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NOT TO BE MISSED

Clinical and Basic Research Papers – March 2011

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Clinical Studies and Drug Effects

Amin S, Kopperdhal DL, Melton LJ 3rd, Achenbach SJ, Therneau TM, Riggs BL, Keaveny TM, Khosla S. Association of hip strength estimates by finite element analysis with fractures in women and men. *J Bone Miner Res*. 2011 Feb 8. [Epub ahead of print] [Abstract]

> *Finite element analysis (FEA) of proximal femur QCT scans was applied to estimate hip strength and load-to-strength ratio during a simulated sideways fall in an age-stratified random sample of community-dwelling adults. Odds ratios for prevalent overall and osteoporotic fractures were similar for FEA measures and hip aBMD and vBMD obtained from QCT images. C-statistics (estimated areas under ROC curves) were also similar (e.g., 0.84-0.85 in women and 0.75-0.78 in men for osteoporotic fractures). In women and men, the association with prevalent osteoporotic fractures increased below an estimated hip strength threshold of ~3000 N.* —DK

Boyer KA, Kiratli BJ, Andriacchi TP, Beaupre GS. Maintaining femoral bone density in adults: how many steps per day are enough? *Osteoporos Int*. 2011 Feb 12. [Epub ahead of print] [Abstract]

> *Walking is the most common weight-bearing activity in older adults. However, the amount and intensity of walking needed to maintain a healthy skeleton is unknown. This study examined the relationship between habitual walking and femoral BMD in 105 healthy individuals (49-64 years of age) using a quantitative theory for bone maintenance: an index of cumulative loading (bone density index, BDI) was examined as a predictor of BMD. In women, BDI was slightly correlated with BMD. For a woman of average body weight, walking at 1.00 m/s requires 4,892 steps/day to maintain a T-score of -1.0. Substantially more steps (18,568 steps/day) are required for a 20%-lighter woman (or an increase in speed to 1.32 m/s at 10,000 steps/day) for the same effect. The study is an important step (no pun intended!) on the way towards individualized exercise programs aimed at mechanical stimulation at the hip, which must also take into account genetic predispositions to exercise and factors affecting walking ability.* —DK

Eastell R, Christiansen C, Grauer A, Kutilek S, Libanati C, McClung MR, Reid IR, Resch H, Siris E, Uebelhart D, Wang A, Weryha G, Cummings SR. Effects of denosumab on bone turnover markers in postmenopausal osteoporosis. *J Bone Miner Res*. 2011 Mar;26(3):530-7. [Abstract]

> *Detailed analysis of 160 women from the FREEDOM trial shows that denosumab suppresses CTX levels below premenopausal levels shortly after injection in 100% of*

subjects and that CTX remained maximally suppressed in 79% of patients at the end of the first 6-month dosing interval and in 51% of women at the end of the last (3-year) dosing interval. —SF

Hadji P, Claus V, Ziller V, Intorcia M, Kostev K, Steinle T. GRAND: the German retrospective cohort analysis on compliance and persistence and the associated risk of fractures in osteoporotic women treated with oral bisphosphonates. *Osteoporos Int*. 2011 Feb 10. [Epub ahead of print] [Abstract]

This database analysis of more than 4,000 women confirms that persistence with both weekly and monthly bisphosphonates at one and two years is low (29% and 13%, respectively) and is associated with a higher fracture incidence (12% vs. 15% at two years in compliant vs. non-compliant women). —SF

Park-Wyllie LY, Mamdani MM, Juurlink DN, Hawker GA, Gunraj N, Austin PC, Whelan DB, Weiler PJ, Laupacis A. Bisphosphonate use and the risk of subtrochanteric or femoral shaft fractures in older women. *JAMA*. 2011 Feb 23;305(8):783-9. [Abstract]

This observational study from a Canadian database over 6 years used diagnostic codes to identify subtrochanteric or femoral shaft fractures following initiation of bisphosphonate therapy in women 68 years or older, excluding diagnostic codes for fractures resulting from trauma, motor vehicle collisions, and falls from a height, as well as fractures treated exclusively with hip replacement surgery. 716 subtrochanteric or femoral shaft fractures and 9,723 women who sustained a typical osteoporotic fracture of the intertrochanteric region or femoral neck were found. By matching each case to 5 controls, the study found bisphosphonate use for more than 5 years, but not less, to be associated with a decreased risk of typical osteoporotic fracture but a nearly 3-fold increased risk of subtrochanteric/femoral shaft fracture. The incidence of these fractures, however, remained low (0.13%/yr.). —SF

