

## **NOT TO BE MISSED**

### **Clinical and Basic Research Papers – April 2008**

**Serge Ferrari, Editor-in-Chief**  
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**Toshio Matsumoto, Associate Editor**  
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#### **Bone Modeling, Remodeling and Repair**

◆Di Iorgi N, Rosol M, Mittelman SD, Gilsanz V. Reciprocal relation between marrow adiposity and the amount of bone in the axial and appendicular skeleton of young adults. *J Clin Endocrinol Metab.* 2008 Apr 1; [Epub ahead of print]

*It has been suggested that bone loss with aging is likely the consequence of a preferential differentiation of mesenchymal stem cells (MSCs) into adipocyte lineage cells, and that osteoporosis may result from an increased number of adipocytes at the expense of osteoblasts. To address this question, the authors examined parameters for the amount of bone and fat, including bone density, cortical bone area and fat density in 255 sexually mature young adults. The results indicate an inverse relation between bone marrow adiposity and the amount of bone in the axial and appendicular skeleton. These results support the notion that a common progenitor cell differentiates into the cell lineages for bone and fat formation in a mutually exclusive fashion. —TM*

◆Squire M, Brazin A, Keng Y, Judex S. Baseline bone morphometry and cellular activity modulate the degree of bone loss in the appendicular skeleton during disuse. *Bone.* 2008 Feb;42(2):341-9. [[Abstract](#)]

*The authors report that unloading produces greater trabecular bone loss in the metaphyses than in the epiphyses, 2-fold greater trabecular bone loss in females than in males, and greater bone loss in trabecular than in cortical bone. The lesson here is that bone loss was inversely related to baseline bone volume fraction and directly related to baseline bone surface to volume ratio. Bone loss determines morphology but morphology modulates bone loss – regions with greater surface lose more bone because remodeling is surface-based. There are other lessons here too. —ES*

◆Tian XY, Zhang Q, Zhao R, Setterberg RB, Zeng QQ, Iturria SJ, Ma YF, Jee WS. Continuous PGE(2) leads to net bone loss while intermittent PGE(2) leads to net bone gain in lumbar vertebral bodies of adult female rats. *Bone.* 2008 Feb 5; [Epub ahead of print] [[Abstract](#)]

*This research is interesting because it addresses morphology and the components of tissue- and BMU-based remodeling. Continuous and intermittent PGE2 increased bone remodeling. Continuous PGE2 stimulated more BMUs to have a negative bone balance, causing bone loss. The authors claim resorption was greater and formation period was shorter in each BMU. Intermittent PGE2 resulted in cancellous bone gain by stimulating bone formation and shortening the resorption period, while endocortical bone gain exceeded the decrease in periosteal bone and increased intracortical bone loss. —ES*

- ◆Tommasini SM, Nasser P, Hu B, Jepsen KJ. Biological co-adaptation of morphological and composition traits contributes to mechanical functionality and skeletal fragility. *J Bone Miner Res*. 2008 Feb;23(2):236-46. [\[Abstract\]](#)

*These investigators provide an insightful approach to whole bone strength because they attempt to coordinate material and structural strength. In this study the authors measured slenderness (area/length) and tissue level mechanical properties from tibias from 14 female (22-46 yr old) and 17 male (17-46 yr old) donors. Ash content correlated negatively with slenderness and marrow area indicating that slender bones were constructed of tissue with higher mineralization. Slender tibias were compensated by higher mineralization and a greater area fraction of bone suggesting that bone adapts by varying the relative amount of cortical bone within the diaphysis and by varying matrix composition. —ES*

