of ALP. Our present data suggests that regulation of the cellular uptake mechanisms for Zn, and possibly alterations in intracellular Zn homeostasis, may modulate the osteoblast differentiation pathway.

Disclosures: ***Z. Tang****, None.*

M194

Insulin-Like Growth Factor I Enhances Osteoblastic Function but not Osteoblastic Differentiation. V. Deregowski*, R. C. Pereira, L. Priest*, E. Gazzerro, E. Canalis. Research, Saint Francis Hospital and Medical Center, Hartford, CT, USA.

Bone marrow cells can differentiate into various lineages, including osteoblasts, myoblasts, chondrocytes and adipocytes. Insulin like growth factor (IGF) I enhances the differentiated function of the osteoblast, but there is no evidence that IGF-I plays a role in the differentiation of stromal cells toward osteoblasts. In contrast, bone morphogenetic proteins (BMPs) induce the differentiation of stromal cells, which, in their presence, mineralize and undergo apoptosis, a terminal event in the process of cell differentiation. To define the role of IGF-I in stromal cell differentiation, murine ST-2 stromal cells were cultured in the presence of 5 mM βglycerophosphate and ascorbic acid for a 4 week period. Previously, we determined the association of IGF-I mRNA levels with the state of stromal cell differentiation. ST-2 stromal cells were transduced with retroviral vectors directing the constitutive expression of CCAAT enhancer binding protein (C/EBP) homologous protein (CHOP), a nuclear factor recently found to enhance BMP-2-induced osteoblastogenesis, or noggin, a BMP antagonist that prevents osteoblast differentiation. CHOP overexpression in the presence of BMP-2 caused an acceleration of osteoblastic differentiation, as determined by the appearance of mineralized nodules assessed by Alizarin Red staining, and apoptosis, as determined by nuclear fragmentation and condensation in Acridine Orange stained cultures. Both events, mineralization and apoptosis, were preceded by a decline in IGF-I mRNA levels. In contrast, overexpression of noggin prevented osteoblastic differentiation and apoptosis, and the levels of IGF-I transcripts did not decline. These results, in conjunction with the known anti-apoptotic effects of IGF-I, suggest that a decline of IGF-I expression is necessary to allow for the terminal differentiation of stromal cells and consequent apoptosis. To investigate further the role of IGF-I in stromal cell differentiation, ST-2 cells were treated with BMP-2 at 1 nM and IGF-I at 100 nM for up to 4 weeks. BMP-2 induced the formation of mineralized nodules, whereas IGF-I had a minor effect. Furthermore, cultures treated with IGF-I for 4 weeks induced the formation of adipocytes as detected by Oil Red staining. However, there was an increase in mineralized nodule formation in cultures treated with BMP-2 and IGF-I for 4 weeks, suggesting that osteoblastic phenotype had been enhanced. In conclusion, IGF-I does not induce stromal cell differentiation toward osteoblasts and a decline in its expression is necessary to allow for apoptosis and terminal osteoblastic cell differentiation.

Disclosures: ***V. Deregowski****, None.*

M195

Gap Junctions and Hemichannels Mediate Effects of Intermittent and Continuous Parathyroid Hormone (PTH) on Mineralizing Cells. P. P. Cherian[1], X. Wang*[1], L. F. Bonewald[2], J. X. Jiang[1]. [1]Biochemistry, University of Texas Health Science Center, San Antonio, TX, USA, [2]Oral Biology, School of Dentistry, University of Missouri, Kansas City, MO, USA.

It is well known that continuous PTH, as occurs in hyperparathyroidism, has a catabolic effect on bone, whereas the intermittent, anabolic effect of PTH on bone formation has been documented both in animals and in humans. Even though $PTH_{(1-34)}$ is now available for the treatment of osteoporosis, the mechanism whereby PTH mediates these opposing effects is not well understood. Intermittent, but not continuous, treatment by $PTH_{(1-34)}$ has been demonstrated to stimulate osteoblast differentiation and new bone formation. As inhibitors of functional gap junctions block mineralization of osteoblasts and deficiency of Cx43 results in the underdevelopment of bone, we sought to determine the role that gap junctions and Cx43 play in the effect of $PTH_{(1-34)}$ on osteoblast mineralization. The cell line MLO-A5, that rapidly mineralizes in culture within 3-6 days, was used. Intermittent PTH accelerates mineralization, whereas continuous PTH inhibits this process. Cx43 is abundantly expressed in MLO-A5 cells but with the greatest expression intracellular and with minor expression on the cell surface. When the cells were treated with 10^{-8} M $PTH_{(1-34)}$ continuously for 48 h, the expression level of Cx43 increased significantly; however, there was no obvious change in the cell surface expression of Cx43. Interestingly, if cells were subjected to an intermittent treatment (4 h-treatment per 24 h culture) of 10^{-8} M $PTH_{(1-34)}$ for a

total of 48 h culture time, the majority of the intracellular Cx43 migrated to the cell surface even though overall total protein expression of Cx43 was unchanged. An increase in Cx43 expression was observed along the entire cell surface, not only at the gap junctional regions, suggesting that an increase in the formation of Cx43 hemichannels is occurring in response to intermittent PTH. We propose that Cx43 in the form of hemichannels, instead of gap junctional channels, is likely to modulate the effect of intermittent PTH on the mineralization process. These studies show the importance of not only determining mRNA and protein expression but also protein localization and function. Ongoing research is to determine the role of hemichannels in mediating the anabolic and catabolic effects of PTH on bone formation.

Disclosures: ***J.X. Jiang****, None.*

M196

Effects of Green Tea Extracts and Polyphenols on the Proliferation and Activity of Bone Cells. H. Park*[1], S. Ko*[2], J. Kim[3], S. Kim[1]. [1]Department of Dental Pharmacology, Dankook University, Cheonan, Republic of Korea, [2]Department of Oral Biochemistry, Dankook University, Cheonan, Republic of Korea, [3]OCT Inc., Cheonan, Republic of Korea.

For many years, it has been recognized that some compounds in several foods have beneficial medicinal effects to health. This study was performed to investigate the effects of green tea extracts and polyphenols on the proliferation and activity of bone cells. Fifty grams of green tea were extracted with 70% methanol. After evaporation of methanol, the extracts were dissolved in 10% ethanol at a concentration of 0.1 g/ml. Effects of green tea extracts (0.2 - 0.0016 μl/ml) and polyphenols (10^{-12} - 10^{-5} M) on the proliferation and activity of osteoblasts and generation of osteoclasts from RAW264.7 cells were examined. Green tea extracts increased the proliferation of osteoblasts at relatively lower concentration. However, they inhibited the proliferation and viability of osteoblasts at higher concentrations. Green tea extracts increased ALP activity of osteoblasts at lower concentrations, and decreased it at higher concentrations. Green tea extracts dose-dependently inhibited the generation of osteoclasts. Polyphenols, such as catechin, epicatechin and epigallocatechin gallate (EGCG), increased the proliferation of osteoblasts at lower concentrations. Epicatechin increased the ALP activities of osteoblasts at lower concentrations. Higher concentrations of EGCG increased the ALP activities of HOS and ROS17/2.8 cells. Polyphenols when treated with lower concentration, stimulated the generation of osteoclasts. However, catechin and EGCG inhibited the generation of osteoclasts at higher concentrations. Taken together, this result indicates that green tea extracts and polyphenols, when used at appropriate concentrations, may stimulate the proliferation and activities of osteoblast, while inhibiting generation of osteoclasts. These compounds could be useful as prophylactic and therapeutic agents for many bone diseases which accompanies massive bone loss.

Disclosures: ***S. Kim****, None.*

M197

Differentiation of Human Mesenchymal Stem Cells at Specific Tissue Sites in Immunodeficient Mice. Z. Xia*, M. Lawson, J. T. Triffitt. The Botnar Research Centre, Institute of Musculoskeletal Science, Nuffield Department of Orthopaedic Surgery , Oxford University, Oxford, United Kingdom.

Bone marrow mesenchymal stem cells (MSC) have great potential to differentiate into various mesenchymal tissues. However, the mechanisms controlling these differentiation processes *in vivo* are still largely unknown. The aim of the study was to investigate the fate of human MSC, encoding enhanced green fluorescent protein (EGFP) as a cell marker, after implantation into different tissue sites in immunodeficient mice. Human bone marrow fibroblastic cells were transfected by a MuLV viral vector encoding EGFP and Neo^r genes with a transfection rate of 70%. The transfected cells expressed EGFP in a consistent manner throughout over 15 passages *in vitro* as confirmed by FACS analysis and fluorescence microscopy. The osteogenic potential of the cells was shown by immunocytochemistry and RT-PCR expression of osteocalcin and alkaline phosphatase before transplantation into specific tissue sites (intramedullary, intramuscular, intraperitoneal, intravenous, periosteal and subcutaneous) in NOD/SCID or CB17 scid beige mice. The results showed that the implanted cells survived for 2 to 12 weeks in these mice and integrated into local tissues. Surviving cells were detected by either EGFP fluorescence or human vimentin immunohistochemistry and EGFP mRNA expression by using RT-PCR. The integrated cells attained the morphology of the local host tissue cells and expressed osteocalcin and alkaline phosphatase when located close to bone tissue or in muscle. However, major proportions of the implanted cell populations were eliminated by four weeks after implantation and, thereafter, the remaining cells were distributed as individual cells or small groups at the tissue sites. No large colonies formed and no extensive intramembraneous or endochondral ossification from the implanted cells was observed in any non-osseus tissue. However, genetically marked cells were observed morphologically to participate in callus formation when the host bone periosteum was injured, and in trabecular bone formation following intramedullary injection. Large numbers of mouse macrophages were observed to invade tissue sites following implantation of human cells, but their role in the survival or elimination of the human cells is unknown. The results of this study suggest that the fate and differentiation potentials of the implanted human cells are dependent on the local environment of the host tissue. The regenerating environment following tissue injury appears to favour participation of the injected cells in tissue repair mechanisms and precise control of their development.

Disclosures: ***Z. Xia****, None.*

M198

TNFα Inhibits Glucocorticoid-Induced Differentiation of Human Osteoblasts by Up-Regulation of GRβ: Slower Cellular Differentiation Results in Increased RANKL Production. D. C. Ireland*, S. Bord, D. O'Gradaigh*, J. E. Compston. University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom.

Mutual antagonism between NF-κB and GR couples cellular responses to inflammatory cytokines and glucocorticoids which are known differentiation factors for osteoblasts. We investigated the effects of TNFα and hydrocortisone (HC) on the differentiation rate of human bone-derived osteoblasts (hOBs) and on their production of RANKL, the primary osteoclastogenic factor.

hOBs, supplied commercially or isolated in our laboratory, were cultured for 8 days in McCoy's medium containing 100μM long-life ascorbic acid, 1% or 10% human serum, 200mM glutamine and antibiotics. For experiments using HC alone, medium was supplemented with either 4μM β-cyclodextrin (carrier) or 0.2μM or 4μM cyclodextrin-encapsulated HC. For experiments using TNFα and HC, medium was supplemented for one day with 0, 1 or 10ng/ml TNFα, followed by additional supplementation with 0.2μM HC. The medium was changed every two days. Duplicate cultures were harvested for total RNA and protein isolation. Levels of mRNAs for GRα, GRβ, ERα, ERβ, COL1A1, ALP, OPG and RANKL were measured using real-time RT-PCR. Polypeptides were separated by PAGE, blotted onto PDVF membrane and immunostained for GRα, GRβ and β-actin.

In hOBs cultured in medium without added TNFα or HC, GRα mRNA and protein remained unchanged from day 3 to day 8 but GRβ mRNA and protein increased significantly ($p<0.05$) during this time. The addition of HC abrogated this increase in a dose-dependent fashion. When TNFα alone was added to cultures for one day, GRβ mRNA and protein and OPG mRNA increased significantly compared to controls. Subsequent addition of HC reversed the change in OPG mRNA levels and resulted, by day 8, in marked TNFα-dose-dependent increases in RANKL mRNA. On day 8, hOB cultures with added TNFα were less differentiated than controls as shown by their lower levels of ALP mRNA and lower ERβ/ERα mRNA ratios.

Thus, TNFα reduces the differentiation rate of hOBs by increasing cellular GRβ which acts as an inhibitor of GRα activity. This slower differentiation results in increased expression of RANKL mRNA. The data suggest a mechanism whereby high levels of TNFα may cause excess bone resorption in pathological states.

Disclosures: ***D.C. Ireland****, None.*

M199

Differentiation of MC3T3-E1 Pre-Osteoblastic Cells on Two-Dimensional Poly(lactide-co-glycolide) (PLGA) Films and Three-dimensional PLGA Scaffolds - A Real-Time RT-PCR Study. W. Huang, G. H. Rudkin*, M. Sukkarieh*, K. Ishida*, D. T. Yamaguchi, T. A. Miller*. VA Greater LA Healthcare System, Los Angeles, CA, USA.

Osteogenic differentiation of MC3T3-E1 cells cultured in vitro in tissue culture dishes has been well characterized. However, their differentiation in three-dimensional scaffolds, which is more relevant to their biology in vivo and useful in creating tissue engineered bone implants, is poorly understood. In this report, we studied mRNA expression of a number of key bone-related genes in MC3T3-E1 cells cultured on two-dimensional (2-D) poly(lactide-co-glycolide) (PLGA) films and three-dimensional (3-D) PLGA scaffolds using quantitative real-time RT-PCR technique. Expression of osteopontin (OPN), bone sialoproteins (BSP) and osteocalcin (OCN) all increased when cells cultured on 2-D PLGA films were subjected to differentiation medium containing ascorbic acid and b-glycerol phosphate for a period of 2 weeks. Treatment of cells with recombinant human BMP-2 resulted in further increases in expression for all three genes. However, when cells were cultured on 3-D PLGA scaffolds, their differentiation, as measured by expression of the above three genes, was significantly retarded. Expression of OPN was essentially unchanged during a two-week period regardless of BMP-2 treatment. Increases in expression of BSP and OCN were significantly lessened when cells were cultured in 3-D scaffolds. Expression of vascular endothelial growth factor (VEGF) increased as differentiation proceeded on 2-D films and was further enhanced with BMP-2 treatment. However, expression of VEGF decreased after a two-week culture of cells in 3-D scaffolds and was not responsive to BMP-2. Interestingly, expression of both OPN and VEGF was markedly induced when cells were transplanted from 2-D films to 3-D scaffolds. The induction occurred 2 hours after transplantation. Our results indicate that MC3T3-E1 cells commit to osteogenic differentiation at a much slower rate in 3-D scaffolds than in 2-D films. The reduced osteoblastic differentiation rate in 3-D scaffolds should be taken into consideration when developing a clinically useful cellular bone implants.

Disclosures: ***W. Huang****, None.*

M200

Hypoxic Conditions of the RWV Decrease Runx2 and Osteoblast Differentiation. C. S. Ontiveros, L. R. McCabe. Physiology, Michigan State University, East Lansing, MI, USA.

Mechanical load is known to be a major regulator in the promotion and maintenance or normal bone homeostasis. Increasing mechanical load leads to increased bone formation, while decreased mechanical load, as in spaceflight or chronic bedrest, leads to decreased bone formation. We have used a rotating walled vessel (RWV) as a model microgravity system to culture cells and address the role of decreased mechanical load on osteoblast growth and differentiation. Previously we have demonstrated that consistent with decreased bone formation during actual spaceflight conditions, alkaline phosphatase (AP) and osteocalcin (OC) expression were decreased by 80% and 50%, respectively following

24 hours of RWV conditions. In addition, Runx2 expression and AP-1 transactivation, key regulators of osteoblast differentiation and bone formation, were reduced by more than 60%. Attempts to understand why these changes were occurring came from an observation of the media from cells grown under RWV conditions. Media from cells grown under RWV conditions appeared more yellow with a pH between 6.5 and 6.7 when compared to a more pink color media with a pH between 7.2 and 7.4 in control cells. Oxygen measurements showed a more than 50% decrease in RWV oxygen concentration conditions compared to cells cultured under normal 10% oxygen incubator conditions. The decrease in oxygen concentration correlated with increased VEGF and GAPDH expression under RWV conditions, consistent with a hypoxic effect. This was an interesting finding since it had previously been demonstrated that decreased mechanical load decreases nutrient exchange and diffusion within bone. To correlate a phenotype with this hypoxic effect, we determined the direct effect of hypoxia on osteoblast phenotype. To this end, osteoblasts were grown under normoxic (10% oxygen) or hypoxic (2%) conditions. After 24 hours under hypoxic conditions, AP, OC, and Runx2 mRNA levels showed decreased expression relative to normoxic controls. Consistent with hypoxic conditions, cells grown under 2% oxygen concentration also showed increased VEGF and GAPDH mRNA levels. To determine whether recovery to a normal osteoblast phenotype is possible, osteoblasts under hypoxia were reoxygenated and markers of differentiation showed signs of reversal. This data leads us to consider that conditions of decreased mechanical load, as in spaceflight conditions, lead to molecular alterations consistent with a phenotype of decreased osteoblast differentiation.

Disclosures: ***C.S. Ontiveros****, None.*

M201

ACTH Alters Gene Expression in Osteoblastic-like Cells and Modulates Osteoblastic-like Cell Proliferation and Differentiation. S. Sridhar[1], Q. Zhong[1], R. Bollag*[1], C. M. Isales[2]. [1]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA, USA, [2]Medicine, Medical College of Georgia and Augusta VA Hospital, Augusta, GA, USA.

We have demonstrated the presence of melanocortin receptors in osteoblastic-like cells (see abstract by Zhong Q., et al). These receptors are activated by fragments derived from a larger molecule, pro-opiomelanocortin (POMC) and include: ACTH, a b and g-MSH, and b endorphin. In an effort to gain some insight into the action(s) of these fragments on osteoblast function experiments involving gene microarrays were performed using the osteoblastic-like cell line MG63. Using the gene microarrays, ACTH 1-24, alpha-MSH, beta-MSH and beta-endorphin (1 nM) altered the expression of 714,1557, 363 and 642 genes over 2-fold respectively. Stearoyl CoA desaturase (SCD)is a membrane bound enzyme which regulates the amount of unsaturated fatty acids in the plasma membrane , altering membrane fluidity, and thus having an impact on cell proliferation and differentiation. In gene microarray experiments ACTH increased expression of SCD by 6.6 fold, alpha-MSH had no effect on SCD while beta-MSH and beta-endorphin decreased SCD expression by 5.4 and 7.2 fold respectively. Northern blots confirmed that ACTH increased SCD expression by 2.5 fold over control, while no increase in SCD expression was observed for alpha-MSH, beta-MSH or beta-endorphin. In 3H-thymidine experiments using MG63 cells ACTH significantly increased thymidine incorporation (Control: 6,225+195; ACTH 0.1 nM: 9148+1901; ACTH 1 nM: 14974+1450; ACTH 10 nM: 16480+1170; ACTH 100 nM: 18785+1234; ACTH 1000 nM: 32,339+5797; cpm+SEM). Thus, ACTH has direct and specific actions which may include effects on osteoblast proliferation or differentiation.

Disclosures: ***S. Sridhar****, None.*

M202

Role of RGD-Peptide in cbfa1/ osf2 Expression Stimulation in Human Bone Marrow Stromal Cells (HBMSC). S. Pallu*[1], M. Dard*[2], A. Jonczyk*[3], B. Guillotin*[1], J. Amédée[1], M. Vernizeau*[4], M. Vernizeau*[4]. [1]INSERM U 577, Bordeaux, France, [2]Biomet-Merck Biomaterials, Darmstadt, Germany, [3]Merck KgaA Preclinical Research, Darmstadt, Germany, [4]Biomet-Merck France, Valence, France.

In order to promote osseointegration of bone substitute materials, we have previously selected a cyclic-RGD peptide which improve HBMSC adhesion and stimulates osteoblastic differentiation (osteocalcin expression). Furthermore, in our previous work, we have shown that culture of HBMSC on RGD-peptide coating induced an increase of protein tyrosine kinase activity which reached a maximum after 30 min and provoked early tyrosine phosphorylation of p125FAK and Erk1 after 15 min of cell seeding. Here, we assessed phosphorylation and expression of the other MAP kinase p38, and expression of transcription factor cbfa1/ osf2.

Human Bone Marrow Stromal Cells have been cultured for 15, 30, 60, 90 min in IMDM alone onto different coatings : cyclic-RGD peptide (100 µM), poly-L-Lysine (PLL) (0,005 % w/v), fibronectin (FN) (10 mg/ml), and plastic culture dishes. Total proteins were extracted using a RIPA buffer containing protease and phosphatase inhibitors. Phosphorylation and expression have been determined by Western Blotting using specific antibodies against p38, phospho-p38 and cbfa1/ osf2 (Sigma Aldrich, Cell Signaling, Santa Cruz Biotechnology). Fixed immunoglobulins were revealed by chemiluminescence. Products have been quantified using NIH 1.62 analyser (Clark Lab Mirco Junker), the ratio phospho-p38/ p38 and cbfa1 /osf2 expression have been normalized using a-tubulin as control.

Culture onto cyclic-RGD peptide promotes tyrosin phosphorylation of p38 after 30 min of cell seeding. Beside, fibronectin coating induce a similar kinetic of phosphorylation according to the engagement of integrins upon adhesion with a upper extent for 30 min. In the same way, adhesion to cyclic-RGD peptide seems stimulate cbfa1 /osf2 expression after 60 min.

In conclusion, adhesion of HBMSC onto cyclic-RGD peptide already shown as involved in early phosphorylation of p125FAK and Erk1/2; promotes activation of p38 which could be responsible of stimulation of osteocalcin expression. Moreover, p125FAK and Erk 1/2 phosphorylation is known to induce activation of transcription factor cbfa1/ osf2 in osteoblastic cells as demonstrated by Gallea and al. (Bone, 2001), and Tamura and al. (J. Bone. Miner. Res. 2001). It will be necessary to complete these preliminary results to determine if the MAP kinase p38 is involved in regulation of cbfa1/osf2 expression, in a first time; and to know its role front of Erk1/2 as for cbfa1/osf2 phosphorylation process, in a second time.

Disclosures: ***S. Pallu****, None.*

M203

Dexamethasone Mediates Osteoblastic Differentiation of Human PDL Cells by Inhibiting Collagenase Expression. T. Hayami*, Q. Zhang*, S. Kapila. Growth & Development, University of California San Francisco, San Francisco, CA, USA.

Although dexamethasone (dex) substantially enhances osteoblastic phenotype in osteogenic cells, including human periodontal ligament (PDL) cells, the basis for this response remains poorly understood. Since the accretion of a collagenous matrix is required for an osteoblastic response and dex is known to decrease collagenase expression, we examined whether osteoblastic differentiation mediated by dex is linked to a decrease in collagenase expression in PDL cells. PDL cells were plated at density of 15,000 cells/cm^2 in αMEM (+ 10% FBS). After 24 hours, serum-free medium was added alone or 50 mg/ml ascorbic acid (AA) or 10^{-7} M dex, or 10 mM β-glycerphosphate (bGP) or AA+dex or AA+bGP or dex+bGP or AA+dex+bGP. The medium was replaced every 24 hours for a total of 5 days. Cell-conditioned medium was assayed by gelatin zymography, collagenase-1 Westerns and collagen degradative assay. The cells were lysed for alkaline phosphatase (AP) assay or mRNA extracted for osteocalcin (OC), osteopontin (OP) and osteonectin (ON) RT-PCR. Cells exposed to dex alone or any combinations of treatments that included dex demonstrated increased AP, OC, OP and ON expression when compared to control cells or those exposed to AA or bGP. The induction of osteoblastic markers was accompanied by a decrease in collagenase-1 expression. A strong negative relationship between collagenase activity and AP (Pearson's r = -0.72), OC (r = -0.74), ON (r = -0.65) were noted. Changes in collagenase activity did not show any relationship to OP mRNA levels (r = 0.26). Dex (10^{-8} to $5x10^{-7}$ M) also produced a dose-dependent increase in AP that was paralleled by a decrease in collagenase activity. The relationship between collagenase activity and osteoblastic phenotype was further dissected by culturing cells in the presence of the collagenase inhibitors GM6001 or TIMP-1. Both 10 nM GM6001 and 50 ng/ml TIMP-1 caused a decrease in collagenase activity that was related inversely to changes in AP expression. Our studies suggest that dex enhances osteoblastic differentiation of PDL cells by decreasing collagenase expression. These findings also implicate the role of endogenous collagenase in regulating osteoblastic differentiation of these cells.

Disclosures: ***T. Hayami****, None.*

M204

Intermittent Treatment with Parathyroid Hormone (PTH) Inhibits Adipocyte Differentiation in Human Bone Marrow Stromal Cells. D. J. Rickard, F. Wang*, B. J. Votta, S. Kumar, M. E. Nuttall. Musculoskeletal Diseases Biology, GlaxoSmithKline, King of Prussia, PA, USA.

Whereas pulsatile PTH treatment leads to a stimulation of bone formation, mass and strength, continuous infusion preferentially increases bone resorption and results in bone loss. The mechanisms believed to account, in part, for the bone anabolic action of intermittent PTH include reactivation of quiescent bone surfaces and a reduction in osteoblast apoptosis. We investigated the possibility that intermittent and continuous exposure to PTH also differentially regulates osteogenic and adipocytic lineage commitment of bone marrow stromal progenitor cells.

To test this, human bone marrow stromal cells cultured under mildly adipogenic conditions in medium supplemented with dexamethasone, insulin, isobutyl-methylxanthine and troglitazone (DIIT), were treated with 50 nM human PTH(1-34) for either 1 hour/day or continuously (PTH replenished every 48 hours). After 6 days, cells treated intermittently with PTH remained fibroblastic whereas those treated continuously adopted a polygonal, irregular morphology. After 12-18 days numerous lipid vacuole-containing, oil red O-positive adipocytes were noted in cultures treated with DIIT alone, or with DIIT and continuous PTH. In contrast, adipocyte number was markedly lower in cultures treated with DIIT and intermittent PTH. These cultures also exhibited increased staining for alkaline phosphatase. Furthermore, intermittent but not continuous PTH treatment dramatically reduced glycerol 3-phosphate dehydrogenase activity in cell lysates as well as the mRNA expression for adipocyte marker genes. The direct PKA activator forskolin, when added to cells for 1h/day, mimicked the anti-adipogenic effect of intermittent PTH. Pretreatment of cells with MAP kinase inhibitor PD098059, failed to prevent the anti-adipocytic effect of intermittent PTH, whereas pretreatment with the specific PKA inhibitor H89 resulted in almost complete conversion to adipocytes.

These results suggest that prevention of adipocyte differentiation by the uncommitted osteoprogenitor cells of bone marrow stroma may represent an additional mechanism for the bone anabolic action of pulsatile PTH, an effect that appears to be dependent upon cAMP.

Disclosures: ***D.J. Rickard****, GlaxoSmithKline 3.*

M205

Endothelial Cells Modulate Bone Marrow Stromal Cell Differentiation Into Osteoblasts: Dependency on Endothelial Cell Maturation. T. Meury*, M. Alini*. Tissue Engineering, AO-Research Institute, Davos-Platz, Switzerland.

Osteoblast precursor cells, which are present within the heterogeneous bone marrow stromal cell population, differentiate into mature osteoblasts in a tightly regulated process. It is known that endothelial cells communicate with osteoblast precursors and also with mature osteoblast cells. However, the interaction and the role of endothelial cells on the differentiation process leading to mature osteoblasts are not well understood. We have therefore investigated the effect of human umbilical vein endothelial cells (HUVEC cell line, Cascade Biologics C-003-5C) on human bone marrow stromal cell (BMSC) differentiation towards the expression of the osteogenic phenotype, by using two types of co-culture systems: indirect contact (2-way communication) and EC-conditioned medium (1-way communication). The cultures were grown with and without the addition of dexamethasone, a known inducer of osteogenesis *in vitro*. In addition, endothelial cells were stimulated with VEGF (a known EC mitogen and suggested to be an important factor for EC maturation) before using them in the co-culture systems. Using the quantitative real-time-RT-PCR technique, we measured at different culture periods the mRNA levels of representative genes expressed at various stages during osteoblastic differentiation like osteopontin, bone sialoprotein II, osteonectin, osteocalcin, collagen I, MMP-13, BMP-2 and cbfa1. Cell proliferation, matrix mineralization, alkaline phosphatase activity and VEGF levels in the medium were also quantified. As expected, BMSC cultures stimulated with dexamethasone differentiate towards the expression of the osteoblastic phenotype, measured by an increased matrix mineralization, an elevated ALP activity and by the expression of specific osteoblastic markers, especially osteopontin, bone sialoprotein II and BMP-2. In both co-cultures, the differentiation process was delayed, independently of the co-culture system used. When HUVEC were stimulated with VEGF before co-culture, the inhibition of osteoblastogenesis was even greater. These results suggest that EC may potentially affect the rate at which BMSC differentiate into osteoblasts and that this modulation is dependent on the maturational stage of EC.

Disclosures: ***T. Meury****, None.*

M206

Adenosine Stimulates Il-6 Secretion By A human osteoprogenitor cell line via the Adenosine A_{2B} Receptor. B. A. J. Evans[1], C. Elford*[1], J. Ham*[2]. [1]Child Health, University of Wales College of Medicine, Cardiff, United Kingdom, [2]Medicine, University of Wales College of Medicine, Cardiff, United Kingdom.

Adenosine has been shown to have a wide range of actions in many tissues, and these actions are mediated by at least four different receptor subtypes: A_1, A_{2A}, A_{2B} and A_3. The presence of functional adenosine receptors in human osteoprogenitor cells and mature osteoblasts, however, has not been described. Using RT-PCR techniques we have demonstrated the presence of these four adenosine receptor subtypes in a human osteoprogenitor cell line (HCC1). In addition we have investigated the effect of 0 - 10 μM of selective adenosine agonists for each receptor subtype on IL-6 secretion by HCC1 cells during 0 - 24 hours. NECA (1 μM), a non-specific adenosine receptor agonist stimulated a 10-fold increase in IL-6 secretion with an EC_{50} of 100 nM. In contrast, 10-fold higher concentrations of adenosine, CCPA (A_1 agonist), and IB-MECA (A_3 agonist) stimulated IL-6 secretion by 2.5-, 2-, and 3.5-fold respectively. CGS21860 (A_{2A} agonist) had no effect, whilst forskolin, a known inducer of cAMP, also stimulated IL-6 secretion. The effects of 100 μM NECA (13-fold), adenosine (5-fold) and CGS21860 (2-fold) on cAMP stimulation paralleled that for IL-6 secretion and suggest that the functionally dominant adenosine receptor in HCC1 cells is the A_{2B} subtype. More recently, we have demonstrated the presence of the four adenosine receptor subtypes in primary human bone marrow stromal cells. These data demonstrate that adenosine can stimulate the release of IL-6 from osteoprogenitor cells via the A_{2B} receptor, and probably a cAMP-dependent mechanism.

Disclosures: ***B.A.J. Evans****, None.*

M207

A Common Stem Cell for Blood and Bone. E. Davis[1], Z. Gugala*[2], F. Camargo*[3], F. H. Gannon*[4], K. J. Jackson*[5], K. A. Kinestra*[6], H. D. Shine*[7], R. W. Lindsey*[2], K. K. Hirschi*[8], M. A. Goodell*[9], M. K. Brenner*[9], A. R. Davis*[10]. [1]Pediatrics/CAGT, Baylor College of Medicine, Houston, TX, USA, [2]Orthopaedic Surgery, Baylor College of Medicine, Houston, TX, USA, [3]Center for CAGT, Molecular & Cellular Biology, Baylor College of Medicine, Houston, TX, USA, [4]Orthopedic Pathology, Armed Forces Institute of Pathology, Washington, DC, USA, [5]Center for CAGT, Molecular Genetics, Baylor College of Medicine, Houston, TX, USA, [6]Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA, [7]Center for CAGT, Neurosurgery, Neuroscience and Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA, [8]Center for CAGT, Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA, [9]Center for CAGT, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA, [10]Center for CAGT, Departments of Pediatrics and Orthopaedic Surgery, Baylor College of Medicine, Houston, TX, USA.

Osteoblasts are continually recruited from stem cell pools to maintain or repair bone. Their immediate precursor is a mesenchymal stem cell (MSC) able to generate a variety of tissues, including muscle, fat, and cartilage, in addition to bone. Increasing evidence suggests the existence of a pluripotent bone marrow stem cell that can differentiate into both hematopoietic and mesenchymal cell lineages, but few candidates for this role have been reported. We tested the hypothesis that primitive side population (SP) stem cells, which reside in bone marrow and possess long-term hematopoietic reconstituting activity, can differentiate through MSC intermediates to become osteoblasts. When transplanted into lethally irradiated mice, a single genetically marked murine SP cell regenerated the depleted MSC population in recipients. Analysis revealed that approximately half of the osteoblasts carried the beta- galactosidase marker and therefore were derived from donor SP cells. Our data support the notion that marrow SP cells are not functionally restricted to hematopoietic reconstitution, but can give rise to MSCs and osteoblasts that actively participate in bone formation. This property of SP cells not only elucidates a previously unrecognized step in osteoblast development, but also has intriguing implications for the use of stem cell therapy in clinical orthopedics.

Disclosures: ***E. Davis****, None.*

M208

Androgen Acts like Estrogen on the Differentiation of the Bone Marrow Stromal Cells. T. Hayashi*[1], S. Kido[2], D. Inoue[2], H. Tanaka*[1], T. Matsumoto[2], R. Okazaki[1]. [1]Third Department of Medicine, Teikyo University, Ichihara, Japan, [2]Department of Medicine and Bioregulatory Science, University of Tokushima, Tokushima, Japan.

Bone marrow stromal cells (BMSCs) are common precursors for bone forming osteoblasts and adipocytes. Because age-related osteopenia is associated with both impaired bone formation and increased bone marrow adipose content, a reciprocal regulation of BMSC differentiation into osteoblasts/adipocytes has been proposed. Consistently, we and others reported that 17β-estradiol (E2) promotes osteoblastic whereas inhibits adipocytic differentiation of BMSCs. Similar to estrogen deficiency, reduced androgen action leads to osteoporosis. However, clinical and experimental evidence has suggested that a large part of androgen action on bone is mediated by estrogen, and contribution of direct androgen action is currently unclear. In the present study, we therefore examined androgen effects on the differentiation of BMSCs using 5alpha-dihydrotestosterone (DHT), a non-aromatizable androgen, and dehydroepiandrosterone (DHEA), an aromatizable adrenal androgen. Cell models used were, mouse stromal cell lines, MC3T3/PA6 (PA6), wild-type ST-2, and ST-2 stably transfected with an human estrogen receptor (ERa)α or ERβ expression vector (ST2ERαa or ST2ERβ, respectively). In all the cell models above, bone morphogenetic protein-2 (BMP-2) increased the number of both alkaline phosphatese (ALP) positive osteoblasts and oil-red O staining positive adipocytes, whereas troglitazone increased the number of adipocytes but not that of osteoblast. Both DHT (100nM) and DHEA (100nM) enhanced ALP induction by BMP-2 in ST2ERαa or ST2ERβ, but not in wild-type ST-2 or PA6 cells, despite comparable level of androgen receptor mRNA expression were detected in all the cells. DHT and DHEA dose-dependently decreased the number of adipocytes in association with a decrease in PPARγ2 mRNA expression in ST2ERαaand ST2ERβ, but not in the other cells. DHT and DHEA effects at 1μM were comparable to 1nM E2 effects, and were completely abolished with an ER antagonist ICI182780, but not with AR antagonists (hydroxy-flutamide or bicalutamide) or aromatase inhibitors. We conclude that DHT and DHEA promote osteoblastogenesis while inhibiting adipogenesis of BMSCs in a manner similar to but independent of estrogen. In light of recent report that not only AR but also ERs can confer anti-apoptotic effect of androgen in osteoblastic cells, our results suggest that these androgen action on stromal cell differentiation may be in part mediated by ER, or influenced by the abundance of ER.

Disclosures: ***T. Hayashi****, None.*

M209

Inhibition of Adipogenesis in Human Bone Marrow Stromal Cells by Hypoxia, TGF-β and Wnt. S. Zhou, J. Glowacki. Department of Orthopedic Surgery, Brigham & Women's Hospital, Harvard Medical School, Boston, MA, USA.

The decrease in bone volume associated with osteoporosis and age-related osteopenia is accompanied by an increase in marrow adipose tissue. Adult human bone marrow stromal cells (hMSCs) have the potential to differentiate to lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, and muscle. Therefore, controlling the differentiation of hMSCs may be useful for treating bone diseases such as osteoporosis or osteoarthritis. Our previous data showed that hypoxia enhanced chondrogenesis (JBMR 2002, 17:S403) and TGF-β/Wnt synergistically stimulated chondrocyte differentiation of hMSCs (JBMR 2002, 17:S432). It is our hypothesis that there is a reciprocal relationship between skeletal and adipogenic differentiation in marrow cells. In this study, we tested the effects of hypoxia, TGF-β, and Wnt signaling pathway on adipocyte differentiation of hMSCs. Mononuclear bone marrow cells were obtained from 42- and 58-year-old women. Adherent stromal cells were expanded in MEM-αa with 10% FBS until 80% confluence. For adipocyte differentiation, the medium was replaced with MEM-α, 1% heated-inactivated FBS and adipocyte supplements (dexamethasone, 1-methyl-3-isobutylxanthine, and insulin). The cells were treated with vehicle control, deferoxamine (DFO, 2.5-15 μM), $CoCl_2$ (25-50 μM), TGF-β1 (1 ng/ml) and/or LiCl (5 mM) for 17-23 days. For hypoxia-inducible factor-1 (HIF-1) expression, the medium of hMSCs in 100-mm dishes was replaced with MEM-α, 1% FBS-HI, and treated with vehicle control, 150 μM DFO, 50 μM $CoCl_2$ or 1 ng/ml TGF-β1 for 16 hours under normoxia (21% O_2) or hypoxia (2% O_2).
In adipocyte differentiation conditions, a portion of monolayer cells developed cytoplasmic lipid droplets. Adipogenesis of hMSCs was inhibited by TGF-β (5% vs. control, $p<0.001$) and Wnt-mimetic LiCl (65% vs. control, $p<0.005$). Synergy between TGF-β and Wnt (0% vs. control) suggested that there was cross-talk between TGF-β and Wnt pathway in the inhibition of adipogenesis of hMSCs. In addition, adipocyte differentiation was blocked by hypoxia-mimetic DFO (39 to1% vs. control, $p<0.001$) and $CoCL_2$ (13 to10% vs. control, $p<0.001$) in dose-dependent manner. Finally, hypoxia (2% O_2) and hypoxia-

mimetics (DFO and $CoCl_2$) up-regulated HIF-1α protein expression in hMSCs, as showed by Western blot. HIF-1α may inhibit adipogenesis via repression of PPARγ gene expression in hMSCs. In conclusion, our previous data showed stimulation of chondrogenesis by hypoxia, TGF-β and/or Wnt, and now we show inhibition of adipogenesis. This study suggests that TGF-β and/or Wnt signaling, hypoxia, and agents that regulate HIF-1 activity may be used to inhibit adipogenesis of hMSCs, and thus control skeletal loss.

Disclosures: ***S. Zhou****, None.*

M210

Periodontal Ligament Fibroblasts Induce Higher Numbers of Osteoclasts than Gingival Fibroblasts. T. J. de Vries[1], A. M. Schoenmaker*[1], M. van den Hoonaard*[2], A. Nieuwenhuijse*[2], W. Beertsen[2], V. Everts[1]. [1]Periodontology (ACTA) and Cell Biology and Histology (AMC), ACTA and AMC, University of Amsterdam, Amsterdam, Netherlands, [2]Periodontology, ACTA , University of Amsterdam, Amsterdam, Netherlands.

Formation and activity of osteoclasts are influenced in the periodontal tissues by a variety of physiological and pathological processes like eruption, and periodontal disease. Recently it was shown that periodontal ligament (PDL) fibroblasts have the capacity to induce the formation of osteoclasts. Whether a second population of tooth-associated fibroblasts, gingival fibroblasts, have a similar capacity, is unknown. It was the aim of the present study to compare the capacity of the two fibroblast populations to induce osteoclast formation from peripheral blood mononuclear cells (PBMCs). To this end, PDL and gingival fibroblasts were isolated and cultured from extracted permanent molars taken from 6 subjects with healthy periodontium. Experiments were performed with cells from the 6th passage. PDL and gingival fibroblasts were cocultured with PBMCs on plastic and on cortical bone slices in the presence and absence of dexamethasone and 1α,25dihydroxycholecalciferol (vitamin D_3). The number of multinucleated TRAP-positive cells (MNC) was assessed after 3 weeks of culturing. Initial mRNA levels for RANK-L and OPG were quantitatively assessed after a culture period of 24 hours.
Our data showed that MNC were formed under all culture conditions tested. Significantly more MNC (1.5x more) were induced by PDL fibroblasts than by gingival fibroblasts, both on plastic and on bone. For both types of fibroblasts the presence of vitamin D_3 and dexamethasone resulted in higher numbers of MNC. The initial mRNA expression of RANK-L and OPG by the two types of fibroblasts was low for RANK-L and high for OPG (200 to 2000 fold excess of OPG). In contrast, high levels of RANK-L and low levels of OPG (1000 fold excess of RANK-L) were expressed by the PBMCs. No differences in expression of RANK-L and OPG could be observed between PDL and gingival fibroblasts. Vitamin D_3 and dexamethasone stimulation for 24 hours did not influence the level of expression of these mRNAs.
We conclude that cultured PDL fibroblasts induce higher numbers of osteoclast-like cells than gingival fibroblasts. This difference could not be attributed to differences in mRNA expression of RANK-L and OPG.

Disclosures: ***T.J. de Vries****, None.*

M211

Separate Populations of Osteoblast (OB) and Adipocyte (AD) Stem Cells Appear in Primary Mouse Bone Marrow Stromal Cells Cultures. T. L. Chen. Natural Sciences, Notre Dame de Namur Universty, Belmont, CA, USA.

Separate Populations of Osteoblast (OB) and Adipocyte (AD) Stem Cells Appear in Primary Mouse Bone Marrow Stromal cells Cultures. T.L. Chen, M. Samant*. Natural Sciences, Notre Dame de Namur University, Belmont, CA
We and others have shown that mouse bone marrow cell lines can be pluripotent with the capability to differentiate into osteoblast (OB) or adipocyte (AD) phenotypes. This finding does not exclude the possibility for the existence of separate OB and AD monopotent stem cells within a mixed population of cells where most of the cell lines originated from. We used a mixed population of cells from long bone marrow (PBMSC) of young (3-6 months) male C57BL/6J mice to test this hypothesis. Marrow cell suspensions in serum-free αMEM were laid on top of a 10 ml discontinuous isotonic OptiPrep™ (iodixanol, Axis-Shield) gradient (ρ = 1.069 – 1.011) and spun in a swinging bucket at 600 x g for 20 min. Two bands of cells were harvested, the top layer at ρ = 1.082 (L1) and lower layer at ρ = 1.088 (L2). The cells were cultured in T-25 flasks for 3 - 4 weeks until about 20-30% of the surface was covered with cell colonies. Cells were removed by collagenase digestion and replated onto 24-well plates. After 2 days, they were treated with $1,25(OH)_2$vitaminD_3 (10 nM), dexamethasone (DEX, 10 nM) or their combination for 5 days. The appearance of OB and AD were examined under microscope after histochemical stainings. The majority (~80 %) of cells in L1 are alkaline phosphatase (ALP) positive indicating their OB lineage. No cell contained lipid granules in L1 cells. In L1 cells, $1,25(OH)_2$vitaminD_3 induced the formation of lacunae lined by mononucleated cells with a few multinucleated osteoclast-like cells (OC) in the center. This was not seen in the DEX treatment. Biochemical measurement of ALP indicated the lowering of ALP activity (to ~60%) by $1,25(OH)_2$vitaminD_3 but not by DEX treatment. The effects of $1,25(OH)_2$vitaminD_3 prevailed upon addition of both steroids. L2 cells contained only ~5% of ALP positive cells. About 15% of cells in L2 were small adipocytes (<1/20 of the size of OB). One to 2 % of the cells were large adipocytes (~1/5 of the size of OB)The number and size of these cells doubled in response to $1,25(OH)_2$vitaminD_3 but not DEX treatment. In conclusion, we have shown that monopotent OB and AD precursors exist in PBMSC. They can be enriched by density gradient centrifugation and each had its unique response to $1,25(OH)_2$vitaminD_3, DEX and their combination.

Disclosures: ***T.L. Chen****, None.*

M212

Transdifferentiation Potentials of Human Mesenchymal Stem Cells Derived from Bone Marrow Stroma. L. Song*, R. S. Tuan*. Niams/cbob, National Institute of Health, Bethesda, MD, USA.

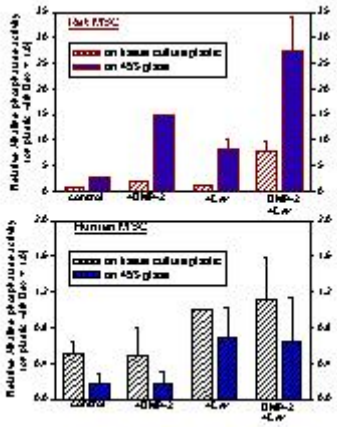
Human mesenchymal stem cells (hMSCs) derived from adult bone marrow stroma are capable of extensive self-renewal and differentiation into cells of multiple mesenchymal lineages, including osteoblasts, adipocytes, chondrocytes, as well as cells with neuroectodermal and endodermal characteristics. However, the regulation of lineage commitment in adult hMSCs is poorly understood. It is unclear if hMSCs that are pre-committed to one cell type maintain their multidifferentiation potential, either within a given developmental lineage or cross the lineage boundary, because of the contamination of other progenitor cells in vitro, the possibility of cell fusion, and/or inefficient cell tracing in the in vivo transplantation studies. In this study, we developed an in vitro differentiation strategy to assess if hMSCs that have differentiated into a given mesenchyme cell lineage can transdifferentiate into other cell types in response to inductive extracellular cues. hMSCs isolated from bone marrow were expanded and divided into three groups. Each group was cultured in osteogenic, adipogenic, or chondrogenic medium, respectively. After exhibiting the lineage specific characteristics, determined by gene expression and histo/immunohistological analyses, cells in each group were then cultured in a different inducing medium to determine if the pre-committed cells can acquire characteristics of another cell type. Our data showed that hMSCs that have differentiated into osteoblasts can transdifferentiate into chondrocytes or adipocytes upon exchange of the inducing supplements from osteogenesis to chondrogenesis or adipogenesis in the medium. In addition, hMSCs that were pre-determined to form chondrocytes or adipocytes were able to re-differentiate into osteoblasts and adipocytes, or osteoblasts and chondrocytes, respectively. To ensure contaminating progenitor cells are excluded from the transdifferentiation assay, we transfected hMSCs with an osteocalcin promoter-driven EGFP construct before being subjected to osteogenesis. The GFP-expressing cells, i.e. fully differentiated osteoblasts, were selected by fluorescence-activated cell sorting and subsequently cultured in either adipogenic or chondrogenic medium. Our data showed that these osteogenically differentiated, GFP-positive cells were able to differentiate into adipocytes. Our results suggest that hMSCs maintain their plasticity after lineage specific differentiation and that cells pre-committed to a specific lineage pathway are able to differentiate along different pathways in response to extracellular cues.

Disclosures: ***L. Song****, None.*

M213

Differential Osteogenic Effects of 45S Bioactive Glass on Rat and Human Mesenchymal Stem Cells. G. C. Reilly[1], S. Radin*[2], G. A. Bhargave*[1], P. Ducheyne*[2], P. S. Leboy[1]. [1]Biochemistry, University of Pennsylvania School of Dental Medicine, Philadelphia, PA, USA, [2]Bioengineering, University of Pennsylvania, Philadelphia, PA, USA.

Bioactive glass (BG) has been shown to increase bone bonding and accelerate bone formation in vivo, and to increase differentiation of osteoblastic cells in vitro. The effect of BG has been hypothesized to be due either to cell attachment to BG or to solution mediated changes in the environment of cells. To test whether osteogenic effects of BG could also be seen in mesenchymal stem cells (MSCs) and to separate attachment effects from solution-mediated effects, we cultured human and rat MSCs on 45S BG and in BG conditioned medium.
MSCs, obtained from the marrow of rat or human femurs, were cultured in alpha MEM with 25 mM HEPES, 1% penn/strep and 10% FBS. Cells were either plated directly on BG disks to assess attachment-mediated effects, or in tissue culture wells separated from BG disks by a 3μm porous membrane, to examine solution mediated effects. On day 1, 100μM ascorbate phosphate was added, with or without 10-7 M dexamethasone (dex) and/or 100ng/ml BMP-2. Cells were harvested at day 7 and assayed for relative cell number and alkaline phosphatase activity (ALP).
Rat MSCs grown on BG disks showed a significant, up to 5-fold, increase in ALP activity relative to controls (seeded on tissue culture plastic in the absence of BG) under all conditions, the effect being larger when dex and BMP-2 were added. Rat MSCs which were exposed to soluble factors from BG, including silicon and calcium ions, also showed elevated ALP activity, as high as cells plated on BG. In contrast human MSCs grown on BG did not differ from controls in ALP activity, under any conditions, although cells from some donors (3 of 5 tested) did respond to BG solution products with a doubling of ALP activity. Combined with our previous observations that the effect of BMP-2 differs between rodent and human MSCs, it seems that there are major differences in their respective osteoinduction pathways.
These results indicate that the bone-forming effects of BG in vivo may be due to increased osteogenesis of MSCs caused by BG dissolution products but that in vitro this effect is much stronger in rat than in human MSCs. The reasons for the limited response of human MSCs in vitro should be investigated in order to understand the mechanisms behind the effects of BG in clinical situations.



Disclosures: ***G.C. Reilly****, None.*

M214

Side Population (SP) Cells Isolated from Fetal Rat Calvaria Are Highly Enriched for Bone-forming Stem Cells. S. Zhang, M. Chan*, S. Uchida, J. E. Aubin. Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada.

Cells termed mesenchymal stem cells reside within the bone marrow stroma and possibly other bone compartments. However, estimates of their frequency are normally reported to be very low and several different kinds of strategies are being used to aid in their rapid enrichment and isolation. In at least some adult tissue types, stem cells are enriched within side population (SP) cells characterized by efficient efflux of a vital dye, Hoechst 33342, but few data are yet available using such an approach for bone cell populations. When we Hoechst-stained and FACS-analyzed freshly isolated 20 or 21-day fetal rat calvaria (RC) cells, a small fraction (0.02-0.04%) comprised a distinct SP. Sorted SP cells were cultured under osteoblast differentiation conditions with or without dexamethasone (Dex). When cells were plated at a density of 30 cells per microtitre well, the percentage of wells containing bone-forming progenitors (CFU-O) was significantly higher in the SP than in the corresponding non-SP and stained but unsorted RC population (13±4% vs. 1.8±0.4% and 0.7±0.4% respectively). The SP was also highly enriched for CFU-alkaline phosphatase (CFU-ALP) and CFU-fibroblast (CFU-F). While Dex increased the number of CFU-O and CFU-F in the SP, it did not modulate the frequency of CFU-ALP. Limiting dilution analysis showed a non-linear relationship between cell plating density (1, 5, 10, 20 or 30 cells/microtitre well) and the frequency of readout CFU-O, CFU-ALP and CFU-F in the SP, non-SP and stained but unsorted cells, suggestive of a cell non-autonomous mechanism for differentiation in all these subpopulations. Notably, Real-Time PCR indicated a similar level of Bcrp1 gene expression between SP and non-SP cells suggesting that other candidate transporter genes may be differentially expressed and participate in dye efflux in fetal RC cells. These data indicate that sorting of a SP of fetal RC cells is a useful strategy for enrichment of CFU-O and possibly other precursor cell types.

Disclosures: ***S. Zhang****, None.*

M215

Isolation of Differentiated Osteoblasts from Human Bone Marrow Stromal Cells Based on Expression of a Retroviral Delivered Col2.3GFP Transgene. L. Peng, M. Stover*, A. Lichtler, D. Rowe. Genetics and Developmental Biology, University of Connecticut, Farmington, CT, USA.

Using marrow stromal cell (MSC) cultures derived from mice transgenic for Col2.3GFP and Col3.6GFP, it is possible to appreciate stages of osteoblast differentiation within the culture in real time and to map the stage of maturation affected by a gene or growth factor that strongly affects bone formation. Expression of the GFP marker genes reveals the cellular heterogeneity of these culture systems and provides an approach for isolating an identified cell population by FACS sorting for subsequent cellular or molecular studies. This study was initiated to test the feasibility of applying the same strategy to human bone marrow stromal cell (hBMSC) cultures. Previously we have demonstrated the high susceptibility of hBMSC to transduction by viral vectors pseudotyped in the VSV packaging cell 293GPG without affecting their subsequent osteogenic potential. In the present study, the Col2.3GFP transgene marker was introduced into freshly plated hBMSC with a self-inactivating (SIN) retroviral vector delivery system. The removal of the viral promoter/enhancer elements of the SIN vector is permissive for stage specific activation of the inserted promoter to drive transgene expression in response to endogenous transcriptional signals. A weak GFP signal expressed in a few hBMSC was evident after 7 days of transduction while the culture was not yet confluent. However with addition of differentiation medium and with increasing cell density, the percentage of GFP positive cells and the intensity of GFP expression were markedly increased. The cells were arranged in small groups that are embedded in GFP negative cells and lack the well-defined nodules found in primary murine MSC cultures. The differentiated culture was disrupted by trypsin/collagenase digestion and subjected to FACS analysis. Approximately 20% of hBMSC were GFP positive, a percentage that is similar to primary murine MSC cultures. The GFP positive and negative cells were then isolated from the heterogeneous culture by FAC sorting. FACS re-analysis of sorted GFP positive population demonstrated >90% purity. Total RNA was extracted from isolated subpopulations for evaluating osteoblast differentiation markers by Northern and PCR. These preliminary data indicated that the cultured hBMSC are quite heterogeneous and that using a bone specific promoter driving GFP is a valuable tool to isolate homogenous sub-populations of hBMSC for subsequent cell and molecular studies. Further refinements to the strategy may make it possible to assess lineage progression in patients with premature osteoporosis.

Disclosures: ***L. Peng****, None.*

M216

Wnt Signaling Promotes Osteogenesis In Vivo and In Vitro. C. N. Bennett*[1], K. A. Longo*[1], W. S. Wright*[1], K. D. Hankenson[2], O. A. MacDougald*[1]. [1]Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA, [2]Orthopedic Surgery, University of Michigan, Ann Arbor, MI, USA.

We have identified Wnt10b as a potent inhibitor of adipogenesis by *in vitro* and *in vivo* analyses. To determine whether Wnt10b regulates cell fate of marrow preadipocytes we investigated the osteogenic and adipogenic potential of marrow stromal cells (MSC) in Wnt10b transgenic mice, where expression is under control of adipocyte specific promoter, 422/aP2. By Faxitron analysis, two transgenic lines have significantly increased bone mass in the hind limbs compared to wild-type littermate controls. This phenotype is present in both sexes and is manifest as early as 10 weeks of age. Wnt10b transgenic femurs, analyzed by micro computerized tomography (microCT), show that in a $1mm^3$ area, distal metaphyseal trabeculae are increased in number (4.71 vs 1.43), thickness (0.033 vs 0.024 mm) and have decreased trabeculae spacing (0.188 vs 0.950 mm) compared to wild-type littermate controls. This increase in trabeculae in the transgenic mice correlates with an overall increase in bone volume fraction of the area (15.75 vs 3.73%). The Wnt10b mice also have increased midcortical bone, as manifested by an increase in cross-sectional area (1.01 vs 0.83 mm^2) and cortical thickness (0.344 vs. 0.213 mm): although these measurements are confounded by an increase in marrow trabeculation. As predicted by the moment of inertia measurements calculated from microCT, mechanical testing by four point bending shows that the Wnt10b mice have increased ultimate load (42.8 vs. 32.1 N) and stiffness (326.6 vs. 235.4 N/mm). Bone marrow RNA was isolated from tibia and femur and RNase protectection analysis was performed to confirm that Wnt10b transgene is expressed in marrow. To further explore Wnts mechanism of action on osteogenesis, isolated wild-type MSC, ST2 and Immortomouse MSC were treated with osteogenic media and lithium chloride, which mimics activation of Wnt signaling through inhibition of glycogen synthase kinase 3. Mineralization, by Alizarin Red staining, is increased in cells treated with osteogenic media in the presence of lithium chloride compared to control cells. MSC treated with an inhibitor of glycogen synthase kinase 3 in non-osteogenic media have increased alkaline phosphatase expression, an early osteoblast marker, compared to controls but do not undergo mineralization. In summary, these data suggest that, Wnt signaling promotes osteogenesis of MSC and increases bone formation *in vivo*.

Disclosures: ***C.N. Bennett****, None.*

M217

Plasminogen Activators Participate in Osteoclastic Bone Degradation. V. Everts[1], W. Korper*[2], E. Daci[3], G. Carmeliet*[3], W. Beertsen[4]. [1]Oral Cell Biology, Academic Centre of Dentistry, Amsterdam, Netherlands, [2]Cell Biology, Academic Medical Centre, Amsterdam, Netherlands, [3]Exp Medicine, Katholieke Universiteit, Leuven, Belgium, [4]Periodontology, Academic Centre of Dentistry, Amsterdam, Netherlands.

Urokinase- and tissue-type plasminogen activators (uPA and tPA, respectively) have been suggested to play a role in resorption of bone. Using a coculture system (osteoblasts and osteoclasts) it was shown that digestion of a nonmineralized bone-like matrix by cells derived from mice deficient for both uPA and tPA (u/tPA -/-) was significantly reduced (Daci et al., JBMR, 14, 946, 1999). The present study was performed to assess the role of the plasminogen activators in the resorbing activity of the osteoclasts.

Calvarial bone explants were isolated from 5-day old wild-type mice or from mice deficient for both uPA and tPA. The explants were cultured for 6 or 24 h in the absence or presence of the cysteine proteinase inhibitor E-64 (40 μM) and subsequently processed for electron microscopic quantitative analysis. Blocking the activity of cysteine proteinases results in the occurrence of a high amount of demineralised non-digested bone matrix adjacent to the ruffled border of osteoclasts. This effect reaches a maximum following 6 hr incubation with E-64 (Everts et al., JBMR, 13, 1420, 1998).

Incubation of control explants for 6 hr with E-64 resulted in the presence of 49 ± 9 μm2 (mean ± SEM) demineralised non-digested bone area per osteoclast, whereas in the u/tPA-/- calvariae this was only 6 ± 2 μm2 (n=6 calvariae; p=0.0043). At the 24 h time point, however, no differences were found in the osteoclast-associated demineralised area between the two groups (WT: 44 ± 11 μm2; u/tPA -/-: 34 ± 16 μm2; NS). Yet, the amount of demineralised area(s) at sites no longer occupied by osteoclasts, was in 24 h control bones almost three times higher than in u/tPA -/- bones (340 ± 124 μm2 versus 126 ± 50 μm2 DA/calvaria). These findings indicate that osteoclastic resorption of calvarial bone is reduced in mice deficient for both uPA and tPA. The absence of the PAs delays but does not block resorption.

Our findings demonstrate for the first time that plasminogen activators participate in the resorption of bone by osteoclasts. This may be due to a decreased acidification at the bone resorption site or to a decreased release/activity of cysteine proteinases.

Disclosures: ***V. Everts****, None.*

M218

Increased Osteoclastogenesis in Cathepsin K Deficient Mice. R. Kiviranta[1], J. Morko*[1], S. Alatalo*[2], J. Risteli[3], E. Vuorio[1]. [1]Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland, [2]Anatomy, University of Turku, Turku, Finland, [3]Clinical Chemistry, University of Oulu, Oulu, Finland.

Cathepsin K is a major protease in osteoclast-mediated degradation of organic bone matrix. Several reports indicate that mutations in human and mouse cathepsin K gene result in severe osteopetrosis due to impaired osteoclast function. Purpose of the present study was to further characterize the skeletal changes in cathepsin K deficient mice.
Right hind limbs of cathepsin K deficient UTURK$^{-/-}$ and control male mice were subjected to in vitro pQCT measurement. Left tibias were embedded in methyl metacrylate and cut into 5µm sections for histomorphometry. Humeri were harvested for molecular biologic analyses and serum samples were collected for measurements of bone degradation products.
pQCT analyses confirmed the osteopetrotic phenotype of UTURK$^{-/-}$ mice. In the femurs, the metaphyseal total bone mineral density (BMD) was 35% higher and trabecular BMD 46% higher in UTURK$^{-/-}$ mice when compared to the control littermates. Northern analysis revealed significant upregulation of matrix metalloproteinase 9 (MMP9), MMP13, MMP14 and TRACP expression in humeri of cathepsin K deficient UTURK$^{-/-}$ mice. Interestingly, also the expression of osteoblast derived receptor activator of NFκB ligand (RANKL) and osteoprotegerin (OPG) were both increased. However, the RANKL/OPG ratio was significantly higher in UTURK$^{-/-}$ mice suggesting increased osteoclastogenesis in these mice. Histomorphometric analysis revealed increased trabecular bone volume and trabecular thickness in UTURK$^{-/-}$ mice when compared to the controls. There was no significant difference in the number of osteoblasts but the number of osteoclasts per bone perimeter was increased by 76% in cathepsin K deficient mice. Also osteoclast surface per bone surface was significantly increased in these mice. Despite impaired bone resorption in UTURK$^{-/-}$ mice, the amounts of serum ICTP and TRACP were increased reflecting the increased number of osteoclasts as well as the upregulation of MMP and TRACP activity.
The present study suggests that impaired bone resorption in cathepsin K deficient mice shifts the balance between OPG and RANKL expression in favour of the latter. This could be responsible for the marked increase in osteoclastogenesis and for the upregulation of MMP and TRACP expression.

Disclosures: ***R. Kiviranta****, None.*

M219

Beta-D-Glucan Suppresses Osteoclast-Recruitment, In Vitro. H. E. Guenther*[1], B. Mueller*[1], N. Sorgente*[2], H. L. Guenther[1]. [1]Clinical Research, University of Berne, Berne, Switzerland, [2]Momentum Pharmaceuticals, Inc, Houston, TX, USA.

Numerous literature reports have shown that β-D-glucans activate neutrophils, macrophages and Natural Killer cells via specific receptors. β-D-glucans are a family of polyglucoses that can have a linear or branched structure. Compounds that have a main chain of glucose units linked by β-(1→3) bonds and side chains linked by β-(1→6) bonds (poly-(1,3)-β-D-glucopyranosyl-(1,6)-β-D-glucopyranose) appear to be the most potent activators of the immune system. Since macrophages and osteoclasts (Oc) both originate from the granulocyte-macrophage colony-forming unit (GM-CFU), we began a series of investigations to examine the effect of β-D-glucans on Oc recruitment and resorption activity. Since in preliminary experiments, we established that a soluble β-D-glucan was the most active, most of our studies were carried out with this soluble β-D-glucan. Osteoclastic cells freshly isolated from1-day-old rat femurs were used to evaluate the effect of β-D-glucan on Oc formation and function, while RAW264.7 cells, a monocyte/macrophage cell line known to differentiate into TRAP+ MNC following treatment with receptor activator NF-κB ligand (RANKL) were employed to assess the effect of the compound on Oc recruitment. Lastly, clonal osteoblastic CRP10/30 cells were used to test the option of osteoblasts acting as assessory cells. The results of this study show that β-D-glucan significantly reduced the number of TRAP+ MNC and resorption pits by 30% and 48 % at doses of 0.1 and 10ng/ml, respectively. Media conditioned by β-D-glucan treated CRP10/30 cells produced no measurable effects on either Oc formation or resorption activity. The fact that the resorption activity (pits formed per osteoclast) of control and treated Oc were almost identical suggests that the inhibition of resorption was the result of a reduction of functional Oc rather than of an inhibition of the Oc resorption activity. This conclusion was supported by the observation that RANKL-induced osteoclast formation of RAW264.7 cells was significantly suppressed by β-D-glucan. Additional experiments revealed that pre-treating RAW264.7 cells with β-D-glucan for 3 days almost totally blocked RANKL-induced Oc formation while Oc that formed during a 3 day pretreatment with RANKL were not affected during a 4-day culture with β-D-glucan and RANKL. These results suggest that the agonist operates at a site upstream of RANKL action. Since we were not able to demonstrate that β-D-glucan elicits apoptosis of Oc, we conclude that β-D-glucan inhibits osteoclastogenesis via a direct, stromal/osteoblast cell-independent action conceivably by decreasing the responsiveness of Oc precursors to RANKL.

Disclosures: ***H.L. Guenther,*** *None.*

M220

TRAF2 is Required for TNFα-induced Osteoclastogenesis. K. Kanazawa*, A. Kudo. Tokyo Institute of Technology, Yokohama, Japan.

TNF receptor-associated factors (TRAFs) are intracellular adaptor proteins that are involved in signal transduction pathways initiated by a variety of TNF receptor family members. Of the six known TRAFs, TRAF2, TRAF5, and TRAF6 activate osteoclast differentiation factors such as NF-κB and JNK. TRAF2 associates with RANK and two tumor necrosis factor receptor isotypes, TNFR1 and TNFR2. We investigated TRAF2 function in osteoclast progenitor cells derived from TRAF2-deficient mice *in vitro*. Because TRAF2-deficient mice show embryonic lethality, we have newly developed *in vitro* stromal free system by employing osteoclast progenitor cells from the fetal liver of 14.5-day-old mouse embryos because no developmental or morphological abnormalities were observed in -/- embryos at this stage. Osteoclast progenitor cells from the fetal liver cultured with M-CSF, which expressed c-fms, Mac-1 and RANK, could differentiate into TRAP-positive (+) multinucleated cells (MNCs) in the presence of sRANKL (50ng/ml) or TNFα (50ng/ml). To examine the ability of osteoclast differentiation, TRAF2 +/+ or -/- osteoclast progenitor cells were induced to be differentiated into TRAP (+) MNCs by treating with sRANKL or TNFα in the presence of M-CSF. The number of TRAP (+) MNCs from TRAF2 -/- compared to TRAF2+/+ was 80% with sRANKL, or <1% with TNFα. This result suggests that TRAF2 is essential in TNFα-induced osteoclastogenesis. After stimulation with RANKL or TNFα, NF-κB and JNK were activated in osteoclast progenitor cells from TRAF2 -/- as well as +/+, however activated signals of NF-κB or JNK in TNFα treated -/- progenitor cells were diminished at the early time, suggesting that other signal pathways are especially present in TNFα-induced osteoclastogenesis. Since we have already demonstrated that TRAF6 and TRAF5 are required for TNFα-induced osteoclastogenesis, we suggest that TRAF5 and TRAF6 compensate TRAF2 function in TNFα signals downstream. Thus, the results first demonstrated that osteoclast progenitor cells obtained from the fetal liver are differentiated into mature osteoclasts and that TRAF2 plays a major role in osteoclast differentiation.This work has been collaborated with Amgen.

Disclosures: ***K. Kanazawa****, None.*

M221

Arsenite Induces Differentiation and Apoptosis of Pre-osteoclastic Cells by a Hydrogen Peroxide-Dependent Mechanism. K. H. Szymczyk*, B. A. Kerr*, C. S. Adams, M. J. Steinbeck. Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA, USA.

Arsenic is a toxicant that is taken up by dust inhalation, by drinking contaminated water, or by direct placement as a dental therapeutic agent. In all cases, arsenic is targeted to bone where it is deposited in the apatite lattice and the bone marrow. The present study investigated the ability of sodium arsenite (AsO_2) at various concentrations and durations of exposure to induce osteoclast differentiation and affect viability of pre-osteoclasts. At low concentrations of AsO_2, both chicken HD-11EM (1.0µM) and mouse RAW264.7 (2.5µM) pre-osteoclastic cell lines displayed initial differentiation characteristics as illustrated by increased numbers of multinucleate and tartrate resistant acid phosphatase (TRAP) expressing cells. These changes were accompanied by a low-level increase in production of H_2O_2 by both cell types. This initial differentiation was prevented by the addition of catalase, which converts H_2O_2 to H_2O and O_2. At higher concentrations of AsO_2, using a neutral red cell viability assay, we found a significant loss (>50%) of cell viability after 8 hours with 2.5-5.0µM AsO_2 treatment for HD-11EM cells which correlated with a significant increase in H_2O_2 production. As well, a significant loss of RAW264.7 cell viability by 24 hours was seen after the addition of 10µM AsO_2. Consistent with previous reports, mouse cells showed a greater resistance to AsO_2, however for both cell types the loss of cell viability was due to apoptosis as demonstrated by TUNEL staining and an increase in caspase-3 activation. Caspase-3 activation was inhibited by pretreatment with the NADPH-oxidase inhibitor, diphenylene iodonium chloride. AsO_2-induced apoptosis, therefore, can be blocked by prevention of H_2O_2 generation. Our findings indicate that AsO_2 activates NADPH-oxidase and induces H_2O_2 production, which at low concentrations promotes initial osteoclast differentiation and at higher concentrations induces cellular apoptosis. Hence, arsenite-induced H_2O_2 production may affect two critical mechanisms of bone maintenance; differentiation and apoptosis of osteoclasts.

Disclosures: ***K.H. Szymczyk****, None.*

M222

Activation of Protease-Activated Receptor-2 Leads to Inhibition of Osteoclast Differentiation. R. Smith*[1], M. Ransjö[2], L. Tatarczuch*[3], S. Song*[3], C. N. Pagel*[3], R. N. Pike*[1], E. J. Mackie[3]. [1]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Australia, [2]Department of Oral Cell Biology, Umeå University, Umeå, Sweden, [3]School of Veterinary Science, University of Melbourne, Parkville, Victoria, Australia.

Protease-activated receptor-2 (PAR-2) is expressed by osteoblasts and activated by a small number of tissue proteases including mast cell tryptase, factor Xa and neutrophil proteinase 3. PAR-2 is also activated by a peptide (RAP) corresponding to the 'tethered ligand' created by cleavage of the receptor's N-terminal extracellular domain. The effect of RAP on osteoclast differentiation was investigated in mouse bone marrow cultures. Osteoclasts were identified as TRAP-positive multinucleate cells, and results confirmed in pit assays. RAP inhibited osteoclast differentiation induced by parathyroid hormone (PTH), 1,25 dihydroxyvitamin D3 (D3) or interleukin-11 (IL-11). RAP did not inhibit PTH-, D3- or IL-11-induced osteoclast differentiation in cultures derived from PAR-2-null mice, confirming that the response to RAP in wildtype cultures is specifically mediated by PAR-2. RAP caused maximal inhibition of osteoclast differentiation induced by all agonists if present for the entire 7-day culture period or only at days 1-3 or 3-5; when present only from days 5-7, RAP was partially effective in the presence of PTH or IL-11, and ineffective in the presence of D3. Cells of the osteoclastogenic RAW 264.7 cell line and bone marrow stromal cells were shown by RT-PCR to express PAR-2. RAP had no effect on RANKL-induced osteoclast differentiation in RAW 264.7 cells, suggesting that osteoclast precursors are not the target cells for the effects of RAP on osteoclast differentiation. Semi-quantitative RT-PCR was used to investigate expression of mediators of osteoclast differentiation in mouse bone marrow and primary osteoblast cultures treated with RAP. In bone marrow or osteoblast cultures treated with PTH, D3 or IL-11, RAP significantly

decreased the ratio of RANKL: osteoprotegerin expression. In bone marrow cultures treated with PTH, D3 or IL-11, RAP suppressed expression of the inducible prostaglandin H synthase-2. RAP inhibited PTH- or D3-induced expression of interleukin-6 in bone marrow cultures. These observations indicate that PAR-2 activation inhibits osteoclast differentiation by acting on cells of the osteoblast lineage to modulate multiple mediators of the effects of osteoclastogenic hormones. The role of PAR-2 may be to protect bone from uncontrolled resorption during inflammation.

Disclosures: ***E.J. Mackie****, None.*

M223

Inhibition of Chaperonin Hsp90 Stimulates Osteoclastogenesis In Vitro and Increases Bone Destruction by Invading Breast Cancer Cells. J. M. W. Quinn[1], A. Nakamura*[1], S. E. Docherty*[1], N. A. Sims[2], M. C. Waltham*[1], M. T. Gillespie[1], E. W. Thompson*[3], J. T. Price*[3]. [1]Molecular Endocrinology, St. Vincent's Institute of Medical Research, Victoria, Australia, [2]Dept of Medicine, University of Melbourne, Victoria, Australia, [3]Dept of Surgery, University of Melbourne, Victoria, Australia.

17-allylaminogeldanamycin (17AAG) is an HSP90 inhibitor that impairs tumour growth in a variety of mouse models and is currently in phase I clinical trials. However, 17AAG effects on tumour metastases in bone have not been investigated. We studied this with a nude mouse model employing intracardiac inoculation by MDA-MB-231 human breast cancer cells, which results in rapid seeding of tumour cells in bone. In mice treated with 17AAG (70mg/kg/day) the size and incidence of osteolytic lesions caused by the tumour cells (observed by Faxitron x-ray analysis) were greatly increased compared to controls. In mice without tumour challenge, despite trabecular bone volume (BV/TV) being already very low in nude mice, 17AAG treatment induced a further reduction in BV/TV.

To investigate whether 17AAG increases tumour-associated bone destruction by stimulating osteoclast formation we studied its effects in vitro. 17AAG enhanced osteoclast formation 2.5- to 4-fold in bone marrow/osteoblast co-cultures (stimulated by 1,25 dihydroxyvitamin D3 and prostaglandin E2) and RANKL stimulated bone marrow or RAW264.7 cells. Like 17AAG, other HSP90 inhibitors, radicicol and herbimycin A showed similar effects on osteoclast formation. Little or no effect of HSP90 inhibitors was noted on osteoclast survival or bone marrow macrophage survival and proliferation.

Our data suggest HSP90 inhibitors powerfully stimulate osteoclast formation by action on osteoclast progenitors. This may underlie the increased osteolysis caused by 17AAG in our in vivo tumor invasion/metastasis model and may also reflect a more general impact of 17AAG on bone remodeling in nude mice.

Disclosures: ***J.M.W. Quinn****, None.*

M224

Peak of Resorption at Day 9 in Tibial Marrow Ablation Model of Bone Turnover Is Local, not Systemic, Event. D. Lovitch, J. P. Gorski. Division of Molecular Biology and Biochemistry, School of Biological Sciences, University of Missouri, Kansas City, Kansas City, MO, USA.

In the tibial marrow ablation (MA) model, marrow below the growth plate is removed surgically from the rodent tibia. Mineralized intramembranous or primary bone then rapidly fills the intramedullary space within 7-8 days. Subsequently, a wave of osteoclastic resorption removes this bone over the next 10 days. Interestingly, serum calcium values rise to a maximum on day 7 (15.9 mg/dl) and remain elevated through day 15 (14.0 mg/ml) ($p<0.05$) [JBMR 12 (1997) pp. 200-209]. The purpose of this study was to determine whether osteoclastic resorption of intramedullary bone was part of a local or a systemic process. Rats were ablated surgically, animals allowed to ambulate normally, and tibias removed for analysis on days 5, 7, 9, 11, and 13 after surgery (n = 6/time point). Representative bones from other non-ablated anatomical sites (calvaria, caudal vertebrae, and humeri) were also taken at each time point. Tissues were fixed, decalcified, and processed for tartrate resistant acid phosphatase (TRAP) staining. TRAP positive mononuclear cells and multi-nucleated osteoclasts were counted in tissue sections in a blinded fashion by two observers; two sections were counted per bone and two fields were counted per section. Results for tibias were separated into three parts representing the proximal, medial, and distal regions. In the proximal region of the tibia, the number of TRAP positive cells peaked on day 9, 1-2 days after mineralized bone had completely filled the proximal intramedullary space. The average number of TRAP positive cells/field on day 9 was 32 ± 4.3 (SEM) versus 11 ± 3.7 and 15 ± 4.9 on days 7 and 11. One-way analysis of variance indicates the differences among the means were significant at $p< 0.001$; by the Tukey pairwise multiple comparison test, the difference between the number of TRAP positive cells on day 9 and the number of cells on other days was significant at $p<0.05$. There were no significant differences among the number of TRAP positive cells on the other days. Importantly, in calvaria, caudal vertebrae, and humeri from ablated rats, the number of TRAP positive cells did not vary significantly over days 5 to 13. In summary, marrow ablation induces a dramatic 3-fold increase in the number of TRAP-positive osteoclasts and precursor cells that peaks on day 9 within ablated tibias. In contrast, no increase occurs in other representative bones. These temporal findings suggest that tibial osteoclastogenesis is controlled by signals arising locally from primary bone within ablated tibias, signals that are strong enough to overcome the negative influence of above-normal serum calcium values.

Disclosures: ***D. Lovitch****, None.*

M225

Dexamethasone Inhibits Multinucleated Osteoclasts Formation via Down-regulation of β_3 Integrin Expression. J. Jun*, Y. Kim*, S. Kim*, G. Kim, J. H. Baek. Dept of Pharmacology and Dental Therapeutics, College of Dentistry, Seoul National University, Seoul, Republic of Korea.

Although glucocorticoids are among the factors of osteotropic activity, previous reports showed the conflicting results regarding the effect of glucocorticoids on osteoclast formation, leading to the speculation that they have biphasic effects according to the culture microenvironments and the target cells. In this study, we observed the regulatory effect of dexamethasone on osteoclast formation and provided the possible regulatory mechanism of it. Osteoclast formation was induced by coculture of mouse osteoblastic cells and bone marrow cells for 7 days in the presence of 10 nM $1,25(OH)_2vitD_3$. In this system, the fusion of tartrate-resistant acid phosphatase (TRAP) positive mononuclear cells started around 4 days. At the end of culture, the number of TRAP(+) cells containing more than 3 nuclei was counted as multinucleated osteoclast-like cells (MNCs). The expression of osteoclast markers (calcitonin receptor, vitronectin receptor, cathepsin K, matrix metalloproteinase-9, carbonic anhydrase 2, RANK, TRAP), RANKL, and OPG was determined by semiquantitative RT-PCR. When dexamethasone was added during the entire culture period, it almost completely inhibited the formation of TRAP(+) MNCs without affecting TRAP(+) mononuclear cells formation. Complete inhibition of TRAP(+) MNCs formation was also observed even when dexamethasone was added only for 1~4 days. Dexamethasone did not change the expression levels of RANKL and OPG. Among the osteoclast markers, dexamethasone decreased the expression of β_3 subunit of vitronectin receptor. Echistatin, a snake venome which specifically binds to and blocks β_3 integrin, also completely inhibited the TRAP(+) MNCs formation. This result suggests that dexamethasone inhibit the formation of multinucleated osteoclasts through down-regulation of β_3 integrin that plays a role in migration of osteoclast precursors.

Disclosures: ***J. Jun****, None.*

M226

A TRAP Promoter Element that Responds Early During RANK-Ligand Induction. D. J. Selski*, X. Qiu*, J. J. Westendorf, D. R. Clohisy. Orthopaedic Surgery, University of Minnesota, Minneapolis, MN, USA.

TRAP expression is one marker of osteoclastogenesis, and analysis of the promoter region of this gene should identify regions activated during osteoclast differentiation. We have generated retroviral constructs with a small element of the TRAP promoter driving expression of a reporter gene. Specifically, a 95 base pair fragment from the TRAP promoter was cloned as a triple repeat into the LTR of the MMLV vector, pBABE-puro, replacing the normal enhancer region of the LTR promoter. The reporter gene, truncated Nerve Growth Factor Receptor (tNGFR), was inserted into the downstream expression position. Virus from this construct was used to infect the macrophage/monocyte precursor cell line, RAW 264.7, and stable cell lines were analyzed for tNGFR expression. In response to RANK-ligand induction, a transient increase in tNGFR expression over the course of two days was observed, with subsequent decrease in expression as cells multinucleated. Confirming this transient response in electrophoretic mobility shift assays, a 27 base pair probe from this TRAP promoter element bound a protein complex in RAW cell extracts. Importantly this protein complex was detected transiently, increasing within the first ten hours of RANK-Ligand treatment then disappearing by 48 hours. Taken together these data suggest that the TRAP promoter contains a short sequence that participates in the *early* induction of osteoclastogenesis in response to RANK-Ligand. Furthermore this element appears *not* to be active after two days of exposure to RANK-Ligand, implying as expected that other regions of the TRAP promoter are responsible for the constitutive expression of TRAP in fully differentiated osteoclasts. Current experiments are aimed at identifying the putative DNA binding proteins involved in this response.

Disclosures: ***D.J. Selski****, None.*

M227

Movie Imaging of Bone-Resorbing Osteoclasts for Spatial Analysis of Cell and Matrix Proteins. S. A. Nesbitt[1], G. T. Charras*[1], C. Laimer*[2], M. Messerli*[2], M. A. Horton[1]. [1]Medicine, UCL, London, United Kingdom, [2]Bitplane AG, Zurich, Switzerland.

Spatial analysis of osteoclast proteins is essential for the understanding of the polarised process of bone resorption. Previously, we have used multicoloured immunofluorescence confocal microscopy with 3D image reconstructions to examine the spatial localisation of osteoclast proteins involved in bone matrix degradation. Herein, we have applied new computer software to convert 3D images of osteoclasts and bone resorption sites into 'interactive' displays using animation and fly-through movies.

Initially, an in vitro model of bone resorption was used. Human osteoclasts, derived from osteoclastoma tissue, were cultured for 24 h on surface biotinylated dentine slices. After fixation and permeabilisation, the resorption cultures were analysed by triple immunofluorescence staining and confocal microscopy. Staining for F-actin (using phalloidin-TRITC) and biotinylated matrix (with streptavidin-Cy5) was used to localise the cells and matrix, respectively. Cathepsin K, proton pump, annexin II and degraded collagens (CTx) were stained using FITC labelled antibodies and cell nuclei stained with propidium iodide. 64 optical sections (thickness 350nm) for each fluorochrome were taken at each site of osteoclastic resorption by confocal microscopy and compiled to generate 3D extracellular isosurface images using Imaris Surpass software (Bitplane AG, Zurich). 3D intracellular views were produced by sectional slicing. The osteoclasts and resorption sites were viewed from above, laterally and below the bone surface. A zoom function facilitated extensive examination of the resorption sites. 300 image frames, each at 512 pixel resolution, were sequentially taken and compiled to generate animation and movie files of 4 MB size. The

animations produced 3D orientations of resorption cultures and the movies provided movement of the viewer with 'fly-through' effects over the cell and bone surfaces and within the resorption pits and inside the osteoclasts. The surface topology of the resorption pits and osteoclast structures were well defined with clear visualisation of the ruffled border, sealing zone, cytoplasmic skirt, basolateral/apical cell surfaces with exocytotic sites and nuclei and transcytotic vesicles within the cell body.
In summary, animation and movie displays appear to be most useful in the spatial and topological analysis of cellular and matrix proteins in osteoclasts and resorption sites.

Disclosures: ***S.A. Nesbitt****, None.*

M228

Calmodulin/CaMKII Regulate Osteoclastogenesis. L. Zhang, J. M. McDonald. Pathology, University Alabama at Birmingham, Birmingham, AL, USA.

The intracellular signaling pathways governing osteoclastogenesis are still not well-characterized. Previously, we reported that inhibitors of calmodulin and CaMKII blocked mouse osteoclast differentiation *in vitro*, independently of apoptosis, protein kinase C and calcineurin. Here we report additional supportive evidence and a potential molecular mechanism for calmodulin/CaMKII-regulated osteoclastogenesis. First, overexpression of calmodulin by recombinant viruses protected osteoclast-like differentiation in RAW264.7 cells from inhibition by trifluoperazine (TFP), an antagonist of calmodulin. TFP treatment (0.06 mg/ml in drinking water for 5 weeks) abolished ovariectomy-induced osteoclastogenesis in intact mice. CaMKIIα mutant mice exhibit a phenotype of reduced osteoclastogenesis (76 ± 8.8% decrease in tibiae, 77 ± 13.1% decrease in femurs. $P < 0.05$, $N = 5$). These observations, both *in vivo* and *in vitro*, further strengthen the concept that the calmodulin / CaMKII pathway plays an important role in regulation of osteoclastogenesis. To identify potential mechanisms by which calmodulin /CaMKII regulates osteoclastogenesis, we have studied the effect of their inhibitors on RANKL signaling pathways, which are known to be critical for osteoclast differentiation. Using RAW264.7 cells, we observed that TFP (10 μM), had no effect on RANKL stimulated-p38 phosphorylation, but dose-dependently inhibited RANKL-stimulated ERK and Akt activation: 38 ± 2.5% and 59 ± 6% decrease in ERK1 activation, 22 ± 2.3% and 46 ± 11.6% decrease in ERK2 activation, and 75 ± 1.8% and 80 ± 5.9% decrease in Akt activation, with treatments of 5 and 10 μM TFP, respectively (all decrements were statistically significant, $N = 3$). KN93, a specific inhibitor of CaMKII, inhibited only the RANKL-Akt pathway but not the other pathways: 52 ± 13.6% and 63 ± 14.2% decrease in Akt activation with treatments of 5 and 10 μM KN93 ($P < 0.05$, $N=4$). Taken together, these data indicate that calmodulin /CaMKII regulates osteoclastogenesis by interfering with specific RANKL-stimulated signaling pathways including ERK and Akt.

Disclosures: ***L. Zhang****, None.*

M229

TRAIL-mediated Apoptosis may Occur in Human Osteoclasts in Conditions Characterized by a High Level of Osteoclastogenesis. S. Roux[1], J. L. Parent*[1], P. Orcel[2], B. Sawan*[3]. [1]Rheumatology, Sherbrooke University, Sherbrooke, PQ, Canada, [2]Rheumatology, Lariboisiere Hospital, Paris, France, [3]Pathology, Sherbrooke University, Sherbrooke, PQ, Canada.

Osteoclast (Oc) is very effective in destroying bone matrix and its activity has to be tightly regulated to maintain normal bone homeostasis. Regulation of Oc numbers through induction of cell death may represent a mechanism by which bone resorption is controlled. TRAIL (TNF-Related Apoptosis-Inducing Ligand) is a pro-apoptotic molecule mainly involved in anti-tumor cytotoxicity. TRAIL can bind to five receptors. TRAIL-R1 and TRAIL-R2 induce apoptosis upon binding of TRAIL. TRAIL-R3 and TRAIL-R4 are membrane-bound receptors that lack death domain, acting as TRAIL decoy receptors. Finally, the fifth TRAIL receptor is the soluble decoy receptor osteoprotegerin (OPG) that inhibits TRAIL-mediated apoptosis. As OPG plays a key role in bone homeostasis by blocking RANKL-RANK interactions, we hypothesize that TRAIL and its receptors could be involved in Oc apoptosis, and thus OPG could differentially regulate Oc survival/apoptosis according to Oc responsiveness to RANKL and TRAIL.
Our objective was to evaluate TRAIL and TRAIL-receptor expression by immunohistochemistry performed on normal bone marrow biopsies (n=5), and a bone biopsy from a patient with primary hyperparathyroidism. In all normal bone marrow biopsies, TRAIL and its receptors, TRAIL-R1, -R2 and -R3, were found to be expressed by hematopoietic cells in the bone marrow. These cells were identified as myeloid cells, especially eosinophils and neutrophils. TRAIL-R4 expression was only detected in few scattered cells. Bone cells, including very few osteoclasts, were not stained with any antibody. In the hyperparathyroidism lesion, characterized by a high bone turn over and numerous osteoclasts close to resorbed bone areas and in stroma tissue next to bone, the pattern of expression of TRAIL receptors differed. TRAIL-R1 and TRAIL-R2 were strongly expressed by resorbing osteoclasts, and osteoclasts present in the stroma. TRAIL-R3 and TRAIL-R4 were also expressed by osteoclasts with a variable intensity, and some osteoclasts were not stained.In conclusion, TRAIL-receptor expression and TRAIL-mediated apoptosis may occur in Ocs in pathological conditions characterized by a high level of osteoclast formation. The strong expression of the 2 death receptors observed in Ocs from bone lesions of hyperparathyroidism could reflect a compensatory mechanism to reduce Oc formation. Although the significance and functional relevance of our results remain to be determined, these findings raise the possibility that TRAIL pathway could play a role in osteoclast apoptosis in humans.

Disclosures: ***S. Roux****, None.*

M230

Up-regulation of Schlafen2 by Tumor Necrosis Factor-Related Activation Induced Cytokine (TRANCE) in Osteoclast Differentiation. N. K. Lee*, H. K. Choi*, S. Han*, S. Y. Lee*. Division of Molecular Life Sciences and Center for Cell Signaling Research, Ewha Womans University, Seoul, Republic of Korea.

Osteoclasts(OCs) are multinucleated hematopoietic cells that resorb bone and are essential for bone homeostasis. Schlafen(Slfn) is a new family of growth regulatory genes that affect thymocyte development. Little is known about a link between Slfn2 expression and differentiation of OCs. Here we show that TRANCE, a TNF family cytokine, up-regulates the expression of Slfn2 through Rac1 GTPase during OCs differentiation. Transient expression of Slfn2 reduced the cell number compared to that of control, indicating that Slfn2 may inhibit cell growth. We also observed that the expression of Slfn2 is gradually induced and translocalized into cytoplasm during OCs differentiation. Taken together, our results indicate that Slfn2 participates in TRANCE-induced OC differentiation, probably by inhibiting cell growth.

Disclosures: ***N.K. Lee****, None.*

M231

Sensitivity Analysis of a Mathematical Model for Cellular Activity Identifies Potential Factors that Are Important in Inhibiting Resorption. M. J. Martin*, C. Buckland-Wright*. Applied Clinical Anatomy Research, King's College, London, United Kingdom.

A new mathematical model based on rates of cellular activity was used to investigate potential factors for inhibiting resorption in cancellous bone.
The mathematical model was based on published histomorphometric data for resorption and the cellular interactions within the bone micro-environment.
The Michaelis-Menten kinetic equations that describe enzyme activity were used to simulate cellular activity during resorption, on a daily time-step, within a representative volume of trabecular bone. During the first of the 2 phases of bone resorption, erosion by osteoclastic activity is dependent on the interaction between osteoblastic RANKL and osteoclastic RANK at cell surfaces, which is limited by the competitive inhibition of OPG for RANKL. This inhibition is represented in the model by the 'effective RANKL', the ratio of RANKL to OPG, which is assumed to limit the substrate available for resorption. The required presence of stromal cell production of M-CSF is represented as factor $F_{M\text{-}CSF}$, which is equal to 1 in normal, healthy bone. The amount of TGFbeta1 released from the matrix during osteoclastic resorption is tracked in the model and used to simulate the decrease in osteoclastic resorption with time, via the negative feedback effect of TGF-beta1-induced OPG production by marrow stromal cells. Apoptosis and cessation of osteoclastic resorption is assumed to occur at a threshold level of TGFbeta1 released from the matrix.
During the second phase of resorption Michaelis-Menten equations are used to describe the activity of mono-nucleated lining cells that remove the collagen fibrils left by osteoclasts.
The sensitivity of resorption depth and duration to changes in individual variable values was assessed by sensitivity analysis.
With changes in most model variables the results of sensitivity analysis show that feedback mechanisms constrain the variation in resorption depth and duration to within 6% of expected values. However, both the TGF-beta1 concentration in the matrix and the apoptosis threshold have greater effects on both duration and depth of resorption. Resorption duration, but not depth, was sensitive to changes in the maximum rate of lining cell activity in the removal of collagen fibrils.
This is the first mathematical model using cellular activity to simulate the rate of resorption, it may also identify those factors that can inhibit resorption.

Disclosures: ***M.J. Martin****, None.*

M232

Differentiating the Mechanisms of Action of Nitrogen-Containing Bisphosphonates. E. R. van Beek*[1], L. Cohen*[2], I. Leroy*[2], F. Ebetino[3], C. Löwik[1], S. Papapoulos[1]. [1]Endocrinology, Leiden University Medical Center, Leiden, Netherlands, [2]Prevention and Health, Gaubius Laboratory TNO, Leiden, Netherlands, [3]Health Care Research Center, Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Bisphosphonates (BPS) are distinguished into two classes. 1) Non N-containing BPS that are of low potency that act via formation of toxic ATP-metabolites. 2) N-containing BPS (NBPS) that act via inhibition of farnesylPP synthase (FPS) and of protein geranylgeranylation. The mode of action of NBPS is, however, studied using primarily the highly potent compounds, while the mode of actions of intermediate or low potency NBPS is not well determined. We addressed this question using alkyl- and heterocycle NBPS with small structural differences resulting in major changes in antiresorptive efficacy. We performed experiments with the following aims: assessment of the effects on resorption, on FPS and on reversibility of the antiresorptive action by geranylgeraniol (GGO). Using cultures of 45Ca-labelled bones, as expected, risedronate and alendronate similary inhibited resorption with EC50's of 0.6 mM. In the presence of 0.1 mM GGO, their action was largely reversed with EC50's of 100 mM; a >100-fold increase in EC50 value. Similar results were obtained with the highly potent NBPS NE-11808 and NE-10575. All these NBPS were also active inhibitors of FPS. These indicate that they suppress resorption via inhibition of FPS and of protein geranylgeranylation. However, the actions of pamidronate (Pam) and NE-21650, that both inhibit FPS, were slightly reversible with GGO. The EC50's of Pam and NE-21650 on resorption were 4 and 2 mM, and 15 and 20 mM, in the presence of GGO; an increase of only 4- and 10-fold. Therefore, despite their effect on

FPS, Pam and NE-21650 act mainly via routes other than FPS and protein prenylation. The heterocycle NBPS NE-58086 and NE-11809 were of very low potency (EC50's of 200 and >500 mM). Their actions were not reversed by GGO and also did not affect FPS. Therefore, these inhibit resorption totally via routes other than the mevalonate pathway. We show that not all NBPS share the same mode of action and can be distinguished at least into three subclasses. 1) Low potency NBPS. These inhibit resorption totally via routes other than the mevalonate pathway. 2) NBPS of intermediate potency, such as Pam, that inhibit FPS, but their action is slightly reversible by GGO. These compounds inhibit resorption mainly via pathways other than suppression of protein geranylgeranylation. 3) Highly potent NBPS that act exclusively via inhibition of FPS and of protein geranylgeranylation.

Disclosures: ***E.R. van Beek****, None.*

M233

The Chloride Channel Inhibitor NS3736 Prevents Bone Resorption in Ovariectomized Rats, while Preserving Bone Formation. M. A. Karsdal[1], S. Schaller*[1], K. Henriksen[1], C. Sveigaard*[1], A. Heegaard*[1], P. Christophersen*[2], M. Stahlhut*[1], N. Hélix*[2], M. T. Engsig*[1], N. Foged*[1], J. Delaissé[1]. [1]Nordic Bioscience, Herlev, Denmark, [2]NeuroSearch, Ballerup, Denmark.

Chloride channel activity, in particular ClC-7, has been shown to be essential for osteoclastic acidification of the extracellular resorption lacuna necessary for bone resorption. Consequently, specific inhibitors of the osteoclastic chloride channel could be used as drugs to prevent bone resorption. Accordingly, we have developed chloride channel (CLC) inhibitors and tested their effect on bone turnover *in vitro* and in *vivo.*

We have identified several compounds that potently inhibit chloride transport, by screening a compound library in resorption and acidification assays. A lead compound, NS3736, blocked chloride ion transport in human osteoclasts measured by patch-clamping, abrogated acidification of the resorption lacunae, and inhibited resorption in cultures of pure human and rat osteoclasts as well as in cultures of mouse tibiae with an IC50 value of 30 microM. When tested in the rat ovariectomy model for postmenopausal osteoporosis, daily treatment with 30 mg/kg p.o. protected bone strength and bone mineral density by approx. 50% 6 weeks postsurgery. Most interestingly, bone formation assessed by osteocalcin, mineral apposition rate and mineralized surface index was not inhibited.

A comprehensive screening of human osteoclasts for chloride channels revealed that ClC-7 and CLIC1 reached by far the highest expression levels, and that ClC-7 was the only chloride channel up-regulated during osteoclastogenesis. Furthermore, immunohistochemical screening of 49 different human tissues showed ClC-7 restricted to bone (osteoclasts), ovaries, appendix and cerebellum (Purkinje cells). This highly selective distribution indicates that ClC-7 inhibition should target quite specifically osteoclasts *in vivo*.

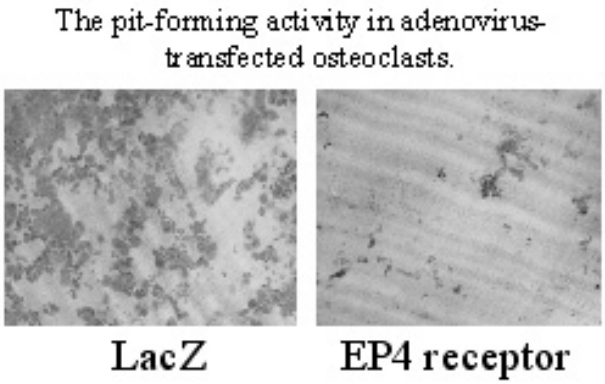
In conclusion, we show for the first time that chloride ion channels inhibitors can be used for prevention of ovariectomy-induced bone loss without impeding bone formation. Treatment with chloride channel inhibitors should therefore lead to a net increase in bone strength. Since NS3736 is an inhibitor of acidification and attenuates bone resorption without affecting bone formation, we speculate that acidification is a key event for proper coupling between bone resorption and formation.

Disclosures: ***M.A. Karsdal****, None.*

M234

Role of PGE_2-Receptors in Osteoclast Differentiation and Function. Y. Kobayashi*[1], I. Take*[1], N. Udagawa[2], N. Takahashi[1]. [1]Institute for Oral Science, Matsumoto Dental University, Shiojiri, Japan, [2]Dept. of Biochemistry, Matsumoto Dental University, Shiojiri, Japan.

PGE_2 is believed to induce bone resorption via the expression of RANKL in osteoblasts. Recently, it was shown that PGE_2 stimulates bone resorption through a direct action on osteoclast precursors with a synergistic effect on RANKL action. Here we studied the precise mechanism of PGE_2 action in osteoclastic bone resorption. Using RT-PCR, expression of PGE_2 receptor subtypes (EP1, EP2, EP3, EP4) was examined in mature osteoclasts and osteoclast precursors such as bone marrow macrophages (BMMø) and RAW 264.7 cells. Both BMMø and RAW cells expressed EP1, EP2 and EP4 mRNAs, while mature osteoclasts expressed only EP1 mRNA. PGE_2 significantly enhanced TRAP-positive multinuclear cell formation induced by RANKL in RAW cell cultures. The synergistic action was mimicked by db-cAMP and inhibited by H-89, a PKA inhibitor, suggesting that the synergistic effect of PGE_2 is mediated by the cAMP-PKA system in osteoclast precursors. RANKL induced degradation of IκB and phosphorylation of p38MAPK, ERK and JNK in RAW cells. PGE_2 enhanced RANKL-induced degradation of IκB and phosphorylation of p38MAPK in RAW cells. These results suggest that PKA-mediated signals crosstalk with NF-κB- and p38 MAPK-mediated signals in osteoclast precursors. We further studied the role of EP1 in osteoclast functions. Calcitonin enhanced intracellular levels of cAMP, but PGE_2 did not. RANKL and calcitonin promoted the survival of osteoclasts. PGE_2, EP1 agonist (ONO-DI-004) and EP4 agonist (ONO-AE1-329) failed to support the survival of osteoclasts, confirming that mature osteoclasts do not express functional PGE_2 receptors. Then EP4 receptor was expressed in osteoclasts using an adenoviral vector system. The expression of EP4 in osteoclasts inhibited their pit-forming activity, though the ligand was not added to the culture. These results suggest that osteoclast progenitors express functional PGE_2 receptors to differentiate into osteoclasts in response to PGE_2, but mature osteoclasts lose EPs 2 and 4 to escape from the inhibitory effects of PGE_2 on bone resorption.



Disclosures: ***Y. Kobayashi****, None.*

M235

Inhibitory Effects of SCOH, a Novel Compound, on Differentiation and Resorptive Activity of Osteoclasts. Z. Lee[1], H. Ha*[1], H. Kwak*[1], H. Kim[2]. [1]Microbiology and Immunology, Chosun University Dental College, Gwangju, Republic of Korea, [2]Cell and Developmental Biology, Seoul National University Dental College, Gwangju, Republic of Korea.

Osteoclasts are multinucleated cells formed by multiple steps of cell differentiation events from the progenitor cells of hematopoietic origin. The intervention of osteoclast differentiation has been considered as an effective therapeutic approach to the treatment for bone diseases involving osteoclasts. In this study, we found that (S)-1-lyso-2-stearoylamino-2-deoxy-*sn*-glycero-3-phosphatidylcholine (SCOH) inhibited osteoclast differentiation. The inhibitory effect of SCOH was observed in mouse bone marrow cell cultures supported either by coculturing with osteoblasts or by adding M-CSF (macrophage colony stimulating factor) and RANKL (receptor activator of nuclear factor κB ligand). The molecular mechanism for this effect of SCOH seems to involve the signaling pathways of ERK, Akt, and NF-κB, since SCOH suppressed the activation of ERK, Akt, and NF-κB by M-CSF and RANKL. SCOH also showed inhibitory effects on differentiated osteoclasts. It attenuated bone resorption function, actin ring formation, and survival of mature osteoclasts. Reduced activation of Akt and NF-κB and decreased induction of XIAP were observed in mature osteoclasts treated with SCOH. Thus, this novel phosphatidylcholine derivative may be useful for treatments of bone-resorbing diseases.

