J Musculoskelet Neuronal Interact 2006; 6(4):391-401

Hylonome

Poster abstracts from the 36th Meeting of the International Sun Valley Workshop on Skeletal Tissue Biology

July 30-August 2, 2006, Sun Valley, Idaho, USA

Program Chairman: David B. Burr

Abstract No.	Topic
P1-26	Poster abstracts (Authors marked with an asterisk (*) are Recipients of the Alice L. Jee Travel Award)

P-1

Changes in Collagen Maturity And Cross-Linking And Their Relationship to Bone Turnover And Mechanical Properties Following One Year Treatment With Anti-Remodeling Agents in Skeletally-Mature Beagles

M.R. Allen*, E. Gineyts, D.J. Lemming, P.D. Delmas, D.B. Burr
*Indiana School of Medicine, Indianapolis, IN, USA
E-mail: matallen@iupui.edu

Collagen cross-links play a significant role in determining the mechanical properties of bone, stabilizing the organic matrix and enhancing bone strength and stiffness. However, recent *in vitro* evidence has shown excessive cross-linking can also make the tissue more brittle, likely by reducing post-yield deformation.

Advanced glycation end-products (AGEs) form spontaneously via the interaction of glucose and collagen amino acid residues and serve to increase collagen cross-linking. Pentosidine, one of several AGEs, increases with tissue age and is significantly correlated to bone brittleness. As we have previously documented a reduction in canine vertebral bone toughness following one-year of treatment with bisphosphonates, we sought to determine if pentosidine content was significantly altered in this same tissue. Skeletally-mature female beagle dogs were treated for one-year with oral doses of saline vehicle, Risedronate (0.05, 0.1, or 0.5 mg/kg/day), Alendronate (0.1, 0.2, or 1.0 mg/kg/day), or Raloxifene (RAL, 0.5 or 2.5 mg/kg/day). Trabecular bone from the fourth lumbar vertebrae was hydrolyzed and assessed for pentosidine, expressed relative to the amount of collagen. Pentosidine was significantly higher in all bisphosphonate-treated animals compared to vehicle (+34 to +58%). Conversely, pentosidine was not significantly different than vehicle in raloxifene-treated animals. Since raloxifene suppressed vertebral bone turnover to a lesser degree than the bisphosphonates, we propose remodeling suppression is intimately linked to AGE accumulation within the matrix. Furthermore, these changes in non-enzymatic collagen cross-links may help explain why bisphosphonates, but not raloxifene, are associated with reduced bone toughness in this dog model.

Drs. Allen and Burr receive research funding from Lilly, P&GP/Alliance for Better Bone Health and Merck. Dr. Burr is a consultant for Lilly and P&GP and has stock ownership in Lilly. All other authors have no conflict of interest.

P-2

The Effect of Tgf-B And Runx2 on Mechanical Properties And Composition of Bone Matrix

G. Balooch*[1,2], M. Balooch[1], G.W. Marshall[1], R.K. Nalla[2], R. Derynck[1], T. Alliston[1], R.O. Ritchie[2]
[1]University of California, San Francisco, CA, USA; [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA
E-mail: gbalooch@lbl.gov

The ability of bone to resist fracture is determined by the bone mass and architecture, and the mechanical properties and composition of the bone matrix. Several signaling pathways, including estrogen, parathyroid hormone, Transforming Growth Factor-β (TGF-β) and other cytokines, have been implicated in the control of bone mass and architecture. Transforming Growth Factor-β (TGF-β) inhibits terminal osteoblast differentiation by binding its cell-surface receptor and activating Smad3, a transcription factor that represses the expression of genes required for osteoblast differentiation. TGF-β-activated Smad3 binds Runx2 at the Runx2 and osteocalcin promoters to repress transcription of genes required for osteoblast differentiation and bone matrix production.

We hypothesized that TGF-β and Runx2 regulate local mechanical properties and composition of bone - including elastic modulus (E), fracture toughness (Kc), and bone mineral concentration.

Existing transgenic mouse models show altered TGF-β signaling, from 16-fold (D4 mice) and 2.5-fold (D5 mice) over-expression of TGF-β in bone, to decreased TGF-β signaling due to osteoblast expression of a dominant negative TGF-β receptor (E1 mice) and targeted deletion of the Smad3 gene (Smad3+/- and -/- mice) and Runx2 gene (Runx2+/- mice). Tibias from these mice were evaluated using Atomic Force Microscopy for local E variation, three-point bending for Kc, and synchrotron X-ray tomography for mineral concentration.

Mice with elevated TGF-β signaling (D4, D5) had up to 23% reduced elastic modulus relative to wildtype/control (WT) littermates. However, mice in which TGF-β signaling was impaired (E1, Smad3+/-, and Smad3-/-) had up to 54% increased elastic modulus. In addition to elevated E, Smad3+/- mice also had elevated Kc, similar to E1 mice that also had partially reduced TGF-β signaling. Histomorphometric, X-ray, and XTM analyses showed that Smad3+/- bone, like E1 bone, possessed increased trabecular volume, cortical thickness and mineral density relative to WT. Runx2+/- mice, like D4 mice, had up to 31% reduced elastic modulus, 29% reduced mineral concentration, and 34% reduced Kc. This phenotype was rescued when Runx2+/- mice were crossbred with partially reduced TGF-β signaling mice (E1/Runx2+/- mice) and more exaggerated when crossed with mice that had elevated TGF-β signaling (D4/Runx2+/- mice).

In conclusion, partial reduction of TGF-β signaling in bone is sufficient to produce bone with increased cortical thickness, trabecular bone volume, mineral content, and local mechanical properties, enabling these bones to

better resist fracture. Furthermore, inhibition of TGF-β signaling rescues the reduction of all measured functional parameters of bone quality caused by decreased Runx2 expression. Finally, our results suggest that reduction of TGF-β signaling should perhaps be considered as a therapeutic target to treat bone disorders. This is particularly interesting as TGF-β inhibitors are in pre-clinical or clinical trials for treatment of cancer metastases. The possible effects of these agents on bone-matrix properties should be investigated.

Supported by the National Institute of Health and Lawrence Berkeley National Laboratory.

The authors have no conflict of interest.

P-3

Elongation of Dendrites in Osteocyte-Like Cells, MLO-Y4, Regardless of Type of Flow Shear Stress, Oscillatory, Pulsatile or Steady

C. Barragan-Adjemian*, M. Dallas, S. Kotha, L. Bonewald
University of Missouri Kansas City, Kansas City, MO, USA
E-mail: barraganc@umkc.edu

Bone fluid appears to diffuse through the bone matrix and to travel through the osteocyte lacuno-canalicular system. It has been hypothesized that osteocytes respond to the shear stress created by this flow in terms of biochemical signaling through the osteocyte syncytium to cells on the bone surface. Osteocytes appear to be more sensitive the fluid flow shear stress than to substrate stretching. However, the exact nature of the flow is still undetermined. Several elegant models have been developed to predict the type and magnitude of flow through the lacuno-canalicular system. In the present study, representative flow rates from these models were compared with regards to effects on dendrite elongation in response to shear stress. Previously we had shown no significant differences between steady and pulsatile shear stress with regards to prostaglandin production and gap junction function (Cheng et al., 2001). In this study, MLO-Y4 cells were subjected to shear stress for 2 hours using a laminar flow chamber at 16 dynes/cm^2 steady flow, 0+8 dynes/cm^2 forward and reverse at 1Hz for oscillatory flow, and 8+8 dynes/cm^2 at 1Hz for pulsatile flow. After 24 hours the cells were fixed and stained with crystal violet and dendrite length quantitated in approximately 125 cells per field. The measured sinusoidal wave for the pulsatile flow was essentially identical to the theoretical. As previously shown the cells subjected to steady shear stress showed a marked dendrite lengthening compared to control. In all three cases, a 2-fold increase in dendrite length was observed compared to control. No significant differences were observed in the effects of the three forms of flow. siRNA to E11, a membrane protein, significantly reduced the increase in dendrite length in response to fluid flow, whereas no effect was observed on dendrite length in static cultures. Therefore E11 appears to play a role in dendrite elongation due to shear stress. These studies suggest that a parameter other than the type of flow in these cultures is responsible for the biological effect.

The authors have no conflict of interest.

P-4

High Bone Mass In Sclerostin-Deficient Knockout Mice

R. Brommage, J. Liu, A. Suwanichkul, D.R. Powell
Departments of Endocrinology and Genetics, Lexicon Genetics, The Woodlands, TX, USA
E-mail: rbrommage@lexgen.com

Mutations in the SOST gene coding the secreted protein sclerostin produce sclerosteosis with extremely high bone mass. Recent data indicate that sclerostin produced by osteocytes is an inhibitor of the Wnt signaling pathway, which is known to be a critical stimulus for osteoblast proliferation and activity. Therefore, SOST mutations leading to defective sclerostin permit elevated Wnt-mediated bone formation to occur. Knockout (KO) mice with targeted deletion of SOST have high BMD and cortical bone area when examined at 4 months of age by pQCT (Sun et al., JBMR 18[Supplement 2]:S7; 2003).

We have employed homologous recombination techniques to generate SOST knockout mice by targeting the entire open reading frames of exons 1 and 2. Bone parameters were examined by PIXImus DEXA and Scanco μCT40.

Male KO mice exhibited extremely high bone mass when examined at 4, 12 and 24 months of age. Extensive trabecularization of the entire femur shaft marrow cavity was observed at 12 and 24 months of age. The boundary between trabecular and cortical bone could not be reliably identified in microCT images.