## Genetics

- ◆van Meurs JB, Trikalinos TA, Ralston SH, Balcells S, Brandi ML, Brixen K, Kiel DP, Langdahl BL, Lips P, Ljunggren O, Lorenc R, Obermayer-Pietsch B, Ohlsson C, Pettersson U, Reid DM, Rousseau F, Scollen S, Van Hul W, Agueda L, Akesson K, Benevolenskaya LI, Ferrari SL, Hallmans G, Hofman A, Husted LB, Kruk M, Kaptoge S, Karasik D, Karlsson MK, Lorentzon M, Masi L, McGuigan FE, Mellström D, Mosekilde L, Nogues X, Pols HA, Reeve J, Renner W, Rivadeneira F, van Schoor NM, Weber K, Ioannidis JP, Uitterlinden AG; GENOMOS Study. Large-scale analysis of association between LRP5 and LRP6 variants and osteoporosis. *JAMA*. 2008 Mar 19;299(11):1277-90. [\[Abstract\]](#)

*Possibly the largest genetic epidemiology study so far, providing definite evidence for association of LRP5 polymorphisms with spine and femur BMD in both men and women, and with any fractures and vertebral fractures in women and overall. —SF*

## Molecular and Cell Biology

- ◆Engin F, Yao Z, Yang T, Zhou G, Bertin T, Jiang MM, Chen Y, Wang L, Zheng H, Sutton RE, Boyce BF, Lee B. Dimorphic effects of Notch signaling in bone homeostasis. *Nat Med*. 2008 Mar;14(3):299-305. [\[Abstract\]](#)

*To investigate the role of Notch signaling in bone homeostasis, the authors created transgenic mice expressing the Notch1 intracellular domain (N1ICD) driven by the type I collagen promoter. Osteoblast-specific gain of Notch function caused severe osteosclerosis due to increased proliferation of immature osteoblasts, by enhancing early osteoblastic proliferation via the upregulation of the cyclin D, cyclin E and osterix genes. N1ICD overexpression suppressed terminal osteoblast differentiation by binding Runx2 and repressing its transactivation function. The authors also created osteoblast-specific presenilin-1 and 2 null mice, and demonstrated that these mice developed late-onset, age-related osteoporosis with enhanced osteoclast activity due to decreased OPG expression. These observations, together with those by Hilton et al. (see below) demonstrate an important role of Notch signaling in bone homeostasis by maintaining early osteoblast proliferation and suppressing osteoclast activity. Manipulation of Notch signaling at different stages of osteoblast differentiation may provide new directions for novel therapeutic applications. —TM*

- ◆Hilton MJ, Tu X, Wu X, Bai S, Zhao H, Kobayashi T, Kronenberg HM, Teitelbaum SL, Ross FP, Kopan R, Long F. Notch signaling maintains bone marrow mesenchymal progenitors by suppressing osteoblast differentiation. *Nat Med*. 2008 Mar;14(3):306-14. [\[Abstract\]](#)

*To investigate the role of Notch signaling in osteoblastogenesis, the authors created mice with conditional disruption of Notch signaling in mesenchymal cells. Conditional knockout of either presenilin-1 and 2, components of the  $\gamma$ -secretase complex, or Notch1 and 2 in mesenchymal cells in the limb and calvaria using Prx1-Cre caused a marked excess of trabecular bone in limb bones but not in calvaria of adolescent 8-week-old mice, and severely reduced the number of mesenchymal cells in the bone marrow. Notch signaling appeared to inhibit osteoblast differentiation through Hes1, Hey1 and HeyL binding to Runx2, which impedes Runx2 function. Disruption of Notch signaling also enhanced RANKL expression and suppressed OPG expression in osteoblasts with an enhancement of osteoclastogenesis. With aging, progressive loss of bone developed in these mice due to a marked decrease in the number of trabecular osteoblasts and an increase in osteoclast activity. This excellent work demonstrates that Notch signaling maintains a mesenchymal progenitor pool by suppressing osteoblast differentiation, and suggests that mesenchymal progenitors can be expanded by enhancing Notch signaling.* —TM