Disclosures: ***Z. Lee****, None.*

M236

Quercetin, a Natural Polyphenol with very Important Antioxidant Activity, Inhibits Osteoclastic Differentiation Induced by RANKL via a Mechanism Involving NFkB and AP-1. A. Wattel*[1], S. Kamel[1], C. Prouillet*[1], M. Gallet*[1], I. El Hajj Dib*[1], R. Mentaverri*[1], E. Offord*[2], P. Fardellone[1], M. Brazier[1]. [1]Laboratoire de Biologie et Pharmacie Cliniques, UMRO, Amiens, France, [2]Bone and Mineral Nutrition Team, Nestlé Research Center, Lausanne, Switzerland.

Nutrition is well known to influence bone metabolism. Several epidemiologic observations, strengthened by *in vivo* and *in vitro* data, have demonstrated that flavonoids, present in fruits, vegetables and beverages of plant origin, could have beneficial effects in prevention of postmenopausal osteoporosis.

We propose to examine the *in vitro* effects of one of the most consumed flavonol, quercetin, on osteoclastogenesis both in human (differentiation of PBMC or Peripheral Blood Monocytic Cells) and murine models (differentiation of the murine-macrophagic cell line RAW 264.7), in presence of RANKL (Receptor Activator of NFkB) and M-CSF (Macrophage-Colony Stimulating Factor).

Quercetin, tested at concentrations at 0.1, 1 and 10 µM, decreases in a dose-dependent manner the number of osteoclasts-like differentiated from PBMC for 14 days with human RANKL (25 ng/mL) and M-CSF (30 ng/mL) on bovine bone slices. Multinucleated TRAP+ cells are evaluated to 50, 45 and 25 % of control in presence of respectively 0.1, 1 and 10 µM of quercetin. Bone resorption is respectively evaluated on these slices to 69, 53 and 20 % of control, and is highly correlated with the number of osteoclastic cells. IC_{50} (inhibitory concentration of 50% of basal resorption) is evaluated to 0.75 µM. Similar experiences conducted with RAW 264.7 cells lead to the same results, ie quercetin decreases the number of multinucleated TRAP+ cells obtained after 5 days of culture with murine RANKL (30 ng/mL).

Electromobility gel shifts (EMSA) performed on RAW 264.7 cells show that quercetin inhibits NFkB and AP-1 activation induced by RANKL. NFkB and AP-1 are two transcription factors highly involved in differentiation and survival of osteoclasts. The decrease in their activation could explain, at least in part, the inhibition of osteoclastic differentiation observed in presence of quercetin.

Our results demonstrate that quercetin, one of the most consumed flavonoids by human, is a potent inhibitor of *in vitro* osteoclastogenesis. They strengthen and complete our previous results recently published (Wattel A. et al. Biochem Pharmacol. 2003 Jan 1;65(1):35-42) which showed that quercetin inhibits bone resorption activity of mature osteoclasts. We conclude that quercetin is a good candidate to explain the *in vivo* effects of fruits and vegetables consumption on bone metabolism.

Disclosures: ***A. Wattel****, None.*

M237

High Extracellular Concentrations of Strontium Directly Stimulates Osteoclast Apoptosis. R. Mentaverri*[1], A. Hurtel*[1], S. Kamel[1], B. Robin*[2], M. Brazier[1]. [1]Unité d'étude des mécanismes de la résorption osseuse, Université de Picardie Jules Verne, Amiens, France, [2]Institut de Recherches Internationales SERVIER, Courbevois, France.

Strontium ranelate induces a decrease in bone resorption and an increase in bone formation, and reduces the risk of vertebral and non-vertebral fracture in women with postmenopausal osteoporosis. As strontium (Sr) has a strong tropism for bone, high concentrations of Sr are likely to occur in the sub-osteoclastic compartment and in the vicinity of the cells during bone resorption. High concentrations of calcium (Ca) are known to inhibit osteoclastic bone resorption, at least partly by inducing osteoclast (OC) apoptosis, thus Sr released during the bone resorption process, may act on these osteoclast activities. Using 10-day-old rabbit OCs, the effects of Sr alone (1.8 to 24 mM) or in combination with Ca (1.8 to 20 mM) were assessed on bone resorbing activity by pits area measurement and on the programmed cell death of purified OCs. Isolated OCs were seeded on bovine bone slices and adherent cells cultured in the presence of Sr for 48 hrs. Osteoclast apoptosis, assessed by Hoechst staining, was confirmed by the electrophoresis of DNA scales.

Sr inhibited bone resorption in a dose-dependent manner with significant inhibition observed from 12 mM (-25%, $p<0.05$) to 24 mM (-50%, $p<0.01$), and dose-dependently induced OC apoptosis. Independently, Sr (24 mM) and Ca (20 mM) induced a similar rate of osteoclast apoptosis (approx. 50%). Tested together, Sr and Ca have additional effects on bone resorption as well as on mature osteoclast apoptosis. The use of specific inhibitors of intracellular Ca signaling pathway (U73122, Caffeine, Ryanodin, 2APB and SKF-96365) indicates that the transduction pathways involved in Sr- and Ca-induced OC apoptosis are different. Although Sr and Ca effects are phospholipase C dependent, only the Ca-induced apoptosis is mediated by IP3-dependent intracellular Ca release and capacitive Ca entry. Sr-induced osteoclast apoptosis seems to be IP3 independent but its intracellular signaling pathway remains to be specified.

Taken together, our data strongly suggest that exposure of OCs to an increasing amount of Sr is responsible for a decrease in the bone resorbing process. This decrease was, at least in part, mediated by the induction of OC apoptosis. Although, Sr and Ca both stimulate a G protein-coupled receptor, which could be the calcium-sensing receptor, they have differential intracellular effects which independently trigger OC apoptosis and could act in a cooperative manner. These results strongly support the reduction in bone resorption observed with strontium ranelate in various *in vivo* and *in vitro* experiments.

Disclosures: ***R. Mentaverri****, None.*

M238

Antisense Oligonucleotides Targeting RANK and RANKL Block Bone Resorption in a Model of PTH-Induced Hypercalcemia. X. Li, C. Thompson*, J. Finger*, M. Guha*, O. Daly*, S. Gregory*, K. Myers*. Isis Pharmaceuticals, Carlsbad, CA, USA.

RANK is a cell surface receptor expressed on osteoclast precursor cells and mature osteoclasts. RANKL is expressed in osteoblasts and bone marrow stromal cells. RANK and RANKL-deficient mice each display an osteopetrotic phenotype, suggesting that signaling through the RANK pathway plays a central role in bone remodeling in vivo. To test the abilities of antisense oligonucleotides (ASOs) to target osteoclast precursors and osteoblasts *in vivo*, we have developed and characterized second generation ASOs specific for both mouse RANK and RANKL, and have tested these ASOs in a short-term model of bone resorption induced by parathyroid hormone mini-pump infusion in young Swiss Webster mice placed on a low calcium diet. In this model, PTH infusion for 24 hrs doubled serum calcium levels, whereas the positive control, salmon calcitonin, provided an average 62.8% inhibition of hypercalcemia in five different experiments. The RANK ASO, ISIS 181071, did not change serum calcium levels in normal (non-PTH-infused) mice, but in mice rendered hypercalcemic by PTH infusion, a 30 mg/kg daily injection of RANK ASO inhibited the hypercalcemia to the same degree as calcitonin treatment. This effect was reproducible, dose-dependent, and also occurred when a second oligonucleotide (ISIS 181080) targeting a different portion of RANK mRNA was used. A reduction in hypercalcemia could be seen with as few as 3 days of daily treatment with the RANK ASO. Analysis of RANK mRNA levels in RANK ASO-treated mice revealed 40-50% reductions in RANK mRNA levels in both metaphyseal bone of the proximal tibia and in unfractionated bone marrow cells, while treatment with an 8-base mismatch control oligonucleotide failed to reduce RANK mRNA in either tissue. To further characterize RANK reductions in bone marrow, RANK expression in bone marrow cells was analyzed by flow cytometry. Mice treated with the RANK ASO displayed 80-90% reductions of RANK protein in total bone marrow, the monomyeloid (CD11b+/GR1+) and monocytic (CD11b +/GR1-) subpopulations. ASOs targeting mouse RANKL also inhibited PTH-induced hypercalcemia, but the inhibition was not as great as that achieved by the RANK ASOs. ISIS 180819 produced a potent, dose-dependent reduction of RANKL mRNA in the C2C12 cell line in which it was screened. This oligonucleotide reduced RANKL mRNA approximately 40% in tibial metaphysis and 50-60% in calvaria in mice challenged with PTH. Our results provide the first evidence that RANK and RANKL ASOs are capable of blocking bone resorption *in vivo*. The results suggest that ASOs targeting the RANK signaling pathway could prove useful in the treatment of osteoporosis and other diseases with increased bone resorption.

Disclosures: ***X. Li****, Isis Pharmaceuticals 1, 3.*

M239

Structure-activity Relationships for Inhibition of Rab Prenylation and Bone Resorption by Phosphonocarboxylate Drugs. F. P. Coxon*[1], F. H. Ebetino[2], M. J. Rogers[1]. [1]Medicine & Therapeutics, University of Aberdeen, Aberdeen, United Kingdom, [2]Procter & Gamble Pharmaceuticals, Cincinnati, OH, USA.

Nitrogen-containing bisphosphonates such as risedronate act by inhibiting farnesyl diphosphate (FPP) synthase, thereby preventing the synthesis of isoprenoid lipids required for prenylation of Ras, Rho and Rab family proteins in osteoclasts. We recently found that a weakly anti-resorptive phosphonocarboxylate analogue of risedronate, NE10790, selectively prevents prenylation of Rabs in cells *in vitro* by inhibiting Rab geranylgeranyl transferase (Rab GGTase). This compound does not inhibit FPP synthase or protein:prenyl transferases other than Rab GGTase, and so has no effect on prenylation of other small GTPases. We have now examined the effect of two additional phosphonocarboxylate analogues of NE10790: PGE1560036, which lacks the geminal hydroxyl group of NE10790, and PGE-5752082, in which the position of the nitrogen in the pyridyl ring of the side-chain is altered (2-pyridyl *vs* 3-pyridyl in PGE1560036).

PGE-1560036, like NE10790, specifically inhibited the prenylation of Rab proteins, demonstrated by metabolically labelling J774 macrophages with [14C]mevalonate and by separating prenylated Rabs from unprenylated Rabs by triton X-114 fractionation. Moreover, PGE-1560036 was of similar potency to NE10790, effectively inhibiting Rab prenylation at concentrations ≥0.5mM. Like NE10790, PGE-1560036 disrupted the golgi-localisation of Rab6 in J774 cells and rabbit osteoclasts cultured on plastic, visualised by immunofluorescence staining. However, unlike NE10790, PGE-1560036 had little effect on the resorptive activity of rabbit osteoclasts cultured on dentine. This is probably due to lack of affinity of PGE-1560036 for bone mineral since, in contrast to NE10790, it did not disrupt the golgi-localisation of Rab6 in osteoclasts cultured on dentine, indicating that Rab prenylation was not inhibited. PGE-5752082, at concentrations up to 1.5mM, had no effect on prenylation of Rabs or other proteins in J774 cells and did not affect bone resorption by osteoclasts *in vitro*.

In summary, these data demonstrate that the geminal hydroxyl group is not essential for inhibition of Rab GGTase by phosphonocarboxylates, but appears to be essential for the anti-resorptive action of this class of drugs *in vitro* by enabling them to bind to bone mineral prior to uptake by osteoclasts. In addition, as with bisphosphonates, the position of the nitrogen in the side chain of phosphonocarboxylates appears to be crucial for their ability to inhibit Rab GGTase and hence to inhibit bone resorption.

Disclosures: ***F.P. Coxon****, None.*

M240

Novel Effect of Natural Prenyl-quinones: Inducing Apoptosis in Mouse Macrophage-like J774 Cells *in vitro*. L. J. Schurgers*, B. A. M. Soute*, C. Reutelingperger*, C. Vermeer*. Biochemistry, University Maastricht, Maastricht, Netherlands.

Several epidemiological studies have shown a close association between osteoporotic bone loss and cardiovascular disease, notably vascular calcification. The vascular calcification is believed to arise from a mineralization process similar to that in bone. N-bisphosphonates, frequently used as drugs to prevent postmenopausal bone loss, were shown to inhibit aortic calcification. The effect is thought to be mediated by a decreased bone turnover, due to osteoclast inhibition. Prenylation of key regulatory proteins such as Ras, Rac, and Rho, is an essential step in osteoclast activation. N-bisphosphonates act as competitive inhibitors in the mevalonate pathway thus blocking the formation of geranylgeranyl pyrophosphate, an essential intermediate in protein prenylation. In a number of reports it has been claimed that one form of vitamin K (menaquinone-4, MK-4) is particularly effective in preventing postmenopausal bone loss. Our group has recently demonstrated that MK-4 (and not other forms of vitamin K) is a powerful inhibitor of artery calcification in an animal model. It is at least noteworthy that MK-4 is the only K-vitamin bearing a geranylgeranyl side chain.

To systematically investigate the potential bisphosphonate-like activity of geranylgeranyl-containing compounds, we have used the mouse macrophage J774 cells in which we measured the induction of apoptosis. Cells were seeded into 6-well plates at a density of 10^5 cells/well. After 24 hours the medium was replaced with fresh medium containing 100uM of either residronate, K_1 (phytol side chain), MK-4, ubiquinone-4 (UQ-4), geranylgeraniol (GG-OH), or phytol. Control cells were treated with fresh medium only. After 48 hours, cells were washed and adhered cells were collected. Apoptosis was measured in an Epics-XL (Beckman-Coulter) using FITC-labeled annexin-V and propidium iodide. Geranylgeranyl-containing compounds such as MK-4 and UQ-4 induce apoptosis in this system which was comparable to the effect of residronate. No apoptosis was observed in control cultures with or without vitamin K_1 or phytol. The different effects of K_1 and MK-4 suggest that MK-4 induced apoptosis is not related to its 'vitamin K-activity'; this conclusion is supported by the apoptosis-inducing activity of UQ-4, a compound lacking vitamin K-activity. Finally, also geranylgeraniol itself induced apoptosis. Our data provide a mechanism for the observed inhibitory effects of natural prenyl quinones in osteoporotic bone loss and vascular calcification, and may provide the basis for a new line of therapeutic drugs in these areas.

Disclosures: ***L.J. Schurgers****, None.*

M241

Identification of Alpha-V Beta-3 Integrin Antagonists with Anti-Resorptive Activity. D. B. Kimmel[1], S. B. Rodan[1], M. E. Duggan*[2], G. Hartman*[2], P. J. Coleman*[2], K. Brashear*[2], C. T. Leu[1], G. Wesolowski[1], T. Prueksaritanont*[1], M. A. Gentile[1], J. G. Seedor*[1], B. Pennypacker[1], P. Masarachia[1], L. T. Duong*[1], L. Lipfert*[1], G. A. Rodan[1]. [1]Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA, USA, [2]Medicinal Chemistry, Merck Research Laboratories, West Point, PA, USA.

$\alpha_{v\beta 3}$ integrin is an abundant cell surface receptor on osteoclasts. Small molecule $\alpha_{v\beta 3}$ antagonists inhibit osteoclast activity. In vitro and in vivo bone efficacy data from two novel and structurally related $\alpha_{v\beta 3}$ integrin antagonists are reported.

^{3}H-labeled Compounds A and B bind with high affinity to purified human $\alpha_{v\beta 3}$ with Kd's of 0.3 and 0.2 nM, respectively. In cell-based assays, A and B: (a) inhibit osteoclast formation and bone resorption with IC_{50}'s of ~15-35 nM; (b) inhibit attachment to vitronectin by $\alpha_{v\beta 3}$-transfected HEK-293 cells, each with IC_{50}'s of 0.8nM and to $\alpha_{v\beta 5}$-transfected HEK-293 cells with IC_{50}'s of 1.8 and 1.3nM, respectively; and (c) inhibit $\alpha_{IIb\beta 3}$-dependent human platelet aggregation with IC_{50}'s of 45 and 133µ M, respectively.

A (1, 3, or 10 mg/kg/d) and B (10, 20, or 30 mg/kg/d) were given p.o. daily to newly-ovariectomized (OVX) seven month old Sprague-Dawley rats for six weeks. Bone resorption markers (urinary deoxypyridinolone/creatinine) were measured and in-life double calcein labeling was done. 3mg/kg/d A suppressed uDPD/Cre and calcein labeling of proximal tibial cancellous bone to Sham levels, and fully prevented bone loss. 20mg/kg/d B behaved similarly. 1mg/kg/d A and 10 mg/kg/d B were partially efficacious on those endpoints.

30, 10, or 3mg/kg/d B was given for six days to OVXd rhesus monkeys. uNTx/Cre (n-telopeptides) was reduced 37% (P<.0001), 28% (P<.0001), or 0%, respectively, when compared to vehicle treatment. Recovery of uNTx/Cre to vehicle levels occurred within 1-2 days after cessation of B.

We conclude that two structurally-related small molecule $\alpha_{v\beta 3}$ integrin antagonists: 1) bind $\alpha_{v\beta 3}$ with sub nanomolar affinity; 2) inhibit osteoclast formation and attachment to vitronectin in appropriate cell-based assays, without affecting platelet aggregation. Both fully block estrogen deficiency bone loss in rats; B suppresses bone resorption in estrogen deficient non-human primates in a rapidly reversible fashion. Small molecule $\alpha_{v\beta 3}$ integrin antagonists have the potential to prevent and/or treat post-menopausal osteoporosis.

Disclosures: ***D.B. Kimmel****, Merck Research Laboratories 1, 3.*

M242

The Purinergic P2X7 Receptor Is an Inhibitor of Bone Resorption in Mice. N. R. Jorgensen[1], T. H. Steinberg*[2], Z. Henriksen*[3], S. D. Ohlendorff*[3], J. B. Jensen[3], I. P. Chessell*[4], O. H. Sørensen[3], R. Civitelli[2]. [1]Dep. of Clinical Biochemistry 339, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark, [2]Dep. of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA, [3]Osteoporosis Unit 545, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark, [4]GlaxoSmithKline, Harlow, United Kingdom.

The purinergic P2X7 receptor is an ATP-gated ion channel primarily expressed in cells of the hemopoietic lineage. We have previously shown that P2X7 mediates intercellular signaling between osteoblasts and osteoclasts, and it is probably involved in modulating osteoclast activity at least partially through regulation of osteoclast apoptosis. To investigate the role of P2X7 in bone *in vivo*, we used mice harboring a null mutation of the P2X7 gene, obtained from GlaxoSmithKline. We examined the bone phenotype of female and male animals at 4 months of age (n=19-31 per group). Whole body bone mineral density in female animals (measured by a PIXImus DXA scanner) was significantly higher in both heterozygote and homozygote null mutants (0.0524 g/cm2, p<0.0001 and 0.0513 g/cm2, p=0.026, respectively) relative to their wild type littermates (0.0502 g/cm2). Heterozygotes had also higher bone mineral content than wild type mice (0.4640 g, vs. 0.4362 g, p<0.001). These differences were not seen in male animals. Histomorphometric analysis confirmed an increased bone volume/total volume in heterozygous and homozygous null mice (15.7 and 16.1 % respectively) compared to their wildtype littermates (14.7 %). These differences in bone mass were associated with increased mechanical strength of the femur. In females, the maximal load before fracture (assessed as a 3-point bending test on a Lloyd Instruments compression device , *ex vivo*) was significantly higher in the heterozygotes compared to wild type littermates (18.89 N vs. 17.69 N, p=0.041). While we found no differences among groups in serum osteocalcin, the bone resorption marker, C-telopeptide collagen type I fragment (RatLaps) was lower in homozygote mutant mice than in wildtype littermates (10.4 ng/ml vs. 13.4 ng/ml, p<0.005). In summary, ablation of the P2X7 gene results in increased bone density and resistance to load, associated with decreased bone resorption. Thus, the P2X7 receptor functions as a negative regulator of osteoclast development and/or function.

Disclosures: ***N.R. Jorgensen****, None.*

M243

Quercetin Suppresses Bone Resorption by Inhibiting Osteoclast Differentiation and Disrupting Functional Structure in Mature Osteoclasts. J. T. Woo, T. Yonezawa*, M. Ohnishi*, N. Kazuo*. Department of Biological Chemistry, Chubu University, Kasugai, Japan.

Rutin (quercetin-3-O-glucose rhamnose) has been shown to inhibit ovariectomy-stimulated bone resorption in rats. However, its target cells and detailed mechanism of inhibition of bone resorption has not been reported. Since rutin is thought to be converted into quercetin in the large intestine by the glycosidase activity of intestinal bacteria, in this study, we investigated the effects of quercetin on the differentiation of osteoclast progenitor cells into osteoclasts, functional structure in mature osteoclasts and bone resorption in bone cultures. Quercetin (1-5 µM, IC_{50}=2 µM) dose-dependently inhibited multinucleated osteoclast formation induced by RANKL in the cultures of TRAP negative MCSF-dependent (MD) cells and RAW 264.7 macrophage (RAW) cells. Quercetin also inhibited the increase of TRAP activity of mononuclear preosteoclasts (pOCs) induced by RANKL in both MD and RAW cell cultures. Quercetin blocked the action of RANKL, but not that of M-CSF, during pOC formation from progenitor cells in MD cell cultures. Quercetin attenuated the phosphorylation of P38 kinase increased by RANKL in RAW cell cultures. Quercetin also reversibly disrupted functional structure such as actin rings in mature osteoclasts. Furthermore it inhibited pit formation by osteoclasts on dentine slices and ^{45}Ca release stimulated by PTH in organ cultures in the same dose-range effective for osteoclast differentiation. These results suggest that osteoclast progenitors and mature osteoclasts are the target cells of quercetin, and that suppressive effect of quercetin on bone resorption results from its inhibitory effect on pOC formation and/or disruptive effect on functional structure in mature osteoclasts.

Disclosures: ***J.T. Woo****, None.*

M244

Effects of Alendronate on the Human Osteoclast Proteome. C. Hughes-Begos[1], S. Neubort*[1], M. A. Gawinowicz*[2], D. W. Dempster[1]. [1]Regional Bone Center, Helen Hayes Hopital, West Haverstraw, NY, USA, [2]Protein Core Facility, Columbia University, New York, NY, USA.

Several mechanisms have been proposed to explain the inhibitory action of alendronate (ALN) on bone resorption. Some mechanisms involve promotion of osteoclast apoptosis, whereas others involve non-apoptotic pathways. The advent of proteomics provides a powerful new tool to shed light on the mechanism of action of drugs. We have applied this approach to determine the effects of ALN on protein expression in a purified population of human osteoclasts in vitro. GM-CSF-mobilized, human CD14-positive mononuclear cells were cultured in plastic culture dishes or on bovine cortical bone slices for 18 days in the presence of RANKL (10 ng/ml) and M-CSF (25 ng/ml). At day 15, ALN (10^{-4}M) or vehicle were added and at day 18, total protein was extracted and subjected to 2D gel electrophoresis. Proteins that were either up- or down-regulated were excised and identified by MALDI-TOF spectrometry. Bone resorption was assessed by measurement of C-terminal telopeptides (CTx) in the medium and osteoclast morphology was monitored by phase contrast light microscopy. ALN treatment resulted in a reduction of bone resorption to less than 20% of control (p<0.001). This was accompanied by a dramatic change in osteoclast morphology from large, flattened cells with numerous cytoplasmic processes to a smaller, spherical configuration without processes. Analysis of the 2D gels revealed that ALN regulated the expression of a number of proteins. Highly up-regulated proteins included vimentin, creatine kinase, cathepsin D, macrophage capping protein, and annexin V. Highly down-regulated proteins included actin, calreticulin, cathepsin B, and galectin 1. In conclusion, ALN-mediated inhibition of bone resorption by human osteoclasts was associated with regulation of a number of proteins that are known to play key roles in osteoclast attachment and motility, calcium binding, and matrix degradation. Regulation of apoptosis-associated proteins was not observed. Application of proteomics in this model promises to provide new insights into the mechanism of action of bisphosphonates and other drugs on human osteoclasts.

Disclosures: ***C. Hughes-Begos****, None.*

M245

Osteoprotegerin Inhibits Loss of Periarticular Bone Mass but Does Not Prevent Cartilage Destruction in the Rat Antigen-induced Arthritis. T. Neumann*[1], P. Oelzner*[1], P. Petrow*[2], K. Thoss*[2], G. Stein*[1], G. Hein*[1], R. Bräuer*[2]. [1]Department of Internal Medicine III, Rheumatology/ Osteology, Friedrich-Schiller-University, Jena, Germany, [2]Institut of Pathology, Friedrich-Schiller-University, Jena, Germany.

Lewis rats with antigen-induced arthritis (AIA) exhibit marked inflammation (paw swelling and massive leukocyte infiltration of the synovium) and skeletal damage (cartilage destruction and loss of periarticular bone). These rats were treated with Osteoprotegerin (OPG) to assess whether blockade of receptor activator of nuclear factor kB ligand (RANKL) would prevent cartilage destruction and loss of periarticular bone mass in the primary and secondary spongiosa. OPG was administered by intraperitoneal injection ten times at a dose of 3 mg/kg/day, beginning at the induction of arthritis. Secondary spongiosa was assessed by histomorphometry on day 23. Bone volume, structure and cellular rearrangement of the periarticular bone (right tibial head – arthritis joint) and axial bone (3rd lumbal vertebra) were determined. Bone structure of the primary spongiosa was analysed by linear scanning. The measured content of mineralized tissue was ploted as a function of distance from the growth plate. The extent of cartilage destruction was assessed by semi-quantitative evaluation of surface erosions, chondrocyte necrosis and depletion of proteoglycans. Sham-treated AIA was associated with a significant reduction of the trabecular bone volume in the secondary spongiosa of the tibial head compared to the healthy animals. The same was found for the primary spongiosa. Treatment with OPG resulted in a significant reduction of bone turnover at the tibial head and at the 3rd lumbal vertebra compared with sham-treated AIA. Osteoid surface with osteoblasts, bone formation rate and resorption surface with osteoclasts were significantly reduced in tibial head as well as in 3rd lumbal vertebra in OPG treated animals. Moreover, OPG had an inhibitory effect on the periarticular arthritis-associated loss of bone mass of the primary and secondary spongiosa. The trabecular bone volume of the secondary spongiosa was significantly higher in OPG treated AIA, but still lower than in healthy animals. The content of mineralized tissue in the primary spongiosa of OPG treated AIA was higher than in sham-treated AIA and in healthy animals. This observation was made starting at a distance of 225 µm from the growth plate. Cartilage destruction in AIA rats was reduced by OPG treatment, however,

the preventive effect was less striking than in bone. The treatment did not influence the inflammatory aspect of AIA.

Disclosures: ***T. Neumann****, None.*

M246

Corticosteroids Stimulate Differentiation of the Monocytic Cell Line RAW264.7 into Osteoclast like Cells, but Inhibit Resorbing Activity; Possible Involvement of p38 MAPK and NFkappaB, but not MEK Pathway. R. J. Arends*, M. van Beuningen - de Vaan*, E. Veen - van den Berk*, M. van Duin*, A. G. H. Ederveen. Pharmacology, N.V. Organon, Oss, Netherlands.

Chronic corticosteroid treatment is known to induce bone loss and osteoporosis in humans. In rodents results are less consistent and vary dependent of experimental conditions. Some studies report bone loss following corticoid treatment whereas others report a prevention of bone loss.

To study the effect of corticosteroids on osteoclast differentiation and function *in vitro,* we used the murine monocytic cell line RAW264.7. These cells differentiate into osteoclast-like TRAP positive cells following treatment with RANK-Ligand (RANKL). TRAP activity and number of osteoclasts is a measure for differentiation.

Osteoclast function was studied on glass coverslips coated with hydroxyapatite (HA). RAW264.7 cells were cultured for 8 days and treated with RANKL. After differentiation, RAW264.7 cells display functional activity by formation of pits in the HA.

Cell signaling inhibitors were tested to establish pathways involved in RAW264.7 differentiation. RANKL-induced differentiation of RAW264.7 cells was completely blocked by OPG (1nM), a soluble decoy receptor for RANK and by an inhibitor of p38 MAPK (SB203580, at 10μM). In contrast to what has been reported in other studies, PD98059, an inhibitor of MAPK extracellular signaling-regulated kinase (MEK) had no effect on differentiation of RAW264.7 cells. The NFκB pathway is involved in osteoclast differentiation and function. SN50, an inhibitor of the NFκB pathway partially inhibited osteoclast differentiation (36μM). These results indicate that the p38 MAPK and the NFκB pathway but not the MEK pathway is involved in differentiation of RAW264.7 cells.

In contrast to the NFκB and the p38 MAPK inhibitors, dexamethasone and prednisolone stimulated differentiation of RAW264.7 cells into mature multinucleated cells. Osteoclast function was studied using RAW264.7 cells cultured on glass coverslips coated with HA. Resorbing activity was determined by quantification of the formed pit area. Dexamethasone and prednisolone inhibited pit formation dose dependently. Dexamethasone was 10 times more potent than prednisolone to completely block pit formation (10nM vs. 100nM).

In conclusion: corticosteroids have a dual effect on RAW264.7 cells by stimulating the differentiation of pre-osteoclasts into multinucleated osteoclasts but suppressing the resorbing activity of mature osteoclasts. The dual effect of corticosteroids on murine osteoclast differentiation and function contributes to the understanding of the different *in vivo* results found in rodent studies with these compounds.

Disclosures: ***R.J. Arends****, N.V. Organon 3.*

M247

Nitric Oxide Regulates Human Osteoclasts via G-kinase and M-calpain Mediated Disassociation of Specific Cytoskeletal Proteins. B. Yaroslavskiy*, A. Mamoune*, Y. Li, R. Bu, S. E. Kalla*, J. I. Oakley*, A. Wells*, H. C. Blair. Pathology and Physiology & Cell Biol, University of Pittsburgh and Pittsburgh VAMC, Pittsburgh, PA, USA.

Nitric oxide (NO) ia a downregulator of osteoclastic activity, but the molecular mechanism is unknown. Osteoclasts resorb bone by acid secretion in a specialized extracellular compartment. A prominent actin ring and integrin attachment are required for this extracellular compartment. The β cytoplasmic tail of integrins, chiefly $\alpha_v\beta_3$, links integrins to the actin cytoskeleton. We studied the effects of NO on osteoclastic attachment and acid secretion in human osteoclasts on bone. Human osteoclasts were produced on bone slices from CD14 monocytes in DMEM containing RANKL, M-CSF, TGFβ, 1,25-dihydroxyvitamin D, and dexamethasone. These osteoclasts have *bona fide* actin rings and produce lacunae labeled as acid lakes by lysotracker. Effects on cellular activity and cytoskeleton were studied with NO donors (SNAP, Na nitroprusside), cGMP agonists including 8-pCPT-cGMP, and NO synthesis or cGMP activity antagonists (L-NMMA, Rp-cGMP, and Ly83583). Changes in cellular attachment and actin distribution were directly related to loss of acid lakes by lysotracker. This included coordinated cytoskeletal rearrangement with loss of actin ring by phalloidin labeling. Thus we hypothesized that NO, via its effect on guanosyl cyclase and cGMP-dependent protein kinase (PKG), modulates cellular cytoskeleton and attachment via actin-integrin association. Immune labeling and Western blot of intermediate protein lead to the discovery that NO donors cause VASP phosphorylation at Ser^{239} which causes dissociation of VASP from the membrane complex. The intermediate protein zyxin was likewise disassociated from the membrane complex and down regulated. These activities were associated with actin depolymerization. In addition to actin depolymerization, our data suggested dissociation of actin cytoskeleton from integrins implicating possible proteinase activity. Zymography revealed proteinase acitivity consistent with m-calpain. BOG, a specific calpain substrate, showed that calpain activity was ~ 3 times higher in cells treated with NO donor compare to treatment with NO antagonist. Western analysis for m-calpain confirmed this result. The relation of G-kinase activation to m-calpain activity will require further analysis but m-calpain was not activated with G-kinase antagonists were present. We conclude that NO modulates osteoclasts function by VASP phosphorylation at Ser^{239}, alteration of zyxin expression, and by activating m-calpain. Alteration of VASP and zyxin results in actin depolymerization. Calpain activation leads to actin integrin dissociation.

Disclosures: ***B. Yaroslavskiy****, None.*

M248

Anti-$\alpha_v\beta_3$ mAb, Vitaxin™, Blocks Attachment and Bone Resorption of Osteoclasts. H. Zhang*[1], S. Shorey*[2], A. Gramoun*[2], J. Heersche*[2], J. Suzich*[1], S. Mao*[1]. [1]Molecular Biology/Biochemistry, MedImmune, Inc., Gaithersburg, MD, USA, [2]Dental Research Institute, University of Toronto, Toronto, ON, Canada.

$\alpha_v\beta_3$ integrin is highly expressed on osteoclasts and is crucial for their resorptive activity. We present here the effects of an $\alpha_v\beta_3$-specific mAb, Vitaxin™, on osteoclast attachment and bone resorption. Vitaxin binds to the $\alpha_v\beta_3$ integrin of human, monkey, guinea pig, rabbit, and hamster origin, but not rat or mouse. Binding studies showed that the antibody has a high affinity (K_D = 2 nM) for human $\alpha_v\beta_3$. Vitaxin binds rabbit and hamster $\alpha_v\beta_3$ with an affinity 3-fold lower than that for the human analog. In this study, we used both human and rabbit osteoclasts to test the effect of Vitaxin on osteoclast function. Human osteoclasts were differentiated *in vitro* from osteoclast precursors. The precursor cells were cultured with MCSF and RANKL on bovine bone slices for 10 days in the presence or absence of Vitaxin. The culture supernatants were collected for measurement of type I collagen peptide. Vitaxin inhibited the bone resorption in a dose-dependent manner, with a maximum of 63% inhibition observed at doses greater than 2 nM. In parallel experiments, Vitaxin was shown to cause detachment of authentic rabbit osteoclasts from plastic culture dishes. Approximately 27% of the osteoclasts became detached after 48 hours in the presence of 0.2 nM of the mAb. Vitaxin also inhibited bone resorption by rabbit osteoclasts cultured on bovine bone slices. Treatment with Vitaxin at 0.7 nM resulted in a 41% reduction in the number of osteoclasts attached to the bone slices, a 45% reduction in pit formation, and a 50% reduction in pit area. Taken together, our results indicate that Vitaxin is effective at inhibiting osteoclast binding and bone resorption, and support the use of this antibody as a therapeutic in diseases involving excessive bone resorption.

Disclosures: ***S. Mao****, None.*

M249

Towards the Rational Design of Therapeutic RANKL Antagonists as Anti-resorptive Agents. R. S. Hubert*, S. Chu*, J. Jacinto*, C. Jordan*, J. R. Desjarlais*, J. Elyazal*, C. Deguzman*, D. Herman*, L. Hyun*, R. Carrillo*, J. Castrellon*, S. Karki*, C. O'Brien*, P. Hammond*, H. S. Cho*, P. Cheung*, B. Dahiyat*. Xencor, Monrovia, CA, USA.

The RANKL/OPG/RANK biochemical axis maintains bone-remodeling homeostasis by modulating osteoclast activity. Soluble RANK-Fc, OPG-Fc, and anti-RANKL antibodies that sequester and block RANK signaling are in development as potential biotherapeutics for the treatment of many bone disorders including osteoporosis, Paget's disease, prosthesis-induced osteolysis, and osteolytic bone cancer and metastases. We present a novel structure-based protein engineering strategy to rationally design biotherapeutic antagonists of osteoclastogenesis based on the human RANKL extracellular domain region (RANKL-ECD). Receptor-binding and cell-based agonism assays identified RANKL variants unable to bind to either RANK receptor or the decoy receptor OPG. We predict that these RANKL-ECD variants do not interfere with OPG decoy receptor activities or get sequestered by OPG. Osteoclastogenesis assays using RAW264.7 cells identified variants with a wide range of activities including potential competitive inhibitors of RANKL which bind to RANK receptor without activating osteoclastogenesis. Moreover, our computational design approach has lead to the first report of high level, soluble human RANKL-ECD expression in *E.coli*. We are currently evaluating the therapeutic potential of these RANKL antagonists using co-culture assays of osteoclastogenesis.

Disclosures: ***R.S. Hubert****, Xencor 1, 3.*

M250

Prevalence of Osteopenia in Pre- and Postmenopausal Women. P. G. Masse[1], J. Dosy*[1], L. Holloway[2], S. M. Donovan[3]. [1]Nutrition, Université de Moncton, Moncton, NB, Canada, [2]Geriatrics Research, VA Medical Center, Palo alto, CA, USA, [3]Human Nutrition, University of Illinois, Urbana, IL, USA.

Healthy, free-living, active Caucasian 30 Pre- and 30 early Postmenopausal (4.0 ± 1.4 y) women from the same northern region and with the same socieconomic background, non-smokers and not taking estrogen, other drugs and nutritive supplements, were compared in a cross-sectional design during a Spring-Summer season. Areal BMD (g/cm2) was measured by Lunar DXA at 13 different axial (Lumbar) and appendicular (Femur) sites and apparent volumetric (BMAD) (g/cm3) was calculated. Fasting morning plasma and urinary markers of bone formation and resorption (expressed per mmol Creatinine) were used to assess bone metabolism in addition to IGF-1. Mean dietary protein, vitamin D, Ca and P intakes and their ratio (as assessed from 3-d records), BMD and BM(A)D of both groups were similar. 60.0 and 88.9% of pre- and post-M women were osteopenic (=-1.1SD and >-2.5SD) at least at one bone site. All markers of bone turnover except for bALP were markedly greater in Post group. The rise in their bone resorption was much greater than that of bone formation, the difference between groups being more pronounced for helical peptide (49.4%) than D-pyr (35.94%). IGF-1, an important regulator of osteoblast-progenitor number and required for the anabolic actions of PTH on bone formation, was reduced ($P<0.01$) in Post subjects. The reduction (-20.4%) was amazingly of the same magnitude to the elevation (+19.3 %) of OC. The highly significant rises in Ca and Pi in the Post group were correlated ($P<0.001$) with both bone resorption markers suggesting a shift of minerals from the skeleton. These early changes occurred independently of the effect of the major calciotropic hormones, suggesting a direct effect of estrogen deficiency on bone but not on intestine (Ca absorption). Both groups had adequate vitamin D status as assessed by plasma 25(OH)D3 (cut-off of 15 ng/mL) despite deficient dietary intakes. Intakes of Post women being more deficient

(P<0.0001), their plasma 25(OH)D3 was surprisingly greater (P<0.01). The present study showed that:1) biochemical markers better discriminate early Post women from Pre controls than BM(A)D; 2) bone resorption starts at an early stage of estrogen deficiency and even preceded the onset of menopause in a high proportion of Pre subjects.

	Pre	Post
Age (yrs)	41.9 (4.8)	54.0 (3.8)***
Estradiol, pmol/L	650 (73)	133 (90)****
BMI (kg/m2)	26.,1 (4.0)	25.5 (3.0)
Ca, mmol/L	2.26 (0.08)	2.40 (0.09****
Pi, mmol/L	1.01 (0.12)	1.11 (0.13)***
iPTH, pmol/L	3.41 (1.40)	3.02 (1.24)
25 (OH) D3, ng/mL	25.7 (13.4)	34.8 (13.5)**
1,25(OH)2D3, pg/mL	30.5 (13.4)	28.1 (11.7)
IGF-1, ng/mL	76.4 (25.6)	60.8 (18.7)**
osteocalcin (OC), ng/mL	36.7 (8.1)	43.8 (6.5)****
bALP, µg/mL	7.2 (3.5)	6.3 (2.4)
D-pyr, nmol/mmol CR	3.9 (1.5)	5.3 (1.3)****
Helical peptide, µg/mmol CR	39.9 (21.3)	59.6 (28.5)***
Mean(SD)****P<0.0001	***P<0.001	**P<0.01

Disclosures: ***P.G. Masse****, None.*

M251

Increased Femoral Neck Bone Loss in Older Black and White Women, but not Men, with Diabetes. A. Schwartz[1], D. Sellmeyer*[1], K. Feingold*[1], E. Strotmeyer[2], F. Tylavsky[3], H. Resnick*[4], R. Shorr*[3], D. Black[1], J. Cauley[2], S. Cummings[1], T. Harris*[5]. [1]University of California San Francisco, San Francisco, CA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA, [3]University of Tennessee, Memphis, TN, USA, [4]MedStar Research Institute, Hyattsville, MD, USA, [5]National Institute on Aging, Bethesda, MD, USA.

Type 2 diabetes (DM) is associated with elevated fracture risk but higher bone mineral density (BMD). A previous study suggested increased bone loss in older white women with DM. To determine if bone loss is increased with DM, we analyzed baseline data from the Health, Aging and Body Composition study of 3,075 white and black, well-functioning men and women age 70-79 years. Hip BMD was measured by DXA at baseline and 4 years later. Those diagnosed with diabetes after baseline (N=134) and those with a missing hip scan (N=833) were excluded. These analyses include 480 (23%) participants with DM at baseline, defined as self-report, use of hypoglycemic medication, or an elevated fasting glucose (FG≥ 126 mg/dl) or 2-hour glucose (OGTT≥ 200 mg/dl), and 1176 (56%) with normal glucose homeostasis (NG), defined as FG<110 mg/dl and OGTT<140 mg/dl.
Those with DM had higher baseline hip BMD and weight, more weight loss during followup, and were more likely to use thiazide diuretics. Use of other bone-active supplements and medications was similar in those with DM and NG. The effect of DM on change in femoral neck BMD differed by gender (p =0.03) but not race. In women, those with DM lost more femoral neck BMD than those with NG (-0.22 %/year; 95% CI: -0.44, -0.001) in models adjusted for age, race, baseline BMD, baseline weight, weight change, calcium or vitamin D supplements, and bone-active medications. In men, change in femoral neck BMD was similar for DM compared with NG participants (+0.11 %/year; 95% CI: -0.09, 0.31). Changes in total hip BMD for DM and NG were not statistically different.
Despite having higher BMD, older black and white women with DM, but not men, had more rapid bone loss at the femoral neck than those with normal glucose homeostasis. This increased bone loss may contribute to the higher fracture risk observed in older diabetic women.

Disclosures: ***A. Schwartz****, None.*

M252

Osteoporosis Risk Assessment Tool (ORAT) Assists Clinicians in Selecting Postmenopausal Women for DXA Testing. J. Valeriano-Marcet[1], T. Johnson*[2], M. Lowenstein*[1], B. Dacchille*[3], A. Stephenson*[4], J. D. Carter*[1], F. B. Vasey*[1]. [1]Department of Internal Medicine, University of South Florida and James A. Haley Veteran's Hospital, Tampa, FL, USA, [2]Proctor and Gamble, Cincinnati, OH, USA, [3]St. Joseph's Hospital, Tampa, FL, USA, [4]Watson Clinic, Plant City, FL, USA.

Introduction: The Florida Osteoporosis Board has developed an osteoporosis risk assessment tool (ORAT) to be used by patients and office staff to help the clinician determine which patients should be sent for DXA scanning.
Methods: The study was designed to validate the ORAT as a screening test for osteoporosis. One thousand seventy postmenopausal females over 45 years old (> 90% Caucasian) undergoing DXA BMD testing at 12 centers in Florida were asked 5 questions from the ORAT (age,height loss, weight, estrogen use, steroid use).
Results: Overall the actual prevalence of osteoporosis in the entire patient population was >34% (any site), 15% (T-hip), 24% (femoral neck) and 22% (LS spine). The ORAT screening tool identified ninety-five percent of the patients who when DXA was preformed did indeed have osteoporosis.

ORAT	Fem Neck (T≤-2.5)	T-hip (T≤-2.5)	LS spine (T≤-2.5)	Any site(T ≤-2.5)
Sensitivity	94%	95%	83%	85%
Specificity	47%	44%	45%	48%
Accuracy	53%	48%	50%	55%

Conclusion: The ORAT, a free and simple risk assessment tool can help increase awareness among patients and encourage the appropriate use of DXA testing in order to diagnose osteoporosis before fractures occur.
Postmenopausal women who present with fractures or other major risk factors should be evaluated with DXA even if their ORAT score does not indicate further testing.
The ORAT is not intended to replace DXA or the practitioner's clinical judgment in determining a patient's diagnoses.

Disclosures: ***J. Valeriano-Marcet****, Procter & Gamble Pharmaceuticals 5, 6.*

M253

Sex Hormone Binding Globulin (SHBG): An Important Predictor of Bone Density In Men and Women. S. Amin, L. J. Melton, S. Achenbach*, A. L. Oberg*, B. L. Riggs, S. Khosla. Mayo Clinic and Foundation, Rochester, MN, USA.

Once considered an inert carrier protein of sex steroids without biologic effects of its own, SHBG has now been shown to effect cellular function directly by acting through a specific membrane receptor (SHBG-R), and this effect may be independent of testosterone or estradiol. Several studies have suggested an association between SHBG and bone mineral density (BMD), but SHBG has also been strongly associated with growth factors and measures of body composition, known to influence bone density. Thus, it is unknown whether SHBG may have direct effects on bone metabolism, independent from sex steroids, or act indirectly through effects on growth factors or body composition.
In an age-stratified sample of the adult community population, we examined the association between serum levels of SHBG and BMD measured at the total hip (DXA). We used regression models to evaluate the associations between SHBG and total hip BMD before and after adjustment for age, bioavailable (non SHBG bound) estradiol (Bio E2) and testosterone (Bio T), insulin-like growth factors (IGF-I, IGF-II) or their binding proteins (IGFBP-2, IGFBP-3) and body mass index (BMI). Analyses were stratified by sex and by menopausal status in women.
We studied 348 men (age range 23-90) and 276 women not on oral contraceptives or hormone replacement (age range 21-93; 166 postmenopausal). In men and women, SHBG levels were positively correlated with age and IGFBP-2, but inversely correlated with other covariates. We present the adjusted Pearson correlation coefficients for the association between SHBG and total hip BMD (see table), representative of results from regression models. Higher SHBG was associated with lower total hip BMD after adjustment for age and sex steroids in men and postmenopausal women, but not premenopausal women. Higher SHBG remained an independent predictor of lower total hip BMD in both men and postmenopausal women after adjustment for growth factors, or their binding proteins, and BMI.

	Pearson Correlation Coefficients Between SHBG and Total Hip BMD		
Covariates in Adjusted Analyses	Men (n=348)	Postmenopausal Women (n=166)	Premenopausal Women (n=110)
(Unadjusted)	-0.39***	-0.50***	-0.30**
Age	-0.26***	-0.46***	-0.28**
Age, Bio E2, BioT	-0.24***	-0.42***	-0.06
Age,BioE2,BioT,IGFBP-2[a]	-0.17**	-0.34***	0.02
Age,BioE2,BioT, IGFBP-2[a], BMI	-0.14*	-0.22**	0.07

*p <0.05; **p <0.01; ***p <0.001 [a] Similar or stronger associations if adjusted for IGF-I, IGF-II or IGFBP-3.

Our data suggest that higher SHBG is a predictor of lower total hip BMD among men and postmenopausal women independent of bioavailable sex steroids, growth factors and body mass. Higher SHBG may be detrimental to bone metabolism when serum estradiol is low as seen in men and postmenopausal women. The biologic role of SHBG on bone metabolism requires further investigation.

Disclosures: ***S. Amin****, None.*

M254

Determinants of Bone Mineral Density are Similar in Older Men and Women. J. A. Cauley[1], K. Stone*[2], R. Fullman*[2], J. M. Zmuda[1], D. C. Bauer*[2], E. Barrett-Connor*[3], K. Ensrud*[4], E. Orwoll*[5]. [1]U of Pittsburgh, Pittsburgh, PA, USA, [2]UCSF, San Francisco, CA, USA, [3]UCSD, San Diego, CA, USA, [4]U of MN, Minneapolis, MN, USA, [5]Oregon Health & Sciences U, Portland, OR, USA.

To determine the anthropometric, historical, medical, and lifestyle factors associated with bone mineral density (BMD) of the spine and proximal femur, we studied 5,995 men, (90% Caucasian) mean age 73.7 ± 5.9 years, enrolled in Mr.OS, a longitudinal cohort study of risk factors for fracture in men. We compared the correlates of BMD with results from the Study of Osteoporotic Fractures (SOF), a study of 9,704 white women mean age, 73.8 ± 5.3 years. All eligible to participate in Mr.OS and SOF were at least 65 years of age or older, ambulatory, and community living. BMD was measured by DXA (Hologic, Inc.). In comparison to Caucasian men, African-American men had 7% (95% Confidence Interval (CI) 5.3, 8.7) higher hip and 5.8% (3.6, 8.0) higher spine BMD even after adjusting for age and body weight. Asian men had 3-5% lower hip and spine BMD than Caucasian men but adjustment for body weight attenuated these differences. The percent difference in femoral neck BMD per unit change (95% CI) in age and weight adjusted models are shown for Mr.OS men and SOF women (Table).

	Unit	Mr.OS Men	SOF Women
Age	5 year	-2.3 (-2.7, -2.0)	-4.3 (-4.7, -4.0)
Weight	10 Kg	4.5 (4.2, 4.7)	5.7 (5.5, 6.0)
Height	10 cm	2.7 (2.3, 3.1)	1.3 (0.7, 1.9)
Fracture History	Yes	-4.6 (-5.6, -3.6)	-5.5 (-6.2, -4.8)
Maternal Fracture History	Yes	-2.6 (-3.6, -1.6)	-2.0 (-2.9, -1.2)
Current Smoke	Yes	-1.7 (-3.5, 0.2)	-1.9 (-3.1, -0.6)
Gait Speed	1 SD (m / sec)	0.3 (-0.1, 0.7)	1.1 (0.8, 1.5)
Thiazide diuretic	Yes	1.0 (-0.2, 2.2)	1.7 (1.0, 2.4)
Grip Strength	1 SD (kg)	0.7 (0.3, 1.2)	1.3 (1.0, 1.7)
Type 2 Diabetes	Yes	3.1 (1.8, 4.3)	3.4 (1.9, 4.8)
Dietary Calcium Intake	1 SD (mg / d)	0.6 (0.2, 1.0)	1.5 (0.5, 2.6)

In conclusion, advancing age was associated with smaller decreases in BMD in men than women. Other correlates of axial BMD were similar in magnitude and direction in older men and women. These factors may identify both men and women with low BMD.

Disclosures: ***J.A. Cauley****, Merck 2, 8; Eli Lilly 2, 8; Pfizer 2; Novartis 2, 5.*

M255

Direct Assessment of Bone Size, Cortical Width, Cortical and Trabecular Bone Density and Biomechanical Indices at the Vertebrae and Hip in Aging Women. B. L. Riggs[1], L. J. Melton[2], R. A. Robb*[3], J. J. Camp*[4], E. J. Atkinson*[5], S. Khosla[1]. [1]Endocrinology, Mayo Clinic, Rochester, MN, USA, [2]Health Sciences Research, Mayo Clinic, Rochester, MN, USA, [3]Physiology, Mayo Clinic, Rochester, MN, USA, [4]Biomedical Imaging, Mayo Clinic, Rochester, MN, USA, [5]Biostatistics, Mayo Clinic, Rochester, MN, USA.

To assess aging effects on skeletal components related to bone strength and fracture risk, we made quantitative computer tomography (QCT) measurements of the lumbar spine (LS) and femoral neck (FN) in an age-stratified, population sample of 75 women (ages 27 to 97 yrs). Data were processed using a powerful new image analysis module within the software program (ANALYZE) that permits 3-dimensional (3-D) reconstruction of the scanned bones. The table gives the change (Δ) in variables over life (age 30 to 80 yr) and the Spearman partial correlation coefficient (r) with age, adjusted for height.

	FN			LS		
Variable	Δ, %	r	P	Δ, %	r	P
Total subperiosteal area	16	.39	<0.001	16	0.36	0.002
Endosteal area	21	0.32	0.005	21	0.38	0.001
Cortical width	3	0.06	0.593	-8	-0.21	0.069
Cortical density	-26	-0.63	<0.001	-33	-0.73	<0.001
Total trabecular density	-56	-0.84	<0.001	-55	-0.80	<0.001
Total volumetric density	-36	-0.80	<0.001	-40	-0.78	<0.001
Section modulus	-15	-0.32	0.005	-62	-0.68	<0.001
Moment of inertia (MOI)	-8	-0.15	0.215	-61	-0.64	<0.001
Index of structural strength (IBS)	-22	-0.20	0.093	-35	-0.45	<0.001

Conclusions: 1) The modified ANALYZE program assesses variables that cannot be determined by standard QCT measurements; 2) with aging, biomechanical indices of bone strength (section modulus, MOI, and IBS) decreased much more at LS than at FN; 3) at both sites, there was more age-related trabecular bone loss than cortical bone loss; 4) surprisingly, we found that cortical width did not decrease with age at either LS or FN. We speculate that this is maintained because with aging endosteal resorption is offset by periosteal apposition as shown by the similar increases in subperiosteal area and endosteal area; 5) despite maintenance of width, the density of cortical bone decreased with aging, presumably because of increased porosity; 6) the effect of age-related decreases in bone density on decreasing bone strength was partially offset by increases in bone area at LS and FN; and 7) in the future, the method for 3-D reconstruction of the LS and FN may allow biomechanical indices of bone strength to replace standard bone mineral density measurements and, thus, to assess fracture risk more accurately.

Disclosures: ***B.L. Riggs****, None.*

M256

Prevalence of Low Bone Density Among Women and Men Self-referred for Coronary Calcium Screening by Electron Beam Computer Tomography (EBCT). E. Barengolts[1], I. Syed*[2], A. Sevrukov*[2], V. Jelnin*[2], J. Hoff*[2], G. Kondos*[2], S. Kukreja[3]. [1]University of Illinois/ VA CHCS, Chicago, IL, USA, [2]University of Illinois, Chicago, IL, USA, [3]VA CHCS, Chicago, IL, USA.

We evaluated prevalence of low BMD among women and men self-referred for coronary calcium screening by EBCT. The EBCT center is located in the middle class community of suburban Chicago with 95% of participants having education more than 12 years. About 10% of patients added bone evaluation to EBCT test. The BMD was measured by 3D QCT. Participants filled in a short health questionnaire. Only people who had both tests were included in this analysis. We designated Normal BMD > 140 mg/cm^3, Low Normal 110-140 mg/cm^3, and Low < 110 mg/cm^3. Majority of 231 postmenopausal women (73.6%) had either Low Normal or Low BMD and were relatively healthy: 14% had arthritis, 25.2% had hypertension, 33.3% had positive calcium score, and 7.4% had diabetes. Comparison of groups did not reveal any statistical differences but for age [Table: Values are Mean ± SD; BMI, body mass index; AgeMeno, age at menopause; HRT, hormonal replacement therapy; * $p < 0.001$ by one-way ANOVA].

Prevalence of Low BMD in overweight (BMI 25-29.9) and obese (BMI ≥ 30) postmenopausal women was 22.6% and 21%, respectively. Among 99 premenopausal women average age was 45 years, BMI was 26.4; 21.2% were smoking, and 41.8% were exercising. Majority (78.8%) had normal BMD and none had Low BMD. Among 127 men average age was 55 years, BMI was 27.7; 25.6% were smoking, and 53.6% were exercising; prevalence of normal, Low Normal and Low BMD was 30.7%, 44.9%, and 24.4%, respectively. We conclude that there is significant prevalence of relatively low BMD in a population self-referred for coronary calcium screening by EBCT, and addition of bone measurement during EBCT may provide valuable information. Follow-up study to validate 3D QCT BMD against fracture outcomes and to determine whether people with low BMD seek intervention leading to fracture prevention is warranted.

Postmenopausal women characteristics:

	Normal BMD	Low Normal BMD	Low BMD
Number	61	107	63
Age, years*	53.7 ± 5.3	57.5 ± 6.8	64.5 ± 8.3
BMI, kg / m^2	26.4 ± 5.0	26.7 ± 5.6	25.8 ± 5.5
AgeMeno, years	47.4 ± 6.3	49.5 ± 4.8	47.6 ± 5.4
Smoking, %	19.7	21.5	17.5
Exercise, %	29.3	43	42.9
HRT use, %	70	68	53.1

Disclosures: ***E. Barengolts****, None.*

M257

The Association of Race/Ethnicity with the Receipt of Bone Mineral Density Measurement in a Population-Based Sample at High Risk for Osteoporosis and Fracture. T. R. Mikuls*[1], K. G. Saag[2]. [1]Department of Medicine, University of Nebraska Medical Center and Omaha VAMC, Omaha, NE, USA, [2]Center for Education and Research on Therapeutics (CERTs), Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA.

Although dual energy x-ray absorptiometry (DXA) measurement has been shown to be a valid and reliable predictor of fracture risk, the association of race/ethnicity with DXA receipt has not been well defined. The purpose of this study was to examine the association of African American and Caucasian race with DXA receipt among community-dwelling older adults at high risk for osteoporosis and fracture.

We conducted a comprehensive Computer-Assisted Telephone Interview (CATI) using a population-based random sample that was drawn from 6 pre-selected Alabama counties: 5 rural and 1 urban. Eligible respondents had self-reported arthritis and were over 50 years of age; 1,424 people responded to the survey. Logistic regression examined the association of race/ethnicity with DXA receipt, adjusting for multiple predisposing, need, and enabling characteristics. To examine predictors of ever DXA receipt among those at highest risk for osteoporosis, we restricted the analysis to older women reporting a history of fracture after the age of 45 years.

Women reporting a prior fracture (n = 251) had a mean age of 68 ± 11 years and were predominantly Caucasian (n = 178, 71%). African Americans in this group (n = 73, 29%) were only 50% as likely (OR = 0.5; 95% CI 0.4-0.6) as Caucasians to have received a DXA after multivariable adjustment. Other factors associated with greater odds of ever DXA receipt included a self-reported physician diagnosis of osteoarthritis (adjusted OR = 2.1; 95% CI 1.6-2.8), a history of kidney disease (1.6; 95% CI 1.0-2.6), urban residence (OR = 1.4; 95% CI 1.0-1.8), existing health care coverage (OR = 2.4; 95% CI 1.1-5.1), and the receipt of arthritis care from a rheumatologist (OR = 2.7; 95% CI 1.9-3.7) or internist (OR = 2.1; 95% CI 1.5-2.8). Socioeconomic status and educational level were not significantly associated with DXA. The association of African American race with DXA was not changed (OR = 0.4; 95% CI 0.3-0.6) when all Caucasian and African American respondents (n = 1,380) were included in the analysis (irrespective of sex or fracture status).

In this population-based study of older adults, African American race/ethnicity was associated with a lower frequency of DXA receipt among those at high risk for osteoporosis and fracture. This association was independent of other important sociodemographic predisposing, need, and enabling factors.

Disclosures: ***T.R. Mikuls****, None.*

M258

Prevalence of Low Bone Mass and Knowledge of Osteoporosis in Residents of Senior Living Facilities. F. M. Gloth[1], T. W. Weiss[2], A. F. Lewis*[3], Y. Chen[2]. [1]Dept. of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]Outcomes Research, Merck & Co., Inc, West Point, PA, USA, [3]Epinomics Research, Inc., Alexandria, VA, USA.

Osteoporosis is prevalent in community-dwelled elderly individuals. Less is known about the prevalence of osteoporosis in institutionalized individuals. The purpose of this study was to describe the distribution of quantitative bone ultrasound (QUS) T-scores among residents of senior living facilities (assisted living and independent living) and to assess their awareness/knowledge of osteoporosis.

A total of 2820 (2418 women and 402 men) age 50 and older residing at 119 facilities of a large national provider of senior living services were included in the analysis. QUS T-score was determined by heel ultrasound (Lunar Achilles Express) performed by an independent vendor who visited each facility during a scheduled 'Bone Health Day' event. Using a validated questionnaire, knowledge of osteoporosis was evaluated in a separate population of

42 residents (33 women and 9 men) at six of the facilities participating in the event.
The mean age of the 2820 participants was 82.0 (SD 10.3) and the mean T-score was -2.0 (SD 1.0). Overall, nearly 80% of the residents had a QUS T-score ≤ –1.0; of those 43% had a T-score ≤ –2.5. Prevalence of low bone mass as estimated by QUS increased with advanced age. Among those 80 years and older, over 85% had a T-score ≤ –1.0 and 53% had T <= -2.5. In general, women had lower T-scores than men, although over half of the men had a T- score ≤ –1.0.
Among the 42 residents participating in the assessment of knowledge of osteoporosis, 71% did not know that osteoporosis is preventable and only one-half correctly identified family history of osteoporosis as a risk factor for osteoporosis. However, 74% correctly identified height loss and post-menopausal status as osteoporosis risk factors. The vast majority of participants (91%) believed that adequate calcium/vitamin D intake and exercise could help slow the rate of bone loss.
Among residents of senior living facilities, there was a high prevalence of low bone mass. While participants were able to identify a number of risk factors associated with osteoporosis, there was a lack of knowledge that osteoporosis is preventable. Efforts to evaluate and educate residents of senior living facilities may help to reduce the future burden of osteoporosis and its complications in this population.

Disclosures: ***F.M. Gloth****, Merck & Co., Inc 5, 8.*

M259

Associations Between Self Assessed State of Health and Postmenopausal Musculoskeletal Health. J. Sirola*[1], T. Rikkonen*[1], H. Kröger[2], R. Honkanen[1], M. Tuppurainen[1], O. Airaksinen*[3], S. Saarikoski*[4]. [1]TULES Research Unit, Kuopio, Finland, [2]Dpt of Surgery, Kuopio University Hospital, Kuopio, Finland, [3]Dpt of Physical Medicine Rehabilitation, Kuopio University Hospital, Kuopio, Finland, [4]Dpt. of Obstetrics and Gynaecology, Kuopio University Hospital, Kuopio, Finland.

Present study investigated the relationship between self assessed state of health and bone density as well as muscle strength.
Grip (GR, kPa) and quadriceps (QA, kg) strength with strain gauge dynamometer as well as DXA absorptiometry measurements for lumbar spine (LS) and femoral neck (FN) were performed on 1016 naturally postmenopausal Finnish women from the OSTPRE study. Self assessed state of health (SAST) was obtained via postal inquiries in five categories: very good, good, moderate, bad, very bad.
There were no differences in LS and FN bone density between SAST categories. In contrast, SAST positively predicted both GR and QA strength in ANOVA (Table) as well as in linear model (p<0.001). Adjustment for anthropometric variables, duration of menopause, use of HRT and other variables did not change these results.
Easily reproducible five categorical SAST is strongly associated with muscle strength in postmenopausal women. Its relationship with fractures and falls remains to be solved.

Mean grip and quadriceps strength according to self assessed state of health

SAST	GR(kPa)	QA(kg)	p (vs. very good)
Very good	76,9	36,9	--
Good	72,0	33,0	NS(GR) / <0.03 (QA)
Moderate	65,7	29,8	<0.001
Bad	61,0	26,5	<0.001
Very bad	61,4	23,6	<0.001

Disclosures: ***J. Sirola****, None.*

M260

Prescreening for Postmenopausal Osteoporosis by Calcaneal Ultrasound, Metacarpal Digital X-ray Radiogrammetry and Phalangeal Radiographic Absorptiometry: A Comparative Study. H. Borghs*, J. Nijs*, F. Luyten, D. Vanderschueren, H. Peeters*, S. Boonen. Center for Metabolic Bone Diseases, Leuven University, Leuven, Belgium.

Identifying women with osteoporosis remains a clinical challenge as it may not be feasible or cost-effective to recommend dual-energy x-ray absorptiometry (DXA) for all postmenopausal women. In this regard, quantitative ultrasound (QUS) has emerged as an attractive screening tool because of the (relatively) low cost.
The objective of this study was to compare the ability of calcaneal QUS to predict osteoporosis with two alternative potential screening methods: digital x-ray radiogrammetry (DXR) and radiographic absorptiometry (RA). We enrolled a total of 221 postmenopausal community-dwelling Caucasian women aged 50-75 years. Bone mineral density (BMD) was measured at the lumbar spine and the total hip regions using DXA. Calcaneal ultrasound attenuation and velocity were assessed using QUS and metacarpal and phalangeal bone density were estimated by the use of DXR and RA, respectively. Receiver operating characteristic (ROC) curves were constructed by calculating the specificity and sensitivity of QUS, DXR, and RA at different cutpoint values in predicting osteoporosis – as defined by a T-score below –2.5 at the spine *or* hip using DXA – and the areas under the curves (AUCs) were computed.
The sensitivity for identifying women with osteoporosis was 67.6% (95% confidence interval [CI], 50.2%-82.0%) using QUS and was 76.9% (95% CI, 60.7%-88.8%) and 82.9% (95% CI, 67.9%-92.8%), respectively, using DXR and RA. The negative predictive value (NPV, the proportion of patients with a negative test who have no osteoporosis) was 90% for QUS, compared to a NPV of 94% for both DXR and RA.
These data suggest that, compared to calcaneal QUS, metacarpal DXR and phalangeal RA can be used more effectively for targeting DXA testing in high-risk postmenopausal women.

Disclosures: ***H. Borghs****, None.*

M261

What Factors Influence Hip Bone Mineral Density (BMD) and Change Over Time in Elderly Female Nursing Home (NH) Residents? K. E. Broe[1], M. T. Hannan[1], D. M. Cheng*[2], D. P. Kiel[1]. [1]Hebrew Rehab Ctr for Aged, Boston, MA, USA, [2]Biostats Dept, BUSPH, Boston, MA, USA.

NH residents have very low BMD and are at high risk for hip fracture, yet little is known about factors associated with hip BMD in these individuals. Our goal was to identify characteristics associated with hip BMD and hip BMD annual percent change in elderly, female NH residents.
110 female long-term care residents (mean age 88 y, range 71-100) had baseline BMD measured between 1992 and 1997 and follow-up BMD measured on average 1.6 years later.
Hip BMD (g/cm^2) was measured at the total hip (TH), femoral neck (FN), and trochanter (TR) using the Hologic, QDR 1000W. BMD change was defined as annualized percent change at each of these sites.
Age, weight, height, cognitive impairment (none/mild/severe), mobility impairment (y/n), falls in the previous year (y/n), and fracture history (y/n) were assessed at baseline and were included simultaneously in multivariate regression models to test associations with BMD and with BMD change. BMD change models also included baseline BMD and weight change (followup - baseline weight).
Age was associated with FN BMD (p=.02). Weight was positively associated at all three hip sites (TH p<.0001, FN p=.0002, TR p=.001). Women in the lowest weight tertile had significantly lower multivariate-adjusted hip BMD than those in the highest weight tertile (i.e. - TH T1 (low) =.550 g/cm^2, T2 =.563 g/cm^2, T3 (high)= .669 g/cm^2, p-trend=.0005). Height was associated only with TR BMD (p=.04). Residents with impaired mobility had lower BMD at the TH (p=.01) and TR (p=.006) as did those with a history of fracture (TH p=.05 TR p=.02). Cognition and falls were not associated with hip BMD.
The table below shows characteristics associated with BMD loss. Additionally, baseline TH BMD was associated with TH BMD loss (p=.05). Age, weight, weight change, height, and fracture history were not associated with hip BMD change. No factors were associated with TR BMD change.
In our population of elderly, female, NH residents low weight, previous fracture, and impaired mobility were associated with low hip BMD; Falling in the previous year, impaired mobility, and cognitive impairment were associated with higher rates of hip BMD loss.
Easily measured characteristics of elderly NH residents can be used to identify those at greatest risk of low BMD and loss of BMD, which may help in targeting therapy in this population.

Multivariate-Adjusted Annualized % Bone Loss

	TH	p-value	FN	p-value
Faller (no)	-2.41	.03	-2.61	.02
Faller (yes)	-3.96		-4.29	
Mob Imp (no)	-1.52	.009	-2.57	.16
Mob Imp (yes)	-4.85		-4.34	
Cog (No)	-2.48	p-trend=.76	-1.62	p-trend=.02
Cog (Mild)	-3.35		-2.98	
Cog (Sev)	-3.72		-5.77	

Disclosures: ***K.E. Broe****, None.*

M262

Weight Change and Forearm Bone Mineral Density in Peri- and Postmenopausal Women - The Health Study of Nord-Trøndelag (HUNT), Norway. S. Forsmo[1], J. Aaen*[2], A. Langhammer*[1], B. Schei*[1]. [1]Dept. of Public Health and General Practice, Norwegian University of Science and Technology, Trondheim, Norway, [2]Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

Bone mineral density (BMD) is strongly associated with body weight. Weight loss has been shown to be a predictor of hip fracture. The relationship between weight change and BMD in non-weight bearing bones, such as the forearm is not clear. The purpose of this study was to investigate the association of weight change over a period of 11-13 years with forearm BMD in peri- and postmenopausal women. In 1984-86 and 1995-97 all citizens in the county of Nord-Trøndelag aged >19 years (about 92,000) were invited to the HUNT study, a multipurpose health survey. The study sample consists of 2749 women born between 1924 and 1941 who met at the first HUNT study and who were invited to BMD measurement at the second HUNT study. A total of 2187 women attended (79.6%) of whom 172 were excluded due to self reported hypo-/hyperthyroidism or other metabolic disorders and eight due to missing weight data, leaving 2007 women eligible for analysis. BMD measurements were performed, at the second HUNT study only, by single X-ray absorptiometry (Osteometer DTX 100) in the non-dominant forearm provided no previous fracture in distal radius. Weight (kg) and height (cm) were measured at both occasions with light clothing and without shoes. Mean age at first and second screening (all were menopausal) was 54.5 and 65.8 years, respectively. There was an overall weight gain of 3.4 kg (95% CI: 3.1; 3.7) during these 11.3 years, inversely related to age at a statistically significant level. A total of 27.1% had lost weight (mean -3.8 kg). About 30% of the women with weight loss were in the age-specific lowest BMD quartile compared to 23.5% of the women with stable or increased weight (p<0.02). In logistic regression adjusted for weight at baseline odds ratio (OR) for BMD in the age-specific lowest quartile was 1.8 (95% CI: 1.3; 2.4) in women with weight loss compared to women having gained >7 kg. In a multivariate linear regression model with weight change, weight at baseline, age, smoking (packyears), age at menopause and estrogen therapy (never, past, current) as independent variables, weight change was a positive and statistically significant predictor of forearm

BMD. A stratified, but similar model of weight loss and weight gain showed a significant association only in the group of weight losers. Replacing weight at baseline with current weight showed no association between weight change and BMD, indicating that body weight at examination explains the relationship.
In conclusion, weight loss in peri- and menopausal women is a predictor of reduced forearm BMD.

Disclosures: ***S. Forsmo****, None.*

M263

Correlation between Carotid Intimal-Medial Thickness and Osteoporosis in Korean Rural Area - Cross-sectional Study. C. Lee*[1], C. H. Kim*[2], D. W. Park*[3], S. B. Park*[4], H. K. Lim*[1], B. Y. Choi*[2]. [1]Department of Internal Medicine, Hanyang University, Seoul, Republic of Korea, [2]Department of Preventive Medicine, Hanyang University, Seoul, Republic of Korea, [3]Department of Radiology, Hanyang University, Seoul, Republic of Korea, [4]Department of Rehabilitation Medicine, Hanyang University, Seoul, Republic of Korea.

It is well known that estrogen deficiency is a common risk factor for osteoporosis and coronary artery disease(CAD) and the women with osteoporosis may have a higher risk of developing coronary atherosclerosis. And a measurement of intimal-medial thickness(IMT) of common carotid artery is a relatively economical noninvasive technique in detection of coronary-vascular disease. Based on these observations, the hypothesis of this study is that the thickening of carotid IMT must be greatest in those individuals with the greatest amount of bone loss in same age group. Aim of this study is to find out the association between change of carotid artery, reflecting CAD and osteoporosis. Subjects of this study were recruited from Yangpyung Hypertension Program (YHP), which is prospective cohort study of cardiovascular disease in Yangpyung area. Random sample of 127 residences in YHP were invited to participate in this study. We measured blood pressure two times on both side in 10 min interval, IMT of common carotid artery two times by Ultrasound Sonograph (128BW, Medison, Korea), and bone mineral density by ultrasound bone densitometer (Mark 2000, Medison, Korea). Table shows IMT and T-score in three age group in both sex. The change of IMT and T-score in three group were compared by one-way ANOVA, and the relationship controlling age between IMT and T score in all both sex populations were determined by partial correlation analysis. Controlling ages, there was a positive correlation beteen IMT and T score in female subjects (r = 0.26, p=0.026) and not in male subjects (r = -0.04, p=0.75). In conclusion, there was relationship between change of carotid artery and osteoporosis. But, to complete the relationship between two pathologic processes, large scale prospective study will be needed.

IMT and T score in age groups

Age (n)	IMT(mm)		T-score	
	Male	Female	Male	Female
~ 45 (31)	0.49 ± 0.11	0.37 ± 0.04	- 1.4 ± 0.7	- 1.3 ± 0.6
46 ~ 60 (42)	0.53 ± 0.15	0.51 ± 0.10	- 2.2 ± 1.3	- 1.6 ± 0.9
60 ~ (39)	0.57 ± 0.11	0.53 ± 0.10	- 2.2 ± 0.9	- 2.2 ± 0.7
	NS	p <0.05	NS	p <0.05

Disclosures: ***C. Lee****, None.*

M264

The Utility of Dual X-ray Absorptiometry in Determining Frailty in the Elderly: How Does it Relate to a Continuous Summary Physical Performance Score (CSPS) in the Elderly? J. W. Nieves[1], E. Vasquez*[1], M. Zion*[1], M. Pahor*[2], R. Williams*[3], T. Li*[3], J. Park*[3], P. Lapuerta*[3]. [1]Clinical Research Center, Helen Hayes Hospital, West Haverstraw, NY, USA, [2]Sticht Center on Aging, Wake Forest University, Winston Salem, NC, USA, [3]Bristol Myers Squibb, Princeton, NJ, USA.

Frailty is clearly a risk factor for falls and fractures in the elderly. While dual x-ray absorptiometry (DXA) is accepted as a useful measure of skeletal fracture risk, it is unknown whether DXA would also be useful for measuring frailty. Physical performance tests in lower extremity functions including 4 meter walk, repeated chair stands, and balance have been shown to predict mortality and risk of nursing home admission, and are used to measure functional status in the elderly. Using these tests, a Continuous Summary Performance Score (CSPPS) has been developed which was sensitive to change in function in an analysis of data from the Women's Health and Aging Study. The goal of the current study is to determine whether total body and regional lean mass and fat mass as determined by DXA are related to these functional measures of frailty. 76 subjects (mean age 76 years, 84% female) who reported at least 2 of 4 functional domains of disability participated in this study. Physical function and general health self-assessments were collected by the SF-36 and Quality of Life questionnaires. Grip strength was measured with a Jamar Dynamometer. Body composition was assessed by DXA (Lunar n=24 and Hologic n=48). Total lean and fat mass and subdivisions of appendicular and trunk lean and fat mass were calculated. To calculate the CSPPS the timed scores of the performance tests (4 m walk, chair stands and balance test) were rescaled to values ranging from 0 (worst performance) to 1 (best performance) and added. Averaging the scores from these 3 tests and multiplying by 100 results in the CSPPS (range from 0 to 100). The partial correlations (controlling for height and age) between CSPPS and %lean mass (r=0.31; p<0.02) and % fat mass (r=-0.32; p<0.02) for the total body were similar to the partial correlations between CSPPS and trunk or appendicular lean or fat mass. When the individual components of the CSPPS were examined in relation to DXA results, the correlations of %lean mass with walking speed and balance score were significant (r>0.27; p<0.03). Grip strength also correlated with CSPPS (r=0.40; p=0.002). DXA of the skeleton remains the gold standard for evaluating hip fracture risk. Frailty an important measure of hip fracture risk may also be measured by DXA body composition or grip strength, although the correlations are modest. The utility of the CSPPS, DXA body composition and grip strength in predicting fracture risk could best be evaluated in a prospective study.

Disclosures: ***J.W. Nieves****, Bristol Myers Squibb 2.*

M265

Osteoporosis in American Indian Males Attending Arthritis Clinics. J. R. Lisse, D. J. Power*, I. Villanueva*, B. S. Botzong*, K. J. Lampert*, J. O. Posever*, D. E. Yocum*. Internal Medicine, University of Arizona, Tucson, AZ, USA.

Introduction: There is less information on osteoporosis in males than in females. Even less is available on male American Indians (AI). No articles to date have been published on this population. We present data here on the largest group of AI males studied.
Subjects: Patients were screened for osteoporosis as part of their clinical care in arthritis clinics for AI run by the Arizona Arthritis Center. A total of 71 men were studied, ranging in age from 17-91 years. The mean age of the population was 48.3 years. These patients had accompanying arthritis, including but not limited to rheumatoid arthritis, osteoarthritis, and seronegative spondylarthropathies.
Methods: Patients were evaluated by dual x-ray absorptiometry (DXA) using a Hologic 4500W, and an Achilles express heel ultrasound unit (HUS) using the same certified technician. 60 patients underwent ultrasound evaluation. 43 had DXA evaluation performed. Statistical analysis was performed using SPSS.
Results: Using DXA, 5/43 (11.6%) men were osteopenic at the hip, 5/20 (25%) over age 46 years. 1/43 (2%), age 85 was osteoporotic. At the femoral neck 11/43 (26%) had osteopenia, 10/20 (50%) over age 46. The same 85 y/o was osteoporotic. Osteopenia in the spine was present in 10/43 (23%), 4/20 (20%) over age 46. Osteoporosis was present in 2/43 (4%), 2/20 (10%) over age 46. Using HUS, osteopenia was present in 20/60 (33%), 7/25 (28%) over age 46, and osteoporosis in 15/60 (25%), 13/25 (52%) over age 46.
Conclusions: Low bone densities do occur in AI men. The magnitude of this varies, depending on site measured and method. It appears that HUS in this population yields lower bone densities than DXA, and will classify more AI men as osteopenic or porotic. DXA should be used to confirm the diagnosis in all AI men with low bone mass indicated by HUS.

Disclosures: ***J.R. Lisse****, None.*

M266

Osteoporosis in Obese African American Women. J. Keith*. Medicine/GI/Nutrition, University of Chicago, Chicago, IL, USA.

The prevalence of osteoporosis in minorities remains widely debated. Obesity and African-American race are thought to be protective against the disease. We report osteopenia and osteoporosis in 4 of 41 obese African American women participating in a medical weight management program. These postmenopausal women were incidentally noted to have abnormal bone mineral density studies during evaluation for participation in the medical weight management program during a three-month period. All four women are postmenopausal with ages greater than 50 years (54-74 years), and have evidence of bone mineral loss on Dual X-ray Absorptiometry (DEXA) (GE/Lunar Prodigy, Madison, WI) using WHO (World Health Organization) criteria.

	Spine BMD	S.D.	Neck BMD	S.D.
Patient 1	0.917	-2.40	0.893	-0.70
Patient 2	1.321	1.00	0.793	-1.60
Patient 3	0.824	-3.10	0.707	-2.30
Patient 4	1.044	-1.30	0.685	-2.50

Silverman[1] et al published epidemiological data in 1986 that first recognized osteoporosis-related fractures in minority women. In the NORA study [2], nearly 40% of post-menopausal African American women screened had low bone density and approximately 4% of the women studied already had unsuspected osteoporosis. Neither the African American individuals in the NORA study nor the four women described above had evidence of the traditional risk factors and would not have been screened for osteoporosis. None of the women described were on medications that would negatively influence bone health. In most studies, obesity is associated with a lower incidence of abnormal bone mineral density. In contrast, Blaauw et al [3] found an increased risk of osteoporosis related to increased central body fat. Increased central body fat is now associated with low dietary calcium intake in studies by Zemel et al [4]. Emerging data[5] links obesity to inadequate dietary calcium from calcium-rich dairy products leading to suboptimal levels of calcium thereby increasing the risk of osteoporosis. These data suggests that osteoporosis is prevalent in African American women over the age of 50 years and that obesity may not be protective. Additional studies are recommended.

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Disclosures: ***J. Keith****, Dairy Management Incorporated 2; International Dairy Foods, Inc 5, 8; Wyeth Pharmaceuticals 8.*

M267

The Osteoporosis Self-Assessment Tool (OST) Performs Differently in Younger Versus Older Postmenopausal Women. E. A. Mossman*[1], M. Luckey[2], M. R. McClung[1]. [1]Oregon Osteoporosis Center, Portland, OR, USA, [2]Saint Barnabas Osteoporosis and Metabolic Bone Disease Center, Livingston, NJ, USA.

The OST is a promising and elegantly simple method for predicting low bone density, requiring only age and weight to calculate. Validation of the OST is ongoing in a variety of populations, and it has proven to perform well against other methods of predicting low bone density by DXA. Various guidelines recommend routine bone density testing for all women 65 years of age and older. It is also generally accepted that BMD testing is appropriate for postmenopausal women with fragility fractures or with medical problems associated with bone loss. A screening tool such as OST would therefore be most useful in women who do not meet these criteria for BMD testing. This study was designed to evaluate the performance of the OST in this population.

We examined the electronic medical records of postmenopausal women referred to our clinic for DXA testing from January 2000 through February 2002 who had record of a hip or spine DXA measurement and a medical history questionnaire. Patients were excluded for secondary causes of osteoporosis such as oral steroid or anti-convulsant use, nutritional or digestive disorders, and conditions such as hyperparathyroidism and rheumatoid arthritis. Patients reporting a low-trauma fracture since age 45 were also excluded. OST was calculated as 0.2 x (weight[kg]-age), truncated to yield an integer. Performance of the OST in predicting T-scores ≤-2.5 at the femoral neck, spine, and at either site by DXA was then examined in patients aged less than 65 years (n=3969), those 65 or older (n=2621), and in the entire group. Sensitivity, specificity, and area under the ROC curve (AUROC) were calculated.

23% of the ≥65 group and 9% of those <65 had osteoporosis at either the spine or hip. While the AUROC was similar for both age groups (0.697 in <65, 0.696 in ≥65 for OP at either site), cutoff-dependent measures such as sensitivity and specificity varied significantly between the two age groups at every threshold. At an OST cutoff of ≤0 in those aged 65 and over, sensitivity at the hip and spine was 94% and 90% respectively, with specificity of 28% for both. Using this same threshold for those under age 65, however, yielded a sensitivity of 62% at the hip and 57% at the spine (specificity 70% for both). Using a higher threshold of ≤3 resulted in a sensitivity of 93% at the hip and 91% at the spine in those under 65 (specificity 31%, 32%), while in those 65 and older sensitivity was 98% at the hip and 97% at the spine using this cutoff (specificity 9%, 10%).

Conclusion: OST can be used effectively as a screening tool for postmenopausal osteoporosis in women under 65 years old, but a higher cutoff is required to maintain adequate sensitivity.

Disclosures: ***E.A. Mossman****, None.*

M268

Patients Staying >3 Days in Hospital May Warrant Evaluation and Treatment to Prevent Fractures. S. R. Cummings[1], F. Harris[1], J. A. Cauley[2], D. Black[1], S. Kritchevsky*[3], E. Simonsick*[4], M. Nevitt*[1], T. Harris*[4]. [1]SF Coordinating Center, San Francisco, CA, USA, [2]Univ of Pittsburgh, Pittsburgh, PA, USA, [3]Univ of Tennessee, Memphis, TN, USA, [4]National Institute on Aging, Bethesda, MD, USA.

Long (> 3 day) hospital stays may indicate frailty and high risk of fractures. Such stays may also be convenient times to start treatments to prevent fractures,

We tested the hypothesis that patients staying > 3 days in hospital (excluding admissions for fractures) have an increased risk of fracture. 3075 white and black men and women age 70 to 79 from two communities reported days of hospital stay during the first year of study. During 3.6 years of 97% complete follow-up, we validated 173 post-discharge fractures, including 91 'frailty' (hip, pelvis, humerus, and spine) and 33 hip fractures. Analyses were adjusted for age, gender and race.

Compared to those not hospitalized, those staying > 3 days had a 4.4-fold (P<.001) risk of hip fracture, 2.1-fold (1.2 to 3.6) risk of frailty fracture and1.6-fold (1.04 to 2.5) risk of any fracture.

Therefore, older patients staying > 3 days in hospital have a high risk of hip and other fractures. After 3 days in hospital, all older patients should be assessed and perhaps treated to prevent fractures. This approach could reach many high-risk patients who are currently not treated for prevention of fractures.

Disclosures: ***S.R. Cummings****, None.*

M269

When Is an Ankle Fracture an Osteoporotic Fracture? J. R. Center, T. V. Nguyen, K. P. Chang*, J. A. Eisman. Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, Australia.

Ankle fracture is not considered an osteoporotic fracture. However, a history of a low trauma fracture is a major risk factor for a subsequent fracture. The aim of this study was to examine whether a history of ankle fracture was a risk factor for future fracture.

Data from 821 men and 1286 women aged 60+ years, who were participants of the Dubbo Osteoporosis Epidemiology Study were analysed. During 13 years of follow-up (April 1989 to May 2002), 29 women and 15 men had sustained an ankle fracture and 431 women and 152 men had sustained a non-ankle fracture.

	No fracture	Non-ankle fracture	Ankle fracture
Women			
Age (yrs)	70 ± 6	73 ± 8b	74 ± 7b
BMD (g / cm^2)	0.81 ± 0.12	0.71 ± 0.13b	0.77 ± 0.09b,c
RR[a] (95% CI)	1	2.9 (2.4, 3.5)	0.9 (0.3, 2.5)
Men			
Age (yrs)	70 ± 6	73 ± 7b	70 ± 6
BMD (g / cm^2)	0.93 ±0.15	0.83 ± 0.16b	0.95 ± 0.17c
RR (95% CI)	1	3.6 (2.4, 5.2)	6.3 (2.94, 13.5)

[a]Relative risk for subsequent fracture; [b]p<0.05 for comparison with no fracture; [c]p<0.05 for comparison with non-ankle fracture.

Thus women and men with ankle fractures behaved differently. Analysis of the type of fracture classified as "ankle" may shed some light on these differences. In women, ankle fractures were not a risk for future fractures, yet women with ankle fractures were older and had lower BMD than those without fracture. Although the men resembled those who had not sustained a fracture, an ankle fracture was a risk for future fracture.

Disclosures: ***J.R. Center****, None.*

M270

Hypertension Increases the Risk of Perimenopausal Fractures. A Prospective Population Based Study. J. A. Huopio*[1], R. Honkanen*[2], S. Saarikoski*[3], E. Alhava*[4], H. Kröger[1]. [1]Department of Surgery, Kuopio University Hospital, Kuopio, Finland, [2]Research Institute of Public Health, University of Kuopio, Kuopio, Finland, [3]Department of Obstetrics and Gynaecology, Kuopio University Hospital, Kuopio, Finland, [4]Department of Surgery, University of Kuopio, Kuopio, Finland.

There are no studies which have examined the risk of common chronic health disorders for perimenopausal fractures. Our aim was to evaluate the effect of common health disorders on fracture rate in a prospective cohort study. 3078 women were randomly chosen from the population base which consisted of 14 220 women aged between 47 - 56 years residing in Kuopio Province, Eastern Finland, in 1989. All study subjects were given a list of chronic diseases where they were advised to mark the diseases they had at the time of baseline. Self-reported chronic disorders were validated, when possible, by comparing questionnaire information with statistics provided by the Social Insurance Institution of Finland. These statistics include code number information of diseases for patients whose medicine expenses for the disease are reimbursed by the state. Thus, we could validate information of self-reported hypertension, coronary artery disease and cardiac insufficiency. The first fracture during the follow-up period was the end point event. Follow-up fractures were validated from medical records. Relative risks were estimated as proportional hazards using the Cox's proportional hazards model. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated. There were 265 women who experienced at least one fracture during the follow-up period of 3.6 years (SD ±0.78). Hypertension (n=563) per se increased the risk of fracture by 40% (HR 1.4, 95% CI 1.0-1.9) in Cox's analyses. Moreover, hypertension together with coronary artery disease (n=39) increased the risk over two-fold (HR 2.2, 95%CI 1.0-4.6). Also, hypertension together with cardiac insufficiency (n=17) increased the risk even 5-fold (HR 5.0, 95% CI 1.9-13.4). However, bone mineral density (BMD) was also an independent predictor of fracture both in healthy and non-healthy groups. Among hypertension patients BMD in fractured women was significantly (p=0.002) lower as compared to those hypertension patients with no fracture. We conclude that perimenopausal women with hypertension have increased risk for fractures.

Disclosures: ***J.A. Huopio****, None.*

M271

Should Women with Osteoporosis without Fracture Be Treated? Geelong Osteoporosis Study. K. M. Sanders*[1], E. Seeman[2], M. A. Kotowicz[1], J. A. Pasco*[1], M. J. Henry*[1], G. C. Nicholson[1]. [1]Clinical and Biomedical Sciences: Barwon Health, The University of Melbourne, Geelong, Australia, [2]Medicine, ARMC, The University of Melbourne, Melbourne, Australia.

This analysis estimates the number of women needed to treat (NNT) to prevent one fracture (Fx) using antiresorptive drugs in all women with osteoporosis (OP) or a previous fracture (incurred after 50 years of age) compared with treating women with both osteoporosis and previous fracture. The proportion of women with osteoporosis (T score <-2.5) and fracture after 50 years of age was estimated from a random sample of the Barwon region population (n=1,151). The prevalence of these risk factors among the fracture group was estimated from 692 women, representing 77% of all eligible women with incident fracture. The median time between fracture and BMD measurement was 57 days. The prevalence of osteoporosis and previous fracture in the population was used to estimate the number in the population receiving treatment. Antiresorptive therapy was assumed to reduce fractures by 50% and the treatment effect was estimated using the age- and sex-specific fracture incidence for the region among those with osteoporosis and/or previous fracture. Among women aged 35 to 49 years, 1.5% of the population had osteoporosis whereas 6% of all fractures occurred in those with osteoporosis resulting in an NNT to prevent one fracture of 167. By contrast, a majority of the population, aged 70 years and over had osteoporosis (43%) and a third of all fractures occurred in women with both osteoporosis and previous fracture (32%, n=103/317).

Number Needed To Treat

Age (yrs)	50-59	60-69	70-79	80+
n=pop vs Fx	*206 vs 108*	*203 vs 162*	*205 vs 219*	*203 vs 98*
OP	91	76	48	28
Previous Fx	139	115	55	27
OP and Fx	36	68	34	27

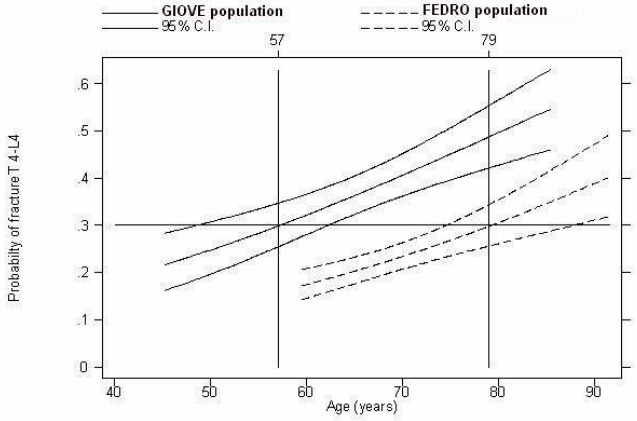
In young women with osteoporosis alone, many must be treated to avert one event because of the low absolute risk of fracture. In older women, drug therapy is worthwhile in those with either osteoporosis or previous fracture, as well as those with both osteoporosis and previous fracture, because of their higher absolute fracture risk.

Disclosures: ***M.A. Kotowicz****, None.*

M272

Age-related Prevalence of Vertebral Deformities in Postmenopausal Women Treated or Untreated with Glucocorticoids: A Comparison Between Two Italian National Studies. A. Angeli[1], G. Capelli*[2], D. de Feo*[3], A. Giustina*[4], G. Guglielmi[5], R. Nuti[6]. [1]Internal Medicine, University of Torino, Torino, Italy, [2]Public Health, University of Cassino, Frosinone, Italy, [3]Procter & Gamble, Rome, Italy, [4]Internal Medicine, University of Brescia, Brescia, Italy, [5]Radiology, Scientific Institute Hospital "CSS", Foggia, Italy, [6]Internal Medicine, University of Siena, Siena, Italy.

The prevalence of vertebral fractures (VFs) in postmenopausal women is thought to be underestimated, mostly due to the limited focus of clinicians and radiologists in recognizing vertebral deformities. Exposure to glucocorticoids (GCs) should significantly increase such prevalence. GIOVE (Glucocorticoid-Induced Osteoporosis Vertebral Evaluation) is a national study aimed at measuring the prevalence of VFs in a sample of postmenopausal Italian women treated with systemic GCs for at least 6 months. FEDRO (Fracture Evaluation by Digital Radiography Observational study) is a national study aimed at measuring the prevalence of VFs in an Italian postmenopausal women population. The data quality revision has already been completed in 494 women (aged +45 yrs) recruited in the GIOVE study and in 885 women (aged +60 yrs) recruited in the FEDRO study. These populations were analyzed by multivariate logistic regression to predict the probability of at least one VF in T4-L4 (vertebral deformity over 20%) by age and participated study. The graph below shows the logistic regression model predictions for fracture prevalence by age and study, with 95% confidence bands. The probability of at least one VF seems to be simply translated in time between the two studies. At a referral point of 30% probability, a 57 year old woman exposed to GCs corresponds to a 79 year old osteoporotic woman without current or prior exposure to GCs. The parallelism between the two populations indicates that GCs therapy and age are not synergistic but additive risk factors, at least for the first fracture. These results confirm the importance of GCs therapy as an independent risk factor for VFs. They also strongly support the wide scale application of strategies for the prevention or early therapy for glucocorticoid-induced osteoporosis.



Disclosures: ***A. Angeli****, None.*

M273

Estrogen-Related Fracture Risk Reduction Is Lost Rapidly after Treatment Withdrawal. J. Yates[1], E. Barrett-Connor*[1], E. Siris*[1], P. Miller*[1], Y. T. Chen*[1], S. Barlas*[2], P. Miller*[1]. [1]US Medical and Scientific Affairs, Merck & Co., Inc., Upper Gwynedd, PA, USA, [2]Outcomes Research & Management, Merck & Co., Inc., West Point, PA, USA.

Estrogen (+/- progestin) use initially increases, and then maintains, bone mass. Data from WHI confirms that continued use of estrogen reduces the incidence of fractures, including those of the hip. However, discontinuation of estrogen use results in rapid bone loss, and it thus seems likely that the anti-fracture efficacy of estrogen might also dissipate following treatment withdrawal. To study this question we evaluated the associations of recency and duration of estrogen use with fracture incidence in postmenopausal women. 140,584 NORA participants had peripheral BMD measurements, self-reported information on estrogen use at baseline and follow-up information on fracture approximately 12 months later. Women were categorized according to estrogen recency (current, quit ≤ 5 years, quit > 5 years) and duration (≤ 5 years, 6-10 years, 10+ years). Odds of osteoporotic fracture (hip, spine, forearm, wrist, or rib) and hip fracture were computed adjusting for important risk factors. 61.8% had ever used estrogen and 48.4% were current users. 2031 women reported ≥1 new osteoporotic fracture, of whom 269 had a hip fracture. Duration of use (current or prior) was not related to fracture incidence. The relationship of recency and fracture is shown in the Table. The only benefit was observed in current users. Fracture risk in past users, regardless of recency, was similar to those who never used estrogen. This study suggests that anti-fracture efficacy associated with current estrogen use may be lost soon after cessation. Such patients should be evaluated for fracture risk and if considered to be at increased risk for fracture, should receive an alternative treatment for prevention of bone loss and fracture risk reduction.

Estrogen usage	Total N	**Osteoporotic fractures**		**Hip Fractures**	
		N	**OR (95% CI)***	**N**	**OR (95% CI)***
Never Used	53,737	947	Reference	149	Reference
Current	67,973	705	0.74 (0.67, 0.83)	66	0.66 (0.49, 0.89)
Quit ≤ 5 years	8,723	127	0.99 (0.83, 1.20)	23	1.69 (1.08, 2.66)
Quit > 5 years	10,151	225	1.13 (0.97, 1.30)	31	0.98 (0.66,1.44)

* Adjusted for age, prior fracture, health status, maternal history of fracture, and cortisone use.

Disclosures: ***J. Yates****, Merck & Co., Inc 3.*

M274

Fracture Occurrence in Women with Lupus. A. B. Chadha[1], E. Shamiyeh*[1], S. Manzi*[2], S. Spies*[1], R. Ramsey-Goldman[1]. [1]Feinberg School of Medicine, Chicago, IL, USA, [2]University of Ptiisburgh, Pittsburgh, PA, USA.

Women with Lupus are at risk for low bone mineral density (BMD) and fracture (fx). The primary aim of this study in 300 women with lupus (225 Caucasian [C] and 75 African-American [AA]) was to assess the relationship between BMD and fx occurring after lupus diagnosis. BMD was measured at the hip, spine, and distal forearm by dual x-ray absorptiometry (DXA). All women completed a self-administered risk factor survey including fx history. Univariate analyses were done on all risk factor variables and BMD scores. Fisher's exact test was used to compare variables in lupus women with and without fx. Thirty-eight lupus (12.7%) women developed a fx after SLE diagnosis (8/75 [10.7%] AA vs. 30/225 [13.3%] C, ns). The different sites of fx were as follows: 7 spine, 7 ankle, 5 wrist, 5 ribs, 3 arm, 3 foot, 3 leg, 3 other, 1 heel, and 1 pelvis. The mean age at first fx was 41.6 years (SD 14.1). Of the 38 fxs, 3 occurred in women with SLE for less than 5 years and 35 in women with SLE for greater than 5 years (<0.0001). When comparing lupus women with and without fxs, these variables differed significantly: age at visit (48.8 vs. 40.7 years, p= <0.001), disease duration (16.5 vs. 8.3 years, p= <0.0001), and BMD raw scores at the hip (0.823 g/cm^2 vs. 0.908 g/cm^2, p= 0.006) and wrist (0.606 g/cm^2 vs. 0.682 g/cm^2, p= <0.0001). BMD at the spine was lower in women with fx vs. women who did not fx after lupus diagnosis and approached significance (0.943 g/cm^2 vs. 0.989 g/cm^2. P= 0.057). Developing a fx after lupus diagnosis was associated with longer disease duration, older age at baseline visit, and lower BMD at the wrist, hip, and spine. Both C and AA women appear to be at risk for fxs suggesting race may not protect against fracture occurrence. Measures to prevent osteoporosis need to be implemented early in all lupus patients.

Disclosures: ***A.B. Chadha****, NIH/NIAMS 2; Arthritis Foundation Clinical Science Grant and Greater Chicago Chapter 2; Proctor and Gamble 2, 5; Merck 2, 5, 8S.*

M275

Predictors of Incident Vertebral Fractures in Men and Women. G. R. Haynatzki, M. R. Stegman*, K. M. Davies, J. M. Lappe, D. Travers-Gustafson*, R. P. Heaney, R. R. Recker. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

A rural population sample, 50+ years of age, was randomly selected for a prospective observational study (The Saunders County Bone Quality Study), consisting of 899 women and 529 men, followed for an average of 3.66 and 3.62 years, respectively. At each visit, they were measured by QUS at the patella and by SPA at distal radius and ulna, and completed a questionnaire on current and past medical history, medication use, dietary calcium intake, alcohol and caffeine consumption, cigarette smoking, fall and fracture history. Spine radiographs were obtained at the first and last planned study visits. Here we report the most current results from the popular "relative and absolute change" method for vertebral fracture, which declares a vertebral body fractured if its height has decreased by at least 20% and by at least 4 mm, between radiograph films. We used logistic and Poisson regressions to assess the significance of the effect of different predictors and their first order interactions on who fractures and, respectively, how many fractures are experienced. The table below shows the number of fractured and, more importantly, the number of fractures per 100 person-years. The analyses revealed that sex was not a significant predictor for either number (also seen from the table) while age, smoking, QUS, radius BMD, ulna BMD, years of diabetes, years of thyroid problems, and arthritis were very significant predictors of one or both dependent variables of interest, at level of significance 0.05. The statistical package SAS was used in all analyses.

VERTEBRAL FRACTURE

	# Fractured	Fxs / 100 person-yrs
Women	97	3.577
Men	51	3.352
Total	148	3.496

Disclosures: ***G.R. Haynatzki****, None.*

M276

Recognizing Osteoporosis and its Consequences in Québec (ROCQ): A Patient Health Management Programme. J. P. Brown[1], L. Bessette*[1], M. Beaulieu*[2], C. Chin*[3], S. Jean*[1], L. Ste-Marie[4]. [1]Laval University, Ste-Foy, PQ, Canada, [2]Merck Frosst Canada, Montreal, PQ, Canada, [3]Aventis Pharma, Montreal, PQ, Canada, [4]University of Montreal, Montreal, PQ, Canada.