Genotype (Males)	Age (months)	N	Body BMD (mg/cm^2)	Femur BMD (mg/cm^2)	Spine BMD (mg/cm^2)	LV5 BV/TV (percent)	Femur Total Area (mm^2)
WT	4	2	51.8	86.2	61.7	22.4	2.70
KO	4	4	68.8	106.0	89.1	43.4	3.03
WT	12	11	61.8	98.4	59.1	17.9	3.37
KO	12	7	81.5	142.4	91.1	29.3	4.19
WT	24	7	56.2	71.8	54.4	11.8	3.64
KO	24	4	103.6	148.2	128.8	46.0	4.65

Female mice at 30 weeks of age underwent sham or ovariectomy surgery and were sacrificed 8 weeks later. At baseline, sclerostin KO mice had elevated body BMD (56%), femur BMD (72%) and spine BMD (94%). Compared to WT mice, sclerostin KO mice had greater declines in whole body BMD (4.6 vs. 1.9 mg/cm^2), femur BMD (10.3 vs. 4.7 mg/cm^2) and spine BMD (21.7 vs. 6.7 mg/cm^2) following ovariectomy.

At 38 weeks of age, sham-ovariectomized sclerostin KO mice had elevated LV5 cancellous bone volume (3.5-fold) and midshaft femur total area (56%), mineralized bone area (2.3-fold) and polar moment of inertia (2.7-fold). Compared to WT mice, sclerostin KO mice had a greater decline in LV5 cancellous bone volume (39 vs. 21%) following ovariectomy. WT mice lost endocortical bone following ovariectomy as marrow area increased by 15% and cortical thickness decreased by 15%. Sclerostin KO mice lost bone in the midshaft femur following ovariectomy as mineralized bone area decreased by 17% and bone marrow area increased by 70%. Ovariectomy did not lead to periosteal bone loss as total femur area remained unchanged in both WT and KO mice. As a consequence of a larger bone diameter, the polar moment of inertia remained 2.9-fold higher following ovariectomy in sclerostin KO mice compared to WT mice.

Sclerostin KO mice maintain greatly elevated bone mass through 2 years of age. Consistent with data that this high bone mass results from increased bone formation and not decreased bone resorption, sclerostin KO mice lose bone following ovariectomy. Since bone loss occurs in the central region of the femur, bone strength remains high in ovariectomized sclerostin KO mice.

The sclerostin knockout mice were produced as part of a collaboration with Genentech to analyze the function of 500 secreted and transmembrane proteins.

The authors all have corporate appointments with Lexicon Genetics.

P-5

The Role of Estrogen Receptor Beta in Bone Mechanotransduction

A.B. Castillo*, J.W. Triplett, F.M. Pavalko, C.H. Turner
Indiana University School of Medicine, Indianapolis, IN, USA
E-mail: alecasti@iupui.edu

Mice lacking estrogen receptor-alpha (ERα) show reduced periosteal bone formation in response to mechanical loading suggesting that ERα plays an important role in bone mechanotransduction. Mice lacking ERβ show they have an enhanced response to mechanical loading, suggesting that ERβ inhibits load-induced periosteal bone formation. Based on these observations, we are studying the role of ERβ in cell growth, survival and mechanotransduction. Preliminary experiments in primary calvarial osteoblasts show that basal levels of proliferation and apoptosis are not significantly different between cell types. Subjecting cells to 30 minutes of oscillatory fluid flow (OFF) resulted in a 2-fold and 2.5-fold increase in

pERK in wild-type and ERβ-/- cells, respectively. Two hours of OFF plus a 24-hour post-flow incubation resulted in a 3.8-fold increase in Cox-2 expression, while no difference was observed in wild-type cells. In addition, ERβ-/- cells appear to have enhanced PGE2 release following flow. Taken together, these results indicate that the enhanced response observed in ERβ-/- mice cannot be explained by differences in cell proliferation or survival. The greater increase in ERK phosphorylation with flow, and an elevated level of Cox-2 expression 24 hours after flow in ERβ-/- cells, which was not observed in wild-type cells, and enhanced PGE2 release may provide a possible mechanism for the enhanced periosteal response in ERβ-/- mice. Thus, ERβ itself, or signaling via ERβ, may normally suppress load-induced periosteal bone formation by suppressing early and intermediate cellular responses in mechanotransduction.

The authors have no conflict of interest.

P-6

The Combined Effects of Alfacalcidol And Exercise Did Not Differ From Alfacalcidol Alone in Vertebral Cancellous Bone of Adult Female Rats

H.Y. Chen[1], X.Y. Tian[1], X.Q. Liu[1], R.B. Setterberg[1], M. Li[2], W.S.S. Jee[1]
[1]Division of Radiobiology, Department of Radiology, University of Utah School of Medicine, Salt Lake City, UT, USA; [2]Osteoporosis Research, Department of Cardiovascular and Metabolic Disease, Pfizer Global Research & Development, Groton, CT, USA
E-mail: haiyan.chen@utah.edu

The current study was designed to investigate the skeletal effects of Alfacalcidol and exercise in eight-month-old intact female Sprague-Dawley rats. Seventy-four, 8-month-old rats were orally treated with 0, 0.005, 0.025, 0.05 or 0.1μg/kg/d of Alfacalcidol, 5 days/week for 12 weeks, and with the same dose of Alfacalcidol combined with raised cage "exercise". The exercise was making the rats rise to a bipedal stance for feeding using a raised cage model designed to increase load to the vertebral column.

There were no body and muscle weight effects. After 3 months of treatment with Alfacalcidol, at 0.05μg/kg/d and 0.1μg/kg/d of Alfacalcidol, the bone volume of these rats was increased from 36-52%, accompanied by increased trabecular thickness and decreased trabecular separation.

Mechanical properties of LV6 were determined by compression testing. A trend of decreased Energy to Peak (unit: N*mm) (-79%) and Toughness (unit: N^*mm/m^3) (-65%) was observed in the rats at 11 months of age compared with the rats at 8 months of age. However, these indices in the Alfacalcidol treatment groups were significantly increased from 148% to 338% in Energy to Peak, and 86% to 294% in toughness with 0.05μg/kg/d and 0.1μg/kg/d of Alfacalcidol, compared to the aging control group.

The two higher doses of Alfacalcidol exhibited improved micro-architecture in increased trabecular plates, trabecular width, decreased trabecular separation, and the connectivity of trabeculae. The elements contributing to connectivity of trabeculae are an increase in number of node to node and the ratio of free end to node, and a decrease in numbers of node to free end, free end to free end. The numbers of node to free end and free end to free end decreased from 37%-61%, and 55%-91%, respectively, with the two highest dose levels compared to aging control. The node-to-node number increased 163% on average.

Histomorphometrically, bone resorption was depressed more than formation resulting in a positive bone balance. Eroded surface was significantly decreased 6x after Alfacalcidol treatments relative to aging control, accompanied with the mineralizing surface lower by 1.8x, and the bone turnover rate significantly lower about 2x, respectively. Mineral apposition rate was increased from 12-31% compared to the aging control. With the decreasing mineralizing surface, there is a decreased trend in bone formation rate, but not significantly. We used percent labeled perimeter compared to percent eroded perimeter as an index of bone balance. There was a positive dose response in bone balance, but only at the highest dose level. Its index of bone balance was 261% significantly greater than control.

In addition, the frequencies and number of "bone buds" emanating from trabecular surfaces increased with increasing Alfacalcidol dose. The bone buds formation rate was different from the bone formation rate at their adjacent bone surfaces. The bone buds MAR was almost the same. Their %L.PM was 3.3x, and BFR was 3.3x greater than the non-bone buds surface. Some bone buds had scalloped reversal lines, indicating remodeling-based formation; while other bone buds had smooth cement lines, indicating minimodeling or mixed remodeling & minimodeling formation.

We measured the periosteal surfaces of left and right transverse processes, the vertebral body and spinal column nerve canal of the lumbar vertebrae. The bone formation was more apparent on the periosteal surfaces of transverse processes and vertebral body than the canal. A significant increasing trend in mineral apposition rate, mineralized surface was observed accompanied with the elevated bone formation rate. Especially in transverse processes, at the 0.1μg/kg alone dose level, the MAR was increased 1.8x, percent label perimeter was increased 1.8x, and bone formation rate was increased 3.3x on average compared to the aging control.

The Alfacalcidol treatment combined raised cage groups were showing similar responses, but no better than that of Alfacalcidol alone in these aged intact female rats. One can speculate that no effects were observed because the mechanical stimuli was inadequate and that Alfacalcidol lacks the same bone anabolic profile possessed by PGE2 that exhibited synergetic effects when combined with RC.

In summary, Alfacalcidol treatment increased cancellous bone mass, improved architecture and bone strength by positive bone balance by depressing bone resorption more than formation, and stimulating "bone buds" formation. The "bone buds" formation may be the result of positive remodeling balance and minimodeling formation (the BFR of bone buds is 3.3x greater than in the non-bone buds surface). At the same time, modeling based bone formation in transverse processes on the periosteal surfaces had increased 3.3x compared to the aging control, in line with the previous studies that Alfacalcidol does promote formation on the periosteal surface. No difference in response was found between the combined effect of Alfacalcidol and raised cage and Alfacalcidol alone. Although raised cage alone has partially prevented OVX-induced cancellous bone loss in previous studies of our lab; this time, in the growth steady state 8-month old rats and under the metabolic mechanism of Alfacalcidol, it showed no better results than normal caged (NC) rats.

M. Li has a corporate appointment with Pfizer. All other authors have no conflict of interest.

P-7

A Proposal For A Standardization of pQCT Data For Evaluation of The Musculoskeletal Status in Forearms And Limbs in Normal Adults

J.L. Ferretti, R.F. Capozza, G.R. Cointry, S. Feldman, S.E. Ferretti, A. Lipsitz, G. Ivancich, G. Marchetti, M.V. Ferretti
Center for P-Ca Metabolism Studies (CEMFoC), National University of Rosario, Argentina
E-mail: jlferretti@arnet.com.ar

The bulk of available pQCT data of the musculoskeletal system of normal and ill individuals has been obtained by different methods and in different anatomical sites. Scarce reference data are available for comparison purposes. This situation claims for some standardization of methods, sites, variables, data management, analysis, and interpretation.