Cancer and Bone

Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, Patrawala L, Yan H, Jeter C, Honorio S, Wiggins JF, Bader AG, Fagin R, Brown D, Tang DG. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med*. 2011 Feb;17(2):211-5. [Abstract]

Dysregulation of microRNAs has been implicated in tumorigenesis and metastasis. Here, miR-34a was found to be underexpressed in CD44+ prostate cancer stem cells in both xenografts (LAPC4, LAPC9, DU145) and primary tumors (n = 18). Enforced expression of miR-34a in CD44+ Du145 and LAPC9 prostate cancer stem cells inhibited orthotopic tumor growth and metastasis in vivo. In addition, systemically delivered miR-34a reduced lung metastasis in animals bearing LAPC9 orthotopic tumors. To determine whether CD44 is a functionally important target of miR-34a for prostate cancer development, CD44 expression was silenced in LAPC4, PC3 and DU145 cells. CD44 silencing phenocopied miR-34a overexpression in inhibiting tumor growth and metastasis formation. Thus, miR-34a is a key negative regulator of CD44+ prostate cancer stem cells, suggesting it could be used as a therapeutic agent against prostate cancer metastases. —PC

Sänger N, Effenberger KE, Riethdorf S, van Haasteren V, Gauwerky J, Wiegratz I, Strebhardt K, Kaufmann M, Pantel K. Disseminated tumor cells in the bone marrow of patients with ductal carcinoma in situ. *Int J Cancer*. 2011 Jan 4. [Epub ahead of print] [Abstract]

Detection of disseminated tumor cells in bone marrow is an independent prognostic factor in primary breast cancer. Here, the authors conducted a proof-of-principle study to evaluate whether this tumor cell spread has already occurred in patients with ductal carcinoma in situ (DCIS). Thirty women with DCIS were included. Bone marrow aspirates were taken at the time of primary surgery. The authors found that hematogenous tumor cell dissemination into bone marrow was an early event in breast cancer development. —PC

Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived Jagged1 promotes osteolytic bone metastasis of breast cancer by engaging Notch signaling in bone cells. *Cancer Cell*. 2011 Feb 15;19(2):192-205. [Abstract]

The authors report that the Notch ligand Jagged1 is expressed by breast cancer cells and promotes osteolytic bone metastasis by activating the Notch pathway in bone cells. Jagged1 directly activates osteoclast differentiation and it stimulates IL-6 release from osteoblasts which, in turn, stimulates tumor growth. In addition, Jagged1 is an essential functional target of the cytokine TGF-β that is released during bone destruction. Importantly, γ-secretase inhibitor treatment reduces Jagged1-mediated bone metastasis by disrupting the Notch pathway in bone cells. Taken together, these findings provide the rationale for using γ-secretase inhibitors for the treatment of bone metastasis. —PC

Genetics

Songpatanasilp T, Chailurkit LO, Chantprasertyothin S, Ongphiphadhanakul B, Taechakraichana N. Effect of GGCX gene polymorphism on the responses of serum undercarboxylated osteocalcin and bone turnover markers after treatment with vitamin K2 (menatetrenone) among postmenopausal Thai women. *J Bone Miner Metab*. 2011 Feb 23. [Epub ahead of print] [Abstract]

Menatetrenone (MK-4) is a vitamin K2 homologue that has been used as a therapeutic agent for osteoporosis. This study evaluated the influence of gamma-glutamyl carboxylase (GGCX) gene polymorphisms (rs699664, Arg325Gln) on the response of serum undercarboxylated osteocalcin (ucOC) and bone turnover markers 3 months after treatment with MK-4. The GGCX gene was chosen since its rare mutations cause defects in vitamin K-dependent proteins. In postmenopausal Thai women, there was a significant reduction of serum ucOC, beta-CTx and P1NP from baseline to 3 months, independent of genotype. Only in a small subgroup of women 65 years and older was there a significant difference in the response to vitamin K2 (reduction of ucOC). —DK

Williams FM, Popham M, Hart DJ, de Schepper E, Bierma-Zeinstra S, Hofman A, Uitterlinden AG, Arden NK, Cooper C, Spector TD, Valdes AM, van Meurs J. GDF5 single-nucleotide polymorphism rs143383 is associated with lumbar disc degeneration in Northern European women. *Arthritis Rheum*. 2011 Mar;63(3):708-12. [Abstract]