In Canada, approximately 20–25% of women presenting with fragility fractures are subsequently investigated for osteoporosis, and only half of these receive treatment.
ROCQ's objective is to improve the use of evidence-based diagnostic and treatment for women 50 years and older, who have suffered a fragility fracture.
The interventions are expected to: 1) double the number of patients investigated for osteoporosis; 2) initiate pharmacological treatment in 70% of osteoporosis-diagnosed women; and 3) reduce recurrent fragility fractures incidence by 12.5%, if applied on a long-term basis.This programme is a prospective cohort study using a randomized control design composed of three phases. At phase 1, subjects will provide informed consent as well as information to classify their fracture as fragility or traumatic fracture. At phase 2 (6-8 months after Phase 1), women with fragility and traumatic fractures will be interviewed by telephone by a non-health professional interviewer to complete two questionnaires. One is collecting data regarding demographics, risk factors for osteoporosis, diagnosis, treatment, and medical history. The other collects subjects' health utility. The questionnaires will allow identification of baseline gaps regarding diagnosis and treatment. Randomization of women with fragility fracture to one of the following 3 intervention groups will occur one week following the assessment: 1) receive a 2-hour learning programme held by their local community health centre, and additional written educational materials directed at patients and at their respective family physician; 2) only receive written educational material by mail or, 3) receive no specific intervention other than the interest raised by the questionnaires. Group assignment will be sent by mail, and all educational materials will be based on the 2002 Clinical Practice Guidelines for the Diagnosis and Management of Osteoporosis in Canada. At phase 3, the same two questionnaires will be administered once again 6-8 months after randomization. Both questionnaires will be compared between phase 2 and 3 and differences between responses will measure the effectiveness of ROCQ's interventions in improving diagnosis and treatment of osteoporosis. Patients will be followed for 20 years using the RAMQ database. The results of a pilot phase during the first 18 months of the Programme will determine the best recruitment strategy to use and will also assess the feasibility of ROCQ's interventions.

Disclosures: ***J.P. Brown****, None.*

M277

Happiness is Associated with Lower Fracture Risk in Older Men and Women: MacArthur Studies of Succesful Aging. A. S. Karlamangla[1], G. A. Greendale[1], B. H. Singer*[2], T. E. Seeman*[1]. [1]Division of Geriatrics, UCLA, Los Angeles, CA, USA, [2]Office of Population Research, Princeton University, Princeton, NJ, USA.

The World Health Organization defines health as a state of complete physical, mental, and social well-being. Well-being and thus health, requires more than just the absence of illness in these spheres. While several studies have demonstrated the negative effects of mental illnesses such as depression on physical health outcomes, including low bone density, few have studied the protective effects of positive mental well-being. In this study, we examined the association between happiness and the risk of incident fractures in a cohort of 885 community-dwelling men and women, 70-79 years of age, who were screened to be in the top one-third with respect to physical and cognitive functioning status, and had no history of previous fractures. We measured happiness at baseline (1988), using a 5-question survey, adapted from work at the University of Michigan's Survey Research Center. Fracture status (and date of fracture) was determined from self-reports obtained at follow up in 1991 and 1995, for 86% (N=763) of the cohort. 22 of the 349 men and 49 of the 414 women reported fractures over the 7 years of follow up. We used proportional hazards modeling to study the association between happiness at baseline and incident fracture risk. Participants in the highest quartile of the happiness measure had lower risk of incident fracture. Compared to individuals in the other 3 quartiles, their hazard ratio for new fractures was 0.41 (95% CI 0.22-0.78, p value 0.007). After adjusting for age, ethnicity, body height, body weight, and body mass index, and stratifying by gender, the hazard ratios were 0.20 in men (95% CI 0.05-0.85) and 0.48 in women (95% CI 0.23 - 1.00). Further adjustment for history of previous strokes and history of previous heart attacks did not substantially alter the primary associations. Our results suggest that happiness, not just absence of depression, can confer protection from physical ill-health. It is unlikely that these findings are confounded by pre-existing physical co-morbidities, since this cohort was chosen to be high-functioning at baseline. Further research is needed to study the effects of positive mental health on bone mineral density and to investigate the physiological mediators of these associations.

Disclosures: ***A.S. Karlamangla****, None.*

M278

Prediction of Fracture Risk using the Osteoporosis Self-Assessment Tool (OST). S. L. Johnson*, V. I. Petkov*, M. I. Williams*, P. Via*, R. A. Adler. Endocrinology, McGuire Veterans Affairs Medical Center, Richmond, VA, USA.

OST has been developed as an osteoporosis (OP) screening tool. It utilizes only two risk factors for osteoporosis, as calculated by the formula: {[weight (kg)-age]*0.2}, truncated to an integer. In women and men, OST is a very good predictor of bone mineral density (BMD) measured by central DXA. The objective of this cross-sectional study was to determine if OST can predict fractures in patients attending an Orthopedic Clinic.
We reviewed electronic medical records of patients who attended a weekly orthopedic clinic at a Veterans Affair Medical Center during a 9-month period. One hundred and ninety patients with fractures (recent and old fractures) were identified. Orthopedic clinic patients without fractures were randomly selected (N=230) from 2019 patients seen during the study period. Age and weight 1 year prior to clinic visit and fracture status at the time of the visit were recorded (fracture location, traumatic or minimal trauma, age of fracture occurrence, recent or old). The operating characteristics of OST were examined at various cut-offs of the index. Odds ratios (OR, crude and adjusted for race) were calculated. Logistic regression was used to estimate the fracture probability. Women were excluded because of small representation (5.5%).
The final data set consisted of 397 men (45.6% with fractures; 56.7% white). The mean (SD) age, weight, and OST index were: 59 (13.6) years, 94 (20.8) kg, and 6.6 (5.0) respectively. At a cut-off of 3 (which provides good sensitivity and specificity for predicting DXA in men), the operating characteristics of OST for minimal trauma fracture were: sensitivity 47%, specificity 78%, positive predictive value 45%, and negative predictive value 79%. The area under the ROC curve was 0.659, 95% CI [0.588, 0.729]. Patients with OST $\leq$ 3 had an increased risk for minimal trauma fracture (crude OR 3.17 [1.84, 5.43], adjusted OR 3.22 [1.84, 5.66]). From the logistic regression model, for every increase of OST of 1 point, the risk for minimal trauma fracture decreased by 14% [7, 21]. OST predicted total fractures less well than it predicted minimal trauma fractures.
Although OST did not predict fracture as well as it predicts DXA, a lower OST score was clearly associated with an increased minimal trauma fracture risk in patients evaluated in an orthopedic clinic. Thus, using OST to predict DXA is supported by the finding that lower OST scores are associated with higher fracture risk.

Disclosures: ***S.L. Johnson****, None.*

M279

Older Women with Hyperkyphotic Posture Are at Increased Risk for Future Self-reported Osteoporotic Fractures: The Rancho Bernardo Study. D. M. Kado[1], M. Huang*[1], E. Barrett-Connor[2], G. A. Greendale[1]. [1]Medicine, University of California, Los Angeles, Los Angeles, CA, USA, [2]University of California, San Diego, San Diego, CA, USA.

It is well known that vertebral fractures predict future osteoporotic fractures, but is unknown whether hyperkyphosis, often considered to be a marker of vertebral osteoporosis, is also associated with an increased risk of future fractures. To answer this question, we performed a prospective cohort study of 588 women, aged 50-92 (mean age 70.7 years), who participated in the osteoporosis visits of the Rancho Bernardo Study. At the 1988-1991 baseline clinic visit, kyphotic posture was measured with participants lying recumbent, as the distance from the occiput-to-table (units = 1.7 cm blocks, placed under participant's heads). Incident fractures of the hip, clavicle, shoulder, arm, wrist, hand, rib, spine, pelvis, leg, and ankle were identified by self-report at the follow-up clinic visit between 1992-1995. Using a cut-off of ≥1 block, 18% of the subjects were classified as hyperkyphotic. Seventy-six fractures were reported at follow-up, of which 9 were of the hip, 14 of the wrist, 7 of the spine, and 36 were of the other sites. In age and baseline fracture adjusted models, women who required ≥1 block had a 2.12-fold increased odds (95% C.I.:1.11 – 4.08) of suffering from a future fracture. After using a backward selection procedure ($p<.10$) considering age, body mass index, physical activity, baseline history of fracture, spine bone mineral density (BMD), hip BMD, smoking, alcohol use, and self-reported difficulty in walking, bending, or climbing, only physical activity and spine BMD remained in the multivariable model. Adjusting for age, baseline fracture, physical activity and spine BMD, women with hyperkyphosis had a 1.85-fold increased odds of sustaining an incident fracture (95% C.I.:0.94 – 3.64) compared to women without hyperkyphosis. Our results suggest that older women with hyperkyphotic posture are at increased risk of future osteoporotic fractures.

Disclosures: ***D.M. Kado****, None.*

M280

Fractures in Older Women: Patient Characteristics, Trends and Factors Associated with BMD Measurement and Treatmeant for Osteoporosis. P. J. Elmer*[1], A. O. Feldstein[1], G. A. Nichols*[1], D. H. Smith*[1], M. Aickin*[1], M. Herson*[2], E. Orwoll[3]. [1]Epidemiology and Disease Prevention, Center For Health Research, Portland, OR, USA, [2]Endocrinology, Kaiser Permanente Northwest, Portland, OR, USA, [3]Clinical Research Center and Endocrinology, Oregon Health Sciences University, Portland, OR, USA.

Clinical Guidelines for osteoporosis management in older women with prior fractures recommend either initiation of pharmacologic treatment, or bone mineral density (BMD) measurement followed by treatment. Many patients are not managed in accordance with these guidelines. This cohort study evaluated, in this high-risk group, trends in osteoporosis management-BMD measurement and/or treatment and patient adherence to medications since publication of major clinical guidelines and identified factors associated with management.
We identified women ≥50 years who were members of a large group-model HMO between Jan. 1, 1998, and June 30, 2001, and who had a diagnosis of new study-defined fracture. The HMO's clinical electronic medical record databases provided demographic, diagnostic, pharmacy, and BMD utilization data. Analytic methods included bivariate comparisons using Student's t-test for continuous measures and Pearson's chi-square tests for dichotomous or categorical variables. The Mantel-Haenszel chi-square tests trends across index years.
At baseline, the population was 3,812 women, mean age 71.3; 14.7% had a hip fracture, 13.8% vertebral, 9.1% wrist, and 62.4% "other". Fewer than 12% had a diagnosis of osteoporosis prior to their index fracture, but 10.6% had increased risk for secondary

osteoporosis and 38.8% had risk for falls due to a diagnosis or medication.
Clinical guideline-specified management was found in only 46.4% of these women. These women were younger (68.5 vs 73.6) and less likely to have the risk factor of weight < 127 pounds (20.1% vs. 25.4%), hip fracture (9.9% vs 18.9%), or wrist fracture (7.5% vs 10.4%). They were more likely to be taking steroids (6.2% vs 2.2%) and have had a vertebral fracture (21.2% vs 7.4%). At baseline <2 % received a BMD, but BMD significantly increased during the study period (1.3% 1998, 10.2% 2001, p<.001). During 1998, 45% had pharmacologic treatment, predominantly HRT, but treatment rates did not increase over the study. In those receiving osteoporosis treatment, 73.6% had good adherence.
Guideline-recommended evaluation and treatment for osteoporosis after fracture did not improve despite wide dissemination of evidence-based guidelines. For re-fracture prevention, it may be fruitful to target high-risk groups for tailored interventions. Methods to enhance clinician education and facilitate improved processes of care will be necessary.

Disclosures: ***P.J. Elmer****, Merck Co 2.*

M281

Relation of Femoral Neck Geometry to Age and Body Composition among Older Men. L. M. Marshall[1], T. F. Lang[2], J. A. Cauley[3], K. E. Ensrud[4], C. E. Lewis[5], M. L. Stefanick*[6], E. S. Orwoll[1]. [1]Oregon Health and Science University, Portland, OR, USA, [2]University of California, San Francisco, CA, USA, [3]University of Pittsburgh, Pittsburgh, PA, USA, [4]VAMC & University of Minnesota, Minneapolis, MN, USA, [5]University of Alabama, Birmingham, AL, USA, [6]Stanford University, Palo Alto, CA, USA.

Femoral neck (FN) geometry changes with age. However, little is known about the distributions of FN geometric measures among older men or the factors that influence these skeletal characteristics. Body composition could affect FN geometry via mechanical or endocrine pathways. We quantified associations of FN bone volumes and section modulus (SM) with age and body composition among 1,902 men aged ≥65 years enrolled in the *Osteoporotic Fractures in Men* (MrOS) cohort. FN total, cortical and medullary volumes (cm^3) and SM (cm^3) were derived from quantitative computed tomography scans of the left hip. Total body lean mass (LM) and fat mass (FM) (kg) were obtained with DXA; height (m), grip strength (kg), and leg power (watts) were directly measured; age and physical activity were self-reported. We estimated the independent associations of age (5-year categories), LM and FM with the FN volumes and SM using multiple linear regression after adjustment for height. We examined potential confounding by physical activity, grip strength and leg power. In the referent category for age (65-69 years), the mean±sd for the total, cortical, and medullary volumes were 23.3±0.3, 10.2±0.1, and 13.0±0.2, respectively, and for the SM was 0.97±0.01. Total and medullary volumes increased by 1-2 cm^3 on average in each age group after adjustment for height, LM and FM (table). Cortical volume did not change significantly with age. SM increased through ages 80-84, but declined among men ages ≥85. The volume measures and SM increased significantly with LM (e.g., total volume increased by 1.8 cm^3 for a 7 kg increase in LM), but were not related to FM. None of the FN volumes or SM was related to physical activity, grip strength, or leg power. These cross-sectional data support previous observations that FN geometry changes with age. FN total and medullary volumes increased with age and cortical volume was maintained. SM increased through ages 80-84. The independent relation of FN volume and SM to LM but not to FM suggests that mechanical loading from skeletal muscle continues to influence FN geometry even among older men.

Table. Change (95% confidence interval [CI]) in FN geometry measures per increase in age (5-year) or a standard deviation in LM or FM*

Variable	Total Volume Change (95% CI)	Cortical Volume Change (95% CI)	Medullary Volume Change (95% CI)	Section Modulus Change (95% CI)
Age 70-74	1.2 (0.5, 2.0)	0.3 (-0.01, 0.6)	0.9 (0.4, 1.4)	0.02 (-0.01, 0.05)
Age 75-79	1.0 (0.2, 1.7)	0.1 (-0.2, 0.5)	0.9 (0.4, 1.4)	0.04 (0.01, 0.07)
Age 80-84	1.7 (0.7, 2.6)	0.05 (-0.3, 0.3)	1.6 (1.0, 2.3)	0.09 (0.06, 0.10)
Age ≥85	1.9 (0.4, 3.3)	-0.2 (-0.4, 0.9)	2.1 (1.1, 3.1)	0.02 (-0.04, 0.08)
Lean Mass (7 kg)	1.8 (1.4, 2.2)	0.8 (0.6, 1.0)	1.0 (0.7, 1.2)	0.11 (0.10, 0.12)
Fat Mass (7 kg)	0.1 (-0.2, 0.4)	0.05 (-0.1, 0.2)	0.1 (-0.2, 0.3)	-0.10 (-0.02, 0.003)

*Variables in the model are age (5-year categories), height, lean mass, and fat mass.

Disclosures: ***L.M. Marshall****, None.*

M282

Differences in Rate of Decline in Bone Mineral Density at the Total Hip Between Older Black and White Men. M. C. Hochberg[1], J. K. Tracy*[2], W. A. Meyer*[2], P. D. Wilson*[2], R. H. Flores*[3]. [1]Medicine, University of Maryland School of Medicine and Maryland Veterans Affairs Health Care System, Baltimore, MD, USA, [2]Epidemiology, University of Maryland School of Medicine, Baltimore, MD, USA, [3]Medicine, University of Maryland School of Medicine, Baltimore, MD, USA.

Older black men have higher adjusted bone mineral density (BMD) at all sites compared with older white men. This may be due to their having attained higher peak bone mass as young adults and/or having a slower rate of bone loss as adults. There are few published longitudinal data on bone loss in older white men and none that we are aware of in older black men. We measured change in BMD at the total hip in a cohort of 455 older men participating in the longitudinal component of the Baltimore Men's Osteoporosis Study (MOST). 379 white and 79 black men aged 65 and above (mean [SD] age at baseline 75 [5.7] and 72 [5.6] years, respectively) returned for a second visit after a mean [SD] of 17.2 [5.9] months. BMD was measured at the total hip at visit 1 using a Hologic QDR-2000 and at visit 2 using a Hologic QDR-4500 by Hologic trained and certified technicians; cross calibration was performed using hip and spine phantoms provided by the manufacturer. Mean total hip BMD at V1 and V2, and absolute change, percent change, and percent change per year in total hip BMD by race are shown in the Table. In bivariate analyses, older age at V1 and weight loss between V1 and V2 were both significantly correlated with absolute, percent and rate of bone loss at the total hip between V1 and V2; current smoking at both V1 and V2 was significantly associated with the rate of bone loss at the total hip between V1 and V2. In multiple variable adjusted linear regression models, black men had greater absolute, percent and rate of bone loss at the total hip than white men: differences (SE) were -.014 gm/c2 (.004), -.1.4% (.5) and -.89%/yr (.39), respectively; P = .005, .004 and .023, respectively. Sensitivity analyses were performed after excluding 67 subjects (61 white and 6 black) taking any bone-active medications (antiresorptive agents [bisphosphonates, calcitonin], fluoride or testosterone); results of these analyses did not differ from those summarized above. These data demonstrate that total hip BMD declines at a greater rate in older black than in older white men. Differences in biochemical markers of bone turnover may, in part, help explain these findings.

Total Hip BMD by Race in Older Men

	Blacks (N = 76)	Whites (N = 379)
Total Hip BMD @ V1 (g/cm2)	1.065 (.15)	0.951 (.13)
Total Hip BMD @ V2 (g/cm2)	1.047 (.16)	0.940 (.14)
Absolute Change (g/cm2)	-0.0185 (.044)	-.0107 (.033)
Percent Change	-1.97 (5.23)	-1.17 (3.36)
Percent Change per year	-1.55 (3.41)	-1.03 (2.82)

Disclosures: ***M.C. Hochberg****, None.*

M283

The Association of Loop Diuretic Use with Bone Mineral Density in Older Men. L. S. H. Lim*[1], H. A. Fink[2], M. A. Kuskowski*[3], J. Cauley[4], K. E. Ensrud[2]. [1]for the MrOS Research Group. Internal Medicine (Preventive Medicine), Mayo Clinic, Rochester, MN, USA, [2]University of Minnesota, Minneapolis, MN, USA, [3]GRECC, VA Medical Center, Minneapolis, MN, USA, [4]University of Pittsburgh, Pittsburgh, PA, USA.

Loop diuretics are postulated to have a negative effect on calcium homeostasis and bone metabolism. However, results from studies of their effect on bone mineral density (BMD) are inconsistent. There are few data regarding the association of loop diuretics and BMD in men.
To address this issue, we utilized data from MrOS, a multi-center, prospective cohort study being conducted to identify determinants of osteoporosis in men aged ≥65 yrs (n=5995). At MrOS baseline, data collected included demographics, medical history, medications, and in-clinic measures of anthropometry and physical performance. BMD was measured by DXA at the lumbar spine (LS) and total hip (TH). Subjects were categorized according to loop diuretic and non-diuretic use. Users of other diuretics or combinations of diuretics were excluded. Analyses compared non-diuretic users (n=4939) with loop diuretic only users (n=250). For each skeletal site, ANCOVA was used to assess the age-adjusted and multivariate-adjusted (MV) association between loop diuretic use and BMD. MV models at both sites included age, alcohol use, BMI, and diabetes.
Mean age-adjusted BMD was approximately 3% higher in loop diuretic users than in non-diuretic users at both LS and TH (p<0.01 for both comparisons). In MV models, mean BMD was slightly higher among loop diuretic users at both sites (approximately 0.5%), but this difference no longer achieved statistical significance.

Mean BMD (g/cm2) by Category of Loop Diuretic Use

	LS BMD		TH BMD	
	*Age-adjusted**	*MV* [+]	*Age-adjusted**	*MV* [#]
Loop diuretic use only	1.099	1.074	0.983	0.960
No diuretic use	1.066	1.068	0.954	0.956

[*]p<0.01 [+]adjusted for age, BMI, alcohol use, diabetes mellitus, hypertension, COPD, arthritis and dizziness [#]adjusted for age, BMI, alcohol use, diabetes mellitus, osteoporosis, smoking, physical activity, height change since age 25

We found no evidence of an independent association between loop diuretic use and bone density in older men suggesting that use of loop diuretics does not place them at increased risk of osteoporosis.

Disclosures: ***L.S.H. Lim****, None.*

M284

Prevalent Vertebral Fractures in Men: Initial Results from the MROS Study. D. M. Black[1], L. Lambert*[2], L. Palermo*[1], S. Cummings[3], K. Ensrud*[4], H. Fink*[5], E. Orwoll*[6]. [1]Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA, [2]Oregon Health and Science University, Portland, OR, USA, [3]Medicine, University of California, San Francisco, San Francisco, CA, USA, [4]VA Medical Center, Minneapolis, MN, USA, [5]Geriatric Research Education & Clinical Center, VAMC, Minneapolis, MN, USA, [6]Endocrinology and Metabolism, Oregon Health Sciences University, Portland, OR, USA.

Little is known about the epidemiology of vertebral fractures in men. The MROS recruited 5995 men ≥ age 65 from 6 US sites. At baseline, vertebral radiographs were collected, as was DXA at the hip and spine. Vertebral morphometry has thus far been performed on a random sample of 781 men.. Prevalent vertebral fractures are defined using a 3 SD cutpoint for vertebral fracture, the same as used in the Study of Osteoporotic Fractures (SOF) in older women.

Age	Prevalence in men			Prevalence in women (SOF)			Relative Risk
	n	%	RR*	n	%	RR*	women vs. men
65-69	207	10.1%	---	4082	14.5%	---	1.4
70-79	403	13.7%	1.4	4531	22.0%	1.5	1.6
80+	123	17.1%	1.7	962	37.6%	2.6	2.2

*Relative risk compared to age 65-70

At each age, the prevalence of fractures in men is lower than in women but the increases with age are steeper in women. This suggests a much higher incidence of vertebral fractures in women compared to men after age 70. There was a significant relationship between BMD and prevalent fracture. For example, the odds ratio per 1 standard deviation decrease in spine BMD was 1.6 (95% CI: 1.1, 2.5); similarly, for total hip BMD it was 1.7 (1.2, 2.6) per 1 SD decrease.

Although our findings from these initial analyses indicate that the prevalence of vertebral fracture is somewhat lower in men than in women and increases with age less steeply than in women, our results suggest that the relationship between BMD and prevalent vertebral fracture is similar in men and women.

Disclosures: ***D.M. Black****, Novartis Pharmaceuticals 2; Merck 2, 8.*

M285

Why Are Men Participating in an Osteoporosis Study? A Study of a Study - Comparing Sweden and Hong Kong. B. Borgström*[1], M. Andersson*[1], E. M. C. Lau[2], O. Johnell[1], K. Akesson[1]. [1]Dept of Orthopedics, Malmö, Sweden, [2]Dept of Community Medicine, Hong Kong, Hong Kong Special Administrative Region of China.

Introduction: For men as for women, ethnic, cultural and life style differences may play significant roles for a geographic variation in the prevalence of osteoporosis and fracture. The ongoing international study on male osteoporosis (Mr Os) including 10 000 men should provide answers to some of these questions, but how shall the results be interpreted?

Aim: The aim of this substudy was to investigate the reason for men to participate in an osteoporosis study and in their knowledge of the condition. Comparison was made between two study centers, Malmö (Mö), Sweden and Hong Kong (HK).

Material and Methods: Mö is including 1000 and HK 2000 men between age 70-80 yrs in the Mr Os study. In Sweden inclusion is population-based, in HK through invitation and advertisement. 153 Swedish men and 100 Hong Kong men participated in this substudy. A specific questionnaire was developed and the investigators visited both sites. The questionnaire (Qe) was given to the participant after completing the baseline investigation and returned by mail.

Results: The main reason for *participating* in Mö was contribution to research 76%, in HK 6%. The main reason in HK was to avoid osteoporosis or to get help for osteoporosis, 46% and 19%, in Mö 13% and 1%. At both sites 80% of the participants found the main study Qe easy to understand and answer and the time spent at the research center agreeable. In Mö 46% had participated in other research studies, and 5% in HK. In order to get to the center, 81% took the bus in HK, 1% walked, 18% other means of transport, in Mö 51% went by car, 17% biked and 17% walked. Swedish men felt well informed about *osteoporosis* (71%) and a similar number new someone with osteoporosis (28% Mö and 25% HK). Fewer Chinese men believed active life style to be preventive of osteoporosis 3% vs 26%, or life style and diet 21% vs 36%, while 11 and 12% favored diet alone. *Health care* is government funded in both cities, the number of doctors similar 3/1000 Mö vs 2.6/1000 HK, the life expectancy 76.7 vs 77.2 yrs and retirement age 65 yrs. Chinese men visit the doctor more often, 30% once a month or more compared to 3% in Mö. **Conclusion:** This is to our knowledge the first study investigating reasons for participating in an osteoporosis studies across cultures and geographic locations. It clearly indicates that local factors and cultural differences may influence the comparability in international multicenter studies, a fact to be considered when evaluating data. Both a wish to contribute to science and medical attention to a condition are important factors for participation among men.

Disclosures: ***B. Borgström****, None.*

M286

Serum Concentration of Estradiol and Testosterone: Diagnostic Relevance in Male Osteoporosis. I. M. Frieling*, F. Rittmeyer*, R. Jung*, H. P. Kruse*. University Hospital Hamburg, Hamburg, Germany.

Like in female osteoporosis primary osteoporosis with & without different known risk factors & secondary osteoporosis is found in male osteoporosis. The influence of low ß-estradiol levels & other gonadotropins are newly discussed. Our primary endpoint of this study is to find out, which gonadotropins are relevant in diagnosis of male osteoporosis.

We examined in this prospective study 86 male patients between January 2001 & May 2002 newly referred to our outpatient clinic with a suspected or diagnosed osteoporosis from 17-81 years, mean age 52 years. We didn't differentiate between already treated & 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, testosterone, ß-estradiol, DHEAS, DHT, LH, SHBG, growth hormone, phosphate clearance, calcium in 24 hour urine, 25-OH-vitamin D, 1.25-(OH)2-vitamin D, bone specific alkaline phosphatase, deoxypyridin crosslinks & PTH. Densitometry control on lumbar-spine & hip with Hologic QDR 1000+ was done. X-ray of vertebra was performed, when no X-ray was present.

Mean DXA values were decreased with T-score –2.35 SD at lumbar spine & – 1.83 SD total hip, although in 12% in lumbar spine & in 13 % in hip no osteoporosis was confirmed. Normal values were found in total alkaline phosphatase (196 ± 88.8 U/l)* & bone specific alkaline phosphatase (17.2 ± 7.1 [IF1] µg/l)*. Deoxypyridin crosslinks (5.9 ± 2.14 mmol/nmol creatinine / normal values: 3.85 ± 1.55)* were only in 52% normal. We found a negative significant correlation to 25-0H-vitamin D levels (r = -0.55). Mean phosphate clearance was elevated (27.9 ± 17.1 ml/min/normal values: 10.75 ± 5.25)*. Testosterone levels were measured in lower normal range (mean 5.1 µg/l) with a decreased level in 7.5% of patients. It showed a significant correlation (r = 0.62) to ß-estradiol levels (24 ± 9.6 ng/l / normal values: 36.5 ± 23 ng/l)*.There was also a significant correlation from testosterone to free testosterone (r = 0.84) and SHBG (r = 0.74). Beta-estradiol levels were decreased in 13% of patients, but there was no correlation to DXA or calcium-phosphate parameters. In patients with prevalent fractures a significant correlation of testosterone (r = 0.42) and free testosterone (r = 0.46) to DXA of the hip was found.

The results of this study show, that a routinely examination of ß-estradiol in the diagnosis of male osteoporosis is of no relevance. In patients with prevalent fractures testosterone levels are correlating with the severity of the disease. These results don't doubt the important role of ß-estradiol in the pathogenesis of male osteoporosis, although no correlation to DXA was found in our patients.

Disclosures: ***H.P. Kruse****, None.*

M287

Correlates of Knowledge About Osteoporosis Among Osteoporotic Men. O. Edelstein*[1], P. Werner*[1], I. Vered[2]. [1]Department of Gerontology, Faculty of Social Welfare and Health Studies, University of Haifa, Haifa, Israel, [2]Endocrinology, Sheba Medical Center, Ramat Gan, Israel.

Osteoporosis, once considered a "women's disease" is prevalent among men, and is associated with large expenditures in health care resources. Knowledge of the disease is important for prevention as well as for treatment of the disease. The present study examined levels and correlates of knowledge about osteoporosis among 100 Israeli-Jewish osteoporotic men (mean age = 63; mean spine t-score = -2.4; 33% had a low-trauma fracture; 20% had glucocorticoid-induced osteoporosis). Participants' knowledge of osteoporosis was assessed by an adapted version of the Facts on Osteoporosis Quiz (FOOQ) (Ailinger & Emerson, 1998). This version consisted of 28 true and false statements related to self-care risk factors and preventive behaviors associated with osteoporosis. In addition to the true and false responses, there was a "don't know" response, which allowed the respondent a choice without guessing. Each item was coded 0 if an incorrect answer or "don't know" answer was given and 1 if the correct answer was given. The correlates examined included participants' socio-economic and health characteristics. Results of the study showed that levels of knowledge were moderate to low (mean 18.4; range 6-28; SD 4.5). On the basis of the median score (median = 19) we found that half of the participants responded correctly to 68% of the statements in the overall index. Especially low levels of knowledge were found for the items assessing men-specific topics. For example, only 41% of the participants responded correctly to the item assessing prevalence of osteoporosis among men, and only 43% responded correctly regarding the role of male sex hormones as a risk factor in the development of osteoporosis. The association to smoking was known to 47% of the participants. In contrast: most of the men were aware of the protective roles of high calcium intake (93%) and physical activity (98%). Having glucocorticoid-induced osteoporosis had no effect on the levels of knowledge. Older age, having had a fracture, lower education, and having no extended health insurance were significantly associated with lower levels of knowledge. Educational programs, geared to the needs and capabilities of male osteoporotic patients, should be encouraged.

Disclosures: ***I. Vered****, None.*

M288

Lifestyle and Hormonal Predictors of Bone Mineral Density in Asian Men. E. Y. N. Cheung*, A. Y. Y. Ho*, A. W. C. Kung*. Medicine, The University of Hong Kong, Hong Kong, China.

Osteoporosis in men is a public health problem that had been overlooked in the past, especially in Asia, as an inevitable and untreatable consequence of aging rather than a disease. 30% of hip fractures in Hong Kong and 50% in Beijing, China, happened in men. Scanty data were published on osteoporosis in Asian men. To derive preventive strategies, a better understanding of the risk factors for fractures and low BMD among Asian men is needed. We performed densitometry measurements on 213 community dwelling southern Chinese men age 50 or above residing in Hong Kong. The social and medical history, lifestyle habits and anthropometric data were obtained using a structured questionnaire. A semi-quantitative dietary questionnaire was administered to determine the calories, calcium, protein and phytoestrogen intake. Blood were analyzed for 25 Vit D, PTH, total and bioavailable estrogen (E) and testosterone (T). Subjects with secondary causes were excluded from study.

The mean age was 66 ± 9 yrs. 6.1% and 6.7% of the subjects have T score below -2.5 at the

femoral neck (FN) and spine respectively. BMD was associated with current or past tobacco smoking (FN, p=0.007; spine p=0.03) but not with alcohol drinking. Both FN and spine BMD were associated with age, height, weight and serum PTH level. FN BMD was borderlinely associated with calcium intake (p=0.058) but not spine BMD. Lignan intake correlated with FN BMD (p<0.03) but not spine BMD. Weight-bearing physical activity for >1 hour per day was positively associated with BMD at FN (p<0.005) and spine (p=0.05). Total E and T were not predictive of BMD at both FN and spine. None of the subjects had Vitamin D deficiency with 25Vit. D level <12ng/ml.
In the linear regression model, weight, age, weight-bearing physical activity, PTH and lignan intake accounted for 44.4% of the total variance in FN BMD. However, majority of the effect was accounted for by weight and age (40%). For spine BMD, serum PTH and weight were the best predictors. They accounted for 20% of the total variance of spine BMD while weight alone could explain 18% of the variance. It is concluded that weight, age and PTH are the best predictors of BMD in men.
Risk factor (per SD reduction) RR (95% CI) for osteoporosis at FN
age 4.2 (1.7-10.6)
weight 0.2 (0.1-0.7)
PTH 2.0 (1.1-3.7)
Risk factor (per SD reduction) RR (95% CI) for osteoporosis at spine
weight 0.2 (0.1-0.5)
PTH 1.8 (1.0-3.0)

Disclosures: ***E.Y.N. Cheung****, None.*

M289

Histomorphometric Analysis of Iliac Crest Biopsies from Healthy Men. D. E. Jewison*, J. A. Burgess*, K. L. Shogren*, R. T. Turner, J. D. Sibonga. Orthopedic Research, Mayo Clinic, Rochester, MN, USA.

Healthy subjects were recruited for a study of age-related bone loss in the male population. Bone biopsies from 42 subjects (ages 20-80 years) were processed and embedded non-decalcified in methylmethacrylate. Serial sections were stained with Goldner Masson Trichrome for static histomorphometry or mounted unstained for tetracycline-based measurements. Tissue slides were measured with the OsteoMeasure image analysis system (OsteoMetrics Inc., Atlanta, GA). Generated values were based upon quantification of the entire biopsy section (11.6 – 55.0 mm² area range) and represented the average data of two measurers. Tissue areas with bone fragments or within the endocortical envelope were excluded. Values for static histomorphometry (% bone volume/tissue volume; % osteoid surface/bone surface, osteoid thickness and % eroded surface/bone surface) and values for dynamic histomorphometry (mineral apposition rate and bone formation rate, surface and volume referants) are provided.

Disclosures: ***D.E. Jewison****, None.*

M290

Endogenous Hormones and Height Loss in Men. A. B. Araujo*[1], A. B. Black*[1], A. E. Morse*[1], S. S. Harris[1], C. Longcope*[2], J. B. McKinlay*[1]. [1]New England Research Institutes, Watertown, MA, USA, [2]Dept ObGyn and Medicine, University of Massachusetts Medical School, Worcester, MA, USA.

Age-related height loss occurs largely as a result of vertebral fractures. Longitudinal data on testosterone and related hormones in relation to height loss or vertebral fracture in men are rare. We sought to address this issue by examining the effect of endogenous hormone levels on height loss in the Massachusetts Male Aging Study (MMAS), a population-based cohort study of older men. MMAS included 1,709 men 40-70 yr assessed between 1987-89 (T1), with follow-up of 1,156 men between 1995-97 (T2) -- interviews were generally conducted in the study participants' homes. Blood was drawn within 4 hrs of awakening; data were available for 18 endogenous hormone measures, all of which were log transformed for analysis. Height was measured with a carpenter's rule according to standard protocols for epidemiologic field studies. Change in height was computed by subtracting height at T1 from height at T2 -- observations with changes outside ± 10 cm (n=2) were deleted, leaving 1,042 men with complete data available for analysis. Differences between T1 and T2 height were examined in the entire cohort using paired t-tests, and by age decade with analysis of variance. Multiple linear regression with height change as the outcome, controlled for T1 height, was used to assess the relation between T1 hormones adjusted for T1 confounders. There was a significant (p<0.001) loss of height over the 8.8-yr study period (mean ± SD: -0.54±1.77 cm), which varied significantly (p<0.001) by age decade (40-49 yr: -0.18±1.73 cm; 50-59 yr: -0.48±1.63 cm; 60-70 yr: -1.10±1.88 cm). Total testosterone, androstenedione, and cortisol were associated with change in height, adjusted for T1 height and age. The apparent effect of cortisol was eliminated by adjustment for global health status, body-mass index, and physical activity. The relation of testosterone and androstenedione to height loss varied by age. In our final model, there were significant interactions between testosterone and age (p=0.0004) and androstenedione and age (p=0.0376) in relation to height loss after adjustment for T1 height, health status, body-mass index, and physical activity. The sign of the parameter estimates for the interaction terms indicate that the relation of both testosterone and androstenedione to height loss becomes more pronounced with age, with lower testosterone and androstenedione levels leading to greater losses in height. In age-stratified models, the effects of testosterone and androstenedione on height loss were significant only among men in their 60s. In summary, there are significant age-dependent effects of testosterone and androstenedione on height loss.

Disclosures: ***A.B. Araujo****, None.*

M291

Men Present with a Higher Rate of Prevalent Fractures than Women When Started on Bisphosphonate Therapy in Canadian Specialty Osteoporosis Practices. A. M. Sawka*[1], A. Papaiouannou[1], J. D. Adachi[1], G. Ioannidis[2], W. Olszynski*[3], J. P. Brown*[4], D. A. Hanley*[5], T. Murray*[6], R. Josse*[6], R. J. Sebaldt*[1], A. Petrie*[2], A. Tenenhouse*[7], C. H. Goldsmith*[2]. [1]Clinical Epidemiology and Medicine, McMaster University, Hamilton, ON, Canada, [2]Clinical Epidemiology, McMaster University, Hamilton, ON, Canada, [3]Department of Medicine, University of Saskatchewan, Saskatoon, SK, Canada, [4]Department of Medicine, Centre Hospitalier de Laval, Quebec City, PQ, Canada, [5]Department of Medicine, University of Calgary, Calgary, AB, Canada, [6]Department of Medicine, University of Toronto, Toronto, ON, Canada, [7]Department of Medicine, McGill University, Montreal, PQ, Canada.

Differences between men and women treated with bisphosphonates in osteoporosis practices are not well-defined. We studied 1588 patients (163 men, 1425 women) over the age of 50 years who were started on cyclic etidronate or alendronate and had at least 2 years of follow-up in the Canadian Database for Osteoporosis and Osteopenia Patients (CANDOO) Study. We found that the baseline femoral neck and lumbar spine bone densities were higher in the men than the women (femoral neck bone density: men, n=96, 0.721, SD 0.123gm/cm^2 versus women, n=824, 0.695, SD 0.109gm/cm^2; p=0.045. L2-L4 bone density: men, n=83, 0.951 gm/cm^2, SD 0.195 gm/cm^2, versus women, n=787, 0.891, SD 0.146gm/cm^2; p=0.008). Rates of previous vertebral fracture were twice as high in men compared to women (men, 44%, 72/163, versus women, 22%, 315/1425; p<0.001) and rates of multiple vertebral fractures were triple in men compared to women (men, 10%, 17/163, versus women, 3%, 37/1425, p<0.001). Rates of alendronate prescription were not significantly different between men (35.0%, 57/163 patients) and women (28.9%, 412/1425; X^2=2.58, p = 0.108). However, bisphosphonate-treated men were more likely than women to be prescribed first-line alendronate therapy (without a previous history of etidronate use) (men, 21.5%, 35/163, versus women, 12.2%, 174/1425; X^2=9.82, p=0.003). Men were more likely than women to sustain an incident fracture within 24 months of starting bisphosphonate therapy than women (men 7/163, 4%, women 24/1425, 2%, X^2=5.21, p=0.033). In conclusion, in a database of bisphosphonate-treated Canadian patients, men presented with more severe disease, as reflected by higher rates of prevalent and incident fractures than women.

Disclosures: ***A.M. Sawka****, None.*

M292

Association Of Mild Lung Disease and BMD. T. L. Dam*[1], S. Litwack*[2], E. Barrett-Connor[3]. [1]Medicine, UCSD, San Diego, CA, USA, [2]Prevention Science Group, UCSF, San Francisco, CA, USA, [3]Family and Preventive Medicine, UCSD, San Diego, CA, USA.

Several studies have shown that osteoporotic fractures are more common in patients with advanced chronic obstructive pulmonary disease, who are apt to be thin, inactive and treated with corticosteroids. Little is known about bone mineral density (BMD) in ambulatory men with mild or moderate lung disease.
We evaluated 5,995 community dwelling ambulatory men aged 65 years or older who were enrolled in the Osteoporotic Fractures in Men study. Men who reported a history of lung disease (asthma, chronic bronchitis, emphysema, or chronic obstructive lung disease) were compared to men who did not report any lung disease. Those who reported lung disease were categorized as mild or moderate. The mild group included men with lung disease but not receiving medical treatment. Moderate lung disease group consisted of men who were currently receiving medical treatment.
Mild to moderate lung disease was significantly associated with increasing age, cigarette smoking, low physical activity, corticosteroid use, and less optimal quality of life, while alcohol use and body mass index were not positively associated with lung disease. Men with mild or moderate lung disease had significantly lower BMD at all sites except total body BMD (Table). After adjusting for all measured covariates, a significant inverse association between severity of lung disease persisted between spine BMD (p<0.006) and trochanter BMD (p< 0.03). Using NHANES normative data for men (available only for the total hip), men with mild-moderate chronic lung disease had a 1.47 odds of having osteoporosis compared to men without lung disease.
Elderly community dwelling ambulatory men with self reported mild or moderate lung disease have decreased bone mineral density compared to men without. These findings are not explained by age, smoking history, alcohol consumption, physical activity or corticosteroid use. We conclude that men with mild or moderate lung disease may be an independent risk factor for low BMD.
Table 1. BMD measurements by Lung Disease

	No Lung Disease N=5355	Mild Lung Disease N=280	Moderate Lung Disease N=360	Mild/Mod Lung Disease N=640	P-value
BMD Total Hip g/cm^2	.96 (±.14)	.94 (±.14)	.93 (±.15)	.94 (±.15)	.0002
BMD Spine g/cm^2	1.08 (±.19)	1.04 (±.18)	1.04 (±.20)	1.04 (±.19)	<.0001
BMD Total Body g/cm^2	1.17 (±.13)	1.18 (±.13)	1.16 (±.14)	1.17 (±.14)	.0555
BMD Intertrochanter g/cm^2	1.11 (±.17)	1.10 (±.17)	1.09 (±.18)	1.09 (±.17)	.0051
BMD Trochanter g/cm^2	.77 (±.13)	.75 (±.12)	.74 (±.13)	.74 (±.13)	<.0001

*p-value for comparing no lung vs. mild lung vs. moderate lung

Disclosures: ***T.L. Dam****, None.*

M293

Risk Factors and Clinical Characteristics of Male Osteoporosis in Korea. Y. Kim*, Y. Rhee*, S. Lee*, Y. Cho*, S. Kim*, S. Lim. Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea.

Osteoporosis has been a leading cause of morbidity and mortality associated with hip fracture as well as vertebral or other major fracture in elderly people especially in men. However, few studies dealt with male osteoporosis in Asians unexpectedly. Therefore, we explored this cross-sectional study of risk factors, clinical characteristics, and treatment response of male osteoporosis in Korea.

A total of 217 male osteoporotic patients from January 1996 to August 2002, who visited Endocrinology and Metabolism Division at Yonsei University Medical Center were enrolled and the risk factors were assessed. The patients with primary osteoporosis were divided into 2 groups according to bisphosphonate use and the change of bone mineral density (BMD) was compared with the mean duration of 1.7±1.4 yrs. Also, the change of BMD was assessed in patients with hypercalciuria who were treated with thiazide.

Secondary osteoporosis was as high as 68.7% and the risk factors were as follows: steroid use; 77.9%, hypercalciuria; 15.4%, endocrine disorder; 3.4%, hypogonadism; 2.0%, and malignancy; 1.3%. The mean age of primary osteoporosis (31.3%) was 56.2±9.7 yrs, where the mean height and weight were 168.0±5.7 cm and 63.7±7.1 kg. The mean smoking history was 12.4±13.5 pys and 3 patients had heavy alcohol history, where 50 patients had social alcohol history. The mean calcium intake was 598.3±233.3 mg/d. The biochemical markers of primary osteoporosis were as follows: testosterone; 522.8±180.2 ng/dl (total), 15.9±3.6 pg/ml (free), 1,25(OH)vitamin D; 20.1±9.8 ng/ml, 24 hour urinary calcium; 181.9±62.1 mg/d, PTH; 28.5±11.9 pg/ml, TSH; 1.73±1.63 uIU/ml, NTX; 45.6±40.2 nM BCE/mMCr, osteocalcin; 19.7±7.2 ng/ml, IGF-1; 203.4±7.2 ng/ml, and E2; 22.3±9.2 pg/ml. The BMD of alendronate group was significantly increased by 11.4% (L2-L4), 2.7% (femur neck), and 3.7% (trochanter). The alendronate group was then divided into the high and low bone turnover groups but the change of BMD was not different between 2 subgroups. On the other hand, among 23 hypercalciuric patients, 9.2% (L2-L4), 3.3% (femur neck), and 4.6% (trochanter) increase of BMD was achieved in 8 patients through thiazide. From our study, the most common causes of osteoporosis in Korean men were undoubtedly secondary causes, especially steroid use and hypercalciuria, which was significantly improved with thiazide. Primary male osteoporosis was ameliorated by bisphosphonate regardless of bone turnover rate. However, whether the increase of BMD in male osteoporosis would decrease fracture-related mortality is still unknown and need to be investigated.

Disclosures: ***Y. Kim****, None.*

M294

Reduced Bone Density, but not Bone Size, Is Associated with Elevated Systolic Blood Pressure in Older Men. R. M. Daly, M. Brown*, S. L. Bass, C. Nowson*. School of Health Sciences, Deakin University, Melbourne, Australia.

Osteoporosis and hypertension are both chronic conditions which are prevalent in older adults. Any disturbance in calcium metabolism can alter extracellular calcium homeostasis leading to increased bone resorption and bone loss. High blood pressure has been shown to be related to increased urinary calcium excretion, elevated PTH levels, and accelerated bone loss. In this study, we asked: 1) is reduced bone density associated with elevated blood pressure in men aged 50+ yrs (mean ± SD, 61.7 ± 7.7 yrs), and 2) if this relationship exists, is it bone- and surface- specific? Men were recruited from the community as part of a dietary intervention. At baseline, we assessed femoral neck (FN), lumbar spine (L3), ultra-distal (UD) and 33% radial BMD by DXA (n=206); L3 cortical and trabecular BMD (mg/cm3) and femoral mid-shaft total, cortical and medullary area (mm2), cortical BMD (mg/cm3), and the polar moment of inertia (Ip, mg/cm) by QCT (n=112). Seated systolic (SBP) and diastolic (DBP) blood pressure was measured under standardized conditions (mean of 3 readings). Those on thiazide diuretics were excluded. Linear regression analysis was used to examine the relationship between each bone trait and blood pressure after adjusting for age, BMI, use of anti-hypertensive medication, smoking status and calcium intake. At the lumbar spine, DXA L3 BMD (coefficients ± SE; -9.07 ± 4.51, $p<0.05$) and QCT L3 trabecular BMD (-0.08 ± 0.03, $p<0.01$) were inversely related to SBP. L3 cortical BMD and vertebral dimensions were not related to BP. At appendicular skeletal sites, DXA FN (-17.07 ± 6.83), UD (-40.59 ± 15.20) and 33% radial BMD (-25.58 ± 11.57) ($p<0.01$ to <0.05) were also negatively related to SBP. Similarly, QCT mid-femoral shaft cortical BMD was inversely related to SBP (-0.07 ± 0.03, $p<0.05$), whereas a positive relationship was detected for medullary area (0.06 ± 0.03, $p<0.05$). No relationships were detected for mid-femur total or cortical area or Ip. DBP was not associated with any bone trait. In summary, these results indicate that reduced lumbar spine trabecular, but not cortical BMD, was associated with elevated SBP, whereas at appendicular sites cortical and trabecular BMD appeared to be similarly affected. No relationships were detected between bone size or strength and blood pressure, but an increase in medullary area at the femur was associated with elevated SBP. In conclusion, these results indicate that reduced bone density, but not bone size or strength, was associated with elevated blood pressure in older men. Although the mechanism remains unknown, it is possible that there is a systemic effect on calcium metabolism leading to both a reduction in bone density and an increase in blood pressure.

Disclosures: ***R.M. Daly****, None.*

M295

See Friday Plenary number F162

M296

The Relationship between Circulating Levels of Estradiol and Leptin and Bone Mineral Density and Body Composition in 70-Year-Old Women – the NORDOS Study. A. G. Nilsson*[1], M. Stenström[2], K. Landin-Wilhelmsen[1], E. Norjavaara*[3], C. Ohlsson[1], D. Mellström[2]. [1]Center for Bone Research at the Sahlgrenska Academy, Dept of Endocrinology, Sahlgrenska University Hospital, Göteborg, Sweden, [2]Center for Bone Research at the Sahlgrenska Academy, Dept of Geriatrics, Sahlgrenska University Hospital, Göteborg, Sweden, [3]Dept of Pediatrics, Sahlgrenska University Hospital, Göteborg, Sweden.

Women in Norway and Sweden have the highest incidence in the world of hip and vertebral fractures. The reason for this is largely unknown, and it has been suggested that differences in body mass index (BMI) and body composition influence bone mineral density (BMD) and fracture risk. The purpose of this study was to investigate the relationship between circulating levels of estradiol and leptin with BMD and body composition in a random population-based sample of 482 seventy-year-old women in Göteborg, Sweden. The study was approved by the Ethics committee at the Sahlgrenska University Hospital and all subjects gave their informed consent to participate. Total serum estradiol was measured by a modified RIA (Orion Diagnostica) with a lowest detection level of 10 pmol/L. Serum leptin was measured by RIA (LINCA Research Inc.) with a detection level of 0.5 μg/L. BMD and body composition were measured by dual energy x-ray absorptiometry (DXA; equipment Hologic 4500A). Serum levels of leptin were strongly correlated to body weight ($r=0.68$, $p<0.001$), BMI ($r=0.74$, $p<0.001$), body fat mass ($r=0.78$, $p<0.001$) and to the hip and waist circumference ($r=0.63$ and 0.72, respectively, $p<0.001$). Serum leptin concentrations were significantly correlated to serum total estradiol ($r=0.15$, $p=0.001$). Estradiol levels were highly correlated to body weight ($r=0.19$, $p<0.001$), BMI ($r=0.22$, $p<0.001$), body fat mass ($r=0.21$, $p<0.001$) and hip and waist circumference ($r=0.33$ and 0.26, respectively, $p<0.001$). BMD of the total body, the hip, and the lumbar spine was significantly correlated to serum leptin levels in a linear regression model ($r=0.13$-0.28, $p<0.01$-0.001) but this was not seen in multiple regression models correcting for differences in BMI. Logarithmic total serum estradiol was significantly correlated to BMD of the total body, the hip, and the lumbar spine ($r=0.14$-0.20, $p<0.01$-0.001). After correction for BMI, total serum estradiol concentrations were positively correlated to BMD of the lumbar spine (partial $r=0.12$, $p=0.01$). The results were similar if women using estrogen substitution therapy (n=54) were excluded. The present study suggests that serum estradiol and leptin may be involved in the endocrine mechanism mediating the variation in BMD with different body composition in elderly postmenopausal women.

Disclosures: ***A.G. Nilsson****, None.*

M297

Withdrawn

M298

Effects of Calcium Absorption and Excretion on Urine Pyridinoline in Postmenopausal Women. A. G. Need[1], P. D. O'Loughlin*[1], H. A. Morris[2], M. Horowitz*[3], B. E. C. Nordin[1]. [1]Clinical Biochemistry, Institute of Med and Vet Science, Adelaide, Australia, [2]Hanson Institute, Institute of Med and Vet Science, Adelaide, Australia, [3]Medicine, Royal Adelaide Hospital, Adelaide, Australia.

Since negative calcium balance may increase bone resorption to maintain the plasma ionised calcium we examined the relationships between radiocalcium absorption, fasting obligatory calcium and the excretion of a biochemical marker of bone resorption, urinary pyridinoline (PYD) in 266 untreated postmenopausal women attending our osteoporosis clinics. All had lateral spine Xrays performed and all attended fasting for blood and urine collection after which they had their hourly fractional calcium absorption (α) and serum 1,25-dihydroxyvitamin D measured [1]. Total PYD was measured by HPLC after overnight hydrolysis. Urine calcium and PYD were expressed relative to creatinine (Ca/Cr and PYD/Cr).

Ca/Cr and α were not significantly related. Multiple linear regression showed that PYD/Cr was related positively to Ca/Cr ($P<0.001$) and inversely to α ($P<0.001$) as shown in the following equation:

$PYD/Cr = 91.9 + 46.8\ Ca/Cr - 28.2\ \alpha$ ($R = 0.32$; $P<0.001$)

The number of spinal compression fractures (score of 1 for a wedge and 2 for a crush fracture) was related positively to PYD/Cr ($P<0.001$) and inversely to α ($P=0.028$). Those with scores greater than 1 had lower α than the others (0.59,0.21SD vs 0.67,0.26; $P=0.017$) but their serum 1,25D values did not differ significantly (111,37 vs 119,36). From the regression of α on 1,25D we estimate that the difference in 1,25D can account for 21% of the difference in α between the two groups. We conclude that both decreased calcium absorption and increased urine calcium increase bone resorption.

1. Nordin BEC et al, J Nuclear Med 1998;39:108-113

Disclosures: ***A.G. Need****, None.*

M299

Reduced Serum Levels of Vitamins D, E, and K in Hypervitaminosis A in Rats. P. M. Lind*[1], A. Gustafsson*[1], H. Melhus[2]. [1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

Interactions between vitamin A, on the one hand, and vitamins D, E, and K, on the other hand were suggested in the 1930's. Research in the past decade has suggested that all fat-soluble vitamins may have an impact on bone. We have recently shown that subclinical hypervitaminosis A is associated with thinner and more fragile bones and our clinical data support this finding. Our aim of this study was to investigate the serum concentration of the fat-soluble vitamins D2 (25-OH vitamin D2), D3 (25-OH-vitamin D3), E (alfa-tocopherol) and vitamin K (phylloquinone) in subclinical hypervitaminosis A in rats. The rats were divided into three groups with fifteen animals in each. They were fed a pellet standard diet containing 12 IU vitamin A/g pellet (control, C), or standard diet supplemented with either 120 IU ("10 x C") or 600 IU vitamin A/g ("50 x C") vitamin A/g pellet. The vitamin A was added to the pellets in the form of retinyl palmitate and retinyl acetate. The serum concentrations of the vitamins were analyzed by AS Vitas Oslo (www.vitas.no) with HPLC. The following table shows mean ± SEM of the vitamins in rat serum. *p<0.05 compared to control, and the number of animals are given in parentheses.

	Treatment		
	Control	"10 x C"	"50 x C"
Vitamin D2 (nM)	15.6 ± 1.2 (11)	12.7 ± 1.0 (15)*	12.1 ± 0.4 (15)*
Vitamin D3 (nM)	27.1 ± 3.4 (15)	22.3 ± 2.7 (15)	15.7 ± 0.8 (15)*
Vitamin E (uM)	33.3 ± 1.9 (15)	26.7 ± 1.2 (15)*	17.3 ± 0.8 (15)*
Vitamin K (ng/ml)	1.6 ± 0.2 (13)	1.9 ± 0.3 (13)	1.2 ± 0.1 (13)

In rats with increased intake of vitamin A, serum levels of vit D2 , D3 and E decreased in a dose-dependent way. Serum levels of vitamin D2, D3 and E were reduced about 20% in the "10xC" group and the levels of vitamin D3 and E about 40-50% in the "50xC" group compared to the control group. The results show that hypervitaminosis A leads to a reduction in the serum levels of all the other fat-soluble vitamins, suggesting that excessive vitamin A may also have indirect effects on bone. Our findings indicate that the interaction among fat-soluble vitamins occur in the intestinal tract prior to or during absorption.

Disclosures: ***P.M. Lind****, None.*

M300

PTH Increases Bone Strength Independently of IGF-I Status in Adult Rats. P. Ammann[1], J. A. Gasser[2], R. Rizzoli[1]. [1]Department of Geriatry and Internal Medicine, Division of Bone Diseases, Geneva-14, Switzerland, [2]Dept. of Arthritis & Bone Metabolism, Novartis Pharma AG, Basel, Switzerland.

PTH is a potent bone anabolic agent. The mechanism by which it increases bone strength, remains unclear. Results suggest that its anabolic effect could be mediated by IGF-I. To address this issue, six-month old female rats were fed isocaloric diets containing recommended casein intake or 50% of the minimal amount of casein necessary to maintain bone mass. After 2 weeks of reduced casein intake, a 31% decrease in circulating IGF-I was found. Under these conditions, there is a marked resistance to exogenous IGF-I administration. Then, PTH(1-34) (5 or 40 µg/kg BW) or its solvent was given subcutaneously to rats on either diet daily for 4 weeks. Proximal tibia bone mineral density (BMD) and ultimate strength using a compression test were measured. Before PTH(1-34) administration, i.e after 2 weeks of low protein diet, plasma IGF-I was 311ng/ml±7 and 224±17 in rat fed a normal or a low protein diet, respectively. After 4 weeks of PTH(1-34) treatment the results were (*p<0.05 vs time control)

	Casein	vehicle	PTH 5µg	PTH 40µg
IGF-I	normal	324±29	303±25	294±25
(ng/ml)	low	219±26	205±15	221±26
BMD	normal	251 ±3	271±5*	299±5*
(mg/cm2)	low	241±4	258±5	275±7*
Bone Strength	normal	167±16	259±24*	328±27*
(N)	low	191±17	199±17	275±25*

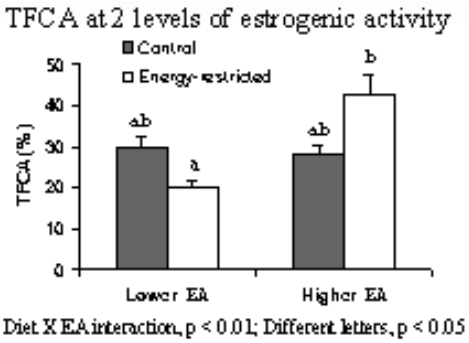
These results indicate that independently of the casein intake an anabolic effect of PTH was detected. These results also point out that despite a low plasma IGF-I and a resistance to IGF-I, PTH increases BMD and bone strength, though this effect was less pronounced than in rat with normal IGF-I status. Thus to achieve the same effect a 8 times higher dose was required in rats fed an isocaloric low protein diet. These results suggest that even in lowered IGF-I, PTH can increase bone mass and strength.

Disclosures: ***P. Ammann****, None.*

M301

Estrogen Prevents the Reduction in Fractional Calcium Absorption Due to Energy Restriction in Mature Rats. S. A. Shapses[1], M. Cifuentes*[2], J. P. Advis*[3]. [1]Nutritional Sciences, Rutgers University, New Brunswick, NJ, USA, [2]Inta, University of Chile, Santiago, Chile, [3]Animal Sciences, Rutgers University, New Brunswick, NJ, USA.

Weight reduction is a risk factor for bone loss. We have previously shown that energy restriction is associated with a decrease in calcium (Ca) absorption and decreased estrogenic activity. We hypothesized that the hypoestrogenic status may be the cause of the decrease in Ca absorption, and that estrogen replacement during energy restriction would prevent it. Six month-old rats were ovariectomized and implanted s.c. with 17ß-estradiol (E2) pellets to maintain levels within physiological range. Rats were allowed ad libitum access to feed (n=12) or were 40% energy restricted (n=12) for 10 weeks. At the end of this study, rats were divided into two groups according to their uterine weight into those having high estrogenic activity (EA) and those with low EA. Data was analyzed by 2-way ANOVA and Tukey's post-hoc test. While rats eating ad libitum gained ~46% weight from baseline, energy restricted rats maintained their weight throughout the study. Energy restriction was associated with lower Ca absorption (5d measurement, 45Ca radioisotope) and Ca balance in low EA but not high EA rats (Fig). True fractional calcium absorption (TFCA) in rats with lower and higher estrogenic activity (EA) after 10 weeks of ad libitum access to food or 40% energy restriction. Similarly, Ca absorption was correlated with both serum E2 (r=0.68, p<0.05) and body weight (r=0.72, p<0.05) and estradiol concentrations correlated inversely with corticosterone (r= -0.66, p<0.05) in rats having low EA but not in those with high EA. Finally, 24-hour corticosterone excretion was higher in energy-restricted rats than in those having ad libitum access to feed, a response that was blunted in high EA rats. Our findings suggest that decreases in estrogen and hyperadrenocorticism with energy restriction play a significant role in the regulation of Ca absorption and balance.



Disclosures: ***S.A. Shapses****, None.*

M302

Suppression of Osteoblastogenesis Overrides the Purported Effects of Hypogonadism in the Mouse Model of Glucocorticoid-Induced Osteoporosis. R. S. Weinstein, D. Jia, C. O'Brien, C. C. Powers*, J. A. Crawford*, S. A. Stewart*, R. L. Jilka, A. M. Parfitt, S. C. Manolagas. Div. of Endo/Metab, Center for Osteoporosis, Central Arkansas Veterans Healthcare System, Univ of Ark for Med Sci, Little Rock, AR, USA.

Loss of sex steroids has been implicated as an aggravating factor in glucocorticoid-induced osteoporosis but evidence for this is limited. In addition, loss of gonadal function stimulates the production of osteoblasts and osteoclasts in the bone marrow resulting in an increase in cancellous osteoblasts, osteoclasts and bone turnover—lesions quite distinct from those found in glucocorticoid-induced osteoporosis. To examine the interaction between glucocorticoid excess and hypogonadism, we used established murine models of glucocorticoid-induced osteoporosis and androgen deficiency. After 28 days, vertebral bone mineral density (BMD) and compression strength decreased to the same extent in mice receiving prednisolone or after orchidectomy (orch), but the changes were no worse when both conditions were combined. Seminal vesicle weight did not change in the sham-operated animals receiving prednisolone but, as expected, was decreased in orch mice receiving placebo or prednisolone. Orch induced the expected increases in osteoblast and osteoclast progenitors, but the increases were prevented by simultaneous administration of prednisolone. Consistent with these findings, the increases in osteoid, osteoblasts and osteoclasts found in the orch animals receiving placebo were prevented or greatly attenuated when these animals received prednisolone. In addition, serum interleukin-6 levels doubled in orch animals receiving placebo whereas this increase was completely abrogated in animals receiving both orchidectomy and prednisone. Animals receiving combined orch and prednisone also failed to show the increased rate of bone formation and activation frequency noted with orch alone. The prevalence of osteoblast apoptosis increased in mice receiving prednisolone or after orch, but again, the increases were not additive when both conditions were combined. In conclusion, the results of this study demonstrate that the increases in osteoblast and osteoclast precursors, incidence of new remodeling cycles at sites on cancellous bone and rate of bone remodeling that occurs after the loss of androgens are abrogated by glucocorticoid excess. This evidence indicates that cells of the stromal/osteoblastic lineage are required mediators of the increase in bone remodeling and loss of bone mass that ensues following loss of androgens. Furthermore, the evidence demonstrates that concurrent hypogonadism does not play a role in the loss of BMD and strength that occurs with glucocorticoid excess.

Disclosures: ***R.S. Weinstein****, Anabonics 4; P & G, Novartis, Hoffman La Roche 2; Merck, P & G 8; Lilly 5.*

M303

Serum Osteoprotegerin as a Predictive Marker for Bone Mineral Densities in Glucocorticoid-Induced Osteoporosis. I. Tanaka[1], H. Oshima[2]. [1]Department of Biofunctional Research, National Institue for Longevity Sciences, Obu, Japan, [2]Department of Laboratory Medicine, Fujita Health University School of Medicine, Toyoake, Japan.

[Objective] It is well known that glucocorticoid causes osteoporosis, butÅ@convenient markers for bone mineral densities in glucocorticoid-induced osteoporosis have not yet established. In this study, we try to clarify a clinical role of serum osteoprotegerin (OPG) level in glucocorticoid-induced osteoporosis.
[Subjects & Methods] Fifty patients (39 females and 11 males) with connective tissue diseases under glucocorticoid therapy were enrolled in this study. The mean of age, daily glucocorticoid dosage (prednisolone equivalent), and total glucocorticoid dosage were 43 years old, 17mg/day, and 4.6g, respectively. Serum OPG and urinary Cross-linked N-telopeptide (NTX) were measured by ELISA, respectively.
[Results] 1) Among patients who started to take glucocorticoids (an average dosage of 37mg/day) but not anti-osteoporosis agents, OPG levels decreased after four weeks significantly (from 3.7+/-1.2 to 2.6+/-1.1 pmol/ml, $p<0.05$). Anti-osteoporotic agents did not change this decrease. On the other hand, NTX values increased after four weeks (an average of 158% of pre-value, $p<0.02$).
3) A significant positive correlation was found between OPG and NTX ($p< 0.01$) before the start of glucocorticoid treatment. Patients under glucocorticoids, however, there was no correlation between these levels.
4) OPG was positively correlated with a change in bone mineral densities of the lumbar spine after 1 year ($p<0.02$).
[Conclusion] These results suggest that 1) bone absorption is increased, at least in part, with suppression OPG by glucocorticoids in early stage of glucocorticoid treatment and 2) a meseament of serum OPG may be a predictive marker of bone mineral densities in glucocorticoid-induced osteoporosis.

Disclosures: ***I. Tanaka****, None.*

M304

Dietary Calcium Affects the Intertrochanteric Region of the Femur. J. M. Welch[1], C. M. Weaver[1], C. H. Turner[2]. [1]Foods and Nutrition, Purdue University, West Lafayette, IN, USA, [2]School of Medicine, Indiana University, Indianapolis, IN, USA.

The intertrochanteric region of the femur is a common site of osteoporotic fracture in the elderly, and we investigated whether this site was preferentially affected by a diet containing suboptimal levels of calcium. We therefore examined the effects of a marginal calcium (Ca) diet on the femur of growing rats. F-344 female rats, aged 6 weeks were fed control diets containing 0.5% Ca (CaCon) or 0.2% Ca (CaLow) for 8 weeks. They were subsequently sacrificed and their femurs scanned by peripheral quantitative computed tomography (pQCT) at 3 sites: intertrochanteric (78% distal), midshaft (50% distal), and distal metaphyseal (10% distal). Scan images were analyzed for geometry, volumetric bone mineral density (vBMD), and bone mineral content (BMC) of total, cortical, and trabecular bone. Body weights and bone lengths were the same for both treatments. However, the moderately low calcium diet detrimentally affected the geometry, mineral density and content of the femurs in the intertrochanteric and midshaft sites, with the greatest differences observed in the intertrochanteric site. At this location, the cross-sectional area of the cortical fraction of the bone (CSAcort) was 9.5% lower in the CaLow rats, the vBMD of the whole bone (vBMDtot) was 5.5% less, and the vBMD of the trabecular fraction of the bone (vBMDtrab) were reduced by 10.8% (all $p< 0.05$). Additionally, both the BMC of the whole bone (BMCtot) and the cortical fraction (BMCcort) were lower in femurs of the CaLow rats by 9.2% ($p<0.01$) and 9.8% ($p<0.05$), respectively, at that site. The midshaft of the femurs were also affected by the CaLow diet. At this entirely cortical site, the CSAcort was reduced by 6.7%, vBMDcort by 0.8%, and BMCcort by 7.4% (all $p<0.01$). In contrast to the 2 more proximal sites, scans at the distal metaphysis did not indicate differences between CaLow and CaCon in any of the parameters examined. The lesser CSA and vBMD in the cortical bone at the midshaft of the femur suggest a loss of strength at that site. In the intertrochanteric site, the loss of cortical bone in the CSA and decreased vBMD in the trabecular bone indicate that both cortical and trabecular fractions of the bone were likely weakened by the low Ca diet. The responsive of the intertrochanteric area to Ca intake suggests that a suboptimal Ca diet may be a factor in the high incidence of fracture at this site.

Disclosures: ***J.M. Welch****, None.*

M305

Glucocorticoid-Induced Alterations in Biochemical Markers of Bone Turnover in Adult Cynomolgus Monkeys Mimic Human Bone Disease. V. Shen[1], R. M. Perez*[2], F. P. De Villa*[2], R. C. Tianzon*[2], T. Hayashi*[2], I. Itagaki*[3], T. S. Bailey*[1], S. Bain[1], C. P. Jerome[1]. [1]SkeleTech, Inc., Bothell, WA, USA, [2]Ina Research Philippines, Inc., Laguna, Philippines, [3]Ina Research, Inc., Nagano, Japan.

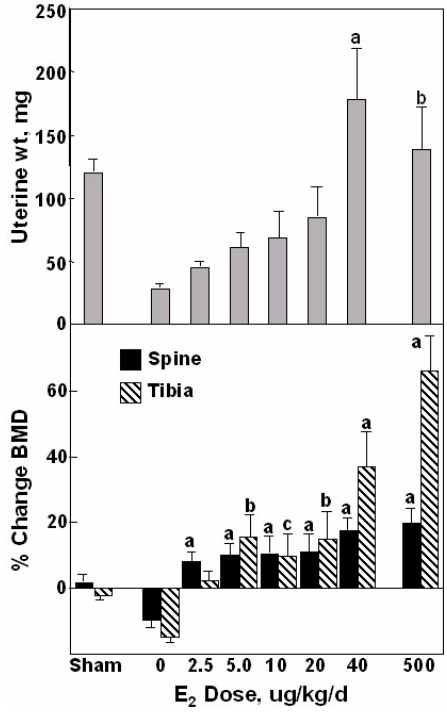
Glucocorticoid use is the most common form of drug-related osteoporosis, and its long-term administration for medical disorders such as arthritis and chronic pulmonary disease is associated with a high rate of fracture. While rodent models have been widely used to investigate the relationship between glucocorticoid (GC) use and bone loss, the utility of the nonhuman primate has been largely ignored. This is despite the close phylogenetic relationship between nonhuman primates and man and the similarities in bone remodeling and responsiveness to hormonal influences. The objective of this study was to therefore investigate the changes in bone turnover in response to intravenous glucocorticoid administration in adult cynomolgus monkeys. Experimentally, the animals were divided into 2 groups, each comprising 3 males and 3 females: one group received intravenous (IV) injections of 1 mg/kg dexamethasone phosphate while the placebo group was injected with distilled water. Serum samples obtained at baseline and on days 2, 4, 8 and 14 were tested for osteocalcin (OC), bone-specific alkaline phosphatase (B-ALP) and type I collagen cross-linked N-telopeptide (NTx). Accumulated 24-hour urine collected at baseline and on days 2, 4, 8 and 13 were analyzed for NTx. Compared to the placebo-treated group, significant reductions in serum OC levels were observed early and throughout the course of dexamethasone treatment (29.61%, 47.53%, 56.69% and 56.27% decline on days 2, 4, 8 and 14, respectively, at $p=0.01$). In contrast, dexamethasone caused significant increases in serum (92.54%, 60.19% and 81.41% on days 4, 8 and 14) and urinary NTx (78.73% on day 14). There were no significant effects of GCs on B-ALP . These findings indicate that IV administration of dexamethasone inhibits bone formation and increases bone resorption in cynomolgus monkeys as evidenced by a decrease in serum osteocalcin, and an increase in the levels of the serum and urinary NTx. These data are essentially identical to the effects of GCs on bone biomarkers in humans and provide evidence that the cynomolgus monkey model can be used effectively in evaluating the skeletal actions of glucocorticoids.

Disclosures: ***V. Shen****, None.*

M306

Dose Response of Estradiol on Bone and the Uterus in Mice: Evidence that Bone Is More Sensitive to Estrogen than Reproductive Tissue. U. I. L. Moedder[1], B. L. Riggs[1], T. C. Spelsberg[2], D. G. Fraser*[1], E. J. Atkinson*[3], S. Khosla[1]. [1]Endocrinology, Mayo Clinic, Rochester, MN, USA, [2]Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA, [3]Biostatistics, Mayo Clinic, Rochester, MN, USA.

Mice are increasingly used for studies of estrogen (E) action on bone, in part due to the availability of numerous transgenic and knock out models, which are usually in a C57BL/6 background. However, doses of estradiol (E_2) used in these studies are often pharmacological, and very high doses of E_2 appear to result in osteosclerosis in mice with evidence of increased bone formation in addition to the physiological actions of E on inhibiting bone resorption. Moreover, there is little data directly comparing the dose response of E_2 on bone and a reproductive tissue such as the uterus. In the present study, we compared the effects of sham surgery or ovariectomy (ovx) with pellets delivering 0, 2.5, 5, 10, 20, 40, or 500 μg/kg/d of E_2 for 60 days in 6 month old C57BL/6 mice (n = 6-12 per group). Bone mineral density (BMD) was measured both by DXA and by pQCT. As shown in the lower panel of the Figure, extremely low doses of E_2 were able to prevent ovx-induced bone loss in these mice (a, $P < 0.01$ and b, $P < 0.001$ vs ovx). The skeletal response to E_2 at the spine (L_1-L_4, black bars) was maximal even at these low doses, whereas BMD at the proximal tibia (hatched bars) appeared to plateau and then increase further at the 40 μg/kg/d and even more so at the 500 μg/kg/d dose, consistent with induction of the pharmacologic osteosclerotic effect of E at the tibial metaphysis. Of note, prevention of ovx-induced bone loss was achieved by doses of E_2 that had minimal effect on uterine weight (Figure, upper panel). These findings thus indicate that: (1) extremely low doses of E_2 can prevent ovx-induced bone loss in mice; (2) the response of the spine versus the proximal tibia to E_2, while similar at low doses of E_2, clearly differs at high doses, with the apparent osteosclerotic effect of E_2 present only at the tibia; and (3) the skeleton is much more responsive to E than the uterus. A better understanding of the cellular and molecular mechanism(s) for the differential sensitivity of bone versus reproductive tissue to E may lead to improvements in the design of selective estrogen receptor modulators or other compounds with beneficial skeletal effects but without adverse side-effects on reproductive and perhaps other tissues.



Disclosures: ***U.I.L. Moedder****, None.*

M307

Importance of Osteoprotegerin and Estradiol Serum Levels on Bone Mass at Menopause. M. Muñoz Torres[1], P. Mezquita-Raya*[2], M. de la Higuera*[1], D. Fernandez*[1], G. Alonso*[1], M. E. Ruiz-Requena*[3], F. Escobar-Jiménez*[1]. [1]Endocrinology, University Hospital San Cecilio, Granada, Spain, [2]Endocrinology Division, Hospital "Torrecardenas", Almeria, Spain, [3]Biochemistry Division, University Hospital "San Cecilio", Granada, Spain.

In vitro and animal studies have revealed the role of OPG as a decoy receptor that neutralizing the effect of RANKL on the differentiation and proliferation of osteoclasts. However, the relationship between serum concentrations of OPG and bone mass is uncertain, with different studies yielding different results.

AIMS: to examine the association between circulating levels of OPG and bone mineral density (BMD) in postmenopausal women.

SUBJECTS AND METHODS: We determined anthropometrics parameters, serum levels of OPG (OPG ELISA BI-20402, BIOMEDICA-GRUPPE, Bensheim, Deutschland) and estradiol (DSL-39100 3rd Generation Estradiol RIA, Diagnostic System Laboratories, Inc, Texas, USA), and BMD by dual X-ray absorptiometry (DXA; Hologic QDR4500) at lumbar spine (LS) and femoral neck (FN) in 204 ambulatory healthy postmenopausal spanish women (age: 61±7, years since menopause (YSM): 13.8±8.5).

RESULTS: Mean OPG and estradiol concentrations were 117±55 pg/mL and 19.9±11.4 pg/mL, respectively. LS and FN BMD (z-score) were -0.59±1.03 and -0.36±1.05. OPG levels were significantly related to age (r=0.204; p=0.003), YSM (r=0.196; p=0.005) and LS Z-Score (r=0.199; p=0.004), but not with estradiol (r=0.051; p=0.472). Estradiol were significantly related to age (r=-0.21; p=0.003), YSM (r=-0.172; p=0.014), LS BMD (r=0.298; p<0.001) and FN BMD (r=0.145; p=0.038). In multiple regression analysis, LS BMD was significantly associated with OPG and estradiol after controlling for age, YSM and body mass index.

CONCLUSION: we conclude that OPG and estradiol serum levels are independently associated with bone mass in postmenopausal spanish women. However, our results dont support a relation between circulating OPG and estradiol at menopause.

(Supported by Research Founding from Eli Lilly & Co)

Disclosures: ***M. Muñoz Torres****, Eli Lilly & Company 2.*

M308

The 'In Vivo' Gene Expression Changes of RANKL/OPG/RANK in Human Mononuclear Cells Following Treatment with Estrogen or Raloxifene : Possible Role in the Reduction in Bone Resorption. A. Bashir*[1], Y. T. Mak*[1], S. Sankaralingam*[1], J. Cheung*[1], I. Fogelman*[2], G. Hampson[1]. [1]Chemical Pathology, St Thomas Hospital, London, United Kingdom, [2]Osteoporosis Screening Unit, Guy's Hospital, London, United Kingdom.

Previous studies have suggested that bone marrow mononuclear cells , including T and B lymphocytes may be implicated in the increases in bone resorption in post-menopausal osteoporosis through the up-regulation of RANKL. There have been no studies, to date, on the *'in vivo'* changes in the RANKL/OPG/RANK pathway in these cells following estrogen replacement therapy (ERT) . We investigated the changes in RANKL, OPG and RANK gene expression in peripheral mononuclear cells (PMNC's) as surrogates for bone marrow cells in a group of post-menopausal women with osteoporosis following treatment with estrogen or the SERM, Raloxifene (RAL). This was a 12-month intervention study. We studied 24 women, mean (SD) age 68.3 (8.2) years. They were divided into 2 groups, ERT group n = 9, RAL group n = 15. Bone mineral density (BMD) was measured at baseline and at 12 month. Serum carboxy-terminal telopeptide (CTX), OPG, RANKL were measured at baseline and at 1, 3, 6 and 12 month by ELISA. In a sub-group of subjects, ERT (n = 5), RAL (n = 11), PMNC's were isolated at baseline, 1, 3 and 6 month following treatment. RNA was extracted for gene expression analysis of RANKL, OPG and RANK by Real-time PCR. Values are expressed as % compared to baseline (100%). We observed a significant increase in BMD at the lumbar spine in both treatment groups(mean [SEM], ERT : 3.7% [1.9] P = 0.047, RAL : 4.1 [1.2] P = 0.0026). A significant reduction in serum CTX was seen following treatment in both groups with a nadir at 3-6 months (percentage change from baseline, ERT : 57 % [5] p < 0.001, RAL : 35% [9] p < 0.01). Serum OPG showed a gradual decrease over time (12 month, ERT : 40 % [6], RAL : 32 [6] P < 0.001). Serum RANKL was detectable in only 7 subjects (ERT n = 4, RAL n= 3) and showed a trend towards a decrease with treatment (12 month ERT : 61% [22], RAL 19 % [17.3] NS). RANKL gene expression decreased gradually following treatment in both groups (6 month, ERT : 30.5 % P = 0.09, RAL : 60 % P= 0.1). OPG gene expression decreased significantly (6 month ERT : 20.7% P < 0.05, RAL : 56.3 % at 3 month P = 0.04 and 21.4 % at 6 month P = 0.003). RANKL:OPG mRNA ratio decreased at 1 month (ERT : 49.2%, RAL : 77.8%) but gradually increased at 6 month (ERT : 140 % , RAL : 200% P = 0.06) compared to values at 1 month. RANK gene expression decreased following treatment (1 month ERT : 68% P = 0.1, 6 month RAL : 68% P = 0.008). The changes in the RANKL/OPG/RANK gene expression in mononuclear cells may contribute to the reduction in bone resorption and slowing of bone remodelling following treatment with estrogen or Raloxifene.

Disclosures: ***A. Bashir****, None.*

M309

Effect of Depot Medroxyprogesterone Acetate on Bone Mineral Density at the Spine, Hip and Forearm. J. S. Walsh, R. Eastell, N. F. Peel. Bone Metabolism Group, University of Sheffield, Sheffield, United Kingdom.

Previous studies of the injectable contraceptive depot medroxyprogesterone acetate (DMPA, Depo-Provera) and bone mineral density have produced differing results; a bone mineral deficit associated with DMPA use has been demonstrated more consistently when the lumbar spine and hip are measured than when the forearm is measured. This study aims to examine for a heterogeneous skeletal response to DMPA. We measured bone mineral density by dual energy x-ray absorptiometry at the lumbar spine, total hip (Hologic Delphi) and distal forearm (Osteometer DTX 200) in 58 women ages 18 to 45 who had been using DMPA for at least 12 months (range 12-90). Results are expressed as Z scores calculated from 99 normal premenopausal Sheffield women ages 20 to 40.

Z scores were analysed by one-way ANOVA with Bonferroni correction. There were significant differences between sites (p= 0.003).

	Mean Z score	95% CI	ANOVA
Lumbar Spine	-0.72	-0.96 to -0.48	a
Total Hip	-0.33	-0.63 to 0.00	ab
Distal Forearm	0.00	-0.32 to 0.17	b

a and b differ at 5% significance level

These results show that long-term DMPA use is associated with a deficit in bone mineral density at the lumbar spine and hip, but not at the forearm. This is an unexpected finding, as experience with post-menopausal osteoporosis suggests that the forearm should be affected by sex steroid deficiency. This challenges the hypothesis that the effects of DMPA on the skeleton are mediated purely through oestrogen deficiency, and it may be that DMPA as a progestagen has a protective effect at the forearm.

Disclosures: ***J.S. Walsh****, None.*

M310

Low Serum Total and Bioavailable Testosterone Levels are Associated with Lower BMD in Elderly Women. P. B. Rapuri[1], J. C. Gallagher[1], G. Haynatzki*[2]. [1]Bone Metabolism Unit, Creighton University, Omaha, NE, USA, [2]Department of Medicine, Creighton University, Omaha, NE, USA.

Recent evidence suggests that very low levels of serum endogenous hormones are related to bone mineral density (BMD) and the rate of bone loss in elderly women. We have earlier demonstrated that low levels of serum total and bioavailable estradiol are related to BMD and the rate of bone loss in elderly postmenopausal women. In the present study, we examined whether serum total (TT) and bioavailable testosterone (BioT) levels are associated with BMD and the rate of bone loss in these women. The study population constitutes 489 elderly women (mean age 71 ± 3 years) recruited for an osteoporosis intervention study. The baseline data of these women were used to test the relation between TT, BioT and BMD, and the data collected on 112 women in the placebo arm followed for 3 years were used to test the relation between the TT, BioT and the rate of bone loss. BMD and the rate of bone loss were compared across the tertiles of TT and BioT. BMD measurements were performed by DEXA. Serum TT was measured by sensitive RIA and BioT was determined as the difference between the total and SHBG bound testosterone. The data were analyzed by Pearson's correlation and procedure Mixed from the SAS statistical package. Adjustments were made for appropriate covariates like age, weight etc. There was a significant correlation between TT and Bio T (r=0.72, p<0.001). The women in the lowest tertile of TT and BioT had significantly (p<0.05) lower BMD at proximal femur, total body and radius compared to those in the highest tertile. There was a trend of increased bone loss in the women in the lowest tertile of TT and Bio T in comparison to those in the highest tertile, but the differences were not statistically significant. In conclusion, these results suggest that endogenous levels of TT and BioT are related to BMD in the elderly and may contribute to pathogenesis of osteoporosis.

Effect of Endogenous Testosterone Levels on Baseline BMD

	Spine	Femoral neck	Total Body
BioT			
Tertile 1	1.0±0.02	0.74±0.01	0.98±0.01
Tertile 3	1.06±0.01	0.79±0.01*	1.04±0.01*
Total T			
Tertile 1	1.0±0.02	0.74±0.01	0.99±0.01
Tertile 3	1.03±0.01	0.78±0.01*	1.03±0.01*

Values are mean±SEM. *p<0.05 compared to tertile1.

Disclosures: ***P.B. Rapuri****, None.*

M311

Low Endogenous Estradiol Levels Maintain Bone Mass in Younger Postmenopausal Women After One Year Follow Up. A. Bagur[1], B. Oliveri[1], S. Mastaglia*[1], A. Cristófari*[1], D. Yankelevich*[2], F. Sayegh*[2], M. Royer*[2], C. Mautalen[1]. [1]Sección Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Sección Climaterio, División Ginecología, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina.

Estradiol levels higher than 10 pg/ml and testosterone were involved in the maintenance of bone mineral density (BMD) in healthy postmenopausal (PMP) women as reported in a previous cross-sectional study.

The aim of this study was to evaluate if the low endogenous estradiol levels protect BMD along one year of follow up without any preventive treatment for osteoporosis. Preliminary results of 34 PMP women aged 55 to 75 years were included. We excluded 3 patients because they started specific osteoporosis treatment. The BMD of L2-L4, Total Femur (TF) and Total Skeleton (TS) was measured by DXA (Lunar Prodigy). Serum calcium,

bone alkaline phosphatase (BAP), crosslaps (CTX), estradiol and testosterone, and urine calcium were measured. Estradiol was measured with a sensitive assay that detects up to 5 pg/ml. The population was divided in two groups: ≤65 (n:19) and >65 years of age (n:15). The preliminary biochemical results of the population were (mean ± DS): Serum calcium 9.4±0.4 mg/dl, phosphate 3.9±0.4 mg/dl, BAP 69.2±23.2 UI/L and urine calcium 152±83.1 mg/24hs.

The groups were also stratified according to baseline estradiol levels: ≤10 and >10 pg/ml and the results were analyzed as a percentage of change of estradiol and BMD from the baseline to one year follow up. Younger PMP women (≤65) who had > 10 pg/ml of estradiol at baseline maintained their BMD at all skeletal sites and estradiol levels. On the other hand PMP women >65 with > 10 pg/ml of estradiol at baseline had a diminution of their estradiol levels (-44%, p<0.01) and also of the BMD of the TF (p<0.05), TS (p<0.01) and L2-L4 (p 0.07,ns). All the population with ≤10 pg/ml of estradiol decreased their BMD at all skeletal areas although the change did not reach statistical significance.

In conclusion, 1- Endogenous estradiol levels more than 10 pg/ml maintained the BMD in younger postmenopausal women along one year follow up without preventive treatment for osteoporosis.

2-Healthy postmenopausal women > 65 years of age had an important changes in the estradiol levels (-44%) accompanied with a decrease in the BMD

Disclosures: ***A. Bagur****, None.*

M312

See Friday Plenary number F331

M313

Safety and Efficacy of Risedronate in Reducing Vertebral Fracture Risk in Elderly Women with Documented Osteoporosis. S. Boonen[1], R. Eastell[2], I. P. Barton*[3], M. R. McClung[4]. [1]Division of Geriatric Medicine, Center for Metabolic Bone Disease, Leuven, Belgium, [2]University of Sheffield, Sheffield, United Kingdom, [3]Procter & Gamble Pharmaceuticals, Egham, United Kingdom, [4]Oregon Osteoporosis Center, Portland, OR, USA.

There is currently little information on the efficacy of antiresorptive agents in preventing osteoporotic fractures in elderly patients, and no published evidence in persons over 80 years of age that these agents lead to greater reductions in fracture risk than calcium and vitamin D alone. The present analysis was conducted to determine the efficacy of risedronate in reducing vertebral fracture risk in elderly women with osteoporosis. The analysis included osteoporotic (femoral neck bone mineral density T-score <-2.5SD and/or at least one prevalent vertebral fracture) women 75 years of age or older in the placebo (n=1507) and RIS 5 mg/day (n=1531) groups from the 3-year Hip Intervention Program (HIP) and the two Vertebral Efficacy with Risedronate Therapy (VERT) studies. All patients received 1000 mg/d calcium and, if baseline levels were low, up to 500 IU/d vitamin D. After 1 year, the risk of new vertebral fractures in women 75 years of age or older was reduced by 65% compared with placebo (95% CI, 46%-77%; $P<0.001$). This early onset of efficacy was consistent across the clinical programs, and antifracture efficacy was maintained over 3 years. Similar risk reductions were observed in individuals 80 years of age or older, both at 1 and 3 years. RIS was well tolerated, with a safety profile comparable to placebo. These findings support the concept that, even in the oldest old, reducing bone resorption rate remains an effective osteoporosis treatment strategy.

Disclosures: ***S. Boonen****, None.*

M314

A Prospective Study of Risedronate on Regional Bone Metabolism and Blood Flow at the Lumbar Spine Measured by ^{18}F-fluoride Positron Emission Tomography. M. L. Frost[1], G. J. R. Cook[2], G. M. Blake[1], P. K. Marsden*[1], I. Fogelman[1]. [1]Guy's, King's & St Thomas' School of Medicine, London, United Kingdom, [2]Department of Nuclear Medicine, The Royal Marsden Hospital, London, United Kingdom.

Quantitative radionuclide studies of bone reflect bone blood flow and regional osteoblastic activity, and the latter should change after treatment with a bisphosphonate, although this has not been previously demonstrated. The aim of this study was to examine regional ^{18}F-fluoride kinetics at the lumbar spine measured by ^{18}F-fluoride positron emission tomography (PET) before and after treatment with risedronate. Eighteen women, with a mean age of 67.0 years and a T-score of less than –2 at the spine or hip, had a dynamic PET scan of the lumbar spine after the injection of 90 MBq ^{18}F-fluoride ion at baseline and 6-months after commencing risedronate therapy. The arterial plasma input function was derived using aorta arterial activity from the PET image. Time activity curves were measured by placing regions of interest over the lumbar vertebrae. A three-compartmental model was used to calculate bone blood flow (K_1) and the net plasma clearance of tracer to bone mineral (K_i). Rate constants k_2, k_3 and k_4, which describe transport between plasma, the ECF compartment and the bone mineral compartment, were also measured. Mean vertebral K_i decreased significantly by 18.4 % from baseline (3.32×10^{-2} ml min^{-1} ml^{-1}) to 6-months post treatment (2.71×10^{-2} ml min^{-1} ml^{-1}), p=0.04. This decrease was similar in magnitude to the decrease observed for bone-specific alkaline phosphatase, a marker of bone formation. There was no significant difference in K_1 from baseline (1.49×10^{-1} ml min^{-1} ml^{-1}) to 6-months after treatment (1.38×10^{-1} ml min^{-1} ml^{-1}) p>0.05. There was a significant increase in k_2, reflecting the reverse transport of fluoride from the extravascular tissue compartment to plasma, after 6 months of treatment (2.90×10^{-1} min^{-1} vs. 4.43×10^{-1} min^{-1}, p 0.01). No significant changes were seen for k_3 or k_4. There was a significant decrease from baseline in the fraction of tracer in the extravascular tissue space that underwent specific binding to the bone matrix ($(k_3/(k_2+k_3))$, decreasing by 18.1%, p=0.02. In conclusion K_i, the net plasma clearance to bone mineral reflecting bone blood flow and regional osteoblastic activity, displayed a significant decrease after 6-months of antiresorptive therapy. This is the first study to show a direct metabolic effect of antiresorptive therapy on skeletal kinetics at the clinically important site of the lumbar spine. The use of ^{18}F-fluoride PET may provide a useful non-invasive tool to assess novel treatments currently being developed for osteoporosis.

Disclosures: ***M.L. Frost****, None.*

M315

Dissociation of the Anti-Apoptotic Effects of Bisphosphonates on Osteocytes/Osteoblasts from their Pro-Apoptotic Effects on Osteoclasts with Novel Analogs. L. I. Plotkin, B. Laska*, S. C. Manolagas, T. Bellido. Endocrinology, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, Univ. Arkansas for Med. Sci., Little Rock, AR, USA.

Bisphosphonates (BPs) induce osteoclast apoptosis, thereby decreasing bone resorption. Additionally, BPs prevent osteocyte apoptosis and thereby preserve the integrity of the osteocyte network. This latter effect may also contribute to their anti-fracture efficacy. Whereas induction of osteoclast apoptosis results from inhibiting the mevalonate pathway, prevention of osteoblastic cell apoptosis is mediated by connexin43 hemichannel opening and activation of the extracellular signal regulated kinases ERKs. In this study, we examined the ability of several BPs, including novel analogs, to exert these two effects. Induction of apoptosis was examined in osteoclasts generated from Raw 264.7 cells treated with M-CSF and soluble RANK ligand for 4 days. Mature osteoclasts were subsequently exposed to 10^{-4}M BPs for 24 h and apoptosis was assessed by measuring caspase3 activity using the fluorogenic substrate AFC-DEVD. Prevention of apoptosis was examined in MLO-Y4 osteocytic cells treated with 10^{-11} to 10^{-5}M BPs for 1h, followed by addition of etoposide for 6h. Apoptosis was quantified by trypan blue uptake and by measuring caspase3 activity with either AFC-DEVD or the caspase3 sensor protein yellow fluorescent protein-DEVD that translocates to the nucleus upon caspase3 activation. All 17 BPs studied inhibited etoposide-induced apoptosis of osteocytic cells with an EC50 (concentration needed to obtain a 50% reduction in apoptosis) of 10^{-11}-10^{-10} M. On the other hand, only 11 analogs induced osteoclast apoptosis. Each of the 6 BPs that lack pro-apoptotic activity in osteoclasts but retain anti-apoptotic activity in osteocytes (designated "osteocyte-selective") has a structural-related analog that is active in both cell types. Specifically, the osteocyte-selective BPs are closely related to alendronate, olpadronate, minodronate, risedronate, or a pyridyl BP. These findings indicate that the structural prerequisites for the anti-apoptotic effect of BPs on osteoblastic cells are less stringent than the ones required to cause osteoclast apoptosis, and confirm that these agents act on the two cell types by distinct mechanisms. The demonstration of osteocyte-selective BPs opens new possibilities for the treatment of bone fragility in conditions in which a decrease in bone remodeling is not desirable. The use of these analogs might be also valuable as a tool in determining the contribution of osteocyte viability and the osteocyte network to bone strength.

Disclosures: ***L.I. Plotkin****, None.*

M316

High Dose Risedronate Treatment Incompletely Preserves Cancellous Bone Mass and Microarchitecture after Long-term Disuse in Older Adult Dogs. C. Y. Li*, D. M. Laudier*, C. J. Hernandez, M. B. Schaffler. Orthopaedics, Mount Sinai School of Medicine, New York, NY, USA.

Bone loss in disuse osteoporosis results principally from extremely elevated bone resorption. Currently, bisphosphonates are the most effective inhibitors of osteoclast activity and have been shown to effectively prevent bone loss in postmenopausal osteoporosis. Their efficacy in preventing long-term disuse osteoporosis is unknown. In the current study, we examined whether risedronate at high dose can prevent bone loss from long-term disuse. Right forelimbs of 5-7 years old retired breeder beagle dogs were immobilized (IM) long-term using a custom designed splint. Age matched, non-immobilized dogs served as controls. Half the animals from each group received risedronate daily (1 mg/kg, P.O.) for the 12 month duration of the study. The remaining dogs received only the sterile water vehicle. Changes of cancellous bone mass and microarchitecture were assessed by histomorphometry on undecalcified sections from the distal metaphyses of second metacarpal of the right forelimb.

At 12 months after IM, there was a significant (p<.0001) reduction of cancellous bone mass (Tb.Ar/T.Ar, -71%), resulting from a marked decrease in trabecular thickness (Tb.Th, -48%) and number (Tb.N, -43%). Bone formation (L.Pm/B.Pm, BFR/B.Ar), resorption (E.Pm/B.Pm, Oc.S/B.Pm, Oc.N/B.Pm) as well as the activation frequency (Ac.f) were significantly higher in IM group compared to the control group (p<.01); these results indicate that disuse bone loss at this long-term period is a "high turnover" type process. Risedronate-treatment in IM animals reduced the amount of bone loss, but this effect was incomplete. Even with the bisphosphonate treatment, IM animals lost nearly 50% of cancellous bone mass, while Tb.Th. and Tb.N were reduced by 31% and 25%, respectively (p<0.006). Activation frequency and bone formation were significantly reduced with risedronate treatment, whereas bone resorption remained elevated in the treated IM animals. These data indicate that high dose risedronate cannot completely protect bone loss from prolonged decrease of mechanical loading. This failure to protect bone mass and suggests that antiresorptive strategy alone is inadequate to preserve bone integrity after long-term disuse osteoporosis.

Disclosures: ***C.Y. Li****, None.*

M317

Minodronic Acid Prevents Bone Loss, Increased Bone Markers and Decreased Mechanical Strength in Ovariectomized Cynomolgus Monkeys. M. Tanaka*, H. Mori*, R. Kayasuga*, Y. Ochi*, N. Kawada*, H. Yamada*, K. Kawabata*. Ono Pharmaceutical Co., Ltd., Osaka, Japan.

Present study examined the effects of an aminobisphosphonate, minodronic acid (ONO-5920/YM529) on bone loss, bone turnover markers and mechanical strength after ovariectomy (OVX) in cynomolgus monkey. Forty-eight adult female cynomolgus monkeys, were assigned into four groups (one sham group and three OVX groups) each consisting of 12 animals. In OVX groups, monkeys were orally administered either a vehicle (lactose) or two doses of minodronic acid (0.015 or 0.15 mg/kg) once a day for 17 months beginning the day after ovariectomy. Bone mineral density (BMD; dual x-ray absorptiometry) and bone turnover markers (urinary NTX and deoxypyridinoline, serum osteocalcin and BAP) were measured every four months. In the vehicle treated OVX group, lumbar spine BMD was significantly reduced and bone turnover markers were persistently increased as compared with the sham group after four month post-surgery. Minodronic acid treatment significantly prevented the loss of lumbar spine BMD and incensement of bone turnover markers in a dose dependent manner. Seventeen months after ovariectomy, monkeys were sacrificed and their mechanical strength of lumber vertebral body, femoral shaft and femoral neck were measured. Minodronic acid significantly prevented loss of the ultimate compressive load of 4th lumbar vertebral body and femoral neck. Microarchitecture of trabecular bone will also be discussed. We conclude that oral minodronic acid treatment prevents loss of BMD and mechanical strength of lumber spine in OVX monkeys.

Disclosures: ***M. Tanaka****, None.*

M318

Osteopenia Induced by Immunosuppressive Drugs: Preliminary Data on Risedronate Effect. L. Dalle Carbonare*, S. Giannini, S. Zordan*, S. Sella*, S. Pavan*, L. Sartori, G. Crepaldi. Dept. of Medical and Surgical Sciences, University of Padova, Padova, Italy.

Transplantation is nowadays the most reliable treatment for many patients with end-stage organ failure. However, a number of complications can still occur after transplant, and among them post-transplantation bone disease is one of the most frequent. Corticosteroids use is one of the most important pathogenic factors, while more contrasting data have been reported about the effects on bone metabolism of cyclosporin A. Besides, there is very few and non consistent information concerning the effects of the combined immunosuppressive therapy on bone. Bisphosphonates are a powerful therapeutic option to prevent osteoporosis and related fractures even in the prevention of osteoporosis caused by immunosuppressive treatment.

The aims of this study based on the rat model were: 1. to verify osteopenia and structural alterations on bone induced by immunosuppressive therapy with glucocorticoids (GC) and ciclosporin A (CsA); 2. to establish the efficacy of Risedronate (Ris) in the prevention of these effects. We studied 70 female Sprague-Dawley rats, maintained at the same standard diet. The rats were divided randomly into 7 groups of treatment (10 rats each) as follows:

1.Control group: CsA vehicle (alcoholic solution 0.5% 3 times a week s.c.) + vehicle of Methyl Prednisolone (100 µl s.c. of sesame oil 3 times a week) + vehicle of Risedronate (saline solution 3 times a week); 2. Group Methyl Prednisolone (MP): 7 mg/Kg 3 times a week s.c. + CsA vehicle + vehicle of Risedronate (Ris); 3. Group CsA: CsA 3 mg/Kg 3 times a week s.c. + vehicle of MP + vehicle of Ris; 4. Group CsA + MP: 3 mg/Kg 3 times a week s.c. + 7 mg/Kg 3 times a week s.c. + vehicle of Ris; 5. Group CsA + Ris: CsA 3 mg/Kg 3 times a week s.c. + Ris 5 µg/Kg body weight 3 times a week + vehicle of MP; 6. Group MP + Ris: MP 7 mg/Kg 3 times a week s.c. + Ris 5 µg/Kg body weight 3 times a week + CsA vehicle; 7. Group CsA + MP + Ris: CsA 3 mg/Kg 3 times a week s.c. + MP 7 mg/Kg 3 times a week s.c. + Ris 5 µg/Kg body weight 3 times a week.

All the rats were weighed once a week to adjust the dose of drugs to body weight and they were treated for 30 days. Then, the rats were sacrificed. All the rats underwent a bone densitometry at baseline and at the end of treatment.

Data concerning densitometric evaluations on the first 35 rats (5 for each group of treatment) showed that risedronate can counteract the negative effects of immunosuppressive treatment on bone (group 6 vs 2 +8.0%, p< 0001; group 5 vs 3 +7.6%, p< 0001). Histomorphometric evaluations are being carried out on the right tibiae of all the rats.

In conclusion, risedronate seems to prevent immunosuppressive drug-induced osteopenia.

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Disclosures: ***L. Dalle Carbonare****, None.*

M319

Alendronate and Risedronate Have Similar Pharmacokinetic Half-Lives When Analyzed Over the Same Time Interval. A. Porras*, A. Denker*, A. Santhanagopal*, A. G. Daifotis. Merck & Co., Inc., Rahway, NJ, USA.

