

## **NOT TO BE MISSED**

### **Clinical and Basic Research Papers – September 2010**

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#### **Clinical Studies and Drug Effects**

◆Pouwels S, Lalmohamed A, Souverein P, Cooper C, Veldt BJ, Leufkens HG, de Boer A, van Staa T, de Vries F. Use of proton pump inhibitors and risk of hip/femur fracture: a population-based case-control study. *Osteoporos Int.* 2010 Jun 29. [Epub ahead of print] [\[Abstract\]](#)

◆de Vries F, van Staa TP, Leufkens HG. Proton pump inhibitors, fracture risk and selection bias: three studies, same database, two answers. *Osteoporos Int.* 2010 Jun 19. [Epub ahead of print] [\[Info\]](#)

*More fuel on the controversial question of the role of PPIs in contributing to fracture risk – is it causal association or selection bias? In a new observational study and a letter to the editor, Dr. de Vries emphasizes that previous findings of an association between PPI use and fractures, according to their own former study, may not hold true with prolonged administration, hence questioning a true pathophysiological relationship between inhibition of gastric acid secretion and bone fragility. —SF*

#### **Genetics**

◆O'Seaghdha CM, Yang Q, Glazer NL, Leak TS, Dehghan A, Smith AV, Kao WH, Lohman K, Hwang SJ, Johnson AD, Hofman A, Uitterlinden AG, Chen YD; The GEFOS Consortium, Brown EM, Siscovick DS, Harris TB, Psaty BM, Coresh J, Gudnason V, Witteman JC, Liu YM, Kestenbaum BR, Fox CS, Köttgen A. Common variants in the calcium-sensing receptor gene are associated with total serum calcium levels. *Hum Mol Genet.* 2010 Sep 2. [Epub ahead of print] [\[Abstract\]](#)

*This paper continues and strengthens the message of the GWAS from Kapur et al. ([PLoS Genet.](#) 2010 Jul 22;6(7):e1001035) that we discussed in last month's Not To Be Missed [here](#). Variants in or near the calcium-sensing receptor (CASR) gene were associated with total serum calcium levels in 20,611 individuals of European ancestry screened for 2.5 million SNPs. Interestingly, the SNP with the lowest p-value – rs17251221 – was also associated with serum magnesium levels ( $p = 1.2 \times 10^{-3}$ ), serum phosphate levels ( $p = 2.8 \times 10^{-7}$ ), and BMD (less strongly). Signals in other genomic loci did not reach genome-wide significance, however, a SNP on 17p13.1, near the genes SERPINF1 (involved in kidney development) and SERPINF2 (glomerulonephritis), was associated at  $6.7 \times 10^{-7}$ . —DK*

#### **Bone Modeling, Remodeling, and Repair**

◆Atesok K, Li R, Stewart DJ, Schemitsch EH. Endothelial progenitor cells promote fracture

healing in a segmental bone defect model. *J Orthop Res.* 2010 Aug;28(8):1007-14. [\[Abstract\]](#)

*It has been shown recently by Lounev et al. (J Bone Joint Surg Am. 2009 Mar 1;91(3):652-63) that Tie-2-positive cells (mostly endothelial with some hemopoietic cells) can make a major contribution to bone formation, not only via forming vessels but by in vivo differentiation down the osteoblast lineage. This paper is thus of particular interest. In a well-characterized critical defect model, local pieces of sterile gel-foam were aseptically impregnated with either 0.3 mL PBS (control), or with 0.3 mL media containing  $1 \times 10^6$  cells (EPC) depending on the study group of the animal. EPC-treated and control animals had a 5 mm defect internally fixed and were examined at multiple time points. At 10 weeks, all the animals in the EPC-treated group had complete union (7/7), but in the control group none achieved union (0/7). Histological evaluation revealed that specimens from EPC-treated animals had abundant new bone and vessel formation compared to that in controls. Micro-CT assessment of the samples from the animals sacrificed at 10 weeks ( $N = 14$ ) showed a 3.5-fold increase in bone volume and significant increases in BV/TV, TbN and TbTh. It remains to be shown whether the cells induced bone via cell signaling, or by differentiation down a vascular or osteogenic lineage. —DGL*