Aiming to offer some preliminary reference with that purpose, we have, **a.** determined pQCT indicators of bone mass (total, cortical and trabecular BMC, cortical CSA, trabecular vBMD), mineralization (cortical vBMD), design (Ix, Ip), and strength (BSIs, SSI), and muscle strength (muscle CSA) in forearms (4 and 66% sites) and legs (4, 14, 38, and 66% sites) of 250 normal adult volunteers (40 men, 60 pre-MP women, 150 post-MP women) aged 20-86 years, and **b.** developed an original software utility, working in Windows, which allows: (1) massive storage, meshing and sorting; (2) analysis of the whole bulk of data into single, manageable databases; (3) automatic Z-scorization of any obtained correlation curve according to the best-fitting equation; and (4) individual Z-score calculation as derived from any of the available reference graphs, specifically for gender and reproductive status.

The following interactions between the obtained data were described that way:

1. **Correlation of the data with age or time since MP** showed only little or no relationship in men and pre-MP women. However, some significant, negative influence of the years elapsed since MP (YSMP) on bone

mineralization > mass > strength > design indicators was observed, in this decreasing order of statistical association.

2. **Associations between design (y) and mass or mineralization indicators (x) ("distribution /mass", d/m, and "distribution / quality", d/q, curves)** showed gender-specific, significant positive (d/m) and negative (d/q) relationships for men and pre-MP women. Post-MP-women data shifted to the left in both cases, indicating a loss in cortical mineralization with relatively minor changes in diaphyseal design. Calculated Z-scores for d/m curves correlated positively, and those for d/q curves correlated negatively, with YSMP.
3. **Variation of total bone mass (BMC) in different sites in the leg** described a 1.5:1.0 relationship between 4% (y) and 14% (x) sites, and a 1.0:1.0 relationship between 4% (y) and 38% (x) sites. Both associations were linear and their 95% ranges of variations of the data comprised statistically the origin of each graph. Post-MP women data tended slightly to shift to the lower region of the graphs in these cases.
4. **Correlations between bone mass (BMC, cortical CSA), design and strength indicators** (y, at the 38% site in the tibia and the 66% site in the radius) **and the regional muscle CSA** (x, at both 66% sites) showed significant, linear relationships for men and pre-MP women in all cases. In particular, the significant, linear relationship between cortical [tibia+fibula] CSA and muscle CSA, both variables determined at the 66% site in the same area units, showed the expected, 0.05 slope and comprised statistically the origin of the graph. Post-MP women data tended significantly to shift to the lower region of the graphs in all these instances. Negative associations of the corresponding Z-scores (y) and the YSMP (x) were observed in every case. Importantly, the statistical significance of these relationships was lower when diaphyseal design indicators (Ix, Ip) were correlated with YSMP than in any other instance.

From the physiological point of view, results provided the following information:

a. They described the normal relationships between the mechanically most significant variables that can be determined by pQCT in the forearms and legs of normal men and pre- and post-MP women.

b. They pointed out a relatively much larger negative influence of MP on cortical bone mineralization (attributable to excessive Haversian remodeling, especially evident through analysis of vBMD data) than that exerted on bone design (as assessed by the CSMIs). This suggests that some modeling reaction could have been induced by the bone mechanostat in these women. The resulting improvement in diaphyseal design would have not been large enough to compensate for the loss of cortical stiffness derived from the impaired vBMD of cortical bone.

c. They verified some interesting hypotheses concerning the predictability of bone indicators as determined by well-known mechanical associations between bone and muscle variables.

d. They determined some interesting differences between some normal muscle-bone interactions concerning their influences on bone mass, design, and strength.

From the practical side:

e. The obtained Z-scored graphs offer a preliminary source of standardization and reference for a number of pQCT variables in men and women of a wide range of ages for diagnostic purposes.

f. The developed software is presented as a preliminary tool for pQCT data management and analysis for application in clinical studies.

The authors have no conflict of interest.

P-8

Insulin Effects on De Novo Bone Formation And Osteogenic Gene Expression *In Vivo* Using a Streptozotocin-Induced Diabetic Mouse Model And Distraction Osteogenesis

J.L. Fowlkes, R.C. Bunn, K.M. Thrailkill, L. Liu, E.C. Wahl, G. Cockrell, C.K. Lumpkin Jr

Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, Little Rock, AR, USA
E-mail: fowlkesjohnl@uams.edu

Type 1 diabetes mellitus (T1DM) is associated with deficits in bone formation and bone regeneration. We have recently developed mouse models to study the consequences of T1DM on direct bone formation using tibial limb lengthening or distraction osteogenesis (DO). DO allows for direct observation of osteoblastogenesis and bone regeneration *in vivo*. Herein, we have determined the impact of chemically-induced T1DM on bone formation using streptozotocin (STZ). Female CD-1 mice were treated with 40 mg/kg of STZ, inducing diabetes in 21 animals (88% conversion). Animals underwent application of a two-ring external fixator to the left tibia, using four transosseous pins and a mid-diaphyseal, low-energy osteotomy (day 1). Daily manual distraction was initiated on day 4, at 0.075 mm BID, for 14 days. Distracted tibiae were analyzed radiographically. The distraction osteogenesis procedure was carried out on 10 diabetic mice treated with insulin (LinBit® sustained release insulin implant pellets), 11 diabetic mice treated with vehicle (vehicle implants), and 11 comparably aged non-diabetic (no STZ) mice. A significant reduction in total new bone formation in the distraction gap was noted in diabetic animals compared with controls (33.4±13.6 vs. 67.9±4.6%; $p<0.05$); treatment with insulin restored NBF to levels, which were not statistically different from non-diabetic animals (65.8±5.4%). To identify genes potentially regulated by the insulin-signaling pathway during insulin-mediated new bone formation, the STZ-induced diabetes + DO experiment was reproduced. At the time of sacrifice, following 14 days of distraction, cellular material from the distraction gaps was harvested from 6 insulin-treated diabetic mice, 6 vehicle-treated diabetic mice, and 7 non-diabetic mice. Biotin-labeled cRNA was prepared from the total RNA using the TrueLabeling-AMP™ kit from SuperArray Corp. Biotin-labeled cRNA was hybridized to nylon arrays (Oligo GEarray® Mouse Osteogenesis Microarray [OMM-026]) which contains >100 genes known to be involved in osteoblastogenesis and bone formation. Bound, biotinylated cRNA was detected with streptavidin-horseradish peroxidase and enhanced chemiluminescence. Normalized gene expression data was obtained by dividing the signal intensity value of specific genes by the average signal intensity of several normalization genes (GAPDH, Rps27a, β2-microglobulin, and HSPβ). Osteogenic gene arrays revealed that a number of genes were altered in their expression in the untreated diabetic state compared to controls, while a significant number were also preserved or even increased in their expression in the insulin-treated animals when compared to diabetic and control animals. Due to threshold limitations of the arrays, only a few genes were present and regulated under all three conditions. These were: MMP-13 (collagenase-3), MMP-9 (72kd-gelatinase), integrin binding sialoprotein (IBSP), and type I collagen. Validation for three genes (MMP-13, MMP-9 and IBSP) by RT-PCR was confirmatory. These results suggest that insulin participates in the regulation of a number of genes involved in bone formation and regeneration, including gene products that contribute to ECM formation and turnover.

The authors have no conflict of interest.

P-9

Hypoxia Increases Cystatin C Expression in MLO-Y4 Osteocytes

D.C. Genetos[1], G.D. DuRaine[1], L.M. Forquer[2], C.E. Yellowley[2], D.P. Fyhrie[1]
[1]Lawrence J. Ellison Musculoskeletal Research Center, Department of Orthopaedic Surgery, UC Davis School of Medicine, Sacramento, CA; [2]Department of Anatomy, Physiology, and Cell Biology, UC Davis School of Veterinary Medicine, Davis, CA, USA
E-mail: dgenetos@ucdavis.edu

Enormous effort has been applied to understanding the effect of systemic and local factors on both the genesis and activity of osteoblasts and osteoclasts. However, relatively little attention has focused on the role of osteocytes in regulating remodeling activity. Indeed, because of their localization and distribution within the matrix, osteocytes are thought to perceive alterations in the skeletal loading environment and to regulate the formation and activity of both osteoblasts and osteoclasts. Two candidate proteins capable of regulating both osteoblast and osteoclast activity are sclerostin and cystatin C, respectively.

Osteocytes, once embedded within mineralized matrix, secrete sclerostin, which antagonizes bone morphogenetic proteins (BMPs) and inhibits bone formation by osteoblasts. The importance of sclerostin in normal skeletal

formation and function is underscored by two pathologic states, sclerosteosis and Van Buchem's disease, that directly result from mutations to *SOST*, the gene encoding sclerostin. There is one report that the cysteine protease inhibitor cystatin C is expressed by osteocytes in a pattern similar to the expression of sclerostin. Cystatin C is known to be an inhibitor of osteoclastogenesis and of osteoclastic resorption. The reduction of resorption is thought to occur by inhibition of cathepsin K activity, but it is not yet clear by what mechanism osteoclast formation is reduced.

It is not yet known whether sclerostin and cystatin C are expressed simultaneously in osteocytes. If so, this would suggest that osteocytes suppress both osteoblast activity (by sclerostin) and also osteoclast formation and activity by cystatin C). We also sought whether expression of sclerostin and cystatin C is regulated by oscillatory fluid flow or alterations in oxygen tension.

