NOT TO BE MISSED

Clinical and Basic Research Papers – November 2010

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Clinical Studies and Drug Effects


40 patients with periodontitis underwent periodontal surgery and received daily teriparatide (20 µg) or placebo for 6 weeks. The patients were followed for 1 year. Compared to placebo, radiographic linear resolution of osseous defects was greater after teriparatide at 6 months, with a reduction in periodontal probing depth of 33% vs. 20% (2.42 mm vs. 1.32 mm) and a gain in clinical attachment level of 22% vs. 7% (1.58 mm vs. 0.42 mm) in target lesions at 1 year (P = 0.02 for both comparisons).
—ES


Seeman E. Bone morphology in response to alendronate as seen by high resolution computed tomography: Through a glass darkly. J Bone Miner Res. 2010 Oct 6. [Epub ahead of print] [Info]

Effects of alendronate (ALN) on vBMD and bone microstructure by HR-pQCT have previously been reported against strontium ranelate on one side, and denosumab or placebo on another side (see recent BoneKEy Commentary by S. Ferrari), with somewhat discordant results (increase in cortical thickness from baseline with ALN in one study, but not in the other). This study reports improvements of vBMD and bone structure at the tibia, particularly in cortical thickness and area, but not at the radius, after 2 years of ALN vs. baseline in osteopenic women, and minor improvements vs. placebo at both sites. In a brilliant accompanying editorial, Dr. Seeman explains the mechanisms by which antiresorptive therapy produces some changes in cortical bone microstructure (porosity) and mineralization that may result in detectable changes by HR-microCT. —SF


It is well-known that oral ALN plus daily sc PTH given simultaneously has no additive effects on BMD gain. Yet once yearly iv zoledronic acid plus daily sc teriparatide (TPT)
given to post-menopausal women with osteoporosis for 1 year produced greater earlier increases of hip and spine BMD than either treatment alone, and greater one-year increases of hip BMD vs. TPT alone. Although unlikely to result in new clinical practice, these results challenge the notion that bisphosphonates necessarily blunt TPT effects in humans and may pave the way to optimize rapid BMD gain at both the spine and hip simultaneously. —SF


In this 3-year, double-blind, randomized, placebo-controlled trial in 50 postmenopausal women with osteopenia taking a single 5-mg dose of zoledronate, mean serum CTX and P1NP were 44% and 40% lower in the zoledronate group (p < .001 vs. placebo for each marker). BMD was higher in the zoledronate group than in the placebo group by an average of 6.8% at the lumbar spine, 4.0% at the total hip, and 2.0% at the total body (p < .001 for each skeletal site). Between-group differences in bone turnover markers and BMD were stable from 12 to 36 months. —ES


Bone biopsies were collected at 24 and/or 36 months from osteoporotic postmenopausal women in the FREEDOM study (45 receiving placebo, 47 denosumab) and at 12 months from women previously treated with alendronate in the STAND study (21 continuing alendronate, 15 changed to denosumab). In FREEDOM, median eroded surface was reduced by > 80% and osteoclasts were absent from > 50% of biopsies. Double labeling in trabecular bone was observed in 94% of placebo bones and 19% of those treated with denosumab. Median bone formation rate was reduced by 97%. In STAND, double labeling in trabecular bone was seen in 20% of the denosumab biopsies and in 90% of the alendronate samples. —ES


13,714 bisphosphonate nonusers were matched to 6,857 bisphosphonate users, at a 2:1 ratio, on cancer type, age, sex, presence of bone metastases, and SEER geographic region. Results suggested that intravenous bisphosphonate use was associated with an increased risk for atrial fibrillation (HR = 1.30; 95% CI: 1.18-1.43), all supraventricular tachycardia (SVT) (HR = 1.28; 95% CI: 1.19-1.38), and stroke (HR = 1.30; 95% CI: 1.09-1.54). The risk for all SVT increased 7% for each increase of five bisphosphonate dose equivalents (HR = 1.07; 95% CI: 1.02-1.12). —ES

Public Health – Epidemiology


1,490 community-dwelling men at least 65 years of age were followed for 7.3 years.
330 (22.2%) died: 97 from cancer, 110 from cardiovascular disease, and 106 from other causes. The adjusted HR per SD decrease in 25(OH)D for all-cause mortality was 1.01 (95% CI: 0.89-1.14); no association between 25(OH)D and cardiovascular or other-cause mortality was seen. Lower 25(OH)D levels were associated with a decreased risk of cancer mortality (HR per SD decrease = 0.80; 95% CI: 0.64-0.99). Higher PTH was associated with an increased risk of all-cause mortality (HR per SD increase = 1.15; 95% CI: 1.03-1.29) and cardiovascular mortality (HR per SD increase in PTH = 1.21; 95% CI: 1.00-1.45). —ES


