MEETING REPORT

Meeting Report from the 29th Annual Meeting of the American Society for Bone and Mineral Research

September 16-19, 2007 in Honolulu, Hawaii, USA

CHONDROCYTES: A FEW PEARLS IN AN OCEAN OF BONES

Ernestina Schipani

Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

Though bone dominated the scene at this year's ASBMR meeting, cartilage made its presence felt in several interesting studies.

The “Sox trio”, a master regulator of chondrogenesis, is still in search of company. By screening chemical libraries, investigators have identified Runx1 as a transcription factor that works cooperatively with the “Sox trio” to induce chondrogenic differentiation in vitro (1). Intriguingly, however, mice lacking Runx1 in cartilage do not display an overt chondrocyte phenotype. All in all, Runx1 could be important for tissue repair and regeneration, but redundant in organogenesis.

In vivo and in vitro models meshed well when p63 was at center stage. p63 is a member of a gene family that includes the p53 tumor suppressor. It has been known for quite some time that mice lacking p63 exhibit severe limb deformities. Investigators have now reported that p63 plays an important role in cartilage development by regulating key genes for chondrogenesis such as Sox6, Sox9 and Col2a1 (2).

The list of transcription factors modulating chondrocyte terminal differentiation and hypertrophy is also growing. Investigators at last year's meeting reported that Hif-2α, one of the hypoxia responsive transcription factors, positively regulates collagen type X expression. Conversely, the transcriptional repressor TRPS1, which is associated with human tricho-rhino-phalangeal syndrome (TRPS), was shown to delay chondrocyte terminal differentiation. For both transcription factors, additional experimental evidence supporting their essential role in chondrocyte hypertrophy has now been presented (3;4).

Speaking of chondrocytes and hypertrophy, PTHrP, a key gatekeeper of terminal differentiation, at least in cartilage, comes to mind. While it is clear and well documented that this ligand delays hypertrophy, its main downstream targets are still uncertain. An elegant study provided evidence that the zinc finger protein Zfp521 antagonizes the transcriptional activity of Runx2, a positive modulator of hypertrophy, and lies downstream of PTHrP at the border between pre-hypertrophy and hypertrophy (5). Moreover, a novel extracellular matrix protein, ECM1 (extracellular matrix protein 1), a protein that has been linked previously to chronic inflammatory conditions, appears to be a direct downstream molecule of PTHrP in cartilage and, consistent with this finding, also a negative regulator of chondrocyte differentiation (6). Upstream of PTHrP is the morphogen Ihh; how is the expression of Ihh itself regulated? A nice piece of work suggested that the transcription factor ATF4 may be one of the physiological regulators of Ihh by directly targeting transcription of the Ihh gene (7).

Regarding established pathways in cartilage, in a real tour-de-force, researchers have discovered that, while a lack of Smad1, Smad5 or Smad8 does not generate any obvious phenotype when the genes encoding these proteins are individually deleted in cartilage, the conditional ablation of both Smad1 and Smad5 is bad news for chondrocytes that, on the contrary, tolerate
well the lack of both Smad1 and Smad8 (8). Moreover, the phenotype generated by the lack of both Smad1 and Smad5 is more severe than the one observed in growth plates in which co-Smad4 was conditionally deleted, thus challenging the dogma that co-Smad4 is required to mediate Smad signaling downstream of TGFβ and BMPs. The story gets even more complicated when inhibitory Smad6 and Smad7 enter the scene, as they appear to impair cartilage development through Smad-independent pathways (9).

Growth plate development has both a prenatal and a postnatal component, but whether chondrocytes follow similar rules before and after birth is something that still needs to be fully established. It has been known for a long time that the endocrine regulation of the postnatal growth plate is critically important. Particularly interesting is the role of thyroid hormones in chondrocytes. Researchers have reported that thyroid hormones promote chondrocyte hypertrophy, at least in part, through Igf1 modulation of canonical Wnt signaling (10); it is a complex but interesting loop. Notably, conditional deletion of the Igf1 receptor postnatally causes a severe chondrodysplasia by impairing both chondrocyte proliferation and differentiation (11). The calcium-sensing receptor is another important candidate for postnatal growth plate biology, as suggested by data that have been generated using an elegant tamoxifen-inducible system (12). All in all, the detailed molecular mechanisms that regulate the formation of the secondary ossification center and the closure of the epiphysis in the postnatal growth plate are still obscure, but progress continues to be made.

During its life, the goal of a chondrocyte is to make a specific matrix. Thus it is not surprising, though it remains extremely interesting, that matrix may feed back and somehow help the chondrocyte. In this regard, researchers have now provided clear evidence for a critical role of perlecan in FGF and VEGF signaling (13). Perlecan is a large, multidomain, heparan sulfate proteoglycan that interacts with extracellular matrix proteins, growth factors and receptors. Its knockout leads to a severe chondrodysplasia that resembles the growth plate phenotype caused by gain-of-function mutations of Fgfr3 in mice. Notably, expression of VEGF in hypertrophic chondrocytes is significantly upregulated in mice lacking perlecan, despite a delay in blood vessel invasion, suggesting that perlecan modulates VEGF activity. The data were confirmed in a transgenic model of perlecan overexpression (13). While perlecan seems to be important for VEGF and FGF signaling, matrilin-3, another matrix protein, could play a negative role in chondrocyte hypertrophy by modulating BMP signaling (14).

The ultimate events in the life of a chondrocyte are mineralization of the matrix and death, probably through apoptotic mechanisms. Experimental evidence supporting a critical role of annexin V (15) in mineralization of the matrix, through regulation of intracellular Ca\(^{2+}\) influx, and of phosphate uptake in the death of the chondrocyte has been presented. Chondrocytes may die not only because the activity of the pro-life protein Bcl2 is downregulated, but also because expression of the pro-apoptotic molecule Bnip3 is dramatically upregulated (16), and the two may be components of a loop that also involves phosphate.

Finally, a challenging question in chondrocyte biology is why the fetal growth plate is an avascular structure for most of its length. A molecule identified as “chondrostatin,” a naturally occurring collagen fragment, has some interesting anti-angiogenic properties (17); is chondrostatin a key factor for the avascularity of the fetal growth plate?

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References:


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GENETICS OF HUMAN BONE DISEASES

Serge Ferrari

Division of Bone Diseases, WHO Collaborating Center for Osteoporosis Prevention, Department of Rehabilitation and Geriatrics, Geneva University Hospital, Geneva, Switzerland

The sessions dedicated to the genetics of bone diseases started with an outstanding symposium entitled "Novel Insights into Bone Metabolism through Genetics." M. Whyte first reviewed disorders associated with mutations in the RANKL/OPG-RANK system. Juvenile Paget’s Disease, JPD (also known as familial hyperphosphatasia, OMIM No #239000), is a recessive disorder caused by OPG (TNFRSF11B gene) null mutations. The skeletal deformities and high bone turnover characteristic of this disorder may be complicated by vascular calcifications, as seen in the OPG null mouse, manifest as retinal exudate and bleeding. As an alternative to bisphosphonates, recombinant OPG is a promising new therapy for these patients. In a few JPD cases, however, OPG mutations were not found. RANK (TNFRSF11A gene) mutations that affect the signal peptide region, resulting in increased signaling (activating mutations), cause familial expansive osteolysis, FEO (OMIM No #174810). Other constitutive RANK mutations were found in some rare cases of early-onset Paget’s disease, PDB2 (OMIM No #602080). Most recently, Sobacchi et al. described RANKL (TNFSF11 gene) loss-of-function mutations in an osteoclast-poor form of autosomal recessive osteopetrosis, OPTB2 (OMIM No #259710, see Not To Be Missed, BoneKEy, 2006 December;3(12):11-29). Of note, an abstract at the ASBMR meeting (1) reported no differences in RANKL gene polymorphisms between subjects with/sporadic Paget’s disease, whereas OPG polymorphisms were reported to be associated with Paget’s in this group.

E. Shore reviewed her own findings concerning the causes of fibrodysplasia ossificans progressiva, FOP (OMIM No #135100), and progressive osseous heteroplasia, POH (OMIM No #166350). These two disorders are characterized by new bone formation independent of the skeleton, i.e., new bone at the wrong place and at the wrong time: endochondral ossification in FOP, intramembranous ossification that occurs in the skin in POH. They are autosomal dominant disorders with a frequency of about 1/million individuals that in the case of FOP can be inherited from either the father or the mother, whereas POH is inherited exclusively from the father. From a phenotypic point of view, these two disorders differ by the presence of big toe malformation in FOP but not in POH. Linkage approaches revealed that FOP is due to mutations in the type 1 BMP receptor (ACVR1 gene, see S. Ferrari, BoneKEy, 2006 December;3(12):11-29). In contrast, linkage approaches have been difficult in POH due to the small number of known cases (about 50). The responsible gene has nevertheless been identified by a candidate gene approach based on the phenotypic similarities that may exist between POH, Albright’s hereditary osteodystrophy, AHO, and pseudohypoparathyroidism PHP1a. In two-thirds of POH cases, this revealed heterozygous mutations of the G protein \( \alpha \) s (GNAS) allele that is paternally-derived (XL-
α-s protein). However, how this mutation and/or paternal imprinting explains the restricted expression of the mutant protein in the skin remains unclear.

J. Marini reviewed autosomal recessive, mostly lethal forms of osteogenesis imperfecta (OI) characterized by white (normal) sclerae, small head circumference, and a round face. In 10 to 15% of OI cases, Col1A1 and Col1A2 mutations are not found. In OI type VII and IIB, for instance, (OMIM No #610682 and #610854), homozygous and compound mutations were found in a cartilage-associated protein (CRTAP gene) that shares 53% homology and forms a complex with prolyl 3-hydroxylase 1 (P3H1 gene, also known as leprecan, LEPRE1 gene). This complex is responsible for hydroxylation of the pro986 residue of Col1A1 necessary for the assembly of the triple helix. A mouse model of the CTRAP null mouse published by Morello et al. (2) demonstrates that this protein is expressed in chondrocytes at the chondro-osseous junction. Interestingly hypomorphic (non-lethal) mutations do exist in both CRTAP and LEPRE1, the latter found in families of African origin with OI type VIII.