◆Devlin MJ, Cloutier AM, Thomas NA, Panus DA, Lotinun S, Pinz I, Baron R, Rosen CJ, Bouxsein ML. Caloric restriction leads to high marrow adiposity and low bone mass in growing mice. *J Bone Miner Res.* 2010 Sep;25(9):2078-88. [\[Abstract\]](#)

*While potential musculoskeletal benefits of caloric restriction (CR) in aging mammals are still debated, much less is known about how CR affects bone mass in young, rapidly growing animals. Young male C57Bl/6J mice (at 3 weeks of age) were fed either a normal diet ad libitum (10% kcal/fat) or the same diet at 70% of normal ad libitum consumption (CR group). CR mice had 33% and 39% lower serum IGF-1 at 6 and 12 weeks of age ( $p < .05$ ); they were smaller, with lower whole-body BMD, trabecular, and cortical bone parameters; and their bone resorption indices were higher. Strikingly, despite having lower percent body fat, bone marrow adiposity was elevated in CR versus normal diet mice ( $p < .05$ ). These results in mice may translate into the skeletal effects of anorexia nervosa in human adolescents. —DK*

◆Hastrup SG, Chen X, Bechtold JE, Kyle RF, Rahbek O, Keyler DE, Skoett M, Soeballe K. Effect of nicotine and tobacco administration method on the mechanical properties of healing bone following closed fracture. *J Orthop Res.* 2010 Sep;28(9):1235-9. [\[Abstract\]](#)

*Effects of smoking on fracture healing are reported to be negative in clinical studies. The full basic science understanding is incomplete. This study compared a new oral administration route for nicotine to the established pump method. Four groups of Sprague-Dawley rats were studied: (1) pump saline, (2) pump saline + oral tobacco, (3) pump saline/nicotine + oral tobacco, and (4) pump saline + oral nicotine/tobacco. Dosing commenced at 1 week pre-fracture (stabilized with intramedullary pin). Compared to saline control, strength for oral nicotine/tobacco was higher ( $p < 0.05$ ), and stiffnesses for pump nicotine + tobacco and oral nicotine/tobacco were higher than control ( $p < 0.05$ ). No differences in energy were found for either nicotine-tobacco group compared to saline control. No difference was found in torsional strength or stiffness between oral nicotine/tobacco or pump nicotine + tobacco groups, while energy absorption with oral nicotine/tobacco was greater than with pump nicotine + tobacco ( $p < 0.05$ ). Mean serum cotinine (stable nicotine metabolite) was in the range of 1-2 pack/day smokers. Nicotine advanced fracture healing in this and previous models, such that the negative clinical effects of smoking remain to be elucidated. —DGL*

- ◆Xing Z, Lu C, Hu D, Miclau T 3rd, Marcucio RS. Rejuvenation of the inflammatory system stimulates fracture repair in aged mice. *J Orthop Res*. 2010 Aug;28(8):1000-6. [[Abstract](#)]

*It is well-documented that age reduces responsiveness to bone repair, although the causes are not fully elucidated. This study shows that the functional status of inflammatory cells contributes to delayed healing in aged animals. Chimeric mice created by bone marrow transplantation after lethal irradiation have been characterized to show that chondrocytes and osteoblasts in the regenerate are derived exclusively from host cells while inflammatory cells are derived from the donor. When the inflammatory system of middle-aged mice (12-month-old) was replaced by transplanted bone marrow from juvenile mice (4-week-old), larger calluses in early healing and faster remodeling were seen compared to age-matched controls. This strongly suggests that inflammatory cells derived from the juvenile bone marrow accelerated bone repair in the middle-aged animals. Transplanting bone marrow from middle-aged mice into juvenile mice did detectably change the process of fracture healing in juvenile mice. Thus, the roles of inflammatory cells in fracture healing may be age-related, opening up the hypothesis that fracture healing could be enhanced in the elderly by manipulating the inflammatory system. —DGL*