1,073 community-dwelling older adults were followed to 10.4 (mean 6.4) years with 111 cardiovascular deaths. After adjusting for age alone or multiple covariates, there was no association between 25(OH)D, 1,25(OH)₂D, or intact PTH and cardiovascular mortality. —ES


Among 1,194 men (mean age 71) followed for 12.7 years, 584 (49%) died. A 50% higher total mortality was observed among men in the lowest 10% (< 46 nmol/L) and the highest 5% (> 98 nmol/L) of plasma 25(OH)D concentrations compared with intermediate concentrations. Cancer mortality was also higher at low plasma concentrations (HR = 2.20; 95% CI: 1.44-3.38) and at high concentrations (HR = 2.64; 95% CI: 1.46-4.78). For cardiovascular death, only low (HR = 1.89; 95% CI: 1.21-2.96), not high (HR = 1.33; 95% CI: 0.69-2.54) concentrations suggested higher risk. Both high and low concentrations of plasma 25(OH)D are associated with elevated risks of overall and cancer mortality. Low concentrations are associated with cardiovascular mortality. —ES

Genetics


These 2 papers illustrate the interest in systems genetics to identify new genes (pathways) that regulate bone mass/turover. By systems genetics, we mean the combination of gene variant association with a phenotypic trait on one side with gene expression data on another side. Hence C. Farber first identified 11 transcribed gene modules (networks) in silico (i.e., using previously published monocyte microarray expression profiles from young Chinese adults with extremely low or high BMD), and then validated his findings by using two publicly available GWAS data sets to perform an in silico association study testing for association of genes in module 9 (immune process viral response!) with aBMD, thereby identifying 6 novel potential

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determinants of bone mass. Suwanwela et al. used a similar approach, but in mice, to link chondrocyte gene expression data with femur bone geometry, and subsequently tested the functionality of the newly discovered genes by inhibiting their expression with siRNAs. Both approaches point to new genes potentially involved in bone strength. —SF


Although the exact effect of adiponectin on bone is incompletely understood, adiponectin was hypothesized to contribute to the pathogenesis of osteoporosis. Recently, several GWAS for adiponectin in Caucasians have identified ADIPOQ and ARL15 as possible causal genes. To date, there have been no GWAS of adiponectin levels in Asians; here are two recent GWAS in two Asian populations. In the first study, 4,001 subjects were genotyped by using a genome-wide marker panel; another 2,304 subjects were used for follow-up replication studies with selected markers. The top SNP associated with mean log adiponectin was rs3865188 in CDH13 on chromosome 16. The meta-analysis p value for this SNP in all 6,305 individuals was \(2.82 \times 10^{-83}\). This gene encodes a receptor for high-molecular-weight forms of adiponectin.

In the second study of 1,776 unrelated Filipino women, the strongest signal for adiponectin was again shown with the gene CDH13 (same SNP, rs3865188, \(P = 7.2 \times 10^{-16}\)). Strong association was also detected near the ADIPOQ gene (rs864265, \(P = 3.8 \times 10^{-9}\)). These signals were also observed in 1,774 young adult offspring of these women. —DK


To replicate recent GWAS of BMD in populations of European ancestry, the authors genotyped 50 markers from 23 genomic loci in samples from Korea (n = 1,397, women, age 59.06 [SD 7.36] yrs.) and two Chinese Hong Kong samples (n = 3,869 and n = 785, men and women, respectively). 14 loci were associated with BMD in East-Asian samples, including: ZBTB40, GPR177, CTNNB1, MEPE, MEF2C, ESR1, STARD3NL, FLJ42280, TNFRSF11B, SOX6, LRP5, TNFSF11, FOXL1, and SOST, but not TNFRSF11A (RANK). The effect of BMD association in each of these loci is very similar to that observed in the European samples; the same alleles were associated with a BMD decrease in both ethnicities. —DK


Lee JS, Suh KT, Eun IS. Polymorphism in interleukin-6 gene is associated with bone mineral

These 2 parallel studies from the same group examined the association between bone mass and polymorphisms in two osteoporosis candidate genes in 198 Korean girls diagnosed with adolescent idiopathic scoliosis (AIS). Mean LS BMD and FN BMD in AIS patients are lower than in age- and sex-matched healthy controls. In the first paper, the VDR Bsml, FokI, and Cdx2 polymorphisms were studied. Only the Bsml polymorphism significantly differed in genotype frequencies between AIS patients and controls; a significant association was found between this polymorphism and LS BMD.