OI cases with unusual presentations were reported by a group in Sweden (3), the most interesting being three girls with multiple fractures but high BMD (+3SD) by DXA (“dense OI” according to the authors) and normal bone turnover markers. In all three cases, novel Col1A1 or Col1A2 mutations were found, in particular at the splice site of the C-terminal peptide. The major limitation of this work was the fact that the pathogenesis of the identified mutations was not directly demonstrated whereas concomitant (HBM) mutations in other genes were not ruled out. Also related to OI was a very important communication (4) reviewing experience with IV pamidronate in children with OI in relation to the potential risk of osteonecrosis of the jaw (ONJ). As a reminder, dentinogenesis imperfecta is also often present in these children who therefore require a number of dental procedures during growth. Despite follow-ups of up to 10 years in some children, extraction sockets were never found to be complicated by ONJ.

A group in France (5) reported its experience with the highly variable response to treatment with active vitamin D (1α vitamin D, 1-2 microg/d) and phosphorus in a large series of patients (n=81) with X-linked hypophosphatemic rickets (XLH). Their results suggested that genetic variation in the vitamin D receptor (VDR), in particular a haplotype that they identified as Hap1, influenced the height and Ca-Pi metabolic response to treatment. Dr. M. Whyte, however, pointed out that the outcome of XLH is primarily influenced by the type of PHEX mutations that are present, i.e., deactivating vs. non-deactivating mutations.

Moving from monogenic disorders to osteoporosis genetics, LRP5 gene polymorphisms previously described to be associated with BMD and/or fractures in several independent cohorts were analyzed in relation to these phenotypes in an extraordinarily large collection of participants (n=37,000) from both European and US cohorts (6). This prospective meta-analysis at the participant level confirmed previous findings in that LRP5 missense SNPs in exons 9 and 18 were associated with spine and femur aBMD and fracture risk, with a maximal 26% increase in risk of vertebral fracture per 667M allele. Another abstract (7) reminded us that heterozygous carriers of LRP5 OPPG mutations have a lower BMD and a higher prevalence of vertebral fractures than controls, further suggesting that heterozygous LRP5 mutations should be suspected whenever osteoporosis is detected in middle-aged subjects. This study also indicated a higher prevalence of diabetes and hypercholesterolemia in these subjects, thereby providing a possible molecular clue to explain the association of these disorders with osteoporosis.

An interesting approach to predict osteoporotic fractures using Col1A1 polymorphisms was taken by Australian investigators (8). They developed models that indicated the TT genotype of Col1A1 (5% of the population) to more than double
the risk of any fracture at all ages independently of femur neck BMD, so that homozygosity for the T allele would be equivalent to +20 yrs of age or -1SD of BMD. A new candidate gene to be associated with BMD is \textit{FNLB}, filamin B, mutations in which cause osteochondrodysplasia (9). This gene is in a region previously mapped for linkage with osteoporosis (3p14) by one group in England. Now the authors report association of \textit{FNLB} SNPs with BMD in two independent cohorts, i.e., they were able to replicate the association they found. Large-scale genotyping methods to simultaneously analyze thousands of SNPs were presented. By analyzing 113 SNPs in 54 selected genes with microarray-based techniques, sex-specific associations with BMD were reported, such as \textit{LRP5} and \textit{ESR2} in men, and \textit{IL6} and \textit{ESR1} in women (10). A genome-wide association study (GWA) using the 100k SNP gene chip method found numerous SNPs associated with BMD and bone geometry at the hip (11). However virtually no SNP shared association with BMD and bone geometric indices, such as femoral neck width, suggesting that these traits are determined by separate genes. A region-wide association study based on 200 tag SNPs covering 6Mb on chromosome 3p21 identified a voltage-dependent calcium channel subunit, CACNA2D2, to be associated with BMD in Chinese subjects (12).

Negative reports are sometimes also useful. By studying genetic variation in the PTH system (i.e., \textit{PTH}, \textit{PTHrP}, \textit{PTH1R} and \textit{PTH2R} genes) in a prospective study of 1000 elderly women, OPRA investigators found no association with BMD, fractures and/or PTH levels, except between some \textit{PTH} haplotypes and fracture risk (13).

A major component of skeletal strength, besides bone mineral density, is the microarchitecture of cortical and trabecular bone. Using high-resolution pQCT, the heritability (h\textsuperscript{2}, %) of human bone microstructure was evaluated at the distal radius and tibia in more than 100 mother-daughter pairs (14). H\textsuperscript{2} was generally high and similar to h\textsuperscript{2} for aBMD. However, some heritability estimates decreased sharply past the menopause, suggesting that intense bone remodeling may overcome additive genetic effects on peak bone microstructure. Also, interestingly, the mean heritability of trabecular and cortical microarchitecture was near 50% once adjusted for body size and BMD at the same site, adding to the evidence that genetic effects may specifically affect bone structure.

Although reviewing the plethora of abstracts on mouse genetics is beyond the scope of this summary, it is worth mentioning an interesting approach that used the Haplotype Association Mapping (HAM) program to extract information from SNPs and BMD in 18-week female mice from 30 mouse strains (15). This technique identified a positional candidate gene, \textit{CER1}, in which a non-synonymous SNP was later found to be associated with low BMD in 1000 Chinese premenopausal women as well. Finally, one of the most interesting abstracts in this field (16) identified a highly polymorphic region in the 3'-UTR of the \textit{Ppar\textgamma} gene by comparing sequences from B6 and C3H mice. Studies in congenic mice revealed that this region regulates the \textit{Ppar\textgamma} transcription level in response to a high fat diet. Moreover, SNPs in the human syntethic region of mouse \textit{Ppar} 3'-UTR were also reported to interact with dietary fat in association with BMD.

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\textbf{References}


14. Ferrari SL, Chevalley T, Bonjour JP, Rizzoli R. Heritability of bone
microstructure in women. J Bone Miner Res. 2007 Sep;22(Suppl 1):S68. [Abstract]


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OSTEOBLASTS: STAYING THE COURSE

Renny T. Franceschi\(^1\) and Guozhi Xiao\(^2\)

\(^1\)University of Michigan School of Dentistry, Ann Arbor, Michigan, USA
\(^2\)University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

This year’s meeting catalogued the steady progress in our understanding of osteoblast biology. Now that many of the key factors controlling this cell lineage are known, studies are increasingly focused on the control of osteoblast activity by a wide range of signal transduction pathways and factors.

They’re Alive!

Early in the meeting, we were treated to beautiful video images showing the dynamic nature of osteoblasts and their osteocyte descendants – a far cry from the static histology we’re used to seeing. Different colored GFPs driven by the 3.6kb \(Col1a1\) and \(DMP1\) promoters were used to visualize calvarial osteoblasts and osteocytes, respectively, in real time (1;2). The continuum between these two cell types was emphasized as we watched an individual osteoblast gradually become surrounded by extracellular matrix, take on the DMP1 marker and mineralize. Interesting studies from the same group also suggest that a function we normally attribute to osteoblasts, induction of mineralization, may, in fact, be a role of the osteocytes that secrete matrix vesicle-like structures associated with hydroxyapatite crystallite nucleation (3).

Signaling and More Signaling

A number of presentations continued the recent trend of focusing on osteoblast regulatory mechanisms. The essential roles of a number of key signal transduction pathways in osteoblast function were revealed using a combination of \textit{in vitro} biochemical analysis and \textit{in vivo} gene deletion/transgenic studies.

G protein-coupled receptors, also known as GPCRs, regulate many pathways, including those required for PTH signaling in osteoblasts. However, the functional roles of specific G proteins in bone are poorly understood. Designer GPCRs provide powerful tools for dissecting the roles of these G proteins in osteoblasts. A transgenic approach was used to selectively express in osteoblasts an engineered GPCR (Rs1) that signals through the Gs/cAMP/PKA pathway. These mice displayed a massive and progressive increase in bone formation and bone mass (4). Likewise, mice lacking Gs\(\alpha\) have impaired bone formation, resulting in marked bone fragility (5). Transgenic expression of Gi in osteoblasts using the \(Col I\) 2.3 kb promoter negatively affected bone formation (6). Lastly, transgenic mice expressing the G\(\alpha q\) subunit in osteoblasts, the major mediator of PTH-dependent phosphoinoside/PKC signaling, have osteopenia due to impaired osteoblast differentiation and reduced matrix formation (7). Taken together, these data suggest opposing roles of Gs vs. Gi or Gq signaling in osteoblasts. The knowledge obtained from these studies will help define new potential therapeutic targets for improved...
treatment of metabolic bone diseases such as osteoporosis.

It is well established that canonical Wnt signaling increases osteoblast activity and bone formation. Wnts signal by blocking GSK3 activity and stabilizing β-catenin, thereby increasing osteoblast proliferation/differentiation and osteogenesis (8). However, much less is known about interactions between Wnt and other signaling pathways. Studies showed the Wnt pathway to be firmly intertwined with other important signals. Notably, Wnt3a stimulated phosphorylation of S6K in osteoblasts, one of the two major downstream targets of mTOR signaling. Furthermore, mTOR activity is markedly increased in Wnt10b transgenic mice. Interestingly, Wnt activated mTOR in a β-catenin-independent manner (9). The non-canonical Wnt signaling pathway component, Wnt5a, also increased osteoblast differentiation of human mesenchymal stem cells in vitro (10). PTH, a major regulator of calcium homeostasis and osteoblast activity, significantly increased levels of β-catenin protein and β-catenin-dependent transcriptional activity in cultured osteoblasts by recruiting LRP5/6, coreceptors of Wnt canonical signaling, to the PTH1R and stabilizing β-catenin. Furthermore, intermittent PTH rapidly increased levels of LRP5/6 phosphorylation and β-catenin protein in vivo (11). Osterix (Osx), a major regulator of calcium homeostasis and osteoblast activity, significantly increased levels of β-catenin protein and β-catenin-dependent transcriptional activity in cultured osteoblasts by recruiting LRP5/6, coreceptors of Wnt canonical signaling, to the PTH1R and stabilizing β-catenin. Furthermore, intermittent PTH rapidly increased levels of LRP5/6 phosphorylation and β-catenin protein in vivo (11). Osterix (Osx), a critical osteoblast differentiation factor, was shown to inhibit cell proliferation by antagonizing Wnt signaling. This was accomplished by induction of the Wnt inhibitor, Dkk1. Osx(-/-) calvarial osteoblasts failed to express Dkk1 while Osx activated Dkk1 gene expression. Consistent with this finding, Osx inhibited β-catenin-dependent TOPFLASH reporter activity and β-catenin-induced secondary axis formation in Xenopus embryos, supporting the notion that Osx inhibits osteoblast proliferation by blocking Wnt signaling, thereby allowing differentiation to proceed (12).

