

## **NOT TO BE MISSED**

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

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

◆ Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ*. 2010 Jul 29;341:c3691. [[Abstract](#)] [[Full Text](#)]

*An effect of calcium supplements on risk of myocardial infarction and cardiovascular events was examined in this meta-analysis. 15 randomized, placebo-controlled trials of calcium supplements ( $\geq 500$  mg/day) were assessed, 5 with patient level data ( $n = 8,151$ , median follow-up 3.6 years), and 11 with trial level data ( $n = 11,921$ , mean duration 4.0 years). In the 5 studies with patient level data, 143 people allocated to calcium had a myocardial infarction compared with 111 allocated to placebo (HR = 1.31, 95% CI, 1.02 to 1.67). Non-significant increases occurred in the incidence of stroke (HR = 1.20, 95% CI, 0.96 to 1.50,  $P = 0.11$ ), the composite of myocardial infarction, stroke, or sudden death (HR = 1.18, 95% CI, 1.00 to 1.39,  $P = 0.057$ ), and death (HR = 1.09, 95% CI, 0.96 to 1.23,  $P = 0.18$ ). The meta-analysis of trial level data showed similar results: 296 people had a myocardial infarction (166 allocated to calcium, 130 to placebo), with an increased incidence of myocardial infarction in those allocated to calcium (pooled relative risk 1.27, 95% CI 1.01 to 1.59,  $P = 0.038$ ). Calcium supplements are associated with an increased risk of myocardial infarction.*

—ES

◆ Cardwell CR, Abnet CC, Cantwell MM, Murray LJ. Exposure to oral bisphosphonates and risk of esophageal cancer. *JAMA*. 2010 Aug 11;304(6):657-63. [[Abstract](#)]

*During a mean of 4.5 and 4.4 years in bisphosphonate and control cohorts (41,826 in each), 116 esophageal or gastric cancers (79 esophageal) occurred in the bisphosphonate cohort and 115 (72 esophageal) in the control cohort. The incidence of esophageal and gastric cancer combined was 0.7 per 1000 person-years in both cohorts; the incidence of esophageal cancer in the bisphosphonate and control cohorts was 0.48 and 0.44 per 1000 person-years, respectively. Among patients in the UK General Practice Research Database from which the data of this study were extracted, bisphosphonate use was not associated with esophageal or gastric cancer.* —ES

◆ Cummings SR, McClung M, Reginster JY, Cox D, Mitlak B, Stock J, Amewou-Atisso M, Powles T, Miller P, Zanchetta J, Christiansen C. Arzoxifene for prevention of fractures and invasive breast cancer in postmenopausal women. *J Bone Miner Res*. 2010 Jul 23. [Epub ahead of print] [[Abstract](#)]

*In a randomized, blinded trial, women with osteoporosis or a vertebral fracture, and women with low bone mass, were assigned to arzoxifene 20 mg or placebo daily. After*

3 years, the incidence of vertebral fractures in patients with osteoporosis was 2.3% lower in the arxofene than in the placebo group, a 41% relative risk reduction (95% CI, 0.45 to 0.77,  $P < 0.001$ ). In the overall population, the incidence of invasive breast cancer over 4 years was reduced by 1.3%, with a 56% relative reduction in risk (HR = 0.44, 95% CI, 0.26 to 0.76,  $P < 0.001$ ); there was no decrease in nonvertebral fracture risk. Arxofene increased the cumulative incidence of venous thromboembolic events by 0.7%, with a 2.3-fold relative increase (95% CI, 1.5 to 3.7). —ES

- ◆ Drake MT, Srinivasan B, Mödder UI, Peterson JM, McCreedy LK, Riggs BL, Dwyer D, Stolina M, Kostenuik P, Khosla S. Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. *J Clin Endocrinol Metab.* 2010 Jul 14. [Epub ahead of print] [\[Abstract\]](#)

Participants included 27 postmenopausal women treated with PTH (1-34) for 14 days and 28 control women. Circulating sclerostin decreased in the PTH-treated subjects by 12.7% but did not change in the controls. Marrow and peripheral serum sclerostin levels were significantly correlated ( $R = 0.64$ ,  $P < 0.0001$ ). Marrow plasma sclerostin levels were 24% lower in PTH-treated subjects compared with control women. —ES

- ◆ Duque G, Li W, Adams M, Xu S, Phipps R. Effects of risedronate on bone marrow adipocytes in postmenopausal women. *Osteoporos Int.* 2010 Jul 27. [Epub ahead of print] [\[Abstract\]](#)