◆Shinohara M, Koga T, Okamoto K, Sakaguchi S, Arai K, Yasuda H, Takai T, Kodama T, Morio T, Geha RS, Kitamura D, Kurosaki T, Ellmeier W, Takayanagi H. Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell*. 2008 Mar 7;132(5):794-806. [[Abstract](#)]

*This paper offers new insights into the molecular pathways shared by immune cells and bone cells, namely the role of non-receptor tyrosine kinases Btk and Tec. These molecules not only play a crucial role in B cell receptor signaling complexes, but are now shown to be essential for RANKL-induced osteoclastogenesis by linking RANK to ITAM signaling. In turn, double Btk and Tec KO mice have increased bone mass. These findings allow for a better understanding of some of the skeletal disturbances accompanying immune deficiency syndromes.* —SF

## Physiology and Metabolism

◆Méndez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. *Nature*. 2008 Mar 27;452(7186):442-7. [[Abstract](#)]

*Hematopoietic stem cells are released from bone marrow niches into the circulation through the downregulation of Cxcl12 and inhibition of osteoblast function. This paper now shows these events to be regulated in circadian cycles through the adrenergic system, thereby expanding the role of the latter in the bone environment. Most interestingly in this case,  $\beta_3$ -adrenergic receptors expressed on adherent stromal cells/pre-osteoblasts, rather than  $\beta_2$ -adrenergic receptors expressed on more differentiated osteoblasts, appear to play a prominent role.* —SF

◆Pioszak AA, Xu HE. Molecular recognition of parathyroid hormone by its G protein-coupled receptor. *Proc Natl Acad Sci U S A*. 2008 Apr 1;105(13):5034-9. [[Abstract](#)] [[Full Text](#)]

*This will look completely exotic to many, but for those who have attempted to model the molecular interface between PTH and its receptor for years, these are fantastic results. Using *E. coli*, the authors eventually managed to synthesize and purify the extracellular domain of the PTH/PTHrP receptor in a form that crystallizes, thereby allowing them to solve the overall structure of the receptor bound to PTH.* —SF

◆Sun L, Vukicevic S, Baliram R, Yang G, Sendak R, McPherson J, Zhu LL, Iqbal J, Latif R, Natrajan A, Arabi A, Yamoah K, Moonga BS, Gabet Y, Davies TF, Bab I, Abe E, Sampath K,

Zaidi M. Intermittent recombinant TSH injections prevent ovariectomy-induced bone loss. *Proc Natl Acad Sci U S A*. 2008 Mar 18;105(11):4289-94. [[Abstract](#)] [[Full Text](#)]

*Intermittent TSH injection is antiresorptive and restores lost bone after ovariectomy. The osteoclast inhibitory action persists ex vivo after therapy is stopped for 4 weeks and is mimicked in cells that overexpress the TSH receptor (TSHR). Loss of function of a mutant TSHR (Pro → Leu at 556) in congenital hypothyroid mice activates osteoclast differentiation, suggesting that TSHRs participate in regulating bone remodeling. —ES*

## Clinical Studies and Drug Effects

◆ Anglen JO, Weinstein JN; American Board of Orthopaedic Surgery Research Committee. Nail or plate fixation of intertrochanteric hip fractures: changing pattern of practice. A review of the American Board of Orthopaedic Surgery Database. *J Bone Joint Surg Am*. 2008 Apr;90(4):700-7. [[Abstract](#)] [[Full Text](#)]

*This paper reviews the practice of young US orthopaedic surgeons in their approach to internal fixation of intertrochanteric hip fractures. It finds an increase toward nail-based over plate-based fixation systems. Nail-based fixation systems increased from 3% in 1999 to 66% in 2006. There were some geographic differences with higher utilization rates in the South, Southeast and Southwest. Overall, patients managed with plate fixation had slightly less pain and deformity compared to those with nail fixation. It is highlighted that this increase is not supported by the literature that in fact attests to possibly more complications in nail fixation. —DGL*