In the second paper, 3 polymorphisms of IL6 (-597 G→A, -572 G→C, and -174 G→A) were studied. IL6-572 G→C showed a statistically significant difference between AIS patients and controls and was also associated with LS BMD. —DK

Bone Modeling, Remodeling, and Repair


Whether sclerostin inhibits Wnt-LRP signaling and/or BMP signaling remains unclear. This study shows that it binds weakly to both LRP6 and BMP7, but inhibits their signaling through different mechanisms, i.e., direct inhibition of Wnt3a-induced signaling in vitro, whereas it prevents BMP7 signaling by inducing its proteosomal degradation when both molecules are produced in the same cell. —SF


Gut-derived serotonin decreases bone accrual, while brain serotonin increases it. Gut-derived serotonin binds to the Htr1b receptor on osteoblasts, culminating in cAMP response element-binding protein (CREB) regulation of osteoblast proliferation. Brain-derived serotonin favors accrual by binding to the Htr2c receptor on neurons of the hypothalamic ventromedial nucleus (VMH). This study reports that after binding to the Htr2c receptor on VMH neurons, serotonin uses a calmodulin kinase (CaMK)-dependent signaling cascade involving CaM KKβ and CaMKIV to decrease the sympathetic tone and increase bone accrual. The transcriptional mediator of these events is CREB phosphorylation on Ser 133 that is increased by CaMKIV following serotonin treatment of hypothalamic explants. A microarray experiment identified two genes necessary for optimum sympathetic activity whose expression is regulated by CREB. These results identify CREB as a determinant of serotonin signaling in hypothalamic neurons to regulate bone mass accrual, although through different mechanisms depending on the cell type, neuron, or osteoblast in which it is expressed. —ES


In vivo microCT has sometimes been called “virtual bone biopsy”, which was
erroneous since it did not provide dynamic indices of bone remodeling...so far. Now the pioneering team of the microCT shows that by superimposing very precisely (by a procedure called registration) a later measurement of an in vivo mouse onto an earlier measurement of the same animal, areas of bone formed and resorbed can be evaluated, and correlate well to histomorphometrical indices of bone turnover. — SF


This is an important study for all of those using mice with the C57/BL6 background to understand the principles of bone turnover and/or the effects of treatments on bone loss. This strain is well-known to spontaneously lose a great deal of trabecular bone with age. This study shows that long-term estrogen-supplementation in OVX C57/BL6 mice does not prevent trabecular bone loss, although it improved cortical bone loss. It also shows that osteoblast numbers in bone decrease with age, but the osteogenic potential of progenitor bone marrow cells increases – rather than decreases – with age. Hence estrogen could play a greater role in the maintenance of cortical than trabecular bone mass. The mechanisms for the profound remodeling of trabecular bone in this strain remain to be elucidated. — SF

Molecular and Cell Biology


Fra-2 (Fosl2), a Fos-related protein of the AP-1 family, is expressed in bone cells, and Fosl2(-/-) newborn mice exhibit defects in chondrocytes and osteoclasts. This study demonstrates that Fosl2(-/-) osteoblasts display a differentiation defect both in vivo and in vitro, while Fra-2-overexpressing mice were osteosclerotic because of increased osteoblast differentiation. The osteoblast-specific osteocalcin and collagen1α2 genes were direct transcriptional targets of Fra-2 in both murine and human bone cells, and Fra-2-ATF-4 dimers and Fra-2-c-Jun or JunB dimers positively regulated this gene transcription. These findings identify Fra-2, in addition to the previously found Fra-1 and ΔFosB, as a novel transcriptional regulator of bone matrix production and osteoblast differentiation. — TM


The authors performed a genome-wide screening of mRNAs for transcription factors that were increased by > 4-fold during osteoblastogenesis, and from bone marrow stromal cells (BMSCs) that were decreased with aging by > 2-fold. The authors identified Maf as the most highly expressed in the BMSCs. Maf regulated mesenchymal cell bifurcation into osteoblasts and adipocytes by cooperating with the osteogenic transcription factor Runx2 and inhibiting the expression of the adipogenic transcription factor Pparg. Results showed delayed bone formation in perinatal Maf(-/-) mice and accelerated fatty marrow formation that was associated with bone loss in
aged Maf(+/−) mice. These observations may provide novel therapeutic strategies against age-related bone loss. Also see a Commentary on this study by L. McCauley in the same issue of JCI. —TM