Paired bone biopsies from patients at baseline and after placebo or risedronate for 3 years ( $n = 14$  per group) were assessed for adipocyte volume/tissue volume (AV/TV), adipocyte number (AD(#)) and diameter (AD(diam)). In the placebo group, AV/TV, AD(#), and AD(diam) increased after 3 years (~15%,  $p < 0.01$ ). AD(diam) remained unchanged and AV/TV and AD(#) were reduced (~20%) in the risedronate group. These changes were associated with a reduction in PPAR $\gamma$ 2 expression in the marrow of risedronate-treated women. Risedronate reduces marrow fat. —ES

- ◆ Khalil N, Sutton-Tyrrell K, Strotmeyer ES, Greendale GA, Vuga M, Selzer F, Crandall CJ, Cauley JA. Menopausal bone changes and incident fractures in diabetic women: a cohort study. *Osteoporos Int.* 2010 Jul 24. [Epub ahead of print] [\[Abstract\]](#)

This study includes 2,171 women, aged 42 to 52 years at baseline (in the year 1996) with 8 years of annual follow up. 1,346 (62%) completed annual visit 7 (in the year 2004). Despite higher baseline BMD the rate of decline in BMD was faster at the hip ( $\beta = -0.45$  vs.  $-0.11$  gm/cm $^2$ /year,  $p < 0.001$ ) for women with diabetes mellitus (DM), compared to non-DM. However, lumbar spine bone loss was slower in women with DM as compared to non-DM women ( $\beta = 0.04$  vs.  $-0.25$  gm/cm $^2$ /year,  $p = 0.004$ ). DM women experienced menopause 3 years earlier than non-DM women ( $p = 0.002$ ), and age-adjusted incident fractures were two-fold higher in women with DM compared to non-DM (RR = 2.20, 95% CI, 1.26-3.85,  $p < 0.006$ ). —ES

- ◆ Rennert G, Pinchev M, Rennert HS. Use of bisphosphonates and risk of postmenopausal breast cancer. *J Clin Oncol.* 2010 Aug 1;28(22):3577-81. [\[Abstract\]](#)

- ◆ Chlebowski RT, Chen Z, Cauley JA, Anderson G, Rodabough RJ, McTiernan A, Lane DS, Manson JE, Snetselaar L, Yasmeen S, O'Sullivan MJ, Safford M, Hendrix SL, Wallace RB. Oral bisphosphonate use and breast cancer incidence in postmenopausal women. *J Clin Oncol.* 2010 Aug 1;28(22):3582-90. [\[Abstract\]](#)

Here are two studies investigating a potential association between use of

*bisphosphonates and risk of postmenopausal breast cancer (also see Commentary in this issue of BoneKEy). In the study by Rennert et al., in 4,039 postmenopausal patients and controls, use of bisphosphonates for longer than 1 year before diagnosis was associated with a reduced risk of breast cancer (OR = 0.61, 95% CI, 0.50 to 0.76), and the association remained significant after adjustment (OR = 0.72, 95% CI, 0.57 to 0.90). Breast tumors in bisphosphonates users were more often ER+ and less often poorly differentiated.*

*In the study by Chlebowski et al., among 154,768 participants, 2,816 were bisphosphonate users (90% alendronate, 10% etidronate). After 7.8 years, invasive breast cancer incidence was lower in bisphosphonate users (HR = 0.68, 95% CI, 0.52 to 0.88,  $P < .01$ ) as was incidence of ER+ invasive cancers (HR = 0.70, 95% CI, 0.52 to 0.94,  $P = .02$ ). The incidence of ductal carcinoma in situ was higher in bisphosphonate users (HR = 1.58, 95% CI, 1.08 to 2.31,  $P = .02$ ). —ES*

## Genetics

◆ Chew S, Mullin BH, Lewis JR, Spector TD, Prince RL, Wilson SG. Homozygous deletion of the UGT2B17 gene is not associated with osteoporosis risk in elderly Caucasian women. *Osteoporos Int.* 2010 Sep 29. [Epub ahead of print] [\[Abstract\]](#)

*The authors attempted to replicate a recent finding of homozygous deletion of the UGT2B17 gene, which was associated with hip fracture (Yang et al. *Am J Hum Genet.* 2008 Dec 12;83(6):663-74). In a sample of 1,347 elderly Caucasian women from the CAIFOS study, they genotyped UGT2B17 copy number variation (CNV) in the gene and assessed the effect of this CNV on BMD and osteoporosis risk. They did not find any significant difference in BMD between genotype groups, nor an increased risk of incident fragility fracture, although they did detect a significant association with SHBG (UGT2B17 encodes an enzyme catabolizing steroid hormones, and therefore might govern serum levels of testosterone and estradiol). The authors propose several sources for this non-replication of UGT2B17 associations: (1) the original study included men and women; (2) in this study, women were approximately 25 years older and were all postmenopausal; and (3) differences between Caucasian and Chinese subjects might partially be to blame. —DK*

