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Abstract No.	Topic
P1-17	Poster abstracts (Authors marked with an asterisk (*) are Recipients of the Alice L. Jee Travel Award)

P-1

FGF Receptor 3 Mutations Increase The Differentiation Potential of Murine Mesenchymal Cell Lines C3H10T1/2 And C2C12

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Fibroblast growth factor receptor 3 (FGFR3) is a tyrosine kinase membrane-spanning protein that regulates chondrocyte proliferation and differentiation during cartilage development. Various activating mutations in the receptor are associated with human dwarfing chondrodysplasias such as Hypochondroplasia (HCH), Achondroplasia (ACH) Thanatophoric Dysplasia Type I (TDI) and Type II (TDII). In these genetic diseases increased signalling of mutant receptor leads to decreased proliferation of chondrocytes through the STAT1 pathway and prolonged prehypertrophic differentiation through the MAPK pathway. Depending on the mutation and the level of receptor overactivation, dysplasia may be mild (HCH), moderate (ACH) or severe (TDI/II). It has previously been shown (Hoffmann et al. 2002) that induced overexpression of wild type (WT) FGFR3 alone in murine mesenchymal cell line C3H10T1/2 is sufficient to bring about chondrogenic differentiation by upregulation of the MAP kinase pathway and SOX9 in a similar way to chondrogenic induction induced by the addition of BMP-2 *in vitro*.

The aim of this study was to overexpress not only WT but also mutant TDII FGFR3 in C3H10T1/2 cells, comparing the effects of the two receptors on the resulting chondrogenic differentiation. As the receptors *in vivo* reduce chondrocyte proliferation prior to differentiation, we initially examined the effect on proliferation of C3H10T1/2 cells overexpressing the WT and mutant TDII receptor over a 72 hour period and found that both receptors reduce cell proliferation, with a significantly greater reduction in the case of the TDII type.

We have also compared the chondrogenic differentiation potential of both receptor types in C3H10T1/2 by Alcian Blue staining and Western Blotting. Alcian Blue staining revealed greater proteoglycan synthesis in cells overexpressing TDII than in cells overexpressing WT FGFR3 and Western Blotting showed an increase in MAPK signalling in the TDII receptor compared to WT.

A different cell line, murine myogenic C2C12, has previously been shown to undergo osteogenic induction by the addition of BMP-2 *in vitro* (Katagiri, 1994). In this study, we also examined the effect of induced overexpression of WT and TDII FGFR3 on the proliferation and differentiation of this cell line to investigate whether or not osteogenic as well as chondrogenic differentiation can be induced by FGFR signalling.

In C2C12 cells, the WT and TDII FGFR3 receptor increased, rather than decreased, cell proliferation, and furthermore induced osteogenic differentiation as measured by both cell staining and a quantitative assay for alkaline phosphatase.

This study shows that depending on the type of primary cell involved, FGFR3 may decrease or increase cell proliferation, whilst the potent and constitutive signalling by the mutant TDII receptor alone is sufficient to induce chondrogenic and osteogenic induction. Further studies are underway to assess whether or not TDII MAPK signalling can increase SOX9 and downstream chondrogenic matrix proteins in T1/2 cells and whether or not the osteogenic transcription factor Cbfa1 and its downstream osteogenic proteins are increased in C2C12 cells expressing the same TDII mutant receptor. These studies will be carried out with the aim of a possible therapeutic intervention for the *in vitro* or *in vivo* production of cartilage or bone matrix proteins.

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The authors have no conflict of interest.

P-2

PTHrP Regulation of Articular Chondrocytes

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In an allelic PTHrP-lacZ knock-in mouse, we detected PTHrP to be deployed at the sites of future joint formation as early as e11.5 during embryonic development, and continue to be expressed in articular cartilage throughout the postnatal life of the mouse. ISHH reveals PTH1R-expressing

cells subjacent to the zone of PTHrP expression in articular chondrocytes.

In the knee joint, PTHrP is expressed at all sites of articular cartilage contact. To examine the question of mechanotransduction of PTHrP in articular cartilage, we unloaded the knee joint and the patella by transecting the suprapatellar ligament. We observed a marked decrease in PTHrP/β-gal activity in the articular chondrocytes, which was accompanied by an increase in the number of alkaline phosphatase-positive hypertrophying chondrocytes that advanced toward the joint surface. Moreover, we found that there was an increase in differentiating chondrocytes at joint sites in the homozygous PTHrP-lacZ (PTHrP-null) mouse and that these cells approached the articular surface more closely than in the heterozygous PTHrP-lacZ mouse. Articular cartilage is a permanent cartilage that remains hyaline cartilage throughout life. We also inquired into putative PTHrP regulation of chondrocyte differentiation in other permanent cartilage structures. In nasal cartilage and costal cartilage, we found PTHrP was deployed in the surrounding perichondrium and that in PTHrP-null mice these cells became terminally differentiated and completely mineralized.