The authors created mice in which a diphtheria toxin (DT) receptor-GFP fusion protein transgene was knocked into the Cxcl12 locus, allowing conditional ablation of CXCL12/SDF-1-abundant reticular (CAR) cells by DT administration. Short-term ablation of CAR cells severely impaired the adipo-osteogenic differentiation potential of marrow cells as well as SCF and CXCL12 production, leading to a marked reduction in circulating lymphoid and erythroid progenitors. Hematopoietic stem cells (HSCs) were more quiescent with reduced number and size, and showed high early myeloid selector gene expression, similar to the phenotype of wild-type HSCs without a niche. These results demonstrate the importance of the niche composed of adipo-osteogenic progenitors for proliferation and maintenance of HSCs and lymphoid and erythroid progenitors. Also see an analysis of this paper by P. Kincade in the same issue of Immunity. —TM


I put these two papers together to illustrate the complexity of cytokine action on bone. On one side the review by Schlesinger et al. on the mechanisms of osteolysis in gout underscores the key role of IL-1β in the activation of osteoclasts. On another side, Lee et al. show that IL-1β added to monocytes inhibits RANKL-mediated osteoclastogenesis, partly by triggering the proteolysis of c-Fms (the M-CSF receptor) that is required for the expression of RANK. Hence, depending on the context in which it is produced (local inflammatory response as in RA or gout, or inflammatory/immune response as in the presence of bacteria or LPS), IL-1β can favor differentiation of monocytes into macrophages or osteoclasts (also see April 2010 BoneKEy Commentary by S. Ferrari-Lacraz and D. Burger). —SF

Pathophysiology


Here are two papers on RANKL, progestins, and breast cancer. Mammary glands of RANK- and RANKL-deficient mice fail to form lobuloalveolar structures during pregnancy because of defective proliferation of mammary epithelium. RANKL causes the proliferative response to progesterone during mammary lactational morphogenesis, and in mouse models, activation of the RANK/RANKL pathway produces mammary proliferation. In the first study by Gonzalez-Suarez et al., accelerated pre-neoplasias and increased mammary tumor formation were observed in mouse mammary tumor virus (MMTV)-RANK transgenic mice after multiparity or treatment with carcinogen and progesterone. Pharmacological inhibition of RANKL attenuated mammary tumor development in hormone- and carcinogen-treated MMTV-RANK and wild-type mice and in the MMTV-neu transgenic spontaneous tumor model. RANKL inhibition acts directly on hormone-induced mammary epithelium at early stages in tumorigenesis, and the permissive contribution of progesterone to increased mammary cancer incidence is due to RANKL-dependent proliferative changes in the mammary epithelium.

Medroxyprogesterone acetate (MPA) increases the risk of breast cancer. In the second paper by Schramek et al., the authors show that the in vivo administration of MPA triggers massive induction of RANKL in mammary gland epithelial cells. Genetic inactivation of the RANKL receptor RANK in mammary gland epithelial cells prevents MPA-induced epithelial proliferation, impairs expansion of the CD49f hi stem cell-enriched population, and sensitizes these cells to DNA damage-induced cell death. Deletion of RANK from the mammary epithelium results in a markedly decreased incidence and delayed onset of MPA-driven mammary cancer. These results show that the RANKL/RANK system controls the incidence and onset of progestin-driven breast cancer. —ES

Reviews, Perspectives and Editorials


◆ Stockton KA, Mengersen K, Paratz JD, Kandiah D, Bennell KL. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. Osteoporos Int. 2010 Oct 6. [Epub ahead of print] [Abstract]

Other Studies of Potential Interest


Oct 21. [Epub ahead of print] [Abstract]


Conflict of Interest: Dr. Ferrari reports that he receives research support from Amgen and Merck Sharp & Dohme, and is an advisory committee member and lectures occasionally at conference symposia for the Alliance for Better Bone Health (sanofi aventis/P&G), Amgen, Merck Sharp & Dohme, Eli Lilly, Servier, and Novartis. Dr. Little reports that he receives royalties, research funds and consultancy fees from Novartis Pharma, as well as research support from Stryker Biotech. Dr. Seeman reports that he is an advisory committee member for sanofi-aventis, Eli Lilly, Merck Sharp & Dohme, Novartis, and Servier, and that he lectures occasionally at conference symposia for those companies. Dr. Matsumoto and Dr. Karasik report no conflicts of interest.