Because type I diabetics exhibit defects in bone formation, it has long been suspected that insulin may have direct actions on bone. However, insulin can cross-react with the IGF-1 receptor, also active in bone, so it has not previously been possible to establish direct actions for insulin. A clever use of IGF-1 receptor (IGF-1R) and insulin receptor (IR)-deficient mice allowed investigators to clearly establish that insulin can stimulate osteoblast proliferation and differentiation in the absence of IGF-1 signaling (13). Effects of insulin, as in other systems, were mediated by Akt and GSK3β and resulted in up-regulation of Runx2. These workers also showed that mice lacking the IR in osteoblasts have decreased bone volume. A related study further explored the basis for Akt actions in osteoblasts. Akt1(-/-) mice have a low-turnover osteopenia associated with increased osteoblast apoptosis. Akt1 was shown to protect osteoblasts from apoptosis by stimulating phosphorylation of cytoplasmic FoxO3a, thereby blocking its nuclear translocation and up-regulation of Bim, a pro-apoptotic factor whose expression is largely dependent upon the active FoxO3a in the nucleus (14).

The central position of GSK3β as a kinase involved in both insulin/Akt and Wnt signaling was further emphasized by a presentation describing the results of GSK3β haploinsufficiency (15). These mice have greater bone mass and formation rates and their osteoblasts show accelerated rates of differentiation in vitro when compared with wild-type littermates. Although levels of the Runx2 transcription factor were not affected by GSK status, transcriproal activity was severely attenuated by GSK over-expression. This regulation may be the consequence of direct phosphorylation of Runx2 by GSK, in that mutation of 3 consensus GSK phosphorylation sites rendered Runx2 no longer sensitive to GSK3β inhibition.

You Gotta Carry That Load

Polycystin-1 (PC1) and connexin 43 (Cx43) have both been implicated in the response of osteoblasts/osteocytes to mechanical signals. PC1 is a component of the primary cilium thought to form a mechanosensing complex in bone, while Cx43 mediates the gap junctional communication linking
osteocytes to each other and to surface osteoblasts. Two presentations described the consequences of PC1 (16) and Cx43 (17) deficiency. PC1-deficient mice have osteopenia and decreased expression of the bone-related type II Runx2 isoform. Furthermore, a genetic link was established between Runx2 and PC1 by showing that double heterozygous PC-1/Runx2 mice have a more severe osteopenia than was seen with haploinsufficiency of either gene. Furthermore, PC1 overexpression stimulated the promoter controlling type II Runx2 via a mechanism involving both PI3K and Akt. Disruption of gap junctional communication via osteoblast-specific deletion of Cx43 also caused an osteopenia that the authors suggest may be due to an inability of knockout animals to properly adapt to ambulatory loads. The canonical MAP kinase pathway is also a component of the response of osteoblasts to mechanical loads/matrix signals and transgenic manipulation of this pathway can alter bone development and osteoblast differentiation (18). This requirement for canonical MAPK signaling in osteoblast differentiation was confirmed by analysis of ERK1/2 inactivation in mesenchymal precursors using a Prx1-Cre (19). ERK-deficient mice showed delays in the formation of primary ossification centers in long bones while Prx1-driven MEK1 over-expression led to accelerated osteogenesis, synostoses of long bones and premature lambdoid suture closure.

I Can't Breathe

As one of the most metabolically active cells in the body, osteoblasts require an adequate blood supply whose formation precedes overt bone formation. This year, a number of studies emphasized the intimate relationship between angiogenesis and bone formation. The pericyte, a cell in intimate contact with the vasculature, has long been suspected of being an osteogenic progenitor (20). A smooth muscle actin-GFP mouse was used to track pericytes into osteoblast and adipocyte lineages in vitro and in vivo, thereby confirming that this cell type is an osteoprogenitor (21). The hypoxia inducible factor-1α transcriptional regulator (HIF-1α) induces angiogenesis genes under conditions of low oxygen tension. Several presentations established the important function of this factor in osteoblasts. HIF-1α-deficient osteoblasts expressed reduced levels of VEGF and osteoblast differentiation markers in vitro. Introduction of a HIF-1α mutation into osteoblasts in vivo reduced bone volume and bone formation parameters and increased osteoclast numbers, effects that were exacerbated by ovariectomy (22). Interestingly, stimulation of HIF-1α activity was also shown to stimulate bone regeneration in a distraction osteogenesis model (23).

Other Players

Calcium receptors (CaRs) play a key role in sensing circulating ionized calcium concentrations. It is now clear that they are also critical for bone development. Mice lacking the CaR in osteoblasts had smaller, undermineralized skeletons and, depending on the stage of osteoblast differentiation at which gene excision took place, either reduced or increased levels of osteoblast differentiation markers (24;25). ATF4, an osteoblast-enriched transcription factor required for normal differentiation and bone formation, was shown to mediate the anabolic actions of PTH in long bones and calvaria in studies using an ATF4-deficient mouse model. This study also demonstrated that ATF4 is a novel downstream target of PTH in osteoblasts; PTH increased Atf4 gene expression and ATF4-dependent transcripational activity through multiple signaling pathways mainly involving PKA and C (26). Ephrin B2, produced by osteoclast precursors, was recently shown to enhance osteoblast differentiation and bone formation via interactions with its osteoblastic receptor, EphB4 (27). Interestingly, mice lacking Ephrin B1 in osteoblasts displayed reduced peak bone mass. Conversely, overexpression of Ephrin B1 enhanced osteoblast proliferation in vitro (28). These results suggest that factors coupling osteoblast-osteoclast communication play important roles in bone formation, an underexplored area of bone biology. A number of new factors interacting with the Runx2 transcription factor were also identified. Among these are Nell-1, a
downstream Runx2 target that can partially compensate for Runx2 haploinsufficiency (29), Zfp521, a FosB interacting protein that antagonizes Runx2 transcriptional activity (30) and TGFβ Inducible Early Gene (TIEG) that directly stimulates expression of Runx2 and its downstream targets (31).

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References


30. Hesse E, Wu M, Rowe GC, Neff L, Horne WC, Baron R. Zfp521, a D2D FosB-interacting protein, is a novel inhibitor of Runx2 activity with opposite effects on osteoblasts and bone formation in vitro and in vivo. J Bone Miner Res. 2007 Sep;22(Suppl 1):S70. [Abstract]


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**OSTEOCLASTS**

Roberta Faccio

*Washington University School of Medicine, St. Louis, Missouri, USA*

Integrin signaling plays an essential role in osteoclast (OC) function. The importance of the αvβ3 integrin in osteoclastic bone resorption is well established (1-3), and now new evidence indicates a critical role for the α9β1 integrin receptor in osteoclast formation and function (4). Studies from α9(-/-) mice and human OC precursors infected with α9 shRNA revealed decreased numbers of mature osteoclasts with disrupted actin rings. α9 was shown to be the only receptor for ADAM8 (A Disintegrin and Metalloproteinase 8). The importance of the ADAM8/α9β1 interaction was demonstrated by increased OC numbers in WT, but not α9 null cultures treated with soluble ADAM8. Mechanistically, the disintegrin motif of ADAM8 formed a complex with the tyrosine kinase Pyk2 and modulated paxillin phosphorylation. Thus, these data indicate that interaction between the α9β1 integrin and its ligand ADAM8 is critical for OC activity by activating a PYK2-dependent signaling pathway. The importance of PYK2 in bone homeostasis was also unveiled through demonstration that PYK2 deletion led to high bone mass through a positive balance between bone formation and bone erosion in aged mice (5). Furthermore, another recent study underscores an important role of Pyk2 in microtubule-dependent podosome organization, bone resorption, and other osteoclast functions (6).

New insights into activation of OC-mediated bone resorption were elegantly presented (7) in work using Cdc42 gain-of-function mice (Cdc42GAP), which lack the GTPase-activating proteins, thereby allowing prolonged activation of Cdc42, die soon after birth. Transplant studies of Cdc42GAP(-/-) bone marrow cells into lethally irradiated WT mice induced lower bone mass, increased OC numbers and higher levels of bone resorption. This in vivo finding correlated with accelerated M-CSF dependent proliferation and RANKL-induced differentiation in vitro. Conversely, bone marrow macrophages (BMMs) from cdc42(flox/flox) mice treated in vitro with retroviral Cre to delete Cdc42 displayed a decreased response to M-CSF, increased apoptosis and diminished OC differentiation. This is the first report indicating an important role for the Cdc42 GTPase in both OC differentiation and bone resorption.

NFκB activation is critical for osteoclast development and survival. Both RANKL and TNFα can induce NFκB activation and both cytokines activate the canonical (p65 and p50) and non-canonical (p52 and RelB) NFκB pathways. However, their effect on osteoclast development is different. RANKL strongly promotes OC differentiation, while TNFα does so only in the presence of permissive levels of RANKL (8). Intriguing findings were presented suggesting that TNFα, independent of RANKL, can promote OC differentiation, albeit to a lesser extent than the osteoclastogenic cytokine (9). A possible mechanism explaining the differential effect of these two cytokines on NFκB-mediated osteoclast differentiation relies on the capacity of TNFα to induce upregulation of both p52 and its precursor...