The pharmacokinetics of alendronate (ALN) elimination have been carefully studied in postmenopausal women with osteoporosis and a terminal half-life ($t_{½}$) of ~11 years, similar to that of calcium and other minerals in bone, has been reported. In contrast, risedronate has been reported to have a $t_{½}$ of 200 to 218 hours (~9 days) based on a 28-day study following single oral and IV doses [DY Mitchell et al. Pharm. Res. 128:2, 166-170, 2001]. The difference in the lengths of the studies can give rise to apparent differences in half-life, since the shorter study might entirely miss the true terminal phase. We reanalyzed the alendronate data to examine the effect that differences in length of followup might have on reported half-life. Twenty-one postmenopausal women with osteoporosis (mean age, 66) received 30 mg intravenous ALN over 4 days (7.5 mg/day), and urinary excretion of alendronate was monitored for 18 months [SA Khan et al., *J Bone Miner Res* 12:1700-1707, 1997]. Data were truncated at 30 days and "observed" $t_{½}$ was estimated from this data set to mimic the risedronate report. Based on 30 days of follow up, the "observed" $t_{½}$ for ALN was 11 days, comparable to the results reported for risedronate. This exercise illustrates that the half-life reported for risedronate is unlikely to be the true terminal half-life, since the length of followup was too short to observe a half-life of years, expected from bone turnover rates and from the observation that competitive binding of radiolabeled risedronate to powdered human bone is similar to that of alendronate.

Disclosures: ***A.G. Daifotis****, Merck & Co., Inc. 1, 3.*

M320

Bisphosphonates In Male Osteoporosis – Long-Term Histomorphologic Changes. B. Muche*[1], M. Hellmich*[1], F. Feyen*[1], G. Delling*[2], J. Semler*[1]. [1]Osteology, Immanuel-Krankenhaus Rheumaklinik, Berlin, Germany, [2]Osteopathology at Inst. of Pathology, Universitaetsklinikum Hamburg, Hamburg, Germany.

Approved therapy of osteoporosis in men includes calcium and vitamine D3, flourides and the bisphosphonate alendronate. We used different bisphosphonate therapies (BT) in severe male osteoporosis since mid 1990s under control of histomorphometry. PATIENTS AND METHODS: We analyzed retrospectively 219 bone biopsies (iliacal Yamshidi punction) in 62 men (mean 53 ± 10 yrs at first biopsy) before and under BT up to 6 years. We differentiated group 0 (before therapy), group 1 = one year, group 2 = 2 to 3 years and group 3 = at least 4 years BT. 48% had preceeding low trauma fractures at the spine, only 30% did not suffer from any fractures before. At time of the biopsy there were lab tests for bone turnover and bone densitometries (lumbar spine and hip, DXA Hologic® QDR 2000) performed. Patients received either Alendronate, Etidronate, Risedronate (at the standard dose for postmenopausal osteoporosis) or Pamidronate (30 mg IV every 3 months). They also took calcium 500-1000 mg/d and vitamine D3 400-1000 IE/d. Some 10 per cent also got substitution according to low testosterone. RESULTS: BMD improved at lumbar spine (T-score –2.04 vs. –2.99; P<0.001) and femoral neck (T –2.27 vs. –2.79; P<0.05). Alkaline phosphatase decreased from 141 to 117 U/l (P<0.001). Unfortunately there were some changes at the lab for CTx and NTx influencing direct comparison; but, parameters of bone resorption decrease and were often very low with BT. In histology there was an increasing suppression of osteoclastic resorption in group 1 (46%) and 2 (53%) vs. group 0 (16%; P<0.05), but as a trend a new osteoclast activity in group 3 (35%; P=0.096). Intertrabecular connectivity and thickness of single trabecules were widely reduced already in group 0 and did not change significantly. There was an increase up to 21% of patients with "giant osteoclasts" during BT (at start only 7%, P=N.S.). There were no severe side effects reported, some patients suffered from gastrointestinal discomfort. There were only 6 new fractures (only 2 at the spine), all in cases with preceeding vertebral fractures. CONCLUSION: BT is secure and effective (improving BMD, only few fractures) in male osteoporosis. Like predicted, there was a reduction of bone turnover in lab tests and histomorphometry. Increasing connectivity or trabecular thickness could not be seen. About 20% of our patients got "giant osteoclasts" in their bone, and there was a new increase in osteoclast activity after >3 yrs of BT. These facts were not reported before and their meanings are unknown. Because the numbers are low (23 patients in group 3), more studies on these phenomena should be done.

Disclosures: ***B. Muche****, None.*

M321

Safety and Efficacy of Zoledronic Acid: A Chart Review. K. H. Mikulec*, M. F. Delaney, S. Hurwitz*, M. S. LeBoff. Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, Boston, MA, USA.

Oral bisphosphonates are effective for the prevention and treatment of osteoporosis, however they are associated with adverse gastrointestinal side effects in some patients. Zoledronic acid (ZA), an intravenous bisphosphonate, is approved for the treatment of hypercalcemia of malignancy, multiple myeloma, and metastatic bone lesions from solid tumors. A randomized trial showed that ZA given once yearly suppresses markers of bone turnover and increases bone density (Reid, NEJM, 2002). We carried out a retrospective chart review in an osteoporosis clinic to investigate the safety and efficacy of ZA for the treatment of osteoporosis. We analyzed data from 45 patients (7 men and 38 women, mean age 64 years, 1 subject with osteogenesis imperfecta) who received at least one infusion of ZA between October 2001 and February 2003. All patients were started on a regimen of 2 mg IV Q 6 months, but 5 subjects have subsequently been switched to a regimen of 4 mg IV Q 1 year. 87% had received previous bisphosphonate therapy. Many patients had serum calcium, phosphate, and creatinine and urinary N-telopeptide (NTx) measurements before and 3 to 8 weeks after ZA infusion. Safety parameters (creatinine, calcium, and phosphate) were similar before and after ZA.

	n	Pre ZA (mean ± SD)	Post ZA (mean ± SD)	P value
Creatinine (mg/dL)	28	0.91 ± 0.25	0.95 ± 0.26	0.09
Calcium (mg/dL)	32	9.5 ± 0.6	9.4 ± 0.6	0.29
Phosphate (mg/dL)	15	3.2 ± 0.6	3.0 ± 0.6	0.15

In general, the infusions were well tolerated. 16% (7) of patients reported side effects (fever/flu-like symptoms most commonly) which were generally mild and transient. However, 2 subjects discontinued treatment with ZA (1 due to worsening of chronic pruritis and 1 due to fever/flu-like reaction). Although most patients had received previous bisphosphonate therapy, median urinary NTx (n=18, subject with osteogenesis imperfecta excluded) decreased by 25% following the initial ZA infusion (pre NTx=32 nmol BCE/mmol cre and post NTx=24 nmol BCE/mmol cre, p=0.02). Our retrospective findings support published data that ZA is safe and well tolerated for patients with osteoporosis and effective in decreasing urinary NTx. While fracture data are needed, ZA may provide a reasonable alternative for patients who are unable to take oral bisphosphonates.

Disclosures: ***K.H. Mikulec****, None.*

M322

Cyclic Intravenous Pamidronate Treatment for Primary Osteoporosis. Y. Chung[1], S. K. Lee*[2], H. Lee*[1], K. W. Lee*[1]. [1]Endocrinology and Metabolism, Ajou University School of Medicine, Suwon, Republic of Korea, [2]Internal Medicine, Dong Eui Medical Center, Pusan, Republic of Korea.

Bisphosphonate treatment increase bone mineral density in osteoporosis. Cyclic intravenous administration might be a convenient alternative for osteoporosis treatment.
Patients diagnosed with osteoporosis by T score of bone mineral density less than -2.5 were included. Subjects who have been diagnosed with secondary osteoporosis or taken any drug affecting bone metabolism were excluded. Thirty miligram of pamidronate with normal saline were administered intravenously every three months. Bone mineral densities were measured by DP-Expert (Lunar Co., WI) before and after treatment.
Lumbar spine (L2-4) bone density was significantly increased after pamidronate treatment for 1yr (n=24, mean±SD, 5.55±5.03%, p<0.05), and 2yrs (n=13, mean±SD, 6.38±8.85%, p<0.05). No clinical hypocalcemia was reported. Acute phase reactions occured in 75% of subjects after first administration.
In conclusion, cyclic intravenous administration of pamidronate significanlty increased lumbar bone mineral density without any serious side effect.

Disclosures: ***Y. Chung****, None.*

M323

Both Oral and Intravenous Ibandronate Effectively Prevent Postmenopausal Bone Loss in Women without Osteoporosis. J. D. Adachi[1], C. Christiansen[2], J. A. Stakkestad*[3], M. McClung[4], A. Burdeska*[5], P. Mahoney*[5]. [1]McMaster University, Hamilton, ON, Canada, [2]CCBR, Ballerup, Denmark, [3]CECOR AS, Haugesund, Norway, [4]Oregon Osteoporosis Center, Portland, OR, USA, [5]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

The already considerable socio-economic costs of postmenopausal osteoporosis (PMO) are predicted to increase in line with the projected growth in the elderly population. Thus, it is important to not only treat patients with established PMO, but also to prevent osteoporosis. Three recent, large-scale, placebo-controlled studies evaluated the efficacy and safety of ibandronate, a potent, nitrogen-containing bisphosphonate, in the prevention of postmenopausal bone loss in women without osteoporosis.[1–3] These studies examined both oral and intravenous (i.v.) formulations, with and without extended between-dose intervals. One study involved 653 women, randomized to 2 years' oral daily ibandronate (0.5mg, 1mg or 2.5mg) or placebo. Another study involved 630 women, randomized to 2 years' treatment with oral weekly ibandronate (5mg, 10mg or 20mg) or placebo. A third study involved 629 women, randomized to 1 years' treatment with ibandronate (0.5mg, 1mg or 2mg) or placebo, given as an i.v. injection once every 3 months. All participants received daily calcium (500mg) supplementation. The primary endpoint was the relative change in lumbar spine (L1–L4) BMD. Ibandronate therapy produced consistent, statistically significant and dose-dependent increases in BMD at the lumbar spine and hip relative to baseline, together with dose-dependent decreases in biochemical markers of bone turnover. The largest gains in lumbar spine BMD versus placebo were seen in women receiving the highest doses of each investigated regimen: at study end, these were 3.1% (p<0.0001), 4.0% (p<0.0001) and 2.9% (p<0.0001) for the oral daily (2.5mg), oral weekly (20mg), and i.v. ibandronate (2mg) regimens, respectively. All three regimens were well tolerated, with no clinically important safety concerns. In conclusion, ibandronate, whether administered orally on a daily or weekly basis, or as an intermittent i.v. injection once every 3 months, is effective in preventing postmenopausal bone loss, and is well tolerated. The development of compatible intermittent oral and i.v. ibandronate regimens will provide physicians with the flexibility to individualize care in response to specific patient circumstances, which may be a significant benefit when ensuring long-term compliance in the preventive setting.
1. J Bone Miner Res 2002;17(Suppl. 1):S157(Abstract 1138).
2. Osteoporos Int 2002;13(Suppl. 1):S16–7(Abstract P42SU).
3. Osteoporos Int 2002;13(Suppl. 1):S17(Abstract P43MO).

Disclosures: ***J.D. Adachi****, None.*

M324

Oral Daily and Intermittent Ibandronate Normalize Bone Turnover and Significantly Reduce Vertebral Fracture Risk: Results from a Large Phase III Study. R. Emkey[1], R. R. Recker[2], J. A. Stakkestad*[3], P. Mahoney*[4], R. C. Schimmer[4]. [1]Radiant Research, Wyomissing, PA, USA, [2]Creighton University School of Medicine, Omaha, NE, USA, [3]CECRO AS, Haugesund, Norway, [4]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

As shown for change in bone mineral density (BMD), increasing evidence suggests a strong relationship between the rate of bone turnover and fracture risk. Hence, measurement of biochemical markers of bone turnover is becoming a widely used endpoint in clinical trials in postmenopausal osteoporosis. Less frequent bisphosphonate dosing is predicted to improve compliance and optimize patient management and benefit. However, the characteristics of bone turnover markers in less frequent dosing regimens are poorly characterized. A recent fracture study (oral iBandronate Osteoporosis vertebral fracture trial in North America and Europe: BONE) demonstrated that oral daily (2.5mg) and intermittent (20mg every other day for 12 doses every 3 months) ibandronate, a potent, nitrogen-containing bisphosphonate, significantly reduce the incidence of new vertebral fractures (VF) by 62% (p=0.0001) and 50% (p=0.0006), respectively, after 3 years.[1] Significant increases in spine and hip BMD were also observed. During the study, change in the rate of bone resorption was assessed by urinary excretion of the C-telopeptide of the alpha chain of type I collagen (CTX/creatinine). Serum osteocalcin concentrations were used to assess bone formation. After 3 months, a highly pronounced reduction in CTX/creatinine and serum osteocalcin was observed in both ibandronate groups versus baseline and placebo, which was sustained throughout the study. After 3 years, CTX/creatinine levels were reduced by 9.3%, 65.3% and 52.7%, and serum osteocalcin levels by 2.1%, 35.8% and 40.9% in the placebo, daily and intermittent arms, respectively, relative to baseline (p<0.0001 vs placebo for all comparisons). Both regimens provided a steady suppression of resorption markers. Although clinically and statistically significant, the magnitude of CTX/creatinine suppression was slightly lower in the intermittent arm versus the daily arm owing to the measurement of residual biomarker values at the end of the between-dose interval. The reduction in osteocalcin levels was comparable between regimens. These results demonstrate that oral ibandronate, whether given daily or with a between-dose interval of >2 months, normalizes the rate of bone turnover to provide significant increases in BMD and strong VF efficacy. This study provides an opportunity to increase our understanding of bone turnover in response to intermittent bisphosphonate therapy.
1. Delmas PD, et al. Osteoporos Int 2002;13(Suppl. 1):S15(Abstract O37).

Disclosures: ***R. Emkey****, None.*

M325

MicroCT Analysis of Transilial Biopsy Specimens from Alendronate (ALN) Phase III Studies. P. Masarachia[1], P. Chavassieux[2], M. Arlot[2], P. J. Meunier[2], A. Santora[1], J. Yates[1], G. A. Rodan[1], D. B. Kimmel[1]. [1]Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA, USA, [2]Faculte de Medecine R. Laennec, INSERM Unite 403, Lyon, France.

Alendronate (ALN), 10mg/d oral for three years, reduced spine fracture incidence by 50% (p<0.05) in Phase III trials of osteoporotic (OP) women (age 62±6yrs). The object here is to compare findings obtained by 2D histomorphometry to those obtained by micro-computed tomography (mCT) in transilial biopsies from a population subset (N=42).
Specimens were embedded and analyzed by histomorphometry (2D) and mCT (3D, 0.02mm resolution) for trabecular bone quantity (bone volume, (BV/TV), structure, (Tb.N and Tb.Th), and density (mCT, Tb.BMD). Degree of mineralization of bone (DMB) was calculated from mCT data (Tb.BMD/mBV/TV). Differences were tested by Mann-Whitney U.
A strong 2D:3D correlation of all common endpoints (BV/TV, Tb.Th, and Tb.N) exists (R=0.77-0.88). By each method BV/TV correlates well with Tb.Th and Tb.N (R=0.73-0.84). The major ALN effect in the ilium (by mCT alone) was increased DMB, as previously reported by microradiography and back scatter analysis, and increased Tb.Th. Additional mCT data are being collected and will be presented.
Conclusions: 2D and 3D histomorphometric methods yield data for bone volume, trabecular number, and trabecular thickness that are highly correlated. Both also show a positive relationship of bone volume to trabecular number and thickness. mCT independently confirms that ALN increased mineralization level of trabecular bone of the ilium in this randomized Phase III trial of early seventh decade OP women.

Endpoint	Units	PBO (N=27)	ALN (N=15)
$SpBMD_i$	g/cm^2	0.756± 0.068	0.749± 0.054
ΔSpBMD	%	+0.65± 2.69	+9.75± 3.23*
ΔuNTxCre	%	-22± 32	-77± 16*
Tb.BMD	mg/mm^3	185± 63	207± 77
DMB	mg/mm^3	9.78± 1.02	10.83± 1.09[x]

Mean±SD; x- P<.05, *- P<.0001 vs. PBO;
SpBMDi- initial spine bone mineral density;
uNTxCre- urinary N-telopeptides/creatinine
Δ - change during study

Disclosures: ***P. Masarachia****, Merck Research Laboratories 1, 3.*

M326

Alendronate Increases Trabecular Bone Connectivity in Elderly Osteoporotic Women. P. Masarachia[1], T. Howard*[2], A. Santora[1], J. Yates[1], G. A. Rodan[1], R. R. Recker[2], D. B. Kimmel[1]. [1]Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA, USA, [2]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Alendronate (ALN) reduces fracture risk and bone turnover in osteoporotic subjects. ALN effects on trabecular bone of the ilium, studied by micro-computed tomography (mCT), are reported here.
Osteoporotic (OP) women (age 69±6yrs) received placebo (PBO) or 5mg/d oral ALN for two years. Transilial biopsy specimens were embedded in methacrylate and analyzed by both histomorphometry (2D) and mCT (3D, 0.02 mm resolution) for trabecular bone quantity, structure, and density (BMD) (Table 1). Degree of mineralization of bone (DMB) was calculated (Tb.BMD/3DBVTV). Differences of ALN and PBO were tested by Mann-Whitney U.
ALN increased bone volume (BV/TV) and trabecular number (Tb.N), and thickness (Tb.Th) (by 2D) (Table 2). Trabecular connectivity (Tb.Conn) (Euler number/mm^3), calculated by mCT was higher in ALN subjects. There was a strong correlation of 2D and 3D endpoints (R=0.70-0.78). By each method, BV/TV correlated well with Tb.Th and Tb.N (R=0.77-0.89). In contrast, the association of Tb.Conn with other endpoints was only moderate (Tb.Th, R=-0.03; BV/TV, R=0.27; Tb.N, R=0.65).
We conclude that in this population: 1) ALN increased bone volume, trabecular number, thickness and connectivity in trabecular bone of the ilium; 2) 2D and 3D provide similar information about traditional trabecular structural endpoints; and 3) bone quantity (BV/TV) by either technique correlates well with Tb.N and Tb.Th, but not with Tb.Conn.

Table 1

Endpoint	Units	PBO (N=33)	ALN (N=14)
SpBMDi	g/cm^2	0.732± 0.090	0.729± 0.101
ΔSpBMD	%	+0.70± 3.85	+6.71± 3.31*
ΔuNTxCre	%	-31± 39	-69± 17*
Tb.BMD	mg/mm^3	113± 55	145± 55
DMB	mg/mm^3	7.96± 2.34	8.29± 1.03

Mean±SD; *- P<.05
SpBMDi- initial spinal bone mineral density
uNTxCre- urinary N-telopeptide/creatinine ratio
Δ- on study change

Table 2

Endpoint	Units	PBO (N=33)	ALN (N=14)
2DBV/TV	%	11.9± 4.9	16.8± 6.0*
2DTb.N	#/mm	1.01± 0.24	1.23± 0.29*
2DTb.Th	μm	115± 32	135± 34*
3DBV/TV	%	13.8± 5.0	17.3± 5.2*
3DTb.N	#/mm	1.21± 0.25	1.46± 0.32*
3DTb.Th	μm	112± 29	118± 25
Tb.Conn	$/mm^3$	3.29± 1.71	4.89± 2.33*

Disclosures: ***P. Masarachia****, Merck Research Laboratories 1, 3.*

M327

Efficacy and Tolerability of Alendronate in Veterans with Renal Insufficiency. K. E. Hansen[1], R. K. Drake*[2], T. R. Good*[2], M. L. Cartledge*[2], M. E. Elliott[2]. [1]Medicine, Veterans Affairs Medical Center, Madison, WI, USA, [2]School of Pharmacy, University of Wisconsin, Madison, WI, USA.

Osteoporosis and fragility fractures are common complications of renal failure. Although oral bisphosphonates are commonly prescribed to preserve skeletal health in such persons, studies investigating the safety and efficacy of bisphosphonates have not been performed, and package inserts state that their use should be avoided in subjects with a creatinine clearance <35 ml/min. To better understand the safety and efficacy of bisphosphonates in patients with renal insufficiency, we initiated a retrospective survey on the tolerability and efficacy of alendronate in male veterans at one Veterans Affairs Medical Center (VAMC). We hypothesized that 1) alendronate is tolerated equally well in subjects with and without renal insufficiency and 2) alendronate is equally effective in both groups. Male veterans who were prescribed alendronate at 10 mg daily or 70 mg/week during the period from 1996 through 2002 were identified from pharmacy records. Patients were included in the study if they had received at least one prescription of alendronate and had at least two bone density measurements. Femoral and lumbar spine bone density was measured by DXA, and creatinine clearance was calculated using the Cockcroft-Gault equation. Controls were defined as those individuals with creatinine clearance ≥ 35ml/min, while renal insufficiency subjects were those with creatinine clearance < 35 ml/min. Medical records were reviewed to assess compliance with therapy, and frequency and indication for discontinuation of therapy. The work was approved by the appropriate institutional review boards of the University of Wisconsin and the Madison, Wisconsin VAMC.
Of the 250 men who met study criteria, results from the first 45 patients are now available. Mean age of veterans was 69.5 years (range 45-87). Twelve study subjects had a calculated creatinine clearance <35 ml/min (mean 28 ml/min), and 33 controls had a creatinine clearance ≥35 ml/min (mean 57 ml/min). Seven of the 12 (58%) subjects with renal insufficiency exhibited stable or improved bone density, compared to 67% of the control group (p>0.5). Fifteen percent of the renal insufficiency group discontinued treatment due to adverse effects, mainly gastrointestinal, compared to sixteen percent of those in the control group (p>0.5).
These results suggest that in patients with renal insufficiency, alendronate is well tolerated and can result in improvement or stabilization of bone density.

Disclosures: ***M.E. Elliott****, Merck & Co. 2.*

M328

Renal Effects of Minimally Nephrotoxic Doses of Ibandronate and Zoledronate Following Single and Intermittent Intravenous Administration in Rats. T. Pfister*[1], E. Atzpodien*[1], F. Bauss[2]. [1]Preclinical Research and Development, F. Hoffmann-La Roche Ltd, Basel, Switzerland, [2]Pharma Research Penzberg, Roche Diagnostics GmbH, Penzberg, Germany.

Rapid, intravenous (i.v.) infusion of high doses of bisphosphonates has been associated with acute renal toxicity. This preclinical study investigated the potential for subclinical renal damage to accumulate to clinically relevant levels when minimally nephrotoxic doses of ibandronate (1mg/kg) or zoledronate (3mg/kg) were given intermittently or as a single dose by i.v. injection. Ibandronate (1mg/kg), zoledronate (1 or 3mg/kg) or isotonic saline was given to groups of six rats by i.v. bolus injection once every 3 weeks for 25 weeks, or as a single i.v. injection in week 25 only. Serum biochemistry was analysed at week 25 and urinalysis performed at weeks 22 (α-glutathione S transferase only) and 25. Rats were killed 4 days after the last dose and the kidneys analysed for histopathological changes. Ibandronate, given as single or intermittent doses resulted in a similar incidence and severity of proximal convoluted tubule (PCT) damage (Table). No accumulation of these histopathological changes occurred over the 25-week study. In contrast to ibandronate, the incidence and severity of renal damage was increased following intermittent dosing of zoledronate (1 and 3mg/kg), compared with a single dose. Interestingly, renal damage accumulated with 1mg/kg zoledronate following intermittent dosing, despite being undetectable after a single dose. The urinary excretion of alpha glutathione S-transferase (marker of PCT injury) was increased with intermittent zoledronate (1 and 3mg/kg) compared with a single dose ($p<0.05$) and confirmed the histopathologically determined accumulation of renal damage. In conclusion, renal damage accumulated when zoledronate was administered at doses that were minimally nephrotoxic or lower, with a between-dose interval of 3 weeks. Renal damage did not accumulate with intermittent dosing of ibandronate. Therefore, a 3-week dose of ibandronate can be regarded as a single and independent administration.

Renal tubular damage after single and intermittent doses of ibandronate and zoledronate.

	Mean severity score		Incidence (per six rats)	
Dosing regimen	Single	Intermittent	Single	Intermittent
Ibandronate (1mg/kg)*	1.0	1.0	1	2
Zoledronate (1mg/kg)*	0.0	1.0	0	3
Zoledronate (3mg/kg)*	1.3	3.0	4	6
Zoledronate (3mg/kg)†	–	1.7	–	3
Zoledronate (3mg/kg)‡	–	1.5	–	6

*degeneration/necrosis of PCT
†degeneration (cytoplasmic swelling/basophilia) of tubules in the outer medulla
‡tubular atrophy with thickened basement membrane

Disclosures: ***T. Pfister****, None.*

M329

Oral Monthly Ibandronate in Postmenopausal Bone Loss: Results from the Monthly Oral Pilot Study (MOPS). J. Y. Reginster[1], C. Wiese*[2], K. Wilson*[2], R. C. Schimmer[2]. [1]University of Liège, Liège, Belgium, [2]F. Hoffmann-La Roche Ltd, Basel, Switzerland.

Prospective evidence has demonstrated a strong patient preference for less frequent bisphosphonate dosing in postmenopausal osteoporosis (PMO). A once-monthly regimen could provide a convenient dosing option that may enhance long-term adherence and outcomes. Ibandronate is a potent, nitrogen-containing bisphosphonate with proven fracture efficacy in regimens with extended between-dose intervals. A randomized, double-blind, dose-finding, phase I study (Monthly Oral Pilot Study: MOPS) explored the tolerability, pharmacokinetics and pharmacodynamics of once-monthly oral ibandronate. A total of 144 postmenopausal women (aged 55–80 years; TSM ≥3 years) with low lumbar spine BMD (mean T-score –1.1) participated. Patients were randomized to one of four oral monthly regimens (n=36 for all arms) for 3 months in a staggered fashion: placebo, 50mg, 100mg, or 150mg ibandronate. To separate the effect of dose from first-time treatment, the 50mg arm was split into two equal groups (n=18) after the first cycle, with patients continuing on 50mg or 100mg ibandronate. Calcium and vitamin D supplementation was not provided to facilitate detection of any effects of high-dose ibandronate. At the studied doses, once-monthly ibandronate was well tolerated, with a similar safety profile to placebo. Adverse events (AEs) were typical of the bisphosphonate class and mild to moderate; no serious AEs or unexpected safety issues were reported despite the considerably higher single doses compared with daily dosing. Systemic exposure (AUC and Cmax) to ibandronate increased with increasing dose and dose-related suppression of biochemical markers of bone resorption relative to baseline was observed (placebo: –12.3% and –5.5% for serum CTX and urinary CTX/creatinine, respectively vs 150mg: –56.7% and –54.1% for serum CTX and urinary CTX/creatinine, respectively) after 3 months. While these results indicate the feasibility of once-monthly ibandronate dosing, the small number of patients and absence of standardized calcium and vitamin D supplementation warrant further exploration of this regimen. However, the feasibility of once-monthly ibandronate dosing is supported by the results from Clinical Trial Simulation (CTS) in which urinary CTX predictions were consistent with the outcomes seen in the MOPS study. A large randomized controlled trial of once-monthly ibandronate is ongoing to further characterize the efficacy and safety of this novel dosing concept in postmenopausal osteoporotic patients. In summary, phase I data support that once-monthly ibandronate appears to be well tolerated and provides dose-dependent reductions in bone resorption.

Disclosures: ***J.Y. Reginster****, None.*

M330

A Reinforcement Message Based on Bone Turnover Marker Response Influences Long-Term Persistence with Risedronate in Osteoporosis: The IMPACT Study. P. D. Delmas[1], B. Vrijens*[2], C. Roux*[3], A. Le-Moigne-Amrani*[4], R. Eastell*[5], A. Grauer*[6], N. B. Watts*[7], H. A. P. Pols*[8], J. D. Ringe*[9], D. Cahall[4]. [1]INSERM Research Unit 403, Lyon, France, [2]AARDEX Ltd., Zug, Switzerland, [3]University René-Descartes, Paris, France, [4]Aventis Pharmaceuticals, Bridgewater, NJ, USA, [5]University of Sheffield, Sheffield, United Kingdom, [6]Procter & Gamble Pharmaceuticals, Geneva, Switzerland, [7]University of Cincinnati, Cincinnati, OH, USA, [8]Erasmus University, Rotterdam, Netherlands, [9]Klinikum Leverkusen, Leverkusen, Germany.

Long-term persistence is important for patients to benefit from treatments for postmenopausal osteoporosis. The IMPACT study assessed the effect of physician reinforcement using bone turnover marker (BTM) data on persistence with risedronate (RIS) treatment. Eligible patients included postmenopausal women aged 65 to 80 years with

spine/hip T-score ≤–2.5 or spine/hip T-score ≤–1.0 with a low-traumatic fracture after age 45 years. 2302 women from 161 centers in 21 countries were enrolled. All subjects received RIS 5 mg/d for 1 year and all were pretreated with calcium 500 mg/d and vitamin D 400 IU/d for 19 days (median) prior to BTM baseline and initiation of RIS. Centers were randomized into either reinforcement (RE+; verbal feedback based on urinary N-telopeptide of type I collagen [NTX] change from baseline) or nonreinforcement (RE–) groups. In the RE+ group, 3 reinforcement messages were delivered based on BTM results from weeks 10 and 22 (good, neutral, and poor BTM response, defined, respectively, by a >30% decrease; a –30% and +30% change; and a >30% increase). Electronic monitoring caps were used to assess daily compliance. Persistence was defined as the number of days from first dose intake until treatment discontinuation. Hazard ratios (HRs) for treatment discontinuation were calculated (persistence increases as HR decreases). In patients reaching the first reinforcement visit at week 13, 1-year persistence was higher in the RE+ than in the RE– group (HR=0.80; *P*=0.056). A significant relationship between the type of message and persistence was observed (*P*=0.017). The message given to patients with a good BTM response was associated with significant improvement in persistence (HR=0.71; *P*=0.02). On the other hand, the message given to those with a poor BTM response (<5% of visits) led to a lower persistence (HR=2.22; *P*=0.005). The message given to those with a neutral BTM response had no effect on persistence compared with the RE– group. We conclude that the message given to patients with a good BTM response improves persistence in postmenopausal women treated with RIS, and that monitoring osteoporosis treatment using BTM data should be encouraged.

Disclosures: ***P.D. Delmas****, Aventis Pharmaceuticals 5.*

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Bone Anabolic Activity of APOMINE™ in a Mouse Model: Comparison to Risedronate. Y. Guyon-Gellin*[1], E. J. Niesor[1], N. Lameloise*[1], M. O. Montjovent*[1], S. van Dijk[2], S. M. Weitman*[3], C. L. Bentzen*[1]. [1]Biosciences and Pharmacology, ILEX Oncology Research, S.A., Geneva, Switzerland, [2]Program Management, ILEX Oncology, Inc., San Antonio, TX, USA, [3]Clinical Development, ILEX Oncology, Inc., San Antonio, TX, USA.

APOMINE is a bisphosphonate ester that has shown bone anabolic activity in preclinical studies. APOMINE is currently being tested in a Phase II clinical trial in postmenopausal women with osteopenia or osteoporosis. The activity of APOMINE is mediated through an increase in the rate of degradation of HMG-CoA reductase concomitant with an increase in bone mineral density (BMD) in both rats and mice. In the current study the effect of orally administered APOMINE was compared to the effect of risedronate, a bisphosphonate acid, on bone trabecular and cortical bone mineral content (BMC). In addition, in a subset of ovariectomized mice the effect of APOMINE on gene expression of bone-specific genes was studied. OF1 mice (6-7 weeks old) were fed 0, 50, 100 or 200 mg/kg APOMINE or 100 mg/kg risedronate (n=6 per group) in their diet for a period of one month. Animals were sacrificed and bone density and bone mineral content were determined at the right femur by peripheral quantitative computed tomograhy (pQCT). Trabecular BMC increased by 164% ($p<0.02$) in mice fed 200 mg/kg APOMINE, whereas no significant change was observed in mice administered risedronate. In contrast, cortical BMC increased by 93% ($p<0.001$) in mice fed 100 mg/kg risedronate, whereas no significant change in cortical BMC was observed in APOMINE-fed mice. In ovariectomized mice treated with APOMINE, expression of the genes for CBFa1, BMP1, COL-1 was significantly increased, indicating an activation of osteoblasts. These results indicate that the mechanism of action of APOMINE differs from the bisphosphonate acids, represented by risedronate, and resembles more the action of statins. Whereas bisphosphonate acids are known to act through inhibition of bone resorption, APOMINE appears to act primarily by stimulating bone formation and increasing bone BMC rather than by inhibiting resorption. These observations suggest that APOMINE may have the potential to be developed as a new non-hormonal therapy for the treatment of osteoporosis.

Disclosures: ***Y. Guyon-Gellin****, ILEX Oncology, Inc. 3.*

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Consequences of Drop-Outs in Clinical Trials: Biased Estimation of Event Rates and Rate Ratios. M. Vaeth*[1], D. E. Thompson[2], N. R. Bohidar*[2]. [1]Department of Biostatistics, University of Aarhus, Aarhus, Denmark, [2]Clinical Development, Merck & Co., Inc., West Point, PA, 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.

Disclosures: ***D.E. Thompson****, Merck & Co., Inc. 3.*

M333

Importance of Examining the Incidence of Fracture in the Placebo Groups in Randomized Clinical Trials. S. Baim*[1], D. Baran[2], C. Teutsch[2], A. Santora[2], A. de Papp[2], D. E. Thompson[2], L. Wang*[2]. [1]St. Mary's Hospital, Milwaukee, WI, USA, [2]Merck & Co., Inc, West Point, PA, USA.

Studies that focus or provide information on the effect of antiresorptive agents on fracture risk have a relatively short history. Prior to the FIT study, the size of the effect of antiresorptive agents on hip fracture was unknown. FIT was the first rigorous attempt to examine the effect of a bisphosphonate (alendronate) on the incidence of hip fracture in community dwelling women. Prior to that study the effects of anti-resorptive therapy on such fractures were known only for the combination of calcium and vitamin D in vitamin D deficient women. In recent years, several studies have reported the effect, or lack of an effect, of various drugs on risk reduction for hip fracture. The question of whether these studies had sufficient statistical power to determine an effect similar to that seen in FIT has been debated. We examine the incidence rates and sample size in 12 studies to address this issue. Using the Black study (FIT I) as the reference, what was the relative power of the other studies to determine an effect similar to that seen in the Black study? We used the reported incidence of hip fractures and number patients in the placebo group in 11 studies. Hip fracture was the primary or a key endpoint in 6 of the studies. We used FIT I as the reference study and computed the relative power of each of the remaining studies to determine the likelihood of finding an effect similar to that seen in the reference study. The number of patients in the placebo groups and the incidence of hip fracture varied among the studies. Five studies (FIT II (T < -2.5), HIP II (T< -2.5), VERT (NA, MN), HERS, and MORE) had the same power as FIT I to determine a treatment effect of 50% reduction in hip fracture. Only FIT II (T < -2.5) demonstrated a significant reduction. Three studies (Pols, Karpf and the Long term care) had lower power and none of them demonstrated a significant reduction. Four studies (HIP I (2.5mg), HIP I (5 mg), HIP II (2.5 & 5 mg) and WHI) had greater power and one (HIP I (5 mg) failed to demonstrate a significant reduction. In RCTs, it is important to examine the properties of the placebo group before drawing conclusions about the similarity of the efficacy of two or more agents. Although many studies had equal or better power than FIT I study to demonstrate reductions in hip fracture, only three (WHI, FIT II (T<-2.5) and HIP (2.5 mg)) demonstrated a significant effect.

Disclosures: ***D.E. Thompson****, Merck & Co., Inc. 3.*

M334

Relative Binding Affinities of Bisphosphonates for Human Bone. C. Leu, G. A. Rodan, A. A. Reszka. Bone Biology and Osteoporsis Research, Merck Research Laboratories, West Point, PA, USA.

The binding of bisphosphonates to bone plays an important role in their mode of action. The object of this study was to determine the relative affinity of bisphosphonates for human bone. Consistent with its short serum half-life during clinical dosing, binding of ^{14}C-alendronate (ALN) to human bone powder was maximal after 10 minutes. In dose-dependence studies, binding of ^{14}C-ALN to 0.25 mg bone powder was saturable, reaching plateau at about 500 uM, with an apparent Kd of 140 uM. Unlabeled ALN displaced ^{14}C-ALN binding in competition assays with an IC50 of 153 uM. Binding of ^{14}C-ALN was also competitively inhibited by pyrophosphate with an IC50 of 284 uM, which is comparable to its affinity for calcium.

We further examined the relative binding affinities for several clinically studied bisphosphonates, including two that lack an hydroxyl group at R^1 and six that bear an hydroxyl at R^1. The latter group has various different substituents attached at R^2. Of the bisphosphonates lacking an hydroxyl, tiludronate (H at R^1) was most comparable to pyrophosphate (IC50: 310 uM), while clodronate (Cl at R^1) was seven-fold weaker (2.1 mM). Meanwhile, all hydroxyl-bearing bisphosphonates displayed similar relative binding affinities to that of ALN, including: etidronate (168 uM), ibandronate (206 uM), pamidronate (170 uM), risedronate (186 uM) and zoledronate (160 uM).

Together these findings suggest that bisphosphonates differ in their binding to human bone, based on the presence or absence of OH at R^1. Most comparable to pyrophosphate is tiludronate, while clodronate binds weakly and all hydroxyl bearing bisphosphonates bind with comparable enhanced affinity. Differences in affinity for bone may account for part of the potency associated with each bisphosphonate, with most dramatic reductions predicted for clodronate. Since all of the clinically tested nitrogen-containing bisphosphonates bind with similar affinity, their individual potencies depend first of all on their IC50 for inhibition of farnesyl diphosphate synthase. Other differences likely relate to pharmacokinetics, ability to cross the osteoclast plasma membrane, cellular extrusion and binding to proteins within the osteoclast cytoplasm.

Disclosures: ***A.A. Reszka****, Merck and Co., Inc. 1, 3.*

M335

Efficacy, Safety and Tolerability of Once Weekly (80mg vs 160mg) Oral Alendronate Over 2 Years in Postmenopausal Osteoporosis. J. P. Brown[1], C. Banville*[1], S. Jean*[1], W. L. Drake*[2], D. L. Kendler[2]. [1]Laval University, Ste-Foy, PQ, Canada, [2]Providence Health Care Center, Vancouver, BC, Canada.

Oral bisphosphonates have become the gold standard for the treatment of postmenopausal osteoporosis. Recently, 70 mg alendronate once weekly was shown to have a similar effect on bone mineral density and bone markers as 10 mg daily. In our study, we evaluated efficacy, safety and tolerability of higher once weekly doses of oral alendronate in postmenopausal osteoporosis.

In this 2-yr blinded randomized parallel group study, 81 postmenopausal women (mean age 69.1 ±5.2 SD) with lumbar spine T-score < -2.5, were randomly assigned to receive oral alendronate 80 mg (n = 39) or 160 mg (n = 42) once weekly calcium carbonate 500 mg twice daily. Treatment was randomly allocated to block randomization by centers (2).BMD

of lumbar spine (LS), femoral neck (FN) and total hip (TH) were measured by DXA (Hologic) at baseline, 6, 12, 18 and 24 months. Adverse events were also collected.
Using an analysis of repeated measurements, we tested the statistical significance of treatment effect over time for each site and each center (see table). The treatment effect was significant for each site with respective Satterwaite p values: p = 0.0039, p<0.001, p = 0.0002. There was a significant effect overt time on LS and TH BMD (p<0.001) but not on FN. The effect at one center on TH was significant (p<0.001). Beta CrossLaps were similarly reduced in the treatment groups as well as BSAP Adverse events possibly related to study drug reported a least once are: nausea, diarrhea, arthralgia, abdominal pain, vomiting, dyspepsia, and constipation. All were of mild severity without significant impact on daily activities and resulted in one subject discontinuation.
We conclude that oral alendronate 80 and 160 mg once weekly are safe and well tolerated. The 160 mg once weekly dose leads to significantly greater increases in BMD at all sites. The clinical significance of these small improvements in surrogate BMD and marker indicators is of uncertain to individual women with postmenopausal osteoporosis.

BMD v. baseline Dose (mg)	6 mo % (SEM)	12 mo % (SEM)	18 mo % (SEM)	24 mo % (SEM)
LS 80 mg	+3.6 (0.6)	+5.5 (0.5)	+7.1 (0.8)	+7.8 (0.9)
LS 160 mg	+4.1 (0.5)	+6.7 (0.6)	+7.5 (0.5)	+8.9 (0.5)
FN 80 mg	-0.4 (1.1)	+0.6 (1.1)	+0.8 (1.3)	+0.6 (1.2)
FN 160 mg	1.6 (0.5)	+3.2 (0.5)	+2.9 (0.5)	+3.7 (0.8)
TH 80 mg	+1.3 (0.4)	+2.2 (0.3)	+2.8 (0.5)	+3.4 (0.4)
TH 160 mg	+2.1 (0.4)	+2.7 (0.4)	+3.4 (0.4)	+3.8 (0.4)

Disclosures: ***J.P. Brown****, None.*

M336

Failure Characteristics of the Thoracic Spine with a Posteroanterior Load. M. M. Sran[1], K. M. Khan[2], Q. Zhu*[3], T. R. Oxland*[3], H. A. McKay[3]. [1]Medicine, UBC, Vancouver, BC, Canada, [2]Family Practice, UBC, Vancouver, BC, Canada, [3]Orthopaedics, UBC, Vancouver, BC, Canada.

Osteoporosis and back pain are common alone and in combination among older adults. Spinal mobilization techniques have been shown to relieve back pain and improve function in various clinical settings. However, whether controlled spinal mobilization can cause vertebral fracture in individuals with osteoporosis is not known. Thus, we investigated: (1) the failure load of cadaveric mid thoracic vertebrae when a posteroanterior (PA) load was applied to the spinous process of T6, (2) the failure site, (3) the influence of BMD and bone geometry on PA failure load, and (4) the difference between applied load, in vivo, and failure load, in vitro.
Twelve T5-8 cadaveric specimens (mean age 72 yrs) were scanned using DXA (Hologic 4500), radiographed, and measured for bone size. We measured failure load, failure site, and intervertebral motion (using a precision opto-electronic camera system) when a PA load was applied at the spinous process of T6 using a servohydraulic material testing machine (Instron 8874). Post test radiography and CT scan were used to verify failure site. These tests were repeated in an intact cadaver, using a Tekscan I-Scan sensor to measure applied loads. We also quantified in vivo applied loads during PA mobilization during seven trials by two experienced physiotherapists.
Mean (SD) failure load and applied load was 479N (162N) and 145N (38N) respectively. Applied loads, in vivo, were significantly lower than failure loads, in vitro (p<.0001). The relationship between failure load and AP BMD was weak (r= 0.18) yet non-significant (p=0.05) (Fig.1). All specimens failed at the spinous process. An experienced radiologist identified fracture site on only 3 of 12 radiographs and 6 of 12 CT scans.
To our knowledge, the failure load and site of normal or osteoporotic vertebrae under a PA load has not been described until now, despite the long term widespread use of this technique in clinical practice. BMD by DXA explained a lesser proportion of the variance in failure load than it has in previous studies. This may relate to BMD values representing the entire vertebra, not the spinous process alone.

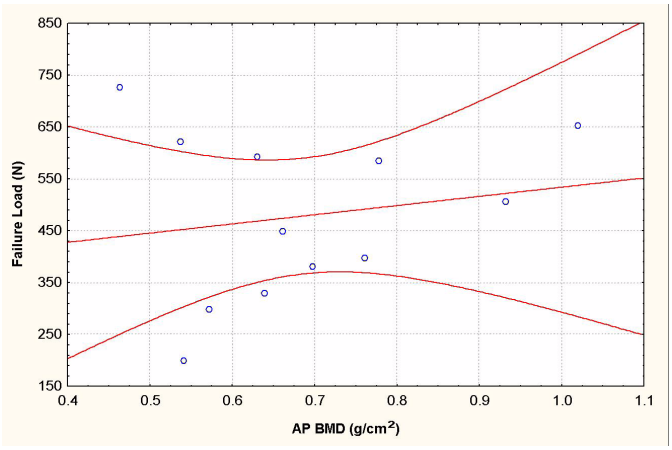


Fig.1. Relationship between AP BMD (g/cm^2) and failure load (N) of osteoporotic T6 vertebrae when a posteroanterior load was applied at the spinous process (and 95% confidence bands) (r=0.18).

Disclosures: ***M.M. Sran****, None.*

M337

Introduction of Dynamic Hyperextension Brace(D.H.B) & Its Effect on Prevention and Correction of Senile Kyphosis in 50-70 Years Old Women. B. Saeed Modaghegh*. Orthopaedic Surgery - Firouzgar Medical center, Iran University of Medical Science, Tehran, Iran (Islamic Republic of).

Purpose: To introduce D.H.B and evaluate its effect on prevention & Correction of senile kyphosis in 50-70y old age women .
Method: We,ve done a prospective cohort randomize control trial study. We selected 58 well matched 50-70y old age women . 25 were Control group and 33 were study group . The treatment program (medication & exercise) was the same in both group, but the study group applied D.H.B with 1000g weight . We've taken standing lateral X ray In both group at the beginning & at the end of study after one year follow up. Lab.exam (Ca,P,Alk.P) and bone mineral densitometry was done as well.
Result: Cobb angle of kyphosis showed mean correction of 1.36 degree in control group & 7.15 degree in study group. P.Value: 0.0004
Conclusion: Our result revealed the D.H.B is an effective Device for preventing & treatment of senile kyphosis. So we advise the D.H.B in women above 50y old age.

Disclosures: ***B. Saeed Modaghegh****, None.*

M338

Aquatic verses Land Exercise to Reduce Fall Risk in Women with Osteoporosis. C. M. Arnold[1], A. J. Busch*[1], C. L. Schachter*[1], E. L. Harrison*[1], W. P. Olszynski[2]. [1]School of Physical Therapy, University of Saskatchewan, Saskatoon, SK, Canada, [2]Saskatoon Osteoporosis Centre, Saskatoon, SK, Canada.

This study compared the effects of aquatic, land and no exercise on fall risk factors in women 60 years or older diagnosed with osteoporosis (OP), according to WHO criteria. Seventy-three women were randomly assigned to control, aquatic or land exercise. Control subjects were randomly assigned to one of the exercise groups following the control period. Both exercise interventions, 3 times/week for 5 months, focused on balance, posture, muscle strengthening and mobility. The Control group met once per month for social interaction. Fall risk factors measured were: balance (Berg balance scale, tandem walk), posture (index of kyphosis), lower extremity muscle strength (chair stands), ankle mobility (goniometer-dorsiflexion), number of falls, function (Osteoporosis Functional Disability Questionnaire), quality of life (Osteoporosis Quality of Life Questionnaire), health satisfaction, movement confidence (Activities and Balance Confidence Scale) and bone status at tibia and radius (quantitative ultrasound). Testers were blinded to group assignment. Average attendance and drop out rates were similar for both programs. There were no significant differences between groups for baseline measurements or demographics.
There was a significant difference (p<0.05) in change scores as measured by a general linear model MANOVA (intention-to-treat analysis, Land = 28, Aquatic = 27, Control = 28), Pillai's trace = 0.60, F = 1.5 (df = 2,36). Univariate differences (Tukey's post hoc analysis, p<0.05) were: Health Satisfaction (Aquatic and Land exercise > Control), Balance - tandem walk (Aquatic > Land) and Functional Disability Questionnaire (Land > Aquatic). There were no significant differences in bone status or number of falls. Two falls occurred during the performance of the land exercise program. There were twice as many reports of increased joint pain during the land exercise as compared to aquatic.
Both exercise programs resulted in improved health satisfaction, with no significant difference in bone status or other fall risk factors when compared to Control. Aquatic exercise was superior to land in improving select balance skills to prevent lateral falling. Land exercise was more effective in improving function, possibly due to specificity of practicing functional movements in an environment similar to home and community. Increased joint pain and falling during the performance of exercise occurred more frequently during the land program suggesting that aquatic exercise may be safer for the older, frailer OP client.

Disclosures: ***C.M. Arnold****, None.*

M339

Site-Specificity of Exercise on Bone Mineral Density in Premenopausal Women. K. M. Winters*[1], C. M. Snow[2]. [1]Population-Based Nursing, Oregon Health & Science University, Portland, OR, USA, [2]Exercise and Sport Science, Oregon State University, Corvallis, OR, USA.

We studied the response of lumbar spine BMD to the addition of upper body resistance exercise in premenopausal women (age = 30-45 yrs) performing thrice weekly lower body resistance + jump exercise. Thirty-five exercisers and 24 age-matched controls (CON) participated in the 12-month study. Women who did not complete > 60% of prescribed sessions were excluded from statistical analyses (N=7). Among the regularly exercising women (N=28), all participated in lower body resistance plus jump exercise (LOWER) while half (N=14) also performed upper body resistance exercise (UPPER + LOWER). Exercisers trained three times per week in a program of 100 jumps and 100 repetitions of lower-body resistance exercises with or without an additional 100 repetitions of upper body exercises at each session. Intensity for lower-body exercise was increased using weighted vests to final values of 10% and 13% of body weight for jump and resistance exercises, respectively while intensity for upper-body exercise was increased using greater levels of resistive tubing to a final resistance of approximately 12-RM. Bone mineral density (BMD) at the greater trochanter, femoral neck, lumbar spine and whole body were measured by dual energy x-ray absorptiometry (Hologic QDR-1000/W) at baseline and 12 months. At baseline, anthropometric variables and BMD were similar between groups. Using ANOVA and analyzing BMD % change scores, significant group differences were observed at the greater trochanter (LOWER: +2.8% ± 2.7%, UPPER + LOWER: +2.6% ± 2.7%, CON: +.7% ± 1.7%; p < .01) and lumbar spine (LOWER: -.6% ± 1.7%, UPPER +

LOWER: +1.4% ± 3.9%, CON: -.6% ± 1.8%; p < .05). Post-hoc analyses for trochanter BMD showed significant differences between each exercise group vs. controls, but no significant differences between exercise groups (p=.822), while lumbar spine BMD showed significant differences between LOWER vs. UPPER + LOWER and UPPER + LOWER vs. CON, but not for LOWER vs. CON (p=.93). We conclude that the lower body and upper body exercise training has site-specific effects on the musculoskeletal system in premenopausal women. These data support the site-specificity of mechanical loading on regional BMD.

Disclosures: ***K.M. Winters****, None.*

M340

Effect of Strength Training on Bone Mineral Density of Young Women: A Prospective, Randomized, Controlled Study. J. A. Singh*[1], K. H. Schmitz*[2]. [1]Division of Rheumatology, VA Medical Center, Minneapolis, MN, USA, [2]Division of Epidemiology, University of Minnesota, Minneapolis, MN, USA.

OBJECTIVE: To assess if twice weekly strength training for 39 weeks increases bone mineral density (BMD) in young women.
METHODS: 60 healthy young women aged 30-50 years (56 pre-menopausal, 4 post-menopausal on hormone replacement therapy) were randomized to either twice weekly supervised strength training followed by 6 months of unsupervised training (treatment group) or control group. Using 2-sided t-test, we compared the baseline characteristics between groups. Percent changes in BMD (measured by DEXA at baseline, 15 and 39 weeks) over 15 and 39 weeks were compared using analysis of co-variance, after adjusting for baseline BMD, fat free mass and % body fat..
RESULTS: Data was analyzed for 56 women (control group: 2 dropped out for personal reasons; treatment group: 1 dropped out for personal reasons, 1 with Grave's disease excluded). The two groups were similar at baseline with regard to following characteristics: age, education level, marital status, ethnicity, body weight, height, body mass index, total body fat, fat free mass, % body fat, waist circumference, bench and leg press strength, and physical activity. The treatment group had higher energy intake at baseline as compared to the control group (1961±371 vs. 1709±457 kilocalories/day; 2 sided t-test p-value = 0.03).
Total BMD gain was higher in exercise group at 15 and 39 weeks compared to control group, with 1% higher gain at 39 weeks (p=0.06; **Table 1**). There was a significant increase in upper torso (upper spine and ribs) BMD in the exercise group compared to control group at 15 weeks. Despite more favorable regional BMD changes in the exercise group (% BMD gain almost twice or more, and % BMD loss less than half than that observed in the control group), none of the 39 week between group differences achieved statistical significance.
Table 1. Comparison of % change in bone mineral density over baseline at 15 and 39 weeks after adjusting for baseline BMD, fat free mass and % body fat

	% BMD change in 15 wks (CONTROL GROUP)	% BMD change in 15 wks (EXERCISE GROUP)	% BMD change in 39 wks (CONTROL GROUP)	% BMD change in 39 wks (EXERCISE GROUP)
Total	+0.20	+0.80	+0.55	+1.50†
Spine	-0.44	+0.38	+0.91	+2.23
Hip	-0.49	-0.11	-0.81	-0.46
Arm	+3.8	+6.55	+4.80	+8.20
Leg	+0.21	+0.50	+1.12	+1.46
Trunk	-0.36	+0.27	-0.64	+0.11
Upper torso	-0.77	+1.46*	-1.23	-0.69

†p-value=0.06, *p-value=0.037; all other differences were statistically insignificant
CONCLUSIONS: Exercise for even a short duration in young women may lead clinically meaningful changes in bone BMD, though these changes were not statistically significant in this small study.

Disclosures: ***J.A. Singh****, None.*

M341

Effects of Reducing the Quantity and Frequency of Back-Strengthening Exercise on the Strength of Back Extensors in Healthy Young Women: A Preliminary Study. M. Hongo*[1], E. Itoi*[1], M. Sinaki*[2], Y. Shimada*[3], N. Miyakoshi[1], K. Okada*[1]. [1]Orthopedics, Akita University, School of Medicine, Akita, Japan, [2]Physical Medicine and Rehabilitation, Mayo Clinic Rochester, Rochester, MN, USA, [3]Rehabilitation, Akita University, School of Medicine, Akita, Japan.

We have reported that back muscle exercise using a specific backpack is effective in increasing the back extensor strength (BES) (Itoi and Sinaki 1994) and decreasing the risk of vertebral fractures (Sinaki et al 2002). The subjects in these studies were healthy postmenopausal women. The same quantity of exercise, when performed by patients with osteoporosis, may cause back pain or even vertebral fractures. Therefore, less quantity of exercise, as long as it is effective in increasing the BES, may be beneficial for osteoporotic patients. The purpose of this study is to determine the effects of reducing the quantity of back strengthening exercise on BES in healthy young volunteers. Fifty-four healthy young nonsmoking female volunteers with a mean age of 21 years were enrolled in this study. Subjects with high activities (physical activity scores ≥ 8 points) were excluded. The exercise protocol was to lift the weighted backpack in prone position. Participants were randomly assigned to one of the following five groups: Group 1 (control group, n=10) without exercise prescription; Group 2 (n=10) with a standard protocol that has been demonstrated to increase the BES (weight lifted = 30% of BES, 10 repetitions/day, 5 days/week); Group 3 (n=12) with the same protocol except the weight lifted (15% of BES); Group 4 (n=12) with the same protocol except the repetition times (5 repetitions/day); and Group 5 (n=10) with the same protocol except the frequency (3 days/week). The BES was measured at baseline, 4, 8 and 12 weeks using an isokinetic dynamometer. The BES increased significantly in all the groups except Group 1. The maximum increase at 8 weeks was observed in Group 2 (39.5%), followed by Group 4 (22.7%), Group 3 (16.0%), and Group 5 (12.5%). The BES in Group 2 was significantly greater than in Group 1 and Group 5 at 8 weeks (p=0.013). In conclusion, reducing the weight or repetition times makes less difference from the standard exercise program than reducing frequency in terms of increasing the back extensor strength in healthy young women.

Disclosures: ***M. Hongo****, None.*

M342

HIP PRO: A Multicenter, Randomized, Controlled Trialof Hip Protectors In Nursing Home (NH) Residents. D. P. Kiel[1], S. J. Birge[2], S. I. Zimmerman*[3], J. Magaziner*[4], B. Barton*[5]. [1]Hebrew Rehab Ctr for Aged and Harvard Medical School, Boston, MA, USA, [2]Washington University, St. Louis, MO, USA, [3]University of NC, Chapel Hill, NC, USA, [4]University of Maryland, Baltimore, MD, USA, [5]Maryland Medical Research Institute, Baltimore, MD, USA.

Although hip protectors appear to be effective at preventing hip fracture in those at high risk, there have been no randomized trials in the United States. Previous European trials have been unable to exclude the possibility that subjects wearing pads might have been treated differently than controls, and compliance has been low. Therefore, "HIP PRO," an NIA-funded, multicenter, randomized, controlled trial of a biomechanically tested hip protector is currently underway in NH residents over age 65 yrs in Boston, Baltimore and St Louis. The primary aim is to compare the rates of hip fracture in protected versus unprotected hips. A secondary aim is to better understand individual and institutional factors influencing compliance. This study uses one-sided hip protector garments so that each resident is her/his own control, eliminating the chance of differential treatment by facilities and caregivers according to group assignment. The hip protector is a unique energy absorbing and dispersing pad tested biomechanically for its ability to reduce the forces commonly delivered to the hip during a fall to the side by 95%. An NIA-funded pilot study of this hip protector revealed greater than 80% compliance among NH residents. In the HIP PRO, NHs are randomly allocated to be either right or left-sided hip protector facilities based on the expected recruitment from that NH. The allocation of residents to side will be approximate due to the cluster randomization, and will vary depending on the distribution of active patients in the NHs at any one time; however the goal is to achieve an equal representation of right and left sided NH residents. This trial also includes a compliance enhancing strategy to achieve better compliance than reported in previous studies (about 50%), and in recognition of challenges that hip protectors present for frail and impaired residents and caregivers. Hierarchical analyses of compliance will consider the clustering of individual factors within NHs. As of April 2003, the study had enrolled 252 subjects, and monthly NH resident compliance averaged more than 80%. Given the nature of NH populations, the expected attrition of participants will require on-going recruitment throughout 3.5 years of follow up to achieve 1500 resident-years of observation. This presentation will overview the design of the HIP PRO, and provides methodological suggestions relevant to other clinical trials in NH settings.

Disclosures: ***D.P. Kiel****, None.*

M343

Good Maintenance of High-Impact Activity-Induced Bone Gain in Premenopausal Women. A 3.5-Year Follow-Up After a Randomized Controlled Trial. S. A. Kontulainen*[1], A. Heinonen[2], P. Kannus*[2], M. Pasanen*[2], H. Sievänen*[2], I. Vuori[2]. [1]UKK Institute, Tampere, Finland and, UBC/ Orthopaedics Engineering, Vancouver, BC, Canada, [2]UKK Institute, Tampere, Finland.

Objective: To assess the long-term maintenance of the musculoskeletal benefits obtained in an 18-month intervention of high-impact exercise in premenopausal women.
Design: Follow-up of the randomized controlled trial.
Participants: 34 former trainees and 31 controls.
Methods: Physical performance and areal bone mineral density (aBMD, g/cm^2) was measured at baseline, after 18 months, and after 5 years.
Results: All significant 18-month improvements in the trainees' neuromuscular performance (isometric leg press, and vertical jump with and without additional 10% weight of the body mass) had been lost at the 5-year follow-up. However, the similarly significant exercise-induced aBMD gains (the intergroup difference average 2% to 3% in favour of the trainees) were well maintained at the lumbar spine, femoral neck, distal femur, patella, proximal tibia, and calcaneus at the 5-year follow-up. At the trochanter and distal radius, the intergroup aBMD difference was statistically insignificant at both the 18-month and 5-year follow-ups.
Conclusions: The significant bone gain that was obtained by 18-month high-impact exercise was well maintained three and half years after the end of the intervention, while the exercise-induced improvements in the neuromuscular performance returned to baseline. These findings emphasise good maintenance of high-impact activity-induced bone gain in premenopausal women despite cessation of the activity, and thus, refer to a possibility of long-term bone benefits of high-impact training.

Disclosures: ***S.A. Kontulainen****, None.*

M344

Effect of Exercise on Long Bone in Ovariectomized Mature Rats. N. Nakamichi*[1], S. Ichimura[2], T. Kikuchi*[1], Y. Aoki*[1], M. Tomiya*[1], J. Iwamoto[3], Y. Toyama*[4], K. Fujikawa*[1]. [1]Dept.of Orthop. Surg., National Defense Medical College, Saitama, Japan, [2]Dept.of Orthop. Surg., Kyorin University, Tokyo, Japan, [3]Sports Clinic of Medicine, Keio University, Tokyo, Japan, [4]Dept.of Orthop. Surg., Keio University, Tokyo, Japan.

The aim of the present study was to examine the effect of running exercise on femur and tibia in ovariectomized (OVX) mature rats. 23 week-old female Wistar rats were divided into 4 groups of 8 animals each; ovariectomy (O), ovariectomy and exercise (OE), sham-operated (S), and sham-operated and exercise (SE). The day after sham and OVX were operated, rats were given a program of exercise with motor driven treadmill. The exercise regimen consisted of a treadmill running at 12 m/min, 5°incline, 1hr per day 5 days a week, for 12 weeks. At the end of the exercise, the femur, tibia, and calf muscles were removed and weighed. Right femur and tibia were measured their bone mineral densities (BMD, mg/cm^2) by dual-energy X-ray absorptiometry (DXA). Serum calcium (Ca), inorganic phosphorus (Pi), alkaline phosphatase (ALP), and osteocalcin (OC) were also analyzed.
OVX caused an increase in the body weight over the experimental period. The average body weight in OE group was lower than that in O group, but it was not significant, and so was SE's to S's
The whole femoral BMD in O group significantly decreased than that in S group (mean decrease of 10%), but the whole tibial BMD didn't statistically differ between these two groups. Compared with O group, the whole femoral BMD in OE group significantly increased. Especially, the proximal and distal femoral BMD were maintained at the same level as S group. The whole tibial BMD in OE group was significantly increased compared with O group, particularly the middle and distal tibial BMD were higher than those in S group. However, there was no significant difference in the proximal BMD. Serum Ca and Pi levels in O and OE groups were significantly lower than those in S and SE groups. Serum ALP and OC levels in OE and SE groups were significantly higher than those in O and S groups respectively.This study showed that exercise had significant influence on femoral and tibial BMD, however, their effect was different at the site of the each bone and that the effect of the running exercise was influenced by conditions such as mechanical loading and bone architecture in the OVX mature rat.

Disclosures: ***N. Nakamichi****, None.*

M345

Physical Activity is Associated with Reduced Bone Loss in the Postmenopausal Period. H. G. Ahlborg*, O. Johnell, M. K. Karlsson. Dept of Orthopaedics, Inst of Orthopaedics, Malmo, Sweden.

Bone strength depends on material properties, such as bone mineral density (BMD) and on skeletal geometry, such as bone size. The accrual of BMD can be enhanced by physical activity during growth, but the purpose of this study was to evaluate whether moderate physical activity also reduces bone loss in the postmenopausal period and/or influence the skeletal geometry.
This 23-year prospective observational study compared bone loss and changes in skeletal geometry and Strength Index, evaluated by single-photon absorptiometry of the distal radius every second year from menopause (MP) until age 72, in 91 moderate physically active and 21 inactive women. Data is presented as mean with 95% CI.
There was no difference in BMD or skeletal geometry when active and inactive women were compared at baseline. Following MP, the annual decrease in BMD was less in physical active [1.6% (1.5-1.8)] than in inactive women [2.1% (1.8-2.3)] (p=0.002). Changes in skeletal geometry did not differ between the groups. The annual reduction in Strength Index was as a result, less in the physically active [0.7% (0.6-0.8)] than in the inactive women [1.1% (0.8-1.4)] (p=0.005). The physically active women had, at age 72, a 0.6 standard deviation (SD) higher BMD (p =0.009) and a 0.3 SD higher Strength Index (p = 0.21) than the inactive women.
Bone loss following menopause is less in moderate physically active than in inactive women, so that active women have higher BMD at age 72 than inactive women. Moderate physical activity in the postmenopausal period may be recommended as a strategy to prevent bone loss.

Disclosures: ***M.K. Karlsson****, None.*

M346

Osteoporotic/Kyphotic (OK) Women Increased Back Strength After Four-Week Trial of Proprioceptive Dynamic Posture (PDP) Training. M. Sinaki[1], E. Preisinger[2]. [1]Physical Medicne and Rehabilitation, Mayo Clinic, Rochester, MN, USA, [2]Physical Medicne and Rehabilitation, Krankenhaus Lainz, Vienna, Austria.

Immobility and pain related to post-vertebral fracture reduce back strength. We hypothesized that proprioceptive muscle re-education techniques can increase back strength in osteoporotic/kyphotic (OK) women with compression fractures and pain.
Sixteen women ages 66 to 83 who had been evaluated in our Rehabilitation of Osteoporosis Program – Exercise (ROPE) were included in this study with each subject serving as her own control. Inclusion criteria consisted of the presence of compression fracture of the spine with thoracic hyperkyphosis (Cobb angle 50 to 65° measured on radiographs of spine). All subjects had baseline bone mineral density, radiographs of the spine, and strength evaluation. In addition, physical activity level was assessed using a standardized Physical Activity Scale. Subjects' level of back pain was assessed with a standardized pain scale, 0 to 10 (0–no pain, 10-severe pain). Following baseline evaluation, all subjects were instructed for use of a proprioceptive dynamic posture (PDP) training program which included a weighted kypho-orthosis (2-2.5 pounds) applied at T10 level of the spine. All subjects were re-evaluated at the end of 4-week trial.
Subjects who had no pain initially, still had no pain while those who had pain at baseline had significantly less pain at the end of 4 weeks (P=0.0005). There was an overall statistically significant improvement in back muscle strength (P = 0.02). Posture improved slightly on clinical evaluation of height. Subjectively, all subjects reported more interest and ability in performing daily physical activities.
We conclude that PDP training is a valuable technique for safely improving back strength and reducing pain in OK subjects at post-vertebral fracture stage.

Disclosures: ***M. Sinaki****, None.*

M347

Does Resistance Training Maintain Bone Mass During Moderate Weight Loss in Older Adults with Type 2 Diabetes? A 12-month Randomised Controlled Trial. R. M. Daly[1], D. Dunstan*[2], N. Owen*[3], J. Shaw*[2], P. Zimmet*[2]. [1]School of Health Sciences, Deakin University, Melbourne, Australia, [2]International Diabetes Institute, Melbourne, Australia, [3]University of Queensland, Brisbane, Australia.

Weight loss (WL) is recommended as a strategy to improve glycaemic control in patients with type 2 diabetes, but it can also lead to significant bone loss in obese adults. The inclusion of exercise into weight loss regimens however, may protect against bone loss, conserve lean mass (LM) and improve glycaemic control. The aim of this study was to examine the effects of moderate weight loss, with and without resistance training (RT), on bone mass, body composition and hormonal parameters in overweight, sedentary older adults aged 67.4 (5.1) yrs with established type 2 DM. During the first 6 mths in the supervised laboratory setting (Phase 1), all participants followed a moderate WL program and were randomised to either RT [3/wk@75-85% of max strength] (RT+WL, n=19) or light training [stretching, 3/wk] (WL, n=17). Thereafter, participants received individualised instructions and equipment to continue training in the home setting (without dietary counselling) for a further 6 mths (Phase 2). Compliance during phase 1 (87%) and 2 (75%) was not different between the groups. TB BMC, LM and fat mass (FM) were assessed by DXA every 6 mths. HbA1c, IGF-1, testosterone, estradiol and SHBG were assessed every 3 mths. Phase 1: Subject retention after 6 mths supervised training was 81% (RT+WL 84%; WL 76%). The RT+WL and WL groups had similar reductions in weight (-2.7% vs -3.3%) and FM (-7.4% vs -5.6%) ($p<0.01$), whereas LM increased in the RT+WL relative to WL group (0.9% vs -0.9%, ANCOVA interaction $p<0.05$). TB BMC remained unchanged in the RT+WL group (mean ± SD: 0.2 ± 1.9%) but decreased in the WL group (-1.5 ± 1.8%) after adjusting for age, height, weight, gender and use of oral hypoglycemic medication (interaction, $p<0.05$). HbA1c decreased in the RT+WL relative to WL group (-1.2% vs -0.4%, interaction, $p<0.05$), but there were no between group differences in any other hormonal traits. Phase 2: Two participants withdrew from the study during phase 2. After 12 mths, the change in TB BMC from baseline was no longer different between groups (RT+WL -0.3 ± 2.2% vs WL -1.3 ± 3.4%). Weight (1.6%) and FM (6.4%) increased similarly in both groups ($p<0.01$); there were no between group differences for any hormonal traits during the home-based training. In conclusion, these results indicate that a supervised, progressive resistance training program should be recommended to older overweight adults with type 2 diabetes to maximise compliance and optimise the benefits associated with weight loss for diabetes, without having a negative effect on bone health.

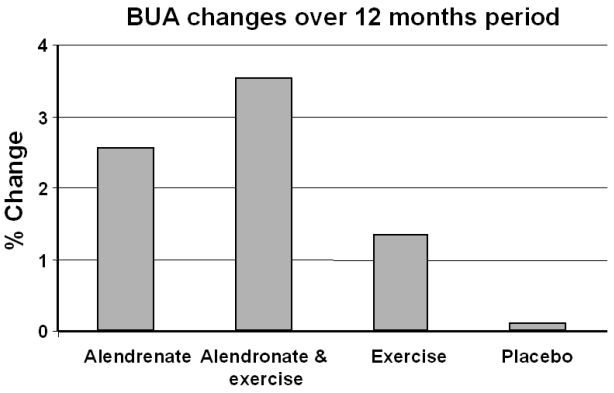
Disclosures: ***R.M. Daly****, None.*

M348

High Impact Exercise and Alendronate Have an Additive Effect on Calcaneus Ultrasound Attenuation in Postmenopausal Women. A. O. Heinonen[1], Q. Wang*[1], P. Nicholson*[1], P. Moilanen*[1], H. Sievänen*[2], S. Cheng[1]. [1]Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [2]UKK Institute, Tampere, Finland.

Our previous report has shown that alendronate was effective in increasing bone mass at the lumbar spine and femoral neck measured by DXA, while exercise was effective in increasing the mechanical properties of the distal tibia measured with pQCT. However, no additive effect of exercise and alendronate was found in any of the measured bone sites. The purpose of this one-year, randomized (concerning alendronate double blind) placebo-controlled intervention trial was to evaluate if there is a combined effect of jumping exercise and alendronate treatment on the calcaneus, the most loaded bone site, in early postmenopausal women. The subjects were 164 women and they were randomly assigned into four groups: 1) 5 mg of alendronate + exercise, 2) 5 mg alendronate, 3) placebo + exercise and 4) placebo. The inclusion criteria were no regular exercise or previous bone fractures, 1-5 years after the onset of menopause, no current or previous use of drugs or illness affecting bone metabolism, no contraindication to exercise and alendronate, femoral neck t-score > -2.5, and the FSH level > 30 IU/l. Measurements were done at baseline and 12-month. The final data were obtained from 131 women. Broadband ultrasonic attenuation (BUA) maps (2300mm2) were obtained at the right calcaneus using a QUS-2 Ultrasonometer (Quidel, USA). BUA was extracted from the heel map using different sizes of the region of interest (ROI=140 mm^2, 100 mm^2, 60 mm^2). Compliance of attendance at all available training sessions was average 1.6 (SD 0.9) times a week and for taking alendronate/placebo pills 93%. Neither five mg of alendronate daily nor exercise alone showed a statistically significant increase in BUA at the calcaneus for any of the ROI sizes. However, in all three sizes of the ROI, exercise had an additive effect with alendronate on BUA at the calcaneus. In the largest ROI the mean increase for the alendronate+exercise group was 3.6% in BUA as compared to 0.1% change in the placebo group (Figure 1). Alendronate concurrent with exercise were effective in increasing BUA at the calacaneus, at the most loaded bone site. Together alendronate and exercise may effectively decrease the risk of

osteoporotic fractures.



Disclosures: ***A.O. Heinonen****, None.*

M349

An Orthopedist-Generated Nurse Practitioner Consultation on Low-Impact Fracture Patients Improves Osteoporosis Evaluation and Management. C. Simonelli[1], J. A. Morancey*[1], L. Swanson*[2], K. K. Killeen*[2], K. A. Grimm*[3], T. Weiss*[4], Y. Chen*[4]. [1]Osteoporosis Service, HealthEast Clinics, Woodbury, MN, USA, [2]Orthopedic Collaborative Practice, HealthEast, St. Paul, MN, USA, [3]Research and Statistics, HealthEast Medical Research, St. Paul, MN, USA, [4]Merck & Co., West Point, PA, USA.

The purpose of this study is to see if an orthopedist-generated nurse-practitioner (NP) consult in addition to bedside education improved osteoporosis care for the fragility fracture patient compared with bedside education alone. In June 1999, we implemented a NP bedside education program on osteoporosis for patients admitted to the hospital with a low-impact fracture (N=184). Beginning August 2001, a request for a NP consultation was added to admitting orders for hip fracture and extremity fracture patients (N=82). The consultation included chart review, physical examination and orders were written for laboratory testing, nutritional supplements and medications as indicated. Baseline characteristics were comparable between the two groups except for age. Logistic regression analysis was used to adjust for age differences between the two groups when evaluating outcomes at discharge and follow-up. In the NP consultation group we found a high incidence (89%) of secondary osteoporosis with 80% of patients vitamin D deficient and 10% of patients with undetectable OH-vitamin D levels (<5ng/ml) and 13% with elevated parathyroid hormone levels. Without NP consultation, laboratory testing was not done. Other results are noted below:

	Education Only N=184	**Education and Consultation** N=82	**p-value***
Calcium≥500mg			
Admission	45.9%	35.4%	NS
Discharge	49.2%	70.4%	0.009
12-month	72.1%	86.2%	0.020
VitaminD ≥400IU			
Admission	25.4%	21.3%	NS
Discharge	28.0%	60.5%	<.001
12-month	68.4%	62.1%	NS
Osteoporosis Med.‡			
Admission	14.1%	12.2%	NS
Discharge	14.1%	29.8%	0.002
12-month	24.4%	32.0%	NS

*p-values based on logistics regression models; NS = not significant (p> .05).
‡Alendronate, risedronate, calcitonin, raloxifene

Patients receiving a NP consultation in addition to education were more likely to be discharged on a calcium supplement, vitamin D, and osteoporosis medicaiton. At 12 months follow-up, more patients in the consultation group were taking calcium supplement. Both the education and the education/consultation study groups demonstrated significant improvements in vitamin D and medication use at 12 months follow-up compared to admission. An orthopedist-generated nurse practitioner consultation for identification, evaluation and management of osteoporosis improves care immediately following a low impact fracture and allows for diagnosis of secondary osteoporosis.

Disclosures: ***C. Simonelli****, None.*

M350

Patient, Physician, and Practice Setting Characteristics Associated with Non-Adherence to Recommended Management of Osteoporosis. D. H. Solomon[1], T. K. Gandhi*[2], A. Karson*[2], S. Gharib*[2], S. Shaykevich*[2], E. J. Orav*[2], D. W. Bates*[2]. [1]Division of Pharmacoepidemiology, Brigham and Women's Hospital, Boston, MA, USA, [2]Division of General Medicine, Brigham and Women's Hospital, Boston, MA, USA.

Background and Objective: Several surveys have suggested that screening and treatment for osteoporosis is sub-optimal, but little is known about patient, physician and practice factors associated with non-adherence to recommendations. We examined the predictive value of patient, physician and practice setting factors with respect to adherence of osteoporosis recommendations.

Methods: Using the electronic medical records from practices affiliated with a large academic medical center, we selected patients in any of 4 at-risk categories for osteoporosis; (1) women > 65, (2) women over 45 who smoke cigarettes, (3) men or women with a history of a fracture as an adult, and (4) men or women taking oral glucocorticoids for 3 months or longer. We determined whether they had undergone BMD testing and/or received a medicine for osteoporosis. Patient, physician, and practice setting characteristics were examined in clustered logistic models to determine potential predictors of which patients were not being managed in accordance with a local adaptation of national osteoporosis guidelines.

Results: We included 6,388 patients being seen in the target primary care practices: 4,511 (71%) women > 65, 626 (10%) women > 45 who smoke, 751 (12%) persons taking oral glucocorticoids > 3 months, and 500 (8%) with a prior fracture. Of these patients, 3,545 (55%) had no record of BMD testing and 4,456 (70%) had no record of receiving a prescription medicine for osteoporosis. Testing and treatment differed across at-risk groups ($p < 0.01$). In multivariable adjusted logistic regression to account for clustering of patients among doctors and doctors within practice sites, we found that several patient and physician characteristics were important predictors of who did not receive testing and/or treatment (see **Table**).

Conclusion: After controlling for patient, physician, and practice site characteristics, we identified several factors that were strong predictors of sub-optimal osteoporosis management for high risk patients, including patient's age, race, and gender, as well as MD gender and training. This knowledge will help plan quality improvement interventions.

Adjusted Predictors of Non-Adherence with Osteoporosis Recommendations

Risk Factor	**Odds Ratio (95% CI)**
Patient Age < 55	2.2 (1.7 - 2.7)
Patient Age 85+	3.3 (2.6 - 4.2)
Male Patient	3.9 (2.7 - 5.6)
Non-White Patient Race	3.5 (2.7 - 4.3)
Male MD	2.0 (1.3 - 2.9)
MD Trainee	2.4 (1.8 - 3.2)
MD Training, non-Endo non-Rheum	2.0 (1.1 - 3.4)

Disclosures: ***D.H. Solomon****, None.*

M351

Clinical Reasons for Prescribing Raloxifene or Alendronate in Brazil. O. L. Bracco*[1], M. Lazaretti-Castro[2], L. A. Russo[3], A. Urbanetez*[4], M. C. Osório*[5], S. Liberman*[6], J. E. Marson*[1], M. J. Kayath[1]. [1]Medical Division, Eli Lilly and Co, São Paulo, Brazil, [2]Endocrinology Division, Paulista School of Medicine UNIFESP, S Paulo, Brazil, [3]Catholic University, Rio de Janeiro, Brazil, [4]Gynecology Division, UFPR, Curitiba, Brazil, [5]Gynecology Division, UFRS, Porto Alegre, Brazil, [6]Geriatric Division, School of Medicine USP, S Paulo, Brazil.

CHOOSE is an observational 12 month-study designed to evaluate compliance, safety and tolerability associated with raloxifene and alendronate. We evaluated the reasons physicians choose either raloxifene or alendronate for postmenopausal osteoporosis prevention or treatment. Thirty-two physicians were included in the study. They provided care for 247 postmenopausal women over 60 years old. Raloxifene was prescribed for 181 women and alendronate (10 mg per day or 70 mg per week) was prescribed for 95 women. Physicians completed a questionnaire to indicate their reason for prescribing either raloxifene or alendronate. The questionnaire provided 9 options (multiple answers were allowed): "well tolerated", "prevents fractures", "stronger bone mineral density data", "cardiovascular safety", "breast safety", "uterus safety", "no thromboembolic disease", and "other, please specify". 87.3% of raloxifene prescriptions and 95.8% of alendronate prescriptions were written to prevent fractures, p = 0024. 84.5% and 54.7 % of prescriptions were written because of raloxifene and alendronate tolerability, respectively, $p < 0.001$. Stronger BMD data was the reason for 51.9 % and 66.3 % raloxifene and alendronate prescriptions, respectively, $p < 0.001$. Breast, cardiovascular and uterine safety were the reasons for prescribing raloxifene in 61.3 %, 49.7 % and 43.6 % of patients and in 25.3 %, 20.0 %, 22.1 % for prescribing alendronate, $p < 0.001$ to all comparisons. No venous thromboembolic risk was the reason for 9.9 and 29.5 % raloxifene and alendronate prescriptions, respectively, $p < 0.001$. Others causes for prescribing were < 2.1% in both groups. Our data indicate that physicians considered fracture reduction as the most frequent reason to prescribed raloxifene or alendronate in our setting. Fracture reduction, BMD data, and tromboembolic safety were more related with alendronate than with raloxifene. Tolerability, breast, cardiovascular and uterus safety was more related with raloxifene than with alendronate. In conclusion, the main reason for prescribing raloxifene or alendronate was to prevent osteoporotic fractures in Brazil. Each drug seems to have specific attributes physicians consider when choosing these anti-resorptive therapies in Brazil.

Disclosures: ***O.L. Bracco****, Bracco OL 3; Kayath MJ 3; Marson JE 3.*

M352

Patient's Clinical Features and Compliance Associated with Raloxifene or Alendronate after a 6 month-observational Brazilian Study. F. Bandeira[1], M. Kayath[2], J. Marques-Neto[3], S. Ragi*[4], J. Danovski[5], S. Radominski[6], L. Ito*[2], O. L. Bracco*[2]. [1]H A Magalhães, Recife, Brazil, [2]Eli Lilly and Co, S Paulo, Brazil, [3]Rheumatology Division, UNICAMP, Campinas, Brazil, [4]CEDOES, Vitória, Brazil, [5]Rheumatology Division, UFRJ, Rio de Janeiro, Brazil, [6]Rheumatology Division, UFPR, Curitiba, Brazil.

CHOOSE is a prospective observational 12 month-study designed to evaluate patient satisfaction and compliance related with raloxifene or alendronate in a natural set. Thirty-two physicians included 247 postmenopausal women over 60 years old with osteopenia or osteoporosis. Raloxifene was prescribed for 181 women and alendronate (10 mg o.d. or 70 mg weekly, 23% and 77%, respectively) was prescribed for 95 women. The median chronological age for raloxifene group was 69.0, and 71.3 years for the alendronate group (p = 0,011). The time since menopause onset was 19.8 ± 8.2 (median ± SD) and 22.5 ± 9.8 years for raloxifene and alendronate groups, respectively (p = 0,016). HRT was the most frequent prior treatment in the raloxifene or alendronate groups (38% and 13% of women, respectively, p < 0,001). The densitometric test result was the most frequent clinical criteria to prescribe an anti-resorptive treatment in both groups (N.S.). Thirteen percent of women in the raloxifene group and 24% in the alendronate group had a previous osteoporotic fracture (p < 0,001) at the beginning of the study. Eighteen percent of the raloxifene patients and 17% of alendronate patients abandoned treatment after 6 months of treatment (N.S.). All patients that stopped alendronate due to adverse events complained of gastrointestinal symptoms, while headache, leg cramps or an endometrial hyperplasia were the reasons for stopping raloxifene. Compliance, assessed by a specific 8-question questionnaire, was not different between raloxifene and alendronate groups. Patient satisfaction, evaluated by a visual analog scale, was not statistically different between groups before and after 6 months of treatment (78.5 ± 21.3 for raloxifene and 76.7 ± 18.8 for alendronate, N.S.). Thus, raloxifene was prescribed for postmenopausal women that were younger and with shorter time since menopause than for those which alendronate was prescribed. Besides, raloxifene was more commonly prescribed than alendronate after HRT, although a great number of patients with prevalent osteoporotic fractures were on alendronate. Raloxifene and alendronate treatments had the same discontinuation and compliance rate although the majority of the patients on alendronate was on weekly dose. In conclusion, patients profile for raloxifene and alendronate were different, discontinuation rate and compliance were not different in an observational Brazilian study.

Disclosures: ***O.L. Bracco****, Márcia J Kayath 3; Lívia T Ito 3.*

M353

Comparison of Raloxifene and Alendronate on Markers of Cardiovascular Health in Postmenopausal Women with Osteoporosis. J. D. Adachi[1], J. Reginster*[2], Y. Qu*[3], S. Siddhanti*[3], C. Keech*[3]. [1]Dept. of Medicine, St. Joseph's Hospital-McMaster Univ., Hamilton, ON, Canada, [2]Bone/Cartilage Metabolism Unit, CHU Brull, Liege, Belgium, [3]Women's Health, Eli Lilly and Company, Indianapolis, IN, USA.

Raloxifene (RLX) and alendronate (ALN) are approved for prevention and treatment of osteoporosis in postmenopausal women. The current analysis compared the effect of RLX and ALN on surrogate markers of cardiovascular health in postmenopausal women with osteoporosis. In all, 331 postmenopausal women were randomized in a double-blind fashion to one of 4 treatment groups: 1) placebo (PLC); 2) RLX 60 mg/day; 3) ALN 10 mg/day; or 4) RLX 60 mg/day plus ALN 10 mg/day (RLX+ALN). Serum lipids and fibrinogen were analyzed at baseline and at 1, 6, and 12 months of treatment. A mixed model repeated measures (MMRM) approach was used to account for the correlation over time. Only women who had a baseline and at least one post-baseline measurement were included in the lipid analyses (n=322). Baseline values were not significantly different among treatment groups, with overall mean LDL-C of 155.3 mg/dl, HDL-C of 59.9 mg/dl, non-HDL-C of 175.7 mg/dl, total cholesterol of 235.6 mg/dl, triglycerides of 102.3 mg/dl and fibrinogen of 3.2 g/L. RLX and RLX+ALN, but not ALN, significantly reduced LDL-C, total cholesterol, non-HDL-C and fibrinogen compared to baseline (p<.0001). The reductions in these parameters observed with RLX and RLX+ALN were significantly different from PLC and ALN (Table 1). There were no significant differences between treatment groups in HDL-C or triglycerides. There were no significant differences either between PLC and ALN, or between RLX and RLX+ALN for any parameter in Table 1 (p>.05). Similar results were obtained for women who did not report use of lipid-lowering drugs during the trial (n=313). There was no difference in incidence of adverse events that may be related to RLX or ALN, including vasomotor symptoms (4%-8%, p=.86). In conclusion, RLX, but not ALN, had favorable effects on cardiovascular markers, and co-administration of the two drugs resulted in lipid levels similar to that seen with RLX alone.

Table 1. Mean percent change from baseline to 12 months

	PLC	RLX	ALN	RLX+ALN
Total-C	1.7*	-9.0**	0.1*	-5.7**
LDL-C	-0.2*	-14.8**	-3.5*	-8.3**
Non-HDL-C	0.1*	-13.5**	-2.3*	-6.8**
HDL-C	7.4	3.6	7.2	6.9
Triglycerides	9.4	1.1	10.8	14.2
Fibrinogen	-0.1*	-6.6**	-1.1*	-8.2**

*p<.005 vs RLX and RLX+ALN; **p<.005 vs ALN and PLC

Disclosures: ***J.D. Adachi****, Eli Lilly 2, 5, 6, 8; Merck 2, 5, 6, 8; Procter & Gamble 2, 5, 6, 8; Aventis 2, 5, 6, 8.*

M354

The Effect of Bone Mineral Density Feedback and Group Education on Osteoporosis Preventive Behaviour and Bone Mineral Density in Premenopausal Women: A Randomized Controlled Trial. T. M. Winzenberg*[1], S. Frendin*[2], B. Oldenburg*[3], G. Jones[1]. [1]Menzies Centre for Population Health Research, University of Tasmania, Hobart, Australia, [2]Department of Health and Human Services, Hobart, Australia, [3]Queensland University of Technology, Brisbane, Australia.

Limited information is available on ways to influence bone density and hence later osteoporosis risk in premenopausal women. The aim of this study was to determine the effects of bone density (BMD) feedback and differing educational interventions on osteoporosis preventive behaviour and BMD in pre-menopausal women.

A total of 467 healthy, non-pregnant women aged 25-44 years were randomly recruited, and then were randomly assigned to receive either an osteoporosis information leaflet or the Osteoporosis Self-management Course (OPSMC). Those with a mean T score < 0 at the femoral neck and lumbar spine were informed that they were at higher risk of fracture in later life, while those with a mean T score ≥ 0 were informed that they were at no higher risk. The following were measured at baseline, 12 months and 2 years: calcium intake and calcium supplement use by food frequency questionnaire; physical activity by self-report and objective measures; and smoking behaviour by self-report. BMD was repeated at 2 years.

During the study period, femoral neck BMD increased significantly in all groups (+1.1% p.a., 95% CI +0.9, +1.4). There was no significant change in lumbar spine BMD (+0.1% p.a., 95%CI -0.1, +0.2). Those with low BMD had a greater increase in femoral neck BMD than those with normal BMD (Difference +0.9% p.a., 95%CI +0.5, +1.4). Both the educational interventions had similar increases in BMD compared with baseline. (Leaflet = +1.0% p.a., OPSMC = + 1.3% p.a., p=0.4). Behavioral changes associated with increases in femoral neck BMD were: starting to take calcium supplements during the study (1.0% p.a., 95% CI 0.1, 1.8), and having self-reported change in physical activity levels at both 12 months and 2 years (0.6% p.a., 95% CI 0.1, 1.1). Smoking cessation, lower limb strength and endurance fitness were not associated with changes in femoral neck BMD.

In conclusion, bone density feedback in premenopausal women is effective at increasing hip but not spine bone density. This effect appears to be mediated by changes in physical activity (but not fitness) and calcium supplement usage. Simple educational interventions appear as effective as more intensive interventions at increasing BMD. As both these health behaviors have implications for the prevention of a number of diseases, the provision of bone density information at a young age may have substantial public health benefits, particularly if these gains can be maintained in the longer term.

Disclosures: ***T.M. Winzenberg****, None.*

M355

Underuse of Osteoporosis Treatment in Postmenopausal Women at High Risk for Low Trauma Fracture: Evidence from National Osteoporosis Risk Assessment (NORA). E. S. Siris[1], T. W. Weiss*[2], S. Barlas*[2], Y. Chen[2], E. Barrett-Connor[3], P. D. Miller[4]. [1]Toni Stabile Osteoporosis Center, Columbia Presbyterian Medical Center, New York, NY, USA, [2]Outcomes Research, Merck & Co., Inc., West Point, PA, USA, [3]University of California San Diego, San Diego, CA, USA, [4]Colorado Center for Bone Research, Lakewood, CO, USA.

Postmenopausal women with low bone mineral density (BMD) and/or additional risk factors are at elevated risk for low impact fracture. Treatment for these high-risk women is strongly recommended to avoid future fracture events. It has been shown that low BMD measured at a peripheral site, though not always as low as central bone density measurements in the same patient, is highly predictive of fracture risk. We evaluated the extent of osteoporosis treatment in postmenopausal women who had low bone density at a peripheral site (with or without additional risk factors) in a large cohort of NORA participants recruited from primary care physician offices.

Women in NORA had no prior diagnosis of osteoporosis and were not on osteoporosis-specific treatment at the time of enrollment. However, hormone therapy (HT) was allowed. In NORA, both participants and their physicians received education on osteoporosis and its management and treatment. Between 5/99 and 6/00, 143,930 participants with baseline BMD measured at one peripheral site reported if they were currently taking osteoporosis-specific medication (calcitonin, raloxifene or alendronate; risedronate was not yet available) at the time of the one-year follow-up survey. HT was not included as osteoporosis treatment since it was not known if participants were specifically prescribed this therapy for osteoporosis. Of the 74,352 women who reported not using HT at baseline, 7,507 (10%) reported HT use at year 1. Indications for treatment were defined as a T-score ≤ -2.5 (World Health Organization diagnostic definition of osteoporosis) or a T-score < -2.0 or < -1.5 with ≥ one risk factors including low body weight, personal or family history of fracture, and current smoker (NOF criteria for treatment).

25,455 (17.7%) participants reported receiving any osteoporosis-specific treatment. 9,367 women had a T-score ≤ -2.5 and of those 55.3% reported receiving treatment; another 32,298 women had a T-score < -2.0 or –1.5 with ≥ one risk factors and of those 42.1% reported receiving treatment.

In NORA, despite physician and patient education on osteoporosis, less than one-half of women at increased risk for fracture, for whom interventions to reduce fracture should be considered, reported receiving treatment. In the primary care setting, there is room for improvement in the management of postmenopausal women at increased risk for osteoporotic fractures.

Disclosures: ***E.S. Siris****, Merck & Co., Inc 2, 5, 8; Procter & Gamble/Aventis (Alliance for Better Bone Health) 5, 8; Eli Lilly 2, 5, 8; Wyeth 5, 8.*

M356

Redesigning Care of Fragility Fracture Patients to Improve Osteoporosis Management. J. T. Harrington*[1], H. L. Barash*[2], S. Day*[1]. [1]Department of Medicine, Rheumatology Section, University of Wisconsin Medical School, Madison, WI, USA, [2]Department of Orthopedics, University of Wisconsin Medical School, Madison, WI, USA.

Fragility fracture patients seldom receive osteoporosis care, even though they are at high risk of fracturing again. In our health system, only 5% of 1999 hip fracture patients were provided DXA and a bisphosphonate (BP). In the last 3 years, we have redesigned our traditional processes to improve this care. Industrial process improvement methods (Plan-Do-Study-Act cycles) were used to complete 3 improvement cycles. In Cycle 1, (2001) hip fracture inpatients were educated about osteoporosis care, and their primary physicians (PCP's) were prompted to provide it. Only 20% were evaluated and treated within the next 6 months in spite of 78% desiring care. In Cycle 2, (2002) a 4-month pilot project was completed wherein all vertebral and long bone fracture patients above 50 years old were directly referred by 3 willing orthopedists to an osteoporosis care service (OCS) with the agreement of PCP leadership. New care processes included nurse management, identifying patients each month for direct referral from orthopedic billing data, registering patients and managing tasks with process management software, and monitoring by telephone. Thirty-seven of 42 patients were enrolled. Two were deceased; 3 lived elsewhere. Their median age was 75, 44% had a previous fracture, 27% a prior DXA, and 14% a BP. Twenty-three (62%) accepted OCS management, 9 preferred PCP care, and 5 were unwilling or unable to participate. All OCS patients had a DXA and consultation, 19 a BP, 2 had hyperparathyroidism, and 2 with normal bone mass were not treated. BP patients are monitored each 3 mos., and all are adherent to treatment at 9-12 mos. Four of 9 PCP patients had DXA and 3 a BP. Of 27 DXA's, 12 showed osteoporosis, 11 osteopenia, and 4 a normal bone density. Of 54 patients managed by 3 nonparticipating orthopedists and PCP's during the same period, none had a DXA and 3 a BP. In Cycle 3, (2003) all orthopedists agreed to participate. After establishing treatment, the OCS returns patients to their PCP and monitors adherence. Conclusions: Osteoporosis care was not provided reliably even after PCP's were prompted. Direct referral by orthopedists to an OCS has done so. Use of monthly billing data has aided reliable referral. Redesigning processes and provider roles is required, and performance data motivates physicians to consider this. Industrial process improvement methods can accomplish this. More effective prevention should also be provided before fractures occur. (Grant support from the Alliance for Better Bone Health.)

Disclosures: ***J.T. Harrington****, Alliance for Better Bone Health (Procter & Gamble and Aventis) 2, 5, 8; Merck Pharma 5, 8.*

M357

Octreotide Increases Bone Mineral Density in a Patient with Carcinoid and Osteoporosis. D. McCommon*, K. Tauer*, G. M. Palmieri. The West Clinic, P.C., Memphis, TN, USA.

We reported enhancement of the positive Ca balance in children by the somatostatin analog Octreotide (OC), suggesting Ca bone accumulation by OC (Am J Med Sci 310:91, 1995). Moreover, receptors for somatostatin and its analogs have been demonstrated in bone cells. A 68 year (y) old Caucasian woman presented with osteoporosis by DEXA [lumbar spine (LS) T score -3.8] but no fractures. From ages 68-74 y, she received Prempro, L-thyroxine for of hypothyroidism and erratic oral Ca because of gastrointestinal intolerance. DEXA was stable. At age 74 y, the diagnosis of malignant carcinoid of the intestine with liver metastasis was made. She underwent partial ileecetomy for intestinal obstruction by carcinoid and received short acting OC for 2 ½ y with some subjective improvement. Also at age 74 y, she underwent splenectomy for a localized lymphoma in the spleen followed by chemotherapy, and high doses of steroids, for 6 months. Between ages 73-75 y, BMD fell: LS -8.3%, total hip (TH) -10.5%. From age 76-79 y, she received slow release OC (Sandostatin LAR), 20 mg, sc every 28 d plus daily L-thyroxine, atorvastatin, lorazepam, verapamil, Prempro; but no Ca supplementation, or calcitonin (she did not tolerate bisphosphonates). Yearly BMD increased progressively: LS +11.4%, TH + 7.9%. Currently the patient is active with moderate short of breath on exertion. She does not have bone pain. The lymphoma is in remission. Serum and urine markers of bone turnover, PTH, creatinine, 25 OH and 1,25 OH_2 vitamin D continue to be normal during 9 years of observation. Urinary Ca was 63-111 mg/24h and 5HIAA fell from 65mg/d, before OC to 22-32 during OC (normal <10). This observation suggests that slow release OC may increase BMD in osteoporosis in agreement with previous studies on a potential effect of OC in bone.

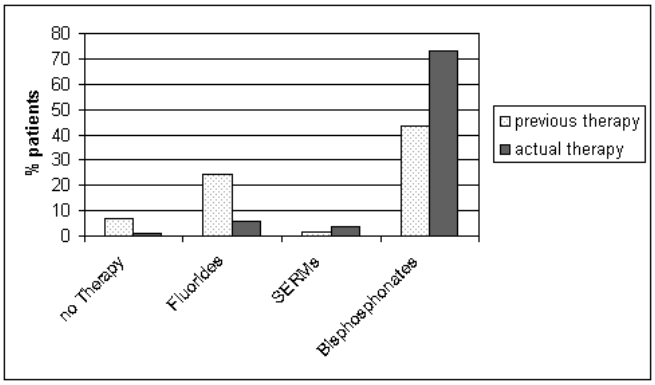
Disclosures: ***G.M. Palmieri****, None.*

M358

Quality Circles Increase the Quality of Treatment of Osteoporosis Patients in Germany. K. Abendroth[1], M. A. Dambacher[2], A. Defèr*[3], E. Bitzer*[4], B. Birkner*[5], K. Wawra*[6]. [1]Regional Experts Groups Osteoporosis (REKO), Jena, Germany, [2]Regional Experts Groups Osteoporosis (REKO), Konstanz, Germany, [3]Regional Experts Groups Osteoporosis (REKO), Dresden, Germany, [4]Institute for Social Medicine, Epidemiology and Health Systems Research (ISEG), Hannover, Germany, [5]Quality Management, Gastroenterology Practice, Munich, Germany, [6]Procter&Gamble Pharmaceuticals, Weiterstadt, Germany.

Osteoporosis is an under-treated disease in Germany - only a minor part of osteoporosis patients in Germany is treated with first line pharmacotherapy according to the recently published Osteoporosis Guidelines (DVO) for German-speaking countries.
With the initiative of REKO (Regional Experts Groups Osteoporosis) 97 Quality Circles Osteoporosis have been established in Germany to improve the quality of care of osteoporosis patients. Quality circles are interdisciplinary groups of physicians working on the improvement of osteoporosis care in their regions. Key elements of quality circle work are:
- Interdisciplinary and intersectoral networking
- Standardized documentation
- Continuing education and training.

A standardized documentation has been set up to deliver data in daily practice of osteoporosis care. The analysis of 4461 patients out of the quality circles documentation shows an increase in the quality of treatment of osteoporosis patients. There was an increase in use of EBM class A rated drugs in patients with diagnosed osteoporosis (n=1387) after visiting a physician working in a quality circle. The treatment with SERMs increased from 1.4.% to 3.7.%, bisphosphonates from 43.6% to 72.9%. The percentage of non-treated patients and therapeutics lower than EBM class A (i.e. fluorides) decreased.
Physicians working in Quality Circles Osteoporosis in Germany are improving the quality of treatment of osteoporosis patients in line with the National Osteoporosis Guidelines (DVO).



Disclosures: ***K. Wawra****, Procter&Gamble Pharmaceuticals 3.*

M359

Exploring Adherence to Calcium Recommendations in a Group of Post-Menopausal Women with Reduced Bone Mineral Density. M. R. French*, K. Moore*, F. Vernace-Inserra*, G. A. Hawker. Osteoporosis Research Program, Sunnybrook and Women's College Health Sciences Centre, Toronto, ON, Canada.

Ensuring adequate calcium intake is an essential component of the prevention and treatment of osteoporosis. No studies to date have provided information regarding adherence to dietary calcium recommendations among patients with reduced bone mineral density (BMD). The objective of this qualitative study was to describe the experiences of post-menopausal women with low BMD with respect to following calcium recommendations. Participants included 30 post-menopausal women diagnosed with low BMD (t score ≤ -1.0) who had attended an initial visit to a multidisciplinary program for osteoporosis treatment in 1999. Using focus group methodology, we explored perceived factors that affect adherence to dietary calcium intake and calcium supplement use. A semi-structured interview guide was used to guide the discussions. Focus groups were audio-taped and transcribed. From the transcripts, key words and concepts were identified and categorised. Several major themes associated with one's ability to obtain adequate calcium intake were identified and include (1) knowledge and confidence in actions, (2) lifestyle and food preferences and (3) side effects and conflict with other health conditions. Patients were aware of the importance of calcium to their bone health and were attempting to follow dietary calcium recommendations. Participants reported that they obtained information in an effort to make a confident decision regarding calcium intake yet were easily confused by conflicting information. Dietary behaviour was often influenced by changes in daily routines, family and personal food preferences. Women indicated that side effects, particularly those associated with perceived lactose intolerance, caused them to restrict their calcium intake. Our data provide important insight into the factors that patients believe affect their ability to reach the recommended intakes of calcium. Dietitians and other health professionals should focus on individualised patient assessments to identify factors affecting adherence to dietary recommendations in order to optimize osteoporosis prevention and treatment.

Disclosures: ***M.R. French****, None.*

M360

Monitoring Response to Osteoporosis Therapy with Alendronate by a Multi-site Ultrasound Device: A Prospective Study. E. Segal[1], I. Yaniv[2], M. Weiss[3], S. Ish-Shalom[1]. [1]Technion Faculty of Medicine, Rambam Medical Center, Haifa, Israel, [2]Research and Development, Sunlight Medical Ltd., Tel-Aviv, Israel, [3]Endocrinology Department, Assaf Harofeh Medical Center, Zerifin, Israel.