◆ Coxon FP, Thompson K, Roelofs AJ, Ebetino FH, Rogers MJ. Visualizing mineral binding and uptake of bisphosphonate by osteoclasts and non-resorbing cells. *Bone*. 2008 Jan 26; [Epub ahead of print] [[Abstract](#)]

*The images here are gorgeous and this paper is a long time in appearing. Fluorescently-labelled alendronate analogue (FL-ALN) was internalized from the surface of dentine by resorbing osteoclasts. Unprenylated Rap1A accumulated whether osteoclasts were cultured on RIS-coated dentine or with RIS in solution. J774 macrophages internalized FL-ALN and RIS from solution, but took up little from dentine because they don't resorb mineral. Osteoclasts take up large amounts of BP, due to their ability to release the BP from the dentine surface during resorption. —ES*

◆ Gates S, Fisher JD, Cooke MW, Carter YH, Lamb SE. Multifactorial assessment and targeted intervention for preventing falls and injuries among older people in community and emergency care settings: systematic review and meta-analysis. *BMJ*. 2008 Jan 19;336(7636):130-3. [[Abstract](#)] [[Full Text](#)]

*This review was performed according to the highest standards for meta-analyses, and serves its purpose well, that is to improve the power of single, size-limited studies in order to provide evidence for, or against, the use of multifactorial risk assessment and interventions to prevent falls in elderly ambulatory patients. The majority of the 18 studies included here were quite homogeneous in terms of falls risk assessment, including evaluation of gait and balance, drug reviews, and home environment. In contrast, they provided a variety of interventions. The number of fallers or fall-related injuries, as usually evaluated on a 12 months' follow-up, was reduced 10%, with a CI above 1, i.e., non-significantly by these interventions. However, more intense interventions could be more effective, as would accounting for the number of falls rather than fallers, and/or for distinct fall-related outcomes, such as peripheral fractures, rather than an ill-defined*

*"injuries" endpoint. The authors appropriately conclude that new studies adequately powered to detect clinically relevant effects are needed. —SF*

- ◆Johansson HR, Skripitz R, Aspenberg P. Bisphosphonates can block the deterioration in implant fixation after withdrawal of intermittent doses of parathyroid hormone. *J Bone Joint Surg Br.* 2008 Mar;90(3):400-4. [\[Abstract\]](#)

*Pull-out force of uncoated stainless steel screws in the proximal tibia was examined in rats. Two weeks treatment of 60 µg/kg/day PTH(1-34) had a positive effect on integration and pull-out strength, but the effect was lost after 16 days. It was subsequently demonstrated that these gains could be maintained and even improved at 5 weeks when pamidronate administration commenced at the cessation of PTH at 2 weeks. Pamidronate treatment alone commenced at 2 weeks had little effect. This paper nicely highlights that synergy between anabolic and anti-catabolic agents, when correctly timed, can produce improvements over either therapy alone in experimental systems. —DGL*

- ◆Nakamura Y, Hayashi K, Abu-Ali S, Naito M, Fotovati A. Effect of preoperative combined treatment with alendronate and calcitriol on fixation of hydroxyapatite-coated implants in ovariectomized rats. *J Bone Joint Surg Am.* 2008 Apr;90(4):824-32. [\[Abstract\]](#) [\[Full Text\]](#)

*Ovariectomized rats received hydroxyapatite or uncoated intramedullary implants. Animals were treated with alendronate, calcitriol or both and compared to sham OVX and vehicle-treated OVX controls. Combined calcitriol- and alendronate-treated groups gave the best result; significantly higher push-out strength and implant contact was noted over controls. There was no difference between treatment given pre- and post-implantation (12 weeks) or post-implantation only (4 weeks). The alendronate alone group was very close to the combined treatment group but did not achieve significant increases over controls. Further evidence is mounting that bisphosphonates, plus or minus concomitant treatments, are good candidates for improving fixation in osteoporotic bone. —DGL*