The authors have no conflict of interest.

P-10

Synergistic Effect of Vitamin K2 And Prostaglandin E2 on Cancellous Bone Mass in Hypophysectomized Young Rats

J. Iwamoto[1], T. Takeda[1], A. Sei[2], J.K. Yeh[3]
[1]Department of Sports Medicine, Keio University School of Medicine, Tokyo, Japan, [2]Hamuri Co., Ltd., Japan, [3]Metabolism Laboratory, Department of Medicine, Winthrop-University Hospital, NY, USA
E-mail: jiwamoto@sc.itc.keio.ac.jp

Hypophysectomy (HX) results in cessation of bone growth and cancellous osteopenia in rats. It has been reported that prostaglandin E2 (PGE2) improves cortical and cancellous bone mass in HX rats. The purpose of the present study was to examine whether the combined administration of vitamin K2 and PGE2 would have a better beneficial effect on bone than either single administration alone in HX rats. Forty-three female Sprague-Dawley rats, 6 weeks of age, were randomized by the stratified weight method into five groups; intact controls, HX, HX + vitamin K2 (30 mg/kg, p.o., daily), HX + PGE2 (0.83 mg/kg, i.m., 5 days a week), and HX + vitamin K2 + PGE2. The duration of the experiment was 4 weeks. There was a reduction in cancellous bone volume/total tissue volume (BV/TV) of the proximal tibial metaphysis and a reduction in total tissue area (Tt Ar) and cortical area (Ct Ar) of the tibial diaphysis. Vitamin K2 did not affect cancellous BV/TV or Ct Ar. On the other hand, PGE2 attenuated the loss of cancellous BV/TV in association with higher bone formation rate/bone surface (BFR/BS) and eroded surface (ES)/BS compared with intact controls. PGE2 also increased percent Ct Ar compared with non-treated HX rats as a result of attenuation of a decrease in periosteal BFR/BS. Vitamin K2 had a synergistic effect with PGE2 on cancellous BV/TV as a result of the suppression of an increase in ES/BS observed by PGE2 treatment. These results suggested that PGE2 had an anabolic action on cancellous and cortical bone and that despite no apparent effect of vitamin K2 on bone, it had a synergistic effect with PGE2 on cancellous bone mass in HX young rats.

The authors have no conflict of interest.

P-11

Compositional Imaging in Bone: Spatial Correlation of FTIR And Density-Calibrated CT Data

G. Kazakia*, A. Burghardt, J. Carballido-Gamio, K. Saldanha, S. Majumdar
UCSF Dept of Radiology, San Francisco, CA, USA
E-mail: galateia.kazakia@radiology.ucsf.edu

Compositional measures of bone quality are increasingly being studied in an effort to understand bone metabolism associated with age, disease, and drug therapy, and in exploring the effects of tissue level factors on whole bone strength. Fourier transform infrared microspectroscopic (μFTIR) imaging is a modality for compositional analysis, providing spatially-resolved measures of mineral quality over a 2-D region of interest. A second emerging modality for tissue-level analysis of bone is density-calibrated micro computed tomography (μCT). The newest generation of μCT scanner provides assessment of both micro-architecture and mineral density within a 3-D volume. Each of these techniques is being used independently to investigate bone quality. However, the relationships between measures derived from these two modalities are unclear. For example, though specimen-averaged mineral to matrix ratio has been shown to be highly correlated with ash density, we do not know how this ratio corresponds to mineral density at high spatial resolution. Additionally, spatial evaluation of μFTIR measures relative to morphological features has not been attempted in trabecular bone.

The goal of this work was to develop a technique for the spatial comparison of μFTIR-derived compositional measures and μCT-derived structure and mineralization measures. This technique enables simultaneous assessment of parameters of structure, mineral density, and chemical composition at high spatial resolution, leading to more complete characterization of trabecular tissue and mineralization mechanisms. In the current study, an automated registration method is created to spatially correlate μFTIR and μCT data sets. Quantitative spatial comparisons between μFTIR-derived and μCT-derived measures of mineralization are performed.

The automated registration technique successfully aligned the μFTIR and μCT ROIs using a combination of rigid and non-rigid transformation parameters. In every case the additional warping function provided by the non-rigid transformation was necessary to fully align the ROIs. Point to point comparisons of mineral density and mineral to matrix ratio yielded a positive relationship (R^2=0.86). However, the distribution patterns of these two measures are visually distinct. μFTIR mineral to matrix maps contain regions resembling resorption pits in size and shape. These features are not evident in the matching μCT images.

The results indicate that at high spatial resolution μFTIR and μCT mineralization measures are not as well correlated as suggested by specimen-averaged data, but rather display distinctive spatial patterns. This may be due to differences in the physics underlying each modality, and must be further investigated and validated. The technique established here may be used to examine relationships between mineral density and measures of mineral maturity/crystallinity, and to correlate mineralization measures with micro-architectural features to develop a detailed description of mineralization within different morphological regions.

The authors have no conflict of interest.

P-12

A Novel Insight Into The Cellular Mechanism of External Apical Root Resorption (EARR)

D. Liu*
Department of Developmental Sciences/Orthodontics, Marquette University School of Dentistry, Milwaukee, WI, USA
E-mail: dawei.kiu@marquette.edu

Introduction: External apical root resorption (EARR) occurs in 75-80% of patients undergoing orthodontic treatment and can be caused by multiple factors[1]. Unfortunately, the cellular mechanism of EARR is unknown. On tissue level, cementum, the shielding layer covering the dental root, bears a dynamic mechanical load during orthodontic tooth movement. Cementoblasts secrete the mineral matrix and ultimately become embedded in cementum[2]. Our preliminary study showed that fatigue loading generated microdamage in the layer of cementum[3], and the micro-damage is known as an important factor for triggering bone remodeling[4]. Therefore, our hypothesis is that a physiological level of mechanical load enhances cementogenesis and prohibits cementum resorption. To test this hypothesis and reveal the cellular mechanism of mechanically modulated remodeling of cementum, we subjected cementoblasts to a physiological level of mechanical load (12 dynes/cm^2 fluid shear stress, FSS) *in vitro*, and examined both anabolic and catabolic markers as well as several signaling pathways.

Materials and methods: OCCM.30 cells - an immortalized murine cementoblastic cell line (from Somerman MJ, U. Washington, Seattle), were seeded onto glass slides coated with type I collagen (10μg/cm^2) and grown in DMEM supplemented with 10% FBS till 90% confluent. After being starved overnight, the cells were subjected to 12 dynes/cm^2 FSS, while static controls were kept in the identical environment without FSS. After the onset of FSS, media samples were collected at 5 and 15 minutes to determine the release of ATP and PGE2, respectively. Following 1 hour of FSS, the cells were post-incubated for either 30 minutes to determine COX-

2 or 6 hours to determine OPN, OPG and RANKL production, respectively. For testing MAPK activation, the cells were sheared for 30 minutes. ATP was measured using ATP EIA Kit and PGE2 was measured using PGE2 ELISA Kit. Proteins were analyzed by western blot. Statistically, two-way ANOVA was used to compare the difference between FSS-treated and static control groups for each variable, with significance considered when p value was less than 0.05.

Results: FSS induced a drastic release of ATP in OCCM.30 cells at 5 minutes followed by increased PGE2 release at 15 minutes. FSS also increased COX-2 and OPN productions. ERK1/2 and P38, but not JNK were activated within 30 minutes by FSS. Inhibiting ERK1/2 activation with PD98059 almost abolished the FSS-induced PGE2 release ($p<0.001$) and reduced the FSS-induced OPN production by 71% ($p<0.01$). Catabolically, FSS significantly reduced RANKL production by 60% ($p<0.05$) (OPG was undetectable). Blocking ERK1/2 with PD98059 completely reversed the FSS-induced reduction of RANKL.

Conclusions: Cementoblasts are mechanosensitive, activating a signaling pathway axis of ATP-PGE2-MAPK, through which anabolic and catabolic markers are co-ordinately modulated. Mechanical load of physiological level enhances cementogenesis and prohibits cementum resorption, which potentially helps prevent and heal EARR.

References

1. Hartsfield JK Jr, Everett ER, Al-Qawasmi RA. Genetic factors in external apical root resorption and orthodontic treatment. Crit Rev Oral Biol Med 2004; 15:115-122.
2. Bosshardt DD. Are cementoblasts a subpopulation of osteoblasts or a unique phenotype? J Dent Res 2005; 84:390-406.
3. Katona TR, Liu D, Roberts WE. Root fatigue damage – a pilot study. In: Davidovitch Z, Mah J (eds) Biological Mechanisms of Tooth Movement and Craniofacial Adaptation. Harvard Society for the Advancement of Orthodontics, Boston, Massachusetts; 2004:1-9.
4. Burr DB. Remodeling and the repair of fatigue damage. Calcif Tissue Int 1993; 53(Suppl.1):S75-80.

The author has no conflict of interest.

P-13

The Effects of Combining Alfacalcidol And "Exercise" on Tibial Cancellous And Cortical Bone in Intact Rats Assessed by Histomorphometry And pQCT Analyses

X.Q. Liu[1], H.Y. Chen[1], X.Y. Tian[1], X.Q. Liu[1], R.B. Setterberg[1], Mei Li[2], W.S.S. Jee[1]

[1]Division of Radiobiology, Department of Radiology, University of Utah School of Medicine, Salt Lake City, UT, USA; [2]Osteoporosis Research, Department of Cardiovascular and Metabolic Disease, Pfizer Global Research and Development, Groton, CT, USA

E-mail: annylxq2005@yahoo.com

The study was designed to investigate the skeletal effects of Alfacalcidol and "Exercise" on the proximal tibial metaphysis and tibial shaft in eight-month-old female intact Sprague-Dawley rats. Seventy-four rats were orally treated with 0, 0.005, 0.025, 0.05 or 0.1 μg/kg/d of Alfacalcidol (ALF), 5 days/week for 12 weeks and with the same dose of Alfacalcidol combined with Raised Cage "exercise" (RC). The exercise was making the rats rise to a bipedal stance for feeding using a raised cage model designed to increase load to the hind legs.