Introduction: Peripheral devices can be used for osteoporosis diagnosis, but their role in long term monitoring of skeletal changes is unclear. The current study evaluated the ability of Multi-site Quantitative Ultrasound (QUS) measurements to follow osteoporotic subjects treated with alendronate 10 mg/day.
Methods: QUS measurements were performed with Sunlight Omnisense™, which determines the bone speed of sound (SOS) in several skeletal sites. Thirty-six postmenopausal

women (mean age 68.9±9.4) with T-scores of –2 or less at least in one QUS measured site were treated with alendronate and followed for two years. Follow-up was done with QUS and DXA (Lunar DPX scanner, Madison, WI, USA) measurements.
Statistical methods: A *repeated-measures* experimental design, also called a *within-subjects* or *treatment-by-subject* method was used i.e. multiple measurements, which performed on the same subject comprised as replicate data. [1]
Results: Precision of SOS measurements with the Omnisense at the radius (RAD) and tibia (TIB) was 0.44%, and 0.59%, respectively. For the efficacy evaluation, we assessed women with a baseline T-score of –2 at the evaluated site. The table presents the increase in T-score at each site after 12 months and 24 months of follow up.
Conclusions: This longitudinal data of alendronate-induced SOS increase are in accordance with a previous report of one-year treatment follow up by Omnisense. Peripheral multi-site QUS measurement may be considered for follow-up on skeletal changes in response to alendronate treatment.
[1] Kepner and Robinson (1988) discuss nonparametric methods for this experimental design.

Change in T-score at 12 and 24 months Follow Up

	Site	12 months period - N; Mean(SD)	24 months period - N; Mean (SD)
Omnisense	Radius	N=21 0.25 (0.11)*	N=14 0.51(0.20)*
	Tibia	N=14 0.25 (0.09)*	N=11 0.34(0.12)*
DXA	Lumbar Spine	N=36 0.42 (0.06)*	N=21 0.34 (0.15)*
	Femoral Neck	N=22 0.13 (0.05)*	N=12 0.27 (0.13)

* p<0.05

Disclosures: ***I. Yaniv****, Sunlight Medical Ltd. 3.*

M361

Unipedicular Kyphoplasty for Treatment of Vertebral Compression Fractures. S. Timon*, M. Gardner*, R. E. Hong*, J. M. Lane. Orthopaedics, Hospital for Special Surgery, New York, NY, USA.

Introduction: Kyphoplasty effectively achieves pain relief and deformity correction in vertebral compression fractures. Standard technique includes perforating each pedicle and inserting two balloons per level. The purpose of this study was to evaluate the efficacy of single-balloon unipedicular kyphoplasty in restoration of vertebral height and patient satisfaction in the treatment of vertebral compression fractures.
Methods: One hundred and twenty-one vertebral compression fractures in fifty-two patients underwent kyphoplasty utilizing a unilateral approach. The entire procedure was performed under dual fluoroscopic guidance. One pedicle was perforated posteriorly and the guide wire was inserted across the midline on the anteroposterior view. Maximal correction was attempted at each level. Each level was measured pre-operatively and serially in the post-operative period. Vertebral bodies were measured at the anterior, mid-portion, and posterior margins and compared to the adjacent normal levels. Each patient filled out SF-36 and Oswestry questionnaires pre-operatively and at three months post-operatively. Two-tailed Wilcoxon signed rank tests were used to analyze changes in vertebral body height and questionnaire scores.
Results: Average restoration of anterior height was 21 percent ($p < 0.01$), mid-body height increased 40 percent on average ($p < 0.01$), and posterior height increased 5 percent ($p < 0.01$). Forty-eight SF-36 questionnaires were available for analysis. The physical functioning subsection score improved an average of 36 percent ($p < 0.01$), bodily pain improved 68 percent ($p < 0.01$), vitality improved 26 percent ($p < 0.05$), and role emotional improved 56 percent ($p < 0.01$). Thirty-one Oswestry questionnaires, specific for back pain, were available and scores improved an average of 30 percent ($p < 0.01$). There were no complications with the procedures. Of the patients whose fracture age was known, 40 percent (46 of 116) had fractures at least three months old.
Conclusions: Single-balloon kyphoplasty provides the surgeon with a viable option for vertebral compression fractures. This technique allows for excellent correction of deformity and provides the patient with significant pain relief and improved functioning. The correction and patient satisfaction achieved in this series is consistent with previous reports using a standard bipedicular technique. By using a unilateral approach to kyphoplasty, it significantly reduces operative time while maintaining the integrity of the contralateral pedicle. This approach may also be useful for chronic vertebral compression fractures.

Disclosures: ***S. Timon****, None.*

M362

The OPAL Study: BMD Data from a Three-year, Double-blind, Randomized Study Comparing the Effects of Tibolone, CEE/MPA and Placebo in Postmenopausal Women. M. R. McClung[1], M. Omizo*[1], R. D. Langer*[2], K. H. McBride*[3], F. A. Helmond*[4]. [1]Oregon Osteoporosis Center, Portland, OR, USA, [2]University of California, San Diego, La Jolla, CA, USA, [3]Organon USA Inc, West Orange, NJ, USA, [4]Organon International Inc, Roseland, NJ, USA.

The Osteoporosis Prevention and Arterial effects of tiboLone (OPAL) study is a three-year study multi-center, double-blind study to determine the effects of tibolone, continuous combined conjugated equine estrogens (CEE) plus medroxyprogesterone acetate (MPA) and placebo on carotid intima media thickness and bone mineral density (BMD) in postmenopausal women. 866 healthy postmenopausal women between the ages of 45 and 59 and who were at least 1 year beyond menopause were randomly assigned to receive daily therapy with tibolone 2.5 mg (Tib), 0.625 mg CEE plus 2.5 mg MPA (EP) or placebo (C). All subjects also received 500 mg calcium supplement daily. Women with severe osteopenia were excluded at the discretion of the local investigator. DXA measurements of the lumbar spine (LS) and in the total hip (TH), femoral neck (FN) and trochanteric (Troch) regions of the proximal femur were obtained at baseline and after 12, 24 and 36 months of treatment. Of those enrolled, 759 subjects had at least one post baseline assessment (ITT group). At baseline, the ITT subjects were, on average, 58.8 years old, 10.6 years beyond menopause, and had a BMI of 25.2. The preliminary BMD data are presented as the mean percent change from baseline after 3 years treatment using the last observation carried forward approach in the ITT group

Treatment group	Number of subjects	Mean baseline BMD (gm / cm^2)		BMD: % change from baseline			
		LS	TH	LS	TH	FN	Troch
Tibolone	247	1.00	0.89	+4.22%	+3.61%	+3.10%	+6.18%
CEE / MPA	255	1.00	0.90	+4.56%	+3.01%	+3.31%	+5.61%
Placebo	257	0.99	0.88	-1.96%	-1.05%	-0.27%	+0.36%

The differences between the active groups and the placebo group at each skeletal site were highly significant ($P<0.0001$). No differences were observed between Tib and EP. A difference of more than 10% between Tib and EP was observed for the following site effects: breast pain (Tib=15.0%, EP=31.3%, C=4.5%) and vaginal bleeding (Tib=10.8%, EP=26.4%, C=3.1%). The differences between Tib and EP were statistically significant $p<0.0001$. In conclusion, Tib and EP therapy for 3 years resulted in significant and similar increases in BMD compared to placebo. Fewer subjects who received Tib experienced vaginal bleeding and breast pain than in the EP group. Tibolone may be an attractive option for the prevention of bone loss in postmenopausal women.

Disclosures: ***F.A. Helmond****, Organon International Inc 3.*

M363

Regulation of Osteoblast Phenotype and Gene Expression by Hop-Derived Phytoestrogens. K. E. Effenberger*[1], S. A. Johnsen*[2], D. G. Monroe[3], T. C. Spelsberg[3], J. Westendorf*[1]. [1]Institute for Toxicology, UKE, Hamburg, Germany, [2]Ctr for Molecular Neurobiology, Hamburg, Germany, [3]Dept of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA.

Loss of estrogen at the onset of menopause results in increased fracture risk and osteoporosis. While hormone replacement therapy (HRT) prevents these effects there are also significant risks associated with it. Recent studies have demonstrated that HRT increases the long-term risk for certain cancers, thus alternative therapies have to be found. Certain plant-derived compounds may be suitable for use as such an alternative to prevent osteoporosis without any undesirable side effects. However, the molecular mechanisms and estrogen receptor (ER) specificity of many of the phytoestrogens in osteoblasts are not known. In the current study we tested the effects of 8-prenylnaringenin (8-PN), 6-PN, xanthohumol (XH) and isoxanthohumol (IXH) and a hop extract (HE) to modulate markers of differentiation and gene expression in osteoblasts. The bone-forming activity of these compounds was initially investigated by measuring alkaline phosphatase (AP) activity in human fetal osteoblast (hFOB) cells, stably transfected with either ERα or ERß, following treatment with the compounds. To further assess the ER specificity of these compounds, and their ability to regulate gene transcription, we treated U-2 OS cells, stably expressing equal levels of ERα or ERß, with these compounds and measured the mRNA levels of AP, IL-6, pS2 and the novel estrogen target gene von Willebrand Factor (VWF) using RT-PCR. Finally, the cytotoxicity of prenylated flavonoids was measured using a colony forming efficiency assay. Our results demonstrate that AP, pS2 and VWF mRNA levels are significantly increased by the compounds in an estrogen-like manner in the order 8-PN = HE > 6-PN > XH > IXH in both ERα and ERß containing cells. Interestingly, while IL-6 is similarly down-regulated by the compounds and estrogen in cells expressing ERα, mRNA levels were unchanged in the ERß-cell line with all treatments. Consistent with our gene expression data, AP activity is upregulated by all compounds and HE in hFOB/ERα9 cells. AP activity was undetectable in the hFOB/ERß6 cells, possibly reflecting a low level of estrogen receptor. Finally, the flavonoids display cytotoxic effects only at high concentrations (10^{-6}M - $3x10^{-5}$M). In summary, we have demonstrated for the first time that specific phytoestrogen compounds found in hop extract regulate markers of bone formation as well as gene expression through both ERα and ERß. These data suggest that hop-derived phytoestrogens exert estrogen-like activities on bone metabolism and display potential for use in osteoporosis-prevention therapy.

Disclosures: ***K.E. Effenberger****, Dr. Willmar Schwabe AG 2.*

M364

Combination Therapy with Micronized Dehydroepiandrosterone and Raloxifene or Placebo: Changes in Markers of Bone Turnover. M. F. Delaney[1], S. Hurwitz*[2], C. K. Chan*[1], M. S. LeBoff[1]. [1]Endocrinology, Diabetes, and Hypertension Division, Brigham and Women's Hospital, Boston, MA, USA, [2]Harvard Medical School, Boston, MA, USA.

Dehydroepiandrosterone (DHEA), an anabolic agent, decreases with aging and is associated with low bone mineral density (BMD). Raloxifene is a selective estrogen-receptor modulator and anti-resorptive agent approved to treat postmenopausal osteoporosis. In this study, we investigate combination therapy with Raloxifene and micronized DHEA (supplied by Belmar Pharmacy, CO) to evaluate the combined effect of an anti-resorptive and an anabolic agent on bone turnover. Twenty-five postmenopausal women with a T score <

–2 and no secondary causes of bone loss were enrolled in this randomized, double-blinded, placebo-controlled study. Subjects were recruited from our osteoporosis clinic and local advertisements. Mean age was 64 years (range 51-84). All subjects received micronized DHEA 25 mg PO BID and Raloxifene 60 mg PO QD or placebo for 12 weeks. Calcium 500 mg QD and vitamin D 400 IU QD was given to all subjects. Baseline and 12-week BMD of femur, lumbar spine and whole body were measured using dual energy X-ray absorptiometry (DEXA; QDR 4500A, Hologic). Levels of serum DHEAS, testosterone (testo), estradiol (E), and markers of bone turnover, serum bone-specific alkaline phosphatase (BSAP) and urine N-telopeptides (NTX), were also measured. One subject dropped out due to constipation. Subjects received placebo (n=13) and Raloxifene (11).
As expected, DHEAS, T and E increased in all subjects. The rise in E was less in Raloxifene-treated subjects than with placebo (5.9 pg/mL vs 9.6 pg/mL; p=0.05). No other significant difference was found between the two groups. We then examine changes in the entire study cohort (n=24) from baseline to 12 weeks. The predicted decrease in urine NTX in response to DHEA and Raloxifene was not observed. Femoral neck BMD increased by 0.01 g/cm^2 (0.02) (p<0.03) and lean body mass increased by 0.68 g/cm^2 (1.2) (p=0.015). There was no change in BMI or fat mass. The increase in BSAP without a decrease in NTX suggests that the anabolic activity of DHEA predominates. Overall, we found a significant increase in bone formation markers, lean body mass and femoral neck BMD in both groups. Further studies are needed to evaluate the long-term effect of DHEA and Raloxifene on bone turnover and bone density.

n=24	Mean change over 12 weeks (SD)	P Value	Normal range
DHEAS ug/dL	206 (104)	< 0.0001	35-450
Testo ng/dL	18 (12)	< 0.0001	30-100
E pg/mL	8 (4.8)	< 0.0001	2-19
BSAP u/L	3 (3.9)	< 0.001	15-44

Disclosures: ***M.F. Delaney****, Eli Lilly and Company 2; Procter & Gamble Pharmaceuticals 2, 5, 8; Roche Pharmaceuticals 2.*

M365

Effect of Raloxifene and Alfacalcidol on Spinal Bone in Ovariectomized Rats by the Simultaneous Administration. S. Higashi*, T. Masaki*, A. Shiraishi*, N. Hayakawa*, N. Kubota*, N. Imai*. Product Research Department, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

The aim of this study was to investigate the effect of Raloxifene (RLX) on ovariectomy (OVX) induced spinal bone loss and fragility when simultaneously administered with Alfacalcidol (ALF). 40 week-old Wistar rats were OVX or sham operated and then treated with vehicle, RLX (0.1, 0.3, 1.0, 3.0 mg/kg/day) and ALF (0.0125, 0.025, 0.05 μg/kg/day) respectively or in combination. Each drug was orally administered 5 times a week for 12 weeks. A significant increase in spinal bone mineral density (BMD) by RLX was already evident at the doses of 0.1 and 0.3 mg/kg (p<0.01 vs. OVX, respectively). As for ALF treatment, a significant rise was shown at the dose of 0.05 μg/kg (p<0.001 vs. OVX). Administration of RLX and ALF, however, markedly suppressed the elevated bone resorption markers; osteoclast surface (Oc.S/BS) and osteoclast number (Oc.N/BS), almost dose-dependently while sustained the increased bone formation markers; mineral apposition rate (MAR) and bone formation rate (BFR/BS), to the similar levels of those of OVX+vehicle rats. These data, however, did not simply explain spinal BMD changes as indicated above. Then, the combination effect was examined. RLX, at the dose of 0.1 or 0.3 mg/kg, sustained significant inhibitory effect on bone loss even by the simultaneous administration of ALF 0.025 or 0.05 μg/kg. In addition, in the combination groups of RLX with ALF 0.05 μg/kg, mean values of bone strength (N) increased and, especially, at the combination of RLX 0.1 mg/kg and ALF 0.05 μg/kg, which corresponded to the optimal dose of each monotherapy, reversed from 435.0±59.1 to 557.1±65.2 (p<0.05 vs OVX), although significant changes were not detected by OVX and any doses of single use. All the combination treatments exhibited significant and optimal anti-resorptive effects while keeping the bone formation markers (MAR, BFR/BS) to the OVX levels. Another important point is that, although 0.05 μg/kg ALF raised urinary Ca excretion, RLX corrected it dose-dependently. In summary, RLX, alone or in combination with ALF, was effective on the spinal BMD, although it was difficult to explain the exact mechanism in this study. The simultaneous administration of RLX and ALF did not produce any new unexpected side effects. Finally, the combination treatment might be effective and somewhat expand RLX effect on spine, although a further analysis is needed.

Disclosures: ***S. Higashi****, None.*

M366

Comparison Study of Fatty Acids between the Pre- and Postmenopausal Women and Pre- and Post-Treatement of 4 Weeks of Raloxifene using GC-MSD. Y. Rhee*[1], M. Paik*[2], K. Kim*[2], S. Lim[1]. [1]Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea, [2]College of Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea.

The incidence of cardiovascular diseases rises in the women especially after the menopause. However, it seems that the phenomena do not occur in the same degree among the different ethnic groups. That is, the Eskimo show much lower incidence than the Western menopausal women. Interesting analysis of fatty acids revealed that Eskimos have lower ratio of ω-3 unsaturated fatty acids(UF) which are the precursors of cyclooxygenase pathway vs. ω-6UF which are the precursors of lipooxygenase pathways, where the Western women had higher ratio. So, in this study we tried to show whether the menopausal status affected the status of fatty acids in the Korean women who were apparently healthy and to see whether there might be some changes of the fatty acids after the treatment of raloxifene. We recruited 10 premenopausal women(average 36.5 y.o.) and 28 postmenopausal women(avg. 56.8 y.o., year since menopause 9 y) without any known chronic diseases. The informed consents were taken. They fasted for 10 hours then the blood samples were taken. Among this group, 5 postmenopausal women were selected to participate in the 4 week trial of raloxifene. They had the blood sampling in the same way after the 4 weeks of 60 mg raloxifene. We removed the proteins in the acquired serum of 0.2mL using acetonitrile. Then the distilled water 1mLand the pentadecanoic acid 5ug as the internal standard were administered. The samples were alkalinized with 5M NaOH and washed with the ether. They were acidified again, then saturated with NaCl and extracted with ether. The extracts were totally dried up under the nitrogen gas flow, tert.-butyldimethylsily derived, then were analysed using the selected ion mode(SIM) by the gas chromatography-mass selective detector(GC-MSD). All analysis were done triplicate. In the premenopausal women, they showed higher ratio of the ω-3 UF/ω-6 UF, than the postmenopausal women(0.70 vs. 0.38, p=0.05). The docsapentaenoic/erucic acid were the polyunsaturated fatty acids which were higher in the premenopausal women(p<0.05). Interestingly the ratio of ω-3 UF/ω-6 UF were higher after the 4 weeks of treatment(0.16 vs 0.14, p=0.06). The rise in the ratio of ω-3 UF/ω-6 UF could result in the increase in eicosanoids associated with strong thrombogenic tendency. In conclusion, the reasons of the increase in the atherosclerosis in the postmenopausal women and the increased risk of thromboembolic event of raloxifene were partially explained by the meticulous analyses of the fatty acids in our study.

Disclosures: ***Y. Rhee****, None.*

M367

Progestogens Influence Action of Estrogen on Bone Turnover. P. L. Selby, J. L. Berry*, M. Davies. Medicine, University of Manchester, Manchester, United Kingdom.

Hormone replacement therapy (HRT) has been seen as one of the major forms of treatment for osteoporosis. In general, its benefits have been ascribed to the oestrogen component and less attention has been paid to the effects of progestogens. In order to assess the effect of various progestogens on the action of oestrogen on bone we have undertaken a randomised controlled crossover trial of three different progestogens in women receiving oestrogen based HRT. 20 postmenopausal women were studied. They were all taking HRT has part of a larger study into the effects of progestogens on the vascular action of oestrogen. All women received unopposed oestrogen in the form of conjugated equine oestrogens 0.625mg daily for three months. Following this they received each of the progestogens medroxyprogesterone acetate (MPA), desogestrel (DG) or norethisterone (NE) for 14 days each month for three months in addition to oestrogen. Each subject received all of the progestogens in random order. Bone turnover was assessed using serum bone specific alkaline phosphatase (BSAP) as a marker of bone formation and serum CTX as a marker of bone resorption.
Bone resorption decreased following oestrogen therapy: CTX was 0.428 (SE 0.059) before oestrogen and 0.286 (0.040)ng/ml on oestrogen alone (p<0.0001). There was no further significant change with the addition of progestogen (MPA 0.286 (0.048)ng/ml; DG 0.267 (0.038)ng/ml and NE 0.236 (0.030))ng/ml. There was a borderline significant trend for a lower CTX with increasing androgenicity of the progestogen (p=0.038). There did not appear to be any influence of the order in which progestogens were given. BSAP showed a smaller change on oestrogen 11.9 (0.8)iu/l off treatment and 11.1(0.7)iu/l on oestrogen alone (p=0.06). Treatment with all of the progestogens suppressed BSAP more than oestrogen alone (MPA 9.3 (0.4)iu/l; DG 9.8 (0.5)iu/l; NE 9.2 (0.4)iu/l all p<0.001 compared with oestrogen). There was no difference in suppression of BSAP between the progestogens nor was there a significant effect of the order in which they were administered.
We conclude that the addition of progestogen to HRT has effects over and above those of oestrogen alone. These effects may be greater with the more androgenic progestogens.

Disclosures: ***P.L. Selby****, Various companies in the field 2, 5, 8.*

M368

Treatment with Raloxifene for Two Years Increases Vertebral Bone Mineral Density as Measured by Volumetric Quantitative Computed Tomography. H. K. Genant[1], T. Lang[1], T. Fuerst[2], K. V. Pinette*[3], C. Zhou*[3], A. Diez-Perez[3]. [1]University of California, San Francisco, San Francisco, CA, USA, [2]Synarc, Inc., San Francisco, CA, USA, [3]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.

Volumetric quantitative computed tomography (vQCT), using multiple thin-slice acquisition, measures 3-dimensional volumetric bone mineral density (BMD, mg/cm^3). vQCT is often used to measure BMD of lumbar vertebrae, and may detect early changes in trabecular, cortical or integral BMD that extend beyond the technical limits of areal dual x-ray absorptiometry (DXA) BMD measurements. The objective of this study was to determine the effect of two years of raloxifene (RLX) treatment on various volumetric BMD measures in a subset of postmenopausal women (n=58) enrolled in the Multiple Outcomes of Raloxifene Evaluation (MORE) trial. Patients in this study were randomized to one of three treatment groups: placebo (n=21), RLX 60 mg/d (n=17), or RLX 120 mg/d (n=20), and all patients received daily calcium (500mg) and vitamin D (400-600 IU) supplementation. The data from both RLX treatment groups were pooled for analysis. Following two-years of RLX treatment there was a significant percent increase in the vQCT regions of interest (ROI) of Mid Integral BMD, Total Trabecular BMD, and Total Integral BMD simulating AP DXA (p<0.05), compared to placebo. A multivariate analysis was also performed and showed an overall significant effect of RLX treatment on all seven vQCT measurements (p=0.02), compared to placebo. The percent change from baseline for all vQCT BMD variables (mg/cm^3), including simulated DXA ROIs and for standard lumbar spine DXA BMD (g/cm^2) are presented in the table.

Variable	Placebo (n=21)	RLX (n=37)	p-value*
vQCT Mid Integral BMD	-1.95	1.06	0.001
vQCT Total Trabecular BMD	-2.39	0.55	0.016
vQCT Total Integral BMD ~AP DXA	-1.42	0.86	0.020
vQCT Integral BMD ~Lat DXA	-1.64	0.42	0.080
vQCT Mid Trabecular BMD	-3.03	-0.06	0.052
vQCT Total BMD ~Lat DXA	-1.08	0.25	0.251
vQCT Cortical BMD	-0.77	0.10	0.391
DXA Spine BMD	1.49	3.46	0.105

* p-values based on an ANCOVA model adjusting for baseline differences

These data provide a longitudinal assessment by vQCT of changes in vertebral bone density after two years of treatment with RLX. vQCT appears to have greater sensitivity compared to DXA to measure the effects of RLX treatment in this population.

Disclosures: ***H.K. Genant****, Eli Lilly and Company 2, 5, 8.*

M369

Effect of Testosterone Enanthate on Whole Body Muscle Mass, Bone Mass and Fat Mass in *Ob/Ob* Leptin Deficient Mice. M. Liu*, Y. Wang*, K. Chen*, A. L. Schmidt*, M. Sato, E. C. Black*, C. A. Frolik, A. K. Harvey*, G. Krishnan. Gene Regulation, Bone & Enabling Biology, Eli Lilly and Company, Indianapolis, IN, USA.

Leptin is a regulator of peak bone mass because of its ability to inhibit bone formation (*Cell*, 100:197-207, 2001). *Ob/Ob* mice represent a leptin deficient genotype and accordingly reflect higher peak bone mass. In this study male *Ob/Ob* mice that were 12 wks old (n=6) were treated with vehicle or TE (s.c. 10 mg/kg/5d) for 16 weeks. Starting at 8 weeks after treatment, the TE-treated *Ob/Ob* mice showed an increase in muscle mass and a decrease in fat mass as measured by DEXA. In contrast, the vehicle group showed the opposite body composition, namely, an increase in fat mass and a decrease in lean mass, while the total body weight was about the same for the two groups. Serum analysis in these animals was performed to examine changes in creatine kinase, and osteocalcin levels after treatment with TE. At the end of the study, femoral BMD, BMC and cross section area were determined by micro QCT. In addition changes in periosteal alkaline phosphatase were measured at the end of the study. Results from these experiments will shed light on the bone anabolic properties of TE in the context of a higher peak bone mass background. In addition, these results measure the impact of alterations in body composition as a result of TE treatment, on bone mineral density and bone mineral content, and bone quality in these animals.

Disclosures: ***M. Liu****, Eli Lilly and Company 3.*

M370

Effect of Raloxifene on Angiotensin Type 1 Receptor and Endothelial Nitric Oxide Synthase Gene Expression Induced by High Glucose Concentration. J. G. Gminski*[1], T. M. Francuz*[1], T. Gawlik*[1], W. Garczorz*[1], E. Kotrys-Puchalska*[1], T. Jurczak*[1], V. Skrzypulec*[2]. [1]Experimental and Clinical Biochemistry, Silesian Medical Academy, Katowice, Poland, [2]Women Health, Silesian Medical Academy, Katowice, Poland.

According to post-hoc analysis of MORE study raloxifene reduces risk of cardiovascular events in high-risk group by 40%. The most important issue is to know mechanisms of action of raloxifene in cardiovascular system. Acute coronary syndrome is a result of the rupture of atherosclerotic plaque as a consequence of its destabilisation. According to the response-to-injury theory – activation of endothelial cells by metabolic factors, e.g. diabetes, hyperlipidaemia, may play an important role in the induction and progression of atherosclerosis. Among proteins involved in the pathogenesis of atherosclerosis crucial roles are played by angiotensin type 1 receptor (AT1R) and endothelial nitric oxide synthase (eNOS).

The aim of the study was to asses the effect of raloxifene and beta-estradiol on AT1R and eNOS gene expression in human umbilical artery endothelial cells (ECs) incubated with high glucose concentration.

Materials and methods: ECs obtained from human umbilical artery were incubated in selective growth medium with supplement containing ECs growth factors. Just before experiment, cells were cultured in serum-free medium containing 5 or 15 mM glucose. Simultaneously beta-estradiol or raloxifene in concentration 1 microM were added. After 48h cells were harvested, and total cellular RNA were isolated. eNOS and AT1 mRNA levels were calculated using real time RT-PCR technique. Expression of mRNA was normalized using mRNA levels of glucose-6-phosphate dehydrogenase as reference gene. As a control, cells cultured in medium containing 5 mM glucose were used. Statistical analyses were performed by ANOVA test.Results: beta-estradiol and raloxifene have beneficial effects increasing eNOS expression and decreasing AT1 expression in cells incubated with high glucose concentrations.

Expression of AT1 receptor gene

Glucose	Control	beta-estradiol	raloxifene
5 mM	100%	53%	61%
15 mM	324%	170%	183%

Expression of eNOS gene

Glucose	Control	beta-estradiol	raloxifene
5 mM	100%	164%	152%
15 mM	64%	80%	83%

Disclosures: ***J.G. Gminski****, None.*

M371

Interaction between Hormone Replacement Therapy and Resistance Training on Spine Bone Mineral Density in Early Postmenopausal Women. G. F. Maddalozzo, Ph.D*, J. J. Widrick, Ph.D*, M. A. Hoffman, Ph.D*, C. M. Snow, Ph.D.. Exercise and Sports Science, Oregon State University, Corvallis, OR, USA.

Evidence suggests that hormone replacement therapy (HRT), specifically; estrogen in combination with resistance training may promote greater increases in bone mass (BMD) than either resistance training or HRT alone. Thus, the purpose of this study was to investigate the interactive effects of HRT and a 1-yr site-specific resistance-training program. Specifically, two free weight exercises (squat and deadlift) two days per week as a strategy to reverse or attenuate accelerated bone loss at the spine in early postmenopausal women, a time when bone loss is accelerated. Participants from a group of self selected HRT or no-HRT (n = 148) were randomly assigned to either 1) no HRT plus resistance training (n=28); 2) HRT plus resistance training (n=33); 3) HRT no resistance training (n=32); or 4) control (no HRT no resistance training group (n=29). Mean age and months post menopause did not differ between groups (52.1 $\pm$ 3.0 yr and 25.8 $\pm$ 9.9 months, respectively). Post-menopausal status of the subjects was confirmed by follicle-stimulating hormone levels $\geq$ 20 mIU/mL. Bone mineral density (BMD) of the spine was assessed by Dual Energy X-Ray Absorptiometry (Hologic), at baseline (T^1) and month 12 (T^2). Data were analyzed using a 4 (group: no HRT plus, HRT plus resistance training, HRT no resistance training, no HRT no resistance training) x 2 (time: baseline and month 12) repeated measures multivariate analysis of variance to determine the effects of resistance training on HRT and Non-HRT early postmenopausal women. Multivariate analysis revealed a main effects for group (p<. 007), time, (p<. 001), and the group by time interaction (p<. 001). One year of resistance training produced increases in spine BMD of (mean + SD; % Δ) (T^1) 0.973 $\pm$ .13, (T^2) 0.974 $\pm$ .13; +.43% and (T^1) 1.015 $\pm$ .12 (T^2) 1.021 $\pm$ .12; +.56%, respectively for the no HRT plus resistance training and HRT plus resistance training groups. In contrast, women in the control groups; HRT no resistance training and no HRT no resistance training experienced declines in spine BMD of (T^1) 1.014 $\pm$ .09 (T^2) 1.006 $\pm$ .09; -1.4% and (T^1) 0.958 $\pm$ .11 (T^2) 0.916 $\pm$ .09; -3.6%. In conclusion, regardless of HRT status, free weight squats and deadlifts performed two d/wk reversed bone loss at the spine. Moreover, resistance training was more effective than hormone replacement therapy in preventing bone loss at the spine in this group of early postmenopausal women.

Disclosures: ***G.F. Maddalozzo, Ph.D****, None.*

M372

Combination of Soy and a Sub-Optimal Dose of 17β-Estradiol may Reverse Bone Loss in a Rat Model of Postmenopausal Osteoporosis. L. Devareddy*[1], D. A. Khalil[1], L. J. Hammond*[1], D. Y. Soung[1], E. A. Lucas[1], B. J. Smith[1], S. Juma[1], S. C. Chai*[1], D. S. Galloway*[2], B. H. Arjmandi[1]. [1]Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Veterinary and Medical Teaching Hospital, Oklahoma State University, Stillwater, OK, USA.

Although sub-optimal dose of estrogen can relieve menopausal symptoms, it cannot prevent bone loss associated with ovarian hormone deficiency. The purpose of the present study was to evaluate whether soy in combination with sub-optimal level of 17βestradiol (E_2) can reverse bone loss in ovariectomized osteopenic rats. Another objective of this study was to evaluate if the addition of fructooligosacchride (FOS), a non-digestible carbohydrate that supports the growth of beneficial bacteria in the gut, can improve the bone protective efficacy of soy. Seventy two 9-mo old female Sprague-Dawley rats were either sham-operated (sham) or ovariectomized (ovx) and then fed a casein-based semi-purified diet for 90 days to establish bone loss. Thereafter, rats were divided into six groups (n=12): sham, ovx (control), ovx + E_2 (E_2; 10 µg E_2/kg body wt. twice per wk), ovx + ½E_2 (5µg E_2/kg body wt. twice per wk), ovx + ½E_2 + soy; ovx + ½ E_2 + soy + fructooligosacchride (FOS). 125 days after treatment the rats were necropsied and the bone mineral density (BMD) and content (BMC) of tibia, femur, third and fourth lumbar vertebrae (L3-L4) were measured using dual energy x-ray absorptiometry. The table below shows the effects of various treatments on selected bone parameters:

Group	Tibial BMD	Femoral BMD	L4 BMD	L3 BMC
Sham	0.1993±0.002^{a}	0.2317±0.003^{a}	0.2342±0.003^{a}	0.1279±0.004^{a}
Ovx+Control	0.1825±0.002^{c}	0.2054±0.004^{c}	0.2055±0.003^{c}	0.1071±0.004^{b}
Ovx+ E_2	0.1892±0.002^{bc}	0.2097±0.003^{bc}	0.2132±0.003^{bc}	0.1163±0.004^{ab}
Ovx+½E_2	0.1862±0.002^{bc}	0.2086±0.003^{bc}	0.2122±0.003^{bc}	0.1065±0.004^{b}
Ovx+½E_2+Soy	0.1907±0.002^{b}	0.2111±0.003^{bc}	0.2155±0.003^{b}	0.1180±0.004^{ab}
Ovx+½E_2+Soy+ FOS	0.1886±0.002^{bc}	0.2170±0.004^{b}	0.2172±0.003^{b}	0.1207±0.004^{a}

Data are mean ± SE (n=12). Values in a column that do not share the same superscript letters are significantly (P<0.05) different from each other.

The results suggest that the combination of sub-optimal E_2 and soy protein has a greater effect on bone than optimal E_2 by itself. Furthermore, the addition of FOS to soy may enhance its effectiveness in reversal of bone loss as evident by improved femoral and L4 BMD and L3 BMC. FOS may have improved calcium absorption, isoflavone bioavailability or both.

Disclosures: ***L. Devareddy****, None.*

M373

The Cost-Effectiveness of Calcium and Vitamin D or Hormone Replacement Therapy in the Prevention of Osteoporosis and Osteoporotic Fractures in Canadian Postmenopausal Women. E. A. Papadimitropoulos[1], R. Goeree*[2], G. Blackhouse*[2], P. Coyte*[3], R. G. Josse[4], J. Adachi[2], C. Greenwood*[3]. [1]Research and Development, Eli Lilly Canada Inc., Scarborough, ON, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]University of Toronto, Toronto, ON, Canada, [4]St. Michael's Hospital, Toronto, ON, Canada.

Background: With continued advances in average life expectancy and a larger proportion of Canadian women reaching post-menopause, there are increasing concerns about the incidence of hip fractures (HF), vertebral factures (VF) and other fractures. Therapies designed to prevent postmenopausal osteoporosis can not only prevent these fractures, but also increase quality of life and potentially free up a significant amount of health care resources.
Methods: We developed a health state transition model to estimate the long-term costs and effects of "no intervention (NI)", calcium and Vitamin D (CVD) and Hormone Replacement Therapy (HRT) for the prevention of postmenopausal osteoporosis. Various data sources were used to estimate the natural progression of postmenopausal women in terms of the development of osteoporosis, osteoporosis-related fractures, cardiovascular disease, breast cancer and venous thromboembolism. The model also incorporated treatment relative risk data, mortality estimates, quality of life estimates, costs of treatments, and costs of managing various health conditions. Probabilistic sensitivity analyses, where probability distributions are defined for key model input parameter, were used to systematically incorporate uncertainty into the study results.
Results: When therapy is intiated at age 55, HRT is dominated by CVD having higher expected costs and lower quality adjusted life years (QALYs). HRT is expected to cost $5,128 over the 5 year treatment period and 25 year follow-up period for fractures. Although NI is not associated with direct treatment costs, the expected cost of NI for the treatment of downstream fractures is still high at $3,244 per patient. Overall, CVD is expected to cost $78,779 per QALY gained compared to NI. However, if treatment is initiated at age 65, the incremental cost per QALY gained decreases to only $38,062. The results of the model were sensitive to age of therapy initiation, the cost of CVD, who bears the cost of CVD and the rate used to discount future costs and effects. Conclusions: According to conventional bench-marks, CVD is a cost-effective management strategy for postmenopausal women, especially when therapy is initiated at around age 65.

Disclosures: ***E.A. Papadimitropoulos****, Eli Lilly Canada Inc. 1, 3.*

M374

Increased Uniformity in Bone Mineralization after Combined Treatment of PTH-(1-34) and OPG in Osteopenic Ovariectomized Rats. P. Roschger[1], A. Valenta*[2], N. Fratzl-Zelman*[1], P. J. Kostenuik[3], C. R. Dunstan[3], P. Fratzl*[2], K. Klaushofer[1]. [1]Ludwig Boltzmann Institute of Osteology, 4th Med. Department, Hanusch Hospital & UKH-Meidling, Vienna, Austria, [2]Erich Schmid Institute of Material Sience, Austrian Academy of Sciences and Institute of Metal Physics,University of Leoben, Leoben, Austria, [3]Metabolic Disorders Research, AMGEN, Inc., Thousand Oaks, CA, USA.

The combination of PTH with OPG have been proposed as potential therapy in patients with severe osteoporosis. In the present study we examined the bone material quality of aged ovariectomized (OVX) rats treated either with PTH-(1-34) or OPG alone or in combination of both. The micro and nanostructural characteristics of the mineralized bone were evaluated using quantitative backscattered electron imaging (qBEI) and small angle x-ray scattering (SAXS). Rats (n=68) were sham-operated or ovariectomized at the age of 3 months and 15 months later OVX animals were treated with vehicle, OPG (10µm/d), PTH (80 µm/kg/d,) or a combination of both during 5.5 months. Secondary metaphysial spongiosa from distal femora were assessed for mineralized bone volume (Md.BV/TV), for the mean Ca-concentration (Ca_{Mean}), for the width of the bone mineralization density distribution (Ca_{Width}) and the average mineral particle thickness parameter (T).
A remarkable increase of Md.BV/TV was found in the PTH treated groups independently of OPG up to 139% ($p<0.001$). Ca_{Mean} was slightly increased (+1.7%, $p<0.05$) in the OPG group. Ca_{Width} was reduced (-6.5%, $p<0.01$, and –9%, $p<0.001$) in animals treated with OPG and PTH+OPG, respectively. In contrast, Ca_{Width} in sham operated rats was 16 % ($p<0.01$) higher than in OVX. The T-parameter was not altered in all the treated and untreated OVX rats. However, there was a significant increase in T (+7%, $p<0.01$) with respect to sham-operated animals. The dramatic increase of bone volume in OVX aged rats could be due to a transient anabolic effect of PTH. The mineralization was rather uniform, indicating that bone turnover was already reduced when the animals were killed. A further (small) increase in homogeneity reflects the antiresorptive property of OPG. This increase would, probably, have been more pronounced in a state of higher bone turnover. In conclusion, qBEI and SAXS data suggest that the effect of OPG on bone remodeling is analogous to other antiresorptive treatments, and may contribute to increased bone quality and reduced fracture incidence.

Disclosures: ***P. Roschger****, None.*

M375

Severe Vertebral Fractures Are Associated with Reduced Quality of Life: Pronounced Reduction in Incidence of Severe Vertebral Fractures by Teriparatide. G. G. Crans*[1], H. K. Genant[2], S. J. Vargas[1], M. D. Rousculp*[1], J. H. Krege*[1]. [1]Eli Lilly and Company, Indianapolis, IN, USA, [2]UCSF, San Francisco, CA, USA.

We investigated the association between baseline vertebral fracture severity and quality of life (QOL) in the Fracture Prevention Trial (Neer et al. 2001), a double-blinded study of postmenopausal women with osteoporosis randomized to placebo or teriparatide [rhPTH(1-34)]. Vertebral fracture severity was assessed by a visual semi-quantitative (SQ) method (UCSF). Individual vertebrae were scored as SQ0 (no vertebral fracture, <~20% height decrease), SQ1 (mild vertebral fracture, ~20-25%), SQ2 (moderate vertebral fracture, ~25-40%), or SQ3 (severe vertebral fracture, >~40%). A subset of 465 patients completed the Osteoporosis Assessment Questionnaire and had a baseline spinal radiograph. Baseline QOL scores were modeled as a function of maximum baseline vertebral fracture grade, number of prevalent vertebral fractures, age, and lumbar spine BMD using ANOVA. The main effect of vertebral fracture grade was statistically significant, while interactions between vertebral fracture grade and the other variables were not statistically significant. SQ3 vertebral fractures were associated with lower QOL scores in physical function, emotional status, and symptom domains. After a median 19 months of therapy, new or worsening SQ3 vertebral fractures occurred in 21 patients (4.7%) in the placebo group (N=448) compared with 3 patients (0.7%) in the teriparatide 20 µg/day group (N=444). Thus, teriparatide 20 µg/day lowered the relative risk of new or worsening SQ3 vertebral fractures by 86% ($P<0.001$). We conclude that compared to fractures of lesser severity, SQ3 vertebral fractures were associated with reduced QOL, and that treatment with teriparatide significantly reduced the risk of new or worsening SQ3 vertebral fracture.

Comparisons of QOL domains between groups defined by maximum vertebral fracture grade

Comparison	Physical Function	Emotional Status	Symptoms	Social Interaction
SQ 3 vs. 0	-14*	-9*	-9*	1
SQ 3 vs. 1	-12*	-10*	-10*	4
SQ 3 vs. 2	-10*	-10*	-9*	5*
SQ 2 vs. 0	-4	0.5	0.02	-4
SQ 2 vs. 1	-2	-0.5	-2	-0.5
SQ 1 vs. 0	-2	1	-2	-3

*P<0.05; Within each QOL domain, lower scores indicate lower QOL

Disclosures: ***G.G. Crans****, None.*

M376

Teriparatide Reduces the Incidence of New or Worsening Back Pain in Women with Osteoporosis. H. K. Genant[1], W. H. Scheele*[2], L. Xie*[2], J. H. Krege*[2]. [1]UCSF, San Francisco, CA, USA, [2]Eli Lilly and Company, Indianapolis, IN, USA.

Administration of teriparatide [rhPTH (1-34)] may reduce the frequency of back pain and of back pain associated with vertebral fracture (VFx). The Fracture Prevention Trial (Neer et al. NEJM 2001) was a double-blinded study of 1,637 postmenopausal women with osteoporosis randomized to receive placebo or teriparatide 20 (TPTD20, the marketed dose) or 40 µg/day. Patients also received supplemental calcium and vitamin D. Patients were defined as having back pain if they reported new or worsening back pain during the trial. VFx severity was assessed by a visual semi-quantitative method at study entry and after a median of 19 months of therapy (UCSF). Compared with placebo, significantly fewer patients treated with TPTD20 reported back pain, moderate or severe back pain, or severe back pain (Table, I). Patients in the placebo group who reported back pain were more frequently found to have new VFx ($P<0.001$, relative risk increased 255%) and more severe VFx ($P<0.001$) than patients not reporting back pain. Compared with the placebo group, fewer patients treated with TPTD20 reported back pain and had a finding of: ≥ 1 new VFx, 1 new VFx, > 1 new VFx, or new moderate or severe VFx (Table, II). We conclude that TPTD therapy of postmenopausal osteoporosis in this study setting meaningfully reduced the overall risk of back pain. Moreover, it nearly eliminated the risk of back pain with a subsequent finding of VFx, consistent with the previously demonstrated effect of TPTD20 to reduce the risk of moderate and severe VFx by 90%.

I. Overall risk of back pain

	Placebo N=544		TPTD20 N=541		Relative Risk Reduction (%)
	n	%	n	%	
Back Pain	123	23	91	17*	26
Moderate / Severe Back Pain	90	17	62	11*	31
Severe Back Pain	28	5	12	2*	57

II. Back pain with a subsequent finding of VFx

	Placebo N=448		TPTD20 N=444		
Back Pain + ≥ 1 VFx	29	6	5	1†	83
Back Pain + 1 VFx	18	4	4	1†	78
Back Pain + > 1 VFx	11	2	1	0.2†	91
Back Pain + Moderate / Severe VFx	23	5	0	0†	100

*P<0.02 vs. Placebo; †P<0.004 vs. Placebo

Disclosures: ***H.K. Genant****, Ei Lilly, Merck, Aventis, GSK, Novartis, BMS, Amgen, Wyeth Ayerst 5; Synarc, Sunlight 8.*

M377

Four Weekly Treatments with Sustained-Duration PTH-Fc Increases Bone Mineral Density in Cynomolgus Monkeys. J. E. Atkinson[1], P. J. Kostenuik[2], S. Y. Smith[3], N. Doyle*[3], P. Cranmer*[1], C. R. Dunstan[4], M. E. Cosenza*[1]. [1]Toxicology, Amgen Inc, Thousand Oaks, CA, USA, [2]Metabolic Disorders, Amgen Inc, Thousand Oaks, CA, USA, [3]CTBR, Senneville, PQ, Canada, [4]ANZAC Research Insititute, Concord, Australia.

We hypothesized that extending the circulating half-life of PTH would permit less frequent dosing while maintaining anabolic activity. To test this, we produced a long-acting form of PTH-(1-34) fused to the Fc portion of IgG1 (PTH-Fc). As part of a safety evaluation study we investigated the effects of short term treatment with PTH-Fc on biochemical markers of bone turnover and bone mineral density and content (BMD and BMC). Forty-eight cynomolgus monkeys, 2 years of age, were assigned to 4 treatment groups (6/sex/group) and received 4 weekly subcutaneous doses of placebo or 10, 100 or 300 ug/kg of PTH-Fc followed by a 1-month treatment-free recovery period (2/sex/group). Routine parameters were assessed for determination of toxicity. Blood samples were drawn for routine clinical pathology determinations, and serum samples were collected at pretreatment, week 2 and 4 of treatment and at the end of recovery for markers of bone turnover [osteocalcin (OC), bone specific alkaline phosphatase (BSAP), and C-telopeptide (CTx)]. pQCT scans were used to measure BMC, BMD, and geometric parameters from the right distal radius and proximal tibia.

PTH-Fc was well tolerated in monkeys, as no definitive signs of toxicity were noted. Males and/or females administered 100 and 300 ug/kg PTH-Fc showed statistically significant ($p<0.05$) dose related increases in total slice BMD (6-9%) and trabecular BMD (18-25%) at the proximal tibia following 4 weeks of treatment. Increases in total slice BMC of 7 to 13% were distributed throughout the total slice area resulting in decreased trabecular area and increased cortical/subcortical area using a threshold analysis. Similar, but less prominent changes were noted at the distal radius. The treatment–related increases observed at 4 weeks on total slice and trabecular BMD were sustained during a 4-week untreated recovery period. The low dose of PTH-Fc (10 ug/kg) had no effect on pQCT-derived parameters nor was there a consistent effect of treatment noted at the tibial or radial diaphysis at any dose.

BSAP and CTx markers were increased at week 2 and 4 of treatment with 100 and 300 ug/kg PTH-Fc, with increases in OC for males at week 2. At the end of the recovery period, OC values in treated groups were comparable to controls while BSAP in males and CTx in females at the 100 ug/kg dose remained elevated from control.

This study demonstrated that a sustained duration PTH given once weekly had strong anabolic activity as evidenced by rapid (1 month) and statistically significant changes in BMD.

Disclosures: ***J.E. Atkinson****, None.*

M378

Sustained-Duration PTH-[1-34] (PTH-Fc) Rapidly Restores BMD and Bone Strength in Aged Osteopenic OVX Rats with and without Estrogen Supplementation. P. J. Kostenuik[1], S. Morony[1], K. S. Warmington*[1], S. Adamu*[1], Z. Geng[1], M. Grisanti*[1], H. Tan*[1], V. Shalhoub[1], T. Boone*[2], C. Dunstan[3], V. Shen[4], D. Lacey[1]. [1]Metabolic Disorders Research, Amgen Inc., Thousand Oaks, CA, USA, [2]Protein Science, Amgen Inc., Thousand Oaks, CA, USA, [3]ANZAC Research Inst., Concord, Australia, [4]Skeletech Inc., Bothell, WA, USA.

The aged osteopenic skeleton poses a significant challenge for restoring bone mass. Anabolic agents like PTH are more efficacious than antiresorptives, but the requirement for daily injections may limit compliance. We therefore created PTH-Fc, a version of PTH-[1-34] with a long circulating half-life conferred by the Fc portion of human IgG. PTH-Fc has optimal efficacy in rodents when injected twice per week. We tested the ability of PTH-Fc to restore BMD and bone strength in aged osteopenic OVX rats, with and without estrogen (E) supplementation. Rats were OVXd or sham-operated at 4-6 months of age. After 9 months, DEXA analysis revealed significant osteopenia at the lumbar vertebra (LV) and the distal femoral metaphysis of OVX rats compared to shams. OVX rats were then treated with either vehicle (PBS), or PTH-Fc (4.5 mg/kg, 2/week SC), or E (0.1 mg/kg, 3/week SC), or with PTH-Fc + E. DEXA was performed weekly, and rats were sacrificed after 4 weeks of treatment. The LV and femur were harvested for destructive mechanical testing. Treatment of OVX rats with PTH-Fc alone, but not with E alone, caused significant gains in BMD at the LV and the femur compared to PBS-treated OVX rats. In the LV and the femur, PTH-Fc + E reversed 100% and 213% of the BMD deficits associated with 10 total months of E ablation. Compressive testing of the LV revealed significant improvements in max load and ultimate strength with PTH-Fc alone, but not with E alone. 3 point bending of the femur revealed significant improvements in max load with PTH-Fc + E, but not with either treatment alone. Compressive testing of the femur revealed significant improvements in max load with PTH-Fc alone, but not with E alone. PTH-Fc + E caused significant improvements in stiffness, max load, ultimate strength and elastic modulus of the compressed femur. These data demonstrate that infrequent dosing with a sustained duration PTH construct is a strong anabolic stimulus. This finding challenges the perception that brief intermittent PTH exposure is critical for anabolism. The addition of E caused even greater improvements in BMD and bone strength at several skeletal sites. 4 weeks of treatment with PTH-Fc + E was capable of fully restoring BMD and bone strength associated with 10 total months of E ablation. The combination of anabolic and antiresorptive agents appears to be an effective means to rapidly restore BMD and bone strength in aged osteopenic bone.

Disclosures: ***P.J. Kostenuik****, None.*

M379

Sustained-Duration PTH-[1-34] (PTH-Fc) Plus Estrogen Restores Bone Mass in Aged Osteopenic OVX Rats. P. J. Kostenuik[1], S. Morony[1], K. S. Warmington*[1], S. Adamu*[1], Z. Geng*[1], M. Grisanti*[1], H. Tan*[1], V. Shalhoub[1], T. Boone*[2], C. R. Dunstan[3], D. Lacey[1]. [1]Metabolic Disorders Research, Amgen Inc., Thousand Oaks, CA, USA, [2]Protein Science, Amgen Inc., Thousand Oaks, CA, USA, [3]ANZAC Research Inst., Concord, Australia.

Restoring bone mass in the aged osteopenic skeleton may require the combination of antiresorptive and anabolic agents. PTH is an effective anabolic agent, but the requirement for daily injections may limit compliance. We therefore created PTH-Fc, a sustained-duration version of PTH-[1-34] that has a long circulating half-life conferred by its fusion to the Fc portion of human IgG. We tested the ability of PTH-Fc to restore bone mass in aged osteopenic OVX rats, with and without estrogen (E) supplementation. Rats were OVXd or sham-operated at 4-6 months of age. After 9 months OVX rats were treated with either vehicle (PBS), or PTH-Fc (4.5 mg/kg, 2/week SC), or E (0.1 mg/kg, 3/week SC), or with PTH-Fc + E. Blood was drawn weekly for serum collection. Rats were sacrificed after 4 weeks of treatment. Tibiae were harvested for dynamic bone histomorphometry. PTH-Fc alone caused a 20% increase in serum calcium at day 3, after which time serum calcium returned to normal levels. Co-treatment with E did not inhibit this transient hypercalcemia. PTH-Fc alone caused significant and sustained increases in serum osteocalcin and alkaline phosphatase, while E alone caused modest reductions. Co-treatment with E partially inhibited the ability of PTH-Fc to increase these bone formation markers. Serum TRAP was significantly suppressed by E alone, while PTH-Fc had no significant effects on this resorption marker. Histologically, OVX led to a significant (70%) reduction in cancellous bone volume in the proximal tibia compared to shams. Treatment of OVX rats with PTH-Fc + E fully restored bone volume within 4 weeks. Interestingly, each agent alone had no significant effect on bone volume, suggesting a synergistic effect with combination therapy. Osteoclast surface was decreased by E alone and was increased by PTH-Fc alone. E co-treatment prevented the increase in osteoclast surface associated with PTH-Fc. PTH-Fc alone increased osteoblast surfaces, and co-treatment with E blunted most of this response. Bone formation and apposition rates were significantly increased by PTH-Fc alone, and co-treatment with E blunted these responses. These data demonstrate that PTH-Fc + E represents an effective and convenient combination for increasing bone volume in aged osteopenic rats. The anabolic effects of PTH-Fc were partially suppressed by co-treatment with E. However, the antiresorptive effects of E appeared to compensate for this suppression and led to greater gains in bone volume compared to either agent alone.

Disclosures: ***P.J. Kostenuik****, None.*

M380

Ostabolin-C™: A Novel Parathyroid Hormone Analogue Uncouples Bone Turnover in Monkeys. J. Jolette*[1], S. Y. Smith[1], J. Mayer*[1], C. Green*[2], B. Rushton*[3], P. Morley*[4]. [1]CTBR, Montreal, PQ, Canada, [2]Covance Labs, N Yorkshire, United Kingdom, [3]Zelos, Stevenage, United Kingdom, [4]Zelos Therapeutics, Ottawa, ON, Canada.

Ostabolin-C™ (Leu^{27}, Cyclo[Glu^{22}Lys^{26}]-hPTH[1-31]amide), a novel parathyroid hormone (PTH) analogue being developed for the treatment of osteoporosis, was administered daily by subcutaneous injection to naïve cynomolgus monkeys (3/sex/group) at dose levels of 0, 10, 25 or 80 µg/kg for 7 weeks to evaluate toxicity. Monkeys were 15 to 24 months of age at the start of treatment. Blood and urine samples were collected to measure biochemical markers of bone turnover and the tibiae were retained for histomorphometry following labeling with calcein green 15 and 5 days prior to euthanasia.

Ostabolin-C™ was well tolerated with no hypercalcemia observed in blood samples collected at Weeks 3 and 6, approximately 24 hours post-dose. Ostabolin-C™ substantially increased cancellous bone mass in the secondary spongiosa of the proximal tibia at all doses. BV/TV was increased >30% compared to controls, attributable to increases in both Tb.Th and Tb.N. This increase in bone mass appeared to be related to increases in bone formation (BFR/BS, <88%) and decreases in bone resorption (Oc.S/BS, <81%). In the tibial mid-diaphysis, periosteal woven bone apposition and increased cortical porosity were the most evident structural changes in high dose animals. Substantial increases in BFR/BS of 2 to 3-fold compared to controls were noted at the periosteal (females only) and endocortical surfaces, and at the Haversian systems, for high dose animals. The endocortical eroded surface was moderately decreased (NS, <97%) in all treated groups, a response consistent with decreases in Oc.S/BS in metaphyseal cancellous bone, and compatible with a reduction of bone resorption. The small number of animals per group precluded observations of a clear reduction of the medullary cavity, the expected outcome of these processes on the endocortex. Increases in the biochemical markers of bone formation, osteocalcin and bone specific alkaline phosphatase, were observed for high dose animals and mid dose females. Biochemical markers of bone resorption, C-telopeptide and deoxypyridinoline,

were comparable to control animals, with the exception of C-telopeptide that showed increases for mid and high dose females.
In conclusion, the results of this study are consistent with the anticipated anabolic effects of Ostabolin-C™ as a PTH analogue. However, the increases in indices of bone formation were associated with decreases in indices of bone resorption, consistent with the uncoupling of these events. This antiresorptive activity and increased bone formation may be of therapeutic value in the treatment of osteoporosis.

Disclosures: ***J. Jolette****, None.*

M381

Effects of Intermittent Administration of Human Parathyroid Hormone on Bone Mineral Density in Rats with Collagen-induced Arthritis. S. Fukata*, H. Hagino, Y. Kameyama*, I. Yamane*, T. Okano, R. Teshima*. Orthopaedic Surgery, Tottori University, Yonago, Japan.

Purpose: We investigated the effects of intermittent administration of human parathyroid hormone (hPTH) on bone mineral density (BMD) in rats with collagen-induced arthritis (CIA).
Materials and Methods: Forty-eight 7-month-old female Sprague-Dawley rats were randomly divided into four groups: control (n= 12), CIA+V (n= 12), CIA+PTH4 (n= 12), and CIA+PTH6 (n= 12). In CIA groups, bovine type II collagen was intracutaneously injected as an initial sensitization. The PTH [h-PTH(1-34)] was subcutaneously injected at 20 g/kg 3 times a week for 6 weeks from 2 weeks after initial sensitization in CIA+PTH6 and for 4 weeks from 4 weeks after initial sensitization in CIA+PTH4. Every 2 weeks, joint score and posterior limb swelling were evaluated, and BMD of the trabecular and cortical bones in metaphysis and diaphysis of the tibia was measured by pQCT. Eight weeks after initial sensitization, the rats were sacrificed and bone histomorphometry of the proximal tibia was perfomed.
Results: Posterior limb swelling was severest at 4 weeks in the three CIA groups. The joint score was the maximum by 6 weeks in all groups, but no significant difference was observed among the groups. At the metaphysis, BMD of cancellous bone began to decrease with the onset of arthritis in the three CIA groups. In CIA+V it became significantly lower than in control at 8 weeks. However, in the PTH-treated groups it did not decrease after 4 weeks. It became significantly higher in CIA+PTH6 than in CIA+V at 8 weeks. In CIA+V, BMD of cortical bone at the metaphysis decreased at 8 weeks with a delay compared with the decrease in cancellous bone. However, it did not decrease in the PTH-treated groups, and it became significantly higher in CIA+PTH6 than in CIA+V at 8 weeks. Histomorphometric analysis showed that BV/TV and Tb.Th were higher in CIA+PTH6 than in CIA+V and Tb.Sp was lower in CONT and CIA+PTH6 than in CIA+V. OS/BS was higher in the three CIA groups than in CONT and MAR and BFR/BV were increased in the PTH-treated groups.
Conclusion: These results suggested that intermittent administration of hPTH activated bone formation, and maintained bone mass in CIA rats.

Disclosures: ***S. Fukata****, None.*

M382

Protein Intake Determines Net Bone Strength Response to PTH or GH, Two Anabolic Agents Increasing Bone Turnover. P. Ammann[1], J. A. Gasser[2], R. Rizzoli[1]. [1]Department of Geriatry and Internal Medicine, Division of Bone Diseases, Geneva-14, Switzerland, [2]Dept. of Arthritis & Bone Metabolism, Novartis Pharma AG, Basel, Switzerland.

Osteoporotic elderly with protein undernutrition are targets for bone anabolic agents. Whether protein intake could influence the response to bone anabolic agents is unknown. To test this hypothesis, we studied six-month old female rats, which were fed isocaloric diets containing 2.5% (low Protein) or 15% (normal Protein) casein for 2 weeks. Then, bGH (0.5 or 2.5mg/kg BW) or solvent were given subcutaneously to rats on either diet twice daily for 4 weeks. In another series of experiment using the same type of protocol PTH(1-34) (5 or 40 µg/kg BW) or solvent were given in rats fed the different diets. Proximal tibia bone mineral density (BMD) and ultimate strength were measured after 4 weeks of either treatment, under either diet. bGH treatment dose-dependently decreased BMD (-5.1% ± 2.5 and -10.7% ± 1.8* in rats treated with 0.5 or 2.5 mg/kg BW) and bone strength (-20.0% ± 4.9* and -44.0% ± 5.6*, respectively) in rats fed a low protein diet. No significant effects were observed in rats fed a high protein diet. PTH(1-34) treatment dose-dependently increased BMD (+10.0% ± 2.1* and +21.5% ± 2.2*, in rats treated with 5 or 40 µg/kg BW) and ultimate strength (+55.3% ± 14.3* and +96.5% ± 16.1*, respectively) in rats fed a high protein diet. Similar effect on BMD (+4.1% ± 2.0 and +11.06% ± 2.7*, respectively) and ultimate strength (+4.2% ± 8.4 and +43.8% ± 13.0*, respectively) were observed in rats fed a low protein diet. Thus to achieve the same effect a higher dose of PTH(1-34) was required in rats fed an isocaloric low protein diet. GH treatment resulted in a negative bone balance when protein intake was reduced in contrast to PTH(1-34) treatment, which induced a positive bone balance independently of protein intake. These results indicate that an isocaloric protein restriction did not prevent the anabolic response to PTH but was associated with marked catabolic effects of GH on bone. These results emphasize the major importance of dietary protein intake in the bone response to GH or PTH administration.

Disclosures: ***P. Ammann****, None.*

M383

Human Parathyroid Hormone 1–34 Reverses Bone Loss in Orchidectomized Adult Rats Mainly by Vastly Increasing Trabecular Thickness. Y. Gabet[1], D. Kohavi*[2], R. Müller[3], M. Chorev[4], I. A. Bab[1]. [1]Bone Laboratory, Hebrew University of Jerusalem, Jerusalem, Israel, [2]Dental Implantology Center, Hebrew University of Jerusalem, Jerusalem, Israel, [3]Institute for Biomedical Engineering, Swiss Federal Institute of Technology (ETH) & University of Zürich, Zürich, Switzerland, [4]Division of Bone & Mineral Metabolism, Beth Isreal Deaconess Medical Center & Harvard Medical School, Boston, MA, USA.

Osteoporosis and the resulting increased fracture incidence are important complications of surgical and chemical castration in prostate cancer patients. Currently, bone loss in these patients is prevented by anti-resorptive treatment. Nevertheless, they could greatly benefit from supplementary or alternative bone anabolic therapy. Intermittently administered parathyroid hormone (PTH) rescues bone loss in sex hormone deprived women and female experimental animals and increases bone mass in elderly men and normal male animals. This study was carried out to assess whether the PTH anabolic activity is also effective in adult castrated males and to gain insight into the associated tissue mechanisms. Four months old rats were subjected to bilateral orchidectomy (ORX). Six weeks later they were treated intermittently with human PTH(1-34), 80 µg/Kg/day or vehicle for 6 weeks. Femora were evaluated by quantitative micro-computed tomography (µCT). The trabecular bone volume density (BV/TV) showed a respective 40% and 56% loss in the distal metaphysis in 6-week and 12-week post-ORX, non-PTH–treated animals. PTH induced complete recovery of this bone loss and further gain above the sham-ORX animals (155% recovery) consequent to a vast increase in trabecular thickness (243% and 174% over ORX rats and sham-ORX controls, respectively). An increase in the trabecular number also contributed to the PTH-induced stimulation of BV/TV, but this parameter showed only partial recovery (62%). ORX did not result in any significant change in the mid-diaphyseal cortical thickness (Cort.Th). By contrast, the PTH treatment induced a highly significant thickening of the cortex (110.0% and 108.3% over ORX rats and sham-ORX controls, respectively). The increase in Cort.Th was partly at the expense of the medullary cavity volume, and partly by increased diameter of the femoral shaft, suggesting a PTH stimulation of both endosteal and periosteal bone formation. These data portray PTH as a highly potent bone anabolic agent in adult ORX rats mainly by increasing both the trabecular and cortical thicknesses. The adult ORX rat model will be useful in future studies investigating the mechanisms involved in the PTH anabolic activity in castrated osteoporotic males and for the development of bone anabolic agents for treating this condition.

Disclosures: ***Y. Gabet****, None.*

M384

Three-Dimensional Microarchitecture of the Trabecular and Cortical Bone in the Iliac Crest of Postmenopausal Osteoporotic Women Treated with Teriparatide [rhPTH(1-34)]: Reproducibility of Micro CT Quantification. Y. Jiang[1], J. J. Zhao[1], E. F. Eriksen[2], H. K. Genant[1]. [1]Osteoporosis and Arthritis Research Group, University of California, San Francisco, CA, USA, [2]Lilly Research Laboratories, Indianapolis, IN, USA.

This study evaluated the precision of micro CT quantitation of 3 dimensional (3D) microarchitecture of the trabecular (Tb) and cortical bone in the iliac crest bone biopsies from postmenopausal osteoporotic women treated with teriparatide (rDNA origin) [rhPTH(1-34)]. Capturing true longitudinal transmenopausal changes in 3D bone micro structure may improve our ability to understand the pathophysiology of osteoporosis and other bone disorders, and to estimate the bone biomechanical properties in terms of fracture resistance because the mechanical competence of bone is a function of its apparent density and 3D distribution. During aging and diseases such as osteoporosis, Tb 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. We examined 52 paired bone biopsies (102 specimens) from the iliac crest, from osteoporotic postmenopausal women with vertebral osteoporotic fracture treated with placebo or teriparatide injection. The specimens were scanned using a Scanco micro CT with isotropic resolution of 17 µ. 3D structural parameters were directly measured without stereological model assumptions as in 2D histomorphometry. Values of 0 and 3 for the structure model index (SMI) represent a perfect plate structure and an ideal cylindrical rod structure, respectively. Over 1 year later, 10 paired bone biopsies (20 specimens) from different groups were re-scanned and re-analyzed. The root mean square coefficient of variation (RMSCV) of the Tb and cortical bone 3D microstructural parameters was calculated as a measure of reproducibility. The RMSCV was 2.6% for Tb bone volume fraction (BV/TV), 3.6% for Tb number, 5.9% for Tb thickness, 4.0% for Tb separation, 3.3% for Tb degree of anisotropy, 2.1% for SMI, 3.9% for Tb connectivity density (CD), 2.7% for cortical porosity (Ct.Po), and 2.9% for cortical thickness (Ct.Th). The annualized median percent change in placebo group was –3.3% for BV/TV, 4.6% SMI, –9.2% CD, and -14% Ct.Po, and in teriparatide treated group 4.7% for BV/TV, -8% SMI, 13% CD, 0.5% for Ct.Po, and 14% Ct.Th. Thus, micro CT can reproducibly quantify 3D microarchitecture of the Tb and cortical bone in the iliac crest biopsies from postmenopausal osteoporotic women treated with teriparatide, which may find application in studying pathophysiology of osteoporosis and other bone disorders and evaluating their therapeutic efficacy.

Disclosures: ***Y. Jiang****, Eli Lilly and Company 2.*

M385

Treatment of Fibrodysplasia Ossificans Progressiva with Pamidronate: Case Report and Review of the Literature. L. A. Rudolph[1], E. M. Lewiecki[1], D. L. Glasser*[2], F. S. Kaplan[2]. [1]New Mexico Clinical Research and Osteoporosis Center, Albuquerque, NM, USA, [2]University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Case Report. This is a case report of the possible improvement of a flare-up of fibrodysplasia ossificans progressiva (FOP) with intravenous pamidronate. A 14-year old female with known FOP has a lifelong history of recurrent flare-ups of acute soft tissue pain and pre-osseous muscle swelling, usually lasting several weeks. Residual effects have ranged from none to permanent deformity due to soft tissue ossification. She presented with an acute flare-up, manifested by the sudden onset of localized chest wall pain and swelling. The patient was treated with an intraveneous pamidronate infusion, 1.2 mg/kg over 4 hours, beginning 24-hours after the onset of symptoms. Symptoms of chest wall inflammation and swelling resolved within 48 hours. This was a marked improvement compared to the natural course of her disease flare-ups since early childhood.

Review of Literature. FOP is a rare autosomal dominant, genetic disorder involving episodic flare-ups leading to progressive and disabling extraskeletal endochondral bone formation. Palliative therapy consists of corticosteroids, cyclo-oxygenase 2 inhibitors, leukotriene inhibitors and analgesics. At the present time there is no proven effective treatment for FOP. Several early changes in cytokine levels, cellular infiltration of lymphocytes, increased presence of mast cells, and new blood vessel formation (angiogenesis) have been identified. These changes include an increase in basic fibroblast growth factor (bFGF) and peri-vascular B-cell and T-cell lymphocyte infiltration into skeletal muscle. Because one of the first pathologic changes in the formation of new bone is angiogenesis, anti-angiogenic agents such as thalidomide have also been used with variable results. Recently, anti-angiogenic effects of bisphosphonates have been documented in vitro. Also, intravenous pamidronate has been shown to decrease vascular endothelial growth factor (VEGF) levels in cancer patients with bone metastasis and bFGF levels in patients with cancer-associated hypercalcemia. Both VEGF and bFGF are potent tumor-associated angiogenesis factors. Intravenous pamidronate has also been shown to reduce various lymphocyte sub-populations. Mast cells are also present but most pronounced at the highly vascular fibroproliferative stage of FOP lesions. SR 41319 and etidronate, both bisphosphonates, have shown activity in inhibiting passive cutaneous anaphylaxis by their action on mast cells. Intravenous pamidronate may potentially be a new treatment for flare-ups of FOP. Further controlled studies are warranted.

Disclosures: ***L.A. Rudolph****, None.*

M386

A Unique Sclerosing Bone Disorder Identified in Three Generations of One Family. J. M. Garcia*[1], W. A. Murphy[2], S. G. Waguespack[3]. [1]Joint Training Program in Endocrinology, Baylor College of Medicine and U.T. M.D. Anderson Cancer Center, Houston, TX, USA, [2]Diagnostic Radiology, U.T. M.D. Anderson Cancer Center, Houston, TX, USA, [3]Endocrine Neoplasia and Hormonal Disorders, U.T. M.D. Anderson Cancer Center, Houston, TX, USA.

Inherited sclerosing bone dysplasias encompass a large number of diseases that have various genetic causes and clinical presentations. We present a family with a disorder that, to our knowledge, has not previously been described. A 71 yo Caucasian female presented for evaluation of a sclerosing bone disorder. Diagnosed incidentally at age 18, the patient had no history of fractures, visual loss, bone marrow suppression, organomegaly or osteomyelitis. She has had dental problems since childhood as well as hearing loss that required a stapedectomy at age 63. On radiographic evaluation, sclerosis was identified only in the skull base, the spine, ribs, and the long bone diaphyses. The long bones contained symmetric medullary sclerosis with homogeneously dense mineralization; they were not expanded and there was no evidence of periostitis or endostitis. The posterior elements of vertebrae were more affected than the bodies and the spine was progressively more involved from bottom to top. We had the opportunity to compare her current radiographs to ones obtained in 1961; this showed a diffuse subtotal resorption of the sclerosis. Laboratory evaluation was unremarkable except for an elevated osteocalcin level. A bone scan showed focal osseous uptake in the sacrum, degenerative changes in most joints and nonspecific tracer uptake in the humeri. A DEXA scan revealed osteopenia in the spine and normal bone mineral density in the hips.

The patient's son and his daughter have similar radiographic and clinical characteristics, although her granddaughter also has associated visual loss and more pronounced radiographic findings, including dense central sclerosis of the scapulae, clavicles, and pelvic bones.

In conclusion, we report a dominantly inherited sclerosing bone disorder with a unique radiographic appearance associated with hearing loss and dental problems. There is no clear increased risk of fracture and the osteosclerosis appears to be attenuated over time, although the contribution of postmenopausal bone resorption in the proband cannot be dismissed. The clinical characteristics observed in this family do not fulfill the criteria for classification into any known sclerosing bone disorder. We postulate that this may represent a novel hereditary sclerosing bone dystrophy with a unique genetic basis or it may be a clinical variant of a previously described disease that will be recognized as such through further genetic testing.

Disclosures: ***S.G. Waguespack****, None.*

M387

Defective BMP Receptor Internalization in Fibrodysplasia Ossificans Progessiva. P. C. Billings*[1], L. Serrano de la Pena*[2], J. L. Fiori*[1], R. Caron*[1], E. M. Shore[1], F. S. Kaplan[1]. [1]Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, USA, [2]Genetics, Rutgers University, Piscataway, NJ, USA.

Fibrodysplasia ossificans progessiva (FOP) is a catastrophic condition characterized by congenital malformations of the great toes and progressive heterotopic ossification of connective tissues. While the precise genetic alteration that induces bone formation in FOP is unknown, BMP4 is upregulated in FOP cells and lesional tissue suggesting that the molecular defect, giving rise to FOP, resides in the BMP signal transduction cascade. We have demonstrated previously that although steady state BMP receptor IA (BMPRIA) mRNA levels are similar in control and FOP cells, BMPRIA protein is elevated ~6 fold in FOP cells compared with cells from unaffected individuals. Using [35S]-Met metabolic labeling and immunoprecipitation, we have now determined that the rate of synthesis of BMPRIA protein is the same in normal and FOP cells. However, in the presence of BMP4 ligand, BMPRIA is internalized and rapidly degraded in control cells, but little or no receptor internalization is observed following exposure of FOP cells to BMP4. These results suggest that BMP-receptor trafficking is defective in FOP and supports the hypothesis that promiscuous BMP signaling in FOP cells results from increased BMPRIA density on the cell surface, thus giving rise to bone formation. Analysis of the molecular pathology of the human BMP4 pathway in FOP will elucidate the mechanism of abnormal ossification in this condition.

Disclosures: ***P.C. Billings****, None.*

M388

Marked Osteopenia and Increased Bone Resorption in Gaucher Disease Patients in Spite of Enzyme Replacement Therapy. B. Oliveri[1], G. Goldstein*[1], M. Rivero*[1], M. S. Parisi*[1], G. Aguilar*[2], C. Mautalen[1]. [1]Sección Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Servicio de Diagnóstico por Imágenes, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina.

Gaucher disease results from mutations that confer a deficit in activity of b glucocerebrosidase, which in turn leads to accumulation of the lipid glucocebrosidase in the macrophages of various organ systems. The organs affected include spleen, liver, lung, kidney, bone and bone marrow. 80 % of patients with Gaucher type I have skeletal involvement (bone pain , bone marrow infiltration, osteopenia, pathologic fractures, impairment of remodelling, and osteonecrosis) which is disabling and has a negative impact on the patient´s quality of life. A cross-sectional study was peformed to evaluate bone mass and mineral metabolism in young patients with Gaucher Disease receiving enzyme replacement therapy (ERT). The study population comprised 8 patients (4 women , 4 men) aged (X± SE) 25 ± 3.0 years (range (r): 18-43) with a body mass index of 19.1 ± 2 . Five patients were splenectomized (total: 4; partial: 1) , 4 had hepatomegaly , 7 referred bone pain, 3 reported "bone crisis" and 4 suffered osteoporotic fractures. All patients had been receiving ERT (imiglucerase) in a dose of 30 ± 1.8 IU/kg/ 2 weeks) during 4.7± 1.6 years (r:1.0-12). Bone Mineral Density (BMD) of lumbar spine (LS) and Total Skeleton (TS) was measured by DEXA. Serum calcium(sCa), phosphate (sP), 25(OH)D, bone alkaline phosphatase (BAP), osteocalcin (BGP), and crosslaps (CTX) were measured (see table below). BMD was found to be significantly diminished: Z score was -1.96 (p<0.001) for TS and Z score was – 1.5 (p<0.01) for LS. Biochemical determinations were within normal range except for bone markers : BAP 117 ± 32 IU/L (normal values (NV) : 31-95) (1/8 above normal level); BGP : 40 ± 21 ng/ml (NV: 11-46) (1/8 above normal level) and CTX 842 ± 224 ng/ml (NV: 40-450) (6/8 above normal level).

Although the patients had been receiving ERT they presented frequent bone symptoms and a significant diminution in BMD associated with high bone resorption. Higher doses of ERT and biphosphonate therapy should be evaluated to improve bone status.

Disclosures: ***B. Oliveri****, None.*

M389

Aspirin-Responsive, Expansile Bone Disease With Asplenism: A New Autosomal Dominant Entity. D. Wenkert[1], W. H. McAlister*[2], S. Mumm[2], M. P. Whyte[1]. [1]Shriners Hospitals for Children, St. Louis, MO, USA, [2]Washington University School of Medicine, St. Louis, MO, USA.

We report a unique autosomal dominant disorder featuring painful, asymmetrical widening of major long bones and asplenism with dramatic aspirin-responsive control of symptoms and improved bone modeling.

A 5-7/12 year-old boy and his 3-2/12 year-old sister were referred for possible chronic recurrent multifocal osteomyelitis (CRMO) in the boy, and hemihypertrophy of a leg in his sister. The boy presented at age 22 months with radial and ulnar fractures and developed chronic shoulder, knee, and sternal pain. CT, MRI, and bone scans raised a concern for CRMO. By 5-7/12 years, pain and 1-2 hours of morning stiffness was unresponsive to acetaminophen and ibuprofen. His sister developed a hard, right thigh at age 8 months. She walked at 15 months, but limped. Evaluation at age 2 revealed a leg-length discrepancy, elevated ESR, and diffuse cortical bone thickening with areas of laminated, smooth, hyperostosis in the right femur. At age 3-2/12 years, she still limped and preferred to be carried. She had morning stiffness, and complained of aching hands. Her pain and stiffness were unresponsive to intermittent acetaminophen and various NSAIDS.

Parameters of mineral and skeletal homeostasis showed modest elevations in serum osteocalcin and urinary calcium levels. BMD was decreased. Radiographs showed asymmetrically expanded long bones with hyperostosis in the diaphyses reminiscent of Caffey disease or neonatal exposure to prostaglandins. Ultrasound for Wilms tumor (associated

with hemihypertrophy) instead revealed asplenism in the sister, her brother, father, and grandfather. Both children mounted normal antibody responses to unconjugated capsular organism vaccines, with no historical predisposition to bacterial infection. The father too was diagnosed with CMRO during childhood. The asplenic grandfather recently succumbed to a myelodysplastic disorder.
To exclude candidate skeletal diseases featuring bone pain and hyperostosis (progressive diaphyseal dysplasia, familial expansile osteolysis, juvenile Paget's disease), we examined 3 genes: *TGFβ1*, *TNFRSF11A* (encoding RANK), and *TNFRSF11B* (encoding OPG). All coding exons and adjacent splice sites were PCR-amplified and sequenced; no mutations were detected. One-year of low-dose aspirin therapy led to remarkable restoration of long bone modeling, diminution of pain, and improved strength. Our patients experience bone pain if aspirin is withdrawn for as little as 24 hours. Studies are underway to define whether this unique condition is a prostaglandin-mediated. Aspirin-responsive expansile bone disease with asplenism is a new autosomal dominant metabolic bone disease.

Disclosures: ***D. Wenkert****, None.*

M390

Dominantly-Inherited Congenital Insensitivity to Pain Manifesting with Recurrent Fractures: A New Entity. D. Wenkert[1], W. H. McAlister*[2], J. G. Coldwell*[3], S. Mumm[2], M. P. Whyte[1]. [1]Shriners Hospitals for Children, St. Louis, MO, USA, [2]Washington University School of Medicine, St. Louis, MO, USA, [3]Children's Medical Center, Tulsa, OK, USA.

Congenital insensitivity to pain with anhydrosis (CIPA) is a rare, autosomal recessive (AR) condition (MIM 256800) characterized by fractures, growth disturbances, avascular necrosis (AVN), Charcot arthropathies, joint dislocations, and clinical anhydrosis (JBJS 84B:252,'02). We report significant CIP (without anhydrosis) in 2 generations where the proband was first evaluated at age 10 for multiple fractures. Despite profound CIP, our patients do sweat (one complains of significant night sweats), make tears, and there is no mental retardation. The affected family members have remarkable hypermobility, suffer recurrent, major fractures without pain (detected by annual radiographic skeletal surveys), AVN, unremitting pruritis leading to severe excoriation (proband is missing one nasal alae, son's tongue is severed), and chronic diarrhea (with non-diagnostic GI evaluations). The patients complain of significant discomfort with exposure to cold stimulus ("like an electric shock") and have preserved hot/cold as well as sharp/dull sensation. Sclerae are not discolored. Skin fibroblast studies of the proband showed no evidence of OI. Investigations of bone quality, including iliac crest histology and biochemical parameters of mineral homeostasis, are unremarkable. The electron microscopy report of a sural nerve biopsy showed absence of unmyelinated axons characteristic of CIP. Molecular analysis is underway of the *NTRK1* (MIM 191315) gene, encoding the receptor tyrosine kinase for nerve growth factor (MIM 162030), that is deactivated in some cases of AR CIPA.
Two families have been reported with dominantly-inherited insensitivity or indifference to pain (MIM 147430). Of these 2 families, one family (also with a disturbance of myelin architecture) manifests many of the same features as the family we are reporting (night sweats, pruritis, blotchy skin), but differs in some of the clinical features (diarrhea, cold intolerance, frequent fractures) as well as in the neurologic exam (sharp/dull and hot/cold discrimination). Our 2-generation family with CIP appears to manifest a unique form of congenital insensitivity to pain responsible for significant recurrent long bone fractures.

Disclosures: ***D. Wenkert****, None.*

M391

Juvenile Paget's Disease: Molecular Analysis of *TNFRSF11B* Encoding Osteoprotegerin Indicates Homozygous Deactivating Mutations from Consanguinity as the Predominant Etiology. S. Mumm[1], S. Banze*[1], J. Pettifor[2], C. Tau[3], K. Schmitt*[4], A. Ahmed*[5], M. P. Whyte[6]. [1]Washington University School of Medicine, St. Louis, MO, USA, [2]Baragwanath Hospital, Johannesburg, South Africa, [3]Hospital of Pediatrics, Buenos Aires, Argentina, [4]Landes-Kinderklinik, Linz, Austria, [5]Institute of Medical Genetics, Glasgow, United Kingdom, [6]Shriners Hospitals for Children, St. Louis, MO, USA.

Juvenile Paget's disease (JPD), also called *hyperostosis corticalis deformans juvenilis* or hereditary hyperphosphatasia, is a rare autosomal recessive osteopathy that presents in infancy or early childhood with bone pain, fractures, and deformities due to accelerated skeletal remodeling (MIM 239000). We have reported, in 2 seemingly unrelated Navajo patients, that JPD is caused by homozygous deletion of *TNFRSF11B* (MIM 602643) encoding osteoprotegerin (OPG) (NEJM: 347:175, 2002). Here, we examine *TNFRSF11B* in additional patients diagnosed with JPD, identifying deactivating mutations in 5 separate families. Patient 1 (of Asian Indian descent) has a remarkable homozygous missense mutation in the ATG start codon. Patient 2 (from Argentina) has a homozygous missense mutation in exon 2 (F42L). Patients 3 and 4, seemingly unrelated but both in Iraq, have homozygous single base deletions in exon 5, that should cause a frame-shift. The final patient, from Scotland, is the only reported to date with compound heterozygosity -- involving a 3 bp deletion in exon 2 (effectively removing an R residue) and a nonsense mutation in exon 4. Hence, in 6 of our 7 probands, JPD is caused by homozygous, inactivating mutation of the OPG gene, presumably reflecting a high occurence of consanguinity. Accordingly, other unknown homozygous genetic influences could contribute to the JPD phenotype. This one case of compound heterozygosity seems to reflect "pure" OPG deficiency. This spectrum of OPG mutations, from missense to complete deletion, is the basis for genotype/phenotype correlations.

Disclosures: ***M.P. Whyte****, None.*

M392

Withdrawn

M393

Similar Effects of Tiludronate and Risedronate on Paget's Disease Activity Assessed by Bone Markers. N. Guañabens, L. Alvarez, D. Cerdá*, S. Vidal*, A. Monegal*, P. Peris, I. Ros*, A. Ballesta*, F. Pons*. Metabolic Bone Diseases Unit, Hospital Clínic, Barcelona, Spain.

Aim: To compare the effects of tiludronate and risedronate on Paget's disease activity assessed by measuring biochemical markers of bone turnover and quantitative scintigraphy.
Patients and Methods: The study has been performed in 53 patients with Paget's disease who received tiludronate (400 mg/d) for 3 months (33 patients) or risedronate (30 mg/d) for 2 months (20 patients). Biochemical markers of bone turnover, including serum total alkaline phosphatase (tALP), bone alkaline phosphatase (bALP) and procollagen type I N propeptide (PINP), and urine hydroxyproline (Hyp) and N-terminal cross-linking telopeptide of type I collagen (NTx) were measured immediately before and at 1, 6 and 12 months after the end of treatment. Quantitative bone scintigraphy for calculating a scintigraphic activity index (SAI) was carried out at baseline and 6 months after treatment discontinuation.
Results: There were no significant differences at baseline between patients treated with tiludronate or risedronate with respect to the demographic variables. Baseline markers of bone turnover as well as SAI were also similar in both groups (tALP 644 ± 101 vs 668 ± 110 U/L; bALP 84 ± 16 vs 76 ± 16 ng/mL; PINP 228 ± 39 vs 177 ± 25 ng/mL; Hyp 217 ± 37 vs 204 ± 22 nM/mg; NTx 269 ± 37 vs 224 ± 42 nM/mM, SAI 9257 ± 1725 vs 10721 ± 2338). The effects of tiludronate and risedronate in reducing the biochemical markers of bone turnover were comparable after one month (tALP: -54 ± 4 vs –54 ± 4 %, bALP: -68 ± 3 vs –65 ± 4%; PINP: -71 ± 3 vs –75 ± 3 %; Hyp: -42 ± 5 vs –47 ± 4%, NTx: -64 ± 4 vs –62 ± 5%) six months (tALP: -51 ± 4 vs –47 ± 5 %, bALP: -68 ± 3 vs –58 ± 6%; PINP: -66 ± 3 vs –68 ± 5 %; Hyp: -47 ± 3 vs –27 ± 9%, NTx: -60 ± 4 vs –52 ± 9%) and twelve months (tALP: -44 ± 5 vs –36 ± 8 %, bALP: -52 ± 7 vs –62 ± 7%; PINP: -53 ± 6 vs –60 ± 9 %; Hyp: -38 ± 6 vs –27 ± 8%, NTx: -46 ± 8 vs –49 ± 10%). The effects of tiludronate and risedronate on SAI were also similar 6 months after the discontinuation of treatment (-45 ± 4 vs –46 ± 4%).
Conclusions: Tiludronate and risedronate given at the currently recommended dosages induce similar decreases in the activity of Paget's disease at short- and long-term follow-up.

Disclosures: ***N. Guañabens****, None.*

M394

Zoledronate in the Treatment of Paget's Disease. A. Bhatia*, G. Chung*, A. Allsworth*, K. W. Keen. Metabolic Bone Unit, Royal National Orthopaedic Hospital Stanmore, Middlesex, United Kingdom.

Background: Bisphosphonates are effective inhibitors of osteoclastic bone resorption and are used clinically in the treatment of Paget's disease. Zoledronate a cyclic nitrogen containing third generation bisphosphonate, is the most potent of the currently available bisphosphonates. We report 5 patients with active and resistant Paget's disease that have been treated with zoledronate (all having recieved previous bisphosphonates).
Methods: To date 5 patients have been treated. Patients 1, 2, 4 and 5 were treated with a single 4 mg intravenous infusion of zoledronate. Patient 3* was treated with two 4 mg intravenous infusions of zoledronate 2 months apart. Response in terms of pain reduction and serum total alkaline phosphatase (SAP) were assessed on follow up.
Results: All five patients achieved both symtomatic relief and full biochemical remission.The overall treatment response was a 93-96% decrease in SAP. The reduction of SAP following treatment is shown in table 1.
Patients 1, 2, 4 and 5 reported no side effects following treatment. Patient 3 experienced transient hypophosphataemia and this may have been related to the higher cumulative dose.
Conclusions: All 5 patients previously resistant to intravenous pamidronate and other bisphosphonates responded symptomatically and achieved full biochemical remission after zoledronate therapy. Therefore zoledronate has a potential role in the managment of patients with active Paget's disease and the results of further randomised studies are awaited.

Table 1: Serial SAP (IU/L) in Five Patients Treated with Zoledronate.

Time after intravenous zoledronate (Months)	SAP [IU/L] (Normal Range 30-130)			
Baseline Characteristics of Patients / Pre-Treatment SAP	Patient 1 (Male A B 65 yrs. old) 569	Patient 2 (Male A B 76 yrs. old) 561	Patient 3 (Male D S 76 yrs. old) 469	Patient 4 (Male G D 47 yrs. old)/ Patient 5 (Female G B 86 yrs. old) Pt. 4: 438 Pt. 5 : 364
One	285	446	417*	
Two	206		162	Pt. 5: 103
Three		211	114	
Five			108	Pt. 4: 129
Eight		116		
Ten	102			

Disclosures: ***K.W. Keen****, None.*

M395

Serum C-Telopeptide and Alkaline Phosphatase Changes Following a Single Intravenous Infusion of Zoledronic Acid in Patients with Paget's Disease of Bone. F. F. Bandeira[1], W. S. Saraiva*[2], W. P. Carvalho*[2], V. A. Rosado*[2], L. H. Griz*[1], G. P. Caldas*[2], C. H. Bandeira*[2]. [1]Endocrine Dept, University of Pernambuco, Osteoporosis Center, Recife-PE, Brazil, [2]Endocrine Dept, Osteoporosis Center, Recife-PE, Brazil.

Zoledronic acid (zolendronate) is a potent bisphosphonate which inhibits bone resorption in low dose intravenous infusions. The aim of this study was to show the effects of a single (1-hour) 4 mg infusion of zolendronic acid on serum C-telopeptide (CTX) and serum alkaline phosphatase (AP) in 10 patients with active Paget's disease of bone. There were 3 males and 7 females (3 with monostotic and 7 with poliostotic disease) aged 50-88 years (mean 73.7+/-10.1 years). Mean+/-SD serum AP were 416.4+/-336 U/L (3.3+/-3 times the upper limit of normal), and serum CTX 1290.4+/-580.6 pg/ml (2.8+/-1.3 times the upper limit of normal). Zolendronic acid was the first therapy in 2 patients, and 8 patients were previously treated with oral alendronate or risedronate. The patients were evaluated at a median of 6 months after zoledronic acid infusion. Serum AP fell to 92.1+/-60.3 U/L (p=0.0001) and serum CTX fell to 406.2+/-319 pg/ml (p=0.0005). The mean reductions in serum AP were 72.6+/-11.9 % and in serum CTX 70.6+/-19.9 %. The differences between AP and CTX changes following zolendronic acid infusion were not statistically significant. Normalization of serum AP levels was achieved in 9 patients, and of serum CTX in 7 patients. One patient with poliostotic disease, in whom the bone markers did not reach the normal range, had 70% and 63% fall in serum AP and CTX respectively. In conclusion, a single 1-h intravenous infusion of 4 mg of zoledronic acid is highly effective for the treatment of patients with active Paget's disease of bone. The reductions in serum alkaline phosphatase and C-telopeptide levels are similar.

Disclosures: ***F.F. Bandeira****, None.*

M396

Risedronate and Pamidronate Therapy in the Management of Patients with Severe Paget's Disease of Bone. G. Mossetti*, D. Rendina*, R. Viceconti*, V. Nunziata*. Clinical and Experimental Medicine, Federico II University of Naples, Naples, Italy.

The therapeutic approach to Paget's disease of bone (PDB) emphasizes the importance of achieving and maintaining normal values in bone turnover markers even after treatment cessation to prevent the disease progression. The aim of this study was to evaluate the efficacy and safety of risedronate and pamidronate, two nitrogen-containing bisphosphonates, in 30 patients (M:F ratio = 17:13; mean age = 57.86 ± 8.90 years) with severe PBD, showing acquired resistance to intravenous (iv) clodronate treatment.
All patients enrolled had been treated with iv clodronate (300 mg/ for five days) from diagnosis. After the last treatment, all patients did not show a reduction in total alkaline phosphatase (tALP) serum levels of > 25% compared to pre-treatment values and had a complete relapse (tALP comparable to the pre-all-treatment values) within six months. Fifteen patients were treated with oral risedronate (30 mg/day for 60 days). Treatment was repeated in patients without evidence of PDB remission (tALP serum levels in the normal range) at 120 day. Fifteen patients were treated with iv pamidronate (30 mg/day for 3 days). Pamidronate treatment (60 mg/day for 3 days) was repeated in patients without evidence of PDB remission at 120 day. Serum levels of tALP, PTH, $1,25(OH)_2D_3$, ionized calcium and phosphate were evaluated at monthly intervals. The patients were instructed to report the occurrence of adverse experiences. The study was conducted according to the Declaration of Helsinki.
At day 60, a significant decrease in tALP serum levels was obtained in all pagetic patients. At day 360, 13 (86.6%) patients treated with risedronate achieved normalization tALP levels, 9 patients during the initial treatment and 4 after re-treatment. Two patients showed a significant decrease in tALP serum levels, without clinical remission after two-risedronate treatment. At the same time, 12 (80%) patients treated with pamidronate achieved PDB remission, 6 patients during the first treatment and 6 after re-treatment. Three patients showed a significant decrease in tALP serum levels, but no clinical remission, after two-pamidronate course. Two of these patients showed a complete relapse during the study.
The incidence of minor side effects (elevation of body temperature, headache and dyspepsia) and secondary hyperparathyroidism related to bisphosphonate treatment, were significantly ($p<0.05$) lower after risedronate.
In patients with severe and resistant PDB, oral risedronate therapy shows comparable efficacy to iv pamidronate treatment with a significantly lower incidence of treatment-related side effects.

Disclosures: ***G. Mossetti****, None.*

M397

P392l Mutation of Sequestosome 1 (sqstm1) Gene Seem to be Less Frequent in Sporadic Italian Cases of Paget's Disease of Bone. A. Falchetti*[1], F. Marini*[1], F. Del Monte*[1], D. Strigoli*[1], A. Amedei*[1], M. Di Stefano*[2], G. Isaia[2], M. L. De Feo*[3], L. Masi[1], M. Matucci*[1], S. Maddali Bongi*[1], D. Melchiorre*[1], G. B. Rini[4], G. Di Fede*[4], M. L. Brandi[1]. [1]Internal Medicine, University of Florence, Florence, Italy, [2]Internal Medicine, University of Turin, Turin, Italy, [3]Azienda Ospedaliera Careggi, Florence, Italy, [4]Internal Medicine, University of Palermo, Palermo, Italy.

Paget's disease of bone (PDB) is a genetically heterogeneous disorder affecting skeleton, with bone abnormalities and increased bone turnover. It is a common disease of bone metabolism affecting up to 3% of Caucasian individuals over 55 years of age. PDB mostly run asymptomatically, while in approximately 30% of patients bone pain, bone deformities, pathological fractures may occur. Evidences of genetic influence in its pathogenesis have been described. At least 7 loci have been reported in literature. In particular the PDB3 locus hosts SQSTM1 gene whose mutations account for most of sporadic and familial PDB cases reported in literature. SQSTM1 gene encodes a component of the NF-kB signaling pathway and the DNA sequence accounting for the ubiquitin-binding domain of protein, exon 8, harbours a mutational hot spot area. Three different mutations of SQSTM1 have been reported in PDB patients: P392L, a T insertion at position 396 and a splice donor site mutation in intron 7. P392L mutation has been reported in 19.1% and 8.9% of familial and sporadic PDB cases, respectively. In order to verify the involvement of this gene and in particular of P392L mutation in Italian PDB cases, we performed mutational analysis in 47 PDB sporadic cases from North (Piemonte), Middle (Toscana) and South (Sicilia) Italy. After informed consent obtainment, DNA from 27 males (age range 44-89 years) and 20 females (age range 55-82), all clinically evaluated, has been collected at our Centre. Twenty-seven cases exhibit a monostotic involvement (14 females and 13 males) while 20 cases have polyostotic localizations of disease (6 females and 14 males). Mutational analysis has been performed according to described methods. P392L mutation has been found in only 1/47 PDB patient corresponding to a 2% rate, which is 4.45 times lower than reported. This preliminary finding may suggest a less important role of this gene in the pathogenesis of sporadic PDB in our Country, although the sample size investigated is 3.5 times smaller than sample analysed by Hocking LJ et al. We are collecting new PDB cases, also in collaboration with other Italian Centres. Moreover, we will perform mutational analysis of other exon 8 and intron 7 mutations in our population. Finally, entire SQSTM1 gene sequencing may add informations on genetics of Italian cases of PDB.

Disclosures: ***A. Falchetti****, None.*

M398

18F-Fluoride Positron Emission Tomography: Preliminary Assessment of Therapy in Paget's Disease of Bone. A. T. Nzeusseu*[1], M. Lonneux*[2], G. Depresseux*[1], J. P. Devogelaer[1]. [1]Rheumatology Unit, St-Luc University Hospital, Brussels, Belgium, [2]Nuclear Medicine Unit, St-Luc University Hospital, Brussels, Belgium.

Monostotic Paget's disease of bone (PDB) may be difficult to quantify by biochemistry and bone scans. 18F-fluoride positron emission tomography (PET) can quantify local skeletal metabolic activity. We assessed, therefore, whether it was capable to demonstrate changes in metabolic activity in localized PDB already shortly after therapy, when total alkaline phosphatase had not yet significantly changed.
Methods: Seven patients with radiologically confirmed focal PDB performed a one-hour dynamic 18F-fluoride PET scan before and one-month after bisphosphonate (BP) therapy. Total serum alkaline phosphatase (TAP) was measured at the same times. Changes in bone metabolism were assessed by a semi-quantitative index such as standardized uptake values (SUV). Wilcoxon signed rank test was used for statistics.
Results: The SUV significantly dropped on average 33 % after one month from [mean (SEM)] 24.6 (8.9) to 15.3 (6.3) ($p = 0.018$). During the same period of time, TAP decreased non significantly from 140.4 (38.5) to 113.2 (32.0) IU/l.
Conclusion: This preliminary data suggests that, better than TAP, 18F-fluoride PET is able to detect already within one month of BP therapy a significant decrease in regional bone remodelling of focal PDB

Disclosures: ***J.P. Devogelaer****, Work supported by The Belgian National Foundation for Scientific Research. 2.*

M399

Interferon-Alpha 2a Responsive Giant Cell Tumors ("Extraskeletal Osteoclastomas") in Paget Bone Disease. W. Read*[1], M. P. Whyte[2]. [1]Department of Hematology/Oncology, Washington University School of Medicine, St. Louis, MO, USA, [2]Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA.

Giant cell tumors (GCTs) infrequently complicate Paget bone disease (PDB). We report a middle-age, black woman with GCTs resembling "extraskeletal osteoclastomas" whose soft tissue masses became refractory to dexamethasone (DEX) therapy but subsequently responded to subcutaneous injections of interferon-alpha 2a (IFN-2a).
Our patient was reported in 1997 (*JCEM 82:3826,'97*) after she developed a large gluteal mass (extraskeletal osteoclastoma) accompanying her familial PBD and neurofibromatosis. Initially, several tumors rapidly regressed over one month during treatment with 8 mg/day of oral DEX, but recurred if DEX was discontinued. The tumors then became incompletely responsive to low maintenance doses of DEX (2 mg every other day). Subsequently, plicamycin and then zoludronate therapy was added, but seemed unhelpful. In 5/02, she developed what we believed was an abscess near the right groin mass. It proved to be another GCT. DEX was increased to 4 mg po qid, but the tumor grew rapidly forming a moist, red, exophytic mass. By 9/02, it measured 13 x 7 cm. CT showed no growth in her old gluteal GCTs during this time.
Because of reports that GCTs may respond to INF-2a, she began treatment with 5 million units INF-2a thrice weekly in 12/02. Within three weeks, the tumor opened and discharged considerable blood. By 1/03, it had decreased to 10.5 x 7.5 cm, and her DEX dose was gradually tapered. In 3/03 it had decreased to 5 cm, and had normal overlying skin, and her DEX dose was reduced further to 2 mg daily.
Reportedly, IFN-2a is effective for mandibular GCTs in young adults, but to our knowledge this is the first case documenting its utility in GCT complicating PBD. Others have hypothesized that IFN-2a acts via an anti-angiogenic mechanism. In cases of GCT and PBD where corticosteroids are ineffective, intolerable, or no longer effective, IFN-2a appears to be a treatment option.

Disclosures: ***M.P. Whyte****, None.*

M400

Truncation of Osteoprotegerin in an Isolated Case of Juvenile Paget's Disease. K. Janssens*[1], M. de Vernejoul[2], F. De Freitas*[1], W. Van Hul[1]. [1]Medical Genetics, University of Antwerp, Antwerp, Belgium, [2]Laboratoire INSERM U 349, Hôpital Lariboisière, Paris, France.

Juvenile Paget's Disease (JPD) (also called Chronic Congenital Idiopathic Hyperphosphatasia or Hyperostosis Corticalis Deformans Juvenilis) is a rare condition with an autosomal recessive mode of inheritance. The disorder is characterised by generalised cortical thickening of the bones combined with sustained elevation of alkaline phosphatase levels. The extremely rapid bone turnover and the failure of primitive fibrous bone to mature into compact lamellar or haversian bone result in osteopenia, fractures and progressive skeletal deformity. Recently, mutations in TNFRSF11B, the gene encoding osteoprotegerin (OPG), have been identified as the cause of JPD.

We describe an isolated case of JPD in a patient of Croatian origin. The patient, a 26-year-old man, was diagnosed with JPD at 2 years of age. His height at present is 130cm. He has a major kyphoscoliosis, a disproportionally large head, coxa vara, curved tibiae and short humeri. X-rays show cortical thinning of the enlarged and bowed diaphyses and demineralisation of trabecular bone. His hearing is decreased. Levels of alkaline phosphatase and D-pyridinoline, markers for bone resorption, are dramatically increased.

By mutation analysis of the five exons and the intron-exon boundaries of TNFRSF11B, we detected a homozygous mutation in this patient. The mutation in exon 5 causes a frame shift at position 966. This induces the formation of a premature stop codon at position 977 and truncates the protein 75 amino acids before the normal stop codon. Despite the deletion of about 20% of the C-terminal region, the mutant protein is normally secreted in plasma. Overexpression of the wild type and mutant cDNA in 293 cells did not show any effect on secretion either. Further functional studies will have to show the impact of the mutation on the binding properties of OPG with RANKL.