Our data indicate that PTHrP is deployed in response to mechanical forces in articular cartilage and regulates articular chondrocyte differentiation. These findings have clear implications for articular chondrocyte biology and for the pathogenesis of osteoarthritis.

The authors have no conflict of interest.

P-3

Three-Dimensional Multiphoton Imaging of Primary Cilia, Cells, And Matrix in Tendon

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Connective tissues such as tendon and bone respond to mechanical forces with changes in metabolism, ultrastructure, and material properties. Primary cilia have been implicated in such mechanotransduction processes, but the mechanisms by which the cells sense loads and convert them to biochemical signals that promote tissue formation and adaptation are poorly understood. We are developing experimental methodologies to investigate primary cilia in tendon, a tissue with a highly oriented extracellular matrix (ECM) that constitutes an ideal model in which to characterize spatial relationships between cilia and matrix.

The objective of this study was to investigate the incidence and orientation of primary cilia in tendon cells in their native ECM. Whole-mount immunohistochemical and multiphoton microscopy techniques were developed to simultaneously image primary cilia, tenocyte nuclei, and collagen. Image stacks were acquired to enable 3-D visualization of the tissue. Oblong nuclei were distributed between parallel bundles of collagen fibers, and primary cilia were observed both in the cytoplasm and projecting into the surrounding ECM at a range of angles (Figure 1). We are refining image-processing analyses to quantify the incidence and spatial orientation of primary cilia in tendon and ultimately to test the hypothesis that primary cilia respond to changes in applied forces by changing their spatial orientation. The development of these methodologies will enable investigation of the role of primary cilia in normal and pathological growth and adaptation in a variety of musculoskeletal tissues.

The authors have no conflict of interest.

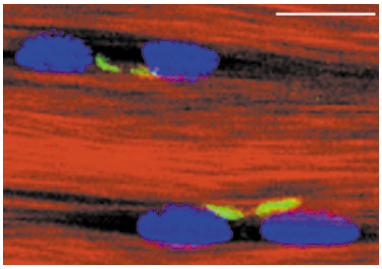

Figure 1. Multiphoton micrograph of rat extensor tendon showing collagen (red), tenocyte nuclei (blue), and primary cilia (green). Scale bar = 10 mm.

P-4

Tomographical (pQCT) Evaluation of The Skeletal Condition And of The Muscle-Bone Interactions in Chronic Cirrhotics as a Function of Their Metabolic Alterations

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Background and aim of the study. The incidence of densitometric osteopenia in chronic cirrhotics rounds 30%. Fracture incidence rate increases, but the biomechanical correlate of the densitometric data is unclear.

We have determined a set of tomographic (pQCT; XCT-2000, Stratec, Germany) indicators of bone mineral mass, material "quality", design and strength, and of muscle strength, in sites at 4%, 14%, 38% and 66% of tibial length proximal to the heel joint line, in 35 chronic cirrhotics (15 men) of alcoholic, viral, cryptogenetic, auto-immune, or cholestatic etiology, aged 18-69 years. The data were compared with those taken from 60 men, 100 pre-menopausal women and 150 post-menopausal women of comparable ages, all of them healthy and free of diseases or treatments which could have affected their skeleton, in order to describe their musculoskeletal condition following current biomechanical criteria. The degree of hepatic insufficiency (*Child-Pugh Score*, *CPS*, which combines all bilirrubinemia, albuminemia, prothrombine time, presence of ascitis, and encephalopaty) and a set of laboratory indicators were also determined. The data were correlated in order to establish whether the biomechanical status of the musculoskeletal system (as described by the tomographic *Z-scores* of the patients with respect to the control values) is or is not affected as a function of either or both, the clinical-metabolic indicators studied and the different etiologies of the hepatic insufficiency.

Results-1. Musculoskeletal alterations. The cirrhotic condition reduced the trabecular BMC and vBMD at the 4% site (ANOVA, $p<0.001$); the proportion between total BMC measured at 4% (a mostly trabecular region) and at 14% or 38% sites (cortical regions; $p<0.01$), and the calf muscles cross-sectional area allometrically adjusted to tibial periosteal perimeter (PoPM) ($p<0.05$). However, the disease did not affect the diaphyseal cortical mass (as evaluated by the cortical area adjusted to tibial PoPm), "quality" (as described by the cortical vBMD corrected from the partial-volume error) or architectural distribution (as assessed by the allometrically-adjusted bending and torsional cross-sectional moments of inertia, CSMIs). The Bone Strength Indices (xBSI, pBSI) and Stress-Strain Index (SSI), which capture both CSMIs and cortical vBMD, were not significantly affected. The proportion between the unadjusted cortical mass (tibia plus fibula data) and the calf muscular cross-sectional area measured at the 66% site tended to diminish ($p<0.05$). The relationships between indicators of tibial diaphyseal design or strength with muscle CSA were unaffected. The relationships between indicators of compact bone tissue distribution (CSMIs, y) and "quality" (vBMD, x) ("d/q" curves, which describe the efficiency of distribution of the available cortical bone as a function of its mechanical quality) were unaltered.