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protein p100 (NFκB2), while RANKL increased protein levels of p52 by promoting p100 degradation (10). Interestingly, deletion of NFκB2 augmented the capacity of TNFα to promote OC differentiation at similar rates to RANKL. Exit from cell cycle is a required step for OC terminal differentiation and involves NFκB. TNFα promoted cell proliferation via activation of cyclinD1 and similarly to NFκB2(-/-) cells, deletion of cyclin D1 stimulated TNF-induced OC differentiation. These data suggest that TNFα may limit OC differentiation through a mechanism involving NFκB2 and cyclinD1.

Interesting results on NFκB-induced osteoclast differentiation were also presented (11). NFκB activity is controlled by 2 upstream kinases, IKKα and IKKβ. In this study, the authors examined the role of IKKβ in in vivo and in vitro osteoclastogenesis by generating myeloid lineage-specific deletion of IKKβ using the Cre-lox system. Deletion of IKKβ in osteoclast progeny was responsible for developmental and survival defects, since knockout bone marrow macrophages formed less OCs in response to RANKL and apoptosis was more sensitive to RANKL and the pro-inflammatory cytokine, TNFα. Interestingly, the deletion of IKKβ in splenocytes was not sufficient to block their differentiation into osteoclasts, suggesting that the microenvironment in the spleen programs OC precursors to differentiate into mature OCs independently from IKKβ activity.

A recent report indicated that RANKL co-stimulatory signals mediated by ITAM-containing receptors FcRγ and Dap12 are critical for osteoclast development in vitro and in vivo (12). FcRγ and DAP12 modulate calcium influx from the ER through the PLCγ2 pathway (4;5) and thereby mediate upregulation of the osteoclastogenic gene NFATc1 (12). Mechanisms mediating the activation of PLCγ are still under investigation. Investigators elegantly demonstrated the need for Tec tyrosine kinases during osteoclast differentiation to modulate PLCγ1 and PLCγ2 phosphorylation (13). Specifically, Tec(-/-) Btk(-/-) mice exhibited an osteopetrotic phenotype due to severe impairment of OC differentiation. In vitro analysis showed that the two Tec family members were recruited to lipid rafts upon RANKL stimulation and formed a complex with RANK, the adapter protein BLNK and the ITAM-harboring adaptors, which mediated PLCγ-mediated NFATc1 upregulation during osteoclastogenesis. Importantly, in vivo studies showed that these mice are protected from ovariectomy-induced bone loss. In light of these findings, results from another group appeared very intriguing (14). In fact, in contrast to Tec(-/-)Btk(-/-) mice, ITAM-containing receptor FcRγ/Dap12 double null mice, which have a severe osteopetrotic phenotype due to a blockade in OC development, responded to ovariectomy with bone loss in both femurs and tibias of approximately 40% relative to basal bone volumes. Thus, this study suggests that whereas ITAM signaling is critical for basal bone remodeling, estrogen deficiency induces an ITAM-independent bypass mechanism allowing for increased osteoclastogenesis and activation in specific bony microenvironments.

The interaction between the immune and bone systems is becoming increasingly recognized. New research demonstrated that T-lymphocytes amplify the anabolic action of intermittent PTH (iPTH) treatment by regulating OC formation (15). Mechanistically, using T cell receptor β (TCRβ)(-/-) mice, researchers demonstrated that the deficient mice had decreased bone mineral density (BMD) measured by DEXA and less of an increase in BV/TV when treated with intermittent PTH than WT mice. 4-point bending tests also showed that iPTH increased femoral stiffness in WT mice but not in TCRβ null animals. These observations correlated with decreased numbers of CFU-ALP colonies, an index of the number of stromal cells (SC) with osteogenic potentials and a 3-fold lower increase in ex-vivo formation of OCs in TCRβ(-/-) mice as compared to WT, indicating that T cells potentiated the capacity of iPTH to stimulate both osteoblasts and OCs. Importantly, the paper showed that iPTH stimulated RANKL expression of T cells by targeting stromal cells. This work is another demonstration of
the importance of T cells in modulating bone cell development.

Overall, several intriguing findings were reported at the 2007 ASBMR annual meeting in Honolulu. These exciting studies will certainly open new roads toward understanding the mechanisms of osteoclast recruitment, differentiation and activation in pathological conditions of bone loss, with the hope of soon finding new effective anti-osteoclastogenic therapeutic targets.

Conflict of Interest: None reported.

References


MEETING REPORT

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OSTEOCYTES EMERGING FROM OBSCURITY

Sarah L. Dallas and Lynda F. Bonewald

University of Missouri at Kansas City, Kansas City, Missouri, USA

Of the three major bone cell types, osteocytes have remained the most elusive and least understood due to their location within the mineralized bone matrix. At this year's ASBMR meeting, the osteocyte truly emerged from its obscurity, with a number of research abstracts and symposium presentations highlighting the key role these cells play in diverse skeletal functions, such as the regulation of bone formation, mechanosensation, glucocorticoid-induced bone loss, and phosphate homeostasis. It was exciting to see that the 2007 ASBMR meeting included for the first time a concurrent oral session devoted to osteocyte biology. The main themes emerging from the osteocyte-related abstracts are summarized below.

Sclerostin – A Potential Approach for the Treatment of Osteoporosis

SOST, and its protein product sclerostin, is highly expressed in osteocytes (1) and acts as an inhibitor of Lrp5-Wnt-β-catenin signaling (2-4). One of the hottest topics at this year’s meeting was the use of antibodies to sclerostin to increase bone formation and prevent bone loss. Treatment with sclerostin antibodies increased markers of bone formation in postmenopausal women and increased bone formation in ovariectomized, aged female or male rats (5-8). This approach looks promising as an anabolic treatment for osteoporosis, which could be particularly useful for the treatment of patients in whom significant bone loss has already occurred. As sclerostin regulates Wnt-β-catenin signaling, which is important in mechanosensation, an advantage of targeting sclerostin therapeutically is that the new bone formed will presumably be laid down in mechanically appropriate locations. However, this remains to be confirmed.

Further insight into the molecular mechanism by which sclerostin inhibits Lrp5-Wnt-β-catenin signaling came from a study showing that mutations in the β-propeller 1 region of Lrp5, including the G171V high bone mass mutation, render Lrp5 resistant to sclerostin binding (9). Mutations in β-propeller 2, 3 or 4 regions had no effect. This identifies β-propeller 1 as the critical region for sclerostin binding. This study further showed that sclerostin inhibits signaling by Wnt1 and Wnt10b but not Wnt3a.

Interactions of PTH with sclerostin may explain, in part, some of the ability of intermittent PTH to enhance bone formation. PTH suppresses SOST expression in UMR-106 cells and in adult bone in vivo. This was confirmed in a study where primary osteocytes from transgenic mice with targeted GFP expression in osteocytes were used (10). Another study further elucidated the mechanism for inhibition of SOST expression by PTH in UMR-106 cells (11). This appears to be mediated via down-regulation of MEF2 transcription factors, which are essential for the activity of the SOST enhancer. Together, these studies provide additional potential targets for therapeutics to prevent bone loss.

Mechanosensation in Osteocytes – The Role of β-Catenin and Hemichannels

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Osteocytes are widely viewed as the main cell type responsible for sensing and coordinating adaptive responses to mechanical loading. This was elegantly demonstrated in studies showing that osteocytes are much more sensitive to mechanical loading compared to osteoblasts (12;13). These investigations revealed that, following in vivo loading, osteocytes are the first cells to respond with a rapid increase in β-catenin signaling. This appears to be mediated via crosstalk of prostaglandin signaling with the β-catenin pathway through GSK phosphorylation. The study authors further proposed that this initial prostaglandin-mediated activation of β-catenin in osteocytes is followed by an amplification of β-catenin signaling, mediated by up-regulation of Wnt/Lrp5 and down-regulation of inhibitors of Lrp5 signaling, such as sclerostin. The authors proposed a unifying model for load-related bone formation that integrates prostaglandin signaling with the Wnt-β-catenin pathway and provides an intriguing molecular explanation for strain signal amplification.

Studies also provided new insight into the mechanism of prostaglandin release in response to shear stress, which appears to occur through connexin 43 hemichannels (14;15). These investigations demonstrated that shear stress causes intracellular assembly of Cx43 channels. Antibodies targeted to hemichannels (not gap junctions) had no effect on ATP release from P2X7 channels but inhibited PGE$_2$ release. In contrast, an inhibitor of the P2X7 channel blocked ATP, but not PGE$_2$ release in response to shear stress. The opening of hemichannels may be mediated by α5 integrins. This provides an intriguing new function for these integrin subunits in osteocytes, which will be very exciting, if confirmed.

Glucocorticoid-Induced Bone Fragility and Osteocyte Apoptosis – Role of Hemichannels and β-catenin

The importance of osteocyte apoptosis in glucocorticoid-induced bone fragility continues to receive support from research presented at this year’s meeting. One interesting study used a bisphosphonate analog, IG9402, which has no effect on osteoclasts but protects osteoblasts and osteocytes from apoptosis (16). Treatment with this reagent maintained bone strength in glucocorticoid-treated mice, without inhibiting resorption, suggesting that preservation of osteocyte/osteoblast viability is an important mechanism for the beneficial effects of bisphosphonates on bone. Investigators from the study had proposed previously that bisphosphonates promote osteocyte viability by interacting with hemichannels. Here they showed that IG9402 had no effect on glucocorticoid-induced bone fragility in mice with targeted deletion of connexin 43 in osteoblasts/osteocytes, supporting a role for Cx43 hemichannels in the protective effects of bisphosphonates.