Dodd AW, Rodriguez-Fontenla C, Calaza M, Carr A, Gomez-Reino JJ, Tsezou A, Reynard LN, Gonzalez A, Loughlin J. Deep sequencing of GDF5 reveals the absence of rare variants at this important osteoarthritis susceptibility locus. *Osteoarthritis Cartilage*. 2011 Jan 31. [Epub ahead of print] [Abstract]

Growth and differentiation factor 5 (GDF5, also known as cartilage-derived morphogenetic protein-1) was previously found to be strongly associated with osteoarthritis (OA) and to play a role in height. The common SNP rs143383 in the 5'

untranslated region (UTR) of GDF5 influences GDF5 expression. The study by Williams et al. investigated whether this variant, beyond being an OA susceptibility locus, is associated with lumbar disc degeneration (LDD). Indeed, an association between LDD and rs143383 was identified in women from 5 European cohorts, with the same risk allele as in knee and hip OA (odds ratio 1.72 [95% CI: 1.15-2.57], P = 0.008).

The second study by Dodd et al. is the first report of deep sequencing of GDF5. Using the Sanger method, the authors sequenced GDF5 in 992 OA patients and 944 controls (from the UK, Spain and Greece; females and males; knee and hip OA cases). They covered the protein-coding region of two of five GDF5 exons, both UTRs, and ~ 100 bp of the proximal promoter, and detected 13 variants. Six were extremely rare, with minor allele frequencies (MAFs) of ~ 0.06%, and seven variants were common (MAFs of 2.5-39%). Three of the extremely rare variants are potentially functional; however, since they are extremely rare (unique), they do not explain the association signal marked by rs143383. Notably, there was a complete absence of variants with frequencies in-between the extremely rare and the common SNPs. —DK

Bone Modeling, Remodeling, and Repair

Aghaloo TL, Kang B, Sung EC, Shoff M, Ronconi M, Gotcher JE, Bezouglaia O, Dry SM, Tetradis S. Periodontal disease and bisphosphonates induce osteonecrosis of the jaws in the rat. *J Bone Miner Res*. 2011 Feb 23. [Epub ahead of print] [Abstract]

Two models of bisphosphonate-related ONJ have been published thus far, both using traumatic teeth extractions. This new rat study is based on the classical tooth ligature periodontitis model. Administration of zoledronate (ZOL) equivalent to the monthly human oncology dose (0.066 mg/kg), but repeated in these rats nine times over three weeks was associated with a reduction of alveolar bone resorption, necrotic bone in 47% and bone protruding into the oral cavity in 21% of ZOL-treated animals. A plausible model is proposed where bone injured by inflammation and infection (periodontitis) is not removed when bone remodeling is inhibited by bisphosphonates, leaving it exposed to even more injury and eventually the tissue dies. Of note, several models of experimental periodontitis showing the beneficial effects of bisphosphonates on alveolar bone loss have been published previously, without evidence of ONJ. Perhaps this means that we find what we look for...or is it the high cumulative dose of ZOL over a relatively short time that does it here? (For a review of recent clinical and preclinical advances in ONJ, see the Perspective by Drs. Allen and Ruggiero in the March 2011 issue of BoneKEy). —SF

Agholme F, Isaksson H, Kuhstoss S, Aspenberg P. The effects of Dickkopf-1 antibody on metaphyseal bone and implant fixation under different loading conditions. *Bone*. 2011 Feb 15. [Epub ahead of print] [Abstract]

A Dkk1 neutralizing antibody was given in various modes of repair. In screw fixation after 28 days, the pull-out force was increased by over 100%. A 70% increase in bone formation in a bone chamber was also noted. Importantly, these studies model intramembranous bone formation and more complex endochondral systems remain to be fully evaluated with Dkk1 inhibition. —DGL

Caetano-Lopes J, Lopes A, Rodrigues A, Fernandes D, Perpétuo IP, Monjardino T, Lucas R, Monteiro J, Konttinen YT, Canhão H, Fonseca JE. Upregulation of inflammatory genes and downregulation of sclerostin gene expression are key elements in the early phase of fragility fracture healing. *PLoS One*. 2011 Feb 11;6(2):e16947. [Abstract]