- ◆Sørensen HT, Christensen S, Mehnert F, Pedersen L, Chapurlat RD, Cummings SR, Baron JA. Use of bisphosphonates among women and risk of atrial fibrillation and flutter: population based case-control study. *BMJ.* 2008 Apr 12;336(7648):813-6. [\[Abstract\]](#) [\[Full Text\]](#)

*13,586 patients with atrial fibrillation and flutter and 68,054 population controls were studied. 435 cases (3.2%) and 1958 controls (2.9%) were current users of bisphosphonates for osteoporosis. The adjusted relative risk of current use of bisphosphonates was 0.95 (95% CI 0.84 to 1.07). New users had a relative risk of 0.75 (95% CI 0.49 to 1.16), similar to continuing users (relative risk of 0.96, 95% CI 0.85 to 1.09). No evidence was found that use of bisphosphonates increases the risk of atrial fibrillation and flutter. —ES*

## Reviews, Perspectives and Editorials

- ◆Garrett RW, Emerson SG. The role of parathyroid hormone and insulin-like growth factors in hematopoietic niches: Physiology and pharmacology. *Mol Cell Endocrinol.* 2008 Mar 2; [Epub ahead of print]

- ◆Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell.* 2008 Apr 4;133(1):38-52. [\[Abstract\]](#)

## Other Studies of Potential Interest

- ◆Brownstein CA, Adler F, Nelson-Williams C, Iijima J, Li P, Imura A, Nabeshima Y, Reyes-Mugica M, Carpenter TO, Lifton RP. A translocation causing increased alpha-klotho level results in hypophosphatemic rickets and hyperparathyroidism. *Proc Natl Acad Sci U S A*. 2008 Mar 4;105(9):3455-60. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆Canaff L, Zhou X, Hendy GN. The proinflammatory cytokine, interleukin-6, upregulates calcium-sensing receptor gene transcription via Stat1/3 and Sp1/3. *J Biol Chem*. 2008 Mar 17; [Epub ahead of print]
- ◆Chang SF, Chang CA, Lee DY, Lee PL, Yeh YM, Yeh CR, Cheng CK, Chien S, Chiu JJ. Tumor cell cycle arrest induced by shear stress: Roles of integrins and Smad. *Proc Natl Acad Sci U S A*. 2008 Mar 11;105(10):3927-32. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. *Proc Natl Acad Sci U S A*. 2008 Apr 1;105(13):5266-70. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆Miller MA, Bare SP, Recker RR, Smith SY, Fox J. Intratrabecular tunneling increases trabecular number throughout the skeleton of ovariectomized rhesus monkeys treated with parathyroid hormone 1-84. *Bone*. 2008 Feb 29; [Epub ahead of print] [\[Abstract\]](#)
- ◆Piper Hanley K, Oakley F, Sugden S, Wilson DI, Mann DA, Hanley NA. Ectopic SOX9 mediates extracellular matrix deposition characteristic of organ fibrosis. *J Biol Chem*. 2008 Feb 22; [Epub ahead of print]
- ◆Vaira S, Johnson T, Hirbe AC, Alhawagri M, Anwisyte I, Sammut B, O'Neal J, Zou W, Weilbaecher KN, Faccio R, Novack DV. RelB is the NF-kappaB subunit downstream of NIK responsible for osteoclast differentiation. *Proc Natl Acad Sci U S A*. 2008 Mar 11;105(10):3897-902. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆Yoshitake F, Itoh S, Narita H, Ishihara K, Ebisu S. IL-6 directly inhibits osteoclast differentiation by suppressing rank signaling pathways. *J Biol Chem*. 2008 Feb 22; [Epub ahead of print]

**Conflict of Interest:** Dr. Ferrari reports that he receives research support from Amgen and consultancy/speaker's fees from Merck Sharp & Dohme, Eli Lilly, and Amgen. 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 reports no conflict of interest.