Mechanical loading has a protective effect on glucocorticoid-induced osteocyte apoptosis and one study proposed a potential molecular mechanism (17). This work revealed that the protective effects of loading on dexamethasone-induced apoptosis of the osteocyte-like cell line MLO-Y4 occur through the rapid production of prostaglandins. This signal enhances cell viability both through the classical cAMP/PKC pathway as well as through crosstalk with the β-catenin pathway via phosphorylation of GSKα and β. Agents that preserve osteocyte viability may therefore be good targets for therapeutics to preserve bone strength.

Osteocytes as Regulators of Phosphate and Calcium Homeostasis

The key role that osteocytes play in the regulation of phosphate and potentially calcium homeostasis was highlighted this year in a number of oral presentations, as well as in a symposium entitled “Osteocytes and the Regulation of Phosphate Homeostasis.” Several genes that play key roles in phosphate homeostasis, including PHEX, Dmp1 and FGF23, are highly expressed in osteocytes. Two studies reported novel mutations in PHEX associated with X-linked hypophosphatemic rickets (18;19).
The 2006 ASMBR meeting saw the first reports that mutations in \textit{Dmp1} are associated with autosomal recessive hypophosphatemic rickets. Follow-up work presented at this year’s meeting showed that the MIV (A1G) Dmp1 mutant protein that lacks the signal sequence fails to be secreted (20). The 1484-1490del mutant, which lacks the C-terminal 18 amino acids and contains 33 novel amino acids, is secreted more rapidly than wild type Dmp1, but is non-functional. The importance of the C-terminus of Dmp1 was elegantly demonstrated in a study in which a 57kDa C-terminal fragment of Dmp1 was re-expressed in \textit{Dmp1}-null mice (21). This fragment rescued the skeletal abnormalities and hypophosphatemia just as efficiently as the full length Dmp1 protein and restored circulating FGF23 levels to normal.

In another study, the 10kb \textit{DMP1} promoter was used to drive a tamoxifen-inducible Cre transgene to selectively and inducibly delete the PTH/PTHrP receptor in osteocytes (22). Inducible expression was confirmed using Rosa26 for newborns and Z/AP for adults. When tamoxifen was administered at 6 weeks of age, low calcium, increased PTH and an improper homeostatic response of serum calcium and phosphate were observed, especially with a low calcium diet. These effects were not observed when tamoxifen was administered at 12 weeks.

Overall, osteocytes are emerging as major regulators of phosphate metabolism and appear to be the main source of elevated serum FGF23 in various types of osteomalacia, suggesting that they may function as an endocrine organ. Similar to phosphate homeostasis, the osteocyte network may also function as an endocrine gland to regulate calcium homeostasis but through other unique mechanisms. It will be exciting to follow developments in this field at future ASBMR meetings.

\section*{Dynamic Properties of Osteocytes}

The first study in which live osteocytes were imaged within their lacunae was reported at the 2006 ASBMR meeting. Two abstracts presented this year extended these observations to show the dynamic properties of both osteocytes and osteoblasts (23;24). These investigators used transgenic mice expressing GFP targeted to osteocytes via the 8kb-\textit{Dmp1} promoter and/or expressing DsRed targeted to osteoblasts via the 3.6kb \textit{col1a1} promoter. Time-lapse imaging of calvarial explants from these mice showed that both osteoblasts on the bone surface and osteocytes within their lacunae are more motile than previously thought and showed that dendritic connections between adjacent osteocytes and between osteocytes and cells on the bone surface may be transient. Imaging of double transgenic mice showed the heterogeneity of cells on the bone surface and identified a Dmp1-GFP-positive, E11-positive surface motile cell that may represent an osteocyte precursor. Dynamic imaging of mineralization in primary bone cell cultures isolated from these transgenic mice integrated mineralization with the transition from osteoblast to osteocyte and suggested that the embedding Dmp1-positive cells are responsible for mineralization. After viewing these movies showing the dynamic nature of cells in bone, we may never view static histological sections in the same way again.

\section*{Conclusions}

The recent explosion in research on osteocytes has been fueled by the availability of new research tools, such as cell lines, reporters targeted to osteocytes, and targeted and timed deletion of genes in osteocytes \textit{in vivo}. With the exciting research that is emerging in this field, it is clear that we can no longer ignore the osteocyte and that it fully deserves to share the limelight with the osteoblast and osteoclast.

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\section*{References}


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PHOSPHATE METABOLISM

Gordon J. Strewler

Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA

Our understanding of phosphate metabolism has moved fast for the past few years. The big step was finding that FGF23 is a phosphatonin, a secreted messenger that directs the kidney to spill phosphate and inhibit synthesis of 1,25(OH)_2D. Two years ago we learned that the klotho protein is a coreceptor for FGF23. Klotho binding converts certain FGF receptors to high-affinity FGF23 receptors (FGFRs), but which ones? Ablation of FGFR3 or FGFR4 does not impair FGF23 action (1), supporting the view that FGFR1c is the principal FGF23 receptor, but more work on this point will be necessary.

Klotho was described as an aging gene but in the mouse, loss of FGF23 and loss of Klotho produce similar phenotypes, which seem to be attributable to impaired FGF23 signaling, as the phenotype is greatly ameliorated by blocking the resultant hyperphosphatemia or the increase in 1,25(OH)_2D. We now learn that the human disorder tumoral calcinosis, which was previously associated with mutations in either FGF23 itself or in the galacosyltransferase GALNT3 that processes FGF23, can also be caused by mutations that disable Klotho (2;3). The affected individual had all the manifestations of tumoral calcinosis but did not have evidence of premature aging. The role of Klotho in aging remains muddy; although many of the "aging" phenotypes seem to be attributable to disordered mineral metabolism, a recent paper suggests that Klotho causes cellular senescence through effects on Wnt signaling (4).

Klotho is expressed at only a few locations, making them prime candidates as sites for FGF23 action. One of these is the parathyroid. FGF23 inhibits the secretion of PTH from isolated bovine parathyroid cells, as subsequently reported by two groups (5-7). Unfortunately we don’t know much about the relation of this effect to calcium signaling for PTH release. It’s also curious that Klotho itself is reported to stimulate PTH secretion in the absence of FGF23 (8).

The principal renal phosphate transporter is NaPi2a. Removal of NaPi2a prevents phosphate reabsorption, and hypophosphatemia results. Crosses of hypophosphatemic NaPi2a null mice and hyperphosphatemic FGF23 nulls have hypophosphatemia (9), hence NaPi2a is the main renal target of FGF23 and therefore the NaPi2a null phenotype is dominant. In bone, rib nodules, increased mineralization of the primary spongiosa and increased osteoid characterize the FGF23 null mouse; these phenotypes are also observed in double knockout mice, which are hypophosphatemic, thus making the important point that some actions of FGF23 in bone are independent of serum phosphate.

Mutations in the NaPi2c transporter gene cause human hypophosphatemic rickets with hypercalciuria (HHRH) (5). Expression of both alleles from a compound heterozygotic HHRH patient showed that one is nonfunctional but the other is hypomorphic, apparently because of an inward-directed sodium leak (10). In the mouse, however, removal of the NaPi2c
gene does not produce rickets, but rather hypercalcemia and an increase in \(1,25(OH)_2D\) levels, with no change in serum phosphate or TmP/GFR (11). FGF23 levels are reduced in NaPi2c null mice, which develop marked hypophosphatemia when treated with FGF23 (12). Relationships between renal phosphate handling, vitamin D activation and FGF23 levels are complex: loss of NaPi2c resets the level of vitamin D synthesis and FGF23 without any net change in phosphate excretion.

Extracellular phosphate levels in cartilage rise as chondrocytes mineralize their matrix, and eventually phosphate induces apoptosis of chondrocytes, ending their life cycle. Phosphate induces several pro-apoptotic molecules in ATDC5 cells (13). One of these is Bnip3. Silencing of Bnip3 by RNAi suppressed phosphate-induced apoptosis, and conversely expression of Bnip3 attenuated the antiapoptotic effect of Bcl-xL. Removal of Bcl-xL from chondrocytes with Cre-loxP produced dwarfism due to massive chondrocyte apoptosis. These abnormalities were largely rescued by a low-phosphate diet, which upregulated Bnip3 in chondrocytes. Another presentation reported that Hyp chondrocytes have reduced phosphate uptake via the Type III transporter Pit-1 (14). Overexpression of Pit-1 increases phosphate uptake, ATP levels, caspase-9 and -3 activation and apoptosis. Phosphate can also signal in renal tubule cells, possibly via NaPi2 (15) and overexpression of Pit-1 produces hyperphosphatemia without abnormalities in vitamin D activation (16).

One of the biggest pieces of news at last year’s ASBMR meeting was that mutations in the SIBLING protein dentin matrix protein 1 (DMP-1) cause renal phosphate wasting and osteomalacia. The business end of DMP-1 is the carboxyl terminal domain (17). Mutations in DMP-1 that cause hypophosphatemic rickets impair processing of the molecule (18). DMP-1 expression patterns in bone are correlated with the pattern of strain (19). The sister SIBLING protein MEPE has a tantalizing but poorly understood relation to phosphate disorders. MEPE levels are high in tumors from patients with tumor-induced osteomalacia but MEPE null mice do not have a demonstrable phosphate disorder, establishing that MEPE is not a potent phosphatonin. MEPE overexpression, in fact, produces hyperphosphatemia and increased levels of \(1,25(OH)_2D\) (20). Bones from these animals have reduced mass, mildly impaired mineralization, and low turnover (21).

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References


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BONE IMAGING AND FINITE ELEMENT ANALYSIS

Yebin Jiang

University of Michigan Medical School, Ann Arbor, Michigan, USA

Finite element analysis (FEA) from bone imaging for bone biomechanical properties continued as a hot topic at this year’s ASBMR meeting. The ultimate goal of bone imaging is to provide non-invasive measures of the likelihood of future fracture. Mechanical testing of machined bone specimens (1) and whole bones (2) have shown that the initial slope of the load-deformation curve is closely related to the material properties and bone architecture and can be estimated with FEA (3), while the breaking point is influenced by non-linear features and may differ by age, disease status, and other factors. Quantitative CT (QCT) can measure clinically relevant 3D volumetric BMD of the cortical and trabecular compartments. QCT BMD values of each bone voxel are converted into elastic modulus values using pre-determined correlations between the elastic modulus and QCT-derived BMD. FEA mechanically integrates geometrical and material property data from CT scans to provide measures and predictions of bone mechanical strength. FEA based on the distribution of the bone material and converting the CT-measured density values to local elastic modulus with a relationship that almost follows a square law (4) can better represent bone strength.