Trabecular bone fragments were collected at the time of surgery for total hip arthroplasty after fragility hip fracture. Gene expression was analyzed at different time points. Inflammatory genes such as IL-6 and TNF-α were elevated early, and then declined. RANKL expression peaked between 4 and 7 days. SOST levels decreased over time. The authors conclude that promoting these effects might be beneficial. —DGL

Claes L, Reusch M, Göckelmann M, Ohnmacht M, Wehner T, Amling M, Beil FT, Ignatius A. Metaphyseal fracture healing follows similar biomechanical rules as diaphyseal healing. *J Orthop Res*. 2011 Mar;29(3):425-32. [Abstract]

In this ingenious experiment, this group shows that the mechanical rules governing cell fate decisions in metaphyseal repair are the same as in diaphyseal repair, rebuking previous dogma. In a clever model of increasing motion in a semi-stable osteotomy, cartilage can be made to form between trabecular surfaces when strain is high. Metaphyseal bone tends to be more stable, but the rules governing its repair may be the same. —DGL

Shahnazari M, Yao W, Wang B, Panganiban B, Ritchie RO, Hagar Y, Lane NE. Differential maintenance of cortical and cancellous bone strength following discontinuation of bone-active agents. *J Bone Miner Res*. 2011 Mar;26(3):569-81. [Abstract]

An innumerable number of studies have shown changes in bone microstructure with various osteoporosis treatments in animal models, but very few have studied the persistence of the effects upon drug withdrawal. In this OVX mouse model, PTH increased vertebral BV/TV and compression strength more than alendronate or raloxifene, but 4 months after treatment cessation, alendronate effects were maintained whereas PTH and raloxifene effects were lost. In contrast, PTH increased cortical bending strength at the tibia up to 4 months after withdrawal. —SF

Molecular and Cell Biology

Duque G, Huang DC, Dion N, Macoritto M, Rivas D, Li W, Yang XF, Li J, Liang J, Marino FT, Barralet J, Lascau V, Deschênes C, Ste-Marie LG, Kremer R. Interferon γ plays a role in bone formation in vivo and rescues osteoporosis in ovariectomized mice. *J Bone Miner Res*. 2011 Feb 3. [Epub ahead of print] [Abstract]

Kohara H, Kitaura H, Fujimura Y, Yoshimatsu M, Morita Y, Eguchi T, Masuyama R, Yoshida N. IFN-γ directly inhibits TNF-α-induced osteoclastogenesis in vitro and in vivo and induces apoptosis mediated by Fas/Fas ligand interactions. *Immunol Lett*. 2011 Feb 19. [Epub ahead of print] [Abstract]

The effects of interferon-γ on osteoclastogenesis and bone resorption are complex and somewhat controversial (see BoneKEy article by Ferrari-Lacraz S, Ferrari S. Is IFN-γ involved in bone loss or protection? Nothing is simple with cytokines. 2007 Feb;4(2):83-87). These two papers bring new evidence that IFN-γ has bone-sparing properties in vivo, both by inhibiting bone resorption and by stimulating bone formation. —SF

Lymperi S, Ersek A, Ferraro F, Dazzi F, Horwood NJ. Inhibition of osteoclast function reduces hematopoietic stem cell numbers in vivo. *Blood*. 2011 Feb 3;117(5):1540-9. [Abstract]

It has been shown recently that the hematopoietic stem cell (HSC) niche is characterized by the interplay between mesenchymal stem cells/osteoblasts and HSCs

that reciprocally control their differentiation. This study now explores the role of osteoclasts and bone resorption in the HSC niche. Alendronate-mediated inhibition of osteoclastic bone resorption reduced the number of primitive HSCs, favoring the expansion of hematopoietic progenitors, and abolished the ability of PTH to increase the primitive HSC pool. —SF

Williams GA, Callon KE, Watson M, Costa JL, Ding Y, Dickinson M, Wang Y, Naot D, Reid IR, Cornish J. Skeletal phenotype of the leptin receptor-deficient db/db mouse. *J Bone Miner Res*. 2011 Feb 15. [Epub ahead of print] [Abstract]

Contrasting results have been published previously concerning the skeletal phenotype(s) of leptin-deficient mice (ob/ob, db/db), i.e., whether or not they have a high bone mass phenotype. This detailed analysis of db/db mice shows that in the absence of the leptin receptor, both trabecular and cortical bone volume are low, arguing for a bone-anabolic effect of leptin that overcomes its bone-inhibitory effects through the central nervous system. —SF