The following *in vivo* and *ex vivo* analyses were performed: (1) *in vivo* peripheral quantitative computerized tomography (pQCT) of the proximal tibial metaphyses at 5 mm and 6 mm distal to the knee joint space (PTM5, PTM6) at 0, 4, 8, and 12 weeks in anesthetized rats; and (2) histomorphometric analyses of the tibial shafts and proximal tibial metaphyses at the end of the study.

pQCT analysis of the proximal tibial metaphyses (5 mm distal to knee joint) showed that Alfacalcidol at a dose of 0.1 μg/kg/d had significantly increased total bone mineral content (BMC), total tissue area (T.Ar) and periosteal circumferences (Ps.Pm), polar moment of inertia and moment of resistance to bending at 4, 8, and 12 weeks when compared to the beginning of the study. When compared with aging control rats at 4, 8, 12 weeks treatment, respectively, Alfacalcidol at a dose of 0.1 μg/kg/d had higher values in total mineral content, periosteal perimeter and moment of resistance to bending, but bone mineral density (BMD) increased significantly only at 8 and 12 weeks of treatment; highest dose of Alfacalcidol BMD had no difference from aging control group at 4 weeks of treatment.

Histormorphometric analysis of proximal tibial metaphyses (PTM) and tibial shafts (TX) showed that the increase in cancellous bone mass (%B.Ar) was accompanied by increased trabecular width (Tb.Wi), and a decrease in trabecular separation (Tb.Sp). Trabecular connectivity parameters, nodes number (Node No.) and node-to-node (NTN) number, had a significant increase and had a decrease in free end to free end numbers (FTF) when intact rats were treated with Alfacalcidol at a dose of 0.1 μg/kg/d. At doses of 0.5 and 0.1 μg/kg/d the mineralized surface (%L.Pm), bone formation rate per unit of tissue area, bone surface and bone area (BFR/TV, BFR/BS, BFR/BV), were much lower than baseline and aging control groups; and the eroded surface (%E.Pm) was decreased at the 0.025, 0.05 and 0.1 μg/kg/d. The ratio of mineralized surface to eroded surface (%L.Pm/%E.Pm) was increased at the highest dose, indicating that Alfacalcidol at a dose of 0.1 μg/kg/d had a positive bone balance on the proximal tibial metaphyses of intact female rats. The 0.1 μg/kg/d Alfacalcidol dose stimulated periosteal bone formation, as well as decreases in bone formation and decreases in resorption index (%En-E.Pm) on the endocortical surface, which resulted in an increase in total area (T.Ar) and cortical area (Ct.Ar).

In this study, we observed "bone buds" emanating from trabecular bone surfaces in proximal tibial metaphyses at doses as low as 0.025 μg/kg/d of Alfacalcidol. Bone buds index elevated with the increasing dose. The bone buds formation rate was three times more than non-bone buds tissue-area based bone formation rate. However, we did not find this phenomenon happening on periosteal and endocortical surfaces of the tibial shaft in all treatment rats. At the same time, we found bone buds labels increased, whereas non-bone buds trabecular bone surface labels decreased with the higher Alfacalcidol doses.

There was a lack of effect of combining Alfacalcidol with raised cage exercise (data not shown).

The histomorphometry analysis showed that Alfacalcidol treatment dose-dependently altered most of the variables measured in the current study. Histomorphometry analysis is very consistent with pQCT analysis. Alfacalcidol at a dose of 0.1 μg/kg/d not only increased bone strength and stiffness, but also increased trabecular bone mass by stimulating bone buds formation, suppressing bone resorption and inhibiting bone turnover rate in the proximal tibial metaphyses and elevating total area and cortical area value by stimulating bone formation on the periosteal surface in the tibial shaft. Alfacalcidol had different degrees of effects on the trabecular bone and cortical bone. On the trabecular bone, bone buds formation was obviously seen, but on the cortical bone, no bone buds were observed on both periosteal and endocortical surfaces, even at the highest dose of Alfacalcidol. This indicated that Alfacalcidol had more anabolic effects on cancellous bone in the proximal tibial metaphyses than cortical bone in the tibial shaft.

Alfacalcidol increased bone buds formation and had predominant bone formation on periosteal surface. It indicated that Alfacalcidol stimulated bone-minimodeling bone formation on the periosteal and trabecular surfaces. Alfacalcidol decreased tissue-based bone formation, and inhibited bone turnover and bone resorption on the trabecular surface of proximal tibial metaphyses. It suppressed bone formation on the endocortical surface of tibial shaft. It indicated that Alfacalcidol inhibited bone remodeling-associated bone formation as well as inhibiting bone resorption at the endocortical surface of tibial shaft. If it depressed bone resorption at the endocortical surface, it will lower bone formation because of A-R-F, with bone formation less depressed than bone resorption, which indicates it had a positive bone balance.

In summary, we concluded Alfacalcidol had a more active effect on the tibial trabecular bone than the tibial cortical bone. It stimulated minimodeling bone formation and inhibited remodeling bone formation and had a positive bone balance when intact rats were treated with Alfacalcidol at a dose of 0.1 μg/kg/d. No effect of combining Alfacalcidol with raised cage exercise was seen.

M. Li has a corporate appointment with Pfizer. All other authors have no conflict of interest.

P-14

Adaptations in The Mandible of High And Low Bone Density Inbred Mice

I.F. Meta[1], S.S. Huja[1,2]
[1]Section of Oral Biology and [2]Orthodontics, College of Dentistry, The Ohio State University, Columbus, OH, USA
E-mail: huja.1@osu.edu

The difference in bone mineral density (BMD) between the C3H/HeJ (C3H) and C57BL/6J (B6) mice makes these inbred strains advantageous to study skeletal genetics. We hypothesize indentation modulus (*E*), microhardness (*H*), and bone mass are not different between the two strains. In addition, there is no difference in *E* and *H* between the mandible and femur within each strain. Mandibles and femurs were dissected from 17-week-old female C3H (n=15) and B6 (n=15) mice. Immediately after dissection, a ~2 mm thick bone block from the distal surface of the 3rd molar and mid femur were obtained from each mouse for indentation testing (Nano-XP, MTS, Oakridge, TN). Indents were made on the mandibular bone at a rate of ~10 nm/s and to a target depth of 500 nm under moist conditions on polished specimens. Mandibular bone mass was estimated from a lateral-view (*LVA*) faxitron image and 4 histologic cross-sections (*CSA*) of the mandible. Statistical significance between groups was evaluated with Mann-Whitney test. *E* and *H* values are based on 2,193 and 1,053 indents in the mandible and femur, respectively.

		C3H (Mean±SD)	B6 (Mean±SD)
Mandible	LVA (n_{C3H}=30; n_{B6}=26)	38.52±1.61 mm^2	35.73±1.83‡ mm^2
	CSA (n_{C3H}=15; n_{B6}=12)	2.02±0.09 mm^2	1.62±0.12‡ mm^2
	E (n_{C3H}=12; n_{B6}=13)	22.90±2.21 GPa	20.71±2.39† GPa
	H (n_{C3H}=12; n_{B6}=13)	0.83±0.07 GPa	0.74±0.07‡ GPa
Femur	E (n_{C3H}=15; n_{B6}=12)	23.91±1.74 GPa	21.49±1.44‡ GPa
	H (n_{C3H}=15; n_{B6}=12)	0.79±0.06 GPa	0.74±0.05† GPa

$^{\ddagger}p<0.001$; $^{\dagger}p>0.05$; $^{\ddagger}p<0.01$

The greater bone mass (~23%) and indentation modulus (~10%) of the C3H mandible is in agreement with trends in the femur of these inbred mice. Relatively smaller differences (~5%) in *E* and *H* were seen within each strain between the mandible and femur. We conclude that the mandibles of the C3H have higher *E, H* and bone mass than the B6.

Funding: AAOF Biomedical Awards.

The authors have no conflict of interest.

P-15

Stretch Activation of Osteocytes Depends on Culture Substrates

Y. Mikuni-Takagaki
Department of Functional Biology, Kanagawa Dental College, Yokosuka, Japan
E-mail: yukomtak@kdcnet.ac.jp

Cells in bone are equipped with mechanisms to sense diverse physical forces and transduce signals so that they adjust themselves to their mechanical environment. Our previous *in vivo* experiments with primary cells and established cell lines showed that certain types of mechanical stress are received only by certain stages of osteogenic cells. In hypotonically stretched young osteocytes, we have reported that PTH-potentiated Ca2+ influxes localized along the cell processes. Upregulation by stretching of the message levels of IGF-I and osteocalcin was also synergized by PTH and was dependent on extracellular Ca2+, suggesting the involvement of stretch-activated cation channels in the anabolic response (Miyauchi et al., 2000). When the osteocytes adhered to osteopontin or vitronectin, which are the αVβ3 integrin ligands, hypotonically induced Ca2+ influx was increased compared to ligands of other integrin types. Pre-treatment with echistatin, which is a soluble αVβ3 ligand, also significantly enhanced the hypotonic Ca2+ influx in addition to an immediate increase in [Ca2+]i.

These results support the hypothesis that αVβ3 integrin signaling in osteocytes interacts with that in stretching, which is downstream of stretch-activated cation channels.

The author has no conflict of interest.