QCT FEA and Treatments for Osteoporotic Patients

PTH (1-34) and alendronate were previously shown to have positive effects on vertebral strength as assessed by FEA of QCT (5). These studies were extended to include analysis of proximal femoral strength for a simulated sideways fall. Total hip strength at 18 months significantly increased (5.9%) in the PTH (1-34) group and did not significantly change in the alendronate group. This significant biomechanical effect for PTH (1-34) was associated with a significant decrease in cortical density (-1%) and an increase in trabecular density (5.1%) (6). In another study, CT examination of the proximal femur indicated that 24 months of PTH (1-34) treatment improved bone strength of the proximal femur with regard to both bending and buckling, with larger protective effects in subjects at higher risk (7). In patients treated with PTH (1-34), DXA loses its ability to estimate bone strength, but QCT and CT image-derived bone structure maintain a high correlation with FEA bone strength (8). High resolution CT combined with FEA of the vertebra showed that bone apposition with PTH (1-34) treatment was not uniform, but directed to skeletal regions of local structural weakness, likely explained by the biomechanical concepts of bone tissue response to local strains, in accordance with Wolff’s law and Frost’s mechanostat (9).

Previously, a QCT-based nonlinear FEA was shown to predict vertebral strength, fracture sites and distribution of minimum principal strain (10). Now, L2 FEA of postmenopausal Japanese women with (n=29) or without (n=75) osteoporotic vertebral fracture showed that the optimal point on the ROC curve as vertebral fracture threshold was 1950 N with 76% sensitivity and 73% specificity. FEA showed a more sensitive response to alendronate therapy than DXA (11).
QCT scans of 36 unembalmed, previously frozen human cadaveric femora, and biomechanical testing that simulated a sideways fall at a rate of 100 mm/s, showed that most of the fracture lines propagated through the superoposterior region of the femoral neck. Hence the superoposterior region may be critical to the strength of the femoral neck as a key site of fracture initiation and propagation in sideways falls (12).

**Micro CT, High Resolution Peripheral QCT, and FEA**

Micro CT examination continues to find application in the assessment of human bone biopsies, and in rodents in vivo and in vitro. High resolution (82 µ) peripheral quantitative CT (HRpQCT) is not yet approved by the FDA, but is used as a research tool in examining the human distal radius and distal tibia.

Micro CT assessment of iliac crest biopsies from postmenopausal women treated with PTH (1-34) demonstrated an increase in both trabecular and cortical thickness, irrespective of whether subjects had received prior alendronate therapy (13). Micro CT examination of iliac crest biopsies at 8 µm isotropic resolution demonstrated that risedronate reduced cortical porosity by reducing the birth rate of new osteons or by filling in the remodeling spaces in osteons that existed prior to treatment (14). FEA of non-linear tissue properties from micro CT scans of human iliac crest biopsies showed that deterioration of trabecular architecture directly affected both strength and bone toughness (15). Examination of cylinders of trabecular bone with micro CT indicated that partial volume, segmentation artifacts, and beam-hardening effects due to the polychromatic source could contribute to errors in micro CT-based measurement of degree of mineralization of bone (16).

Overall moderate relationships were found between comparable measures performed on iliac crest biopsies by 2D histomorphometry and 3D micro CT, and between most 2D as well as 3D parameters at the iliac crest and HRpQCT of the distal radius, while parameters from distal tibia did not correlate well with biopsies (17).

HRpQCT and DXA examinations of 200 women showed that HRpQCT better discriminated fracture risk in osteopenic and osteoporotic patients, while the T-score seemed to underestimate fracture risk (18). FEA on HRpQCT images of the distal radius in 33 postmenopausal women who previously sustained a fragility fracture of the wrist and 33 age-matched controls demonstrated that the load distribution between cortical and trabecular bone seems promising for improving wrist fracture prediction, independent of BMD and microarchitecture (19).

HRpQCT measuring the ulradistal radius showed a significant 6.9% increase in BV/TV at 1 year post-treatment with PTH (1-34), similar in magnitude to the 5.7% change at the iliac crest in women treated for 3 years with PTH (20;21). Sustained increases in the more differentiated osteoprogenitor cells appear to be predictive of larger gains in trabecular bone volume assessed by HRpQCT (22).

HRpQCT of healthy girls without a prior history of fracture showed marked but transient decreases in cortical thickness during puberty, with no significant differences in trabecular parameters (23), which was a mirror image of the rise in distal forearm fractures in girls that peaks between ages 8 and 11, during the time of maximal pubertal growth (24). The trabecular parameters may be established very early in life, while the temporary cortical thinning may relate to increased calcium demands during maximal growth. Examination with pQCT and DXA showed that before and after menarche, bone growth in length and width were influenced differently by hormones and mechanical loading, with mechanical loading as the dominant factor throughout the pubertal period (25).

HRpQCT has various other applications. HRpQCT and QCT examination in men demonstrated that the prevalence of aortic calcification rapidly increased after age 50 and was correlated with lower vertebral and femur neck vBMD and with lower distal

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radius BV/TV and trabecular thickness (26). HRpQCT scans of the radius and tibia in 8 premenopausal women with idiopathic osteoporosis showed fewer, more widely separated trabeculae of similar thickness, and decreased thickness (only in the tibia), compared to controls (n=9) (27). The loss of entire trabecular elements may underlie the more heterogeneous trabecular network. Examination of the distal radius and tibia in 103 European-Caucasian mother-daughter pairs demonstrated that heritability for BMD, cross-sectional area and trabecular thickness were more robust at weight-bearing sites, suggesting a genetic influence on the skeletal response to loading (28). HRpQCT examination of moderate chronic kidney disease and end-stage kidney disease demonstrated that lower eGFR was associated with both cortical and trabecular deterioration independent of gender and age, which may contribute to the increased susceptibility to fracture, due in part to the catabolic effects of secondary hyperparathyroidism (29).

DXA, Controversial Hip Structural Analysis, and Other Imaging Modalities

Current DXA strength calculations assume a linear relationship of density and elastic modulus and also assume a circular structure of the complex 3-D femoral neck structure from 2-D projection images with limited spatial resolution. Results might be improved by an appropriate assumption of a power-based relationship and non-circular structure, respectively (2). Cortical thickness, cross-sectional area, shape, cortical area, and section modulus measured directly from micro QCT images of postmortem specimens from 13 Caucasian females showed that the narrowest neck was not a constant referent predictive of the diverse structure of the femoral neck. Thus, use of DXA indirect estimates of femoral neck structure using hip structure analysis should be viewed with skepticism (30).

Precision may degrade in thicker subjects due to decreased x-ray flux and the effect of thicker tissue on edge detection. Lunar iDXA provided excellent precision for total body measurements, including BMD, BMC and body composition, with root-mean-square standard deviation < 1% from obese subjects (31). Mid-body fat has been shown to be more predictive of cardiovascular risk factors than total body fat. Comparison of corresponding tape-measured and iDXA total body scan-analyzed body fat in android (abdominal) and gynoid (hip) regions in 37 postmenopausal overweight women with mean BMI 32 showed Pearson’s correlation r ranging from 0.7 to 0.9 (32).

3D images constructed from helical CT scan (n=1280) and fluoroscopy images (n=2600) showed that the endplates of fractured vertebral bodies were irregular with multiple Schmorl’s nodes and endplate perforations (33). CT would be better than conventional x-ray for fracture detection, and such changes may not be captured with morphometry.

Prolonged alendronate use (average duration of use of 7.3 years) may actually increase the risk of low energy subtrochanteric and shaft fractures, as x-rays of patients taking alendronate revealed a pattern of a simple or oblique fracture with cortical thickening and breaking of the cortex on one side (34). Examination of biopsies from osteoporotic women treated for 3 years with strontium ranelate (n=6) or placebo (n=6) using x-ray microanalysis of small selected areas (10Å~10 µm) within individual bone packet areas revealed that Sr was present in a molar fraction up to 6%, exclusively in bone packets newly formed during strontium ranelate treatment (35).

A compact peripheral 1.0 T permanent magnet MRI system was used to perform trabecular bone micro-architectural assessment of the distal radius in 5 volunteers, with reproducibility 2-14% as the root mean square CV (36).

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References

1. Cole JH, Myers ER, van der Meulen MCH. Finite element models more accurately predict structural behavior of human cancellous bone when using specimen-specific tissue properties. J


15. Gross GJ, Hong H, Borah B, Phipps RJ, Dufresne TE. Bone strength and toughness are reduced by loss of


27. Cohen A, Recker RR, Guo XE, Zhang XH, Lappe J, Eisenberg HF, McMahon DJ, Shane E. Abnormal trabecular microarchitecture and mechanical competence in premenopausal women with idiopathic osteoporosis (IOP) can be detected by high resolution peripheral quantitative computed tomography (HRpQCT). *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S194. [Abstract]

28. Ferrari SL, Chevalley T, Bonjour JP, Rizzoli R. Heritability of bone...


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BONE ACQUISITION AND PEDIATRIC BONE

Heather M. Macdonald

University of British Columbia, Vancouver, British Columbia, Canada

Childhood and Adolescence – Critical Periods for Bone Development

A continual struggle in pediatric bone research is how best to control for individual variability in growth and maturation. In prospective studies spanning the adolescent growth spurt, multi-level modeling techniques can be applied to compare children according to biological age (years from age at peak height velocity). At this year’s ASBMR meeting, results from the longest follow-up study of bone mineral accrual, which were generated using such modeling techniques, provide further evidence that the adolescent growth period is crucial for skeletal development, as more than 98% of bone mineral is accrued by 4 years beyond the age of peak height velocity (PHV) (1). Although up to 40% of total body bone mineral is accrued in the 2 years before and 2 years after PHV, the post-adolescent period also influences skeletal development as a further 18% of adult bone mineral is laid down during this time.