◆ Lango Allen H, Estrada K, Lettre G, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature.* 2010 Sep 29. [Epub ahead of print] [\[Abstract\]](#)

*This largest genome-wide association study of a complex human trait to date analyzed 183,727 individuals of European ancestry. The trait is adult height, a highly heritable and classic polygenic trait, and, making it especially interesting, a skeletal phenotype. Indeed, hundreds of genetic variants (at least 180 new and known loci) identified were enriched for genes that are connected to biological pathways that underlie skeletal growth defects ( $P < 0.001$ ). Among top signals, genes involved in ossification, osteoblast differentiation, and skeletal myogenesis were obvious ones such as aggrecan, BMP2 and BMP6, CYP19A1, ESR1, growth hormone, IGF1R, insulin receptor, MC4R (melanocortin 4 receptor), MEF2C (myocyte enhancer factor 2C), noggin, RUNX2, and TGF- $\beta$ 2. Despite the fact that the results of this GWAS explain “only” approximately 10% of the phenotypic variation in height, the finding of multiple biologically relevant genes and pathways should contribute to our knowledge of bone, cartilage, and muscle biology. —DK*

## Bone Modeling, Remodeling, and Repair

- ◆ Koob S, Torio-Padron N, Stark B, Hannig C, Stankovic Z, Finkenzeller G. Bone formation and neovascularization mediated by mesenchymal stem cells and endothelial cells in critical-sized calvarial defects. *Tissue Eng Part A*. 2010 Aug 28. [Epub ahead of print] [\[Abstract\]](#)

*This study examined the effect of human umbilical vein endothelial cell (HUVEC) coimplantation on mesenchymal stem cell (MSC)-mediated bone regeneration in a calvarial bone defect model in immunocompromised mice. Neovessel formation was considerably higher in the coimplantation group, suggesting that implanted MSCs supported HUVEC-triggered neovascularization. Implanted MSCs effectively supported bone formation in calvarial defects. However, the human HUVEC-derived neovasculature did not improve MSC-triggered bone regeneration in this model.*

—DGL

- ◆ Garcia P, Speidel V, Scheuer C, Laschke MW, Holstein JH, Histing T, Pohlemann T, Menger MD. Low dose erythropoietin stimulates bone healing in mice. *J Orthop Res*. 2010 Aug 25. [Epub ahead of print] [\[Abstract\]](#)

*Expanding upon the theme of vascularity in bone repair, this study looked at erythropoietin (EPO). EPO treatment significantly accelerates bone healing in this model (a femoral 0.25 mm osteotomy gap was stabilized with a pin-clip technique). This was validated by significantly greater biomechanical stiffness and higher radiological density of the periosteal callus at 2 and 5 weeks after fracture and stabilization. Histological analysis demonstrated significantly more bone and less cartilage and fibrous tissue in the periosteal callus. The number of circulating endothelial progenitor cells was significantly greater in EPO-treated animals, possibly indicating a cellular effect.* —DGL

- ◆ Kumar S, Wan C, Ramaswamy G, Clemens TL, Ponnazhagan S. Mesenchymal stem cells expressing osteogenic and angiogenic factors synergistically enhance bone formation in a mouse model of segmental bone defect. *Mol Ther*. 2010 May;18(5):1026-34. [\[Abstract\]](#)

- ◆ Zhou J, Lin H, Fang T, Li X, Dai W, Uemura T, Dong J. The repair of large segmental bone defects in the rabbit with vascularized tissue engineered bone. *Biomaterials*. 2010 Feb;31(6):1171-9. [\[Abstract\]](#)

*The study by Kumar et al. determined the potential of mesenchymal stem cells (MSCs), transduced ex vivo with a recombinant adeno-associated virus 6 (rAAV6) encoding bone morphogenetic protein 2 (BMP2) and vascular endothelial growth factor (VEGF) in a mouse model of segmental bone defect created in the tibiae of athymic nude mice. Effects of the therapy were determined by enzyme-linked immunosorbent assay measurements for BMP2 and VEGF, dual-energy X-ray absorptiometry (DXA) for bone density, three-dimensional microcomputed tomography (microCT) for bone and capillary architecture, and histomorphometry for bone remodeling. Results of these analyses indicated enhanced bone formation in the group that received BMP2+VEGF-expressing MSCs compared to other groups.*