Zhang J, Tu Q, Bonewald LF, He X, Stein G, Lian J, Chen J. Effects of miR-335-5p in modulating osteogenic differentiation by specifically down-regulating Wnt antagonist DKK1. *J Bone Miner Res*. 2011 Feb 23. [Epub ahead of print] [Abstract]

This study found that DKK1 protein levels were regulated via DKK1 3'UTR by miRNA control: miR-335-5p specifically targets DKK1 3'UTR. In vivo studies showed high expression levels of miR-335-5p in osteoblasts and hypertrophic chondrocytes of mouse embryos. The effects of endogenous miR-335-5p were reversed by anti-miR-335-5p treatment. When site-specific mutations were introduced into the sequence of the predicted binding site of miR-335-5p in the DKK1 3'UTR sequence, the inhibitory effect of miR-335-5p disappeared. As a result of down-regulation of DKK1 protein levels, Wnt signaling is significantly enhanced as indicated by elevated phosphorylation of GSK-3β and increased β-catenin transcriptional activity; expression levels of Wnt target genes (Runx2, BSP and OC) are significantly up-regulated, promoting osteogenic differentiation. —DK

Reviews, Perspectives and Editorials

Li WF, Hou SX, Yu B, Jin D, Férec C, Chen JM. Genetics of osteoporosis: perspectives for personalized medicine. *Personalized Medicine*. 2010 Nov;7(6):655-8. [Abstract]

Mitchell BD, Yerges-Armstrong LM. The genetics of bone loss: challenges and prospects. *J Clin Endocrinol Metab*. 2011 Feb 23. [Epub ahead of print] [Abstract]

Other Studies of Potential Interest

Alam I, Koller DL, Sun Q, Roeder RK, Cañete T, Blázquez G, López-Aumatell R, Martínez-Membrives E, Vicens-Costa E, Mont C, Díaz S, Tobeña A, Fernández-Teruel A, Whitley A, Strid P, Diez M, Johannesson M, Flint J, Econs MJ, Turner CH, Foroud T. Heterogeneous stock rat: A unique animal model for mapping genes influencing bone fragility. *Bone*. 2011 Feb 18. [Epub ahead of print] [Abstract]

Alexander K, Chang M, Maylin E, Kohler T, Müller R, Wu A, Van Rooijen N, Sweet M, Hume D, Raggatt L, Pettit A. Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model. *J Bone Miner Res*. 2011 Feb 8. [Epub ahead of print] [Abstract]

Hughes-Fulford M, Li CF. The role of FGF-2 and BMP-2 in regulation of gene induction, cell proliferation and mineralization. *J Orthop Surg Res*. 2011 Feb 9;6(1):8. [Abstract]

Lei SF, Papasian CJ, Deng HW. Polymorphisms in predicted miRNA binding sites and osteoporosis. *J Bone Miner Res*. 2011 Jan;26(1):72-8. [Abstract]

Reilly MP, Li M, He J, et al. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. *Lancet*. 2011 Jan 29;377(9763):383-92. [Abstract]

Rossi F, Bellini G, Luongo L, Torella M, Mancusi S, De Petrocellis L, Petrosino S, Siniscalco D, Orlando P, Scafuro M, Colacurci N, Perrotta S, Nobili B, Di Marzo V, Maione S; Endocannabinoid Research Group (ERG), Italy. The endovanilloid/endocannabinoid system: A new potential target for osteoporosis therapy. *Bone*. 2011 Jan 13. [Epub ahead of print] [Abstract]

Tolonen S, Mikkilä V, Laaksonen M, Sievänen H, Mononen N, Hernesniemi J, Vehkalahti K, Viikari J, Raitakari O, Kähönen M, Lehtimäki T. Association of apolipoprotein E promoter polymorphisms with bone structural traits is modified by dietary saturated fat intake - The Cardiovascular Risk in Young Finns Study. *Bone*. 2011 Jan 23. [Epub ahead of print] [Abstract]

Yancovitch A, Hershkovitz D, Indelman M, Galloway P, Whiteford M, Sprecher E, Kılıç E. Novel mutations in GALNT3 causing hyperphosphatemic familial tumoral calcinosis. *J Bone Miner Metab*. 2011 Feb 25. [Epub ahead of print] [Abstract]

Conflict of Interest: Dr. Clézardin reports receiving research support from Novartis (Basel, Switzerland) and honoraria for advisory work and speaking engagements from Novartis and Amgen. 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.