

NOT TO BE MISSED

Clinical and Basic Research Papers – March 2010

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Clinical Studies and Drug Effects

◆ Barnes AM, Carter EM, Cabral WA, Weis M, Chang W, Makareeva E, Leikin S, Rotimi CN, Eyre DR, Raggio CL, Marini JC. Lack of cyclophilin B in osteogenesis imperfecta with normal collagen folding. *N Engl J Med.* 2010 Feb 11;362(6):521-8. [\[Abstract\]](#)

We recently mentioned a study showing that mice deficient for cyclophilin B, which is one of the molecules of the collagen hydroxylation enzyme complex, exhibit phenotypic features of OI (see January 2010 Not To Be Missed). This paper now reports two siblings from a consanguineous family suffering from recessive OI due to a homozygous start-codon mutation in the peptidyl-prolyl isomerase B gene (PPIB), which results in a lack of cyclophilin B. —SF

◆ Finkelstein JS, Wyland JJ, Lee H, Neer RM. Effects of teriparatide, alendronate, or both in women with postmenopausal osteoporosis. *J Clin Endocrinol Metab.* 2010 Feb 17. [Epub ahead of print] [\[Abstract\]](#)

◆ Stepan JJ, Burr DB, Li J, Ma YL, Petto H, Sipos A, Dobnig H, Fahrleitner-Pammer A, Michalská D, Pavo I. Histomorphometric changes by teriparatide in alendronate-pretreated women with osteoporosis. *Osteoporos Int.* 2010 Feb 5. [Epub ahead of print] [\[Abstract\]](#)

The first study confirms that initiating teriparatide (TPT), even at a higher dose (40 µg/d), and alendronate (ALN) concomitantly in postmenopausal women with low bone mass does not improve BMD at the spine or hip better than TPT alone after 30 months; actually, ALN reduces the BMD gain with TPT. In contrast, the second study indicates that TPT increases indices of bone remodeling at the tissue level similarly in women pre-treated with alendronate, i.e., with a low baseline bone turnover, and treatment-naive women. —SF

◆ Gooi JH, Pompolo S, Karsdal MA, Kulkarni NH, Kalajzic I, McAhren SH, Han B, Onyia JE, Ho PW, Gillespie MT, Walsh NC, Chia LY, Quinn JM, Martin TJ, Sims NA. Calcitonin impairs the anabolic effect of PTH in young rats and stimulates expression of sclerostin by osteocytes. *Bone.* 2010 Feb 23. [Epub ahead of print] [\[Abstract\]](#)

Calcitonin co-treatment decreased by about 50% the effects of PTH on trabecular BV/TV, without influencing its effects on trabecular number. PTH increased osteoblast surfaces, osteoid volume and non-significantly the bone formation rate after 3 weeks, independent of calcitonin. Hence there were no signs of blunting PTH anabolic activity at the tissue level by this weak anti-resorptive, i.e., in the absence of a marked reduction of remodeling surfaces, PTH activity on osteoblasts seems to be maintained. What is more surprising and interesting are the direct effects of calcitonin on

osteocytes shown here, which increased Sost expression. In turn, PTH mildly decreased sclerostin expression in the presence of calcitonin, as expected, however, the absolute percentage of sclerostin+ osteocytes remained higher in the presence than in the absence of calcitonin. Whether an increase of sclerostin expression by calcitonin may impair PTH anabolic effects on osteoblasts remains to be clarified. —SF

- ◆Harding AK, Toksvig-Larsen S, Tägil M, W-Dahl A. A single dose zoledronic acid enhances pin fixation in high tibial osteotomy using the hemicallotaxis technique. A double-blind placebo controlled randomized study in 46 patients. *Bone*. 2010 Mar;46(3):649-54. [\[Abstract\]](#)

A single zoledronic acid dose increased pullout strength of non-coated pins in hemicallotaxis procedures. Interestingly, there was no detectable effect on healing time or BMD of the regenerate bone. —DGL

- ◆Tägil M, McDonald MM, Morse A, Peacock L, Mikulec K, Amanat N, Godfrey C, Little DG. Intermittent PTH(1-34) does not increase union rates in open rat femoral fractures and exhibits attenuated anabolic effects compared to closed fractures. *Bone*. 2010 Mar;46(3):852-9. [\[Abstract\]](#)

PTH is well-known to increase strength of healing of fractures, albeit at relatively high doses. However, models examined thus far were simple fractures (that may not require any intervention). In this study, these fractures are compared to open fractures with the periosteum stripped. Here the effects were very muted with no increase in the rate of union. PTH may need intact wound repair anabolic systems in place to have beneficial effects on fractures. —DGL