*The second study by Zhou et al. from earlier this year also examines combination MSC/endothelial cell (EC) treatment in a segmental defect model. In a 1.5-cm ulnar rabbit defect model, co-seeding MSCs and MSC-derived ECs resulted in vascularization that was able to promote osteogenesis and improve mechanical properties. Combination treatment seems better than either treatment alone.* —DGL

- ◆Li X, Warmington KS, Niu QT, Asuncion FJ, Barrero M, Grisanti M, Dwyer D, Stouch B, Thway TM, Stolina M, Ominsky MS, Kostenuik PJ, Simonet WS, Paszty C, Ke HZ. Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass and bone strength in aged male rats. *J Bone Miner Res*. 2010 Jul 16. [Epub ahead of print] [\[Abstract\]](#)

*Sclerostin antibody (Scl-AbII, 5 or 25 mg/kg, twice per week, for five weeks) given to male Sprague-Dawley rats resulted in a marked increase in areal bone mineral density of the lumbar vertebrae (LV) and long bones (femur and tibia) in both Scl-AbII-treated groups compared with baseline or vehicle controls at 3 and 5 weeks post-treatment. Ex vivo microCT showed improved trabecular and cortical architecture at the fifth lumbar vertebral body (LV5), femoral diaphysis (FD), and femoral neck (FN) in both Scl-AbII dose groups compared with vehicle controls, and the increased cortical and trabecular bone mass was associated with a higher maximal load of LV5, the FD, and the FN in the high dose group. Bone formation parameters at the proximal tibial metaphysis and tibial shaft were greater on trabecular, periosteal, and endocortical surfaces in both Scl-AbII dose groups compared with controls. Pharmacologic inhibition of sclerostin may represent a promising anabolic therapy. —ES*

- ◆O'Neal JM, Diab T, Allen MR, Vidakovic B, Burr DB, Guldborg RE. One year of alendronate treatment lowers microstructural stresses associated with trabecular microdamage initiation. *Bone*. 2010 Aug;47(2):241-7. [\[Abstract\]](#)

*Trabecular cores from the distal femur of beagles treated for 1 year with alendronate were subjected to uniaxial compression to induce microdamage. von Mises stress for trabeculae exhibiting severe and linear microcrack patterns was decreased by 25% whereas there was no reduction in the von Mises stress state for diffuse microdamage formation. Severely damaged trabeculae were thinner, more aligned with the loading axis, and less mineralized than undamaged trabeculae in alendronate-treated samples. Changes in bone's architecture and matrix properties with alendronate reduce trabecular bone's ability to resist loading-induced severe and linear microcracks. —ES*

## Muscle and Bone

- ◆Nakagaki WR, Bertran CA, Santo-Neto H, Matsumura CY, Camilli JA. Mechanical, biochemical and morphometric alterations in the femur of mdx mice. *Bone*. 2010 Sep 17. [Epub ahead of print] [\[Abstract\]](#)

*Fractures are a significant problem in patients with Duchenne muscular dystrophy (DMD), which is attributed to a mutation in the gene encoding the protein dystrophin. Mdx mice lack dystrophin, and therefore are an experimental model widely used for the study of DMD. In this study, the authors hypothesized that alterations observed in bone tissue may not be due exclusively to muscle impairment, and therefore they investigated the changes that occur in the femurs of mdx mice at 21 days of age when muscle impairment/damage is still not significant. Indeed, compared to the control group, mdx femurs showed a reduction of bone intrinsic stiffness (elastic modulus) and bone strength (stress) and reduced mineralization. Thus, mdx mice developed femoral osteopenia even in the absence of significant muscle fiber degeneration. The authors attribute the weakness of mdx femurs to metabolic changes that are directly or indirectly related to dystrophin deficiency. It might be shown in the future that the mutation affects bone properties independently of the muscles. —DK*

## Public Health

- ◆Nelson HD, Haney EM, Dana T, Bougatsos C, Chou R. Screening for osteoporosis: an update for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2010 Jul 20;153(2):99-111. [\[Abstract\]](#)