P-16

Reduced Mechanical Loading Increases The Anabolic Response of Bone to Intermittent PTH

M.I. Nilsson*, J.M. Swift, J.L. Stallone, K. Baek, S.A. Bloomfield
Texas A&M University, College Station, TX, USA
E-mail: mats@klkn.tamu.edu

Purpose: Skeletally mature rats subjected to 28 days of hindlimb unloading (HU) experience a significant loss of proximal tibia volumetric bone mineral density (vBMD) and plantar flexor muscle strength. Although muscle strength recovers within 14 days of re-ambulation, there is no sign of vBMD recovery through 28 days. Our previous work demonstrated that daily administration of parathyroid hormone (PTH), a systemic regulator of calcium, effectively accelerated recovery of bone parameters during recovery from HU. We have now tested PTH administration in normally weight-bearing (WB) rats of the same age, gender and strain in order to test the hypothesis that a previous period of disuse will increase the anabolic response of bone to PTH.

Methods: Six-month-old male Sprague-Dawley rats were randomized into 2 groups, WB+VEH (n=9) and WB+PTH (n=10), and were given daily IP injections of saline VEH or PTH (1-34, 80μg/kg/day), respectively, for 28 days following 28 days of single housed cage activity. *In vivo* peripheral quantitative computed tomography (pQCT) scans of the proximal tibia metaphysis, providing vBMD for cortical and cancellous compartments, as well as total vBMD, were obtained at baseline, after 28 days of WB and after 28 "recovery days". *In vivo* peak isometric torque (ISO) and mass of plantar flexor muscles were obtained at sacrifice. All results were analyzed by 2-way ANOVA with repeated measures.

Results: PTH treatment had no detectable effects on body mass in WB rats nor on muscle weights and peak torque, but resulted in significant gains in total (+17%) and cancellous (+24%) vBMD at the proximal tibia; no significant effects were noted in cortical shell vBMD. Interestingly, the gain in cancellous vBMD in WB rats was significantly less than that observed in our previous study with rats exposed to 28 days HU (+24% vs. +37%), with a similar trend observed for gains in total vBMD (+17% vs. +22%).

Conclusions: These findings suggest that a previous period of disuse may enhance the responsiveness of bone to intermittent PTH as judged by the greater gain in cancellous and total vBMD in rats previously exposed to HU at a bone site sensitive to alterations in mechanical loading.

The authors have no conflict of interest.

P-17

Transdermal Delivery of Lovastatin in a Fracture Model Improves Biomechanical Properties at The Callus

J.S. Nyman[1], J.R. Edwards[1], I.R. Garret[2], G.R. Mundy[1], G.E. Gutierrez[2]
[1]Vanderbilt University Medical Center, Nashville, TN, USA; [2]Osteoscreen Ltd., San Antonio, TX, USA
E-mail: jeffry.s.nyman@vanderbilt.edu

Statins have been shown to stimulate bone formation through the activation of a potent osteogenic protein BMP-2, but previous reports on the effect of statins on promoting fracture repair have shown variable results. This is possibly due to the route of administration (when administered orally, statins undergo extensive hepatic first-pass metabolism). Therefore, the effectiveness of transdermal Lovastatin (TD) in fracture healing was compared to no treatment or oral Lovastatin (PO) using a well-established closed femoral fracture model in the rat.

Two-month-old virgin Sprague-Dawley rats were randomly divided into five treatment groups: vehicle only, TD at 1 and 2.5 mg/kg/day and PO at 10 and 25 mg/kg/day. The right femur was transversely broken by impact bending. Treatment was started on the day of fracture and continued for five days. In a subsequent experiment, low-dose TD (0.1 and 5 mg/kg/day) was compared to low dose PO (5 mg/kg/day) and vehicle only. After six

weeks of free cage activity, the rats were euthanized by cervical dislocation following anesthesia. Harvested femurs were subjected to three-point bending, giving structural strength, stiffness, and work-to-fracture.

The structural strength of calluses treated with TD at 0.1 and 1 mg/kg/day for 5 days was significantly greater than those treated with the vehicle only. Irrespective of dosage, stiffness for TD-treated femurs was significantly greater than control. Oral delivery of statins also increased the stiffness at the higher doses (10 and 25 mg/kg/day). Statins did not however improve work-to-fracture, although the failure of fracture calluses (with or without treatment) was more brittle-like than the failure of non-fractured femurs.

The authors have no conflict of interest.

P-18
Osteocytes Survive Freeze-Thaw And -80˚C Storage in Unprocessed Rat Long Bones

M. Ohashi[1,2]*, Y. Mikuni-Takagaki[3], K. Naruse[2]
[1]Kitasato University School of Medicine, [2]Department of Functional Biology, [3]Kanagawa Dental College
E-mail: loiseaubleu_o8c@yahoo.co.jp

Recent observations that some bone cells survive bone bank freezing protocols has led others to isolate and culture such cells (Weyts et al., 2003). In this study, we have examined similar rat cell populations by electron microscopic analysis of both frozen and defrosted bone tissue samples and by a cell culture method, to differentiate osteocytic cells from osteoblasts (Mikuni-Takagaki et al., 1995). Dissected long bones of 10-week-old Wistar rats were cooled at an average of 6.4˚C/min and preserved for three weeks at -80˚C. Electronmicroscopic pictures of defrosted bone suggested that osteocytic cells in certain areas of cortical bone and subchondral bone retained intact cellular organella as well as nuclear chromatins with little influence of ice crystals. On the other hand, chondrocytes in articular cartilage and growth plate as well as bone marrow cells were severely damaged by ice crystals. Serial collagenase digestions of chips prepared from defrosted bone recovered no cells in pooled fractions No. 3 through 5. Identical fractions prepared from fresh bone grew colonies of polygonal cells, typical of osteoblasts. Out of remaining chips of both frozen and fresh long bones, flattened cells grew in culture. These cells extended radial processes and made contact to each other when transferred onto type I collagen or Matrigel. From confirmation by bone protein expression profiles of these cells, we concluded that these cells are osteocytes and are resistant to bone bank freezing protocols.

The authors have no conflict of interest.

P-19
Indentation Properties of Young And Adult Canine Condylar Bone

A.M. Rummel, F.M. Beck, S.S. Huja
College of Dentistry, The Ohio State University, Columbus, OH, USA
E-mail: rummel.14@osu.edu

Objectives: The purpose of the study was to determine if differences existed between the indentation modulus (IM) and hardness of condylar bone in young and adult dogs.

Methods: Mandibular condyles were obtained from adult (1-2-year-old) and young (~5-month-old) dogs. From each dog (n=6, adult; n=4, young) a 3-4mm thick bone section was obtained. The condyles were sectioned anteroposteriorly resulting in 19 (7 young and 12 adult) bone sections for indentation testing. The frozen specimen was thawed, mounted on a specimen holder and polished on the day of testing. The condylar bone was divided into two regions (near and far) based upon proximity to the articular cartilage. In adult dogs, the distinct subchondral plate of bone was also quantified. A total of 2,706 indents (young=902, near=423, far=479; old=1,804, near=769, far=818, plate=217) were made on moist condylar trabecular bone to a depth of 500 nm at a loading rate of 10 nm/s using a custom-made hydration system. IM and hardness values were analyzed using a repeated measures factorial analysis of variance and Tukey-Kramer method.

Results: Overall, the IM of the adult condyles (10.0±3.4GPa, Mean±SE) were significantly ($p<0.0001$) higher than in young dogs (5.6±2.6GPa). There was no significant ($p=0.14$) difference in IM between near and far region in adult condyles. However, the far region (6.2±2.6GPa) had a significantly ($p=0.0007$) higher IM than the near region (5.0±2.4GPa) in young condylar bone.

Discussion: The effect of age on material properties of condylar bone has not been reported. This data suggests that the trabecular bone within the young condyle has lower indentation properties than adult condylar bone. This data also provides the baseline information needed to compare healthy to diseased bone.

Conclusion: The relative IM of adult condylar bone is nearly two-fold greater than young condylar bone.

P-20
Modulation of Vascular Endothelial Growth Factor Expression in Rat Bone Marrow Stromal Cells by Growth Differentiation Factor-5

K. Sena*, D.R. Sumner, A.S. Virdi
Department of Anatomy and Cell Biology, Rush University Medical Center, Chicago, IL, USA
E-mail: kotaro_sena@rush.edu

Vascular endothelial growth factor-A (VEGF-A), plays a central role in the development and modulation of angiogenesis and its expression is modulated by factors that stimulate or suppress osteoblast differentiation. Growth Differentiation Factor-5 (GDF-5) belongs to a sub-group of the bone morphogenetic protein family. Previous study revealed that GDF-5 induced angiogenesis *in vivo*, suggesting that it may also play a role during bone formation and repair via angiogenesis. However, the mechanisms of GDF-5-induced angiogenesis or its relation to VEGF-related molecules has not been studied in detail. The aim of this study was to determine the effects of GDF-5 on the expression profiles of VEGF-related molecules in rat bone marrow stromal cells (rBMSC). Cells were cultured with or without GDF-5 for 3, 6 and 24 hours and gene expression was analyzed by real-time PCR for VEGF-A, B, C, and D and their receptors (VEGF-R1, R2, R3, Neuropilin1 [Nrp1] and 2 [Nrp2]). Additionally, expressed splice variants of VEGF-A were identified. We report that GDF-5 treatment upregulated VEGF-A gene expression level at 3 hours that was accompanied by a decrease in its receptor VEGFR2 at 6 hours; VEGF-D and its receptor VEGFR3 showed peak expression at 6 hours. This observation implies that the effects of GDF-5 are mediated by co-regulation of VEGF ligands and receptors. In conclusion, our findings that GDF-5 enhanced gene expression level of selective VEGF isoforms as well as their receptors in rBMSC provides evidence for the role of GDF-5-induced angiogenic factors in bone formation.

The authors have no conflict of interest.