Genetics

- ◆Cauchi S, Byrjalsen I, Durand E, Karsdal MA, Froguel P. PLCL1 rs7595412 variation is not associated with hip bone size variation in postmenopausal Danish women. *BMC Med Genet*. 2009 Dec 23;10:145. [\[Abstract\]](#)

- ◆Chen Y, Jiang XY, Xu L, Li X, Cao FF, Li L, Lu M, Jin L, Wang XF. Association of ER-alpha gene polymorphism with metabolic phenotypes in Chinese Hans. *Lipids*. 2009 Aug;44(8):719-23. [\[Abstract\]](#)

These two studies recently attempted to replicate findings of genome-wide association studies. Thus, Chen et al. did not confirm the association of ESR1 SNPs with BMD, originally found in the Framingham Study (Kiel et al., 2007). More recent GWAS (Liu et al., 2008) identified polymorphisms associated with hip bone size (BS) variation in the PLCL1 locus; however, Cauchi and colleagues found “no obvious effect on hip BS or BMD” in older women. Such replication studies, performed in the same or different ethnic groups, genders, and age strata, are most welcomed since they serve an important goal of filtering false-positive results. —DK

Bone Modeling, Remodeling, and Repair

- ◆Hamrick MW, McNeil PL, Patterson SL. Role of muscle-derived growth factors in bone formation. *J Musculoskeletal Neuronal Interact*. 2010 Mar;10(1):64-70. [\[Abstract\]](#)

This is one of the articles from a terrific special issue of JMNI on muscle-bone interactions (see table of contents by clicking [here](#)). In this paper, the authors show the physical relationship between myocytes and muscle fibers and the periosteal bone surface in mice. Using immunostaining, they show expression of IGF-1 and IGF-1

receptors, as well as of FGF-2 and FGF-2 receptors, on muscle and periosteal cells, respectively, suggesting a paracrine coupling between these two tissues. They discuss the role of these and several other local factors in the coupling of muscle to bone in response to exercise and injury/regeneration. —SF

- ◆Kettunen JA, Impivaara O, Kujala UM, Linna M, Mäki J, Rätty H, Alanen E, Kaprio J, Videman T, Sarna S. Hip fractures and femoral bone mineral density in male former elite athletes. *Bone*. 2010 Feb;46(2):330-5. [\[Abstract\]](#)

Osteoporotic hip fractures were sustained at a significantly older age among former athletes compared with control subjects. Clear skeletal benefits of long-term physical loading were also observed in comparative DXA measurements of areal BMD. Athletes participated more in exercise in their later life. —DGL

- ◆Nakamura A, Akahane M, Shigematsu H, Tadokoro M, Morita Y, Ohgushi H, Dohi Y, Imamura T, Tanaka Y. Cell sheet transplantation of cultured mesenchymal stem cells enhances bone formation in a rat nonunion model. *Bone*. 2010 Feb;46(2):418-24. [\[Abstract\]](#)

Confluent mesenchymal cells transplanted into an acellular (periosteally-stripped) environment resulted in union of a model which went on to non-union in controls. Some donor Y chromosome cells were apparent at 8 weeks in the callus, but we still do not know if the cells themselves healed the bone or if they signaled to host cells that did so. Similar models in rodents heal with BMP application, so the true place of cell therapy in this circumstance remains unclear. —DGL

- ◆Park BH, Yoon SJ, Jang KY, Kim MR, Lee HS, Kim KB, Park H, Lee SY, Park HS, Lim ST, Song KJ, Kim JR. COMP-angiopoietin-1 accelerates bone formation during distraction osteogenesis. *Bone*. 2010 Feb 10. [Epub ahead of print] [\[Abstract\]](#)

- ◆Jeong BC, Kim HJ, Bae IH, Lee KN, Lee KY, Oh WM, Kim SH, Kang IC, Lee SE, Koh GY, Kim KK, Koh JT. COMP-Ang1, a chimeric form of Angiopoietin 1, enhances BMP2-induced osteoblast differentiation and bone formation. *Bone*. 2010 Feb;46(2):479-86. [\[Abstract\]](#)

- ◆Park BH, Jang KY, Kim KH, Song KH, Lee SY, Yoon SJ, Kwon KS, Yoo WH, Koh YJ, Yoon KH, Son HH, Koh GY, Kim JR. COMP-Angiopoietin-1 ameliorates surgery-induced ischemic necrosis of the femoral head in rats. *Bone*. 2009 May;44(5):886-92. [\[Abstract\]](#)