This is the first report to describe a truncating mutation in OPG as the cause for JPD indicating that the absence of the C-terminal region has, in vivo, a clear effect on the potency of OPG to inhibit osteoclastogenesis.

Disclosures: ***W. Van Hul****, None.*

M401

Correlation between Serum Markers of Osteoblast/Osteoclast Interactivity, Collagen Formation and Resorption in Elderly Pagetic Patients. B. H. Durham, J. J. Dutton*, W. D. Fraser. Clinical Chemistry, Royal Liverpool University Hospital, Liverpool, United Kingdom.

Recently assays have become available for the measurement in serum of the receptor activator of nuclear factor kappa B ligand [sRANKL] and its decoy receptor [OPG] which are messenger molecules produced in osteoblasts that signal to pre-osteoclasts. We have measured these two markers together with markers for collagen formation, N-terminal extension peptide of type 1 procollagen [P1NP], collagen resorption, the beta form of the C-terminal telopeptide of type 1 collagen [beta-CTX] an osteoclast marker the osteoclast derived tartrate – resistant acid phosphatase 5b[TRAP5b] and total alkaline phosphatase [TAP] as an osteoblast marker in sera from a cohort of 64 elderly patients with Paget's disease[F31, M33, mean age 74.5yr range 55-88 yr]. After venepuncture serum was separated within 30 minutes and stored at –70 degree Celsius until analysed , all assays were run at the same time so that the sera did not need re-thawing. OPG and sRANKL ELISA kits were from Biomedica ,[Vienna, Austria] and TRAP5b ELISA kit from SBA Sciences,[Turku, Finland], P1NP and beta- CTX kits for the Elecsys [Roche Diagnostics, Germany]. There were no significant differences between men and women for any of the measurements so the cohort of patients was analysed as one group. Mean, SD, range for OPG was 3.6,1.7, 0.7-8.9 pmol/L, for sRANKL 7.4,4.7, 1.3-25.9 pmol/L, for TRAP5b 5.7,1.8,2.2-15.2 U/L, for P1NP 133, 227, 17-1421 mcg/L, for beta-CTX 0.57,0.55,0.07-3.55mcg/L and for TAP 150,122,48-859 U/L. The ratio OPG/sRANKL had a mean of 0.65,SD 0.42, range 0.13-1.50. Pearson's correlation coefficient yields significant correlations between sRANKL and beta-CTX, TRAP, and TAP p=0.020,0.025,0.024 respectively whereas OPG correlates significantly only with P1NP, p=0.043.TRAP correlates with TAP, p<0.01 as well as sRANKL. The inverse correlation between OPG/sRANKL ratio and TAP is not significant p=0.054. In Paget's disease there are significant correlations between RANKL and markers of osteoblast and osteoclast activity as well as bone resorption.

Disclosures: ***B.H. Durham****, None.*

M402

Recurrent SQSTM1 Gene Mutation at Codon 392 in Paget's Disease of Bone. L. Gennari, V. De Paola*, A. Calabrò*, D. Merlotti, G. Martini, A. Avanzati*, B. Galli*, S. Salvadori*, R. Nuti. Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Siena, Italy.

Paget's disease of bone (PDB) has a clear hereditary component. The disease often segregates as an autosomal dominant trait with incomplete penetrance. In the past few years several studies demonstrated linkage heterogeneity indicating that more than one gene is involved in the predisposition of the disease. A recurrent C/T transversion at exon 8, position +1215 of the SQSTM1 gene, resulting in a proline-leucine substitution at codon 392 (P392L) has been recently identified as a cause of PDB in 46% of French-Canadian families as well as in 16% of sporadic affected subjects. The same mutation was observed in 19% and 9% of respectively familial and sporadic PDB of British origin.

The aim of the present study was to evaluate the prevalence of the P392L mutation of SQSTM1 gene in sporadic and familial PDB cases from Italy. Blood samples were obtained from 80 sporadic PDB patients and 6 affected subjects from 3 PDB families as well as from 100 unrelated individuals used as control group. The diagnosis of PDB in affected individuals was confirmed by bone scintigraphy and x-ray examination of areas of increased isotope uptake. Controls were age and sex-matched and all had bone specific alkaline phosphatase levels within the normal range. Since the mutated aminoacid at position +1215 eliminates a SacII restriction site and originates a BspMI site, the presence of the P392L mutation was investigated by double restriction endonuclease analysis, after PCR amplification of part of exon 8 of the SQSTM1gene. The P392L mutation was found in 3 affected individuals from one of the 3 families as well as in 8(10%) of the 80 PDB sporadic cases, giving a total of 11 (13%) patients in which PDB was associated with the investigated SQSTM1 mutation. The mutation was excluded in the 100 analysed individuals in the control group.

In conclusion, results from the present study confirm that the P392L mutation affecting the ubiquitin-binding domain of SQSTM1 is a common cause of familial and sporadic Paget's disease of bone in subjects of Italian descent.

Disclosures: ***L. Gennari****, None.*

M403

Echocardiographic Assessment of Cardiac Function in Paget's Disease of Bone. G. Martini, A. Palazzuoli*, C. Pondrelli*, L. Gennari, R. Valenti*, D. Merlotti, B. Galli*, F. Cipolli*, F. Iovine*, N. Dal Canto*, R. Nuti. Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Siena, Italy.

To identify the mechanisms which influence the development of cardiovascular complications of Paget's disease of bone (PBD), we performed carotid artery doppler ultrasonography and non-invasive assessment of cardiac size and function by clinical criteria, standard electrocardiography, and echo-cardiography in 15 patients with Paget's disease of bone and in 20 control subjects matched by sex, age and body mass index. The PDB patients were divided into two groups on the basis of the degree of skeletal involvement (6 monostotic and 9 polyostotic disease). Left ventricular mass was calculated by the formula of Deveroux and Reichek, modified with Penn convention. Relative wall thickness (RWT) was calculated as the ratio between the sum of the end-diastolic thickness of interventricular septum and posterior wall, and half of left ventricular diastolic diameter. Diastolic ventricular function was assessed by evaluation of the following parameters: ratio between peak E- and A- wave velocities (E/A), left ventricular isovolumetric relaxation time (IVRT) and E-wave deceleration time (DT). Serum calcium, phosphate, creatinine, osteoprotegerin (OPG), RANKL and bone specific alkaline phosphatase were also determined. An alteration in diastolic function was observed in PDB patients with respect to age-matched controls. E/A ratio was significantly reduced (0.79 +/- 0.23 vs 1.11+/-0.29, p=0.008), while DT (249 +/- 70 vs 182+/-17, p=0.0009) and IVRT (119 +/- 30 vs 89+/-12, p=0.0009) were significantly increased in PDB patients than in controls. These differences were greater when the subgroup with more extensive polyostotic skeletal disease only was considered. Moreover, polyostotic PDB patients showed increased IVRT and RWT and reduced E/A ratio with respect to monostotic PDB subjects. A moderate correlation was observed between OPG or BALP concentrations and ejection fraction or diastolic ventricular function parameters in PDB patients but not in controls. Finally, an above normal incidence of cardiac valvular calcifications was observed in PDB patients with respect to controls, while no significant differences were observed in carotid artery ultrasonography parameters.

In conclusion, results from the present study suggest that in the early phases of PDB together with an increase in ventricular mass there is a trend towards a diastolic dysfunction that could be influenced by the extension of the disease and by the degree of bone turnover.

Disclosures: ***G. Martini****, None.*

M404

Pharmacokinetics of Oral Gallium Maltolate Administered in a Single or Multiple Dose Schedule in Patients with Paget's Disease of Bone or Primary Hyperparathyroidism: A Pilot Study. B. L. Lum*[1], A. Gottlieb*[2], R. Altman*[3], P. H. Sayre*[4], F. Valone*[4]. [1]Stanford University School of Medicine, Palo Alto, CA, USA, [2]Robert Wood Johnson Medical School, New Brunswick, NJ, USA, [3]University of Miami School of Medicine, Miami, FL, USA, [4]Titan Pharmaceuticals, Inc., South San Francisco, CA, USA.

Gallium maltolate is a novel orally available formulation of gallium being developed as an agent for the treatment of metabolic bone disorders. Gallium when delivered as low dose subcutaneous gallium nitrate has demonstrated anti-resorptive activity in Paget's disease of bone. This study evaluated the pharmacokinetics of oral gallium maltolate administered at three dose levels as a single and as three consecutive daily doses in patients with advanced Paget's disease of bone or primary hyperparathyroidism. Gallium maltolate (200, 400, or 600 mg) was administered as a single dose and 14 days later, as three consecutive daily doses. Serial gallium ion serum concentrations were measured over 350 hours for the single dose and over 480 hours for the multiple dose schedule. Samples were assayed for total gallium ion concentration using inductively coupled plasma mass spectroscopy. Eight of 12 subjects were male. The median age was 83 years (range: 55-88 years). Ten of the 12 subjects completed the intended course of treatment. One serious adverse event, possibly related to study drug, was reported during the study. This subject had an episode of congestive heart failure after discharge from the study unit, while in the washout period. No deaths were observed. Adequate pharmacokinetic data were available for 9 subjects during the single dose and for 7 subjects during the multiple dose portion of the study. Serum gallium ion concentrations increased in linear fashion with increasing dose over the dose range examined. For single or multiple dosing, a three-fold increase in gallium maltolate dose resulted in an increase of the mean serum total gallium ion Cmax and AUCtotal values by 2.8-fold and 3.0-fold, respectively. Apparent clearance (CL/F), T1/2, Tmax, and terminal phase apparent volume of distribution (Vz/F), were similar across all three doses of oral gallium maltolate. Steady-state

concentrations were not achieved during the three-dose schedule. At the 600 mg/day, total serum gallium ion Cmax following the third dose averaged 1128 ng/mL (S.D. +/- 533) and concentrations observed 24 hours following this dose ranged from 420 to 934 ng/mL. These concentrations are similar to those expected to inhibit bone resorption in vivo. Thus gallium maltolate achieves serum gallium concentrations that may be therapeutic. Further testing of this agent in Paget's and other metabolic diseases is warranted.

Disclosures: ***P.H. Sayre****, Titan Pharmaceuticals, Inc. 3.*

M405

Oxyphil Parathyroid Adenoma: Malignant Presentations of a Benign Disease. J. B. Fleischer, T. L. Breen*, C. B. Becker, S. J. Silverberg. Medicine, College of Physicans & Surgeons, Columbia University, New York, NY, USA.

Clinical manifestations of primary hyperparathyroidism (PHPT) have changed dramatically over the past several decades. Today, most patients have no signs or symptoms typically associated with hypercalcemia or parathyroid hormone (PTH) excess. Kidney stones and overt skeletal disease are rare. When patients do present with severe biochemical and clinical disease, parathyroid carcinoma is suspected. We describe two cases of benign PHPT in which the magnitude of biochemical abnormalities and severity of clinical features raised such suspicions.

Case 1: A 43 year old premenopausal woman from the Dominican Republic with a history of nephrolithiasis, was evaluated in the Emergency Room for posterior cervical neck pain. Neck CT showed a very large heterogenous mass adjacent to the left lobe of the thyroid with substernal extension and displacement of the trachea. The serum calcium was 12.4 mg/dl (8.4-9.8), phosphorus was 1.6 mg/dl (2.5-4.3) and total alkaline phosphatase was 209 U/L (33-96). Intact PTH was dramatically increased at 888 pg/ml (12-72). 25 (OH) and 1,25 $(OH)_2$ vitamin D levels were 18 ng/ml (10-68) and 166 pg/ml (15-60) respectively. DXA showed T-scores of -4.4 at the lumbar spine, -1.8 at the total hip, -2.6 at the femoral neck and -5.0 at the distal radius. At surgery, a large parathyroid mass was resected measuring 6 x 3.8 x 2.1 cm and weighing 24.8 grams. Pathology revealed a benign oxyphilic adenoma.

Case 2: A 63 year old woman from Barbados, with no prior medical history, presented with fatigue and hoarseness. Work-up revealed a serum calcium of 13.9 mg/dl, phosphorus of 1.6 mg/dl, and an intact PTH of 616 pg/ml (8-51). Bone turnover markers were elevated: total alkaline phosphatase: 597 U/L; bone specific alkaline phosphatase:1445 U/L (24-146); and osteocalcin:189.6 ng/ml (7.2-27.9). 25 (OH) vitamin D was 9 ng/ml and1,25 $(OH)_2$ vitamin D was 43 pg/ml. There was mild subperiosteal resorption on hand X-rays. Neck exploration revealed a well-encapsulated and non-adherent parathyroid mass measuring 4.7 x 2.7 x 1.0 cm. Pathology showed a benign oxyphilic adenoma.

In summary, 2 patients presented with marked hypercalcemia, striking PTH elevations, large tumor size, clinical symptoms and overt skeletal disease. Although parathyroid cancer was the presumptive clinical diagnosis, pathology revealed oxyphil adenoma. Most parathyroid adenomas are composed primarily of chief cells, and oxyphil cells are usually non-functional. Oxyphil adenoma must be in the differential diagnosis of PHPT patients with severe biochemical and clinical abnormailites and large tumors that mimic parathyroid carcinoma.

Disclosures: ***J.B. Fleischer****, None.*

M406

Does the Location of the Parathyroid Adenoma Influence Kidney Stone Formation in Primary Hyperparathyroidism? E. Csupor*[1], E. Tóth[2], V. Ferencz*[3], J. Szucs*[3], P. Lakatos[3], J. Horányi*[4], F. Perner*[5], E. V. McCloskey[6], C. Horváth[3]. [1]Budavar Local Authorities, Budapest, Hungary, Budapest, Hungary, [2]Department of Rheumatology, Ferenc Flor County Hospital, Kerepestarcsa, Hungary, [3]1st Department of Internal Medicine, Semmelweis University Medical School, Budapest, Hungary, [4]1st Department of Surgery, Semmelweis University Medical School, Budapest, Hungary, [5]Department of Transplantation and Surgery, Semmelweis University Medical School, Budapest, Hungary, [6]WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, Sheffield, Hungary.

The majority of patients with primary hyperparathyroidism (pHPT) recurrently produce kidney stones, while the rest have other clinical manifestations (metabolic bone disease, acute pancreatitis, depression, etc.). The aim of this study was to examine the association between the clinical symptoms and the location of adenoma.

This was a retrospective evaluation in the records of 91 patients (10 males, 81 females, mean age: 61.9 years (20-70 ys) operated for primary hyperparathyroidism between 1995 and 2000. Kidney stones were presented in 55 cases and other clinical symptoms in 35 cases. The diagnosis of pHPT was proved by surgery and histology. The adenoma was accurately located by operation.

The adenoma of patients with kidney stones was located in 50 cases (91 %), (chi^2=67.5, p<0,00001) in the left inferior parathyroid gland and in 2 patients in the left superior one, while in 3 patients suffered from multiple hyperplasia. In patients without kidney stones the adenoma was localized in the right inferior parathyroid gland in 24 cases (69 %), (chi^2=43.9, p<0,0001), however, in 3-3 patients it was detected in the left or right superior parathyroid gland. Less frequently multiple hyperplasia (3 patients) and ectopic location (2 patients) was also observed.

These results raise the possibility that the clinical manifestation of the pHPT with or without kidney stones could be influenced by the location of the adenoma. Would it be possible that the biologic effects of parathyroid hormone sourced from one or another of the four glands could be different or different biologically active fragments are produced? From a practical point of view: in patients with kidney stones due to pHPT the left inferior parathyroid gland should be the first target of choice for the surgeon if the preoperative procedures leave any debate for the exact location of the adenoma.

Disclosures: ***E. Csupor****, None.*

M407

Kinetics Analysis of Parathyroid Hormone During Parathyroidectomy in the Assessment of Curative Surgery. M. Tommasi*[1], F. Locchi*[1], M. L. Brandi[2], A. Brocchi*[1], S. Raspanti*[1], R. Panconesi*[3], F. Tonelli*[1]. [1]Clinical Physiopathology, State University, Firenze, Italy, [2]Internal Medicine, State University, Firenze, Italy, [3]Surgery Division, AOC Hospital, Firenze, Italy.

Rapid intraoperative parathyroid hormone (IOPTH) test is used to guide adequacy of resection during surgery for primary hyperparathyroidism (HPT). Commonly, a 50 % decrease versus baseline PTH at 10 min after resection of the suspected parathyroid adenoma indicates "cure". For this rule to be valid, the time interval of 10 min would be stated starting from an instant at which the hormone efflux into circulation totally stopped ("ideal" clamping). Nevertheless, sometimes, at the "actual" clamping the vessels of the affected gland have partially been clamped before excision, and this situation, modifying the decay curve, may cause false negative results. Our goal was the definition of a "virtual" clamping, for the development of a new monitoring protocol. Forty-four patients with primary with single-adenoma had blood sampled at anaesthesia induction (basal value), manipulation, clamping, and thereafter at intervals of 5 min until 20 min. Intact PTH (iPTH) was determined using two consecutive rapid assays (IRMA and ICMA) and a standard assay. For 23 patients, an iPTH under the threshold of 50 % of the clamping value at 10 min, confirmed the cure, while 21 patients failed to exibit the same pattern of decrease. By a simulation study on the behaviour of the iPTH disappearance curve, we showed that the shifting in time of the origin of the curve produced a behaviour like to that one obtained in false negatives. Using the time interval between manipulation and clamping (Tmc), 17 patients were tested. Using a 50 % reduction versus clamping in the levels at 10 min after clamping, n 10 patients with Tmc <=10 min were correctly classified as being cured, and n 4 with Tmc <= 10 min were incorrectly classified as not being cured (false negatives); of these four patients, 2 patients were correctly reclassified using the 50 % reduction, versus manipulation value, in the level at 10 min after manipulation, and 2 patients were correctly reclassified by additional monitoring. Additional monitoring was also required in the remnant 3 patients with Tmc > 10 min. In conclusion analysis of PTH kinetics is more accurate in assessing adequacy of resection by taking into account the substantial variations in relative values of clamping to manipulation, as well as their different time intervals.

Disclosures: ***M. Tommasi****, None.*

M408

Characterization of the *GCMB* Promoter by Transient Transfection of Parathyroid Cell Primary Cultures. A. Maret*[1], C. Ding[1], D. Goldenberg*[2], M. Shamblott*[3], M. A. Levine[4]. [1]Pediatrics, Johns Hopkins University, Baltimore, MD, USA, [2]Otolaryngology-Head and Neck Surgery, Johns Hopkins University, Baltimore, MD, USA, [3]Gynecology-Obstetrics, Johns Hopkins University, Baltimore, OH, USA, [4]Pediatrics, Cleveland Clinic Foundation, Cleveland, OH, USA.

GCMB, a recently discovered transcription factor, is uniquely expressed in developing and mature parathyroid cells and is critical for the development and function of parathyroid glands in mice as well as in humans. In order to investigate the mechanism for transcriptional regulation of this gene, we have cloned and sequenced 2.8 kb of the 5' untranslated region of the human *GCMB* gene. Total RNA from a parathyroid adenoma was subjected to RACE using 4 exonic oligonucleotide primers to identify the transcription start site. We generated plasmids in which a luciferase reporter gene was coupled to the 2.8-kb *GCMB* promoter or nested deletion products. The ability of these sequences to activate luciferase expression was analyzed 48 hours after transient transfection of COS7 cells and primary cultures of bovine parathyroid glands and human parathyroid adenomas using Fugene 6. Analysis of the RACE amplicons revealed a single transcription start site located 247 bp upstream of the translation start site. The promoter contains a conventional TATA box and GC box, as well as potential regulatory elements for many transcription factors, including AP2 and Pax1, within the first 200 bp. A Pax9 binding site is located 400 nt upstream of the TSS. The 2.8-kb *GCMB* promoter region strongly stimulated luciferase activity in bovine (40-fold greater than vector) and human parathyroid gland primary cultures (15 to100-fold), but stimulated luciferase activity only weakly (5 to10-fold) when transfected in COS 7 cells. Progressive deletion analysis of *GCMB* promoter-luciferase constructs indicated that the core minimal promoter is located between nt -186 and +83, which stimulates luciferase activity 20-fold over vector. These results demonstrate the utility of parathyroid primary cell cultures in the analysis of transcriptional regulation of genes expressed in the parathyroid. Moreover, the identification of binding sites in the *GCMB* promoter for Pax1 and Pax9, which are critical for parathyroid gland development, suggests that these two transcription factors may promote parathyroid cell development via regulation of *GCMB* expression.

Disclosures: ***A. Maret****, None.*

M409

Gene Expression Profiling in Patients with Primary Hyperparathyroidism Before and After Treatment. S. Reppe*[1], L. Stilgren*[2], O. K. Olstad*[1], K. Brixen*[2], K. M. Gautvik[1], B. Abrahamsen*[2]. [1]Department of Medical Biochemistry, University of Oslo, Oslo, Norway, [2]Department of Endocrinology, Odense University Hospital, Odense, Denmark.

Primary hyperparathyroidism (PHPT) is characterised by increased and sustained secretion of parathyroid hormone (PTH), most frequently due to an adenoma. PTH is known to increase the rate of remodelling where both bone formation and resorption are greatly accelerated, reflecting a hyperactive state of osteoblasts and osteoclasts. The up-regulation of bone turnover in PHPT results in a net catabolic state where a decrease in bone mass is

encountered representing the metabolic osteodystrophy of hyperparathyroidism. Augmented resorption of bone combined with increased renal re-absorption of calcium results in hypercalcemia which may lead to severe kidney, cardiovascular complications and decreased bone mass if undiagnosed. Early diagnosis and removal of pathological glands restore normal calcium balance and bone mass. We have studied the effect of chronic PTH excess on transcriptional activity in bone biopsies taken from the same patients before and after successful treatment for PHPT one year after the operation. Gene expression in bone biopsies have so far been studied in 4 patients using the U133A chip from Affymetrix containing more than 22 000 gene probes. Approximately 11 000 of these were found to be expressed in bone. PTH levels were normalized and all bone and clinical parameters decreased one year after operation. More than 100 mRNAs were altered in patients with PHPT and more than 70 % of these mRNAs were up-regulated. Some of the consistently up-regulated genes are shown below.

Gene	Fold regulation (median)
Collagen, type I, alpha 1	6.7
Collagen, type I, alpha 2	3.8
Collagen, type V, alpha 1	3.1
Collagen, type V, alpha 2	2.7
Osteocalcin	2.2
Osteonectin	2.7
Osteopontin	1.5
osteoblast specific factor 2	2.1

Increased serum levels of Collagen type I and osteocalcin were also detected in the diseased state. In conclusion, we describe the changes in patterns of mRNA expression occurring in bone during PHPT, and their reversibility upon treatment. Furthermore, chronic exposure to PTH reveals that a limited number of genes are affected and involved in bone remodelling.

Disclosures: ***S. Reppe****, None.*

M410

Effective Management of Severe Hypercalcemia with the Calcimimetic Cinacalcet HCl in Patients with Parathyroid Carcinoma. M. Rubin[1], J. Sliney[1], L. C. McCary*[2], S. J. Silverberg[1], J. P. Bilezikian[1]. [1]Columbia University College of Physicians & Surgeons, New York, NY, USA, [2]Amgen Inc., Thousanc Oaks, CA, USA.

Management of parathyroid carcinoma (PT Ca) following unsuccessful parathyroid surgery presents a challenge because effective medical therapy is not available. Progressive morbidity and eventual mortality results from persistent, severe hypercalcemia. The calcimimetic, cinacalcet HCl (AMG 073), reduces parathyroid hormone (PTH) and normalizes hypercalcemia in patients (pts) with primary hyperparathyroidism by specifically binding to and modulating the calcium sensing receptor on the parathyroid gland. It is unknown whether similar salutary effects occur in pts with PT Ca. We now report our single site experience with 6 pts with inoperable PT Ca given cinacalcet HCl.

The 2 women and 4 men (age: 50 ± 6 yr) had undergone initial parathyroid surgery 8.3 ± 3.8 yrs prior to study. Metastatic parathyroid disease had been previously resected in all pts from lung (n=4), neck (n=1) or esophagus (n=1). Both women had increased ß-hCG levels, although pregnancy was excluded in each. Before treatment with cinacalcet HCl, serum calcium was 15.3 ± 1.2 mg/dL (range: 12.7- 19.9; normal: 8.4-10.3) and intact PTH was 889 ± 238 pg/mL (range: 229-1778; normal: 10-65). Hypercalcemia had not resolved with prior bisphosphonate therapy in 5 pts. Cinacalcet HCl was initiated at 30 mg bid, with dose increases every 2 weeks to a maximum of 90 mg qid in order to achieve a serum calcium (S Ca) ≤ 10.3 mg/dL. S Ca fell in all pts as the dose was increased. One patient discontinued drug because of intractable nausea and vomiting, while 2 others experienced moderate nausea, and were able to continue receiving drug. Of the 5 ongoing pts, 2 are still in the dose-titration phase.

	BL S Ca (mg/dL)	S Ca after Cinacalcet HCl (mg/dL)	Cinacalcet HCl Dose
Patient #1	12.7	9.7	70 mg bid (final)
Patient #2	17.7	11.1	90 mg qid (final)
Patient #3	14.3	12.2	90 mg qid (final)
Patient #4[a]	13.3	10.7	70 mg tid (discontinued)
Patient #5	19.9	16.6	90 mg bid (ongoing)
Patient #6	14.0	13.1	50 mg bid (ongoing)

a Patient discontinued the study due to nausea and vomiting. BL = baseline, before study drug

Pre-dose intact PTH was generally unchanged from baseline to final dosing (688 to 714 pg/mL) in the 3 pts who have completed the titration phase. Sense of fatigue improved coincident with reductions in S Ca.

In summary, the calcimimetic agent cinacalcet HCl was effective in reducing serum calcium in pts with inoperable PT Ca and is associated with a subjective improvement in symptoms. Although the effects on tumor burden are unknown, cinacalcet HCl significantly ameliorates states of severe PTH-dependent hypercalcemia.

Disclosures: ***M. Rubin****, None.*

M411

Parathyroid Hormone Is Modified in Parathyroid Carcinoma: A Novel N-Terminally Intact Hormone with Different Immunoreactivity from PTH(1-84). M. Rubin[1], P. Gao[2], T. Cantor[2], J. P. Bilezikian[1], S. J. Silverberg[1]. [1]Columbia University College of Physicians & Surgeons, New York, NY, USA, [2]Scantibodies Lab Inc., Santee, CA, USA.

Although parathyroid hormone (PTH) comprises 84 amino acids, circulating PTH is immunochemically heterogeneous. Newly synthesized PTH undergoes cleavage in the gland and the periphery, resulting in co-secretion of PTH(1-84) and N-truncated fragments of uncertain length and biological activity. Some fragments, among them PTH(7-84), are measured along with PTH(1-84) by "intact" parathyroid hormone (iPTH) IRMAs. The newer IRMA (wPTH), using antigenic determinants at the extreme N-terminal end of the PTH molecule (the first amino acid "Ser"), is believed to be specific only for whole PTH(1-84) containing the complete N-terminal sequence. In normal subjects and in patients with both primary and secondary hyperparathyroidism, iPTH is higher than wPTH. We have shown that PTH levels are 1.3-fold higher by the iPTH assay (132 ±14 pg/ml) than the wPTH assay (96 ±10 pg/ml) in primary hyperparathyroidism. Thus, a substantial percentage of circulating PTH is in the form of large N-truncated fragments. It is unknown whether the ratio of fragment production is similar in pts with parathyroid carcinoma (PT Cancer), the most severe form of primary hyperparathyroidism.

We have just observed 2 patients with metastatic PT Cancer in whom a greater amount of wPTH than iPTH was detected. The patients (1 male, 1 female, 60 and 42 yrs) had a diagnosis of PT Ca of 26 and 9 yrs duration respectively. Serum calcium was 13.4 and 19.6 mg/dl (nl: 8.4-10.3). In the first patient; wPTH was 1.2-fold greater than iPTH (wPTH 946 pg/ml, iPTH 797 pg/ml). In the second patient, wPTH was 1.6-fold greater than iPTH (wPTH 1619 pg/ml, iPTH 1020 pg/ml).

The results suggest that in these 2 patients with PT Cancer, an abnormal fragment of PTH is recognized by the wPTH but not the iPTH assay. It should be N-terminally intact, and contain the first amino acid of PTH (1-84). The species may be a post-translational form of PTH, possibly modified by phosphorylation or glycosylation so it is detected by the wPTH but not by the iPTH assay. Identification and characterization of this newly recognized form of wPTH, and assessment of its potential biological activity, could provide insights into the mechanisms of abnormal PTH processing as well as furthering our understanding of the biology of PT Cancer.

Disclosures: ***M. Rubin****, None.*

M412

Relative Hyperparathyroidism of Unknown Etiology in Older Men. C. A. Clay*, P. S. Coates, J. M. Wagner, M. E. Shoup*, S. L. Greenspan. Univ. of Pittsburgh, Pittsburgh, PA, USA.

In an ongoing study of bone loss in men with and without prostate cancer, we observed a subset of patients with normocalcemic hyperparathyroidism. We postulated this was secondary to a decrease in calcium intake, vitamin D intake, or vitamin D insufficiency. To address this question, we compared these patients to a group of age-matched controls in the same ongoing study. We assessed dietary calcium, vitamin D, indices of bone mineral metabolism, bone turnover, and bone mineral density (BMD). The normal range for Nichols intact PTH was 10-65 pg/mL. All patients had 25-hydroxyvitamin D levels ≥20 ng/mL.

PTH, Calcium, and Other Clinical Characteristics

	Cases (n=21)	Controls (n=42)
Age (years)	67±8.6	67±6.7
BMI (kg/m^2)	31±5.3	29±3.2
Dietary calcium (mg/d)	1105±1109	813±499
Dietary vitamin D (IU/d)	640±473	523±406
PTH (pg/mL)	91±19*	43±14
Calcium (mg/dL)	9.3±0.3	9.2±0.9
Albumin (g/dL)	4.2±0.3	4.1±0.3

Vitamin D, Bone Markers, and BMD

	Cases (n=21)	Controls (n=42)
25-hydroxyvitamin D (ng/mL)	31±7	29±8
Alkaline phosphatase (IU/L)	89±20	78±15
Urine NTx (nmBCE/nmCr)	41±14	34±16
BMD-spine (g/cm^2)	1.02±0.19	1.09±0.20
BMD-total hip (g/cm^2)	0.97±0.13	0.99±0.13
BMD-total body (g/cm^2)	1.10±0.12	1.13±0.11
BMD-forearm (g/cm^2)	0.62±0.67	0.64±0.07

Other than the 2-fold higher PTH (*p<0.0001) for cases vs. controls, there were no statistically significant differences in any of these parameters. In each group, 76% of patients had prostate cancer. In a subset of subjects, 24-hr urinary calcium was within normal limits and was not statistically significantly different between cases and controls. We conclude that the etiology of this relative hyperparathyroidism is unknown and does not appear to be related to low calcium or vitamin D intake, vitamin D insufficiency, or prostate cancer. Furthermore, it was not associated with any statistically significant increases in bone turnover or decreases in bone mineral density. Further studies are pending to determine the etiology of this finding.

Disclosures: ***C.A. Clay****, None.*

M413

Short-term Recovery of Bone Density after Parathyroidectomy for Primary Hyperparathyroidism. L. Cianferotti, E. Ambrogini*, E. Vignali*, F. Cetani, A. Picone*, M. Zaccagnini*, A. Pinchera*, C. Marcocci. Department of Endocrinology, University of Pisa, Pisa, Italy.

Primary hyperparathyroidism is characterized by various degrees of activation of bone remodeling that can be quantified by bone markers and regional BMD measurements. The increased bone remodeling results in reduction in BMD particularly at the appendicular skeleton, rich in cortical bone, rather than in the axial skeleton. Parathyroidectomy (PTX) partly restores these skeletal abnormalities and the main recovery of bone mass is usually seen within the first year after the operation, mostly at sites rich in cancellous bone. Aim of our cohort study was to describe changes in bone turnover markers and in regional BMD 1, 3, 6 and 12 months after successful PTX. The study group was composed of 32 hyperparathyroid patients (mean age 58,5±12,6 yr), 12 men and 23 women (18 postmenopausal). Each evaluation included measurement of several parameters of bone turnover, such as total and ionized serum calcium, PTH, ostecalcin (BGP), bone alkaline phosphatase (BALP), S-CTX, U-CTX and assessment of BMD at lumbar spine, femur and distal radius. The data were analyzed using the Friedman repeated analysis of variance on ranks. Immediately following PTX total and ionized serum calcium were normalized. All bone markers, high at baseline, declined significantly within the 12 months of observation. However, we observed some difference between the bone resorption markers and the bone formation ones. Thus, U-CTX and S-CTX dropped within the normal range in the first month after PTX ($p<0,005$ respect to the baseline), while the decrease of the "anabolic" markers BALP and BGP was blunted and sustained within the 6 months after PTX ($p<0,005$ between each observation). This was in favour of an "anabolic" phase of bone turnover after PTX, expecially in the first 3 months after PTX. This "uncoupling" between resorption and formation was reflected by a faster bone mineral gain at sites rich in cancellous bone as lumbar spine. In fact, we observed a substantial increase of lumbar spine in the first 6 months (5,8% at 6 months, 6,7 % at 1 year, p=NS), while the recovery of femur BMD was slower and sustained over the 1 year of observation (3,1% at 6 months, 5,4% at 1 year, $p<0,05$); no real increase in cortical BMD was observed during the entire period of observation. These results might be explained, at least in part, by the hypothesis of the "bone remodeling transient", a period during which the anabolic phase of bone turnover refills the bone remodeling units which had been activated during the active phase of the disease. Obviously, areas rich in cancellous bone, having more remodeling sites, show an "apparent" faster recovery in bone density after PTX, consistently with our results.

Disclosures: ***L. Cianferotti****, None.*

M414

Does Radial Shaft BMD Compared with Other Regions Help Identify Patients with Primary Hyperparathyroidism? S. Sehgal*, M. Kleerekoper, D. A. Nelson. Department of Internal Medicine, Wayne State University, Detroit, MI, USA.

Primary hyperparathyroidism (PHPT) preferentially affects cortical bone and thus may be suggested by a disproportionately low bone mineral density (BMD) of the radial shaft compared with spine and hip. We examined 403 consecutive bone density reports (Hologic QDR 2000) prepared by a single reader for an urban Veteran's Administration Medical Center. The reader typically had no information about the patients' medical history. The reader noted the possibility of PHPT as a secondary cause of low BMD if the radial shaft BMD was disproportionately low compared to the spine and hip (Table 1). A retrospective review of medical records for all patients whose results had been flagged by the reader for possible PHPT was performed in order to answer the following: 1) What percentage of those patients received follow-up for PHPT by the referring physician? 2) Of those who received follow-up, how many had confirmed PHPT? The objective was to evaluate the utility of BMD measurements at the three sites for predicting the presence of PHPT.

In 23 patients the report indicated PHPT as a possible cause of low radial shaft BMD. This group comprised 19 males and 4 females, ages 51-88 yrs; 87% were white and 13% were African-American. Twelve of these 23 reports were followed up by the referring physician. Of the 11 patients not receiving follow-up, 2 had died, and 4 had other conditions, unknown to the reader, that may have influenced the decision not to pursue PHPT (e.g. metastatic cancer, Grave's disease). Of the 12 who were followed up, 5 (42%) had PHPT confirmed biochemically. Table 1 shows mean age-, sex- and ethnicity-specific Z-scores for each of the following: the total group flagged for PHPT follow-up ("Total"); the subgroup followed up but found negative for PHPT ("No PHPT"); the subgroup followed up and confirmed for PHPT ("Yes PHPT); the subgroup not followed up for PHPT ("No F/U").

In summary, approximately half of the patients flagged for possible PHPT received relevant follow-up. Of these, 42% were diagnosed with PHPT. Table 1 indicates no apparent difference in radial Z-scores between "Yes PHPT" and "No PHPT". However, because 42% of those followed up for PHPT were confirmed, these findings support the utility of DEXA for identifying possible PHPT cases. These observations also emphasize the importance of routinely measuring spine, hip and forearm BMD.

Table 1: Mean Z-scores

Region	Total	No PHPT	Yes PHPT	No F/U
Radial Shaft	-1.93	-1.92	-1.97	-2.19
Femoral Neck	0.08	0.18	1.00	0.37
Lumbar Spine	0.82	0.63	1.85	0.44

Disclosures: ***D.A. Nelson****, None.*

M415

Evaluation of Cortical Bone by Peripheral Quantitative Computed Tomography (pQCT) in Peritoneal Dialysis (PD) Patients. A. L. Negri*, C. Lombas*, E. Del Valle*, C. E. Bogado*, J. R. Zanchetta. Nephrology, Instituto de Investigaciones Metabólicas, Buenos Aires, Argentina.

Peripheral QCT allows the non-invasive evaluation of cortical and trabecular bone separately as well as the geometrical properties of bone. We investigated cortical bone in 22 patients (6 males and 16 females; mean age 45.5 ± 12 years) on maintenance PD; comparisons were made with 28 normal controls (9 males 19 females; mean age 32,7 ± 12.5 years). Patients had been on PD for 48.09 ± 27.5 months; 16 patients had been previously in hemodialysis; total time in dialysis was 78.36 ± 52.6 months. Patient's calcium in PD bath was 3.5 meq/l. Peripheral QCT was performed with a XCT 960 scaner (Stratec, Pforheim, Germany) at the radius of the non-dominant forearm (15% the length of the ulna from endplate). We evaluated Total and cortical bone mineral density (TBMD,cBMD), Total (cross-sectional) and cortical area (TA, cA), cortical thickness (cThK) endosteal and periosteal perimeters and buckling ratio(r/cThk). Intact PTH serum levels were measured by IRMA (Nichols). Correlations were made with total time in dialysis, age and serum iPTH. DP patients had a marked decrease in cThk(1.90 vs 2.95 mm; $p<.0001$)and marked increase in endosteal perimeter (31.2 vs 23.9 mm;$p<.0001$), buckling ratio (3.81 vs 2.21; $p<.0001$) and TA. TBMD and cBMD correlated negatively with total time in dialysis ($p<.01$); no correlations were found between cA, cBMD and cThK with iPTH. Age correlated positively with TA, endosteal and periosteal perimeter and negatively with cBMD. Our results show:1-cortical thinning of the radius with cortical parameters correlating predominantly with time in dialysis; 2- increase in buckling ratio that predisposes to fracture risk; and 3-bone adaptations to aging (peripherization) ocurring despite disturbances in endocrine-metabolic environment of dialysis.

Disclosures: ***A.L. Negri****, None.*

M416

Changes in Bone Mineralization Following Chronic High-Dose Treatment of Uremic Rats with Lanthanum Carbonate are Normalized by Phosphate Supplementation. S. Damment*[1], V. Shen*[2], I. Webster*[1]. [1]Shire Pharmaceutical Development Ltd, Basingstoke, United Kingdom, [2]Skeletech Inc, Seattle, WA, USA.

Lanthanum carbonate (LC) is a non-calcium-, non-aluminum-based phosphate binder for the treatment of hyperphosphatemia in chronic renal failure (CRF). As with another phosphate binder, sevelamer hydrochloride, prolonged administration of high doses of LC can lead to reduced bone mineralization in 5/6th nephrectomized rats – a validated animal model of CRF. We have investigated the ability of phosphate supplementation to prevent this effect with LC.

Adult male Sprague-Dawley rats were randomized into 5 groups (n = 10 per group) as follows: [1] Sham:vehicle treated, [2] CRF:vehicle treated, [3] CRF:low-phosphate diet, [4] CRF:LC treated, [5] CRF:LC treated plus phosphate supplementation. Partial (5/6th) nephrectomy was performed under general anesthetic. Sham controls underwent similar surgery except that their kidneys were left intact. Vehicle or LC were administered daily by oral gavage at a dose of 1000 mg/kg/day (equivalent to 9 times the maximum anticipated human dose) for 6 weeks. Phosphate-supplemented rats received inorganic phosphate (22 mg/day per rat) via subcutaneous osmotic mini-pumps; other rats received 2.5 mol/L sodium chloride via the same method. Phosphate-deficient diet contained 0.03% of the phosphate contained in the normal (phosphate-sufficient) diet.

Chronic high-dose treatment with LC produced similar but less marked effects on serum and urine markers of phosphate homeostasis to those seen with a phosphate-deficient diet. Urine phosphate output was reduced by 80–90% and serum phosphate by 30–40% in LC-treated rats, indicating effective reduction of dietary phosphate absorption. Increases in osteoid parameters in LC-treated rats mimicked those seen in the low-phosphate diet group, and were reversed by dietary phosphate supplementation. The number of rats with increased osteoid volume, osteoid surface and osteoid thickness was higher in the groups receiving LC or phosphate-deficient diet, but were unaltered (*vs.* controls) in the group receiving LC with phosphate supplementation. Similar trends were seen for cancellous bone volume and trabecular thickness. There were no clear effects of LC with or without supplemental phosphate on dynamic bone parameters.

We conclude that the effect of high-dose LC treatment on bone mineralization in CRF rats is due to excessive reduction of dietary phosphate absorption in the gut (i.e. phosphate depletion) as opposed to any direct action of lanthanum on bone. This is unlikely to occur in renal dialysis patients treated for hyperphosphatemia.

Disclosures: ***S. Damment****, Shire Pharmaceuticals 3.*

M417

Comparative Study of the Effects of Lanthanum Carbonate and Calcium Carbonate on Renal Bone Disease in Patients on Dialysis Over 1 Year. P. C. D'Haese*[1], M. E. De Broe*[1], I. Webster*[2], G. B. Spasovski*[3]. [1]Dept. of Nephrology, Antwerp University, Antwerp, Belgium, [2]Shire Pharmaceutical Development Ltd, Basingstoke, United Kingdom, [3]Dept. of Nephrology, Skopje University, Skopje, Yugoslavia.

Hyperphosphatemia is an independent risk factor contributing to mortality in dialysis patients. There is an ongoing need for non-hypercalcemic phosphate-binding agents that effectively reduce and maintain control of serum phosphorus within recommended clinical limits, without adverse long-term effects on bone. Here, we compare the effects of the new non-calcium-, non-aluminum phosphate binder, lanthanum carbonate (LC), on bone with those of calcium carbonate (CC) over 1 year.

Ninety-eight patients starting hemodialysis treatment were randomized to receive open-label LC (n = 49) or CC (n = 49) at a dose titrated to achieve optimal control of serum phosphorus – up to 3750 mg/day for LC and 9000 mg/day for CC. Tetracycline-labeled bone biopsies were taken at baseline and after 1 year of LC or CC therapy for histologic and histodynamic investigation. Phosphorus control and safety/tolerability were also evaluated.
Sixty-eight patients completed the study, 34 from each group. Serum phosphorus levels were well controlled at median doses of 1250 mg/day LC and 2000 mg/day CC during treatment. Bone biopsies from baseline and follow-up were available from 33 LC- and 30 CC-treated patients. ROD sub-types were similarly distributed in both groups; mixed ROD was the most common. Of the patients with adynamic bone disease or osteomalacia at baseline, 71% receiving LC had progressed towards normalization after 1 year *vs.* 42% receiving CC. Of patients with hyperparathyroid bone, 80% receiving LC *vs.* 50% receiving CC progressed towards normalization. One patient (4%) in the LC group developed adynamic bone disease *vs.* 6 patients (26%) in the CC group. The mean lanthanum content of bone was 2.1 ± 1.3 µg/g wet weight (*vs.* 0.1 ± 0.17 µg/g wet weight in the CC group) after 1 year of treatment. There were no differences in bone alkaline phosphatase activities or vitamin D levels between the LC and CC groups. A tendency towards reduced parathyroid hormone levels was seen in the CC group. LC appeared to be well tolerated and showed a good safety profile. The most frequent adverse events were gastrointestinal (typically mild in severity), occurring in 53% of patients with LC *vs.* 49% with CC. Hypercalcemia (serum calcium > 2.65 mmol/L) was significantly lower with LC (6%) *vs.* CC (48%).
These results show no tendency for LC to lower bone turnover over 1 year (unlike CC), and that LC is not associated with any aluminum-like effects on bone. LC is only minimally absorbed, has good tolerability and provides effective phosphate control.

Disclosures: ***P.C. D'Haese****, None.*

M418

Bone Alkaline Phosphatase Activity as a Predictor of Bone Histomorphometric Parameters of Bone Turnover a Year after Successful Kidney Transplantation. N. Bravenboer[1], P. Lips[1], P. Holzmann*[1], J. W. de Fijter*[2], N. A. T. Hamdy[3]. [1]Endocrinology, VU Medical Center, Amsterdam, Netherlands, [2]Nefrology and Transplantation, LU Medical Center, Amsterdam, Netherlands, [3]Endocrinology and Metabolic Diseases, LU Medical Center, Leiden, Netherlands.

We performed histomorphometry on 31 evaluable transiliac bone biopsies obtained from transplant recipients 12 to 18 months after successful renal transplantation. There were 12 women and 19 men aged 50 ± 12 years. All patients had a creatinine clearance >50 ml/min. Biopsies were mildly fixed in 70% alcohol and sent for evaluation to the Free University Medical Center. Sections were stained with Goldners trichrome and tartrate resistant acid phosphatase. Histomorphometric data were obtained for trabecular bone by a semi-automatic technique using Osteomeasure software (Osteometrics Inc, Atlanta, USA). All data are expressed as mean ± standard deviation. Pearson's correlation coefficients were used to calculate correlations between histomorphometric and biochemical indices of bone formation (bone alkaline phosphatase) and between histomorphometric indices of bone resorption and serum PTH concentrations. There was a wide range of variation in the histomorphometric parameters studied: BV/TV 17.43 ± 4.91%, BS/TV 3.44 ± 0.63 mm2/mm3, TbTh 101.05 ± 22.5µm, OS/BS 22.78 ±13.16 %, ES/BS 12.01 ± 4.25 %, NOc/BPm 0.32 ± 0.24 /mm. Despite the universal use of glucocorticoids by all patients, high bone turnover as reflected by increased osteoid and erosion surfaces was the most frequently encountered abnormality. There was no significant correlation between erosion surfaces (ES/BS) and number of osteoclasts (Noc/BPm) as histomorphometric indices of bone resorption and serum PTH concentrations. There was however a significant correlation between the osteoid surface as parameter of bone formation (OS/BS) and bone alkaline phosphatase activity as biochemical marker of bone formation (r=0.4, p=0.048). It is of note that an increase in histomorphometric parameters of bone resorption was not always explained by a concomitant increase in serum PTH concentration. In this respect, the role of immunosuppressive agents other than glucocorticoids is as yet to be elucidated. Our data suggest that in the presence of mild hyperparathyroidism and/or as a result of the use of immunosuppressive agents, long-term use of glucocorticoids does not appear to unduly suppress histomorphometric parameters of bone turnover a year after successful kidney transplantation.

Disclosures: ***N. Bravenboer****, None.*

M419

Effects of Renal Failure and High Calcium Diet, Alone and in Combination, on the Structural Strength of Rat Femur. J. J. Jokihaara*[1], P. Jolma*[1], P. Koobi*[1], J. Kalliovalkama*[1], J. Tuukkanen[2], H. Saha*[1], H. Sievanen*[3], P. Kannus[1], I. Porsti*[1], T. L. N. Jarvinen[1]. [1]University of Tampere and Tampere University Hospital, Tampere, Finland, [2]University of Oulu, Oulu, Finland, [3]UKK Institute, Tampere, Finland.

Chronic renal insufficiency (CRI) with the associated secondary hyperparathyroidism (2ºHPT) is considered the most complex and least predictable form of metabolic bone disease. The current mainstay in the treatment of CRI / 2ºHPT is the control of phosphorus with phosphate binders (calcium-containing salts), which can prevent the development/progression of 2º HPT. However, the situation is somewhat paradoxical regarding bone strength: High levels of PTH have been shown to be detrimental, but oversuppression of PTH could virtually halt bone turnover, and also result in compromised bone strength.
Fifty 8-week old male Sprague-Dawley rats were randomly assigned to either bilateral sham- (Sham) or 5/6 nephrectomy-surgery (NTX). After a 4–week post-operative (CRI-development) period the rats were randomized to receive either standard chow (Sham, NTX) or high-calcium chow (Sham+Calcium, NTX+Calcium) for the subsequent 8 weeks (treatment period). After 12 weeks, the animals were sacrificed, plasma samples were collected, and both femora were excised for comprehensive analysis of femoral neck and midshaft (dimension, peripheral quantitative computed tomography, dual-energy x-ray absorptiometry, and biomechanical analysis).
NTX resulted in clear 2ºHPT ($p<0.05$), whereas PTH levels were suppressed to 32-33% of that in the respective control (Sham) groups in both high-calcium groups ($p<0.05$). Total bone mineral content (TBMC) was decreased by -6% ($p<0.05$) in the NTX group, an effect that was reversed by high-calcium diet. In the proximal femur, a moderate decrease in bone mineral mass was observed in the NTX (–8%, $p<0.05$) but not in the NTC+Calcium group. The NTX-induced loss was attributable to a decrease in volumetric bone density (vBMD) (-7%, $p<0.05$), while the structural strength of femoral neck was maintained through a compensatory increase in the cross-sectional area (+15%, $p<0.05$). In the midshaft, a similar NTX-induced bone loss was observed (-6%), the mechanism also being a decrease in vBMD (-1%, $p<0.05$). Despite the observed changes, no differences in midshaft strength were found between the groups.
In summary, bones can preserve their mechanical integrity despite moderate CRI, apparently through a geometrical adaptation. Although treatment with high-calcium chow effectively suppressed PTH levels, it completely prevented the CRI-induced loss of bone mass without having an influence on the strength of the femoral neck or midshaft.

Disclosures: ***J.J. Jokihaara****, None.*

M420

Osteoprotegerin (OPG) and Receptor Activator of NF-kB Ligand (RANK-L) Serum Levels in Patients on Chronic Hemodialysis. S. Gonnelli[1], A. Montagnani*[1], C. Cepollaro*[1], M. Franci*[1], B. Lucani*[1], E. Gaggiotti*[2], R. Nuti[1]. [1]Department of Internal Medicine, Endocrinologic-Metabolic Sciences and Biochemistry, University of Siena, Siena, Italy, [2]Unit of Nephrology and Dialysis, University of Siena, Siena, Italy.

The mechanisms underlying the skeletal resistance to parathyroid hormone (PTH) in patients on chronic hemodialysis (CHD) are not yet fully clarified. The accumulation of mid molecules with inhibitory effect on bone turnover is one of the most accredited hypothesis. OPG and RANK-L, which are regulated by cytokines, PTH and other hormones, have been shown to modulate the genesis and activity of osteoclasts.
The aim of the present study was to evaluate serum levels of OPG, RANK-L and biochemical markers of bone turnover in patients on CHD.
In a fasting blood sample, obtained from 60 patients (mean age: 67.2±3.5 yrs) on CHD for at least 2 years (mean dialytic age: 7.8±3.2 yrs) and from 40 healthy subjects (mean age: 67.7±2.8 yrs) (CTRs) comparable for sex and age, we measured serum levels of OPG (Biomedica, Austria), RANK-L (Biomedica, Austria), PTH (IRMA, Technogenetics, Italy), bone alkaline phosphatase (BALP, Hybritech), N-terminal fragment of type I collagen (NTx - ELISA, OSTEOMARK Ostex International), calcium and phosphate.
OPG resulted about sixfold higher in CHD patients than in CTRs (38.7 ± 16.2 pgl/ml vs 6.3±0.17 pgl/ml), on the contrary RANK-L serum levels were not significantly different with respect to CTR. As expected PTH, BALP and NTx were significantly higher in CHD in comparison with healthy subjects. In CHD patients both OPG and RANK-L did not correlate with PTH, BALP and NTx serum levels. In CHD patients with a high osteoclastic activity (in the upper quartile of NTx) RANK-L was significantly ($p<0.01$) higher and OPG/RANK-L ratio significantly ($p<0.01$) lower than in patients with low osteoclastic activity (in the lower quartile of NTx). In patients with higher osteoclastic activity, we found a closer relationship between OPG/RANK-L ratio and NTx serum levels ($r= -0.41$, $p<0.01$).
On the contrary, taking into account OB activity (BALP serum levels), we did not find any significant difference for OPG and RANK-L.
Although our study is lacking of histological analysis of bone and of a large sample of patients, we can point out some conclusions:
1) both OPG and RANK-L serum levels are significantly higher in CHD patients than in healthy subjects; 2) OPG and RANK-L do not seem closely related to PTH, 3) OPG/RANK-L ratio is higher in CHD patients with a greater osteoclastic activity, 4) OPG/RANK-L ratio, but not OPG, seems to be related to osteoclastic activity.
However, further studies are needed to clarify the possible role of OPG accumulation in the pathogenesis of renal osteodystrophy.

Disclosures: ***S. Gonnelli****, None.*

M421

Chronic Acidosis-Induced Alteration in Bone Bicarbonate and Phosphate. D. A. Bushinsky[1], S. B. Smith*[1], K. L. Gavrilov*[2], L. F. Gavrilov*[2], J. Li*[2], R. Levi-Setti*[2]. [1]Nephrology/Medicine, University of Rochester School of Medicine, Rochester, NY, USA, [2]Fermi Institute/Physics, University of Chicago, Chicago, IL, USA.

Chronic metabolic acidosis increases urine calcium excretion without altering intestinal calcium absorption, suggesting that bone mineral is the source of the additional urinary calcium. *In vivo* and *in vitro* studies have shown that metabolic acidosis causes a loss of mineral calcium while buffering the additional hydrogen ions. Previously we have studied changes in femoral, mid-cortical ion concentrations after seven days of *in vivo* metabolic acidosis induced by oral ammonium chloride. We found that compared to mice drinking only distilled water, ammonium chloride induced a loss of bone sodium and potassium and a depletion of mineral bicarbonate and phosphate. In the current *in vitro* study we utilized a high resolution scanning ion microprobe with secondary ion mass spectroscopy to test the hypothesis that chronic acidosis would decrease bulk (cross-section) bone phosphate to a greater extent than bicarbonate by localizing and comparing changes in bone bicarbonate and phosphate after chronic incubation of neonatal mouse calvariae in acidic medium. Cal-

variae were cultured for a total of 51 hr in medium acidified by a reduction in bicarbonate (HCO_3^-) concentration (pH ~ 7.14, [HCO_3^-] ~ 13mM) or in neutral medium (pH ~ 7.45, [HCO_3^-] ~ 26 mM). Compared to incubation in neutral medium, incubation in acidic medium caused no change in surface bicarbonate, but a significant fall in cross-section bicarbonate, with respect to the carbon-carbon bond (C_2) and the carbon-nitrogen bond (CN). Compared to incubation in neutral medium, incubation in acidic medium caused no change in surface total phosphate, but a significant fall in cross-section phosphate, with respect to C_2 and to CN. The fall in cross-section phosphate was significantly greater than the fall in cross-section bicarbonate. The fall in phosphate indicates release of mineral phosphates and the fall in bicarbonate indicates release of mineral bicarbonate, both of which would be expected to buffer the additional protons and help restore the pH toward normal. Thus, a model of chronic acidosis depletes bulk bone proton buffers, with phosphate depletion exceeding that of bicarbonate.

Disclosures: ***D.A. Bushinsky****, None.*

M422

Establishment of Novel Experimental Osteoarthritis Models in Mice. S. Kamekura, K. Hoshi, T. Shimoaka*, H. Chikuda, U. Chung, K. Nakamura, H. Kawaguchi. Orthopaedic Surgery & Tissue Engineering, University of Tokyo, Tokyo, Japan.

Due to significant development of mouse genomics and the availability of transgenic and knockout mice, the mouse is now the most ideal animal model for the study of molecular backgrounds of physiological and pathological conditions. Although osteoarthritis (OA) is clinically one of the most common skeletal disorders and is induced by accumulated mechanical stress in joints, little is known about the molecular mechanisms by which the stress leads to cartilage degeneration and abnormal ossification. This may be because animal models of stress-induced OA have been limited to larger species. In the present study we succeeded in creating reproducible experimental OA models in mice (8-week-old C57black/6) by producing instability in knee joints using a microsurgical technique. The models were of three types: severe, moderate and mild, depending on the severity of joint instability imposed by combinations of transection of ligaments and menisectomy. In radiographic and histological analyses of the knee joint, the severe model exhibited cartilage defect and osteophyte formation 4 weeks after surgery, which is characteristic of late OA pathology in humans. The mild model demonstrated proliferation and clustering of chondrocytes 6 weeks after surgery, which is compatible with the early OA pathology. In the moderate model, the early change was observed within 2 weeks, and the late change over 6 weeks after surgery. Mankin's score (0-14), a histological index of OA severity, increased as a function of time after surgery and as that of severity of instability. Combining these three models, we performed cellular and molecular analyses of the OA articular cartilage by immunohistochemistry and in situ hybridization. In the early stage, proliferation of chondrocytes led to formation of clusters including hypertrophic chondrocytes that expressed type X collagen. Among MMPs (MMP-2, 3, 9, 13 and 14), MMP-13 was most strongly induced with OA progression. Although in the growth plate MMP-13 was expressed much later than type X collagen, in the OA cartilage the MMP-13 expression was seen simultaneously with that of type X collagen from the early stage of the chondrocyte hypertrophy. These findings suggest that the chondrocyte differentiation steps, i.e. the earlier hypertrophy and the later degradation/calcification, are disorganized in the OA cartilage. We speculate that MMP-13 may play a pivotal role in these cellular disorders, and might be a therapeutic target for OA.

Disclosures: ***S. Kamekura****, None.*

M423

Effect of Alendronate in Patients with Rheumatoid Arthritis on Chronic Treatment with Low Dose Prednisone. W. F. Lems[1], M. C. Lodder*[1], P. T. M. Lips[2], J. W. J. Bijlsma[3], P. Geusens[4], N. Schrameijer*[5], C. M. van de Ven*[5], B. A. C. Dijkmans[1]. [1]Rheumatology, Free University, Amsterdam, Netherlands, [2]Endocrinology, Free University, Amsterdam, Netherlands, [3]Utrecht Medical Centre, Utrecht, Netherlands, [4]Rheumatology, Academic Hospital, Maastricht, Netherlands, [5]MSD, Haarlem, Netherlands.

Objective: A bone sparing effect of alendronate has been described in patients treated with moderate and high dosages of prednisone for heterogeneous diseases. Up until now, there have been no trials specifically designed to study only patients with rheumatoid arthritis (RA) chronically treated with low dose prednisone. Therefore, we studied the effect of alendronate on BMD of the lumbar spine and hip in patients with RA on chronic treatment with low dose prednisone.
Methods: In a double-blind, placebo-controlled trial 144 patients with RA were enrolled. All patients were treated with low dose prednisone (<10 mg/day) for at least three months. The patients were randomised to receive alendronate or placebo: in men and premenopausal women 5 mg alendronate (or placebo) was given, while in postmenopausal women not on HRT 10 mg alendronate (or placebo) was administered. All patients received daily calcium (500 mg, or 1000 mg determined by questionnaire) and vitamin D (400 IU) supplementation. BMD of the lumbar spine and the total hip was measured at baseline, and after 6 and 12 months. The primary endpoint was change in BMD of the lumbar spine after 12 months (ITT). At baseline and after 3, 6 and 12 months, serum bone specific alkaline phosphatase (SAP) and urinary excretion of NTX was measured.
Results: At baseline, BMD of the lumbar spine was 1.064± 0.17 (mean, SD) g/cm2 for the alendronate group and 1.098±0.214 (mean, SD) g/cm2 for the placebo group (NS). After 12 months BMD at the lumbar spine was increased by 3.55% in the alendronate treated patients and –1.06% in the placebo-treated patients (p<0.0001). At the total hip, the changes were +1.00% and +0.14% respectively (p = 0.20).
After 3 months, SAP was decreased by 17% in the alendronate group versus 3.3% for the placebo group (p = 0.0005), while urinary NTX was decreased by 46% in the alendronate group versus 12% in the placebo group (p <0.0001). Adverse effects were observed in 69% of the alendronate and 74% of the placebo patients, while withdrawals occurred in respectively 16% and 30% of the patients (NS).
Conclusion. We observed a positive effect of alendronate on BMD of lumbar spine and markers of bone turnover in patients with RA treated with low dose prednisone. These data support that the prescription of alendronate is not only useful in patients treated with high dose prednisone but also in patients treated with low dose prednisone.

Disclosures: ***W.F. Lems****, MSD 2, 5, 8.*

M424

Changes in Bone Mineral Density in Patients with Rheumatoid Arthritis Treated with Infliximab. M. Vis*, G. J. Wolbink*, B. A. C. Dijkmans, W. F. Lems. Rheumatology, VU university medical center, Amsterdam, Netherlands.

Background: Secondary osteoporosis is a well-recognized feature of rheumatoid arthritis (RA). The pathogenesis of osteoporosis in RA is multi-factorial. Disease activity, immobility and steroid-use are factors that contribute to bone loss. High disease activity in RA is associated with a low BMD. It is suggested that active treatment of RA could prevent this bone loss. Infliximab (anti-TNF) is effective in reducing disease activity. In a previous study we found that bone formation increases and bone resorption decreases in patients with RA during short-term treatment with infliximab.[1] From this we hypothesized that long-term treatment with infliximab might arrest loss of bone in patients with RA.
Patients and methods: In this open cohort study all patients, who were treated with infliximab in the Slotervaart Hospital and had BMD measurements at baseline and after 1 year, were included. All patients had RA according to the ACR-criteria. Infliximab was administered intravenously at 0, 2, 6, 14 and then every 8 weeks in a dosage of 3 mg/kg. At all visits disease activity was measured by swollen-, tender joint counts, ESR and visual analogue scale for disease activity. From these 4 variables the disease activity score (DAS-28) was calculated. BMD measurements of the hip and lumbar spine (L1-L4) were performed at baseline and after 54 weeks. Differences in means were compared by means of a paired sample t-test.
Results: Thirty-two patients with the following baseline characteristics were enrolled: 80% female, mean age 54 years (SD: 12,8), median disease duration 10 years (range: 1-47), IgM-Rheumatoid-Factor positive: 69%. At baseline nine patients (40%) used corticosteroids mean dose 8,3 mg/day. Mean disease activity (DAS28) changed from 5,8 at baseline to 3,9 at 14 weeks to 3,6 at 30 weeks and was decreased to 3,8 at 54 weeks. The changes in bone mineral density of the lumbar spine and hip are shown in table1.
Conclusion: During one-year treatment with infliximab bone loss in the lumbar spine seems to be arrested, however in the hip bone-loss continues. Although we only included a small number of patients these data could suggest that infliximab (anti-tnf) has a beneficial effect on bone in rheumatoid arthritis.
Literature: 1. Vis et al. Ann Rheum Dis 2002 (suppl I) : S51 (abstract)

Table1: BMD in 32 patients at baseline and after one year treatment with infliximab.

	Baseline	54 Weeks	Change %	
BMD L1-L4 g/cm² mean (SD)	1.025(0.219)	1.030(0.144)	+0.6	p=0,49
BMD total-hip g/cm² mean (SD)	0.870(0.207)	0.864(0.130)	-0.7	p=0.48

Disclosures: ***W.F. Lems****, None.*

M425

Role of Dendritic Cells Stimulated with RANKL for Modulation of Autoimmune Arthritis in MRL/*lpr* Mice. T. Izawa*[1], K. Moriyama[1], Y. Hayashi*[2]. [1]Department of Orthodontics, School of Dentistry, University of Tokushima, Tokushima, Japan, [2]Department of Pathology, School of Dentistry, University of Tokushima, Tokushima, Japan.

Receptor activator of NF-kB ligand (RANKL) is a regulator of the immune system and of bone development. In vitro, RANKL promotes the survival of mature dendritic cells (DCs), and induces the production of proinflammatory cytokines, such as IL-1 and IL-6, that stimulate and induce T cell differentiation. The aim of this study was to analyze the effect of cell transfer of DCs stimulated with RANKL on the development of autoimmune arthropathy in MRL/*lpr* mice, and to evaluate the possible relationship with RANKL-mediated osteoclastogenesis. Freshly isolated bone marrow cells were stimulated with IL-4 and GM-CSF for seven days and induced differentiation into bone marrow DC (BMDC) by further stimulation of RANKL and type II collagen (CII) for three days. BMDC ($1x10^6$) activated with RANKL were subcutaneously injected one or three times into inguinal region. These mice were analyzed at eight weeks after the cell transfer. We tested surface markers on T cells, proliferation assay, anti-CII antibody, rheumatoid factors (RF), cytokine production, and osteoclastogenesis. Acceleration of autoimmne arthritis accompanied by bone destruction was observed in one time DC-transferred mice compared with age-matched controls. We detected a significant increase in T cells bearing memory type and DCs expressing MHC class II ($I\text{-}A^k$). In addition, an increase in osteoclast formation and pit formation was observed in bone marrow culture of DC-transferred mice, suggesting that RANKL induced osteoclast formation and activation by direct stimulation of osteoclast progenitors. In contrast, three times transfer of CII-pulsed RANKL/DC protected the mice from autoimmune arthritis. These results indicate that RANKL pathway plays a crucial role for immunomodulation of autoimmune arthropathy in a murine model for rheumatoid arthritis.

Disclosures: ***T. Izawa****, None.*

M426

Hormone Replacement Therapy Decreases Markers of Cartilage and Bone Metabolism in Rheumatoid Arthritis. H. Forsblad d'Elia[1], S. Christgau*[2], L. Mattsson*[3], T. Saxne*[4], C. Ohlsson[5], E. Nordborg*[6], H. Carlsten*[1]. [1]Department of Rheumatology and Inflammation Research, CBS, Göteborg University, Sweden, [2]Nordic Bioscience A/S, Herlev, Denmark, [3]Department of Obstetrics and Gynecology, Göteborg University, Sweden, [4]Department of Rheumatology, Univerity of Lund, Sweden, [5]Department of Internal Medicine, CBS, Göteborg University, Sweden, [6]Department of Rheumatology, Huddinge University Hospital, Sweden.

Rheumatoid arthritis (RA) is characterized by cartilage destruction, bone erosions, periarticular and generalized osteoporosis with subsequent increased risk of fractures. This prospective study aims to evaluate the effects of hormone replacement therapy (HRT), known to prevent osteoporosis, on markers of bone and cartilage metabolism and to investigate if changes in these markers correspond to alterations in bone mineral density (BMD) and radiographic joint destructions evaluated by Larsen score.

Eighty-eight postmenopausal women with RA were randomly allocated either to receive HRT, vitamin D_3 and calcium or vitamin D_3 and calcium alone for two years. The effects of HRT on bone metabolism were investigated by measuring serum levels of the collagen type I degradation products, C-terminal telopeptide fragments of type I collagen (CTX-I) and C-terminal telopeptide of type I collagen (ICTP), bone sialoprotein (BSP) and of the formation marker C-terminal propeptide of type I procollagen (PICP). Cartilage turnover was studied by analyzing urinary levels of collagen type II C-telopeptide degradation fragments (CTX-II) and the cartilage-remodelling marker, cartilage oligomeric matrix protein (COMP) in serum. Correlation analyzes of the markers and BMD and Larsen score were studied. HRT resulted in a decrease in CTX-I ($p<0.001$), ICTP ($p<0.001$) and COMP ($p<0.01$) compared to controls. PICP was reduced the first year ($p<0.01$) in comparison with the controls and within the group also after two years ($p<0.05$). BSP remained stable in the HRT group but increased in the controls ($p<0.05$). CTX-II decreased within the HRT group ($p<0.05$). Serum levels of CTX-I fell by an average of 53 ± 6 % in 91 % of the HRT treated patients after two years. Decrease in CTX-I, ICTP and PICP were associated with improvement in BMD. Changes in serum levels of estradiol were correlated with alteration in CTX-I ($p<0.01$) and with ICTP ($p<0.05$). Baseline measures of CTX-II and COMP were correlated with Larsen score.

In conclusion, HRT in women with RA reduced markers of bone and cartilage metabolism. The decrease in CTX-I, ICTP and PICP was associated with improved bone mass after two years. CTX-I and COMP seemed to be the most sensitive biochemical markers, reflecting bone and cartilage turnover, respectively.

Disclosures: ***H. Forsblad d'Elia****, None.*

M427

Osteoprotegerin (OPG) Serum Levels as Regulator of Vascular Function: Role in the Pathogenesis of Kawasaki Disease Vasculitis. L. Masi[1], E. Piscitelli*[1], G. Simonini*[2], F. Falcini*[2], A. Falchetti*[1], A. Amedei*[1], E. Colli*[1], R. Imbriaco*[1], V. Ghinoi*[1], F. Del Monte*[1], G. Leoncini*[1], A. Tanini*[1], M. Brandi[1]. [1]Department of Internal Medicine, University of Florence, Florence, Italy, [2]Department of Pediatrics, University of Florence, Florence, Italy.

Kawasaki Disease (KD) vasculitis is characterized by a progression of arterial lesions and a number of immunoregolatory changes, including a deficiency of circulating CD8+ suppressor/ cytotoxic T cells,abundance of circulating B cells and activated monocytes. Biochemical and immunological evidence suggests endothelial cells activations and injury. The most important complicance is represented by the coronary aneurysms. OPG, a member of the TNF receptor family has been identified as regulator of bone resorption and it is produced by a variety of tissues including vascular endothelial cells. OPG deficient mice develop a severe osteoporosis and arterial calcifications. In addition, serum OPG concentration is 30% higher in women with diabetes, which showed a higher risk of cardiovascular mortality. In the present study we evaluated the levels of serum OPG in a group of 26 children (6 female and 20 male) with a mean age of 3.4±1.6 years affected by KD and 46 age-matched healthy children as control. Serum was obtained by centrifuging blood collected by venipuncture during routine laboratory test. Serum OPG concentration was measured using a highly sensitive, commercial sandwich enzyme immunoassay provided by Immunodiagnostik (Bensheim, Germany). Statistical analysis was performed using STATISTICA 5.1 program. Mann-Whitney U test showed that patients affected by KD had statistically significant higher levels of serum OPG in comparison with the control (93.8±33.1 vs. 39.9±7.8 pg/ml; p=0.001). Considering only patients affected by KD we observed a higher serum OPG concentration in children with coronary complicances (119.3±28.5 vs. 87.66±33 pg/ml; p=0.01). In conclusion, the increase of serum OPG in patients affected by KD could reflect a compensatory self-defence mechanism for keeping under control the mechanisms that contribute to vascular injury and may represent a marker in the identification of patient affected by KD with a higher risk to develop coronary complicances.

Disclosures: ***L. Masi****, None.*

M428

Etidronate Specifically Inhibits Periarticular Bone Loss Accompanied with Joint Destruction in Adjuvant Arthritis Rats. T. Tanaka*, T. Nakayama*, T. Katsumata. Research Division, Sumitomo Pharmaceuticals, Osaka, Japan.

Periarticular bone loss accompanied with joint destruction is serious problem for the patients with rheumatoid arthritis. However, no therapeutic drugs have been sufficiently available for these pathogenic changes. In the present study, etidronate (EHDP), non-aminobisphosphonate was examined on periarticular bone loss and joint destruction in adjuvant arthritis (AA) rats, which were produced by a single subcutaneous injection of *Mycobacterium butyricum* into the right hind paw in 6-week-old male Lewis rats. We compared the effect of EHDP with that of aminobisphosphonate, alendronate (ALN). After 17 days of adjuvant immunization, when secondary inflammation had developed in the left hind paw, EHDP (5, 10 mg/kg) and ALN (0.025, 0.05 mg/kg) were subcutaneously administered for 2 weeks. As the index of periarticular bone loss with joint destruction, BMD of the left distal tibia was measured by DXA method. Also BMD of the proximal tibia was measured as the index of systemic bone loss. Joint destruction of the left hind paw was examined in the histopathological assessment. EHDP treatment inhibited BMD reduction not only of the proximal tibia but also of the distal tibia. ALN induced higher BMD of the proximal tibia, however this compound exhibited insufficient effects on BMD reduction of the distal tibia. EHDP, but not ALN, reduced AA-induced joint destruction score in the talus or calcaneus. Also immunohistochemical analysis revealed that EHDP reduced the number of ED1 (+) cells, monocyte/macrophage linage cells, observed in the bone marrow of the talus or calcaneus in AA rats. ALN treatment showed no effect on the number of ED1 (+) cells. These results indicate that EHDP is effective on periarticular bone loss and joint destruction, whereas the effect of ALN is specific for systemic bone loss in AA rats. Moreover, it is suggested that the effects of EHDP not only on osteoclast but also on monocyte/macrophage lineage cells, lead to prevent periarticular bone loss. EHDP may be a preferable therapeutic drug for the treatment of periarticular bone loss accompanied with joint destruction in the patients with rheumatoid arthritis.

Disclosures: ***T. Tanaka****, None.*

M429

Mechanism of Anti-Inflammatory Effects of the Aminobisphosphonate (Incadronate) in Adjuvant-Induced Arthritis. T. Shuto, A. Matsuo*, G. Hirata, Y. Iwamoto*. Dept. Orthopaedics, Kyusyu University, Fukuoka, Japan.

Incadronate (disodium cyclohepeptylaminomethylenediphosphonate monohydrate) is a newly developed third-generation bisphosphonate with a potent inhibitory activity toward osteoclastic bone resorption. Recently, some bisphosphonates have been shown to inhibit bone destruction in experimental arthritis including rat adjuvant arthritis (AA), an animal model of rheumatoid arthritis. We reported that incadronate inhibited bone destruction and joint inflammation in rat AA, when it is given before and after the onset of arthritis. The aims of this study are (1) to compare the anti-inflammatory effects of incadronate against dexamethazone in adjuvant-induced arthritis (AIA), (2) to clarify the mechanisms by which these compounds exert their anti-inflammatory effects.Lewis rats were given an intradermal injection of heat-killed Mycobacterium butyricum for induction of AIA. The bisphosphonates were injected subcutaneously three times a week after the onset of arthritis. The dexamethazone were injected. The severity of joint inflammation was evaluated according to the hind paw volume, radiological and histological examination.Incadronate suppressed the hind paw volume in rat AIA in a dose-dependent manner. Incadronate at dose of 0.1 and1 mg/kg inhibited the hind paw volume by 73.5% and 76.5% on day 14 day, respectively, compared to that of the positive control ($p<0.01$). Incadronate at a dose of 0.1 or 1.0 mg/kg/day suppressed hind paw volume as well as dexamethazone at a dose of 0.25mg/kg/day. Incadronate appeared to suppress this synovial tissue formation in a dose-dependent manner. Incadronate decreased the number of ED1-positive cells infiltrating the synovial tissue. The chemotaxis assay revealed that incadronate suppressed the migration of these cells in a dose-dependent manner. Treatment with a high dose (10-4 M) and a low dose (10-8 M) of incadronate inhibited the chemotaxis of the cells to 45.8% and 64.2% of the control value, respectively. These results suggest that incadronate has an inhibitory effect on the migration of these cells.We demonstrated that incadronate inhibited the macrophage migration induced by MCP-1, in vitro. The histological sections were stained with TUNEL staining, however, macrophages of apoptosis were not observed in the synovium of AIA rats. A high dose treatment of incadronate did not induce macrophage cell death. These findings strongly suggest that the inhibitory effect of incadronate on inflammation might be mediated, at least in part, through the regulation of the migration of macrophage.

Disclosures: ***T. Shuto****, None.*

M430

Effect of Vitamin D and Calcium on Percent True Calcium Absorption Using Ca-46 and Ca-48 in Children with Juvenile Rheumatoid Arthritis (JRA). L. S. Hillman[1], J. D. Robertson*[2], B. J. Higgins*[2], M. F. Popescu*[1], F. Chanetsa*[1], J. T. Cassidy*[1]. [1]Child Health, U. of Mo., Columbia, MO, USA, [2]Chemistry, Mo. Research Reactor, U. of Mo., Columbia, MO, USA.

Children with JRA have impaired bone mineralization associated with decreased bone formation and resorption markers, decreased PTH, and low serum calcium. Animal models have postulated decreased 1,25 dihydroxy vitamin D receptors in the intestine. The study was approved by U.of Mo.IRB and all parents gave written consent. Eighteen children of mean age 10.4±2.6 years were given supplements of a) placebo; b) 1000 mg calcium; c) 1600 IU vitamin D; and d) 1000 mg calcium plus 1600 IU vitamin D for 6 month periods, with a 3 month washout in between, in a randomized order. Order of treatment had no effect so total periods on each treatment were compared. No effect was seen on bone mineral density, markers of bone formation and resorption, or PTH. However a striking effect on serum calcium (mg/dl) was documented over the 6 month period with a fall on placebo (9.20±.47 to 8.90±.39); no change on calcium (9.00±.42 to 9.15±.66); an increase with vitamin D alone (8.97±.36 to 9.43±.72); and a further increase with vitamin D and calcium (8.98±.36 to 9.92±.79). Treatment with vitamin D increased serum 25 hydroxy vitamin D but did not change serum 1,25 dihydroxy vitamin D. To assess the effect of vitamin D and calcium on the percent true calcium absorption (α) subjects were studied at the end of each period using two stable isotopes of calcium, Ca-48 given intravenously and Ca-46 given orally in a milk carrier. Ca-46 and Ca-48 were measured in a 24-hour urine as a ratio to Ca-42 using a VG-Axiom HR-ICP-MS. Δ% excess for both Ca-46 and Ca-48 was calculated

as (measured ratio – natural abundance ratio)/natural abundance ratio. Percent true calcium absorption (α) was calculated as:
α = 0.0035 (Ca-48 dose) (Δ% excess Ca-46)/0.186 (Ca-46 dose) (Δ% excess Ca-48)
Thirty-two studies were completed. Mean$\pm$SD (n) α for the four treatments were: placebo 28.3$\pm$20.0(n=9), calcium 26.0$\pm$11.9(n=8), vitamin D alone 19.2$\pm$11.7(n=6), vitamin D plus calcium 28.9$\pm$17.9(n=9). No significant differences were seen between groups. Additional calcium did not depress α and vitamin D did not increase α. A high degree of variability in α was observed as is reported for children. This was not accounted for by differences in Tanner staging. Mean values without supplementation of 28.3$\pm$20.0% are consistent with published pediatric normals, but included sporadic low values. Whether failure to increase α with vitamin D reflects 1) a down regulation of α after correction of serum calcium by 6 months of treatment, 2) a primary bone effect of vitamin D, or 3) a primary effect of vitamin D on the underlying JRA is unclear.

Disclosures: ***L.S. Hillman****, None.*

M431

Simvastatin Prevents Joint inflammation and Joint Destruction in Lewis Rats with SCW-Induced Arthritis. J. Chen, K. J. Downey*, R. A. Clark*, J. L. Funk. Department of Medicine, University of Arizona, Tucson, AZ, USA.

Inflammatory cytokines and activated T cells are thought to mediate joint inflammation in rheumatoid arthritis (RA). HMG-CoA reductase inhibitors (statins), such as simvastatin, have been reported to prevent both cytokine release and T cell activation. We therefore postulated that statin treatment would prevent joint inflammation in RA. Moreover, even in the absence of an anti-inflammatory effect, we hypothesized that statin treatment, by virtue of its reported anabolic and anti-resorptive effects on bone, could ameliorate the bone loss and joint destruction that accompany RA. To test these hypotheses, the effect of simvastatin on disease progression was tested in streptococcal cell wall (SCW)-induced arthritis, an animal model of RA. Female Lewis rats were treated with: (1) vehicle alone (control); (2) simvastatin (20 mg/kg/d); (3) SCW (15 or 25 mg/kg ip); or (4) SCW + simvastatin. Hydrolyzed simvastatin was administered subcutaneously 5-7 days/week, beginning 4 days prior to SCW injection and animals were followed for 28 days subsequent to SCW administration. SCW-treated animals rapidly developed a typical acute phase of joint swelling, as assessed clinically by daily arthritic index, and subsequent nadir in disease activity, followed by a persistent chronic phase of joint swelling with associated joint destruction. Simvastatin treatment significantly inhibited joint swelling as early as day 3 (70% inhibition), an effect that persisted throughout the subsequent course of acute and chronic arthritis. Joint destruction during the chronic phase of SCW arthritis (day 27-28) was also significantly inhibited by simvastatin treatment, as determined by its ability to block (1) increases in serum pyridinoline (Metra Biosystems), a marker of cartilage and bone destruction; (2) erosion of articular cartilage, as assessed histologically (3) decreases in distal femur bone mineral density, as measured by dual energy x-ray absorptiometry (GE Lunar PIXImus); (4) and increased number of osteoclasts in eroded periarticular bone, as identified by tartrate-resistant acid phosphatase (TRAP) staining. These results suggest that inhibition of HMG-CoA reductase may be an effective treatment for the prevention of joint inflammation and joint destruction in RA.

Disclosures: ***J.L. Funk****, Merck 2.*

M432

CTGF Expression Is Elevated in Rheumatoid Arthritis and Down-Regulated by a Synthetic TSP1-Derived Peptide. F. F. Safadi, J. M. Manns*, M. C. Rico, M. F. Barbe*, R. A. Aswad*, A. B. Uknis*, R. A. DeLa Cadena*, S. N. Popoff. Anatomy and Cell Biology, Physiology, and Medicine, Temple University School of Medicine, Philadelphia, PA, USA.

Rheumatoid Arthritis (RA) is a chronic, inflammatory disease associated with leukocyte infiltration of the synovial tissues. We have shown that human PMNs produce thrombospondin-1 (TSP1) and factor V (FV), and that TSP1 and human neutrophil elastase (HNE) are co-localized on the surface of activated PMNs, leading to the generation of activated factor X (FXa) and thrombin. FXa and thrombin stimulate the production of connective tissue growth factor (CTGF) in fibroblasts. CTGF has a potent effect on fibroblast proliferation and matrix production, and CTGF and has been implicated as a key regulatory molecule in angiogenesis. Our hypothesis is that CTGF mediates the angiogenesis and fibrosis that are associated with the pathogenesis of joint destruction in RA, and that up-regulation of CTGF expression is linked to the TSP1-dependent production of FXa and thrombin by activated PMNs in the synovial tissues. We tested this hypothesis using a genetically susceptible animal model of RA. Female Lewis rats were injected intraperitoneally with peptidoglycan-polysaccharide (PG-APS) to induce RA. Prior to the induction of the disease, some animals received an intravenous injection (daily and for four days) of a synthetic peptide that represents a region within the TSP1 type-3 repeats. This peptide interact with PMNs and inhibits the activity of HNE, and thereby, should prevent the generation of FXa and thrombin by activated PMNs. This, in turn, should prevent the up-regulation of CTGF and joint destruction in RA. Peptide treatment was associated with decreased circulating levels of HNE, neovascularization, neutrophil infiltration and thickening of the synovial lining in the joint when compared to peptide-untreated animals. *In situ* hybridization and immunohistochemical analyses showed that CTGF was highly up-regulated in RA when compared to peptide-treated rats. In peptide-treated animals, CTGF expression in the synovial lining was significantly decreased when compared to the peptide-untreated group and these results were correlated with a decrease in angiogenesis and fibrosis. These results demonstrate for the first time a link between TSP1 and CTGF expression and disease course in an experimental animal model of RA. The up-regulation of CTGF expression during the chronic phase of the disease is likely to be a key mediator to the angiogenesis and fibrosis that are characteristic of erosive joint destruction. The TSP1-derived peptide appears to have a potential therapeutic effect that is linked to its ability to prevent the up-regulation of CTGF expression in RA.

Disclosures: ***F.F. Safadi****, None.*

M433

Longitudinal Analysis of Bone Mineral Density (BMD) in Women with Recent Onset Systemic Lupus Erythematosus (SLE). N. M. Leong*[1], E. Shamiyeh*[1], A. H. Chung*[1], C. Langman[2], H. Price*[2], S. Spies*[1], R. Ramsey-Goldman[1]. [1]Medicine, Feinberg School of Medicine Northwestern University, Chicago, IL, USA, [2]Pediatrics, Childrens Memorial Hospital, Chicago, IL, USA.

This 2-year longitudinal study assessed the changes in BMD of women with SLE of less than 2 years duration and evaluated the impact of lupus related and traditional osteoporosis risk factors on BMD changes.
Lumbar spine, total hip, and distal forearm BMD were measured by dual x-ray absorptiometry (DXA) and traditional osteoporosis risk factors, including current smoking history, alcohol and caffeine intake, calcium and vitamin D, and physical activity, were assessed with a self-administered survey at 0 and 24 months. Lupus disease activity was measured using the revised Systemic Lupus Activity Measure (SLAM-R) and disease severity was scored using the American College of Rheumatology/Systemic Lupus International Collaborative Clinics Damage Index (ACR/SLICC-DI). Bone biochemical markers (BBM) including urinary N-linked telopeptides and serum bone alkaline phosphatase were also measured. Statistical analyses were performed using Student's t-test for paired values, Spearman correlations and Wilcoxan rank-sum test.
27 women completed DXA scans a mean of 25.3 (± 2.6) months apart. 13 women had never received corticosteroids (CS) prior to study entry. Baseline BMDs did not significantly differ between women who had and never had received CS prior to baseline. Median daily dose of CS over the study period was 2.08 (0, 9.38) mg/day. Median cumulative dose of CS during the study period was 1.52 (0, 6.85) gm. Mean SLAM-R score at baseline was 6.9 (± 4.4). Change in BMD was significant at the distal forearm, but not the spine or hip. Change in BMD at the distal forearm, spine or hip during follow-up was not correlated with CS cumulative intake or average daily dose. Change in BMD was not correlated with SLAM-R or ACR/SLICC-DI scores or traditional osteoporosis risk factors. BBM did not significantly change during the study period.
In this population of lupus patients with mild disease, disease duration of less than 2 years at the time of enrollment, and low exposure to CS, no significant decreases in BMD were observed. It is possible that the first sign of decline in BMD in this group of women with mild lupus may be loss of BMD at the distal forearm.

	Baseline BMD g/cm2	24 Month BMD g/cm2	Mean % Change	p-value
Hip (n=26)	0.946+/-0.168	0.944+/-0.176	-0.099+/-5.885	0.83
Spine (n=26)	1.031+/-0.148	1.035+/-0.137	0.761+/-4.988	0.63
Distal Forearm (n=26)	0.685+/-0.066	0.665+/-0.076	-2.9+/-6.592	0.03

Disclosures: ***N.M. Leong****, Amercian College of Rheumatology/REF 2; Arthritis Foundation Greater Chicago Chapter and Arthritis Founadation Clinical Science Grant 2; NIH/NIAMS 2; Proctor and Gamble 2, 5.*

M434

Contrasting Mammalian PTH Promoters: Identification of an NF-Y Binding Site Unique to the Human PTH Promoter. A. P. Alimov, M. C. Langub, H. H. Malluche, N. J. Koszewski. Division of Nephrology, Bone & Mineral Metabolism, University of Kentucky Medical Center, Lexington, KY, USA.

The identification of a highly conserved Sp1 DNA element in mammalian PTH promoters was recently reported. This element was primarily bound by the Sp3/Sp1 transcription factors present in parathyroid gland (PTG) nuclear extracts. However, an additional, novel DNA-binding complex was observed exclusively with the human PTH (hPTH) Sp1 element in mobility shift studies. Selective mutational analysis comparing the bovine and human PTH Sp1 elements revealed that the unknown PTG nuclear factor recognized a 'CAAT'-like sequence resulting from a single nucleotide change unique to the human sequence and contiguous to the highly conserved Sp1 recognition motif: CCGCCCAATGG (hum) vs. CCGCCCCATGG (bov/mouse; rat = G at this position). A consensus NF-Y element was able to specifically compete for formation of the novel complex, while an NF-1 sequence failed to displace it. Antiserum directed against the 'B' subunit of NF-Y resulted in the selective supershift of this PTG complex without disturbing binding by the Sp3/Sp1 complexes. Moreover, incubation of PTG nuclear extracts with a consensus NF-Y oligonucleotide probe resulted in formation of a strong, specific DNA-binding complex. Immunocytochemistry confirmed the nuclear localization of the NF-Y 'B' subunit in PTG cells. Transfection studies in opossum kidney (OK) cells using the hPTH promoter (-177 to +21) that included the Sp1/NF-Y binding sites exhibited vitamin D-dependent repression of reporter gene activity. Simultaneous mutation of both binding sites within the context of the natural promoter reduced basal activity by 63%. Mutation of just the Sp1 site produced a similar 64% reduction in basal promoter activity. However, mutation of the NF-Y binding site did not substantially alter basal or hormone-dependent activities compared to the wild-type element, although mobility shift experiments demonstrated that NF-Y was present in nuclear extracts prepared from OK cells and specifically interacted with the hPTH Sp1/NF-Y DNA element. Introduction of an expression vector for a dominant negative form of NF-Y into OK cells had no effect on basal hPTH promoter activity, but it did significantly impair the repression of reporter gene activity in response to calcitriol. In summary, a unique NF-Y DNA element is present in the hPTH promoter that is not observed in other mammalian PTH promoters. This element adjoins the previously characterized Sp1 binding site and may play a role in the repression of promoter activity by vitamin D.

Disclosures: ***N.J. Koszewski****, None.*

M435

Regulation of OPG and RANKL In Vitro by PTH (1-34) and PTH (1-31): What is the Best Model System? J. Glover*, F. Si*, L. J. Fraher, A. B. Hodsman, P. H. Watson. Medicine, University of Western Ontario, London, ON, Canada.

Receptor activator of NF-kappaB ligand (RANKL) and osteoprotegerin (OPG) are important factors in the regulation of osteoblast to osteoclast communication during bone remodelling. Parathyroid hormone (PTH) is a key regulator of bone remodelling and affects osteoblastic expression of these molecules. It is well known that clinical use of PTH (1-34) in daily intermittent doses is highly anabolic while continuous exposure is catabolic to bone. Recently, PTH (1-31) and its derivatives have also shown promise as selective bone anabolic agents. Understanding the time course, pathways and transducers involved in PTH analogue regulation of OPG and RANKL should aid us to better understand the actions of PTH. In these studies, UMR106, MC3T3-E1 and ROS 17/2.8 cells were cultured in the presence of PTH (1-34) or PTH (1-31) (0-100 nM) for 0-24 hr. Levels of RANKL protein were measured by ELISA or Western blot, and OPG protein was assayed by ELISA. Gene expression was assessed by RPA. In UMR106 cells exposed to PTH (1-34) or PTH (1-31) (0, 10, 50 or 100 nM) for 24hr, there was no significant difference in RANKL mRNA level. In these same cells, RANKL protein levels were significantly increased by 50 and 100 nM PTH (1-34) while there was a trend to decreasing RANKL protein in cells cultured in increasing concentrations of PTH (1-31) which was significant at 100 nM. Similar results were seen with ROS 17/2.8. In time-course experiments, the increase in RANKL induced by 50 nM PTH(1-34) was not noted until 8 hr of treatment and was significant after 24 hr of exposure. There was no detectable OPG mRNA or protein in either UMR106 or ROS17/2.8. In MC3T3-E1 cells cultured in the presence of 50 nM PTH (1-34), a significant increase in OPG protein was seen after 2 and 8 hr of treatment which dropped back to baseline at 24 hr. cAMP production in MC3T3-E1 was significantly enhanced in the presence of 50 nM PTH (1-34). RANKL was not detectable in samples from the MC3T3-E1 experiments.

Cell Type	RANKL Expression	OPG Expression
UMR106	++	-
ROS 17/2.8	++	-
MC3T3-E1	-	++

It is important to note that co-expression of OPG and RANKL was not detectable in UMR106, MC3T3-E1 or ROS 17/2.8 cells in culture suggesting that they are not suitable for studies of the effects of PTH on the OPG/RANKL system. While several studies have employed cell culture models with the missing components transfected in, these tell us little about the intrinsic regulation of OPG and RANKL by PTH analogues.

Disclosures: ***J. Glover****, None.*

M436

Parathyroid Hormone Stimulation of IL-18 in Osteoblastic Cells. L. J. Raggatt, L. Qin, N. C. Partridge. Physiology and Biophysics, Robert Wood Johnson Medical School, Piscataway, NJ, USA.

IL-18 was initially shown in bone to inhibit osteoclast formation. We have identified, using cDNA microarray technology, that IL-18 was dramatically upregulated in UMR 106-01 rat osteoblastic cells in response to parathyroid hormone (PTH) treatment. Confirmation of these data using real time RT-PCR showed that steady state levels of IL-18 mRNA increased by 1 h (1.9 fold), peaked by 4 h (21.2 fold) and had diminished after 12 h (4.4 fold). IL-18 protein, assessed by Western analysis of whole cell lysates, was regulated by PTH in a dose- and time- dependent manner via the PKA intracellular signaling pathway and did not involve the PKC pathway. Analysis of differentiating primary rat calvarial osteoblasts confirmed the regulation of IL-18 mRNA and protein by PTH and also showed that basal expression of IL-18 increased with osteoblastic differentiation. Northern blot analysis of mRNA from UMR 106-01 cells shows that PTH treatment increases steady state levels of the 1.1 kb transcript of IL-18, which may be controlled by the AP-1- and Cbfa1- containing promoter 1. Cycloheximide did not affect expression of IL-18 mRNA in response to PTH treatment, and PTH treatment did not change the degradation rate of IL-18 mRNA, suggesting that the increase in steady state mRNA levels was due to transcriptional activation of the gene. Our findings, that PTH stimulates IL-18, taken together with the known actions of IL-18 to inhibit osteoclastogenesis suggest that IL-18 may be involved in the anabolic actions of PTH in bone.

Disclosures: ***L.J. Raggatt****, None.*

M437

UNR Is a Member of the Protein-RNA Complex that Regulates PTH mRNA Stability in Response to Calcium and Phosphate. R. Kilav*[1], M. Dinur*[1], A. Sela-Brown*[1], H. Jacquemin-Sablon*[2], J. Silver[1], T. Naveh-Many[1]. [1]Minerva Center for Calcium and Bone Metabolism, Hadassah Hospital, Jerusalem, Israel, [2]Laboratoire de Pharmacologie des Agents Anticancéreux, Institut Bergonié, Bordeaux, France.

Calcium and phosphate regulate PTH gene expression post-transcriptionally through the binding of *trans* acting factors to a defined *cis* acting instability element in the PTH mRNA 3'-untranslated region (UTR). We have previously defined AUF1 as a member of the protein-binding complex that protects PTH mRNA from degradation. In hypocalcemia there is increased binding of the AUF1 containing complex to the PTH mRNA 3'-UTR that stabilizes the PTH mRNA leading to increased PTH mRNA and serum levels. Hypophosphatemia leads to opposite effects. Affinity purification using 3'-UTR PTH RNA identified additional proteins that bind the PTH mRNA 3'-UTR. We have now characterized the binding and stabilizing properties of one of these proteins, UNR. UNR (Upstream of N-ras), is a cytoplasmic RNA binding protein which has been characterized as an IRES trans acting factor for two picornaviruses. Recombinant UNR protein specifically interacted with full-length PTH mRNA or its 3'-UTR in RNA electrophoretic mobility shift assays. Addition of UNR antibodies to the binding reaction with parathyroid (PT) extracts led to a super-shift of the protein-RNA complex, showing that UNR was part of the PTH mRNA binding complex. Furthermore, GST-AUF1 pull-down experiments in the presence of either PT extracts or purified UNR proteins demonstrated that UNR and AUF1 interact directly and also in the absence of RNA. There is no PT cell line and therefore the in vivo role of UNR in stabilizing PTH mRNA was investigated by cotransfection experiments, using expression vectors for PTH and flag-UNR. In two cell lines, opossum kidney (OK) and HEK293, over-expression of flag-UNR (detected by Western blot) led to a stabilization of the full-length PTH mRNA plasmid but not of a truncated PTH mRNA that excluded the 63 nt protein-binding site. Similar results were obtained when UNR was cotransfected with a chimeric reporter expression plasmid for growth hormone that contained the PTH mRNA 3'-UTR 63 nt binding element. These results show that UNR stabilizes the PTH mRNA and that this effect depends upon the regulatory element located in the PTH mRNA 3'-UTR. By interacting specifically with this element, UNR as part of a protein complex, stabilizes PTH mRNA. Thus as AUF1, UNR stabilizes PTH mRNA: this represents a novel function for UNR. Altogether our results show that UNR, together with the other proteins in the RNA binding complex, determines changes in PTH mRNA levels and hence PTH synthesis and secretion in response to changes in serum calcium and phosphate.

Disclosures: ***T. Naveh-Many****, None.*

M438

A Regulated Cleavage Pattern of PTH mRNA that Determines mRNA Stability in Response to Hypocalcemia and Hypophosphatemia. O. Bell*, R. Kilav*, J. Silver, T. Naveh-Many. Minerva Center for Calcium and Bone Metabolism, Hadassah Hospital, Jerusalem, Israel.

PTH mRNA levels are increased in dietary induced hypocalcemia and decreased in hypophosphatemia. The effect of Ca^{2+} and phosphate (P) on PTH gene expression is post-transcriptional due to binding of trans acting factors to a cis acting instability element in the PTH mRNA 3'-UTR. The balance between protection by RNA-protein binding and degradation determines the half-life of the PTH mRNA. mRNA decay is a result of endonuclease and 5' or 3' exonuclease activity. We have now characterized the PTH mRNA degradatory mechanism. To identify degradation intermediates total parathyroid (PT) RNA from rats fed the different diets was run on urea acrylamide gels for enhanced separation of the RNA fragments. Hybridization identified full-length PTH mRNA (800 nt), which was increased by low Ca^{2+} and decreased by low P. In the low P rats the full-length PTH mRNA was very faint but interestingly there was a major transcript of ~300 nt. Hybridization with different fragments of the PTH cDNA showed that the 300 nt transcript consisted of <100 nt of the 3'-end of the PTH mRNA 3'-UTR and the poly A tail. This element is preserved when the PTH mRNA is degraded and may be due to either 5'-exonucleolytic cleavage that pauses at this point or an initial endonucleolytic cleavage followed by degradation of the 5'-portion of the mRNA or a combination of both. Addition of antisense RNA transcripts for the terminal 100 nt prevented the in vitro degradation of the full-length PTH transcript by PT proteins. This suggests that the antisense transcript blocked the recruitment of ribonucleases by the 3'-end of the PTH mRNA. We then in vitro transcribed RNA for the PTH mRNA 100 nt terminus, radiolabeled it at the 5' or 3' end and subjected it to in vitro degradation with PT cytosolic proteins. In both cases the label remained intact in the degradation intermediates indicating that the 5' and 3' ends were stable in this reaction. This suggests that endonucleolytic cleavage within the terminal 100 nt of the PTH mRNA is the first step of degradation. The 3'-end of the PTH mRNA includes the protein-binding region but also mediates degradation of the PTH mRNA. Paradoxically this end decays last when PTH mRNA is degraded. Our results suggest that protein-binding prevents PTH mRNA degradation, but in the absence of binding such as with low P the degradation machinery is recruited by this region to initiate cleavage at a more proximal site. This is immediately followed by complete degradation of the functional PTH mRNA and finally the 3' terminus itself.

Disclosures: ***T. Naveh-Many****, None.*

M439

Identification and Characterization of Two PTH-like Molecules in Zebrafish. R. C. Gensure[1], B. Ponugoti*[2], Y. Gunes*[1], M. Papasani*[2], M. Bastepe[1], D. A. Rubin[2], H. Jüppner[1]. [1]Endocrine Unit, Harvard University and Massachusetts General Hospital, Boston, MA, USA, [2]Department of Biological Sciences, Illinois State University, Normal, IL, USA.

Zebrafish have receptors homologous to the human PTH/PTHrP receptor (P1R) and PTH-2 receptor (P2R), and an additional receptor (P3R) which, despite high homology to P1R, responds more efficiently to human PTHrP (hPTHrP) than PTH (hPTH). Zebrafish ligands for these receptor have not been described. To find natural ligands for zP1R and zP3R, we searched the zebrafish genomic database for PTH homology and discovered two distinct regions that, when translated (zPTH1 and zPTH2), showed high homology to hPTH. We isolated cDNAs corresponding to each of these genomic regions, which verified that they are transcribed and allowed us to determine the intron/exon structure of both genes. Similar to mammalian and chicken PTH, each peptide contained a predicted signal sequence and two basic residues to allow cleavage of the pro-sequence. To determine if the zPTHs could activate PTH receptors, peptides consisting of the first 34 amino acids of the secreted zebrafish PTHs were synthesized and tested with human P1R (hP1R), zebrafish P1R (zP1R), and zebrafish P3R (zP3R) expressed in COS-7 cells. zPTH1(1-34) and zPTH2(1-34) activated hP1R with equal efficacy to hPTH(1-34) and hPTHrP(1-36). On the

other hand, zPTH2(1-34) showed ~30-fold lower potency at zP1R, compared to the other three peptides. zPTH1(1-34) activates zP3R with a similar potency to that of hPTHrP(1-36), and the potency of zPTH2(1-34) was only ~3-fold reduced. We next sought to determine if other fishes have multiple PTH-like peptides. Search of the genomic database of the Japanese pufferfish using zPTHs as probes led to the identification of zPTH1 and zPTH2 homologs, each with a putative intron/exon structure similar to those of other PTH genes. Phylogenetic analysis showed that zebrafish PTHs and putative pufferfish PTHs are more related to each other than to any of the known mammalian homologs. Thus, the evolution of two PTH-like peptides in teleosts appears to have occurred after the evolutionary split between fishes and mammals. Regarding the physiological significance of these peptides, the different receptor selectivity patterns of zPTH1 and zPTH2 suggests they may have different roles. Overall, the PTH endocrine system appears more complex in fish than in humans, providing evidence of continued evolution in non-tetrapod species. The multiple forms of fish PTH and their receptors provide additional tools for PTH structure-function studies.