It is a common assertion that sex differences in fracture risk are determined, in part, by sex differences in bone mass and strength that emerge during growth. However, questions remain as to when this sexual dimorphism emerges and what factors influence the magnitude of the difference in bone parameters between boys and girls. Data from Australian twins suggest that boys have greater bone mass and periosteal width (by DXA) than girls in prepuberty (2). Similarly, at the tibia, pQCT outcomes are greater in boys at both the metaphysis and diaphysis in prepuberty (3), although greater muscle cross-sectional area in boys may explain this difference at the tibial shaft. Together with previously published pQCT findings at the tibia in prepubertal children (4), these findings challenge the traditionally held belief that sexual dimorphism is driven only by differences in sex hormones that become more apparent in early puberty.

Another common notion, based on the early work of Stanley Garn and colleagues (5), is that increasing levels of estrogen in girls leads to greater endosteal apposition compared with boys. Prospective pQCT data for the tibial shaft (6) challenge this theory, as do previously published pQCT results on the same cohort (7). However, pQCT data for Finnish girls suggest a negative relationship between time relative to menarche and area of the marrow cavity, suggesting increasing endosteal apposition with advancing maturity (8). Thus, it appears this theory is still up for debate.

Is Dietary Protein a Friend or Foe of Bone Mineral Accrual?

Calcium took a back seat to dietary protein intake at this year’s meeting. Two abstracts reported associations between dietary protein and bone mass by DXA. In Chinese girls, there is a negative relationship between BMC accrual over 5 years and protein intake (9). In contrast, cross-sectional data on Swiss boys (mean age 7 years) indicated a positive relationship between BMC and protein intake (10). Further, high levels of protein intake in combination with high levels of physical activity were associated with greater BMC.
than that observed in boys with high physical activity but low levels of protein intake. The discrepancy in these findings is likely related to dietary calcium intake, which was low among the Chinese girls (~440 mg/day) compared with the Swiss boys (~750 mg/day). Thus, the calcium-protein ratio was lower among Chinese girls, and this may have a negative effect on bone accrual (due to higher levels of calcium excretion). Further investigation of these relationships is required in prospective cohort studies and randomized controlled trials.

Moving Beyond Standard pQCT Analyses

As researchers become more knowledgeable in the assessment of bone geometry with pQCT, it is not surprising that this modality is being used to address more specific questions relating to skeletal development. However, often additional software is needed (other than the standard Stratec software) to answer these questions. One abstract (11) presented pQCT results that were obtained with the free NIH software ImageJ in combination with customized macros. Bone bending strength (Imax) at the tibial shaft increased significantly more in boys who participated in a school-based physical activity intervention (11). Two abstracts presented pQCT data analyzed with Bonalyse software that described changes in the distribution of bone material at the tibial midshaft during growth and how these changes influence bone bending strength (Imax, Imin) (12;13). Although an individual’s skeletal structure is largely genetically predetermined and is thus established before puberty (13), adaptation to loading occurs throughout growth and appears to differ between sexes such that boys demonstrate greater increases in bone bending strength compared with girls.

Although standard pQCT offers many advantages over DXA technology, it lacks the resolution to evaluate trabecular microstructure and to obtain accurate measures of cortical thickness at metaphyseal sites. At this year’s meeting, the first high-resolution pQCT data for children and adolescents were presented (14). Across puberty in girls there were minimal changes in trabecular microstructure (i.e., bone volume/total volume, trabecular thickness) at the distal radius. In contrast, a transient decrease in cortical thickness was apparent during puberty, and this decrease mirrored the temporary increase in forearm fractures that was previously reported in this population (15).

Physical Activity During Growth – Do the Benefits Persist?

The age-old question in studies of physical activity and pediatric bone is whether the benefits of intervention persist once the stimulus is removed. At this year’s meeting, the longest follow-up data from a school-based trial were presented (16). The BUGSY study (Building Growing Skeletons in Youth) found that almost 8 years after completion of the jumping program, children who were in the intervention group maintained a 1.4% advantage in total hip bone mineral accrual compared with the control group. The question is now whether this skeletal advantage will be maintained into adulthood.

There also appears to be some debate on whether general physical activity has a sustained effect on bone mineral accrual. Data from the Finnish Calex Study (17) indicate that over almost 7 years of follow-up, the significantly higher BMC in high-active compared with low-active girls observed after 2 years was no longer apparent, suggesting that the benefits of physical activity during puberty may be temporary. In contrast, when multi-level modeling techniques were applied to data from the UBC Healthy Bones Study (18) to account for variability in growth and maturation, leisure-time physical activity was found to be a significant predictor of bone mineral gain over 7 years at the femoral neck and total proximal femur in girls and boys, respectively.

The Muscle-Bone Relationship

At the last several ASBMR meetings, a focus within the pediatric abstracts has been the muscle-bone relationship and how this relationship changes during growth and
differs between sexes. This theme continued at the 2007 meeting. In cross-sectional and longitudinal studies, muscle cross-sectional area (MCSA, by pQCT) and lean mass (by DXA) were consistently identified as predictors of bone geometry, strength and bone mass (3;8;13;19-21). Interestingly, growth in bone width (total cross-sectional area) precedes growth in MCSA in Finnish girls. This finding does not agree with previous longitudinal data showing that the peak in lean mass precedes the peak in bone mineral accrual (22). Further, it appears that lean mass by DXA may not fully account for skeletal loading associated with physical activity. Even after adjusting for differences in arm lean mass, gymnasts have greater pQCT-estimated bone strength at the radius compared with non-gymnasts (23). What is not clear is whether similar results would be obtained with pQCT-derived measures of MCSA or more functional measures of muscle force and power.

Muscle-bone indices are also useful indicators of bone development in clinical populations (24). In adolescent girls with Type I diabetes, the ratio of tibia BMC to MCSA is lower than that of healthy girls, suggesting a possible “bone-muscle disconnect” in this clinical group due to compromised bone mineral acquisition (25). In contrast, muscle-bone indices (by MRI) in children with cerebral palsy were not significantly different from healthy controls, suggesting that bone strength is adapted to muscle force in this group despite an inability to ambulate independently (26).

**Bisphosphonate Therapy for Pediatric Patients with Low Bone Mass**

Evidence-based recommendations for bisphosphonate therapy in pediatric groups with low bone mass, other than those with osteogenesis imperfecta, are not well-established (27). Two abstracts presented results demonstrating the efficacy and safety of intravenous pamidronate (28) and oral alendronate (29) in pediatric patients with glucocorticoid-induced osteoporosis. A concern of bisphosphonate use in the treatment of bone disorders is the reported relationship between bisphosphonate therapy and osteonecrosis of the jaw (ONJ) in adults (30). The question is whether bisphosphonates have similar effects on the jaw in pediatric patients. The answer appears to be no, as long-term pamidronate exposure was not associated with any cases of ONJ in a large cohort of pediatric patients (31). It may be that oral hygiene is better in pediatric patients as this is highlighted as the primary preventive strategy for ONJ (30).

In summary, the pediatric abstracts at this year’s meeting highlight both how far the field has come in a short time and also how much we have still to learn. The expanding use of pQCT and other imaging modalities and software applications to investigate bone geometry and strength indices is encouraging and helps to further our understanding of skeletal development in both healthy children and in clinical groups.

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**References**


MEETING REPORT

Meeting Report from the 29th Annual Meeting of the American Society for Bone and Mineral Research

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TREATMENT OF OSTEOPOROSIS

Ego Seeman

Austin Hospital, University of Melbourne, Melbourne, Australia

Advances have taken place in therapeutics. The most impressive are new forms of therapy that seem to have bone-forming effects on one or both periosteal and endosteal surfaces and no anti-resorptive effect or an inhibitory effect on resorption. Some of these studies are summarized below. Inferences must await scrutiny of the published work.

Anti-Sclerostin Antibodies

One study reported that antisclerostin antibodies (5 or 25 mg/kg twice weekly for five weeks) given to rats increased BMD by 16 and 27% (lumbar spine), 15% and 20% (distal femur), and 9% and 11% (femur-tibia) (1). Osteocalcin increased with no change in CTX, and trabecular bone volume increased by 133% and 166%, respectively. Osteoblast but not osteoclast surface increased with other evidence of increased bone formation, including mineralizing surface, apposition rate and bone formation rate. Greater increases in mineralizing surface (383% versus 130%) and bone formation rate (852% versus 285%) were found on the endocortical than on the periosteal surfaces.

Sclerostin inhibition was also found to increase periosteal and endocortical bone formation and to decrease cortical porosity in six-month-old female rats oophorectomized and left for 13 months and then treated with sclerostin antibody 25 mg/kg twice weekly for five weeks (3). Marrow cavity area decreased by 17% and cortical bone area increased by 14%, compared with oophorectomized controls. Mineralizing surfaces increased by 155%, mineral apposition rate by 84%, bone formation rate by 339%, and on the endocortical surface the respective increases were 372%, 145% and 913% compared with oophorectomized controls. Cortical porosity was examined and expressed as a percentage of total cortical area. Porosity decreased relative to oophorectomized controls with increased intracortical surface bone formation parameters.

In a study of 48 healthy postmenopausal women, treatment with varying doses of a sclerostin antibody resulted in increases in P1NP, osteocalcin and bone specific alkaline phosphatase of 60-100% at 3 mg/kg by 21 days (4).

Remodeling Suppressants

Several advances have taken place using drugs that reduce the birth rate of new remodeling units. It is a little misleading to call these drugs resorption inhibitors – they are of course – but whether they reduce the volume of bone resorbed in the reduced numbers of remodeling units remains unclear. Evidence for this is best for estrogen but no data is available for bisphosphonates. One of the most powerful remodeling rate suppressants appears to be denosumab, a drug that inhibits the
synthesis of bone-resorbing osteoclasts and the activity of existing osteoclasts.