- ◆Yao W, Dai W, Shahnazari M, Pham A, Chen Z, Chen H, Guan M, Lane NE. Inhibition of the progesterone nuclear receptor during the bone linear growth phase increases peak bone mass in female mice. *PLoS One*. 2010 July 1;5(7):e11410. [[Abstract](#)]

*A high cancellous bone mass with increased bone formation rate was reported in female progesterone receptor knockout mice (PRKO). However, if mature female mice were treated with a PR antagonist, RU486, bone formation was not enhanced. Thus, the authors hypothesized that the timing of PR inhibition is critical for enhancing bone formation. Administration of RU486 in female WT mice at rapid bone growth age (1-3 months) enhanced bone formation and increased peak bone mass (PBM). These results demonstrate that the inhibition of the PR during the rapid bone growth period increases bone formation with acquisition of higher PBM, and suggest that temporary inhibition of the PR can become a novel approach to augment PBM and reduce the burden of osteoporosis. —TM*

## Molecular and Cell Biology

- ◆Kawamori Y, Katayama Y, Asada N, Minagawa K, Sato M, Okamura A, Shimoyama M, Nakagawa K, Okano T, Tanimoto M, Kato S, Matsui T. Importance of Vitamin D receptor for neuronal control of hematopoietic stem cell niche. *Blood*. 2010 Sep 2. [Epub ahead of print] [[Abstract](#)]

*Because hematopoietic stem/progenitor cells (HSPCs) in their osteoblastic niche are released by G-CSF via osteoblast suppression mediated by the sympathetic nervous system (SNS), HSPC trafficking may be regulated by cooperation between calcium-regulating hormones and the SNS. The authors demonstrate that G-CSF-induced osteoblast suppression and HSPC mobilization is severely impaired in vitamin D receptor (VDR)-deficient mice. In osteoblasts,  $\beta$ 2-adrenergic receptor (AR) agonists transiently increased mRNA expression of the VDR and its downstream gene, *Rankl*, and  $1\alpha,25(\text{OH})_2\text{D}_3$  sustained  $\beta$ 2-AR-induced *Rankl* expression at high levels by stabilizing the VDR protein. These results demonstrate that the VDR is essential for durable  $\beta$ 2-AR signaling in the stem cell niche, and suggest the presence of an SNS-mediated bone remodeling mechanism through the VDR. —TM*

- ◆Maes C, Kobayashi T, Selig MK, Torrekens S, Roth SI, Mackem S, Carmeliet G, Kronenberg HM. Osteoblast precursors, but not mature osteoblasts, move into developing and fractured bones along with invading blood vessels. *Dev Cell*. 2010 Aug 17;19(2):329-44. [[Abstract](#)]

*The authors generated tamoxifen-inducible transgenic mice bred to Rosa26R-LacZ reporter mice to follow the fates of stage-selective subsets of osteoblast lineage cells. The results demonstrate that osterix-expressing osteoblast precursors, labeled in the perichondrium prior to vascular invasion of the cartilage, give rise to trabecular osteoblasts, osteocytes, and stromal cells inside the developing bone. Some precursors are intimately associated with invading blood vessels like pericytes. A similar co-invasion occurs during endochondral healing of bone fractures. In contrast, perichondrial mature osteoblasts do not exhibit pericyte properties, remain in the outer cortex of developing bones and are destined to generate cortical bone. These findings reveal the specific involvement of immature osteoblast precursors in vascular and osteogenic transformation for endochondral bone development and repair. —TM*

- ◆Tawfeek H, Bedi B, Li JY, Adams J, Kobayashi T, Weitzmann MN, Kronenberg HM, Pacifici R. Disruption of PTH receptor 1 in T cells protects against PTH-induced bone loss. *PLoS One*. 2010 Aug 20;5(8):e12290. [[Abstract](#)]