Disclosures: ***R.C. Gensure****, None.*

M440

Influence of Amino-terminal PTH Assay Epitopes on Normal Parathyroid Function Measurements. A. Räkel*[1], L. Rousseau*[1], C. Albert*[1], J. H. Brossard[1], T. Cantor[2], P. Gao[2], P. D'Amour[1]. [1]Department of Medicine, Centre de recherche du CHUM, Hopital Saint-Luc, Montreal, PQ, Canada, [2]Scantibodies Laboratory Inc., Santee, CA, USA.

Using hPTH(1-84), hPTH(7-84) and [tyr^{34}] hPTH(19-84) standards, we have identified the epitope of 4 different amino-terminal PTH assays. We have then studied the influence of each epitope on the parathyroid function of 8 normal individuals. $CaCl_2$ and Na citrate infusions over 2 hr were performed and ionized calcium (Ca^{++}) and PTH values were measured every 5 to 15 min. Pools of serum obtained at various Ca^{++} concentrations were analyzed by HPLC. A four parameters mathematical model fitting the sigmoidal relationship between Ca^{++} and PTH concentrations was used to analyze data. Results are means ± SD. The cyclase activating (CA) PTH assay (Scantibodies Lab.) had its epitope in region 1-4, reacted only with hPTH(1-84), recognized a major peak of immunoreactivity coeluting with hPTH(1-84) (P1) and a slightly less hydrophobic minor peak (P2) believed to be [PO_4 Ser^{17}] hPTH(1-84). Basal (2.39±0.87 pmol/L), stimulated (7.37±1.65 pmol/L) and non suppressible PTH (0.52±0.18 pmol/L) values were the lowest with this assay ($p<0.05$). The Elecsys (E) PTH assay (Roche Diagnostic) had its epitope in region 26-32, reacted equally with all three standards, and recognized P1, P2, as well as the CA-PTH assay, and a large more hydrophylic region corresponding to non-(1-84) PTH (P3). It also gave the highest basal (3.98±1.13 pmol/L), stimulated (11.99±2.21 pmol/L) and non suppressible PTH (0.84±0.28 pmol/L) values ($p<0.001$). The total (T) PTH assay (Scantibodies Lab.) and the intact (I) PTH assay (Nichol's Institute) both reacted equally with hPTH(1-84) and hPTH(7-84) but [tyr^{34}] hPTH(19-84) was not reactive in the T-PTH assay and only about 30% as reactive as hPTH(1-84) in the I-PTH assay. P1 was identified by both assays while P2 was less reactive. The amount of P3 detected was identical in both assays. Epitope is believed to be 15-20 in the T-PTH assay and slightly more distal in the I-PTH assay. Both assays gave intermediary results compare to the other two. These results indicate that PTH epitope 1-4 reacts only with hPTH(1-84) molecular forms while more distal epitopes with PTH fragments as well. More molecular forms of PTH are recognized by the 26-32 than the 15-20 epitope explaining higher results with the E-PTH assay.

Disclosures: ***P. D'Amour****, Scantibodies Laboratory Inc. 5.*

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Osteopontin-Deficiency Induces Inhibitory and Enhances Stimulatory Parathyroid Hormone Actions on Cortical and Cancellous Bone Mass Respectively upon "Continuous" Administration of the Hormone I*n Vivo*. K. Kitahara[1], K. Tsuji[1], S. R. Rittling[2], M. Ishijima[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.

Intermittent parathyroid hormone (PTH) treatment has been proven to be an efficacious measure for osteoporosis. In spite of the clear PTH enhancement in cancellous bone mass, PTH effects on cortical bone mass may vary. However, molecules responsible for such diverse actions of PTH in cortical bone vs. cancellous bone are not fully understood. Recently, we observed that in the case of intermittent PTH administration, osteopontin (OPN)-deficiency induces PTH-dependent cortical bone formation. Continuous presence of PTH would be catabolic in cortical bone while it may be anabolic in cancellous bone. In this paper, we examined whether OPN-deficiency alters cortical bone response to *continuous* PTH administration. Continuous PTH treatment was conducted in wild type mice and OPN-deficient mice by subcutaneously implanted osmotic pumps infusing 80 μg/kg/day PTH (1-34) for four weeks. In wild type mice, continuous PTH treatment neither altered bone mineral density (BMD) of whole bone (femora and tibiae) nor changed cortical bone mass while it enhanced cancellous bone volume (approximately 2-fold). In contrast, OPN-deficiency induced reduction in whole bone BMD after continuous PTH treatment even though PTH-dependent increase in cancellous bone volume was further enhanced. Micro CT analysis revealed that cortical bone mass, cortical bone area and cortical bone perimeter were not altered upon continuous PTH treatment in wild type while they were all reduced in OPN-deficient mice. *In vitro* cell culture experiments using bone marrow cells obtained from mice after continuous PTH or vehicle treatment revealed no major alteration by OPN-deficiency with respect to PTH-induced enhancement of TRAP-positive cells developed in the presence of vit D and DEX. The levels of deoxypyridinoline excretion in urine and serum calcium were increased by continuous PTH treatment similarly in wild type and OPN-deficient mice. These observations indicated that the OPN-deficiency induced inhibitory effect on the cortical bone mass in the case of *continuous* PTH treatment was in contrast to its induction of a stimulatory effect on cortical bone mass upon intermittent PTH treatment. Stimulatory effects of both continuous and intermittent treatment on cancellous bone mass were enhanced by the OPN-deficiency. Thus, OPN acts as a negative and positive modulator of PTH actions in cortical and cancellous bone subjected to continuous treatment.

Disclosures: ***K. Kitahara****, None.*

M442

The Effects of hPTH(1-34) on Fracture Healing of Femoral Shaft in Rats. S. Komatsubara*[1], S. Mori[1], T. Mashiba[1], T. Akiyama*[1], K. Nonaka*[2], A. Seki*[3], K. Miyamoto*[1], J. Kawanishi*[1], Y. Cao*[1], Y. Kaji*[1], K. Iwata*[1], H. Norimatsu[1]. [1]Orthopedic surgery, Kagawa Medical University, Kagawa, Japan, [2]Healthcare Division, Elk Corporation, Tokyo, Japan, [3]Hamri Corporation, Tokyo, Japan.

The aim of this study was to investigate the effect of lower dose hPTH(1-34) on fracture healing in rat. Female SD rats (5 weeks old) were injected subcutaneously with either two doses of hPTH(1-34) (10 microg/kg and 30 microg/kg) or vehicle three times a week for 3 weeks, then bilateral femora was fractured and fixed with intramedullary wires. PTH treatment was stopped in pretreatment groups (P10, P30 groups); while the treatment was continued in continious treatment groups (C10 and C30 groups). Animals were sacrificed at 3, 6 and 12 weeks after surgery. Soft X-ray of all fracture was taken to evaluate fracture line. Bone mineral density in fracture callus was analyzed using DXA and pQCT. After densitometrical analysis, three-point bending mechanical test was performed. To derive the data of intrinsic material properties, such as ultimate stress, elastic modulus, toughness, we normalized structural mechanical properties by cross-sectional moment of inertia calculated by pQCT. Number of osteoclast was evaluated using TRAP (tartrate-resistant acid phosphatase) staining. In soft X-ray findings, there was no significant difference in fracture line disappearance ratio among the groups. Bone mineral density in fracture callus increased in C10 and C30 compared with other groups, which were not significantly different among CNT, P10 and P30 at each sacrifice. Ultimate load increased in C30 compared with CNT, P10 and P30 at 12 weeks after surgery. There were no significant differences in stiffness and work to failure among each group. Intrinsic material properties were not significant different among each group at each sacrifice. Number of osteoclast increased significantly in C30 compared to other group at 3, 6, 12 weeks. These results suggests that low dose hPTH(1-34) treatment could lead to accelerate mineralization in callus, and then increase the mechanical strength of fracture site.

Disclosures: ***S. Komatsubara****, None.*

M443

Comparison of Four Immunometric PTH Assay Results in Primary and Secondary Hyperparathyroidism. An HPLC Validation. J. Brossard[1], A. Räkel*[1], L. Rousseau*[1], C. Albert*[1], T. Cantor[2], P. Gao[2], P. D'Amour[1]. [1]Department of Medicine, Centre de recheche du CHUM, Hopital Saint-Luc, Montreal, PQ, Canada, [2]Scantibodies Laboratory Inc., Santee, CA, USA.

We have compared results obtained with four commercial PTH immunometric assays in 33 normal individuals (N), 15 patients with primary hyperparathyroidism (PHP), 21 patients with progressive renal failure (PRF) and 32 hemodialyzed (HD) patients. Each assay used a revelation antibody oriented against a different amino-terminal epitope of the PTH molecule. Sera from three individuals in each group were separated by HPLC and analyzed in all PTH assays. The cyclase-activating (CA) PTH assay (Scantibodies Lab. Inc.) recognized the region 1-4, the total (T) PTH (Scantibodies Lab. Inc.) the region 15-20, the intact (I) PTH (Nichol's Institute) a slightly more distal region and the Elecsys (E) PTH assay (Roche Diagnostic), the region 26-32 of hPTH(1-84). Serum ionized calcium, creatinine and four PTH measurements were obtained in each subject. Results are means ± SD and were compared using an ANOVA for repeated measures followed by a student-Newman-Keuls multiple comparisons test. HPLC analysis disclosed that the CA-PTH assay recognized two immunoreactive peaks, a major one comigrating with hPTH(1-84) (P1) and a minor one believed to be [PO_4-Ser^{17}] hPTH(1-84) (P2). T-PTH and I-PTH both had similar HPLC profiles and reacted with P1, much less with P2, and with a non-(1-84) PTH region (P3). The E-PTH assay recognized P1, P2 and P3. Thus, HPLC analysis explained why CA-PTH results were lowest in all populations, the assay reacting only with hPTH(1-84) molecular forms. It also suggested that E-PTH results should have been the highest values (as seen in normals), the E-PTH assay reacting with more PTH molecular forms than the others. But, I-PTH results were higher in HD and PRF possibly because of calibration differences or for other reasons not currently identified on HPLC profiles. Nonetheless, these results indicate an important role of amino-terminal epitopes in PTH immunometric assay results.

Parameters	N	PHP	PRF	HD
Ca^{2+}	1.22±0.03	1.47±0.04	1.15±0.10	1.14±0.11
Creat.	82±11	70±8	302±141	808±221
CA-PTH	3.0±1.2	6.7±2.9	7.4±3.9	21.7±22.5
T-PTH	3.6±1.5[b]	8.5±4.7[a]	9.7±5.5[b]	29.7±27.0[a]
I-PTH	3.6±1.6[b]	9.6±5.2[b]	10.3±6.0[b]	39.1±40.7[b]
E-PTH	4.1±1.5[b,c,e]	8.2±5.0[b]	9.6±5.7[b,d]	33.3±33.9[a,d]

CA-PTH vs others: a=p<0.01, b=p<0.001. T-PTH vs I- or E-PTH: c=p<0.001. I-PTH vs E-PTH: d=p<0.05, e=p<0.001.

Disclosures: ***J. Brossard****, None.*

M444

Identification and Characterization of the Zebrafish Gene Encoding Tuberoinfundibular Peptide of 39 Residues. M. R. Papasani*[1], R. C. Gensure[2], J. H. Postlethwait*[3], Y. Gunes*[2], B. Ponugoti*[1], H. Jüppner[2], D. A. Rubin[1]. [1]Biological Sciences, Illinois State University, Normal, IL, USA, [2]Endocrine Unit, Massachusetts General Hospital, Harvard University, Boston, MA, USA, [3]Institute of Neuroscience, University of Oregon, Eugene, OR, USA.

Zebrafish express three non-allelic parathyroid hormone (PTH)-like receptors. The PTH type-1 receptor (PTH1R) and type-3 receptor (PTH3R) show similar efficacies for zebrafish and human PTH and PTH-related peptide (PTHrP), although PTHrP has a higher efficiency at the PTH3R than PTH. Thus it appears that the zebrafish and human PTH1R have similar functional properties *in vitro*. Although the PTH type-2 receptor (PTH2R) has been isolated from mammals and zebrafish, only the mammalian ligands for this receptor (i.e. tuberoinfundibular peptide of 39 residues, TIP39) have been isolated and characterized. To determine if zebrafish TIP39 (zTIP39) functions similarly with the zebrafish and human PTH2Rs (zPTH2R and hPTH2R), and to determine its tissue-specific expression, zebrafish genomic databases were screened with a rat TIP39 sequence and a single zTIP39 was identified that showed significant homology to mammalian TIP39. Using standard molecular techniques, we isolated and translated zTIP39 cDNA sequences. The zTIP39 precursor is encoded by a gene comprising at least three exons. It contains a hydrophobic signal sequence and predicted pro-sequence with a dibasic cleavage site, similar to the mammalian TIP39 ligands. Preliminary phylogenetic analyses suggest that TIP39 forms the basal group from which PTH and PTHrP have been derived. Synthetic human TIP39 (hTIP39) and zTIP39 showed similarly high potencies with COS-7 cells transiently expressing the hPTH2R [EC_{50}: 0.11 ± 0.04 nM (hTIP39) and 0.31 ± 0.07 nM (zTIP39)] or zPTH2R [EC_{50}: 4.2 ± 0.3 nM (hTIP39) and 7.8 ± 2.0 nM (zTIP39)], whereas cells expressing the zPTH2R splice variant (zPTH2R#43) showed [EC_{50}: 3.8 ± 0.7 nM (hTIP39) and 1.3 ± 0.3 nM (zTIP39)]. Subtle differences in potency could, however, be discerned; the hPTH2R and zPTH2R were more efficiently stimulated by either human or zebrafish TIP39, while zTIP39 has a higher potency at the zPTH2R splice variant. *In situ* hybridization revealed a strong TIP39 expression in the hypothalamus and portions of the heart in 24h and 48h old embryos. Because zebrafish PTH2R expression was previously shown to be highest in brain and vasculature, these TIP39 studies suggest that there is a conservation of physiological roles for the TIP39-PTH2R endocrine/paracrine system between mammals and fishes. These may affect nociception and hemodynamics, or may have significant roles for cardiovascular and brain development.

Disclosures: ***M.R. Papasani****, None.*

M445

Performance of Different PTH Assays in the Clinical Diagnosis of Primary Hyperparathyroidism: A Preliminary Clinical Study. S. Ayad*[1], N. Parikh*[1], T. Eskridge*[1], E. R. Phillips*[1], S. Qiu[1], T. Cantor[2], P. Gao[2], D. S. Rao[1]. [1]Bone & Mineral Metabolism, Henry Ford Hospital, Detroit, MI, USA, [2]Scantibodies Lab, Inc, Santee, CA, USA.

There has been a growing number of "new & improved" commercially available PTH assays for clinical and/or research use. As part of our continuous quality improvement process, we had the opportunity to evaluate 7 different PTH assays and correlate with parathyroid adenoma weight, and to assess their usefulness as a clinical diagnostic tool.

Serum PTH was measured by 7 different methods in 39 patients with surgically verified primary hyperparathyroidism (PHPT) and 27 apparently healthy individuals. Four of the 7 assays were chemiluminescent (1 of 4 was "bioactive") and 3 were IRMA (1 of 3 was "bioactive") assays. Sample sizes for Pearson's correlation with adenoma weight were 39 for assays PTH1-5 and 35 for PTH 6 & 7. Sensitivity, specificity, and area under the curves (AUCs) were based on samples from 35 patients. The results are in the Table.

Assay #	PTH*	r=	p=	Sensitivity	Specificity	AUC
PTH1	135 ± 72	0.378	0.0176	74.3	96.3	0.910
PTH2	69 ± 37	0.330	0.0403	80.0	92.6	0.919
PTH3	126 ± 67	0.347	0.0305	80.0	92.6	0.913
PTH4	77 ± 39	0.452	0.0039	71.4	96.3	0.892
PTH5	69 ± 34	0.370	0.0224	71.4	88.9	0.837
PTH6	72 ± 43	0.448	0.0069	82.9	92.6	0.925
PTH7	84 ± 49	0.312	0.0679	74.3	92.6	0.878

* mean ± SD; r= Pearson's correlation coefficient with adenoma weight; p is for r values

All assays performed well in the clinical diagnosis of PHPT and correlated significantly with parathyroid adenoma weight. PTH1 & 3 had mean values significantly higher than the other 5 assays. There were no significant differences in correlation coefficients with adenoma weight between the assays, but the AUC for PTH5 was significantly lower than the others. The proportion of bioactive PTH by PTH6 assay was significantly higher in PHPT patients compared to controls (89 ± 33% Vs 77 ± 0.07%; p=0.03). In addition, this assay appears to perform the "best", but the AUCs (range 0.837 – 0.925) of all assays were "good to excellent" for clinical diagnostic purpose.

We conclude that for clinical diagnosis of PHPT all 7 PTH assays performed reasonably well. This is the first study to directly correlate multiple PTH assays with adenoma weight. Our results do not apply to patients with uremia in whom other circulating PTH fragments are present. Further validation studies of intact and bioactive PTH levels with adenoma weight in a larger number of patients with PHPT would be desirable.

Disclosures: ***S. Ayad****, None.*

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Parathyroid Hormone Rescues Disturbed Bone Growth Induced by Mutated FGFR3. K. Ueda*, Y. Yamanaka, E. Yamagami*, D. Harada*, Y. Seino, H. Tanaka. Pediatrics, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan.

Achondroplasia (ACH) and its severe type, thanatophoric dysplasia (TD), are caused by constitutively activated mutations in FGFR3. The excessively activated FGFR3 is known to inhibit the proliferation of chondrocytes, resulting in disturbing the growth of long bones. Recently, we have reported that an excessive activation of FGFR3 by the mutations accelerated differentiation and increased apoptosis of chondrocytes, and that exogenous addition or endogenously controlled expression of PTH or PTHrP rescued cells from these conditions. To explore further mechanisms and new therapeutic applications, we performed organ culture experiments.

Methods: By using an in vitro organ culture system and adenoviral vectors carrying FGFR3 mutation, K650E (Ad-TD) and wild type (Ad-WT), we examined bone growth and the influence of PTH on bone growth. **1.** The fetal mouse femurs which were dissected from ICR mice embryo at d.p.c.15 were infected by incubating with vehicle, Ad-WT or Ad-TD at MOI 18 for 2.5 days. After infection, they were transferred into serum-free medium. Before and after the 3d- and 6d-culture, we measured each longitudinal length and performed histological analysis on the infected femurs. **2.** We added PTH on Ad-WT infected femurs, and compared the length of femur cultured with PTH to femur length without PTH at 3day, and 6day after culture.

Results: **1.**There were significant differences ($p<0.05$) in an increase in bone length at 3d and 6d after culture between Ad-TD and Ad-WT infected femurs (32.9plus/minus 10.5 % at 3day and 66.9plus/minus 14.4% at 6day of the growth rate of the Ad-WT infected femur). Ad-TD infected femurs were significantly disturbed in longitudinal bone growth compared with Ad-WT infected ones. **2.** Axial growth rates of the Ad-WT infected femurs cultured with 10^{-8}M PTH were 1.2 times at 3day ($p=0.036$), 1.16 times at 6day ($p<0.01$) higher than those of the Ad-WT infected femurs without PTH.

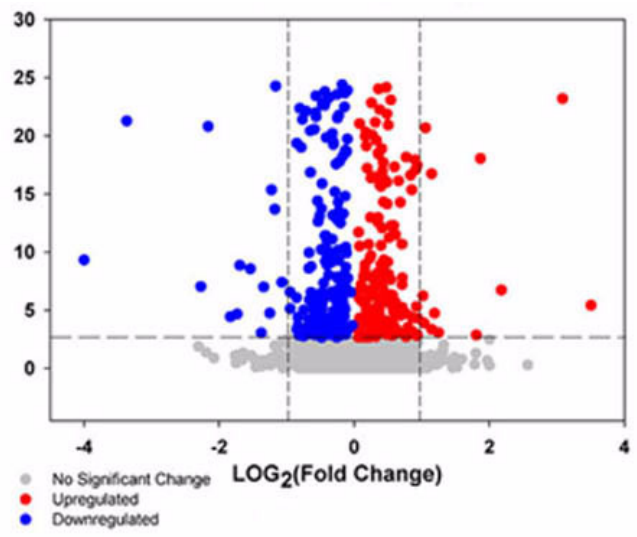
Conclusion: We confirmed that constitutive active FGFR3 mutation suppressed bone growth in organ culture experiments and that PTH partially rescued bone from the growth disturbance. Our results suggest that PTH may be effective for bone growth disturbance of chondrodysplasias such as ACH and TD.

Disclosures: ***K. Ueda****, None.*

M447

Human Osteoclast Response to PTH: Global Gene Expression and Phenotypic Profiling. M. Langub[1], L. Hao*[2], A. Stromberg*[2], H. Malluche[1], N. Koszewski[1]. [1]Internal Medicine, University of Kentucky, Lexington, KY, USA, [2]Statistics, University of Kentucky, Lexington, KY, USA.

Osteoclasts (OCs) are key to bone remodeling. We localized the type-1 PTH receptor (PTH-1R) in OCs of patients with renal osteodystrophy and found that in high-turnover bone, PTH-1R expression was elevated compared to controls and associated with increased erosion depth. To explore the significance of PTH-1R in OCs, we studied their response to PTH 1-84 using derived OCs from peripheral blood monocytic cells (PBMCs) devoid of osteoblastic support cells. PBMCs at ca. $5X10^6$/well were seeded onto coverslips or devitalized bone slice. After four hrs, non-adherent cells were removed and remaining cells given media (α-MEM; human serum; 25 ng/ml M-CSF; 10nM 1,25 vit. D; 10nM Dex; 50 ng/ml RANKL) twice weekly for 21 days. Cells were assessed for resorption pit formation and either stained for TRAP, PTH-1R, and VDR localization or processed for microarray analysis. Briefly, 6 hrs after PTH 1-84 [10 nM] treatment, RNA was harvested, in vitro transcribed, labeled into cRNA, and applied to Affymetrix® HG-U133 GeneChip® arrays and analyzed. After 21 days, >80% of the cells contained three or more nuclei, were TRAP+, PTH-1R+ and VDR+. mRNAs for PTH-1R and VDR as well as other OCs-related genes were confirmed by microarray. Microarray results indicated 8,094 transcripts present at least in one gene chip while 6,244 were absent in all gene chips. Out of the 8,094 genes, 298 genes (ca. 1.35%) were detected with high probability between treatment and control ($p<0.01$). Insightful Array Analysis showed increased expression of 141 genes (see volcano plot). Examples of significantly up-regulated genes include FAS ligand and RGS2, already known to be a PTH target in osteoblasts. In contrast, expression of 157 genes decreased after PTH-(1-84) treatment. One of the significantly down-regulated genes is the transcription factor RUNX2. Biological pathways analyses using GenMAPP software showed cross-relational links to various cellular functions including apoptosis, metabolic, gene transcription, and signal transduction pathways. In summary, these data confirms our in vivo observation using in situ hybridization and immunocytochemistry in patients that OCs express PTH-1R and VDR. The positive and negative effects on gene expression profiles by PTH 1-84 open a wide avenue for further studies.



Disclosures: ***M. Langub****, None.*

M448

Differential Effects of PTH 1-84 and PTH 53-84 on Intacellular Calcium Mobilization and 3H-Thymidine Incorporation in Vascular Endothelial Cells. K. Ding[1], Q. Zhong[1], C. M. Isales[2]. [1]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA, USA, [2]Medicine, Medical College of Georgia and the Augusta VA Hospital, Augusta, GA, USA.

We have previously reported that functional parathyroid hormone (1-84) receptors are present in vascular endothelial cells and that signal transduction events may vary depending on the concentration of parathyroid hormone utilized(1, 2). Although the amino-terminal fragment of PTH was long thought to be the bioactive portion of the intact molecule, it has become increasingly clear that carboxy-terminal PTH fragments are also bioactive. In an effort to define the portion of the PTH molecule responsible for the observed effects we utilized the PTH fragment 53-84. In osteoblastic cells, PTH 53-84 has been shown to stimulate type I procollagen, IGF binding protein 5, osteocalcin and alkaline phosphatase, actions consistent with an anabolic function. Using freshly isolated human umbilical vein endothelial cells (HUVEC) loaded with the calcium sensitive probe, Fura-2, changes in intracellular calcium could be detected at PTH 1-84 concentrations as low as 1 x 10-15M. As previously observed, the changes in intracellular calcium were biphasic with a dose-dependent increase in intracellular calcium observed up to a dose of PTH 1-84 of 1 x 10-11 M followed by a gradual drop in calcium concentration (increase in 340/380 ratio over baseline: PTH 1-84 10-15M: 0.254; 10-14M: 0.499; 10-13M: 0.755; 10-12: 0.854; 10-11M: 1.104; 10-10M: 0.828; 10-9M: 0.487; 10-8M: 0.387). Changes in intracellular calcium with PTH 53-84 were very similar to those of PTH 1-84 (increase in 340/380 ratio over baseline: PTH 53-8410-15M: 0.261; 10-14M: 0.502; 10-11M: 1.125). We next examined the effects on these peptides on 3H-thymidine incorporation in HUVEC. PTH 1-84: 10-14M: 171+5; 10-13M: 172+5; 10-12M: 146+0.9; 10-11M: 183+4.9; 10-10M: 186+8.8 % change over control+SEM. PTH 53-84: 10-14M: 163+4; 10-13M: 162+4.7; 10-12M: 146+4.2; 10-11M: 180+45.1; 10-10M: 141+2.3 % change over control+SEM. Taken together, these data suggest that the PTH fragment 53-84 is (or contains) the bioactive fragment responsible for the changes in intracellular calcium and thymidine incorporation in HUVEC stimulated with PTH 1-84.

1. C. M. Isales et al., Am J Physiol Endocrinol Metab 279, E654-62 (2000).
2. D. Throckmorton et al., Peptides 23, 79-85 (2002).

Disclosures: ***K. Ding****, None.*

M449

Anabolic Actions of PTH: Role of Early Events in Mineralization in a Novel Regeneration Model. G. J. Pettway*[1], A. J. Koh*[2], A. Schneider[2], E. Widjaja*[3], M. Morris*[3], L. K. McCauley[2]. [1]Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA, [2]Perio/Prev/Geriatrics, University of Michigan, Ann Arbor, MI, USA, [3]Chemistry, University of Michigan, Ann Arbor, MI, USA.

Skeletal responses to parathyroid hormone (PTH) may be anabolic or catabolic depending on the dosing regime. The catabolic actions of PTH are well accepted, whereas the mechanisms of the anabolic effects remain unclear. An innovative ectopic ossicle model was used to gain a better understanding of the anabolic actions of PTH. Ectopic ossicles containing cortical and trabecular bone with a hematopoietic marrow were generated from bone marrow stromal cells (BMSCs) implanted in athymic mice. Injections of PTH (1-34) (40μg/kg/d) or vehicle were initiated 1 wk after cell implantation and administered to mice for; 1 wk (group 1), 3 wks (group 2), and 7 wks (group 3). Alternatively, injections were initiated 12 wks after cell implantation, and PTH or veh administered for 3 wks (group 4). Microradiography and histomorphometry were used to qualitatively and quantitatively analyze the ossicles and endogenous vertebral bone. Raman spectroscopy was utilized to determine phosphate mineral content of ossicles from group 1. Northern blot analyses were also performed on total RNA from group 1 ossicles and calvaria of recipient mice to evaluate early events in PTH action. PTH had prominent anabolic actions in ossicles from mice in group 2 (trabecular bone: 37.9% ±5.8% (PTH) vs. 13.0% ±1.9% (vehicle), $p<0.01$). A longer time course of PTH (group 3) did not increase anabolic effects of PTH. Furthermore, PTH was not anabolic in ossicles that were more fully developed prior to initiation of PTH (group 4). Interestingly, vertebral trabecular bone was not significantly affected in any of the groups. These data suggest that anabolic effects of PTH are more pronounced in growing bone than in mature bone. PTH inhibited early mineralization of ossicles as evidenced by Raman spectroscopy of group 1 ossicles where the incidence of phosphate mineral was 100% in vehicle treated but only 25% in ossicles from PTH-treated mice. Gene expression studies confirmed the immaturity of bone in 1 wk ossicles since OCN mRNA levels were lower than in calvaria. In addition, matrix γ-carboxyglutamic acid protein (MGP) mRNA expression levels were upregulated by PTH confirming the mineralization inhibitory impact of PTH. These results indicate that PTH inhibits mineralization early in osseous development likely via the upregulation of mineralization inhibitors. This novel system suggests that regenerating bone is more responsive to the anabolic actions of PTH and that these actions may depend on early events that inhibit mineralization and later lead to increased bone mass.

Disclosures: ***A.J. Koh****, None.*

M450

Parathyroid Hormone and Forskolin Regulate RANKL mRNA Expression in UMR-106 Osteoblastic Cells Through a Cycloheximide-inhibitable Mechanism: Evidence for an Intermediate Factor. D. A. Dossing*, P. H. Stern. Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

Parathyroid hormone (PTH) is thought to exert its bone resorptive effects by inducing the expression of Receptor Activator of NF-kappaB Ligand (RANKL) in osteoblasts. RANKL has been shown to be necessary and sufficient for the maturation, activation and survival of osteoclasts. The signaling pathways and molecular mechanisms responsible for the induction of RANKL expression in osteoblasts following PTH treatment are not well understood. We find that PTH increases RANKL mRNA and RANKL protein in UMR-106 cells osteoblastic cells in a dose and time dependent manner. RANKL mRNA expression is not detected until the cells have been exposed to PTH for 8 hours. RANKL protein is significantly increased at 24 hours, with a maximum response to 10 nM PTH seen with a 48 hour exposure. The delayed responses, especially in mRNA, could indicate that the effect of PTH is indirect and possibly related to the initial synthesis of an intermediate protein. To determine whether the delayed induction of RANKL mRNA expression by PTH was due to a requirement for intermediate gene expression, the UMR-106 cells were pretreated with the protein synthesis inhibitor cycloheximide. Cycloheximide (100ng/ml – 15μg/ml) blocked the PTH- induced increase in RANKL mRNA expression, suggesting that the synthesis of an intermediate protein was required. Cycloheximide did not inhibit RANKL mRNA in control cells, and occasionally increased basal RANKL mRNA levels. There were no apparent morphological changes in the cycloheximide treated cultures and no effects on cell viability as shown by an MTT assay. An increase in cAMP/PKA has been shown to be a mediator of PTH effects on RANKL expression. Studies were therefore carried out to determine the effect of cycloheximide on this signaling pathway. Treatment of UMR-106 cells for 16 hours with 10μM forskolin, a stimulator of PKA, increased RANKL mRNA levels to a similar extent to that seen with a 16 hour exposure to 10nM PTH. Pretreatment with 1 μg/ml cycloheximide also inhibited the forskolin-induced expression of RANKL mRNA, indicating the requirement for the synthesis of an intermediate protein subsequent to the activation of the cAMP/PKA pathway by PTH.

Disclosures: ***D.A. Dossing****, None.*

M451

Acute Regulation of PTH by Dietary Phosphate Is Mediated by a Novel Signal Emanating from the Gastrointestinal Tract. D. R. Martin*, C. S. Ritter*, E. Slatopolsky, A. J. Brown. Renal Division, Washington University, Saint Louis, MO, USA.

Secondary hyperparathyroidism (2°HPT) due to chronic renal failure can be improved by dietary phosphate (Pi) restriction. In uremic rats with 2°HPT, a low Pi diet can restore CaR expression, correct the Ca set point, reduce PTH to normal and arrest parathyroid hyperplasia. Previous time course studies revealed that restoration of CaR was slow (7-14 days) while arrest of hyperplasia and correction of PTH were more rapid (2 days and 1 day, respectively) (Ritter CS et al, JBMR 17:2206-13, 2002). In the present studies, we further investigated the rapidity of the response of serum PTH to changes in dietary Pi. Uremic rats were trained to consume a high Pi diet (HPD = 0.9% P) during a 2-hour feeding period each morning. Feeding of a meal of low Pi diet (LPD = 0.2% P) reduced PTH by 80% by the end of the 2-hour feeding period and PTH levels remained at this minimum for the remaining 12 hours of the study. The LPD meal reduced plasma P by more than 1 mg/dl but did not alter total plasma Ca. To examine earlier time points, uremic rats adapted to the HPD were fasted overnight and gavaged with 2 ml of a 50% aqueous slurry of either the HPD or the LPD. The LPD gavage reduced PTH to a nadir of 40% of basal within 15 minutes, with a concomitant decrease in plasma P (-3.0 mg/dl) but no change in total plasma Ca. In contrast, HPD gavage increased PTH by 80% within 15 minutes with no change in plasma P or Ca, suggesting that the acute response was independent of changes in blood P. This is supported by the observation that infusion of uremic rats on the HPD with dextrose over a 2-hour period produced a decrement in plasma P similar to that seen with the LPD gavage (- 2.5 mg/dl), but had no effect on serum PTH or ionized Ca. Further studies showed that gavaging uremic rats adapted to the LPD with sodium Pi solution (250 mM) increased PTH within 10 minutes, but that this effect was abolished by ligating the pylorus. Introduction of sodium Pi directly into the duodenum via a catheter also increased PTH

within 10 minutes, with no change in ionized Ca despite a modest increase in plasma P. In contrast, duodenal infusion of an equimolar amount of sodium chloride had no effect on any of these parameters. These studies indicate that Pi can acutely regulate PTH within 10 minutes by a mechanism independent of changes in serum Pi and Ca. We propose that oral Pi elicits a signal emanating from the small intestine that stimulates PTH release by the parathyroid glands. This novel regulatory mechanism may provide a new pharmacologic target for the treatment of 2°HPT in renal failure patients.

Disclosures: ***A.J. Brown****, None.*

M452

The Characterization of Nuclear Transport of the Vitamin D Receptor Using In Vitro Transport Assay and Yeast Two-Hybrid System. Y. Miyauchi*[1], T. Michigami[1], T. Sekimoto*[2], Y. Yoneda*[2], K. Ozono[3]. [1]Department of Environmental Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan, [2]Department of Frontier Biosciences, Osaka University Graduate School of Frontier Biosciences, Osaka, Japan, [3]Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan.

The vitamin D receptor (VDR) belongs to the nuclear receptor superfamily and functions as a ligand-dependent transcription factor. The VDR must be located in nucleus to access its target genes. The recent advances in understanding of the nuclear transport of proteins are remarkable. In brief, cargos containing nuclear localization signal are transported by carrier proteins including importin alpha, beta or beta-like factors via nuclear pore complex. In the present study, we have attempted to characterize the nuclear transport of the VDR by an in vitro transport assay. This assay using semi-intact cells is an well-established and extremely useful strategy. The semi-intact cells prepared by digitonin treatment have permeable plasma membrane and intact nuclei. Then the nuclear transport can be reconstructed in vitro by exogenous additives, such as recombinant proteins or cytoplasmic extract. In this assay, the recombinant VDR entered nuclei in the presence of cytosol obtained from HeLa cells. It was of interest that the VDR could be transported to nuclei in the absence of ligand and that N-terminal fragment of the VDR lacking ligand binding domain entered nuclei in the same way. To further investigate the molecular mechanism of the step, we have explored the interactive factors which potentially facilitate the nuclear transport of the VDR with the yeast two-hybrid approach where the N-terminal fragment of the VDR was utilized as the bait. In the screen, we have found that a member of the importin beta-like family, importin 4, interacts with the VDR. The GST pull-down assays demonstrated that the interaction was carried out between the DNA binding domain of the VDR and the C-terminal region of importin 4. In conclusions, 1) importin 4 is the first molecule shown to bind to the VDR in the members of carrier proteins involving in nuclear transport, and 2) its role in the nuclear transport of the VDR can be examined in the in vitro transport assay.

Disclosures: ***Y. Miyauchi****, None.*

M453

Opposing Regulation of 1,25-(OH)2D3 Receptor Transcription by Extracellular Calcium and Parathyroid Hormone Signaling in Human Proximal Kidney (HK-2) Epithelial Cells. M. J. Beckman[1], R. L. Horst[2], S. Christakos[3]. [1]Biochemistry, Virginia Commonwealth University, Richmond, VA, USA, [2]Natl. Animal Disease Center, Ames, IA, USA, [3]Biochemistry, UMDNJ-New Jersey Medical School, Newark, NJ, USA.

Production of 1,25(OH)2D3 by 1alpha-hydroxylase involves a PTH signaling action on 1alpha-hydroxylase promoter and an effect of PTH to decrease the stability of the 24-hydroxylase transcript, thus reducing the potential for catalysis of newly synthesized 1,25(OH)2D3. This study examined the regulation of 1,25(OH)2D3-receptor (VDR) by PTH and Ca in human renal proximal epithelial (HK-2) cells. These cells were previously shown to be PTH responsive and express VDR. The cells were cultured and passaged in DMEM/F12 (50:50) medium containing 10% FBS. Experiments were started by switching to synthetic Keratinocyte serum free medium with or without Ca, and followed by treatment with either vehicle or PTH 1-34 (100 ng/ml). Real-Time RT-PCR using a specific TaqMan probe quantified VDR transcript. The initial effect of PTH showed a slight (10%) decrease in VDR transcript. In contrast, low Ca medium decreased VDR transcript by 20%, and the combination of low Ca and PTH treatment decreased VDR transcript below 50% of control. The PTH-mediated down-regulation of VDR transcript in the low Ca condition was confirmed to be dose-responsive, with the maximal effect reaching an 80% decrease in VDR transcript at 200 ng/ml of PTH 1-34. Treatment of HK-2 cells with either cAMP or PMA lead to decreased VDR transcript only with cAMP, indicating that the PTH regulation at the level of mRNA was specific to G-protein coupled adenylate cyclase activity. By comparison, both PTH and dibutyryl cAMP increased VDR transcript in fetal osteoblast cells, suggesting that the effect of PTH on VDR regulation was specific to the proximal kidney cell type. A VDR promoter-luciferase construct was transfected into HK-2 cells. In low Ca medium, PTH reduced basal promoter activity consistent with the mRNA data; however, graded increases of Ca to a concentration of approximately 4 mg/dL or higher in the medium could completely block the regulation of VDR by PTH. Blockade of the PKA pathway by the synthetic peptide, St-Ht31, abolished the PTH-mediated decrease in VDR mRNA and the PTH-mediated decrease in basal luciferase activity. This study demonstrates opposing regulation of VDR transcription that involves down-regulation by PTH, and a blockade of the down-regulation by normal or elevated Ca concentration in the culture medium. The PTH effect appeared to be elicited through its receptor-G(alpha)s coupling to adenylate cyclase, whereas, the Ca effect may work by an opposing Gqi coupling to the extracellular Ca sensing receptor on these cells.

Disclosures: ***M.J. Beckman****, None.*

M454

Activation of Mutant Vitamin D Receptors from Patients with HVDRR by Phosphorylation. Y. Liu*[1], P. Malloy*[2], E. Soliman*[1], M. Link*[1], D. Feldman[2], S. Christakos[1]. [1]Dept. of Biochemistry, UMDNJ-New Jersey Med. School & GSBS, Newark, NJ, USA, [2]Dept. of Medicine, Stanford University School of Medicine, Stanford, CA, USA.

Hereditary 1,25dihydroxyvitamin D ($1,25(OH)_2D_3$) resistant rickets (HVDRR) is a rare genetic disorder caused by inactivating mutations in the vitamin D receptor (VDR). In this study we examined the VDR from patients with HVDRR with mutations in the ligand binding domain (LBD) (F251C, I268T, H305Q, E420K) that affect coactivator binding and/or ligand binding. Since inhibition of phosphatase has been reported to enhance VDR mediated transcription, at least in part by altering coactivator interaction, we examined a possible role of phosphorylation in restoring transcriptional activity of the mutant VDRs. Mutant VDRs (H305Q, F251C, I268T) activated 24(OH)ase gene transcription 2-3 fold at 10^{-8}M $1,25(OH)_2D_3$ compared to 25 fold for wild type VDR. The E420K mutant, which prevents coactivator binding, was unresponsive even at high concentrations of $1,25(OH)_2D_3$. The transcriptional response of H305Q, F251C and I268T mutants was enhanced by cotreatment of VDR mutant transfected COS-7 cells with $1,25(OH)_2D_3$ and okadaic acid (OA, inhibitor of phosphatase; 50nM). There was a 2–3 fold enhancement of $1,25(OH)_2D_3$ induced 24(OH)ase transcription in experiments using mutants I268T and F251C and a 7-fold enhancement in experiments using mutant H305Q. The E420K mutant was unresponsive to $1,25(OH)_2D_3$ in the presence or absence of OA. The responsiveness of the mutants I268T, F251C and H305Q to enhanced activation in the presence of OA correlated to increased interaction between DRIP and mutant VDR, providing a mechanistic link between activation of the mutant and increased VDR-DRIP interaction. Interaction between DRIP and E420K was not observed using increasing concentrations of $1,25(OH)_2D_3$ (10^{-9} – 10^{-7}M) in the presence or absence of OA. Treatment of COS-7 cells transfected with I268T, F251C or H305Q with hexafluoro $1,25(OH)_2D_3$ analogs (RO-26-2198 and RO-4383561), that are more potent than $1,25(OH)_2D_3$ resulted in at least partial rescue of the transcriptional responsiveness of the mutant VDRs and increased interaction between the mutant VDR and DRIP205. Mutant E420K was unresponsive to the analogs even at high concentrations. Thus both phosphorylation and hexafluoro analogs can enhance the function of the mutant VDRs at least in part by increasing interaction between mutant VDR and DRIP205. In summary we show for the first time that transcriptional activity of VDR from patients with HVDRR can be enhanced by phosphorylation and that at least partial rescue of transcriptional activity is correlated with enhanced coactivator binding.

Disclosures: ***Y. Liu****, None.*

M455

NCoA62/SKIP, a Vitamin D Receptor Coactivator That May Couple Transcription Regulation To RNA Splicing and the Splicesome. C. Zhang, D. R. Dowd*, C. Gu*, P. N. MacDonald. Department of Pharmacology, Case Western Reserve University, Cleveland, OH, USA.

NCoA62/SKIP was co-discovered as a nuclear protein that interacts with the vitamin D receptor (VDR) and the SKI oncoprotein. NCoA62/SKIP expresses properties consistent with other nuclear receptor transcriptional coactivator proteins, such as those in the steroid receptor coactivator (SRC) protein family. For example, NCoA62/SKIP interacts selectively with the VDR-RXR heterodimer, it forms a ternary complex with liganded VDR and SRC proteins, and it synergizes with SRCs to augment 1,25-dihydroxyvitamin D3 [1,25(OH)2D3]- and VDR-activated transcription. Chromatin immunoprecipitation studies examining the rat 24-hydroxylase promoter in ROS17/2.8 cells clearly indicated that native NCoA62/SKIP associates with the VDREs in this promoter and it enters the promoter complexes after VDR and SRC entry. These data suggest that NCoA62/SKIP functions at a distal step in the transactivation process, although the mechanisms are unknown. To explore potential mechanisms, HeLa cell nuclear proteins were incubated with GST-NCoA62/SKIP, protein complexes were purified on glutathione-agarose, and proteins were identified following SDS-PAGE and mass spectrometry. Ten individual human proteins have been identified thus far which represent components of the spliceosome machinery as well as putative nuclear matrix components. These data support our observation of NCoA62/SKIP targeting to the nuclear matrix and previous studies by others showing NCoA62/SKIP in isolated spliceosome complexes. However, a functional role for NCoA62/SKIP in RNA splicing has not been reported. To test this role, a dominant negative inhibitor of NCoA62/SKIP (dnNCoA62/SKIP) was expressed together with a vitamin D responsive growth hormone (GH) mini gene cassette in COS7 cells. A selective, 1,25(OH)2D3-dependent accumulation of unspliced GH transcripts was observed in cells expressing dnNCoA62/SKIP. Such RNA splicing defects were not observed in cells expressing a dnSRC or an inactive dnNCoA62/SKIP derivative. These are the first studies indicating a functional role for NCoA62/SKIP in RNA splicing. Collectively, these data show that NCoA62/SKIP expresses properties consistent with those of NR coactivators and with spliceosome components, thus suggesting a pivotal role for NCoA62/SKIP in coupling transcriptional regulation by VDR to RNA splicing. They further solidify an important role for VDR/NR-interactors downstream of the transcription process in determining the overall response of vitamin D and steroid hormone regulated genes.

Disclosures: ***C. Zhang****, None.*

M456

Regulation of Vitamin D Receptor (VDR) Function by Mitogen Activated Protein Kinase (MAPK) Is Cell Type Specific. R. Narayanan*, N. L. Weigel. Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA.

The active form of vitamin D, 1, 25 (OH)2D3 (1,25D), acts through an intracellular vitamin D receptor (VDR) to alter transcription of target genes as well as through a less well characterized membrane receptor that induces activation of MAPK. Earlier studies had shown that activation of the MAPK pathway in Ras transformed keratinocytes led to an inhibition of the VDR activity. However, in our studies of the role of the MAPK pathway in VDR function and activated MAPK by 1,25D, we find that the response is cell line specific.. Inhibition of the activation of MAPK by treatment with a MEK inhibitor, U0126, increased the endogenous VDR levels in MG-63 and MC3T3 human osteoblastic cells as well as in HeLa (human epithelial cervix carcinoma) cells. However, treatment with U0126 reduced the ligand dependent transactivation of endogenous VDR in MG-63 or HeLa cells as well as the activity of VDR transfected into COS cells. However, treatment with U0126 increased the VDR dependent transactivation in MC3T3 cells. This cell type specific difference in VDR transactivation was also observed with an endogenous target, Alkaline Phosphatase (ALP). U0126 blocked the 1,25D induced ALP activity in MG-63 cells whereas it increased activity in MC3T3 cells. Consistent with these findings, activation of the MAPK pathway by constitutively active Raf-1 or MEKK1 increased the VDR activity in MG-63 and HeLa cells but inhibited it in MC3T3 cells. Because MC3T3 are less differentiated than MG-63 osteoblastic cells, we asked whether differentiation status played a role. We differentiated the MC3T3 cells in the presence of ascorbic acid and β-glycerophosphate for 28 days, but found that subsequent treatment with U0126 still led to an increase in the 1,25D induced ALP activity. U0126 inhibited the activity of progesterone receptor transiently transfected into either MC3T3 or Hela cells indicating that the differential response was not common to all nuclear receptors. Since earlier studies attributed the resistance to 1,25D induced growth inhibition to down regulation of RXRα, we evaluated the levels of RXRα in different cells. Treatment of MC3T3 cells but not MG-63 or HeLa cells with 1,25D led to the down regulation of RXRα and this was partially reversed by U0126 treatment. Moreover, initial studies show that treatment with MG132, a proteasome inhibitor, increased the ligand dependent activity of VDR with a concomitant stabilization of RXRα in MC3T3 cells, but activity was unaffected in HeLa cells. Thus, the 1,25D-induced down-regulation of RXRα limits activity of VDR in MC3T3; this can be blocked by inhibition of the MAPK pathway or of proteasome activity resulting in enhanced activity of VDR.

Disclosures: ***R. Narayanan****, None.*

M457

Glucocorticoid Induces Low Turnover Osteoporosis in Growing Minipigs. S. Akahoshi*[1], S. Ikeda*[1], Y. Morishita*[2], H. Tsutsumi*[3], M. Ito*[4], A. Shiraishi*[2], S. Arita*[1], M. Nagashima*[1], A. Sakai[1], T. Nakamura[1]. [1]Orthopaedic Surgery, Department of Orthopaedic Surgery School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan, [2]Chugai Pharmaceutical Co., Ltd., Tokyo, Japan, [3]Chugai Research Institute for Medical Science, Inc, Nagano, Japan, [4]Department of Radiology Nagasaki University School of Medicine, Nagasaki, Japan.

The purpose of the present experiment is to evaluate osteoporotic changes induced by glucocorticoids in young Gottingen minipigs, an otherwise often used animal model for human disease.

Thirty Göttingen minipigs were respectively assigned to 6 experimental groups (n=5/each group) at the age of 8 months: Baseline control group (BC), Vehicle control group (VC), Glucocorticoid group (GC) , and three groups as treatment groups : GC+alfacarcidol(alf), GC+alendronate(ALN) and GC+alf+ALN.

The minipigs in Group GC were administrated with predonisolone at dose of 0.5 mg/kg bw 5 times a week for 6 months. The minipigs in treatment groups were administrated with alfacarcidol at dose of 0.1É g/kg bw and/or alendronate at dose of 0.1mg/kg bw 5 times a week for 6 months.

Body weight, serum Ca, urine Ca, and bone metabolic markers, such as serum bone alkaline phosphatase (BAP), serum osteocalcin (OC), and urine NTX, were measured at 0, 4, 12, and 24 weeks after the start of the experiment. The minipigs were sacrificed in Group BC at day 0 and in other groups at 24 weeks. BMC and BMD were measured by DXA and pQCT. Three-dimensional microarchitecture was evaluated by micro-CT. Ultimate compressive load was obtained by the materials-testing machine. Specimens of lumber body were assessed with histomorphometric analysis.

The values of serum BAP, serum OC and urine NTX in Group GC significantly decreased compared with those in Group VC. Histomorphometric analysis revealed that bone formation rate (BFR/BS) decreased in Group GC. DXA and pQCT showed that BMD value of lumber vertebra significantly decreased in Group GC. In the results of micro CT, BV/TV and Tb.Th decreased, and SMI increased in Group GC compared with those in Group VC. The values of ultimate load and maximum energy in Group GC significantly reduced compared to Group VC. Bone density and bone strength in treatment groups were similar values in Group GC.

In conclusion, glucocorticoid induces low turnover osteoporosis, leading to deterioration of cancellous bone and reduction of bone strength, in growing Göttingen minipigs. The administration of vitamin D and/or alendoronate didn't prevent the reduction of bone density and bone strength in osteoporosis induced by glucocorticoid in young minipig.

Disclosures: ***S. Akahoshi****, None.*

M458

Pleiotrophin and N-syndecan are Upregulated During Osteoblast Differentiation and Down Regulated by Glucocorticoids. C. A. Ohlsson*[1], A. Bäckman*[2], U. H. Lerner[3], M. Lorentzon[4]. [1]Oral Cell Biology/Sports Medicine Unit and Center for Bone Research at the Sahlgrenska Academy (CBS), Umeå/Göteborg, Sweden, [2]Sports Medicine Unit, Umeå, Sweden, [3]Oral Cell Biology, Umeå, Sweden, [4]Center for Bone Research at the Sahlgrenska Academy (CBS), Göteborg, Sweden.

Glucocorticoids are known to have deleterious effects on bone and calcium metabolism, including stimulation of bone resorption, inhibition of osteoblasts, secondary hyperparathyroidism and calcium loss via reduced intestinal absorption and increased renal excretion. Pleiotrophin (PTN), acting trough its receptor N-syndecan, is an extracellular matrix-associated protein, believed to play an important role in osteoblast recruitment and attachment. Transgenic mice overexpressing PTN develop increased bone thickness.

We investigated effects of the glucocorticoid dexamethasone (DEX) on mRNA expression in mouse primary osteoblasts and in mouse calvarial explants. Mineralization in mouse primary osteoblasts was studied in control cultures and in cultures treated with PTN, PTN + DEX or DEX alone.

Osteoblast-like cells, isolated from mice calvarial bones were cultured for 4, 8, 12, 16, 20 and 24 days and exposed to DEX (10 –7M), with or without the glucocorticoid receptor antagonist RU 38486 (10-6M). Gene expression was measured by cDNA microarray and Quantitative RealTime PCR (QRT PCR). Calcium concentration in bone noduli was determined using atomic absorption spectrometry. Calvarial explants were cultured for 24h, with or without test substances.

Results: Using cDNA array, approximately 50 genes were found to be regulated by DEX, of which PTN and N-syndecan were most highly downregulated (4-6x). QRT PCR showed that, in both calvarial osteoblast cultures and bone explant cultures, DEX decreased, concentration- and time-dependently, PTN and N-syndecan expression. The effects were inhibited by RU 38486.

Mineralization in mouse osteoblast cultures increased over time. PTN and N-syndecan mRNA expression was elevated during osteoblastic differentiation/mineralization, in parallel to increased ALP mRNA expression. Interestingly, mineralization was enhanced by addition of PTN to the media, an effect inhibited by DEX.

Conclusions: The downregulation of PTN and N-syndecan gene expression following DEX treatment in both mice primary osteoblasts and mice calvarial bones, together with the observed stimulatory effect of PTN on bone mineralization, suggest a role for PTN and N-syndecan in osteoblastic differentiation/mineralization and glucocorticoid-induced bone loss.

Disclosures: ***C.A. Ohlsson****, None.*

M459

A Histomorphometric Study of the Effects of Long-term Glucocorticoid Treatment on Cortical Bone Structure. S. Vedi*[1], S. L. Elkin*[2], J. E. Compston[1]. [1]Medicine, University of Cambridge, UK, Cambridge, United Kingdom, [2]Cystic Fibrosis, Royal Brompton Hospital, London, United Kingdom.

Administration of glucocorticoids for the treatment of a variety of disorders is associated with increased bone loss and fracture risk. The effects of glucocorticoids on cancellous bone remodelling and structure are reasonably well documented but there are no reported histomorphometric studies in human cortical bone. The aim of this study was to investigate the effect of long-term glucocorticoids on iliac crest cortical bone in 18 patients, 12 female, aged 27-79 years (mean 41.7years) and 6 males, aged 18-48 years (mean 30.7 years). Results were compared to those obtained in a group of premenopausal women with untreated endometriosis aged 23-40 years (mean 31.1). Cortical bone structure was assessed by image analysis. Cortical porosity was expressed as the percentage of total cortical area occupied by Haversian systems and the number and density of Haversian systems were also assessed.

Cortical width and area were similar in the two groups. However, cortical porosity was significantly higher in patients treated with glucocorticoids (10.5 ± 11.8 % ; mean ± SD) compared with the untreated group (5.1 ± 3.9 %; p=0.006) and the Haversian canal number was also significantly higher in glucocorticoid treated patients (46.1 ± 23.7 vs 27.8 ± 18.6 respectively; p=0.0003). Haversian canal density was significantly greater in the treated patients compared to the untreated group (16.6 ± 13.0 vs 6.6 ± 3.5 /mm^2; respectively p=0.00002), but Haversian canal area did not differ significantly between groups (0.33 ± 0.29 vs 0.26 ± 0.24 μm^2 respectively; p=0.07)

These results demonstrate that cortical porosity is increased in patients treated with long-term glucocorticoid therapy, due to an increase in the number rather than size of Haversian canals. This may reflect a combination of increased activation frequency early in the course of glucocorticoid therapy together with long-term impairment of bone formation Effects on cortical width were not demonstrated, possibly as a result of the relatively small sample size.

M460

Evidence for a Feedback Mechanism in the Control of Glucocorticoid Action in Human Osteoblasts. M. Eijken*[1], M. S. Cooper*[2], M. Hewison*[2], H. Chiba*[3], A. Hagendorf*[1], J. W. Koper*[1], H. A. P. Pols*[1], J. P. T. van Leeuwen[1]. [1]Internal Medicine, ErasmusMC, Rotterdam, Netherlands, [2]Division of Medical Science, University of Birmingham, Birmingham, United Kingdom, [3]Pathology, Sapporo Medical University School of Medicine, Sapporo, Japan.

Glucocorticoids have profound effects on bone and when administered in pharmacological doses cause osteoporosis, mainly by suppressing bone formation. Paradoxically, gluco-

corticoids are crucial for human osteoblast differentiation. Recent data show that glucocorticoid effects on bone are regulated by autocrine actions of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD type 1 primarily displays reductase activity and converts cortisone into active cortisol, while 11β-HSD type 2 converts cortisol into cortisone. We studied glucocorticoid metabolism and glucocorticoid receptor (GRα and GRβ) expression in relation to osteoblast differentiation and mineralization. For this we used human osteoblasts (SV-HFO) for which glucocorticoids are essential for differentiation and mineralization. Real-time PCR was used to quantify mRNA expression. Cortisol levels were measured by an immunoassay.
In the absence of DEX but not in the presence of DEX (10^{-8}–10^{-6} M) 11β-HSD1 expression strongly increased during differentiation starting around day 12. This resulted in a 12-fold higher expression at day 19 compared to the expression in DEX-treated cells. Interestingly, this increase coincides with the start of mineralization in DEX-treated cultures. 11β-HSD2 mRNA expression was not affected by DEX. Cortisol measurements showed that human osteoblasts can produce cortisol. After incubation of osteoblasts at day 19 for 24h with 1 μM cortisone significant amounts (100-200 nM) cortisol were measured in the culture medium. These amounts are sufficient to induce differentiation/mineralization. These measurements confirmed that increased 11β-HSD1 mRNA is reflected in increased enzyme activity: 141±17 and 722±40 pmol/mg/24h in DEX-treated and non-treated cells, respectively. Receptor analysis showed GRα mRNA expression to be about 1.5 fold higher in the absence of DEX. GRβ mRNA could not be detected.
In conclusion, the current study shows that human osteoblasts activate several means to secure glucocorticoid pathway-mediated stimulation of differentiation/mineralization. Moreover, this study demonstrates that in osteoblasts an autocrine feedback mechanism exists to control glucocorticoid levels and activity. This feedback mechanism is dependent on osteoblast differentiation and is most prominent during mineralization. Thereby these data provide new insights into the versatile and paradoxical actions of glucocorticoids in bone.

Disclosures: ***M. Eijken****, None.*

M461

Role of the 1,25(OH)2D3 Membrane Associated, Rapid Response Steroid (1,25D3-MARRS) Binding Protein in Hormone Stimulated Phosphate Movement in Intestinal Epithelial Cells. I. Nemere[1], M. C. Farach-Carson[2], S. Safford*[3], B. Rohe*[2]. [1]Nutrition and Food Sciences, Utah State University, Logan, UT, USA, [2]Biological Sciences, University of Delaware, Newark, DE, USA, [3]Biology, Lincoln University, Lincoln, PA, USA.

We have reported (1) that isolated chicken intestinal cells respond rapidly to $1,25(OH)_2D_3$ with enhanced ^{32}P uptake, but the response is abolished in mature animals. We have confirmed that a similar age-dependent effect occurs for hormone stimulated phosphate transport in the perfused duodenal loop system. In 7-wk animals, control preparations yielded a transport level of 1.02 ± 0.02 (treated/av basal) at T= 40 min, while 130 pM $1,25(OH)_2D_3$ enhanced transport to 1.87 ± 0.28 at T= 40 min. In 14- and 28-wk animals, steroid hormone responsiveness was abolished. Reduced responsiveness to hormone was accompanied by decreased levels of $1,25D_3$-MARRS protein, as judged by Western analyses (1), as well as reduced mRNA levels, as judged by RT-PCR. Ratios of $1,25D_3$-MARRS/GAPDH were 1.28 ± 0.07 in 7-wk birds, dropping to 0.91 in 14-wk birds and 0.95 ± 0.07 in 58-wk birds. The isolated chick intestinal cell system was therefore judged to be suitable for studies on ribozyme ablation of the $1,25D_3$-MARRS protein. For these experiments, isolated intestinal cells were seeded into 35-mm plastic petri dishes with RPMI in the absence of FBS. The following morning, adherent cells were either untransfected, or transfected by lipofectamine with either MARRS ribo6 or the control plasmid, MARRS ribo8 containing a scramble nucleotide sequence in the substrate recognition site. The cells were cultured for an additional 22 h prior to analyses. Phosphate uptake was allowed to proceed for 7 min in vehicle controls (0.01% ethanol, final concentration) or cells exposed to 130 pM steroid. Values (cpm/μg protein) were 162 ± 20 and 270 ± 29 (Con 0 and D 0, respectively), 120 ± 13 and 102 ± 17 (Con ribo6 and D ribo6, respectively), and 146 ± 18 and 242 ± 34 (Con ribo8 and D ribo8, respectively). Specific $[^3H]1,25(OH)_2D_3$ binding was measured in the same cells by the perchloric acid precipitation procedure, which does not detect the nuclear receptor. Specific binding (fmols/mg protein) was observed to be 489 ± 86, 178 ± 30, and 409 ± 62 in untreated, ribo6-, and ribo8-treated cells, respectively. We conclude that the protein identified as $1,25D_3$-MARRS is indeed the cell surface binding moiety and mediator of $1,25(OH)_2D_3$ rapid responses.
(1) Zhao B, Nemere I (2002) J Cell Biochem 86:497.

Disclosures: ***I. Nemere****, None.*

M462

Hypovitaminosis D in a Racially Mixed Sample of Female Adolescents. L. S. Harkness*[1], B. A. Cromer[2]. [1]Nutrition, Case Western Reserve University, Cleveland, OH, USA, [2]Pediatrics, Case Western Reserve University, Cleveland, OH, USA.

To estimate prevalence of both, vitamin D deficiency and vitamin D insufficiency, in a large sample of adolescent females, as well as across racial groups.
Healthy, postmenarcheal females (12 – 19 years) were recruited to participate in the study. Subjects were excluded if they had a history of bone, kidney or liver disease, thromboembolic disorder, current medications that affect bone metabolism, or previous treatment with sex hormones. Serum samples were collected on 370 subjects and assayed for 25-hydroxyvitamin D [25(OH)D] with competitive binding assay (Nichols Institute, San Clemente, CA).
The mean age of the study population was 15.5 ± 1.6 years with a mean body weight of 65.6 ± 16.9 kg. Mean serum 25(OH)D was 53.7 nmol/L (95% CI: 50.7, 56.7). Vitamin D deficiency was defined as serum 25(OH)D ≤ 27.5 nmol/L and vitamin D insufficiency as 25(OH)D ≤ 50 nmol/L. In the total sample, 17% of the female adolescents were vitamin D deficient and 54% were vitamin D insufficient. Racial groups were categorized as Black and Non-black. In the Black and Non-black groups, mean serum 25(OH)D was 43.0 nmol/L and 72.2 nmol/L, respectively (Table 1). There was a significant difference between mean serum 25(OH)D levels across racial categories ($p < 0.0001$). Seasonal variations did exist for the total sample population. Mean vitamin D levels were higher in the Spring/Summer months (60.2 nmol/L) compared to those obtained during the Fall/ Winter months (52.8 nmol/L) ($p < 0.01$) Weight was negatively correlated with vitamin D levels ($r = -0.19$) ($P = 0.0002$). There was no difference in mean body weight between the racial groups, and weight did not have significant effect on vitamin D levels when adjusted for race or season.
The prevalence of vitamin D deficiency and vitamin D insufficiency was high in this population. For adolescent females living in the northeastern U.S., especially black female adolescents, hypovitaminosis D is a noteworthy concern since bone acquisition is crucial during adolescence. Further investigation needs to examine subclinical markers of altered vitamin D status, potential detrimental effects on bone accrual, and racial differences.

Table 1: Serum 25 - Hydroxyvitamin D in Adolescent Females by Race and Season

Variable	Mean 25(OH)D (95% CI) (nmol/L)	No. (%) with 25(OH)D ≤ 27.5 nmol/L	No. (%) with 25(OH)D ≤ 50 nmol/L
Black	43.0 (39.7, 46.2)	50 (21%)	167 (71%)
Non-Black	72.2 (67.9, 76.5)	1 (<1%)	33 (24%)
Winter	52.8 (48.2, 57.3)	28 (22%)	75 (59%)
Summer	60.2 (56.8, 63.4)	33 (14%)	125 (51%)

Disclosures: ***L.S. Harkness****, None.*

M463

1,25Dihydroxyvitamin D3 Is Photoprotective in Human Skin Cells. R. Gupta*, C. J. Holliday*, G. Wong*, M. Slater*, G. M. Halliday*, R. S. Mason. Physiology, University of Sydney, Sydney, NSW, Australia.

Vitamin D is produced in skin by the action of UVB on 7-dehydrocholesterol. The hypotheses that the active metabolite of vitamin D, 1,25dihydroxyvitamin D3 (1,25(OH)2D3) protects keratinocytes, melanocytes and skin fibroblasts from UV-induced cell death and that surviving cells after 1,25(OH)2D3 treatment have no increase in DNA damage were tested. Exposure to 200mJ/cm2 UVB reduced vehicle-treated keratinocyte, melanocyte and fibroblast numbers at 24h by a mean of 19%, 35% or 21% respectively, depending on cell type and donor. Pretreatment with 1,25(OH)2D3 (10-12 to 10-8M) dose-dependently reduced keratinocyte, melanocyte and fibroblast losses by over 50% compared to vehicle control. This was due to a significant reduction in UV-induced apoptosis and was seen in cells treated with 1,25(OH)2D3 24h before irradiation or immediately after irradiation. Since a failure to induce apoptosis in cells with irreparable UV-induced DNA damage would potentially be an adverse outcome, the presence of cyclobutane pyrimidine dimers (CPDs) in irradiated skin cells treated with 1,25(OH)2D3 or vehicle was measured. Treatment with 1,25(OH)2D3 significantly and dose-dependently (10-12-10-8M) reduced CPDs in all skin cell types to around 40% of the levels measured in vehicle-treated wells in all the skin cell types examined. A decrease in CPDs was also seen when 1,25(OH)2D3 treatment was added immediately after UVR. There is evidence for a novel mechanism, reduction in formation of nitric oxide or its derivatives after UVR in the presence of 1,25(OH)2D3. These results are consistent with the proposal that the vitamin D system in skin is part of an intrinsic protective mechanism against UV damage.

Disclosures: ***R.S. Mason****, None.*

M464

Phospholipase A2 Activating Protein (PLAP) is Required for Membrane Activated Signaling by 1,25-Dihydroxy Vitamin D3. L. Wang[1], E. J. Graham*[2], S. Lossdoerfer*[3], V. L. Sylvia*[4], B. D. Boyan[1], Z. Schwartz[1]. [1]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [2]Periodontics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [3]Orthodontics, University of Bonn, Bonn, Germany, [4]Orthopaedics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Steroid hormones act via nuclear receptors as well as through rapid membrane-associated signaling pathways. Phospholipase A2 (PLA2) is pivotal in the action of 1,25(OH)2D3, mediating rapid increases in specific activities of phospholipase C (PLC) and protein kinase C (PKC), and downstream effects on chondrocyte proliferation and differentiation. Microarray analysis shows that PLA2 activating protein (PLAP) is upregulated by factors that induce PKC-sensitivity of rat growth plate (RGP) chondrocytes to 1,25(OH)2D3. This suggests that PLAP couples the membrane receptor to PLA2. Here, we evaluated PLAP's role in 1,25(OH)2D3 signal transduction. RT-PCR showed that PLAP mRNAs are present in confluent cultures of RGP cells from the prehypertrophic and upper hypertrophic cell zones (GC, growth zone) of costochondral cartilage. Northern blot analysis demonstrated that 1,25(OH)2D3 had no effect on PLAP mRNA levels at 10 minutes or at 12 or 24 hours. In situ hybridization of rat costochondral cartilage showed that PLAP was specifically expressed in the growth zone in vivo, but not in the resting zone (RC). RC cells do not exhibit a rapid response to 1,25(OH)2D3 unless induced to do so, although they possess immuno-reactive membrane receptor protein that binds 1,25(OH)2D3, supporting the hypothesis that PLAP is required for coupling the membrane receptor to its signaling pathway. PLAP rapidly increased PLA2 specific activity and increased [14C]-arachidonic acid turnover of GC cells but not RC cells. PLC and PKC specific activities were increased by PLAP within 3 minutes, as noted for 1,25(OH)2D3. The PLAP effect on

PKC was blocked with PLA2 inhibitors quinacrine and AACOCF3, the cyclooxygenase inhibitor indomethacin, the G-protein inhibitor GDPbetaS, and EP1 antagonists, as well as by the PLC inhibitor U73122, indicating that PLAP uses the same signaling pathways as 1,25(OH)2D3. PLAP decreased proliferation ([3H]-thymidine incorporation) and increased alkaline phosphatase specific activity and proteoglycan production ([35S]-sulfate incorporation) in a manner similar to 1,25(OH)2D3. PLAP's effects were additive with those of 1,25(OH)2D3 and both were were inhibited by quinacrine. These results show that PLAP is a critical and early mediator of the rapid response of RGP growth zone chondrocytes to 1,25(OH)2D3.

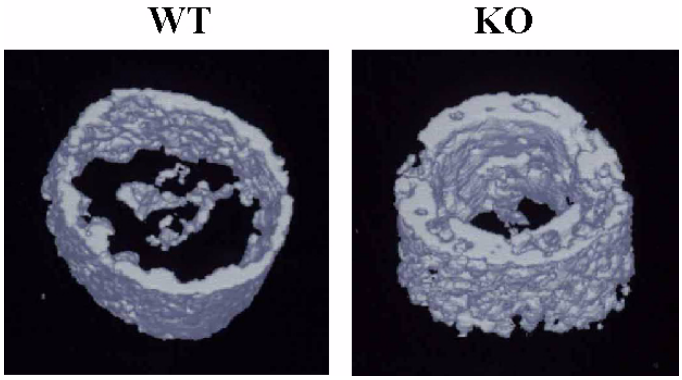
Disclosures: ***L. Wang****, None.*

M465

Direct Action of 1,25-dihydroxyvitamin D on Bone ; VDRKO Bone Shows Excessive Bone Formation in Organ Culture. H. Tanaka[1], N. Inoue*[1], K. Hasegawa*[1], S. Kato[2], Y. Seino[1]. [1]Department of Pediatrics, Okayama University, Okayama, Japan, [2]Tokyo University, Tokyo, Japan.

The vitamin D receptor null mutant mouse (VDRKO) has provided new insights into vitamin D metabolism and its role in vivo. The bone in VDRKO seemed to be indistinguishable from that in wild (WT) mice at birth. Moreover, calcium supplementation showed an apparent cure of rickets. However, calcium-supplement experiments aimed at establishing physiological direct functions of VDR in many organs including bone have been inconclusive owing to the essential roles of calcium in biological function. To clarify the direct roles of Vitamin D in bone physiology, we performed organ culture experiment. As there have been no quantitative analyses in VDRKO bone at birth, we first analyzed the femur of VDRKO at birth with microfocus X-ray, micro-CT, pQCT and histology. As previously shown, VDRKO bone showed no rachitic signs and was indistinguishable from WT bone at metaphysis. But all morphometorical analyses clearly indicated that VDRKO bone showed increased cortical bone at diaphysis. The total bone mineral density (BMD) was 208.5 mg/cm^3 in VDRKO bone and 146.3 mg/cm^3 in wild (WT) bone. The cortical bone volume was 0.059 mm^3 in VDRKO and 0.032 mm^3 in WT, and the cortical thickness was 213.4 μm and 92.6 μm, respectively.

Seven days organ culture of the femur enhanced the differences. Moreover, in the presence of 10^{-8} M 1,25-dihydroxyvitamin D_3, the cortical BMD of the WT femoral diaphysis decreased by 54% of that in vehicle control. The osteoclast number counted on the longitudinal section did not show any differences between in VDRKO and in WT either at birth or during the 7 days culture. On the other hand, VDRKO bone after 7 days culture showed higher number of osteoblast than WT bone. Quantitative PCR showed higher expression of *cbfa1* in VDRKO than in WT.These results suggest that VDRKO bone has higher bone forming potential and that vitamin D may play a role to regulate bone formation



Disclosures: ***H. Tanaka****, None.*

M466

Vitamin D Exerts its Anti-apoptotic Effect in Osteoblasts through a Fas Related Mechanism. G. Duque[1], K. El-Abdaimi*[2], J. Henderson[2], A. Lomri[3], R. Kremer[2]. [1]Geriatric Medicine, McGill University, Montreal, PQ, Canada, [2]Centre for Bone and Periodontal research, McGill University, Montreal, PQ, Canada, [3]INSERM Unite 349, Paris, France.

Apoptosis plays an important role in the regulation of bone turnover. Previously, we showed that 1,25$(OH)_2D_3$, the active form of vitamin D, may increase osteoblast survival by inhibiting apoptosis induced by serum deprivation (SD)[1]. Human osteoblasts express the Fas receptor on their surface and its interaction with Fas ligand is necessary for human osteoblast apoptosis. To investigate the mechanism of 1,25$(OH)_2D_3$ inhibition of apoptosis in osteoblasts isolated from human calvaria, cells were subjected to either SD or addition of Fas antibody. Visualization of apoptotic cells using annexin V revealed a significant decrease in apoptosis at 48 hours in the presence of 1,25$(OH)_2D_3$ (16% ± 2%, p<0.04) compared with non-treated cells (45% ± 3%). Furthermore, flow cytometric analysis of TUNEL labelled osteoblast showed a significant decrease in apoptotic cells in 1,25$(OH)_2D_3$ treated cultures (21% ± 2%) at 48 hours compared with non-treated cultures (44% ± 4%, p<0.04). To further explore the mechanism of 1,25$(OH)_2D_3$ mediated inhibition of apoptosis we examined the changes in activation of death domain proteins, cleavage of caspases and mitochondrial regulators of apoptosis by western blot analysis. A significant inhibition of caspase-8 cleavage and activity in 1,25$(OH)_2D_3$ treated cells was observed in conjunction with a decrease in the ratio of the proapoptotic protein Bax to the antiapoptotic protein Bcl-2. Furthermore, the levels of $p21^{Cipi/WAF1}$, which inhibits the cleavage of caspase-8, was found to be highly induced in 1,25$(OH)_2D_3$ –treated cells. In summary, these results demonstrate that the anti-apoptotic effect of 1,25$(OH)_2D_3$ in human osteoblasts is mediated by the regulation of components of the Fas-related pathway.

[1] Duque G, Abdaimi KE, Macoritto M, Miller MM, and Kremer R. Estrogens (E2) regulate expression and response of 1,25-dihydroxyvitamin D3 receptors in bone cells: changes with aging and hormone deprivation. *Biochem Biophys Res Commun* 299:446-454, 2002.

Disclosures: ***G. Duque****, None.*

M467

Prevalence of Vitamin D Insufficiency in Patients Attending an Outpatient Cancer Care Clinic in Boston Massachusetts at the End of Summer. V. Tangpricha*[1], R. Blanchard*[2], T. C. Chen[1], M. F. Holick[1]. [1]Vitamin D, Skin & Bone Research Laboratory, Boston University School of Medicine, Boston, MA, USA, [2]Section of Hematology and Oncology, Boston University School of Medicine, Boston, MA, USA.

Vitamin D is a steroid hormone important for maintaining normal calcium homeostasis and mineralization of the skeleton. Recent epidemiologic studies and prospective studies have suggested that vitamin D may also be protective against many common cancers in humans including prostate, breast and colon cancer. Furthermore, studies using cancer cell cultures and animal models of cancer have supported the protective role of vitamin D in inhibiting the growth of many types of cancer cells. Therefore, it is important to maintain adequate vitamin D nutrition in patients with cancer not only for good bone health but also for its potential anti-cancer activity. In addition, patients with cancer frequently complain of muscle aches and bone pain, which may be symptoms of vitamin D insufficiency. A study was conducted to determine the prevalence of vitamin D insufficiency in an outpatient cancer care clinic at Boston University Medical Center. After IRB approval, subjects who attended a outpatient cancer care clinic during the summer months of July, August and September were enrolled into our study. A control group of healthy individuals without cancer older than 40 years was recruited the previous year during the same months. Subjects gave written informed consent for determination of serum 25(OH)D and PTH. Both 25(OH)D and PTH assays were performed using the Nichols Advantage System. Subjects with cancer were a mean age of 59 ± 10 years old compared with healthy controls, 51 ± 10 years old (p<0.05, t-test). Twenty-seven (48.2%) out of 56 subjects with cancer were vitamin D insufficient (25(OH)D <= 20 ng/ml) compared to only 6 (12%) out of 50 healthy controls were vitamin D insufficient, (p<0.05, $\chi 2$ test). The mean 25(OH)D of cancer subjects was 21.3 ± 10 ng/ml vs 33.9 ± 10 ng/ml in healthy controls (p<0.05, t-test). This study reveals that a high percentage of subjects with cancer are vitamin D insufficient during the summer, a season where vitamin D insufficiency should be less prevalent. Reasons for the high prevalence of vitamin D insufficiency among cancer patients include malnutrition due to illness, nausea from cancer therapies or infrequent sunlight exposure. Vitamin D insufficiency is easily corrected in these patients by prescribing vitamin D 50,000 IU once a week for 8 weeks then placing them on a daily multivitamin with 400-600 IU of vitamin D . Restoring adequate vitamin D nutrition should improve the muscle aches and bone pains of these patients and potentially be beneficial in slowing the growth of the cancers.

Disclosures: ***M.F. Holick****, None.*

M468

Sulfate Wasting in VDR Knockout Mice: Regulation of Sodium-Sulfate Co-transporter by Vitamin D. M. J. G. Bolt, G. Qiao*, J. Kong*, Y. C. Li. Medicine, University of Chicago, Chicago, IL, USA.

Sulfate is the fourth most abundant anion in mammalian plasma. It is essential for numerous important physiological functions including sulfation of proteoglycans, the largest group of sulfoconjugates required for normal structure and function of bone and cartilage. Mutations in a sulfate transportor gene (DTDST) have been found to be associated with human congenital chondrodysplasia. In mammals, sulfate homeostasis is largely regulated by the kidney, where the majority of filtered sulfate load is reabsorbed in the proximal tubules. Transcellular transport of sulfate from the tubular lumen to the blood depends on a sodium-sulfate co-transporter (NaSi-1), located on the brush border membrane. Recent studies have shown that vitamin D modulates serum sulfate concentration, renal sulfate handling, and the expression and activity of NaSi-1. To further investigate the role of vitamin D in sulfate homeostasis, we studied sulfate excretion in mice lacking the vitamin D receptor (VDR). Urinary sulfate content was measured by a micro turbidometric assay using BaCl precipitation method, and data were expressed as the ratio of sulfate to creatinine. A 45% increase in urinary sulfate excretion was observed in VDRKO mice compared to wild-type controls (62.0±13 vs. 42.9±14, n=15, p<0.0006). NaSi-1 expression in the kidney was determined by quantitative Northern blotting, using a cDNA probe cloned by RT-PCR based on published mouse NaSi-1 cDNA sequence. Consistent with the urinary data, an ~80% reduction in NaSi-1 transcript levels was seen in VDRKO mice. Furthermore, in wild-type mice, injection of 1,25-dihydroxyvitamin D3 significantly induced NaSi-1 expression. The impact of VDR inactivation on sulfate economy in sera and bone is currently being investigated. These results agree with previous work in the vitamin D-deficient rat, and suggest that the vitamin D endocrine system plays an important role in sulfate homeostasis by regulating renal sulfate reabsorption.

Disclosures: ***M.J.G. Bolt****, None.*

M469

Serum Levels of the Fetuin-Mineral Complex Correlate with Artery Calcification in the Rat. T. N. Than*, M. K. Williamson, T. M. T. Nguyen*, P. A. Price. Division of Biology, University of California San Diego, La Jolla, CA, USA.

Our working hypothesis is that a causative agent for artery calcification arises in bone resorption, travels in blood, and then induces calcification in the artery wall. We have tested this blood-born theory of artery calcification by analyzing blood levels of an agent that is plausibly associated with artery calcification, the fetuin-mineral complex (FMC) [JBC (2002) 277:3926-34], in rats in which artery calcification has been initiated by vitamin D doses that increase bone resorption, and in rats in which artery calcification has been prevented by doses of ibandronate and osteoprotegerin that inhibit bone resorption [ATVB (2001) 21:817-24 & 1610-6].

Serum samples were obtained from 49-day-old rats with on-going, vitamin D-induced artery calcification, and from vitamin D-treated rats in which artery calcification was prevented by doses of ibandronate or osteoprotegerin that inhibit bone resorption. Artery calcification was assessed by Alizarin staining, and the presence of a FMC was evaluated by centrifugation of the serum samples and by analysis of the resulting pellets for the characteristic constituents of the FMC, namely calcium, phosphate, fetuin, and matrix Gla protein.

These tests showed that there is a FMC in the blood of rats with on-going, vitamin D-induced artery calcification but not in the blood of rats where calcification has been inhibited by ibandronate or osteoprotegerin. There is also a 2.7-fold reduction in total serum fetuin in rats with on-going artery calcification, but not in the blood of rats where calcification has been inhibited by ibandronate or osteoprotegerin. This massive reduction in serum fetuin appears to be caused by the clearance of the FMC from serum. It is of interest to note that death occurs at this dose of vitamin D within 24h of the time of serum sampling and at the approximate time of serum fetuin exhaustion.

In conclusion, the present experiments demonstrate that the presence of a FMC in serum is indeed associated with on-going artery calcification in the rat, and therefore support the blood-born theory of artery calcification. The biochemical basis for this association, however, is presently unclear. One possibility is that the FMC is formed in the course of the fetuin-mediated inhibition of calcium phosphate precipitation, and that serum FMC is a marker for an increased tendency to form mineral nuclei in rats treated with toxic doses of vitamin D. This possibility is supported by recent evidence that a FMC is formed when fetuin inhibits the precipitation of mineral *in vitro* [JBC (2003) epub:M30074420]. Another possibility is that the mineral core of the FMC may, at high serum FMC, actually seed artery calcification.

Disclosures: ***P.A. Price****, None.*

M470

Seasonal Changes in Serum 25(OH)D in Adolescent Girls in Maine. S. S. Sullivan[1], C. J. Rosen[2], T. C. Chen[3], M. F. Holick[3]. [1]Food Science and Human Nutrition, University of Maine, Orono, ME, USA, [2]Maine Center for Osteoporosis Research and Education, St. Joseph Hospital, Bangor, ME, USA, [3]School of Medicine, Boston University, Boston, MA, USA.

Vitamin D plays a key role in calcium homeostasis and bone mineralization. The purpose of this project was to assess the seasonal variation in serum 25(OH)D levels in adolescent girls residing at a northern latitude (44°N) in the United States where milk is fortified with vitamin D. Twenty-four adolescent girls between the ages of 10 and 13 years were followed for 3 consecutive winter and summer seasons. Serum 25(OH)D and PTH levels were measured each September and March. The samples were stored at -70°C and analyzed using the Nichols Advantage System. The subjects also completed four-day food and activity records each winter and summer, which were analyzed for dietary vitamin D intake and for minutes spent outdoors between 8 AM and 6 PM in the summer. Mean serum 25(OH)D levels decreased significantly ($p<0.001$) from September to March each of the three years with an average decline of 7.7 ng/mL or 26.5%. Mean (SD) levels in September were 29.1 (8.6) ng/mL, while mean levels in March were 21.4 (7.7) ng/mL. The prevalence of 25(OH)D levels below 20 ng/mL was 20% in September and 43% in March. PTH levels did not correlate with 25(OH)D levels. The mean dietary vitamin D intake was 5.06 (2.18) mcg per day. In winter, 43% of subjects had dietary vitamin D intakes below the Adequate Intake (AI). The mean reported outdoor time in summer was 121 (61) minutes per day. Suboptimal serum vitamin D levels were prevalent at the end of winter, indicating the need for higher dietary vitamin D intakes.

Disclosures: ***S.S. Sullivan****, None.*

M471

Hypovitaminosis D in Japanese Diabetic Population. A. Suzuki, M. Kotake*, Y. Ono*, K. Nishiwaki*, S. Imamura*, K. Yamamoto*, T. Kato*, K. Fujiwara*, M. Makino*, T. Mokuno*, N. Oda*, Y. Sawai*, N. Hayakawa*, M. Itoh*. Department of Internal Medicine, Fujita Health University, Toyoake, Japan.

It has been documented that major circulating metabolite of vitaminD is 25(OH)D of which concentration directly reflects the repletion status of vitamin D. European and American studies show high prevalence of hypovitaminosis D and the correlation between osteoporotic fracture and vitamin D deficiency. However, there is only a few report concerning about hypovitaminosis D in Japanese population. Recent studies suggest that relatively higher 25(OH)D level is necessary for preventing secondary hyperparathyroidism and ostepoporosis in elderly. In addition, several studies revealed that abnormalities in calcium, phosphate and vitamin D metabolism in diabetic populations. In the present study, we conducted observational study in 587 diabetic patients in order to assess the prevalence of hypovitaminosis D and the relationship between hypovitaminosis D and clinical manifestations in diabetes mellitus. The samples were collected in March and April 2002. Mean 25(OH)D level assured by direct RIA was 17.0±7.1 ng/ml (Mean ± SD). In Men, 25(OH) D level is higher than in women. 25 (OH) D levels were significantly lower in diabetic patients with apparent diabetic microvascular complications. Interestingly, mean 25(OH)D level in the group treated with insulin is significantly lower than that in the group treated only with diet and exercise. These results suggest that higher prevalence of hypovitaminosis D in the patients with insulin deficiency and extended diabetic complications.

Disclosures: ***A. Suzuki****, None.*

M472

Occult Vitamin D Deficiency, and its Relationship with Bone Mineral Density, Among Post Menopausal Women in Recife, Brazil. F. A. F. Bandeira[1], C. H. Bandeira*[2], E. C. Freese*[3]. [1]Endocrine Unit, University of Pernambuco, Osteoporosis Center, Recife-PE, Brazil, [2]Endocrine Dept, Osteoporosis Center, Recife-PE, Brazil, [3]Osvaldo Cruz Foundation, Aggeu Magalhaes Research Center, Recife-PE, Brazil.

Vitamin D deficiency has been reported to be more prevalent than previously thought even in sunny countries with arid and semiarid climates along the Mediterranean. Recife has a humid tropical climate with a latitude of 12 degrees south. The aim of this study was to determine vitamin D status, and its relationship with bone mineral density, in 91 post menopausal women (aged 50-89 years) attending an endocrine clinic for routine evaluation. Mean+/-SD serum 25OHD levels were 31.3+/-7.4 ng/ml. Mean+/-SD BMD values were at lumbar spine: 0.952+/-0.172 g/cm2 (t score -1.73), and at femoral neck: 0.769+/-0.104 g/cm2 (t score -1.56). Eight percent had 25OHD levels below 15ng/ml, 24% below 20ng/ml, and 43% below 25ng/ml. Patients with 25OHD levels below 25ng/ml were significantly older than patients with 25OHD levels above 25 ng/ml (68.7+/-8.8 *vs* 64.7+/-7.1 years; p=0.02), had a longer period since menopause (21.0+/-8.4 *vs* 16.2+/-8.4 years ; p=0.01), had a lower BMD at femoral neck (0.738+/-0.102 *vs* 0.793+/-0.115 g/cm2; p=0.03), and higher PTH levels (52.9+/-14.5 *vs* 39.7+/-10.8 pg/ml; p=0.002). Calcium intake was similar between patients with 25OHD levels below or above 25ng/ml. The prevalence of vitamin D deficiency at the cutoff of 25 ng/ml, increased significantly with age: 30% in patients at 50 - 59 years of age, 32.5% at 60-69 yr, 54.5% at 70-79 yr, and 83% at 80-89 yr (X2=8.4, p=0.03). In conclusion, we found a high prevalence of occult vitamin D deficiency in post menopausal women attending an endocrine outpatient clinic.

Disclosures: ***F.A.F. Bandeira****, None.*

M473

Evaluation of 25 OH Vitamin D Concentrations in Samples from Subjects with Chronic Renal Failure or End Stage Renal Disease. G. D. MacFarlane, J. L. Sackrison*, D. L. Ersfeld*, A. B. Miller*, A. Bucklen*. Research and Development, DiaSorin Inc, Stillwater, MN, USA.

Analysis of laboratory samples from chronic renal failure (CRF) and end stage renal disease (ESRD) patients can be problematic due to altered serum chemistries, and the presence of multiple medications. Current HPLC and RIA methods for the determination of 25 OH Vitamin D involve sample extraction, where sample matrix influences would be expected to be minimized. However, the differences between a normal serum matrix and the CRF or ESRD matrix can lead to interference or inaccuracy in the determination of 25 OH Vitamin D concentrations in these samples in the non-extracted, automated methods now available. The objective of this study was to assess the accuracy of the non-extracted LIAISON® 25 OH Vitamin D assay in the analysis of CRF and ESRD samples as compared against an extracted sample RIA as reference. Clinical samples (118 CRF/49 ESRD) were collected from regional reference laboratories and analyzed side-by-side in both the LIAISON® 25 OH Vitamin D assay and the DiaSorin 25 OH Vitamin D RIA. The data were analyzed by Student's t test, and linear regression analysis. By Student's t test, no significant difference was observed between the RIA values and the LIAISON® values ($p = 0.07$ CRF; $p = 0.28$ ESRD). The linear regression analysis resulted in the equations: CRF: LIAISON = 0.91(RIA) + 0.6; $r = 0.82$ and ESRD: LIAISON = 0.93(RIA) – 0.6; $r = 0.78$. From these data we conclude that the LIAISON® 25 OH Vitamin D assay correctly assesses the 25 OH Vitamin D status of chronic renal failure and end stage renal disease patients.

Disclosures: ***G.D. MacFarlane****, DiaSorin Inc 3.*

M474

Vitamin D Status in Tanners Compared to Non-Tanners: Consequences on Calcium and Bone Metabolism. V. Tangpricha*, J. S. Mathieu*, T. C. Chen, M. F. Holick. Vitamin D, Skin & Bone Research Laboratory, Boston University School of Medicine, Boston, MA, USA.

Vitamin D insufficiency is a common healthcare problem for adults and children living in areas with inadequate sunlight exposure. Vitamin D is rare in foods and often does not provide the necessary amount of vitamin D to maintain adequate levels year round. Individuals who use indoor tanning beds may have a benefit of maintaining 25-hydroxyvitamin D (25(OH)D) levels throughout the year. Adequate serum 25(OH)D is important for optimal bone health. We compared the blood levels of 107 male and female subjects aged 21 to 61 who use a tanning bed at least once a week to subjects who do not use tanning beds. The study was approved by our institutional review board at Boston University School of Medicine. Subjects were excluded if they had any chronic medical illness or were pregnant. Subjects were divided into two categories: those who used a tanning bed and those who did

not use a tanning bed. A bone mineral density (BMD) was performed at the hip, spinal and total body and blood was collected for 25(OH)D and PTH and urine for N-telopeptide (NTX). The 25(OH)D and PTH assays were performed using the Nichols Advantage™ System. Subjects who used a tanning bed had 270% higher 25(OH)D levels than non-tanners. Tanners had a mean serum 25(OH)D of 48.2 +/- 20 ng/ml compared to non-tanners with a mean of 17.4 +/- 4 ng/ml ($p<0.05$, t-test). We will report the relationship between serum 25(OH)D and serum PTH, urine NTX and BMD. Indoor tanning is associated with higher 25(OH)D levels. The higher 25(OH)D levels may result in better bone density.

Disclosures: ***M.F. Holick****, Indoor Tanning Association 2.*

M475

Prevalence of Hypovitaminosis D (Vitamin D Insufficiency and Deficiency) in Southern Elderly Osteoporotic Patients. J. C. Parker*[1], D. Kelling*[2], T. J. Weber[1]. [1]Medicine, Duke University, Durham, NC, USA, [2]Medicine, Northeast Medical Center, Concord, NC, USA.

Hypovitaminosis D (vitamin D insufficiency and deficiency) is an increasingly recognized problem in the United States. Due to higher solar radiation, one would expect a lower risk for hypovitaminosis D in the southern United States. In this retrospective study, we sought to ascertain the prevalence of vitamin D deficiency by assessing the serum concentration of 25-hydroxyvitamin D (25-OH D) in osteoporotic individuals (N=995: 794 women, 201 men; average age 71.75 years) from a single outpatient clinic in Concord, North Carolina. Vitamin D deficiency, insufficiency, and sufficiency were defined as 25-OH D concentrations <12 ng/mL, 12-20 ng/mL, and >20 ng/mL, respectively. The prevalence of vitamin D deficiency, insufficiency and sufficiency was 17.2 %, 31.7 % and 51.2 % respectively. In addition, Vitamin D levels were compared with levels of calciotropic hormones, including serum parathyroid hormone (iPTH), ionized calcium, and phosphorus concentrations, as well as bone mineral density (BMD) of the lumbar spine, proximal femur and distal forearm. Serum iPTH was inversely correlated with 25-OH D ($r= -0.156$, $p<0.0001$). Serum ionized calcium was correlated with 25-OH D ($r = 0.090$, $p = 0.0049$), while serum phosphorus was not ($r= -0.057$, $p= 0.1575$). Interestingly, the relationship of 25-OH D to forearm BMD was not significant in this patient sample ($r=0.069$, $p = 0.159$). In conclusion, there is a greater prevalence of hypovitaminosis D in the southern United States than has been previously recognized, and concomitant frank hypocalcemia or hypophosphatemia may or may not be present. Although the presence of hypovitaminosis D did not appear to contribute significantly to low BMD in this outpatient population, repletion of Vitamin D may lead to a reduction in fracture risk, based on results from previous studies. Recognition and treatment of occult hypovitaminosis D may therefore provide significant skeletal health benefits in our progressively aging population.

Disclosures: ***J.C. Parker****, None.*

M476

Soluble Form of Recombinant Receptor Associated Protein (RAP) as a Tool to Elucidate Megalin Function. M. Yamagata*[1], Y. Hashimoto*[1], Y. Miyauchi*[1], H. Kondou*[1], K. Ozono[2], T. Michigami[1]. [1]Department of Environmental Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan, [2]Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan.

Megalin is a multifunctional endocytic receptor expressed in apical membrane of renal proximal tubules, and plays critical roles in renal uptake of various proteins. Although the observations in megalin knockout mice indicated its involvement in the renal uptake of 25-hydroxyvitamin D, the functions of megalin are still not fully understood, partially because most of the knockout mice die perinatally from holoprosencephaly. In the present study, to elucidate the function of megalin in kidney, we administered soluble form of receptor-associated protein (RAP) to mice. RAP is a 39-kD protein that is important for normal processing of megalin in proximal tubule cells. It binds to the members of the LDL receptor family including megalin, and inhibits the binding of all other ligands to these receptors. Although native RAP contains an endoplasmic reticulum (ER) retention signal and is mainly located in relation to the ER, here we utilized His-tagged soluble form of recombinant murine RAP [a.a.39-365] which lacked N-terminal signal peptide and the C-terminal ER retention signal. Direct interaction between soluble RAP (sRAP) expressed in E. coli and megalin was confirmed by a ligand blot analysis. Male ICR mice (7-8 wk old) were given i.p. administration of sRAP (3.5 mg/dose, 3 times with 4-hr intervals). Immunostaining using anti-His antibody demonstrated the apical localization of sRAP in the proximal tubules, suggesting that intraperitoneally administered sRAP entered the circulation, followed by glomerular filtration and reabsorption via binding to megalin. Urinary excretion of low-molecular-weight proteins was increased by administration of sRAP, which was consistent with the previous observation in megalin knockout mice. Western blot analysis using antibody against vitamin D binding protein (DBP) confirmed the increased urinary loss of DBP by sRAP administration. We then constructed a mammalian expression vector for sRAP, using pCAGGS vector that possesses a CAG promoter. The immunoglobulin-kappa chain secretion signal was fused to the N-terminus of sRAP [a.a.39-365], which facilitated the secretion of the protein into media when the expression plasmid was introduced to mammalian cells such as COS7. A ligand blot analysis confirmed the interaction between sRAP expressed in COS7 cells and megalin. These results indicated that administration of recombinant sRAP might provide a useful in vivo model to investigate the roles of renal uptake of proteins in mineral metabolism.

Disclosures: ***M. Yamagata****, None.*

M477

Expression of Vitamin D_3-Related Genes in the Dahl Rat. M. Thierry-Palmer[1], C. Demers*[2], J. Bolduc*[2], D. Eatman*[1], M. Bayorh*[1], M. Gascon-Barré[2]. [1]Department of Biochemistry and Pharmacology/Toxicology, Morehouse School of Medicine, Atlanta, GA, USA, [2]Department of Pharmacology, Centre de recherche du CHUM, Hopital Saint-Luc, Montreal, PQ, Canada.

The Dahl salt-sensitive rat (S) is a widely studied genetic model of salt-induced hypertension which is characterized by low plasma renin and an altered calcium endocrine system. Our aim was to evaluate the response of key D_3-related genes in salt-resistant (R) and S Dahl rats in the resting state and following a single calcitriol (CT) dose (120 pmol/kg) under conditions of low (0.3 g/kg) or high (80 g/kg) salt intake for two weeks. S and R rats responded to high salt by a highly significant reduction in hepatic (-62%, $p<0.002$) and intestinal (-74%, $p<0.009$) *CYP27A* (D_3 25-hydroxylase) mRNA levels but CT had no effect on *CYP27A* expression in either group. Expression of the resting kidney *CYP27B1* (1α-hydroxylase) was significantly higher in S than R ($p<0.0002$) on both low (+44%) and high (+290%) salt intake. Moreover, *CYP27B1* was significantly down regulated by CT in R on low salt. High salt also significantly decreased *CYP27B1* in R but abolished the response to CT. Despite increased *CYP27B1* mRNA levels, S rats were completely insensitive to CT on both low and high salt. Resting renal 24-hydroxylase (*CYP24*) was similar in both groups and responded to CT by significant increases in expression levels ($p<0.001$). However, S had an inappropriately high response compared to R ($p<0.02$) during both low and high salt intake. Intestinal *VDR* mRNA levels were significantly higher in S ($p<0.0002$) than in R but contrary to R, CT did not lead to any increases in *VDR* mRNA during low salt intake. High salt led, in both groups, to a resistance to the effect of CT. Thus high salt intake alone perturbs several D_3-related genes (*CYP27A*, and the response of *CYP27B1* and *VDR* to CT) in R rats. Salt sensitivity on the other hand, not only increases *CYP27B1* and *VDR* mRNA levels but leads to a complete insensitivity in the gene response to CT while increasing the *CYP24* response to CT on both low and high salt intake. Our data illustrate that in addition to inappropriate responses to high salt, the S rat exhibits several defects in D_3 endocrine system genes in the resting state.

Disclosures: ***M. Gascon-Barré****, None.*

M478

Prostate 25-Hydroxyvitamin D-1α-Hydroxylase is not Influenced by Parathyroid Hormone and Calcium: Implications for Prostate Cancer Chemoprevention by Vitamin D. L. Wang*[1], G. G. Schwartz*[2], J. N. Flanagan*[1], D. P. Jamieson*[1], M. F. Holick[1], T. C. Chen[1]. [1]Medicine, Boston University, Boston, MA, USA, [2]Cancer Biology, Wake Forest University, Winston-Salem, NC, USA.

The hormonal form of vitamin D, 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D] promotes the differentiation and inhibits the proliferation, invasiveness and metastasis of prostate cells. However, 1α,25$(OH)_2$D is not suitable as a chemopreventive agent because its administration can cause hypercalcemia. Serum levels of 1α,25$(OH)_2$D are tightly regulated by the renal enzyme, 25-hydroxyvitamin D-1α-hydroxylase [1α-OHase], which synthesizes 1α,25$(OH)_2$D from the prohormone, 25-hydroxyvitamin D [25(OH)D]. We have shown previously that normal prostate cells in primary culture, as well as several prostate cancer cell lines, also express 1α-OHase and synthesize the hormone intracellularly. We now investigated the regulation of the prostate 1α-OHase by the three most important regulators of the renal 1α-OHase: calcium, 1α,25$(OH)_2$D, and parathyroid hormone (PTH). The 1α-OHase activity in the prostate cultures was inhibited by 1α,25$(OH)_2D_3$ at 10 and 100 nM. Conversely, PTH at 10 and 100 nM and calcium at 1.2 mM had no effect on enzyme activity, a finding consistent with data on other extra-renal 1α-OHases. Furthermore, PTH at 100 nM and calcium at 1.2 and 2.4 mM had no effect on the 1α-OHase gene promoter activity, whereas the promoter activity was inhibited 48±5 % by 100 nM 1α,25$(OH)_2D_3$. Our findings suggest that, unlike the renal enzyme, the prostate 1α-OHase is largely unregulated by serum levels of PTH and calcium. These findings support the hypothesis that vitamin D or 25(OH)D may be useful as a chemopreventive agent for prostate cancer because its administration should cause an increased synthesis of 1α,25$(OH)_2$D within prostate cells.

Disclosures: ***L. Wang****, None.*

M479

Age-Related Changes in the Action of Parathyroid Hormone and Calcitriol: Discordance between mRNA and Protein Levels. H. J. Armbrecht, M. A. Boltz*. Geriatric Center, St. Louis VA Medical Center, St. Louis, MO, USA.

It is generally assumed that an increase in mRNA levels in response to hormonal stimulation leads to a parallel increase in the level of the corresponding protein. However, in our studies of age-related changes in the vitamin D metabolism of rats, we have found two hormonal systems where this is not the case. First, the capacity of parathyroid hormone (PTH) to stimulate renal production of calcitriol (1,25-dihydroxyvitamin D_3) declines with age. However, the capacity of PTH to increase the mRNA levels for the renal protein that makes calcitriol does not decline with age. This protein is the cytochrome P450 component of the renal 1-alpha-hydroxylase enzyme complex (CYP27B1). Second, the capacity of calcitriol itself to stimulate intestinal absorption of dietary calcium declines with age. This decreased stimulation is due to decreased induction of key proteins involved in calcium transport. These proteins include intestinal calbindin and the plasma membrane calcium pump. However, the capacity of calcitriol to increase the mRNA for these proteins does not decline with age. These changes in PTH and calcitriol action with age have important nutritional

consequences. Old rats fed a low calcium diet go into negative calcium balance while young rats do not. There may be several reasons for this discordance between mRNA and protein levels in old animals. It may be due to decreased translation of mRNA into protein, to increased protein degradation, and/or to the production of non-functional proteins due to free radical damage. These studies point out the importance of using a "proteomic" as well as a "genomic" approach to studies of age-related changes in hormone action.