**Denosumab**

Studies in oophorectomized monkeys found that denosumab (25 or 50 mg/kg for 16 months) increased bone mass by ~20-25% at the femoral neck and spine and increased peak tolerated loads by 54% at the spine and by 19-34% at the femoral neck, compared to oophorectomized controls (5). Stiffness increased 39-46% at the vertebra and by 20-25% at the femoral neck, compared to oophorectomized controls.

Another group reported the effects of denosumab after 48 months in postmenopausal women (6). In 229 patients, 48 months of continuous treatment increased spine bone density by 10.6% and hip bone density by 5.8%. Cessation resulted in a fall in bone density to near baseline while rechallenging increased bone density. Bone turnover markers were decreased with continuous therapy and increased upon discontinuation.

In male mice, denosumab was also reported to prevent cortical bone thinning, induced by prednisolone, which was the result of increased resorption as reflected in increased DPD excretion and increased serum and bone TRAP5b activity (7).

**Bisphosphonates**

Advances in the study of the bisphosphonates have also been made. In the first study of the prevention of fractures in women with hip fractures, 1065 patients were assigned to yearly intravenous zoledronic acid (5 mg), and 1062 patients were assigned to placebo during a median of 1.9 years (8). The respective rates of any new clinical fracture were 8.6% vs 13.9% (a 35% risk reduction, \( P = 0.001 \)), rates of new clinical vertebral fracture were 1.7% vs 3.8% (\( P = 0.02 \)), and rates of new non-vertebral fractures were 7.6% vs 10.7% (\( P = 0.03 \)).

101 of 1054 patients (9.6%) and 141 of 1057 patients (13.3%) died, a reduction of 28\% (\( P = 0.01 \)).

In another study, minodronate was given to 359 postmenopausal Japanese women aged between 55 and 80 years, while 345 received placebo for 26 months (9). Vertebral fracture rate was reduced by 58.9\% (CI 36.6%-73.3\%). Fractures occurred in 10.4\% of treated patients versus 24\% of placebo recipients. Effects on non-vertebral fractures were not reported.

Differences in bisphosphonates that affect the response to anabolic therapy were also reported (10). In 146 post-risedronate- and 146 post-alendronate-treated subjects treated with 20 \( \mu \)g of \textit{PTH}(1-34) for 12 months, the former had a greater response in bone turnover markers (before and after adjusting for higher baseline values in the post-risedronate group). There was a 76\% greater increase in QCT of trabecular bone at the spine (24.1\% versus 13.7\%, \( p = 0.02 \)).

A greater absolute increase in P1NP for the post-risedronate than post-alendronate group during months 1-5 was also reported (11). Similar results were found for other markers. The increases post-risedronate occurred earlier but became similar after 12 months.

If a drug makes BMU balance positive, it is of interest to increase the rate of remodeling, or to at least avoid suppressing it, as the net effect should be reconstruction of the skeleton. It was reported that a bisphosphonate analog (IG9402) without remodeling suppressant activity prevented osteocyte and osteoblast apoptosis and the loss of strength induced by corticosteroid therapy (12). IG9402 did not reduce markers of remodeling while alendronate did. Alendronate decreased bone formation, but this was not found with the drug.

**SERMS**

In a phase 3 study, women with osteoporosis with or without prevalent fractures were treated with 20 mg or 40 mg/day of bazedoxifene (BZA), compared with 60 mg of raloxifene or placebo (13). Among 7492 women after three years, incidences of new vertebral fractures were 2.3\%, 2.5\%, 2.3\% and 4.1\% in the BZA 20 mg, BZA 40 mg, raloxifene 60 mg and placebo groups, respectively, with statistically significant risk reductions for new vertebral fracture of 42\%, 37\% and 42\%, respectively, compared with placebo. There was no effect on non-vertebral fractures. In the post hoc analysis of those patients with a T score \( \leq -3 \) SD or one or more moderate or multiple vertebral fractures (n=1782), nonvertebral fracture incidence was 3\%, 3.8\%, 5.9\% and 6.3\% in the BZA 20 mg, BZA 40 mg, raloxifene 60 mg, and placebo groups, respectively. This resulted in the 20 mg dose of BZA reducing
nonvertebral fractures by 52%, a significant reduction.

Finally, in 1583 postmenopausal women with a mean age of 57 years treated with BZA, prevention of bone loss and a reduction in bone remodeling markers in the order of around 20-25% was found, similar to that found with raloxifene (14).

Cysteine Protease Inhibition

The cysteine protease inhibitor MK-0822 was evaluated in a dose-ranging study in 399 postmenopausal women randomized to placebo or one of four doses (15). The highest dose increased spine BMD by 3.4% and femoral neck BMD by 2.5%, and was associated with a 58% reduction in urinary NTx.

Parathyroid Hormone

In 7 women with osteoporosis, 12 months of 20 µg of parathyroid hormone increased BV/TV by 6.9%. Two-thirds of of the increase was due to an increase in trabecular thickness and one-third of the increase was due to an increase in trabecular number (16). There was a trend towards a decrease in cortical thickness and cortical vBMD.

An increase in microcrack density was reported in patients treated with alendronate compared to untreated controls (17). Sixty-six postmenopausal women with osteoporosis were treated with 20 µg daily of PTH(1-34) for two years. Thirty-eight stopped alendronate and were treated with PTH while 28 were treatment-naive. Paired biopsies were available in 13 treatment-naive and 18 alendronate-treated subjects. Crack surface density and crack length decreased in previously alendronate-treated patients while only crack length was reduced in formerly treatment-naive patients. The authors infer that PTH(1-34) reduced microdamage accumulation.

Histomorphometric results following 20 µg of parathyroid hormone given to treatment-naive and previously alendronate-treated subjects were also reported (18). After 2 years of treatment, bone biopsies demonstrated that activation frequency increased by 130% and 359% for 16 treatment-naive and 29 alendronate-treated subjects, respectively. 3-D micro CT indicated an increase in trabecular and cortical thickness with no difference in the two groups, suggesting that prior alendronate treatment does not impair the morphological response to PTH. Trabecular thickness increased by 30% in both groups. For previously treatment-naive and alendronate-treated groups, there were increases, respectively, of 36.7% and 12.7% in trabecular number, increases of 37.8% and 31.7% in cortical thickness, and increases of 28.2% and 42.8% in total cortical area.

The effects of cyclic and daily parathyroid hormone combined with OPG in 20-week-old mice treated for seven weeks was also reported (19). All treatments increased bone density. Daily PTH increased periosteal circumference by 4.9%, and the combination of daily PTH and OPG increased it by 4.2%. Cyclic PTH produced a 3.8% increase in periosteal circumference, while the combination of cyclic PTH and OPG produced an increase of 2.8%. OPG reduced endosteal circumference by 1.8%, and there was no effect on this measure from daily PTH. The combination of daily PTH and OPG reduced endosteal circumference by -5.4%, whereas cyclic PTH therapy increased it by 2.5%, and combination therapy of cyclic PTH plus OPG reduced it by -3.2%. Cortical thickness increased most with the combined daily PTH plus OPG treatment (+22%). Cyclic PTH plus OPG increased cortical thickness by 10.9%, daily PTH alone increased it by 14%, and cyclic PTH increased it by 6.2%.

Ostabolin-C, a cyclic analogue of PTH(1-31), has been shown to increase bone density in preclinical studies, and it is also now reported that, in 261 postmenopausal women treated by subcutaneous injection in a dose-ranging study, treatment with Ostabolin-C resulted in an increase in bone density within four months of treatment, with mean increases of around 11% within 12 months of treatment in the 45 µg group (20). These changes were accompanied by increases in biochemical measures of bone formation, with an increase in P1NP of over 120% and an increase in osteocalcin of over 100%.

Calcium-Sensing Receptor

Antagonism of the calcium-sensing receptor in the parathyroid gland results in an increase in endogenous PTH release, and investigators have now reported results in
healthy male volunteers given the calcium-sensing receptor antagonist SB-423557 (21). In a study comparing a range of doses, the authors report elevations in plasma PTH that lasted under eight hours. At doses of 100 mg of SB-423557 and above, PTH exposure was 10%-48% higher than placebo.

Activin Fusion Protein

A single dose of ACE-011 was reported to increase bone formation and decrease bone resorption markers in postmenopausal women (22). This fusion protein consists of an extracellular domain of human type II activin receptor IIA linked to the Fc portion of human IgG1. The fusion protein binds to activin and has been reported to improve architecture and bone strength as a result of an anabolic effect on bone. In this randomized, double-blind study of 48 women given a single dose of ACE-011 or placebo and followed for 120 days, treatment resulted in an increase in bone-specific alkaline phosphatase and a dose-dependent decrease in C-terminal type I collagen telopeptide and TRAP-5B.

ACE-011 was also reported to increase bone density and improve microarchitecture in monkeys given a single subcutaneous injection of up to 30 mg/kg, and monkeys given multiple doses at 10 mg/kg over three months. (23). Both doses were well-tolerated. Treatment bi-weekly for three months using 10 mg/kg per day increased BMD, trabecular bone density and structure. BMD of L5 by DXA increased by 13%, and micro-CT revealed that bone volume increased by 16% and trabecular number by 13%, and there was also a 7-fold decrease in structure model index. At the distal femur, similar observations were made, as there was an increase in trabecular density of 79%.

Finally, a study reported that a fusion protein combined with PTH prolongs PTH hormone action (24). This protein was synthesized by combining PTH(1-33) with the collagen binding domain of the ColH collagenase. The new drug resulted in stimulation of cyclic AMP accumulation, similar to that seen with PTH(1-34) in cell lines. Weekly injections in young female mice increased spinal bone density by 16% versus 7% for PTH(1-34). There was no evidence of hypercalcemia in the animals.

References