*Continuous parathyroid hormone (cPTH) treatment causes bone loss, and T cells have been shown to be required for cPTH to induce bone loss in mice. The authors now show that silencing of PTH receptor 1 (PPR) in T cells blocks the osteoclastic expansion and bone loss induced by cPTH. PTH activation of the T cell PPR stimulates tumor necrosis factor  $\alpha$  (TNF) production, and disruption of T cell TNF production prevents PTH-induced bone loss. PTH induces osteoclast formation via the upregulation of CD40 expression in stromal cells (SCs) by TNF, which sensitizes SCs to the T cell costimulatory molecule CD40L and increases their RANKL/OPG production ratio. T cell production of TNF and the resulting upregulation of CD40 signaling in SCs are potential therapeutic targets for hyperparathyroidism-induced bone loss. —TM*

## Public Health/Epidemiology

- ◆Baird J, Kurshid MA, Kim M, Harvey N, Dennison E, Cooper C. Does birthweight predict bone mass in adulthood? A systematic review and meta-analysis. *Osteoporos Int*. 2010 Aug 4. [Epub ahead of print] [[Abstract](#)]

*A meta-analysis of 14 studies that assessed the association between birthweight and bone mineral content (BMC) or bone mineral density (BMD) in adulthood demonstrated that a 1 kg increase in birthweight was associated with an ~1.49 g and 1.41 g increase in lumbar spine and hip BMC, respectively; notably, no association with BMD was found. These associations were similar in boys and girls. Furthermore, a subset of studies showed a similar positive relationship between weight at 1 year and adult BMC of the lumbar spine and hip. These results suggest that intrauterine programming of skeletal development and early postnatal growth trajectory are important for predicting mineral status late in life (however, the influence of skeletal size and the contribution to clinical outcomes such as fracture risk remain open questions). —DK*

- ◆Tsuboi M, Hasegawa Y, Matsuyama Y, Suzuki S, Suzuki K, Imagama S. Do musculoskeletal degenerative diseases affect mortality and cause of death after 10 years in Japan? *J Bone Miner Metab*. 2010 Aug 17. [Epub ahead of print] [[Abstract](#)]

*This study has examined whether musculoskeletal degenerative diseases predict mortality in Japanese adults. 930 persons who were 60+ years of age at baseline underwent musculoskeletal examinations for osteoarthritis of the knee, lumbar spondylosis, and heel ultrasound for osteoporosis. 10-year mortality was compared between the “affected” and “normal” groups for each condition; 125 participants died. Multivariate analysis adjusted for age, gender, body mass index, and lifestyle factors (smoking, drinking, and exercise) found high odds ratio of death for those with knee osteoarthritis or low BMD (ORs of 2.32 and 2.33, respectively). —DK*

### Other Studies of Potential Interest

- ◆ Ashley EA, Butte AJ, Wheeler MT, Chen R, Klein TE, Dewey FE, Dudley JT, Ormond KE, Pavlovic A, Morgan AA, Pushkarev D, Neff NF, Hudgins L, Gong L, Hodges LM, Berlin DS, Thorn CF, Sangkuhl K, Hebert JM, Woon M, Sagreiya H, Whaley R, Knowles JW, Chou MF, Thakuria JV, Rosenbaum AM, Zaranek AW, Church GM, Greely HT, Quake SR, Altman RB. Clinical assessment incorporating a personal genome. *Lancet*. 2010 May 1;375(9725):1525-35. [[Abstract](#)]
- ◆ Engelman CD, Meyers KJ, Ziegler JT, Taylor KD, Palmer ND, Haffner SM, Fingerlin TE, Wagenknecht LE, Rotter JI, Bowden DW, Langefeld CD, Norris JM. Genome-wide association study of vitamin D concentrations in Hispanic Americans: The IRAS Family Study. *J Steroid Biochem Mol Biol*. 2010 Jun 26. [Epub ahead of print] [[Abstract](#)]
- ◆ Manolio TA. Genomewide association studies and assessment of the risk of disease. *N Engl J Med*. 2010 Jul 8;363(2):166-76. [[Info](#)]
- ◆ Thompson WR, Modla S, Grindel BJ, Czymbek KJ, Kirn-Safran CB, Wang L, Duncan RL, Farach-Carson MC. Perlecan/Hspg2 deficiency alters the pericellular space of the lacuno-canalicular system surrounding osteocytic processes in cortical bone. *J Bone Miner Res*. 2010 Sep 2. [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.