Disclosures: ***H.J. Armbrecht****, None.*

M480

The Expression of 25-hydroxyvitamin D 24-hydroxylase and 25-hydroxyvitamin D 1 Alpha-hydroxylase in Human Osteoblasts. G. J. Atkins[1], P. H. Anderson*[2], H. A. Morris*[2], A. C. W. Zannettino*[3], P. Kostakis*[1], D. M. Findlay[1]. [1]Orthopaedics and Trauma, University of Adelaide, Adelaide, Australia, [2]Clinical Biochemistry, Institute of Medical and Veterinary Science, Adelaide, Australia, [3]Haematology, Institute of Medical and Veterinary Science, Adelaide, Australia.

1,25-dihydroxyvitaminD3 (1,25vitD3) is a major regulator of calcium homeostasis through its actions on the intestine, kidney, and bone. Although a major role for 1,25vitD3 in bone formation *in vivo* is still uncertain, the 1,25vitD3 receptor, VDR, is known to be expressed by osteoblasts. Ligation of 1,25vitD3 with VDR induces the expression in osteoblasts of a variety of osteogenic genes, such as osteocalcin and osteopontin. In addition, 1,25vitD3 appears to play a direct role in the osteoclastogenic response by the induced expression in osteoblasts of RANKL. We have recently shown in primary normal human osteoblasts (NHBC) that the induced expression of RANKL mRNA in response to 1,25vitD3 correlates with an increase in the proportion of immature (STRO-1$^+$) cells (1). In this study, we used real-time RT-PCR to examine the expression in NHBC of mRNA encoding VDR, the 25-hydroxyvitaminD 24-hydroxylase (CYP24), and the 25-hydroxyvitaminD 1 alpha-hydroxylase (CYP27B1). We found that NHBC express abundant mRNA for VDR, and that the expression of CYP24 mRNA increases dramatically in response to treatment with 1,25vitD3. Preliminary evidence suggests that the level of CYP24 induction in NHBC varies according to their differentiation state. Our results suggest that human osteoblasts regulate their response to 1,25vitD3 through the induction of CYP24. Furthermore, NHBC express CYP27B1 mRNA, implying that they may synthesise biologically active 1,25vitD3. Therefore 1,25vitD3 may play a more complex and intrinsic role in human osteoblast biology than has hitherto been recognised.

1. Atkins GJ *et al.*, RANKL expression is related to the differentiation state of human osteoblasts. J Bone Miner Res 18, 2003.

Disclosures: ***G.J. Atkins****, None.*

M481

Identification, Cloning and Expression of a Novel Splice Variant of the CYP24 (Vitamin D-24-Hydroxylase) Gene in Macrophages: A Novel Mode for Regulation of 1,25-Dihydroxyvitamin D Production. S. Ren[1], L. Nguyen*[1], M. Hewison[2], J. S. Adams[1]. [1]The Burns and Allen research Institute and Division of Endocrinology, Diabetes and Metabolism, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA, USA, [2]Division of Medical Sciences, The University of Birmingham Queen Elizabeth Hospital, Birmingham, United Kingdom.

Extrarenal production of the active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25-D), occurs in human health and disease. In human granuloma-forming and lymphoproliferative disorders such as sarcoidosis and lymphoma, respectively, the site of hormone synthesis is the macrophage and the responsible enzyme the CYP27B1 (1-hydroxylase) gene product. In contrast to the renal 1-hydroxylase, macrophage production of 1,25-D is characterized by relative 1] unresponsiveness to stimulation by parathyroid hormone and 2] lack of 1,25-D-directed catabolic 24-hydroxylating activity despite expression of the CYP24 gene. We now report that the failure to respond to the negative feedback signals of 1,25-D on the 24-hydroxylase results from tissue-specific alternative splicing of the CYP24 gene. In the chick, the alternative splice product is initiated in exon III of the CYP24 gene. Expression of products of the hologene (55 kDa; 508 aa in length) and the splice variant (36 kDa; 351 aa) were induced to the same degree by incubation of cells with 200 nM 1,25-D, indicating both were under positive control by the same VDRE-containing promoter elements. The splice variant was expressed in the macrophage, heart, and brain but not in kidney. The truncated splice variant lacked the N-terminal mitochondrial targeting domain of the hydroxylase but maintained an intact substrate (i.e. 25-D and 1,25-D) binding domain. As such, this splice variant encoded a protein that is capable of binding substrate but is unable to be inserted in the inner mitochondrial membrane and function catalytically. Transient transfection of the truncated sense cDNA into the chick HD-11 macrophage cell line, which constitutively expresses the CYP27B1 gene and possess abundant 1-hydroxylating activity but no basal or 1,25-D-stimulatable 24-hydroxylating activity, reduced 1,25-D synthesis by 53% ($p<0.01$) and did not induce 24,25-D synthesis. On the contrary, compared to vector alone-transfected cells, stable and transient transfection of the antisense cDNA increased 1,25-D production in HD-11 cells 3-7-fold ($p<0.01$). We propose that alternative splicing of the CYP24 gene can reostatitically control 1,25-D synthesis and catabolism by limiting vitamin D substrates and differentially-spliced CYP24 gene products access to the inner mitochondrial membrane.

Disclosures: ***S. Ren****, None.*

M482

Disruption of Exocytosis Enhances 25-Hydroxyvitamin D–Driven 1,25-Dihydroxyvitamin D Synthesis in Human Kidney Cells. S. Wu, L. Nguyen*, J. S. Adams. The Burns and Allen research Institute and Division of Endocrinology, Diabetes and Metabolism, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA, USA.

The CYP27B1 gene product, the mitochondrial enzyme P450c1, catalyzes the 1-hydroxylation of 25-hydroxyvitamin D (25-D) to the hormone, 1,25-dihydroxyvitamin D (1,25-D). The means by which internalized substrate 25-D finds it way to the inner mitochondrial membrane for catalysis has been under investigation in our laboratory. Recent studies (J Bone Miner Res 17:S292, 2002; Endocrinology 143:4135-4138, 2002) have shown that intracellular vitamin D traffic is controlled by members of the heat shock protein (hsp)-70 family of chaperone proteins. Both hsp "client" proteins and ligands, like 25-hydroxylated vitamin D metabolites, appear to be "sorted" in the endoplasmic reticulum prior to distribution of to specific intracellular destinations or re-secretion from the cell by the process of exocytosis. Two members of the hsp70 family, constitutively-expressed heat shock protein-70 (hsc70) and glucose regulated protein-75 (grp75), can increase cellular 1,25-D synthesis. We have theorized that augmented 1,25-D production is the result of increased delivery of substrate 25-D to the mitochondrial P450c1. To investigate this hypothesis, blockade of golgi formation and exocytosis in P450c1-expressing human kidney cells (HKC-8) was achieved with brefeldin A (BFA) to "close the exits" in an attempt to redirect substrate 25-D traffic to the mitochondrial 1-hydroxylase machinery. HKC-8 cells were transiently transfected with hsc70, grp75 or vector (pcDNA3.1) alone. After transfection (24 h), cells were incubated with 200 nM 25-D for up to 5 h, with or without increasing amounts of BFA. Conditioned medium and cells were extracted with acetonitrile for 1,25-D assay. Total RNA extracted from a separate cell pellet was used to measure CYP27B1 and CYP24 (24-hydroxylase) gene expression by Northern blot analysis. Compared to vector-alone transfected, untreated cells, BFA elicited a time- and concentration-dependent increase (+13.2.1 ± 0.6 % to +43.6 ± 2.9 %; p=0.04 to 0.008) in 1,25-D synthesis; within 2 h of removal of BFA from cells reversal of 1,25-D synthesis to basal levels was achieved. Transfection of hsc70 or grp75 further increased 1,25-D synthesis (+41.4 ± 0.5 % and +28.4 ± 4.2 %, respectively compared to vector-alone transfected, untreated cells; p=0.002 and 0.04). Changes in 1,25-D synthesis occurred independently of a change in hydroxylase gene expression. We propose that hsc70- and grp75-stimulated 1,25-D synthesis in human kidney cells can be enhanced by blocking exocytosis and redirecting substrate 25-D traffic to the mitochondrial P450c1.

Disclosures: ***S. Wu****, None.*

M483

Establishing the Role of CYP24 in the Metabolism of Natural Vitamin D Compounds and their Synthetic Analogs. M. Kaufmann*[1], S. Masuda[1], V. Byford*[1], R. St-Arnaud[2], A. Arabian*[2], G. Jones[1]. [1]Biochemistry, Queen's University, Kingston, ON, Canada, [2]Genetics Unit, Shriner's Hospital, Montreal, PQ, Canada.

Recently, we have developed several in vitro and in vivo model systems for the study of the physiological and pharmacological role of the cytochrome P450, CYP24, in the action of all vitamin D compounds. In this study, we used two hCYP24 cell overexpression systems (hCYP24-V79 and HPKIA-ras) to study the metabolism of four compounds: $1,25(OH)_2D_3$, 25-OH-D_3, 20-methyl-$1,25(OH)_2D_3$ (20-MeD) and 20-epi-$1,25(OH)_2D_3$ (20-epiD). We observed CYP24 expression-dependent metabolism of analogs to 23- and 24-modified metabolites as identified by LC-MS/MS. Interestingly, the two natural compounds: $1,25(OH)_2D_3$ and 25-OH-D_3 were metabolized to water-soluble acids at similar rates when DMEM containing no FCS was present in the culture medium. The rate of metabolism was significantly reduced for 25-OH-D_3 but not for $1,25(OH)_2D_3$ when hDBP (0.1-0.5 mM) was added, suggesting an important role for DBP in imposing altered specificity on CYP24. Alternately, experiments performed in vivo with CYP24-knockout mice administered [26,27-^{3}H]25-OH-D_3 intravenously confirmed that animals are incapable of 24-hydroxylation, are devoid of serum $24,25(OH)_2D_3$ & show higher residual 25-OH-D_3, whereas their heterozygous and wild-type littermates exhibit measurable serum $24,25(OH)_2D_3$ & metabolize administered 25-OH-D_3 more rapidly. In addition, primary keratinocytes from the same three groups of mice also express the same CYP24-dependent phenotype of target cell metabolism of $1,25(OH)_2D_3$ & 25-OH-D_3. We conclude from the studies of natural compounds that the inherent specificity towards $1,25(OH)_2D_3$ over 25-OH-D_3 is through differential sequestration of 25-OH-D_3 on the outside of cells, rather than any profound specificity of pure CYP24. When we incubated CYP24 expression systems with the potent analogs: 20-MeD & 20-epiD, we observed similar lipid- and water-soluble products as with $1,25(OH)_2D_3$, including 20-methyl-calcitroic acid and a novel 20-epi-24-carboxy, 25,26,27-trinor-1α-OH-D_3. In these studies, we noted a reduced rate of water-soluble metabolite production and evidence of a metabolic block at C-23 hydroxylation steps. This confirms with well-defined CYP24 expression systems, the metabolic hesitation at the 24-oxo-intermediate stage that others have documented for 20-MeD and 20-epiD previously using various cell lines. We conclude that these CYP24-associated metabolic nuances partially explain the increased potency of 20-MeD, 20-epiD and possibly other analogs. We conclude that CYP24 plays a major role in the metabolism of certain synthetic analogs as well as natural vitamin D compounds.

Disclosures: ***M. Kaufmann****, None.*

WORKING GROUP ON MUSCULOSKELETAL REHABILITATION IN PATIENTS WITH OSTEOPOROSIS ABSTRACTS

WG1

Introduction and Overview of Current Working Group Activities. M. Sinaki. Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN, USA.

Our 1st Annual Working Group was held in San Francisco in December 1998 during the Joint Meeting of the American Society for Bone and Mineral Research (ASBMR) and International Bone and Mineral Society (IBMS). The Annual Meeting of the ASBMR provides a perfect setting for our Working Group since scientists, researchers and clinicians of various specialties from around the world attend the meeting. Here we can "link up" with our colleagues to join forces in resolving the issues confronting us in the overall management of osteoporosis by combining pharmacotherapy with non-pharmacotherapy and other rehabilitative options for proper management of osteoporosis.

The reduction of bone mass and the occurrence of osteoporotic fractures create specific musculoskeletal challenges that cannot be met with pharmacotherapy alone. Osteoporosis affects the "whole person" lifestyles change, quality of life declines and self-esteem suffers. As the skeletal frame shrinks and posture fails, osteoporotic individuals often suffer psychologically as well. They are afraid to participate in and enjoy physical activities for fear of fall or fracture.

The objectives of our Working Group are to address the musculoskeletal challenges of osteoporosis through education, exercise, therapeutic modalities for pain control, and technical devices for maintenance of posture and prevention of falls. Through our publications, clinicians can help their patients to adjust to the musculoskeletal challenges of osteoporosis and learn to alternate – not terminate their daily physical activities.

Although, some evidence-based studies are available on the efficacy of non-pharmacotherapy in the management of osteoporosis, further controlled trials are needed in this area. As core members of this Working Group, we are committed to the promotion of further controlled trials to confirm the benefits of non-pharmacotherapy in the management of osteoporosis. The core group is also committed to the publication of a review paper addressing the role of non-pharmacotherapy alone and/or combined with pharmacotherapy for prevention/management of osteoporosis.

WG2

Vitamin D and Musculoskeletal Disability in the Elderly. H.A. Bischoff. Robert B. Brigham Arthritis and Musculoskeletal Diseases Clinical Research Center, Brigham and Women's Hospital and Clinical Epidemiology Research and Training Unit, Boston University School of Medicine, Boston, MA and Division on Aging, Harvard Medical School, Boston, MA, USA

There is increasing evidence that higher levels of 25-hydroxyvitamin D may improve or help prevent musculoskeletal disability in older persons. Areas discussed are lower extremity function, falls, and osteoarthritis (OA) of the hip and knee. One basic concept appears to be the direct effect of vitamin D on muscle strength. Highly specific receptors for 1,25-dihydroxyvitamin D are expressed in human muscle tissue and it has been suggested that these nuclear receptors promote protein synthesis in the presence of 1,25-dihydroxyvitamin D, eventually leading to improved strength.

Randomized controlled trials (RCT) indicate that vitamin D supplementation could reduce body-sway in ambulatory elderly women and improve musculoskeletal function in institutionalized elderly women. These finding are supported by several cross-sectional observations documenting a positive association between lower extremity strength and 25-hydroxvitamin D or 1,25-dihydroxyvitamin D levels in institutionalized and ambulatory elderly. A population-based study in the US found that in both active and inactive ambulatory subjects aged 60 years and older, higher 25-OHD levels are associated with better musculoskeletal function in the lower extremities.

This beneficial effect of vitamin D on muscle strength and function appears to translate into a reduced risk of falling in institutionalized elderly women, as observed in a RCT comparing a combined supplementation with vitamin D and calcium to calcium alone. Subjects in the vitamin D plus calcium group had a 49% [95% CI 14-71%] reduced risk of falling within three months of treatment.

Recent epidemiological studies indicate that vitamin D may play an important role in OA prevention and progression. There are two independent epidemiological studies demonstrating an inverse association between vitamin D and the risk for radiographic OA of the hip (SOF Study) and radiographic progression of OA at the knee (Framingham Study).

In summary, due to documented associations between 25-hydroxyvitamin D and muscle strength, lower extremity function, falls and radiographic OA of the hip and knee, vitamin D supplementation may be warranted in the prevention and treatment of musculoskeletal disability in the elderly.

WG3

Interaction Between Falls and Clinical Fractures. P. Geusens. Limburgs University Centrum, Diepenbeek, Belgium & Department of Rheumatology, University Hospital, Maastricht, The Netherlands

Decreased bone strength is a major risk factor for fractures. In the elderly, falling appears to be another determinant of fractures for appendicular fractures, independent of bone density. The risk for fractures therefore is associated with the risk for low bone density and for falls. Only limited studies are available on the interaction between osteoporosis and fall-related factors.

Compared to patients without osteoporosis and without a fall history, the risk for a recent clinical fracture was increased in patients with osteoporosis (Odd's ratio and 95% confidence interval (OR & CI): OR: 1.9 (CI: 1.4, 2.5) per one standard deviation decrease in BMD in the distal forearm) and in patients reporting a recent fall (OR: 6.0, CI: 3.1, 11.5). The age and BMI adjusted risk for a recent fracture was highest in patients with osteoporosis who reported a recent fall (OR: 24.8, CI: 6.9, 88.6)[1]. In the EPIDOS study, low bone density and risk for falls were related to the risk for proximal humerus fractures. However, during a follow-up of 4 years an association of increased fracture risk and fall-related risk factors (falls, physical activity, balance, lower limb pain) was only found in patients with osteoporosis (OR: 4.4, CI: 2.0, 9.9), but not in patients with normal BMD (OR: 1.1, CI: 0.3, 3.3)[2]. In the study of osteoporotic fractures, the risk of incident forearm fractures was highest in elderly (age >75 years) recurrent fallers (OR: 1.8, 95% CI: 1.2, 2.7), but was not significantly increased in women between 65-74 years of age who had one fall during the past year (OR: 1.2, CI: 0.9, 1.6)[3]. In the 'Hip Intervention Program' study, risedronate decreased the risk for hip fractures in patients with low bone density[4]. However, in elderly patients selected on the base of the presence of mainly fall-related risk factors for hip fracture, no significant anti-hip fracture effect could be demonstrated. In the absence of a prevalent vertebral fracture, bisphosphonates have been shown to reduce the risk of fractures only in patients with documented low bone density[4,5].

These observations indicate that: a) there is interaction between osteoporosis and falls in the occurrence of appendicular and clinical fractures in the elderly; b) in addition to BMD, risk evaluation for falls contributes to identifying patients at highest risk for fractures; and, c) prospective studies are needed to evaluate the effect of combined bone- and fall-directed preventive measures to prevent fractures in the elderly.

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WG4

Kyphosis and Osteoporosis. E. Itoi.[1], M. Hongo[1], M. Sinaki[2]. [1]Department of Orthopedic Surgery, Akita University School of Medicine, Akita, Japan, [2]Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN USA

Increased thoracic kyphosis is a typical postural deformity observed in patients with osteoporosis. Disadvantages of increased thoracic kyphosis are well known such as chronic back pain, decreased vital capacity, reflux esophagitis, and increased risk of fall. Falls may cause a new vertebral fracture, which in turn aggravates the kyphotic deformity. In order to stop this vicious cycle, prevention and treatment of malposture is required. We have demonstrated that back strengthening exercise is effective not only in increasing the back extensor strength but also in reducing the risk of vertebral fractures in healthy postmenopausal women. Using a backpack that contained a weight equivalent to 30% of the maximal isometric back extensor strength, each subject was instructed to lift the backpack 10 times in prone position. The exercise was conducted at home once a day, 5 days a week. With increasing back extensor strength, the subjects with kyphotic deformity showed improvement of the deformity, whereas those without an increase in muscle strength showed aggravation of kyphotic deformity in 2 years. Eight years after cessation of the exercise, the subjects still had stronger back extensors compared to the control group because the level of their activities of daily living had been increased. The relative risk for compression fracture was 2.7 times more in the control group than in the exercise group. Our next goal is to prescribe this exercise program to women with osteoporosis. We need to know the safe quantity of exercise for osteoporotic patients in accordance with the advancement of osteoporosis. As a first step, we reduced each of the weight of the backpack, repetition times, or frequency of the exercise in order to determine the relationship between the quantity of exercise and obtained muscle strength. This study is ongoing.

WG5

Recovery from Hip Fracture. J. Magaziner. School of Medicine, University of Maryland, Baltimore, Maryland, USA

Hip fractures occur in over 1.6 million persons age 65+ worldwide each year and the numbers experiencing hip fracture in the future are likely to increase substantially. Among the consequences of hip fracture are increased risk of death, loss of ambulatory ability, a precipitous loss of bone mineral density and muscle mass, and an increase in body fat. Other consequences of hip fracture include loss of ability to perform tasks of daily living independently, decline in neuromuscular function, depression, cognitive loss and a changes in social functioning. Data from 20 years of work in the Baltimore Hip Studies provides clues to understanding the multifaceted losses that occur following a hip fracture and how much of the loss observed can be attributed to the hip fracture, beyond what would not have otherwise been seen in frail older persons. In addition to describing consequences of hip fracture, this presentation will discuss the recovery process and how a more thorough understanding of the way older persons recover from hip fracture may be useful in targeting rehabilitative efforts at different times following a fracture. A sequence of recovery will be presented based on data on patterns of recovery in many of the domains affected by hip fracture. The status of evidence related to our understanding hip fracture consequences will be provided along with a general model that may be useful in understanding recovery and in designing interventions to optimize recovery from hip fracture. Promising areas for intervention will be highlighted along with areas where additional research is needed.

PEDIATRIC BONE AND MINERAL WORKING GROUP ABSTRACTS

WG6

The Development of Bone Strength in Children and Adolescents: A Longitudinal Analysis. C.B. Ruff. Department of Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Age changes in bone strength during growth have important consequences for predicting fracture risk and identifying metabolic bone diseases in children. Previous investigations of bone strength changes in juveniles have been limited to a cross-sectional study design. The present study utilizes longitudinal data collected over the entire growth period to examine age changes in femoral and humeral strength in a sample of 20 individuals (10 males, 10 females). The sample was obtained from the archives of the Denver Child Research Study, a longitudinal study carried out in the 1940's-1960's. The original study included radiography of the limbs performed at 6 month-1 year intervals from near birth through late adolescence (average 34.5 measurements/individual). Periosteal and cortical bone breadths were taken at femoral midshaft and at 40% of bone length from the distal end of the humerus, and used to calculate section moduli, measures of bending and torsional strength. Soft tissue breadths, also derived radiographically, were used to calculate muscle areas in the upper and lower limbs. Body weights and statures at each examination were available from the study archives. A mechanically appropriate "body size" parameter was calculated as body weight x bone length (femoral or humeral). Raw data were smoothed using LOWESS, and growth velocities calculated between each age interval. Growth velocities for femoral strength are strongly correlated with those for body size (r^2 = .65-.80), but very poorly correlated with those for stature (r^2<.06). Growth velocities for humeral strength are moderately correlated with body size (r^2=.40-.73), and again very poorly correlated with stature (r^2<.05). Bone strength and muscle area velocities are weakly but significantly correlated (r^2=.10-.25), except in the male upper limb, where the correlation is much stronger (r^2=.65). Age at adolescent peak growth velocity for stature is significantly earlier than those for body size, muscle areas, or bone strengths, which are not significantly different from each other. Thus, relative to a mechanically appropriate measure of body size (body weight x bone length), there is no apparent "lag" in bone strength during early adolescence. If bone strength is expressed relative to body size, only a minority of the sample shows evidence of a minimum reached in early or mid-adolescence. These results are different from those obtained in a study of the distal radius, and suggest region-specific variability in growth, perhaps mechanically mediated. Overall, the results here argue strongly for the importance of mechanical factors – body weight and (especially in the male upper limb) muscular loadings - in the development of bone strength prior to adulthood.

WG7

Time Dependent Effects of Physical Activity on Bone Mineral Content Accrual. A 7-Year Prospective Study in Children and Adolescents. A.D.G. Baxter-Jones[1], R.L., Mirwald[1], R.A. Faulkner[1], K.C. Kowalski[1], D.A. Bailey[1,2]. [1]College of Kinesiology, University of Saskatchewan, Saskatoon, Canada; [2]Dept Human Movement Studies, University of Queensland, Brisbane, Australia.

Physical activity may be one of the most important modifiable factors for accretion of bone mass in childhood and adolescence. However, the effects of normal growth and maturation may mask the effects of physical activity. To distinguish the time dependent effects of physical activity from those of growth and maturation, individual growth trajectories must be identified; this can only be achieved using longitudinal data. The purpose of this study was to characterize patterns of individual's total body bone mineral content (TBBMC) accrual and to investigate the independent effects of physical activity at each measurement occasion on TBBMC accrual. Subjects were part of the Saskatchewan Bone Mineral Accrual Study (BMAS). Eighty-five boys and 67 girls were repeatedly assessed during the circumpubertal years. At study entry they were aged between 8 and 15 years (i.e. 8 age cohorts). During seven years of annual data collection the composition of these cohorts remained the same. As there were overlaps in ages between the clusters it was possible to estimate a consecutive 13-year developmental pattern (8 to 21 years). Height and physical activity were assessed biannually. TBBMC, lean and fat mass were assessed annually by dual energy X-ray absorptiometry (DXA) (Hologic 2000). Physical activity was determined using the physical activity questionnaire for children (PAQ-C – 1 low, 5 high). Biological age, a measure of maturity, was defined as years from age of peak height velocity. Data were analyzed using random effects models. The fixed coefficients that significantly predicted TBBMC were biological age (years) (69.1g ± 6.7g, p>0.05), height (cm) (6.5g ± 1.2g, p>0.05), lean mass (g) (0.031g ± 0.001g, p>0.05), fat mass (g) (0.009g ± 0.001g, p>0.05) and physical activity (11.2g ± 5.2g, p>0.05). Sex however was not a significant predictor of TBBMC (-20.6g ± 18.9g, p>0.05). For children of the same biological age, height, lean and fat mass, TBBMC is predicted to be 44.8g higher in the most active child (physical activity = 5) compared to the least active one (physical activity = 1). In conclusion a longitudinal growth model has shown that when the confounders of sex, growth and maturation are controlled, physical activity has a significant independent effect on TBBMC accrual during childhood and adolescence.

WG8

Resolution of Severe, Adolescent-Onset Hypophosphatemic Rickets Following Resection of an FGF23-Producing Tumour. L.M. Ward[1,2], K.E. White[3], G. Filler[1], M. Matzinger[1], M. Econs[3] and F.H. Glorieux[2]. [1]Department of Pediatrics, University of Ottawa, Canada; [2]Genetics Unit, Shriners Hospital for Children, McGill University, Montréal, Canada; [3]Department of Medicine, Indiana University, Indianapolis, IN, USA.

We describe an 11 year-old, previously healthy girl with a two-year history of lower extremity pain and progressive loss of ambulation. Height was normal and there was no skeletal deformity. X-rays revealed a fracture of the right femoral neck, rickets and multiple Looser zones. Biochemical parameters were consistent with severe hypophosphatemia (ionized calcium 1.12 mmol/L, N: 1.1-1.3; phosphate 0.6 mmol/L, N: 1.0-1.7; TMP/GFR 0.4, N: ≥0.78; alkaline phosphatase 646 U/L, N: 105-420; intact PTH 3.5 pmol/L, N: 1.1-6.8). Serum $25(OH)D_3$ was normal (44 nmol/L, N: 20-90) while $1,25(OH)_2D_3$ was very low (14 pmol/L, N: 40-140). The FGF23 serum level was markedly elevated (FGF23 = 359.5 Reference Units (RU)/ml, N: <142). An iliac crest bone biopsy revealed severe osteomalacia, with poor and diffuse uptake of tetracycline labels. Periosteocytic lesions, as are typical for X-linked hypophosphatemic rickets, were not seen, and sequence analyses of the PHEX and FGF23 genes were normal. Oncogenic hypophosphatemic osteomalacia was suspected and the search for an occult tumour was undertaken. Total body imaging by magnetic resonance revealed a small exostosis of the lateral aspect of the left, distal ulnar metaphysis. A 2 x 1.2 x 0.8 cm lesion was removed from the distal ulna and the pathology was consistent with non-specific fibro-osseous tissue. Serum FGF23 was normal at 7 hours post-op (44.5 RU/ml), and remained normal thereafter. Serum phosphate reached the lower limit of normal by 16 days post-op, and settled in the mid-normal range. Alkaline phosphatase was near-normal at 12 months following surgery (120 U/L, N: 45-116). There was a rise in serum PTH after excision of the lesion (peak 19.6 pmol/L at 3 weeks post-op), with normalization by 5 months after surgery. Serum $1,25(OH)_2D_3$ rose to normal 24 hours after tumour removal, rebounded to 2.5 times the upper limit of normal at 1 week post-op, and normalized by 12 months after surgery. At 5 months post-op, the patient walked with a normal gait and no longer complained of pain. An iliac crest bone biopsy at this time showed improvement in the osteomalacia, with rapid mineralization of the remaining osteoid. In summary, the rapid normalization of FGF23 following removal of a benign tumour and the subsequent improvement in the biochemical and histological parameters of bone metabolism suggested that FGF23 played a key role in this patient's disease. Despite rapid normalization of circulating FGF23, healing of bone tissue was relatively slow and still incomplete at 8 months after surgery.

WG9

Cyclical Pamidronate Treatment Does not Affect Bone Mineralization Density Distribution in Children Suffering from Osteogenesis Imperfecta. P. Roschger[1], M. Weber[2], N. Fratzl-Zelman[1], P. Fratzl[2], K. Klaushofer[1]. [1]Ludwig Boltzmann Institute of Osteology, 4th Med. Department, Hanusch Hospital and UKH Meidling, Vienna, Austria; [2]Erich Schmid Institute of Materials Science, Austrian Academy of Sciences and Institute of Metal Physics, University of Leoben, Leoben, Austria.

Cyclical pamidronate administration leads to an increase of trabecular bone volume and cortical thickness in children suffering from osteogenesis imperfecta (OI), resulting in a decrease of fracture rate. However, it is unknown if pamidronate treatment has also a beneficial effect on the bone material quality, which in OI is characterized by extreme brittleness associated with hypermineralization of the matrix. Therefore, we measured the bone mineralization density distribution (BMDD) of iliacal spongiosa in paired biopsies of children with OI before and after pamidronate treatment using a quantitative backscattered electron imaging method. Bone biopsies from sixteen patients aged between 4 and 18 years affected by OI types I, III and IV were studied before and after treatment. Pamidronate was administered intravenously on 3 consecutive days at age dependant doses. The period of treatment varied from 1.2 to 3.4 years. Additionally, BMDD data from the OI age group between 10 to 15 years (n=5) were compared to vertebral trabecular bone of an age matched control collective (n=6). We calculated the mean Ca-concentration (Ca_{Mean}), the most frequent Ca-concentration (Ca_{Peak}) and the width of the distribution (Ca_{Width}), which correlates inversely with the homogeneity of the mineralization density of the bone matrix. Interestingly, pamidronate treatment did not show any significant effect on the BMDD in OI patients. This result is in sharp contrast to the effects on osteoporotic patients treated with bisphosphonates, where the antiresorptive treatment moderately increases the Ca_{Mean} and Ca_{Peak}, but markedly reduces Ca_{Width}. In agreement with previous observations, the OI patients, independently of treatment, had significantly increased values of Ca_{Mean} (+7 % versus control values of 21.36, p=0.004) and of Ca_{Peak} (+5 % versus control values of 22.3 wt % Ca, p=0.004). Ca_{width} was unchanged (3.5 wt % Ca) versus normal controls. In conclusion, these data show that the antiresorptive treatment does not further increase the mineralization in the already hypermineralized bone matrix in OI. In consequence, we suggest that the matrix quality of OI bone is not affected by pamidronate treatment.

WG10

Melatonin Signaling Dysfunction in Osteoblasts of Patients with Adolescent Idiopathic Scoliosis. B. Azeddine[1], D.S. Wang[1], S. Forget[1], H. Labelle[1,2], B. Poitras[1,2], C-H. Rivard[1,2], G. Grimard[1,2], A. Moreau[1]. [1]Bone Molecular Genetics Laboratory, Centre de recherche, Hôpital Sainte-Justine, Université de Montréal; [2]Orthopedic division, Hôpital Sainte-Justine, Montréal Qc, Canada.

The aetiology of adolescent idiopathic scoliosis (AIS), the most common form of scoliosis, is unknown. Pinealectomy in chicken has led to the formation of a scoliotic deformity, thereby suggesting that a melatonin deficiency may be at the source of AIS. Indeed, treatment of pinealectomized animals with melatonin, the major hormone of the pineal gland, prevents the formation of scoliosis. Interestingly, bone tissue is well known to respond to melatonin and the persistent osteopenia associated with AIS suggested that osteoblast and osteoclast differentiation and/or function could be affected in AIS. However, the relevance of melatonin in the etiopathogenesis of that condition is controversial since most studies have reported no significant change in circulating levels of melatonin in AIS patients. These considerations led us to look beyond the melatonin deficiency hypothesis to assess whether or not melatonin signal transduction is impaired in AIS. Primary osteoblast cultures prepared from bone specimens obtained intraoperatively during spine surgeries were used to test the ability of melatonin and Gpp(NH)p, a GTP analogue, to block cAMP accumulation induced by forskolin. In parallel, melatonin receptor and Gi protein functions were evaluated by immunohistochemistry and by co-immunoprecipitation experiments. The cAMP assays demonstrated that melatonin signaling was impaired in osteoblasts isolated from AIS patients (n=41) to different degrees allowing their classification in 3 distinct groups based upon their responsiveness to melatonin or Gpp(NH)p. Co-immunoprecipitation assays with MT1 and MT2 antibodies revealed in normal osteoblasts the presence of two forms of coupled Gi proteins, one unphosphorylated (43kDa, active) and one phosphorylated form (60kDa, inactive). Similar assays with osteoblasts isolated from AIS patients revealed distinct phosphorylation patterns, most of the patients exhibiting only the phosphorylated form of Gi proteins. Taken together these results indicate that melatonin signaling dysfunction in osteoblasts from AIS patients could be mediated by a selective hypofunctionality of Gi proteins in this syndrome. Post-translational modifications affecting Gi protein function, such as serine residues phosphorylation, should be considered as one possible mechanism in the etiopathogenesis of AIS. These results are clinically relevant and should lead to the development of innovative pharmacological approaches to prevent and cure AIS.

WG11

Infantile Hypercalcemia Associated with Williams Syndrome Treated Successfully with Bisphosphonate. A. P. Cagle[1], S. G. Waguespack[2], B. A. Buckingham[3], R. Shankar[1], L. A. DiMeglio[1]. [1]Section of Pediatric Endocrinology and Diabetology, JW Riley Hospital for Children, Indianapolis, Indiana, 46202; [2]Department of Endocrine Neoplasia and Hormonal Disorders, University of Texas M.D. Anderson Cancer Center, Houston, Texas, 77030; and [3]Section of Pediatric Endocrinology and Diabetology, Stanford University School of Medicine, Stanford, California, 94305.

Infantile hypercalcemia has been reported in ~15% of infants and children with Williams Syndrome (WS). The cause of hypercalcemia is unknown. The hypercalcemia is typically mild and transient, but can be severe and life threatening. In these cases, bisphosphonate therapy may be appropriate. We report 2 children with WS, confirmed by FISH probes, with severe hypercalcemia treated with intravenous (IV) pamidronate after conservative measures were unsuccessful. The first patient was an 11-month-old male presenting with a several month history of decreased appetite, constipation, decreased tone and increased irritability. Initial investigations included total calcium 17.7 mg/dL, phosphorus 4.7 mg/dL, intact PTH 5 pg/mL, 25-OHD 27 ng/mL, $1,25(OH)_2D$ 9 pg/mL, and urine Ca/Cr ratio 0.75. A renal ultrasound demonstrated nephrocalcinosis. After 48 hours of IV fluids, furosemide, dietary calcium restriction, and calcitonin, the symptomatic hypercalcemia persisted (ionized calcium 7.6 mg/dL). He received IV pamidronate, 1 mg/kg over 6 hours, and his calcium normalized in 2 days. Ten days after this treatment, he developed asymptomatic hypocalcemia (ionized calcium 3.4 mg/dL). This resolved after liberalizing dietary calcium. By age 2 years, he was on an unrestricted diet with normal serum calcium. The second patient was a 14-month-old male who presented with several months of increased irritability, feeding problems, and total calcium of 13.9 mg/dL. Oral furosemide therapy was begun. After 3 weeks his symptoms had not improved. At this time his total calcium was 13.5 mg/dL, phosphorus 5.1 mg/dL, intact PTH 4 pg/mL, 25-OHD 30 ng/mL, $1,25(OH)_2D$ 13 pg/mL, and urine Ca/Cr ratio 1.5. He received IV pamidronate, 1 mg/kg over 6 hours. Three weeks later his calcium normalized and symptoms resolved. Subsequently, he became irritable with total serum calciums >11.5 mg/dL at ages 15 months, 20 months, and 23 months. Each time, he received additional pamidronate infusions with serum calcium normalization and symptomatic improvement. Currently, he is 30 months of age with normal calciums on a low calcium, vitamin D restricted diet. In conclusion, WS associated hypercalcemia can be severe and symptomatic. It can be successfully and safely treated with IV bisphosphonate.

WG12

Gender Differences in the Maturation Rate of Bone Size and Mineral Accumulation. N.J. Crabtree[1,2], M. Kibirige[3], J. Fordham[3], C.B. Boivin[1], N.J. Shaw[2]. [1]Queen Elizabeth Hospital, Birmingham UK, [2]Birmingham Children's Hospital, UK, [3]James Cook University Hospital, Middlesbrough, UK.

Bone growth occurs both by increasing size and by accruing bone mineral. However, these processes are dissociated in time such that a child's bone is always closer to its adult size than its peak bone mass. This pattern of development has implications for the impact disease timing will have on peak adult bone health. The aim of this work was to examine the patterns of bone maturation in a cross sectional group of healthy school children. 637 healthy white children aged 5-18 years, had whole body & lumbar spine DXA. Percentage of peak values was calculated for bone mineral content (BMC), bone area, and bone mineral density (BMD) for the arms, legs, lumbar spine & total body, using gender specific mean values at age 18 years. From as early as age 5 years girls were closer to their peak values than boys for BMC, BMD and bone area in all regions except the lumbar spine. Additionally, girls demonstrated a faster maturation rate such that by age 11-12 years the separation in maturation level was at its greatest. In relation to puberty, girls demonstrated their greatest linear growth between pubertal stages 1 & 2. This was closely followed by their maximum increase in bone mineral accrual by stage 3 such that by this stage (13.8 years) they had reached 83.5% of their peak bone mass and 90.0% of their peak bone size. However, boys at the same pubertal stage (13.6 years) had only reached 62.7% of their peak bone mass and 80.3% of their peak bone size. Also observed in boys was a slow down in maturation rate between pubertal stages 2 & 3 before a final growth spurt during pubertal stages 3 to 4.

	Girls		Boys	
	5-6 years	11-12 years	5-6 years	11-12 years
Total BMC	*33.6 (0.7)	*68.1 (1.9)	*29.2 (0.5)	*55.6 (1.6)
Total Area	*46.5 (0.8)	*79.2 (1.5)	*42.2 (0.6)	*68.7 (1.4)
Total BMD	*71.8 (0.5)	*85.4 (0.9)	*68.8 (0.4)	*80.6 (0.9)
L2L4BMC	25.8 (0.5)	*58.7 (2.2)	24.8 (0.6)	*47.1 (1.6)
L2L4Area	45.5 (0.7)	*75.7 (1.6)	44.9 (0.7)	*69.6 (1.4)
L2L4BMD	55.9 (0.7)	*75.7 (2.2)	55.1 (0.8)	*67.4 (1.4)

* Difference between age matched sexes p<0.001

This data is limited by its cross sectional nature. However, the significant difference between the more constant linear growth & mineral accrual for girls compared to the slightly bi-phasal pattern observed in boys may have great importance when assessing the impact of disease timing, duration & proposed bone therapy when assessing a child's bone health.

WG13

Dual Energy X-ray Absorptiometry is a Valid Tool to Reflect Long Bone Calcium Content in Piglets. R.C. Mollard*, H.R. Kovacs*, U.R. McCloy*, H.A. Weiler*. Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada.

Dual energy x-ray absorptiometry (DXA) is commonly used to measure bone mass in infants and pediatric animal models. Recently, the role of long chain polyunsaturated fatty acids in bone has revealed that bone Ca is elevated using mineral analysis. Our laboratory has shown that long chain polyunsaturated fatty acids elevate bone mass using the piglet model. Previously, DXA has been validated for measurement of whole body and femur bone mass in piglets but not specific mineral content. As a result, this study was undertaken to compare bone mass of the femur using DXA, total ash weight, Ca and P content. Femurs were excised from 21-d old male piglets (n=35, 5-7 kg) and cleaned of soft tissue. Following measurement of weight and length, femurs were placed in a plastic container with water at a content depth and measured for bone mineral content (BMC) using a 4500A Hologic DXA in the sub-region hip scan mode. Femurs were then ashed using a muffle furnace followed by measurement of Ca and P using ICP emission optical spectrometer. Values were expressed per femur and also corrected to weight or length of femur or whole body. Relationships between variables were detected using Pearson product moment correlations. Correlation coefficients are reported in the table below.

	BMC (g)	BMC/whole body length (g/cm)	BMC/whole body weight (g/kg)	BMC/femur length (g/cm)	BMC/femur weight (g/g)
Dry Ash Weight (g)	0.87*	0.23	0.80*	0.84*	-0.31
Total femur Ca (g)	0.88*	0.25	0.81*	0.85*	-0.30
Total g P/femur (g)	-0.86*	-0.21	-0.80*	-0.83*	0.30
Total Ca/g femur (g/g)	0.93*	-0.61*	0.86*	0.84*	-0.61*
Total P/g femur (g/g)	0.92*	0.20	0.86*	0.83*	-0.61*
Total Ca/cm femur (g/cm)	0.88*	0.24	0.81*	0.77*	-0.50*
Total P/cm femur (g/cm)	0.86*	0.21	0.78*	0.74*	-0.51*

*p<0.003.

Based on this data, DXA is a valid tool to reflect long bone ash weight and Ca content. The relationship of BMC with bone ash and Ca is comparable after consideration of femur length or whole body weight. In practice, measurement of femur weight and length in vivo is not possible for longitudinal studies. Thus, correction of BMC to body weight is advisable if growth is not consistent among treatment groups and ex vivo measurement is not possible.

WG14

Correlation between Impact-Loading Activities and Bone Strength Indices in Premenarcheal Girls. P. Nanyan[1,2], C. Jaffré[1,2], L. Benhamou[2], E. Lespessailles[2] and D. Courteix[1,2] . [1]Laboratoire de la Performance Motrice, Université d'Orléans;[2]Inserm ERIT-M 010, CHR d'Orléans, France.

This investigation compares the effects of impact-loading activities on bone geometry and indices of bone strength. 68 healthy premenarcheal girls (age 10.69 ±1.58 years), 25 actives and 43 controls participated in this study. The actives were involved in impact-loading sport such as gymnastics and judo. Standard radiographs of the proximal phalanx (third digit) of the non-dominant hand were obtained in those girls and scanned with a 100 µm resolution at an 8 bit pixel grayscale resolution (256 shades of gray) by the same observer. Our program automatically extracted bone edge of the phalanx, using the Deriche's recursive filtering applied to the gradient norm of the digitized radiographs' image. The geometric variables, outer (D) and inner (d) diameters, total area (ToA), cortical thickness (CT) at the ulnar (u) and radial (r) were assessed at the phalanx shaft using an iterative contour detection approach by detecting the gray levels peaks. Bone strength indices, cross-sectional moment of inertia (CSMI) and section modulus (Z) were derived as follows:

$$\text{ToA} = \pi * D^2/4,\ CSMI = \pi\left[\left(\frac{D}{2}\right)^4 - \left(\frac{d}{2}\right)^4\right] \text{ and } Z = 2\,\frac{CSMI}{D}$$

The root mean square coefficient of variation was 0.083% for our technique. Bone age and height correlated significantly ($p < 0.05$) with ToA, CSMI and Z with coefficient of correlation between 0.26-0.38. CT on the radial side correlated significantly ($p < 0.01$) with bone age ($r = 0.32$), height ($r = 0.30$) and weight ($r = 0.33$). A significant correlation was obtained between the duration of training and CSMI ($r = 0.27$, $p < 0.05$) as well as Z ($r = 0.27$, $p < 0.05$) and ToA ($r = 0.32$, $p < 0.01$). These correlations suggest that impact-loading activities could enhance bone strength in premenarcheal girls.

WG15

Multicenter Study of Carpal Bone Density Measured by Norland DXA Scanners to Assess Skeletal Maturation in Chinese Boys and Girls. J. M. Wang[1], F. W. Guo[2], D.M. Luo[3], J. H. Mei[4], and T. V. Sanchez[5]. [1]Norland--a Cooper Surgical Company, Beijing, China, [2]Hai Dian Hospital of Beijing, Beijing, China, [3] Beijing University of Physical Education, Beijing, China, [4]Harbin Orthopedic and Trauma Hospital, Harbin, China, [5]Norland--a CooperSurgical Company, Socorro, NM, USA.

The assessment of carpal bone density by DXA has been shown to be a precise and effective method of assessing skeletal maturation in boys and girls. The present study aims to develop a reference set relating skeletal maturity to carpal bone density by expanding earlier work to a larger population of Chinese boys and girls from four facilities in China. A population of 450 males and 450 females between the ages of 5 and 22 years old from five areas of China underwent examination of carpal bone density on a Norland table scanner or the Norland pDEXA system. All scans were performed using Research Scan Software with a point resolution of 1.0 x 1.0 mm and a scan speed of 45 mm/s on table scanners and 40 mm/s on the pDEXA system. All studies in this report were audited and analyzed by the same factory trained operator (JMW). Carpal bone density results from different areas of China did not differ significantly from each other. Carpal bone density measured by the table scanners and the pDEXA did not differ significantly from each other. Carpal bone mineral density showed a significant correlation with chronological age in both male ($r = 0.95$) and female ($r = 0.95$) subjects. As with earlier studies, boys and girls did not differ in carpal bone density between the ages of 5 and 12 years. Beyond the age of 12, Chinese males were shown to have progressively greater carpal bone density than females so that by the age of 21 years, females had 80% of the density seen in males. In conclusion, this report provides a reference set with which to assess skeletal maturity in Chinese boys and girls when using the Norland table scanner or pDEXA. As with earlier studies, male subjects over the age of 12 were found to develop significantly greater carpal bone density than females.

ADULT BONE AND MINERAL WORKING GROUP ABSTRACTS

WG16

High Prevalence of Hypovitaminosis D and Osteoporosis in North Indian Women with Rheumatoid Arthritis Despite Adequate Sunshine. A. Aggarwal[1], S.K. Gupta[2], R.N. Misra[1], A. Lawrence[2], U. Singh[2], M.M. Godbole[2]. [1]Department of Medical Endocrinology, Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India, [2]Biostatistics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

Hypovitaminosis-D and osteoporosis is common in subjects with rheumatoid arthritis (RA) residing in temperate climate. No information is available in Indian subcontinent, region with abundant sunshine. We studied calcium metabolism, calcium intake, sun exposure, serum 25(OH)D3 and bone mineral density (DXA) at lumbar spine (L1-4-AP) and hip region in 95 women with RA (age 48.0 „b10.4yrs, disease duration 9.1 „b6.5yrs). Dietary calcium intake and average sun exposure were 914„b513 mg/day and 16.3„b12 min/day respectively. Hypocalcemia (S.Ca<8.5mg/dl), hypophosphatemia (S.iP<3mg/dl), elevated s. alkaline phosphatase (>150 IU/L) were present in 18%, 15%, and 46% respectively. Vitamin-D deficiency (S.25(OH)D3 <15ng/ml) and severe vitamin-D deficiency (<8ng/ml) was present in 44% and 18% respectively. Osteoporosis (T score <2.5) was observed at LS-AP (39%), total hip (25%), femoral neck (40%). Glucocorticoids treated women had lower T-score ($p>0.05$) and Z-score ($p<0.05$) at all sites. Calcium supplemented group (50%) had higher serum 25(OH)D3 levels ($p<0.03$) but had similar BMD as compared to non-supplemented group. BMD at LS and total hip had negative correlation ($p<0.05$) with age, disease duration, age of onset but not with glucocorticoids, S.25(OH)D levels and sun-exposure. This study emphasizes high prevalence of hypovitaminosis-D and osteoporosis in women with RA from Indian subcontinent, thereby needing preventive measures.

WG17

A Lytic Bone Lesion after Biliopancreatic Bypass Surgery for Morbid Obesity. K. Laga, A. Van den Bruel, D. Vanderschueren, M. Bex, C. Mathieu, I. Samson, F. Dedeurwaerdere, R. Bouillon, E. Muls. Departments of Endocrinology, Orthopedic Surgery and Histology, University Hospital, Leuven, Belgium.

Brown tumours due to chronic secondary hyperparathyroidism are well known as a consequence of chronic renal failure but other etiologies are rare.

A 54-year old women with bone pain in her left ankle caused by a lytic lesion, was referred to the orthopedic surgeon. A bone biopsy revealed a giant cell tumour, which was subsequently radically resected and cemented. Histology of the resected lesion showed numerous giant cells in a background population of mononuclear cells, compatible with the diagnosis of a brown tumour .

Laboratory examination revealed secondary hyperparathyroidism (whole PTH value of 60.2 ng/l), due to a severe vitamin D deficiency (25OHD levels below 2 ng/ml). Bone turnover was increased as reflected by osteocalcin and urinary pyridinolines. There were no other deficiencies of vitamins and minerals. Two years before she had a biliopancreatic diversion for morbid obesity. She was not taking any vitamin supplements. Bone mineral density at diagnosis showed 0.503 g/cm² in the femoral neck(T score of –3.92 or 66% of expected value). For the lumbar spine the BMD at diagnosis was 0.887 g/cm²(T score : -1.75). We started with 25OHD supplements orally(calcifediol 2000 IU/day, 3000 IU/day after 1 month) and calcium(1000 mg/day). Four weeks later, serum 25OHD was detectable (13 µg/L), serum alkaline phosphate (481 U/L)lower, and serum PTH normal (11.8 ng/L). However the bone-turnover parameters remained high. Bone mineral density one year after normalisation of the vitamin D level showed a further decrease for the femoral neck : 0.457 g/cm² (T score of – 4.38 and a Z-score of 61%) and a steady state for the lumbar spine : 0.860 g/cm². Therapy with alendronate was started, and 10 months later the bone mineral density was increased up to 0.904 g/cm² for the lumbar spine and 0.486 g/cm² for the femoral neck. Finally osteocalcin and serum pyridoline normalised and remained stable with therapy with alendronate, calcifediol and calcium supplements. Radiologic follow-up of the cemented lesion remained stable, now 3 years after diagnosis.

Vitamin D deficiency as well as secondary hyperparathyroidism are well known as long term complications of biliopancreatic diversion. Fat malabsorption but also the diversion of the usual absorption site (proximal small bowel) and reduced enterohepatic recirculation may all be responsable for the vitamin D deficiency.

Biliopancreatic bypass, while producing excellent weight loss, caries a high metabolic price. Here we present a unique case of a brown tumour in the left ankle due to secondary hyperparathyroidism and vitamin D deficiency 2 years after such procedure.

WG18

Unilateral Bony Lesions and Hypophosphatemia. A. Bloch[1], A. Peyser[1], T. Yamashita[2], S. Fukumoto[3], J. Silver[1]. [1]Minerva Center for Calcium and Bone Metabolism, Hebrew University Hadassah Medical Center, Jerusalem, Israel, [2]Pharmaceutical Research Laboratories KIRIN Brewery Co. Ltd., Takasaki, Japan, [3]Department of Laboratory Medicine, University of Tokyo, Tokyo, Japan.

At the age of 20 years the patient presented with metatarsal stress fractures. Two years later he developed a limp on walking and hard nodules appeared on his right hand and knee together with nevi on the left side of his chest, mild gynecomastia and maxillary bone resorption. For the subsequent 10 years he had a serum phosphate of 0.3 mM (0.8-1.4), with a marked phosphaturia TRP=40-60% (85-90%), normal serum calcium (2.4 mM), alkaline phosphatase of ~240 u (40-130), PTH 30 pM (1.2-6.8) and serum $1,25(OH)_2D$ 10 pg/mL (20-50). CT showed subcutaneous calcification in the fingers of the right hand and knee with no evidence of a tumor. A maxillary bone biopsy showed osteomalacia. Total body CT and skeletal surveys did not demonstrate a tumor that would have substantiated the diagnosis of tumor-induced osteomalacia. The patient was treated with oral phosphorus and 1-alpha-hydroxy-vitamin D_3. A scan with octreotride for the somatostatin receptor that had been described as positive for tumors secreting FGF-23 detected hot spots in his right hip and knee. A lesion was removed from right knee (fibrous tissue with cartilage) with no improvement in clinical or biochemical state. The serum FGF-23 level was 350 pg/ml (normal 10 – 50). In summary, a patient presented right sided bony lesions, left sided skin lesions, hypophosphatemia, hyperphosphaturia and gynecomastia and elevated FGF-23 levels. The diagnosis of McCune-Albright Syndrome was made on the basis of the unilateral skeletal and atypical skin changes, gynecomastia and hyperphosphaturic hypophosphatemic osteomalacia which may occur in this syndrome. This case establishes that the changes in phosphate metabolism in McCune-Albright Syndrome are due to elevated levels of FGF-23. They raise the questions as to the source of the FGF-23 and therapeutic options such as somatostatin therapy and IV bisphosphonate for his fibrous dysplasia.

WG19

Case Report: Long Term Effects of Estrogen Replacement Therapy in a Male with Congenital Aromatase Deficiency Presenting in Late Adolescence. M. Bex, D. Vanderschueren, R. Bouillon. Department of Endocrinology, University Hospital Gasthuisberg, Leuven, Belgium

Aromatase deficiency was suspected in a 17 $^{10/12}$-year-old XX female of consanguineous parents of Northern African descent, presenting with primary amenorrhea, lack of breast development, cliteromegaly and a history of ambiguous genitalia at birth. Serum concentrations of testosterone and gonadotropins were markedly elevated while the estradiol level was very low. Her bone age was greatly delayed at 10.3 years. MRI imaging of the pelvis demonstrated two hypoplastic ovaries adjacent to a small uterus, in contrast to previous published but younger female cases that had polycystic ovaries as a major feature.

Evaluation of her two male siblings demonstrated a delay in bone age of 4 years in the younger, aged $16^{4/12}$.

Genetic analysis of the CYP19 gene (J. Deladoëy, C. Flück and P.E. Mullis, Bern, Switzerland) revealed a homozygous C-base deletion in exon 5 in the propositus and her younger brother. This frame-shift mutation resulting in a prematurely terminated inactive protein has also been identified in a Swiss consanguineous family [(1)].

Anthropometric and bone data of the affected brother are listed in table 1. At age $16^{4/12}$ he had Tanner stage 5 pubic hair and genital development after experiencing normal puberty. He had elevated testosterone levels with high normal gonadotropins and low estradiol and estrone. Despite a bilateral high normal testicular volume an extreme asthenoteratozoospermia was diagnosed. No improvement of sperm quality was observed during replacement treatment with Estradiol valeras 1 mg orally daily from age 17. Follow up of body composition and bone for 3.5 years are summarized in table 1.

TABLE 1	Baseline		1.3 years of E2 (age 18 $^{5/12}$)		3.5 years of E2 (age 20 $^{6/12}$)	
Bone Age (TW2)	12 y 5 m		15 y 1 m		16 y 8 m	
Height (cm)	176,3		184,6		191	
Weight (kg) (BF%)	91 (35.3)		111 (38.6)		129 (40.4)	
Hologic QDR 4500	T-score	BMAD	T-score	BMAD	T-score	BMAD
L1-L4	- 2.31	0.120	-0.51	0.142	-0.55	0.136
Femoral Neck	-1.01	0.156	-0.07	0.147	0.44	0.147
Radius 1/3	-3.63	0.255	-2.58	0.244	-1.84	0.247
Total body	-1.92		-0.70		0.28	

(BF%: Body Fat %; BMAD: bone mineral apparent density)

This adolescent boy with aromatase deficiency responded to estrogen replacement therapy with a normalisation of bone maturation and improvement of BMD. Spermatogenesis was not influenced and obesity worsened over time.

Conclusion: the low BMD observed in untreated aromatase deficient patients is due to a delay in skeletal maturation. Estrogen replacement therapy induces the increase in BMAD that is normally observed during puberty.

Reference 1: J. Deladoëy, C. Flück, M. Bex et al. JCEM 84: 4050-4, 1999

WG20

Resolution of Persistent Bisphosphonate-resistant Idiopathic Hypercalcemia following an Empiric Trial of Itraconazole. R. Christian, R. Wermers. Departments of Endocrinology and Metabolism, Mayo Clinic, Rochester, MN, Rodney Stout, MD, Holzer Clinic, Gallipoli, Ohio

Objective: To present a case of chronic hypercalcemia of unknown etiology that was unresponsive to glucocorticoid and bisphosphonate therapy but resolved after empiric treatment with itraconazole.

Methods: We present a case report, which includes clinical, laboratory, pathology and radiology data.

Results: 59 yo white male foundry worker was referred for two year history of mild to moderate (11-14 mg/dl) hypercalcemia of unknown etiology despite thorough investigation. Prior to referral he had been treated twice with iv pamidronate with a transient lowering of serum calcium , but subsequent iv bisphosphonate therapy (pamidronate and zoledronate) and iv glucocorticoids had no effect. He had a 25 year history of type 1 diabetes mellitus complicated by retinopathy, nephropathy and peripheral neuropathy, hypertension and tobacco abuse. He had no history of kidney stones or fractures, thyroid disease, head or neck irradiation, pancreatitis, tuberculosis or malignancy. There is no history of hypercalcemia, kidney stones, or osteoporosis in his parents, 11 siblings or 4 children.

Laboratory evaluation revealed renal insufficiency (creatinine 2.7), anemia with normal SPEP, low PTH (0.8 pmol/L), undetectable PTH-rp, normal 1,25 vitamin D (50 pg/ml) and free retinol, elevated calcium (11.2 mg/dl) with normal phosphorus and albumin, calcium to creatinine clearance ratio of 0.05, normal thyroid function tests and am cortisol, no hypercalciuria, low levels of N-telopeptides in a 24 hour urine collection, and normal serum ACE level. Fungal serologies in blood and urine were negative with the exception of low titer histoplasmosis mycelial antibody.

Radiology evaluation included a negative PET scan, and a chest x-ray showing calcified hilar and mediastinal lymph nodes and granulomas. BMD at the radius was in the osteopenic range. Bone biopsy revealed reduced bone and osteoid volume, greatly reduced eroded surface and no osteoclasts.

Conclusion: Our investigation failed to reveal a definitive cause for his hypercalcemia. However, we felt granulomatous disease remained in the differential due to hilar adenopathy and granulomas on chest x ray and inappropriately normal 1,25 vitamin D in the face of renal insufficiency. An empiric trial of antifungal therapy was recommended and his referring physician treated him with itraconazole for 4 weeks with prompt and persistent normalization of his serum calcium.

WG21

Oncogenic Rickets Due to a Phosphaturic Tumor. S. De Geronimo[1], A. Scillitani[2], E. Romagnoli[1], F. Paglia[1], J. Pepe[1], A Corsi[3], M. Riminucci[3], S. Minisola[1]. [1]Department of Clinical Science, "La Sapienza" University, Rome, [2]Department of Endocrinology, Casa Sollievo della Sofferenza Hospital, S.Giovanni Rotondo, Foggia, [3]Department of Experimental Medicine and Pathology, "La Sapienza" University, Rome, Italy

A 23-year-old patient was admitted to our hospital because of muscle weakness, bone pain and spontaneous fractures previously developed during the past 6 years. The patient's clinical history began in 1996, when he started to complain of bone pain and walking difficulties. Therefore a x-ray of lower limbs was performed, revealing a fracture of the left distal femoral epiphysis, ascribed to a previous car accident. During the following three years he experienced several spontaneous fractures. Waddling gait and worsening symptoms were reported and eventually he was forced to use a wheelchair. A x-ray performed in 1999 showed fractures of both femoral necks, pelvis deformation and the persistence of growth plates. On this occasion, a metabolic evaluation was carried out which showed severe hypophosphatemia (0.8 mg/dL; n.v. = 2.6-4.5) and increased total alkaline phosphatase (309 U/L, n.v. < 230). At the age of 22 years, he was bedridden; x-rays showed several pseudofractures (Looser zones), kyphosis due to several vertebral fractures, chest and pelvis deformation with persistence of growth plates; a widening of the previous fracture of the left distal femoral epiphysis was also noted. A bone scintigraphy, performed in this period, showed generalised increased uptake throughout the skeleton with a prominent activity at the costochondral junctions. Main biochemical parameters of mineral metabolism, performed upon admission in our hospital, revealed hypophosphatemia (0.85 mg/dL), reduced tubular phosphate reabsorption ($TmPO_4$/GFR = 0.58 mg/dL; n.v. 2.5-4.2), increased total alkaline phosphatase (970 U/L, n. v. < 279) and low 1,25-dihydroxyvitamin D (14.4 pg/mL; n.v. = 19.9-67). Lumbar BMD was severely reduced (0.340 g/cm^2, T-score = - 6.83). A spiral CT scan revealed a focal lesion measuring 3 cm at its largest dimension located in the left ethmoid, that was visualized by Indium-111octreotide. The tumor was completely removed at surgery; histology was consistent with mesenchymal phosphaturic tumor, hemangiopericytoma like. The biochemical picture completely reversed as soon as 1 month after surgery (P = 2.9 mg/dL; $TmPO_4$ = 2.97 mg/dL; $1,25(OH)_2D$ = 31.5 pg/mL). To our knowledge, this is one of the few reported cases in which a phosphaturic tumor prevented the closure of the growth plate until the adult age.

WG22

Severe Hypercalcemia in Pregnancy. Familial Hypocalciuric Hypercalcemia? M.F. Delaney[1], C. Magee[2], G. Hendy[3], E.M. Brown[1]. [1] Endocrine, Diabetes, and Hypertension Division, and [2] Renal Division, Brigham and Women's Hospital, Boston, MA; [3] Medicine, McGill University, Montreal, Canada.

Hypercalcemia is rarely seen during pregnancy, and usually is caused by hyperparathyroidism (80%). Typically, the serum calcium and PTH decrease during the second trimester and the ionized calcium remains normal. We describe a 37yo caucasian female who was admitted to Brigham and Women's Hospital 30 weeks pregnant with nausea, vomiting, and severe hypercalcemia. Her calcium was 23 mg/dl with a BUN of 27 mg/dl and creatinine of 1.8 mg/dl, PTH of 17 pg/ml, phosphate of 1.0 mg/dl, PTH-rp <assay pmol/L, 25-OH vitamin D of 25 ng/ml, 1,25 vitamin D of 25 and normal thyroid function tests. She described a 1 week history of gastroenteritis with nausea and vomiting for which she took TUMS and milk. Daily, she took in excess of 12 grams of elemental calcium for almost 2 weeks. On admission, she was hydrated with intravenous fluids, and given sq calcitonin, furosemide, and betamethasone (for fetal lung maturation). She became hypocalcemic once her renal function improved (creatinine 0.8 mg/dl), calcium dropped to 7.0 mg/dl with a magnesium of 1.2 mg/dl, phos 2.1 mg/dl, and PTH of 464 pg/ml. Her serum calcium was maintained with calcium 500mg tid and vitamin D supplementation on discharge.

She has a history of mild gastritis, mild hypertension, and takes no other medications. She is married and has two children. No family history of malignancy, sarcoidosis, TB, but there is a family history of renal disease and hypercalcemia.

For the remainder of her pregnancy, she decreased her calcium intake and her ionized calcium remained high (6.0 mmol/L; normal range: <5.3). Peripartum, her ionized calcium remained high at 5.8 mmol/L with PTH 17 pg/ml, PTH-rp <assay, phos 3.3 mg/dl and magnesium 1.6mg/dl. Postpartum, her ionized calcium has remained elevated 5.6 mmol/L with PTH 33 pg/ml, 1,25 vitamin D 40 pg/ml, serum protein electrophoresis normal, and low normal range magnesium. She did not breast feed. Chest x-ray, serum Ace level, and breast examination were all normal. During the severe hypercalcemia, her 24 hour urine calcium was 353mg/24 hour. Postpartum, 24 hour urine calcium was very low with a urine calcium/creatinine ratio of 0.009, PTH 85 pg/ml, and calcium 9.9 mg/dl.

Her son was born with hypercalcemia 11.6 mg/dl (birth), and 11.0 mg/dl (2 months). Also, her mother has mild hypercalcemia and renal disease, and sister has hypercalcemia (no lab data available).

In summary, this 38yo female developed severe hypercalcemia during pregnancy, and remains hypercalcemic postpartum with a low urine calcium. We discuss the differential diagnosis, including Hyperparathyroidism, Milk-Alkali Syndrome, and Familial Hypocalcemic Hypercalciuria.

WG23

Fibrogenesis Imperfecta Ossium – Symptomatic and Histologic Improvement with Melphalan Therapy. P.R. Ebeling, R. Smith*, M.A. Brown. Botnar Research Centre, Oxford University Institute of Musculoskeletal Sciences, Nuffield Orthopaedic Centre, Oxford, U.K.

A 70 y.o. man presented with low trauma fractures, increased bone turnover and IgG paraproteinemia. There was no family history of bone disease or parental consanguinity. The first fractures occurred at 49 yrs and involved the ribs, right patella, and 2nd lumbar vertebra. Further fractures followed rapidly at the 12th thoracic and 4th lumbar vertebrae, resulting in inability to walk or stand for 7 years.

X-rays revealed coarse trabecular patterns in the pelvis, proximal femora and vertebral bodies. Subsequently, new bone formation occurred between vertebrae and at the lesser trochanters. Plasma total alkaline phosphatase (TALP) was increased, calcium, phosphate, iPTH, 25(0H)D and $1,25(0H)_2D$ were all normal. Two serum IgG paraprotein bands were detectable; IgG kappa and lambda at 4.7 g/L; urine Bence Jones proteins were normal. Bone marrow immunofluorescence staining showed normal plasma cell numbers but an increased percentage (65%) stained for IgG heavy chains. Serum immunoglobulins were all normal. A bone biopsy showed thickened trabeculae covered with wide unmineralized osteoid seams. Increased osteoclastic and osteoblastic activity resulted in a mosaic rather than lamellar bone appearance. Scanning electron microscopy, showed a disrupted collagen fibre network with altered collagen fibres and scattered masses of calcification occurring within abnormal matrix.

Treatment with alfacalcidiol and melphalan 10 mg daily for 5 days/month was given for two periods of 15 and 60 months. During the second melphalan course fractures stopped, bone turnover decreased but remained elevated, and bone histology returned to normal. Eight years later fractures recurred and bone turnover increased. Bone turnover markers (TALP, PINP and NTx) decreased partially after intravenous therapy with pamidronate and zoledronate, and oral calcitriol treatment, but fractures continued. Melphalan therapy was restarted, but caused severe nausea and weight loss. Fibrogenesis imperfecta ossium (FIO) is a rare acquired disorder where normal bone is replaced by structurally unsound collagen-deficient tissue. Paraproteinemia is a striking and unexplained feature. More than eighteen cases of FIO have been described and will be reviewed.

WG24

The Gatrointestinal Link to Osteoporotic Fractures. L. Eck, C. Jachna. Kansas University Medical Center, Kansas City, KS.

LEARNING OBJECTIVES: To recognize that celiac disease may contribute to low peak bone mass and adult osteoporosis leading to disabling fractures.

CASE INFORMATION: A 49 year old white male with a history of calcium oxalate kidneys stones and atraumatic fractures including a hip fracture at age 22, rib fractures and a recent wrist fracture presented for osteoporosis evaluation. Bone mineral density studies revealed a BMD of 0.868 gm/cm^2 at L3-4 (t-score of –3.1) and a BMD of 0.962 g/cm^2 at the total hip (t-score –1.0). On history, the patient reported a history of kidney stones treated with Urocit, HCTZ, and dietary oxalate restriction, and a long-standing history of multiple loose bowel movements per day associated with abdominal cramping. Lab studies were notable for an elevated IgG antigliadin antibody of 132 Units and hypercalciuria. Vitamin D and PTH levels were normal. Duodenal biopsy was obtained and revealed partial villous atrophy consistent with celiac disease.

DISCUSSION: Celiac disease is a disease caused by a genetically based inability to digest gluten, a major protein commonly found in grains. Due to the availability of new highly sensitive and specific serologic diagnostic tests, it is increasingly identified in the United States. Celiac disease is often asymptomatic but symptoms suggesting the diagnosis include bloating, flatulence, and chronic diarrhea. Other than gastrointestinal symptoms, celiac disease also has a wide spectrum of extra intestinal manifestations including iron deficiency anemia, low bone mineral density, and dermatitis herpetiformis. It can also result in calcium oxalate nephrolithiasis secondary to hyperoxaluria. Studies have shown that patients with celiac disease have an increased risk of fractures. Men may be more severely affected than women with bone manifestations due to estrogen's protective effects on the bone. The pathogenesis of low bone mass and osteoporosis in celiac disease is multifactorial. The reduction in surface area of the intestinal mucosa may contribute to calcium malabsorption and subsequent secondary hyperparathyroidism leading to increased bone resorption. Vitamin D deficiency is also believed to be common. Those who are undiagnosed during childhood and young adulthood may never achieve peak bone mass. Some researchers suspect that systemic effects of inflammatory cytokines involved in the intestinal mucosa may also contribute to the development of bone disease. Not only should patients with celiac disease be screened for osteoporosis, but patients with severe osteoporosis and few risk factors should be considered for evaluation of celiac disease. A long-standing history of undiagnosed celiac disease most likely contributed to our patient's development of osteoporosis.

WG25

High Prevalence of Hypovitaminosis D in Community Dwelling Ambulatory Postmenopausal Women in Northern India Despite Adequate Sunshine. S. Gupta, N. Mittal, M. Dubey, K.K. Chaudhary, M.M. Godbole. Department of Medical Endocrinology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India.

Vitamin D deficiency is an important risk factor for osteoporosis. There is no information about vitamin D status in North Indian postmenopausal women. We assessed vitamin D and bone mineral density (BMD) in postmenopausal women. We estimated serum 25(OH) D, intact parathyroid hormone (iPTH, IRMA) AND BMD (DXA, lumbar spine L1-L4 and hip) in 83 postmenopausal women (mean age 52.4 „b9 yrs, range 34-75 yrs). Corrected serum calcium was normal in all subjects. Low phosphorous (<1.2 mmol/dl) and elevated serum alkaline phosphatase (ALP>125 IU/L) were observed in 20%and 52% of the subjects respectively. Mean serum 25(OH) D (n=83) and iPTH (n=33) were 47.5 „b30.8 nmol/L. (range 12.5-165) and 70.4„b46.1 ng/L (range 19-180) respectively. Vitamin D deficiency was observed (serum 25(OH) D < 37.5 nmol/L) in 45(49%) subjects, and severe vitamin D deficiency (serum 25(OH) D<20 nmol/L) in 16.0(19%) subjects. Elevated serum PTH (>55 ng/L) was seen in 15(45%) subjects. Serum iPTH levels co!

rrelated inversely with log transformed serum 25(OH) D levels(r=0.11,p=0.01). Z score was <-1 at total hip in 42(50%), at femoral neck in 31(37%), lumbar spine L1-4 (LS) anteroposterior in 51(61%) and LS lateral in 30(36%) subjects. There was no significant difference in corrected serum calcium, ALP, 25(OH) D and iPTH levels between patients with Z score<-1 and those with Z score>-1.No correlation of serum 25(OH) D or iPTH levels with BMD at any skeletal site was seen. So, hypovitaminosis D is present in 49% of urban ambulatory Indian postmenopausal women despite adequate sunshine, necessitating adequate preventive measures.

WG26

A Study of Identical Twins with Fibrodysplasia Ossificans Progressiva (FOP): The Role of Environment In the Progression of Heterotopic Ossification. N. Hebela[1,2], E.M. Shore[1,2], F.S. Kaplan[1,3]. [1]The Center for Research In FOP & Related Disorders, [2]Departments of Orthopaedic Surgery, University of Pennsylvania School of Medicine, Philadelphia, PA, USA, [2] Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA, [3] Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Fibrodysplasia ossificans progressive (FOP) is a rare and disabling genetic disorder characterized by congenital malformation of the great toes and by progressive heterotopic ossification in characteristic anatomic patterns. Disease severity varies greatly among unrelated individuals as well as among individuals within the few multigenerational families that exist worldwide. Environmental triggers for heterotopic ossification such as blunt trauma, intramuscular injections, surgery, and viral illnesses have long been suspected as causing flare-ups leading to heterotopic ossification in patients with FOP, but the relative effects of environmental triggers on this genetic background are unknown. Evaluation of identical twins with FOP provides an opportunity to identify genetic versus environmental factors in disease progression. We compared the medical histories, physical examinations, and radiographs of three pairs of identical twins with FOP and found that within each pair, the congenital toe malformations were identical, but the natural history of heterotopic ossification varied greatly depending upon life history. We found that various types of cellular and tissue trauma have the most significant effects on the temporal and spatial progression of heterotopic ossification postnatally. Influencing factors include blunt trauma, immunizations, operations, and viral illnesses. We conclude that people with FOP have both a genetic predisposition to the condition and history of environmental exposures that modifies the phenotypic expression of genetic susceptibility. Our study confirms the importance of environmental factors in triggering heterotopic ossification in a genetically susceptible population.

WG27

Physiologic Evaluation of a Patient with Oncogenic Osteomalacia Before and After Cure. H. J. Heller, J. E. Zerwekh, C. V. Odvina, J. Raisanen*. UT Southwestern Medical Center, Dallas, TX.

57 year-old WM was referred for osteomalacia. He first noted pain at the left lower ribcage. Alkaline phosphatase was > 300. Resection of a rib suspicious for metastasis was "benign." Bone scan revealed increased uptake at 10 areas in the ribcage. He developed right hip pain and lower back pain and was diagnosed with a stress fracture of the right femur. Serum phosphate and 1,25-(OH)2 D were low, and Rocaltrol 1 mcg/day was started. Pain improved slightly but alkaline phosphatase remained elevated and he had difficulty walking.

He was then referred to our center. His only risk factor for osteomalacia was poor dairy intake. Labs at baseline (2 weeks off Rocaltrol) and follow-up are noted in the table below. In addition, TmP/GFR was low at 1.8, and intestinal calcium absorption (dual stable calcium isotope, 100 mg calcium carrier) was low at 28%. Bone histomorphometry confirmed osteomalacia (O.Th. 58 mcm; increased MLT). Clinical symptoms markedly improved on treatment with phosphate and Rocaltrol, but he still had some hip and rib discomfort and walked with a limp. Trabecular bone mineral density greatly increased on treatment, but cortical BMD trended downward. Review of previous bone scans later localized a small lesion to the skull. MRI of the skull, but not plain x-rays, revealed destruction of the right greater wing of the sphenoid by a 2.8 X 1 cm mass. Curative resection of the mass revealed cytologic and architectural features reminiscent of hemangiopericytoma. Off medications, serum phosphate rose to 3.0 mg/dl within 24 hours of surgery. He had rebound calcitriol production, which resulted in hypercalciuria. Poor intestinal calcium absorption with suppressed synthesis of calcitriol and treatment with phosphate combine to accentuate the risk of tertiary hyperparathyroidism in patients with oncogenic osteomalacia.

Labs	Baseline	On Treatment, Prior to Cure	After Cure, 7 wks
Serum Ca, mg/dl	9.3	9.2	9.5
Serum P, mg/dl	2.1	1.3	3.6
Serum AP, IU/L	420	131	104
Serum PTH, pg/ml	37	22	18
Serum 25OHD, ng/ml	33	18	27
Serum 1,25-(OH)2 D, pg/ml	9	34	55
Urinary Ca, mg/d	102	206	283
Free DPD, nM/mM Cr	16	4.6	6.8

WG28

Elevated 1,25-dihydroxyvitamin D in Patients with Crohn's Disease: A Novel Risk Factor for Low Bone Mineral Density. M. Hewison[1], M. T. Abreu[2], V. Kantorovich[3], E. A. Vasiliauskas[2], U. Gruntmanis[4], R. Matuk, K. Daigle[4], S. Chen[4], D. Zehnder[1], Y-C. Lin[4], H. Yang[4], J. S. Adams[3]. [1]Division of Medical Sciences, University of Birmingham, Birmingham, United Kingdom, [3]Division of Endocrinology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA, and [2]Division of Gastroenterology, Inflammatory Bowel Disease Center, [4]Department of Pediatrics, Division of Medical Genetics, Steven Spielberg Pediatric Research Center, Burns and Allen Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA.

Many patients with Crohn's disease (CD) have low bone mineral density (BMD) and this does not appear to be solely attributable to corticosteroid use. We hypothesized that low BMD in patients with CD is associated with elevated circulating levels of the active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). We further hypothesized that this was secondary to increased synthesis of $1,25(OH)_2D_3$ by inflammatory cells in the intestine. The aim of this study was to examine the relationship between $1,25(OH)_2D_3$ levels and BMD in patients with CD and ulcerative colitis (UC). Based on an IRB-approved, retrospective review of medical records from patients with CD (n=138) or UC (n=29), measurements of vitamin D metabolites, intact parathyroid hormone (iPTH) and BMD were carried out. Corticosteroid use was categorized as none, low (<6 months of exposure) or high (>6 months of exposure). Lastly, immunohistochemistry for the vitamin D-activating enzyme 1α-hydroxylase was performed on colonic biopsies from patients with CD and normal colon. Inappropriately high levels of serum $1,25(OH)_2D_3$ (> 60 pg/ml) were observed in 42.0% of patients with CD compared to only 7% in UC, despite no significant difference in circulating iPTH levels. Mean serum $1,25(OH)_2D_3$ levels were higher in CD (57 pg/ml) versus UC (41 pg/ml) (p=0.0001) or controls (42 pg/ml) (P<0.0001). There were no significant differences in $1,25(OH)_2D_3$ levels between patients with low and high corticosteroid use. However, in patients with CD, there was a negative correlation in $1,25(OH)_2D_3$ levels and lumbar BMD (r= -0.301, p =0.005). This was independent of corticosteroid use suggesting independent association with BMD. Immunohistochemistry revealed intestinal macrophages expressing 1α-hydroxylase in patients with active colonic CD but not controls. These data indicate that extra-renal synthesis of $1,25(OH)_2D_3$ in the diseased intestine of patients with CD can lead to elevated circulating levels of the hormone. This in turn appears to be a risk factor for osteoporosis and may serve as an additional marker of CD.

WG29

Bartter's Syndrome in a Patient with Pseudohypoparathyroidism type I. D. Inoue, I. Endo, Y. Ito, Y. Sumitomo, M. Kato, T. Matsumoto. Department of Medicine and Bioregulatory Sciences, University of Tokushima, Tokushima, Japan.

Bartter's syndrome is a heterogenous disorder characterized by deficient renal reabsorption of sodium and chloride, hypokalemic metabolic alkalosis, and elevated renin and aldosterone levels. Mutations in at least four different genes have been identified as causes of the disorder: sodium-potassium-chloride cotransporter (*NKCC2*), renal outer medullary potassium channel (*ROMK*), chloride channel (*CLCKB*), and β-subunit of CLCKB (*BSND*) gene in type1, 2, 3 and 4 Bartter's syndrome, respectively. We report here a case of sporadic, adult-onset pseudohypoparathyroidism (PHP) type I presenting Bartter's syndrome. A 32 yr-old Japanese female developed generalized convulsion and was referred to Tokushima University hospital. The initial laboratory examination revealed hypocalcemia (Ca 1.1 mmol/L; reference range: 2.1-2.6), hyperphosphatemia (P 1.6 mmol/L; 0.8-1.5), hypokalemia (K 3.1 mol/L; 3.5-4.8), hypomagnesemia (0.6 mmol/L; 0.66-0.94) and metabolic alkalosis ($HCO3^-$ 31.6 mmol/L; 22-26). Serum PTH levels were high (intact PTH 102 ng/L, 13-55), and Ellsworth-Howard test revealed no responses of urinary phosphate or cAMP excretion to exogenous PTH. The patient also showed typical features of Albright's hereditary osteodystrophy including short stature, round face and shortened fourth fingers. Serum Ca and P levels of her parents were normal. Hypokalemia was accompanied by increased plasma renin activity (3.7 ng/ml; <2.3) and high normal serum aldosterone levels (130 pg/ml, 30-159). Hypokalemia was spironolactone-sensitive, and the absence of PTH response persisted even after serum potassium levels were normalized, excluding a possibility that hypokalemia itself blunted PTH effects. Based on these data, we diagnosed her as a rare combination of PHP type Ia and Bartter's syndrome. Search for a Gsα mutation is under way. Watanabe et al. reported association between activating mutations of calcium-sensing receptor (CaSR) and Bartter's syndrome (Lancet 360;692, 2002). Although they hypothesized that CaSR-dependent signals inhibit ROMK activity, our case appears to involve a distinct mechanism. Assuming that GNAS1 gene imprinting occurred in the thick ascending limb of Henle in our case, defective cAMP signaling pathways could lead to decreased ROMK activity, which is known to be modulated by PKA, or reduced NKCC2 expression, whose promoter has cAMP response element (CRE). Severely lowered extracellular calcium concentrations may also negatively affect activation of CREB, a transcription factor acting downstream of cAMP. Further analysis will be necessary to determine the molecular basis of association between hypoparathyroidism and Bartter's syndrome with hypokalemia.

WG30

Hypervitaminosis D Caused by Overexposure to UV Light From A Tanning Bed. M. Kamil, A. Hanbali, M. Honasoge, D. S. Rao. Bone & Mineral Division, Henry Ford Hospital, Detroit, MI, USA.

It is well known that tanning beds are a good source of UV light, which enhances the endogenous cutaneous production of vitamin D. However, it is not known if excess tanning would lead to hypervitaminosis D. We report a case of presumed hypervitaminosis D caused by overexposure to UV light from a tanning bed.

A 57 year old Caucasian woman was seen for evaluation of refractory osteoporosis with multiple fractures (right foot, left radius and left ulna) and lack of change in bone density despite receiving residronate for over a year. She was on 945 mg of calcium citrate and 600 IU of vitamin D2. In addition, she gave a history of multiple kidney stones over the past 4 years requiring lithotripsy. Physical exam was unremarkable except for dark complexion, which she attributed to regular tanning for 25 to 30 minutes twice a month for 4-5 years and a walking cast on the left foot because of recent metatarsal fracture.

Laboratory evaluation revealed a serum albumin adjusted calcium of 10.6 mg/dl (N=8.2-10.4 mg/dl), normal creatinine (0.9 mg/dl), elevated 25-hydroxyvitamin D of 66 ng/dl (N= 12-60), and suppressed PTH of <2.0 pg/ml (N= 10-53). A 24 hr urine calcium was 363mg/day (N= <250 mg/day), and normal 1,25-dihydroxyvitamin D level of 26 pg/ml. Serum electrolytes showed hyperkalemia and mild metabolic acidosis. Bone density showed a T-score of –1.1 SD at the lumbar spine, –2.73 SD at the total hip, and –2.49 SD at the femoral neck. An IVP showed bilateral kidney stones suggestive of medullary sponge kidney. She was advised to discontinue the artificial tanning and repeat laboratory tests in 1 month. Commensurate with lightening of her complexion, both serum and urine calcium levels normalized (9.5 mg/dl and 158mg/d), 25-hydroxyvitamin D declined to 45 ng/ml, 1,25-dihydroxyvitamin D increased to 35 pg/ml, and PTH to 44 pg/ml.

To the best of our knowledge, this is the first case report of hypervitaminosis D induced by artificial tanning. Since hypervitaminosis D is associated with hypercalcemia, hypercalcuria, and bone loss, the frequency of kidney stones and metabolic bone disease in the artificial tanning population need to be studied. In addition, the duration and intensity of artificial UV light exposure that would produce hypervitaminosis D needs to be determined.

WG31

Study on Osteoporosis Risk Factors and some Bone Metabolism Parameters in Female Residents of Tashkent-city. .Y. Kandilyotu, F.M. Gafurova, L.B. Nugmanova, K.Y.Agababyan. Institute of Endocrinology of Ministry of Health , Uzbekistan.

We studied osteoporosis risk factors and some bone metabolism parameters in female residents of Tashkent-city aged from 45 to 60. We examined and interviewed 347 females aiming at detecting osteoporosis risk factors. All patients underwent ultrasound osteometry of four bones, such as, collarbone, lower jawbone, elbow bone and tibial bone. Calcium content was measured in blood serum and 24-hour urine. 212 (56.7%) of 347 women were in perimenopause (1st group) and 162 (43.3%) in postmenopause (2nd group). Mean age of menopause onset was 47.7 years. The most spread osteoporosis risk factors, such as, hypodynamia (37%), artificial (surgical) menopause (13%), early menopause (8%), late menarche (10%), asthenic constitution (height less than 157cm, body mass less than 58kg) (8%), coffee addiction (6%) and smoking (9%) were identified in the patients above. Decrease in the rate of ultrasonic spread was registered in 89% of the examinees, in 18% in one bone, in 53% in two bones, in 18% in three bones and in 11% in four bones examined. The lowest rate of ultrasonic spread was observed in lower jawbone and collarbone. Calcium content in blood serum of perimenopausal patients was found normal, in the postmenopausal females it was reduced by 6.4%. In the 1st group women calcium urine excretion was found increased in 43% and decreased in 16%. In the 2nd group patients the hypercalcinuria was seen in 19%, hypocalcinuria in 41%. The questionnaire data analysis showed that hypodynamia (37%) and artificial (surgical) menopause (13%) were the most frequent risk factors of osteoporosis in the region examined. The assessment of menopause duration by bone tissue density parameters is performed. Biochemical parameters of calcium level in blood serum and urine are found not strictly specific and should be considered individually.

WG32

DiGeorge/Velocardiofacial Syndrome Diagnosed in a 32-year-old Man with Hypocalcemia. N. M. Maalouf, K. Sakhaee, C. V. Odvina. UT Southwestern Medical Center, Dallas, TX.

The association of hypocalcemia with abnormal morphological features was first described by Albright as part of the syndrome of pseudohypoparathyroidism. Another congenital cause of hypoparathyroidism associated with different dysmorphic traits is rarely diagnosed in adults.

A 32-year-old man was referred to our clinic for treatment of hypocalcemia. His history was significant for stunted growth and pulmonary valvular stenosis. A seizure disorder was diagnosed at the age of 14 years. He was diagnosed with hypocalcemia shortly afterwards, which for years was presumed to be related to his anti-convulsants. Examination revealed a thin man, with mild dysmorphic facial features, including a prominent forehead, sparse thin eyebrows and eyelashes, hypertelorism, a flattened nasal bridge and a high-arched palate. He had a grade 1 Chvostek's sign, normal metacarpal bones, and no cataracts. Laboratory analysis was consistent with hypocalcemic hypoparathyroidism (Table 1).

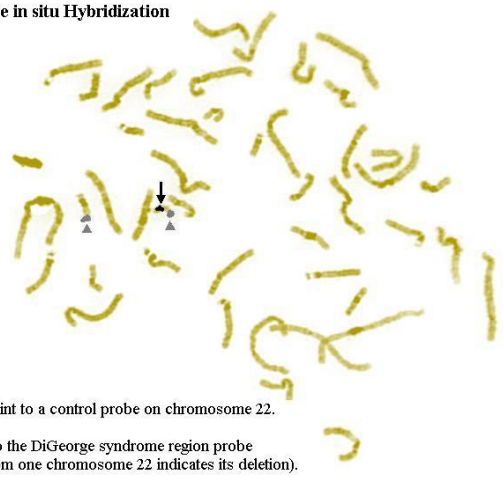
Laboratory Testing

Serum Tests	Normal Range	
Calcium	7.0 mg/dl	8.4-10.2 mg/dl
Phosphorus	4.7 mg/dl	2.4-4.5 mg/dl
Intact PTH	11 pg/ml	12-72 pg/ml
1,25-$(OH)_2$- vitamin D	< 10 pg/ml	15-50 pg/ml
Urinary Tests		
Urinary Calcium	11 mg/ 24 hours	50-300 mg/ 24 hours
Creatinine Clearance	124 ml/min	90-130 ml/min

Since his dysmorphic features and hypoparathyroidism were suggestive of, but not diagnostic for the DiGeorge/Velocardiofacial Syndrome (DGVS), the diagnosis was confirmed by fluorescence in situ hybridization testing, that revealed a submicroscopic deletion in the DGVS critical region on chromosome 22 (Figure 1).

DGVS is characterized by parathyroid and thymus aplasia/hypoplasia, craniofacial anomalies, and conotruncal heart defects. Most cases are caused by a *de novo* microdeletion of chromosome 22q11.2. A spectrum of clinical findings is seen, but DGVS is usually diagnosed perinatally, with findings of hypocalcemia, congenital heart disease, and/or dysmorphic features. Clues to the diagnosis in adults are more subtle, with most cases identified as asymptomatic parents of affected children. Hypoparathyroidism as the presenting finding leading to the diagnosis of DGVS has been reported in very few adult cases. The diagnosis should be considered in adults with idiopathic hypoparathyroidism, since there is a potential benefit from genetic counseling.

Fluorescence in situ Hybridization



Arrowheads point to a control probe on chromosome 22.

Arrow points to the DiGeorge syndrome region probe
(Its absence from one chromosome 22 indicates its deletion).

WG33

Sarcoidosis Presenting with Severe Hypercalcemia and Multi Organ Involvement. N. Napoli, R. Armamento-Villareal. Division of Bone and Mineral Diseases, Washington University, St Louis, Mo, USA

A 42-year old female was admitted to the hospital for persistent nausea and vomiting, and mental status changes. On admission she was found a severe hypercalcemia (14.6 mg/dl), and renal failure (creatinine 3.7 mg/dl), splenomegaly, leukopenia and severe anemia. Hypercalcemia and renal failure improved after IV fluid hydration and zolendronate infusion (4 mg IV). Given the clinical presentation, hypercalcemia was thought to be secondary to malignancy. The diagnosis of multiple myeloma was excluded on the bases of a normal serum and urine protein electrophoresis and a negative bone marrow biopsy. Other chemistries include a normal serum PTH, 1-25 dihydroxy vit.D at the upper limit of normal, ANA positive at 1: 160, and an elevated angiotensin converting enzyme level (70 IU/L, nl 10-55). CT of the abdomen and pelvis showed splenomegaly, and lymphoadenopaty at the splenic hilum and superior mesenteric artery, while that of the chest showed opacity at the lingula, infiltrates at the right middle lobe, nodules in the right upper lobe and right paratracheal lymphadenopathy. Because of these findings, the diagnosis of lymphoma was suspected. She underwent transbronchial lung and lymph node biopsy. Histopathology showed noncaseating granulomas consistent with sarcoidosis. She was started with prednisone 40 m.g. q.day. Her serum calcium was normalized and was 9.7 mg/dl on the day of the discharge. In conclusion sarcoidosis can rarely present with severe hypercalcemia with multiple organ involvement that may mimic malignancy.

WG34

Mastocytosis Associated with Osteosclerosis. A. Rastelli[1], H. Khoury[2], N. Napoli[1], R. Villareal[1], S. Teitelbaum[3]. [1]Division of Bone and Mineral Diseases, [2]Division of Oncology, [3]Dept. of Pathology. Washington University School of Medicine, St. Louis, MO, USA.

We report a case of mastocytosis associated with increased bone mineral density. A 74-year old man with a reported history of osteomalacia was referred to our clinic for further evaluation of persistent bone pain affecting primarily the lower extremities. Seven months before the referral he had been diagnosed with myelodysplastic syndrome, after complaining of chronic weakness and progressive weight loss. Standard radiographs showed diffuse mottled osteosclerosis of the entire skeleton. Bone mineral density measured by DEXA confirmed above normal values, with Z-scores of +9.5 at the spine and +4.7 at the proximal femur. Serum calcium, phosphorus, intact-PTH, 25(OH)D, and 1,25$(OH)_2$D were within the normal range; free testosterone level was below normal whereas alkaline phosphatase was increased at 279 U/L. Hemoglobin, hematocrit, and platelets were below normal. Hepatitis C antibody and heavy metals blood screen were negative. Two months after the initial visit the patient began complaining of pruritus and developed a petechial rash involving the lower abdomen, groins and buttocks. He denied diarrhea or flushing. A skin biopsy revealed mastocytoma. The patient underwent a transiliac crest bone biopsy that was also consistent with mastocytosis. The specimen consisted of markedly sclerotic bone in which virtually 90% of the marrow space was occupied by bone matrix. The number of osteoblasts and osteoclasts appeared unremarkable. Pockets of mast-cell proliferation were identified throughout. Further studies revealed increased serum tryptase (297 ng/ml; normal levels <11.5 ng/ml) and histamine (5.4 ng/ml; normal levels 0.0 and 0.9 ng/ml). Review of the original bone marrow biopsy revealed that mastocytosis was already present at that time. Mastocytosis is a rare disease that affects bone metabolism and can be associated with either osteoporosis or osteosclerosis, but more so with the former. The reason for such contrasting bone disease in mastocytosis is unknown, although others have suggested that when osteosclerosis is present extensive marrow infiltration by mast cell with fibrosis is present with abundant histamine metabolite excretion. Thus, mastocytosis should be considered in the differential diagnosis of sclerosing bone diseases when no other etiology is identified.

WG35

Vitamin D Deficiency Masquerading as Pseudohypoparathyroidism Type II. M.Shriraam, A.Bhansali, P.Velayutham, Department of Endocrinology, PGI Chandigarh, India.

Severe vitamin D depletion characteristically presents with hypocalcemia, hypophosphatemia, increased alkaline phosphatase, and parathyroid hormone (PTH). However, occasionally, vitamin D deficiency may be associated with PTH resistance leading to hyperphosphatemia, mimicking pseudohypoparathyroidism type II (PHP-II). We report a case, which presented with clinical and biochemical features of PHP-II induced by vitamin D deficiency.

A 23 y old man presented with 5 y history of generalized seizures treated with phenytoin. He had proximal myopathy of the lower limbs for 6 months. Denied any other medical illness or malabsorption. Nutritional history and sunlight exposure were judged to be adequate. Physical examination revealed Chvostek's sign (grade 2/4) and diminished deep tendon reflexes, but no features of Albrights Hereditary Osteodystrophy. Laboratory data showed severe hypocalcemia (5.3 mg/dl; N= 9-11 mg/dl), hyperphosphatemia (7.5mg/dl; N= 3-5mg/dl), and mildly elevated alkaline phosphatase (14.6 KAU; N= 8-14 KAU) but normal serum magnesium (2.4 mg/dl; N=1.5-3.0 mg/dl). 24 hour urine calcium was 62.5 mg and phosphate 290 mg. Tubular reabsorption of phosphate (TRP) was 95.6%, but TmP/GFR could not be calculated from the nomogram because of very high serum phosphate. Serum 25-OHD was low at 4.6 ng/ml (N= 9.1 - 41.3 ng/ml) and PTH was high (92 pg/ml; N= 7-52 pg/ml). X-rays of the skeleton and a non-contrast CT of the head were normal. A diagnosis of PHP-II related to vitamin D deficiency was considered and was treated with calcium carbonate (1500 mg/d) and cholecalciferol (600,000 IU i.m. initially, followed by oral cholecalciferol 60,000 IU fortnightly). In 3 months there was both clinical and biochemical improvement. Serum calcium rose to 8.3 mg/dl, but phosphate *declined* to 4.0 mg/dl. Serum 25-hydroxyvitamin D level increased to 23.7 ng/ml and PTH fell to 32 pg/ml with restoration of phosphaturia (290 to 780 mg/d) and TRP (declined from 95.6% to 75%).

We conclude that severe hypocalcemia due to vitamin D depletion may blunt the phosphaturic action of PTH and thus may mimic the biochemical features of PHP-II. In addition, many reported cases of PHP-II had either risk factors for vitamin D insufficiency or frank deficiency. It is, therefore, unclear whether PHP-II is a distinct entity or simply a consequence of severe hypocalcemia causing blunted phosphaturic response to endogenous PTH.

PHYSICAL ACTIVITY WORKING GROUP ABSTRACTS

WG36

Physical Activity and Fall Prevention T.Masud, Clinical Gerontology Research Unit, City Hospital, Nottingham, UK.

The effects of exercise and physical activity on fall risk are complex. Lack of walking and being on one's feet for 4 hours or less per day were shown to be risk factors for hip fracture (Cummings SR et al, 1995), but 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), other data suggested 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). Some multi-factorial studies have included exercise as one of several intervention components and assessing the independent effects of exercise has been 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 advice 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 behavioural 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). Studies from New Zealand have shown that physiotherapist- taught individualised programs of strengthening exercises in older people can reduce fall rates (Campbell AJ et al 1997). The same group subsequently showed that similar programs delivered by nurses in primary care centres and at home can also reduce fall rates in the over 75 year age group, and have the potential of being cost-effective (Robertson MC et al, 2001). Interim data from the UK FaME study suggests that community based exercise (combination of various parameters including muscle strengthening, balance training and co-ordination work) for high risk older women can reduce falls (Skelton et al 2003). Thus, although earlier studies assessing the relationship between physical activity and falls were conflicting, more recent data suggest that targeted individualised exercise and activity programs can reduce fall risk in older populations and may be cost-effective in certain sub-groups.

WG37

The Achilles Heel of Exercise – Its Cessation. M.K. Karlsson. Department of Orthopaedics, Malmö University Hospital, SE –205 02, Malmo, Sweden.

There are a few, and only short-term, longitudinal studies evaluating the effect on BMD with cessation of exercise. Michel et al. reported a decrease of 16% in the BMD of the spine in 9 middle-aged male runners who ceased their running career, compared to no loss in 3 individuals who continued running over a 5-year period and Vuori et la. reported in 12 women, aged 19-27 years that the increase in BMD during 12 months returned to its pre-training level after no more than three months of detraining. No long-term studies evaluating the structural changes in the skeleton with reduction or cessation of exercise exist. Only 3 cross-sectional studies evaluated the BMD effects of cessation of exercise after age 65 years, the age when fragility fractures exponentially increase. Leg BMD was reported 10% higher than age-matched controls in retired male soccer players retired for 5 years, 5% higher in players retired for 16 years, but no higher in players retired for 42 years. The BMD decrease with age was, in the legs 0.33 % /year in the former soccer players compared to 0.21% /year in the controls. This indicate that after 3 to 5 decades of retirement, no residual BMD benefits could be found. Similar data have previously been presented, evaluating both male weight lifters and female ballet dancers.

A lower level of activity may retain some BMD benefits achieved during an active career. The male soccer study supports this when showing a correlation between current activity level and femoral neck BMD (r = ~0.25). The notion is also supported in a 4-year longitudinal study of 13 formerly competitive male tennis players by Kontulainen et al. No changes were seen in the discrepancies in bone mineral content between the playing and the non-playing arm after the detraining period of 2 years, but the athletes had only reduced the activity from 8 to 3 hours/week. Maybe continued activity, but on a lower level, preserves the exercise-induced, beneficial skeletal effects achieved during growth and adolescence.

WG38

Physical Activity, Targeted Bone Loading and Bone: The Effect of Exercise on Bone Mineral Mass, Size and Estimated Mechanical Strength A. Heinonen. Department of Health Sciences, University of Jyväskylä and UKK Institute, Tampere, Finland

The structural behaviour of bone in response to a given load or set of forces is a function of both its geometric and architectural properties and possibly the material properties of bone tissue itself. During physical loading activities such as jumping, running and walking, the whole bone is responding as a structure in which overall changes vary along bone length or cross-section. This illustrates the site specificity of adaptive changes. Controlled studies in rats and humans showed that mechanical loading improves bone strength mostly through changes in bone geometry and redistribution of bone mass. However, not all exercises are equally effective on bone. Results from a number of animal experiments have suggested that either intensive or unusual exercise programs lead to adaptation in bone architecture. Application of the basic rules from findings of animal studies to practical exercise regimen include: high-magnitude, repetitive, high-impact and uncustomary loading. Physical activities generate loads on bone by means of the magnitude of the physical force and the rate at which this force is applied. Any change in the movement conditions affects the kinetics of the movement and probably also the mechanical stress in different exercise regimens, and thus the training response may vary. In terms of skeletal loading, weight training, for instance, creates high-magnitude loading and more specifically, extreme torques in upper extremities whereas lower extremities experience large compressive stresses. The most commonly practised exercise is walking that involves a large number of repetitive loading in lower limbs but is more suited to cardiovascular performance than local bone loading. Exercises that involve speed and power are characterised by fast, forceful, acceleratory, impact producing movements often in multiple directions seems to be an appropriate type of training with which to influence osteogenic response, as it would place a variety of forces on bone. Previous studies have reported that athletes engaged in sports producing high strain rates via versatile impact-type movements have much higher bone mass (9-40%), better geometry and stronger structure (the largest differences over 50%) than their sedentary controls. Adaptation to this loading seemed to occur in a site-specific fashion by gross-geometric changes, structural or architectural changes or by their combination. Cumulative evidence shows that the important components of osteogenic exercise stimulus are high-impact and high-magnitude loading in versatile movements. Physical activity is highly recommended (including weight-bearing, resistance and impact components) through the young and adult years, but as inevitable age-related degenerative changes occur, it might become necessary to modify the exercise regimens by age from an impact-type loading to more pliant exercise programs. The optimal program for older women would, in addition to weight-bearing activity, include activities that improve balance, strength, flexibility, and co-ordination that may indirectly decrease the incidence of osteoporotic fractures by reducing the likelihood of falling.

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