*The purpose of this study was to determine the effectiveness of osteoporosis screening in reducing fractures for men and postmenopausal women without previous fractures. Randomized, controlled trials of screening or medications with fracture outcomes published in English; performance studies of validated risk-assessment instruments; and systematic reviews and population-based studies of bone measurement tests or medication harms were selected. Risk-assessment instruments were modest predictors of low bone density (area under the curve, 0.13 to 0.87; 14 instruments) and fractures (area under the curve, 0.48 to 0.89; 11 instruments). Trials of screening with fracture outcomes are lacking. —ES*

- ◆Siris ES, Pasquale MK, Wang Y, Watts NB. Estimating bisphosphonate use and fracture reduction among U.S. women age 45 and older, 2001-2008. *J Bone Miner Res*. 2010 Jul 26. [Epub ahead of print] [\[Abstract\]](#)

*From 2001-2008, 460,584 women in two medical claims databases initiated treatment with bisphosphonates for 2.4 years. Fracture rates declined with improved medication possession ratio (MPR) from 1.52% for the lowest MPR category to 1.18% for the highest MPR category for ages 45-64 and from 5.12% to 3.75% for those 65 and older. Extrapolating to the U.S. population of female bisphosphonate users, over 27.9 million person years of bisphosphonate treatment with MPR 50% or greater prevented 144,670 fractures. —ES*

## Reviews, Perspectives and Editorials

- ◆Cheung CL, Xiao SM, Kung AW. Genetic epidemiology of age-related osteoporosis and its clinical applications. *Nat Rev Rheumatol*. 2010 Sep;6(9):507-17. [\[Abstract\]](#)

- ◆Heard E, Tishkoff S, Todd JA, Vidal M, Wagner GP, Wang J, Weigel D, Young R. Ten years of genetics and genomics: what have we achieved and where are we heading? *Nat Rev Genet*. 2010 Oct;11(10):723-33. [\[Abstract\]](#)

- ◆Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. *J Biol Chem*. 2010 Aug 13;285(33):25103-8. [\[Abstract\]](#) [\[Full Text\]](#)

## Other Studies of Potential Interest

- ◆Burra S, Nicolella DP, Francis WL, Freitas CJ, Mueschke NJ, Poole K, Jiang JX. Dendritic processes of osteocytes are mechanotransducers that induce the opening of hemichannels. *Proc Natl Acad Sci U S A*. 2010 Aug 3;107(31):13648-53. [\[Abstract\]](#) [\[Full Text\]](#)

- ◆Cao H, Yu S, Yao Z, Galson DL, Jiang Y, Zhang X, Fan J, Lu B, Guan Y, Luo M, Lai Y, Zhu Y, Kurihara N, Patrene K, Roodman GD, Xiao G. Activating transcription factor 4 regulates osteoclast differentiation in mice. *J Clin Invest*. 2010 Aug 2;120(8):2755-66. [\[Abstract\]](#)

- ◆Darwech I, Otero JE, Alhawagri MA, Abu-Amer Y. Tyrosine phosphorylation is required for I $\kappa$ B kinase-beta (IKK $\beta$ ) activation and function in osteoclastogenesis. *J Biol Chem*. 2010 Aug 13;285(33):25522-30. [\[Abstract\]](#) [\[Full Text\]](#)

- ◆Fromigué O, Haÿ E, Barbara A, Marie PJ. Essential role of nuclear factor of activated T cells (NFAT)-mediated Wnt signaling in osteoblast differentiation induced by strontium ranelate. *J Biol Chem*. 2010 Aug 13;285(33):25251-8. [[Abstract](#)] [[Full Text](#)]
- ◆Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. *J Biol Chem*. 2010 Aug 13;285(33):25221-31. [[Abstract](#)] [[Full Text](#)]
- ◆Ma T, Sadashivaiah K, Chellaiah MA. Regulation of sealing ring formation by L-plastin and cortactin in osteoclasts. *J Biol Chem*. 2010 Sep 24;285(39):29911-24. [[Abstract](#)] [[Full Text](#)]
- ◆Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*. 2010 Aug 12;466(7308):829-34. [[Abstract](#)]
- ◆Ortuno MJ, Ruiz-Gaspa S, Rodriguez-Carballo E, Susperregui AR, Bartrons R, Rosa JL, Ventura F. p38 regulates expression of osteoblast-specific genes by phosphorylation of Osterix. *J Biol Chem*. 2010 Aug 3. [Epub ahead of print] [[Abstract](#)]
- ◆Thi MM, Suadicaní SO, Spray DC. Fluid flow-induced soluble vascular endothelial growth factor isoforms regulate actin adaptation in osteoblasts. *J Biol Chem*. 2010 Oct 1;285(40):30931-41. [[Abstract](#)] [[Full Text](#)]

**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.