- *3 recent papers on a novel molecule, COMP-Ang1. This consists of cartilage oligomeric matrix protein (COMP)-Angiopoietin 1 fusion protein. This Ang1 variant is more potent than native Ang1 in phosphorylating Tie2 receptor and AKT. In vitro, expression of Tie2 receptor in osteoblasts was significantly increased in the course of differentiation. Treatment with COMP-Ang1 enhanced BMP2-induced ALP osteocalcin and mineralization dose-dependently. With BMP2, it increased p38, smad 1/5/8 and AKT phosphorylation over BMP2 alone. In vivo injection of COMP-Ang1 increased the amount of heterotopic bone formation.*
- *Impressively, a single injection of 100µg at the end of the distraction period in rats increased both angiogenesis and bone formation by factors of at least 2-fold.*
- *In a further study, a model of osteonecrosis was induced in the femoral head of rats. Comp-Ang1-treated animals showed increased BV/TV, trabecular number and less deformity than BSA-treated animals. Vascularity was improved. Larger animal experiments are required but this chimeric protein shows more promise than previous pro-vascular approaches reported thus far. —DGL*

Molecular and Cell Biology

- ◆ Ambrogini E, Almeida M, Martin-Millan M, Paik JH, Depinho RA, Han L, Goellner J, Weinstein RS, Jilka RL, O'Brien CA, Manolagas SC. FoxO-mediated defense against oxidative stress in osteoblasts is indispensable for skeletal homeostasis in mice. *Cell Metab.* 2010 Feb 3;11(2):136-46. [\[Abstract\]](#)

Oxidative stress enhances nuclear retention and activation of forkhead box O (FoxO) transcription factors that defend against oxidative stress by activating genes involved in free radical scavenging and apoptosis. Conditional deletion of FoxO1, FoxO3, and FoxO4 in mice at 3 months of age increased oxidative stress in bone and osteoblast apoptosis, with a decrease in osteoblast number, bone formation and bone mass. Overexpression of a FoxO3 transgene in mature osteoblasts decreased oxidative stress and osteoblast apoptosis, with an increase in osteoblast number, bone formation rate, and vertebral bone mass. Thus, FoxO-dependent oxidative defense provides a mechanism to protect against constantly generated oxidative stress in osteoblasts and is thereby indispensable for bone mass homeostasis. —TM

- ◆ Rached MT, Kode A, Xu L, Yoshikawa Y, Paik JH, Depinho RA, Kousteni S. FoxO1 is a positive regulator of bone formation by favoring protein synthesis and resistance to oxidative stress in osteoblasts. *Cell Metab.* 2010 Feb 3;11(2):147-60. [\[Abstract\]](#)

In this report, the authors employ osteoblast-specific deletion of three FoxO proteins, and showed that only FoxO1 is required for proliferation and redox balance in osteoblasts. They also demonstrated that FoxO1 regulates osteoblast proliferation via interacting with ATF4, which is shown to regulate amino acid import, as well as via influencing p53 signaling. A decrease in oxidative stress or an increase in protein intake normalizes bone formation in mice lacking FoxO1 in osteoblasts. Thus, this study identifies FoxO1 as a crucial regulator of osteoblast proliferation and bone formation. —TM

- ◆ Guo J, Liu M, Yang D, Bouxsein ML, Saito H, Galvin RJ, Kuhstoss SA, Thomas CC, Schipani E, Baron R, Bringham FR, Kronenberg HM. Suppression of Wnt signaling by Dkk1 attenuates PTH-mediated stromal cell response and new bone formation. *Cell Metab.* 2010 Feb 3;11(2):161-71. [\[Abstract\]](#)

The authors examined whether the suppression of Dkk1 by PTH is essential for PTH-mediated stimulation of Wnt signaling and bone formation. In Dkk1 transgenic mice, osteoblast activity was decreased and osteoclast number was increased with decreased osteoblastic OPG production. Despite these effects of Dkk1 overexpression, PTH still activated Wnt signaling in Dkk1 mice and in osteoblastic cells from these mice. In MC3T3E1 cells, PTH not only suppressed Dkk1 expression, but also induced PKA-mediated phosphorylation of β -catenin and enhanced Lef1 expression. These observations suggest that PTH can activate the Wnt pathway despite overexpression of Dkk1, and that PKA-mediated β -catenin phosphorylation and enhanced Lef1 expression by PTH may be important for the full activation of Wnt signaling by PTH. —TM

Conflict of Interest: Dr. Ferrari reports that he receives research support from Amgen and is an advisory committee member and lectures occasionally at conference symposia for Merck Sharp & Dohme, the Alliance for Better Bone Health (sanofi aventis/P&G), Amgen, Eli Lilly (Switzerland), Servier (Switzerland), and Novartis (Switzerland). 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.