P-21
Fibronectin Assembly And Reorganization in Living Osteoblasts is a Dynamic Cell Mediated Process

P. Sivakumar*[1], E.A. Zamir[2], B.J.Rongish[2], A.Czirok[2], N. Thiagarajan[1], S.L. Dallas[1]
[1]Dept of Oral Biology, School of Dentistry, University of Missouri-Kansas City, Kansas City, MO, USA; [2]Dept of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, USA
E-mail: sivakumarp@umkc.edu

The extracellular matrix (ECM) of skeletal tissues is a dynamic structure that provides the structural framework for mineral deposition and modulates a variety of functions such as cell migration and growth factor signaling. Fibronectin (FN) is a fibrillar ECM protein that plays critical roles in osteoblast differentiation and mineralization. Several lines of evidence show that FN regulates the assembly of multiple bone ECM components such as type I collagen, latent TGF-beta binding protein-1 (LTBP1), fibrillin-1, decorin and biglycan.

The goal of these studies was to determine the dynamic mechanisms of assembly and reorganization of FN in living osteoblasts by time-lapse molecular imaging with fluorescent probes. FN assembly was imaged in primary osteoblasts under "de novo" conditions or in the presence of an established matrix. Cell and ECM fibril dynamics were quantified and

correlated using computational and motion analysis techniques.

Timelapse imaging showed that FN assembly either "de novo" (subconfluent cells) or in the presence of an established matrix initiated as small patches/short fibrils on the cell surface that progressively coalesced to form large fibrillar structures. FN Assembly was facilitated by co-operative contributions from multiple motile cells that were far apart and appeared to be driven by cell motility. Quantitative analyses showed a progressive increase in FN fibril size with time and a decrease in the number of smaller FN particles, consistent with a hierarchical assembly model in which fibrils form by coalescence of smaller units. An *in vitro* cell-scoring model was used as an alternative approach to simulate de novo conditions. In this model, migrating cells appeared to carry the existing matrix with them partway (approximately 30 μm) into the scored area and then rapidly assembled a de novo fibronectin matrix via a hierarchical assembly mechanism. The assembly appeared to initiate approximately 15 μm behind the advancing cell front. Particle Image Velocimetry (PIV) analysis of cell and FN movement revealed that the displacement of cells was tightly correlated with that of newly forming FN fibrils, and showed that the newly incorporated (immature) FN matrix was more stable than the pre-existing (mature) FN matrix. In cells with an established matrix, pulse chase imaging with red and green fluorescent FN probes showed that newly synthesized FN assembled on small cell surface fibrils distinct from pre-existing fibrils but was actively redistributed by motile cells to be deposited onto existing fibrils. FN reorganization appeared to be primarily mediated by motile cells shunting and exchanging (i.e., physically reorganizing) "packets" of fibrillar material. Quantitative analyses showed that FN redistribution was progressive and was maximal at 12hours. These studies suggest novel cell-mediated mechanisms for the initiation and propagation of ECM assembly and its subsequent remodeling in living osteoblasts and highlight a critical role for cell-generated mechanical forces in shaping ECM fibrils. These findings may have important implications in our understanding of ECM regulation during normal bone physiology and in disorders associated with defective ECM assembly such as osteogenesis imperfecta.

The authors have no conflict of interest.

P-22

Skeletal Phenotyping in Neurofibromatosis Type 1 Using Dual Energy X-ray Absorptiometry And Urinary Pyridinium Cross-Links

D.A. Stevenson*[1], L.J. Moyer-Mileur[1], M. Pasquali[2,3], E. Schwarz[2,3], M. Murray[1], X. Sheng[1], J.C. Carey[1], H. Slater[1], D.H. Viskochil[1]
[1]Department of Pediatrics, University of Utah, [2]Department of Pathology, University of Utah, [3]ARUP, Salt Lake City, UT, [4]Department of Family and Preventive Medicine, University of Utah, SLC, Utah, USA
E-mail: david.stevenson@hsc.utah.edu

The common skeletal dysplasias of neurofibromatosis type 1 (NF1) include long-bone dysplasia, scoliosis, and sphenoid wing dysplasia. The pathogenesis of the mesodermally derived osseous defects in NF1 is unknown. We hypothesize that disordered bone resorption contributes to an intrinsic osseous dysplasia. Dual energy X-ray absorptiometry (DXA) imaging and urinary pyridinium cross-links were used in a case control design to better understand the pathogenesis.

We evaluated 84 NF1 individuals (age 5-18 years). 24/80 of the NF1 individuals had an osseous dysplasia (long-bone dysplasia, scoliosis, and/or sphenoid wing dysplasia). Measurements of the whole body, hip, femoral neck and lumbar spine using DXA (Hologic 4500A) were obtained and compared to healthy controls without NF1 (N=293). NF1 individuals had decreased areal bone mineral density of the hip ($p<0.0001$), femoral neck ($p<0.0001$), lumbar spine ($p=0.0025$), and whole body subtotal ($p<0.0001$) using analysis-of-co-variance with a fixed set of co-variates (age, height, Tanner stage, and gender). When NF1 individuals were separated into groups with and without osseous dysplasias, the NF1 group without osseous dysplasias had statistically significant decreases compared to controls. A trend ANOVA between all groups showed a downward trend. Total (free + peptide-bound) pyridinium cross-links from the first morning urines from 60 NF1 children were compared to healthy age-matched controls. Preliminary analysis of the first 14 showed increases in NF1 individuals ($p<0.001$).

These data suggest that NF1 individuals have a unique bony profile with increased bone resorption. DXA and pyridinium cross-links may prove useful in identifying individuals at risk for osseous complications, and monitoring therapeutic trials.

P-23

Assessing The Efficacy of Multiple Dosing Regimens of a ß-Agonist Agent For Bone And Muscle Loss During 28-Day Hindlimb Unloading

J.M. Swift*[1], F. von Walden[2], T. Elman[2], J.D. Fluckey[1], S.A. Bloomfield[1]
[1]Dept of Health and Kinesiology, Texas A&M University, College Station, TX, USA; [2]Dept of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden
E-mail: jms1017@hlkn.tamu.edu

Previous studies have shown that dobutamine (DOB) given during simulated microgravity significantly blunts decreases in femoral midshaft cortical bone area and cross-sectional moment of inertia (CSMI). Our objective was to test the efficacy of 3 dosing regimens of DOB given during 28-day hindlimb unloading (HU), in reversing the decrease in bone mass, area and strength observed during simulated microgravity, and to test for any effects on muscle mass, strength, and protein synthesis. Male Sprague-Dawley rats (n=12), 6-months-old, were hindlimb unloaded for 28 days and injected IP either once daily with saline (VEH) or with one of the following dosing regimens of DOB: 1 mg/kg body weight (BW) 2x/d (DOB 1); 2 mg/kg BW 2x/d (DOB 2); 4 mg/kg BW 1x/d (DOB 4). Bone mineral density (BMD) and bone geometry were determined by peripheral quantitative computed tomography (pQCT; Stratec XCT-M, Norland Corp) at both proximal and midshaft tibia. Peak isometric strength (P_0) of left plantar flexor muscles was measured *in vivo* prior to sacrifice. Soleus (SOL) tissue samples were assessed for rates of muscle protein synthesis, with and without dobutamine, *in vitro* by incorporation of radioactive phenylalanine into muscle protein. DOB during HU resulted in 4% higher total BMD (DOB 2) and 7% smaller marrow area (DOB 4) at the proximal tibia vs. VEH. CSMI at the tibial midshaft was 3% higher in DOB 4 vs. VEH, but cortical bone area was only minimally increased. P_0 was 13%, 18%, and 23% higher in DOB-treated rats (DOB 4, DOB 2, and DOB 1, respectively) vs. VEH. In addition, SOL protein synthesis rates were 24% higher in DOB 4 and 23% lower in DOB 2 rats during HU. Our results indicate a beneficial effect of DOB on tibiae geometry, density, and strength. The modestly higher total BMD and lowered marrow area at the proximal tibia in DOB rats imply reduced endocortical resorption. These preliminary results need to be confirmed in a study involving a larger number of animals.

The authors have no conflict of interest.

P-24

The Effects of Graded Doses of Alfacalcidol And Exercise in The Femurs of Adult Female Rats

X.Y. Tian[1], X.Q. Liu[1], H.Y. Chen[1], R.B. Setterberg[1], Mei Li[2], W.S.S. Jee[1]
[1]Division of Radiobiology, Department of Radiology, University of Utah School of Medicine, Salt Lake City, UT, USA; [2]Osteoporosis Research, Department of Cardiovascular and Metabolic Disease, Pfizer Global Research and Development, Groton, CT, USA
E-mail: xyt65@yahoo.com

The current study was designed to investigate the skeletal effects of Alfacalcidol and exercise on the femur in eight-month-old intact female Sprague-Dawley rats. Seventy-four rats were orally treated with 0, 0.005, 0.025, 0.05 or 0.1μg/kg/d of Alfacalcidol, 5 days/week for 12 weeks, with the same dose of Alfacalcidol combined with raised cage "exercise". The exercise was making the rat rise to a bipedal stance for feeding using a raised cage model designed to increase skeletal loading[1].

The following *ex vivo* analyses were performed to date: (1) pQCT measurements of the distal femoral metaphysis and shaft; (2) three-point bending strength test of the femoral shaft; and (3) cancellous bone histomorphometry of the proximal femur.

Significant changes in pQCT measurements compared to Aging Controls were limited to the 0.1μg/kg/d dose. The distal femoral metaphysis exhibited significant increased total trabecular and cortical content, total and

trabecular density and total area, while only an elevated value of cortical content in the femoral shaft compared to Aging Control was seen.

Only significantly increased maximal load and stiffness and a non-significant increased energy to peak were noted with the 0.1μg/kg/d dose level compared to Aging Controls.

Compared to Aging Controls, the cancellous bone histomorphometry profile of rats treated with Alfacalcidol included increased cancellous bone mass (%B.Ar), improved architectures: decreased trabecular separation (Tb.Sp), increased density of node to node/total tissue area (NTN/T.Ar), and increased ratio of node to free end (N/F) at the 0.1μg/kg/d dose only. All other parameters were depressed: bone formation rates - surface, tissue area, and bone area based (BFR/BS, BFR/T.Ar, BFR/B.Ar), percent labeled perimeter (%L.Pm), percent osteoblast perimeter (%Ob.Pm), and percent osteoclast perimeter (%Oc.Pm). Furthermore, the indices of bone balance (%Ob.Pm/%Oc.Pm) were non-significantly increased from Aging Controls; depressed resorption index (%change of %Oc.Pm) was greater than formation index (%change of %Ob.Pm).

The stimulation of bone buds from positive remodeling balance and/or minimodeling occurred at the 0.05 and 0.1μg/kg/d doses with bone formation parameters that were as high as 9-fold greater in percent labeled perimeter (73.3±10.3 vs. 8.3±4.4), and 7-fold higher than for bone surface-based bone formation rate (63.6±12.3 vs. 9.6±6.0) in the tissue area.

There was a lack of effect of combining Alfacalcidol with raised cage exercise (data not shown).

In summary, the three types of analyses performed showed increases in cortical and cancellous bone mass and strength of the proximal femur, only at the highest Alfacalcidol dose (0.1μg/kg/d). This dose also showed improved architecture. The histomorphometric analysis was the most informative. It showed the vitamin D analog induced a positive bone balance from a combination of decreased bone turnover, depressed resorption greater than formation and stimulation of bone buds formation. Bone buds formation contributed little to the total bone, only 0.008-0.085% of total area by improving connectivity. The effect of superimposing raised cage exercise upon Alfacalcidol treatment lacked effect. Lastly, we found the proximal femoral metaphysis to be 2-3 times less responsive (%L.Pm, BFR/BS, BFR/B.Ar, BFR/T.Ar, and resorption index- %E.Pm or %Oc.Pm) than that for the lumbar vertebral body[2]. Studies to characterize the cortical bone histomorphometric response are in progress.

References

1. Yao W, Jee WS, Chen JL, Li CY, Frost HM. A novel method to "exercise" rats: making rats rise to erect bipedal stance for feeding – raised cage model. J Musculoskelet Neuronal Interact 2001; 1:241-247.
2. Chen HY, Tian XY, Liu XQ, Setterberg RB, Li M, Jee WSS. The combined effects of alfacalcidol and exercise did not differ from alfacalcidol alone in vertebral cancellous bone of adult female rats. Sun Valley Workshop on Skeletal Tissue Biology, Poster Abstract, 2006.

M. Li has a corporate appointment with Pfizer. All other authors have no conflict of interest.

P-25

Pharmacokinetics And Biodistribution of Bone-Targeting N-(2-Hydroxypropyl)Methacrylamide (HPMA) Copolymers in BALB/C Mice

D. Wang[1,4], M. Sima[1], R.L. Mosley[5], J.P. Davda[4], N. Tietze[1], S.C. Miller[3], P.R. Gwilt[4], P. Kopečková[1], J. Kopeček[1,2]

Departments of [1]Pharmaceutics and Pharmaceutical Chemistry/CCCD, [2]Bioengineering and [3]Division of Radiobiology, University of Utah, Salt Lake City, Utah, USA; [4]Department of Pharmaceutical Sciences, College of Pharmacy and [5]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, Nebraska, USA

E-mail: dwang@unmc.edu

Introduction: During the past few decades, the advancement of biomedical research has dramatically improved our understanding of musculoskeletal diseases. Many molecular targets have been identified and numerous new therapeutic agents have been developed for the treatment of diseases such as osteoporosis[1]. However, the lack of tissue specificity may hamper the clinical applications of these new drugs. We believe that novel drug delivery technologies, which can enhance the osteotropicity of drugs may help to solve the problem[2]. Recently, we have developed a polymeric bone-targeting drug delivery system based on N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer[3]. Alendronate (a bisphosphonate) and D-aspartic acid octapeptide (D-Asp8) were used as targeting moieties, which could favorably recognize and strongly bind to biomineral (e.g., carbonated hydroxyapatite) surfaces. In previous *in vivo* studies, the delivery system was given to young BALB/c mice. Histomorphometric analyses demonstrated very strong accumulation of the targeted HPMA copolymers at the high bone turnover sites, such as metaphyseal spongiosa, and moderate deposition at sites of slower turnover, such as endosteum and periosteum of diaphyseal shaft. In the present study, we report the first systemic pharmacokinetic and biodistribution (PK/BD) study of the HPMA copolymer-based bone-targeting delivery system on healthy young BALB/c mice.

Results and Discussion: To investigate the pharmacokinetics and biodistribution of bone-targeting HPMA copolymer, tyrosine amide was introduced into the copolymer. It was labeled with 125I and administered intravenously to BALB/c mice. Biodistribution of the copolymer to different organs and tissues were followed using a gamma counter and single photon emission computed tomography (SPECT). Both the invasive gamma counter data and non-invasive SPECT data further confirmed that D-Asp8 is a strong bone-targeting moiety. HPMA copolymer containing D-Asp8 as bone-targeting moiety were detected in the entire skeleton, especially within the high bone turnover sites (e.g., distal femur, proximal tibia, lumbar vertebral bodies, mandible and incisor root, etc.). To evaluate the influence of molecular weight on their pharmacokinetics and biodistribution, three fractions (Mw of 24, 46, and 96 kDa) of HPMA copolymer–D-Asp8 conjugate were prepared and evaluated. Pharmacokinetic analysis indicated that the elimination half-lives (t1/2) of the three HPMA copolymer conjugates showed a strong correlation with molecular size, with the smallest copolymer demonstrating the shortest half-life (8.7 h). Assuming elimination is facilitated primarily by renal filtration, the copolymers with higher molecular weight were eliminated at a much slower rate than the smaller ones, as reflected by the long half-life observed for the 96 kDa conjugate. Furthermore, the distribution of the 96 kDa conjugate appeared to be almost entirely restricted to the blood compartment as reflected by the low1 steady-state volume of distribution (Vdss=5.37 mL). By contrast, the smallest molecular weight copolymer conjugate, 24 kDa, had a pronounced distribution phase, reflecting rapid initial tissue uptake. For biodistribution, higher molecular weight of the conjugate enhanced their deposition to bone due to the prolonged half-life in circulation, but also weakened the boneselectivity of the conjugate. A higher content of bone-targeting moiety (D-Asp8) in the conjugate may help to achieve superior hard tissue selectivity.

Conclusion: Biodistribution and pharmacokinetic studies of D-Asp8-containing HPMA copolymer conjugates demonstrated that the conjugate could target to all parts of the skeleton. Higher content of D-Asp8 in the copolymer may enhance its specificity to the skeleton. The effectiveness of the delivery system should be further verified in diseased animal models such as the ovariectomized rat model of osteoporosis.

Acknowledgement

We thank Dr. H.E. Gendelman for valuable discussion and for permission to use the SPECT. Dr. Wang deeply appreciates the financial support he received as a new faculty from the College of Pharmacy, UNMC. This work was supported in part by NIH Grant GM069847.

References

1. Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. Science 2000; 289:1508-1514.
2. Wang D, Miller SC, Kopeček J. Drug delivery for musculoskeletal diseases: challenges and opportunities. Adv Drug Deliv Rev 2005; 57:935-937.
3. Wang D, Miller SC, Sima M, Kopečkova P, Kopeček J. Synthesis and evaluation of water-soluble polymeric bone-targeted drug delivery systems. Bioconjug Chem 2003; 14:853-859.

DW, SCM, PK and JK are inventors of a patent application partially related to this work. All other authors have no conflict of interest.

P-26
GENES DECREASE OSTEOPOROSIS RISK ALSO DECREASE RISK OF OBESITY

L. Zhao*, Y. Liu, R.R. Recker, H-W. Deng
Osteoporosis Research Center, Creighton University, Omaha, NE, USA
E-mail: lanjuanzhao@creighton.edu

It is widely known that increasing body weight (and thus higher risk to obesity) is associated with higher bone mineral density (BMD) (and thus lower risk to osteoporosis). This dogma has been widely held and creates an apparent dilemma since some molecular, cell developmental and intervention studies have suggested otherwise. We performed this large-scale quantitative genetic analysis to tackle this dilemma.

We measured whole body fat mass, lean mass, BMI, and bone mass in two samples: 1,988 unrelated Chinese subjects and 4,489 subjects from 512 Caucasian pedigrees. We first evaluated the Pearson correlation, then dissected the phenotypic correlations into genetic and environmental components, with bone mass unadjusted or adjusted for body weight (to control for its mechanical loading effects on bone mass).

In both Chinese and Caucasians, fat mass was, positively correlated with weight-unadjusted bone mass, in agreement with the dogma. However, when bone mass was adjusted for body weight, the phenotypic correlation (including its genetic and environmental components) between fat mass and bone mass turned out to be negative. Our results indicate that, when the mechanical loading effect of body weight on bone mass is adjusted for, increased fat mass is actually associated with decreased bone mass.

Our study, for the first time, may provide a solution to the dilemma by showing that both genes and environmental factors that tend to decrease risk to osteoporosis may also decrease risk to obesity if the effect of mechanic loading on bone mass due to body weight is adjusted for.

R.R. Recker has consultancies with Merck, Lilly Wyeth, P&GP, Amgen, Roche, GSK, Novartis, NPS, and Allelix. All other authors have no conflict of interest.