Results-2. Metabolic correlations. If the alcoholic group of patients was excluded from the analyses, the trabecular bone loss correlated significantly with the CPS, specially with the albuminemia data (always $p<0.001$), and with the degree of cholestasis, as indicated by plasma bone alkaline phosphatase activity or by bilirrubinemia values (always $p<0.01$). Muscle mass deterioration correlated with *CPS* ($r=-558$, $p=0.02$) but not with the biochemical indicators of cholestasis. Every alcoholic patient showed the described alterations (ANOVA, $p<0.01$) independently of the *CPS* and the degree of cholestasis. Serum calcium concentration did not correlate with the osteomuscular data.

Interpretation and conclusions. Results indicate that the chronic metabolic alteration induced by the hepatic disease in these patients: (1) reduced the mass of trabecular bone, and hence its resistance to deformation and fracture under compression at the heel (and presumably so in other skeletal regions with a predominantly trabecular structure) correlatively with both the severity of the hepatic disease and the degree of

cholestasis; (2) reduced also the muscular mass, independently of the biochemically-assessed degree of cholestasis; and (3) tended to affect the efficiency of the muscle-bone interactions in terms of cortical bone mass, but not so concerning diaphyseal bone "quality", architectural distribution, or bending or torsional strength in the studied sample. Alcohol abuse appeared to be a significant independent determinant of these alterations.

This is the first pQCT approach to a biomechanical analysis of the musculoskeletal condition in chronic cirrhotics.

The authors have no conflict of interest.

P-5

Direct Bone Formation During Distraction Osteogenesis Does Not Require TNF Receptors And Elevated Serum TNF Fails to Inhibit Bone Formation in TNFR1 Deficient Mice

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Distraction Osteogenesis (DO) is a process that induces direct new bone formation (direct osteoblastogenesis) as a result of the stimulating effects of mechanical distraction. Tumor necrosis factor-α (TNF) is a cytokine that can modulate osteoblastogenesis. The direct effects of TNF on direct bone formation in rodents are hypothetically mediated through TNF receptor 1 and/or 2 (TNFR1/2) signaling. Neither the effects of TNF receptor deficiency on distraction osteogenesis (DO) in mice nor the effects of recombinant mouse TNF (rmTNF) administration to TNF receptor deficient mice during DO have been reported. We utilized a unique model of mouse DO to assess both the effects of TNFR homozygous null gene alterations on direct bone formation and the effects of rmTNF on wild type (WT), TNFR1 -/- (R1KO), and TNR2 -/- (R2KO) mice. Both radiological and histological analyses of direct bone formation in the distraction gaps demonstrated no significant differences between the WT, R1KO, R2KO, or TNFR1 -/- & R2 -/- (R1&2KO) mice. R1&2KO mice had constitutively elevated levels of serum TNF but demonstrated no inhibition of new bone formation. Systemic administration by osmotic pump of rmTNF during DO (10 ug/kg/day) resulted in significant inhibition of gap bone formation measures in WT and R2KO mice, but not in R1KO mice. No significant weight differences were noted in any group. Collectively, we conclude that exogenous rmTNF and/or endogenous TNF act to inhibit new bone formation during DO by signaling primarily through TNFR1.

The authors have no conflict of interest.

P-6

Remodeling Rates in The Jaws And Femurs in Two Age Groups of Beagle Dogs

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Remodeling is a primary homeostatic and healing mechanism in osseous tissues. The purpose of this study was to examine secondary osteonal remodeling in two age (young and adult) groups and at 3 sites (maxilla, mandible, and femur). Beagle dogs (5 m; n=4 and 1.1 yrs old; n=6) were given a pair of calcein labels (5 mg/kg) 16 and 2 days prior to sacrifice. Bone sections were obtained from 3 sites. The specimens were embedded in resin and sectioned using routine histological techniques. Standard hit/intercept methods were used to evaluate dynamic histomorphometric (MAR-mineral apposition rate, BFR- bone formation rate) parameters.

In both age groups the BFR was ~2 fold higher in the mandible than the maxilla. In the adult dogs, the bone turnover in the jaws was 3 to 6 ($p<0.01$) fold higher than the femur. In contrast, the young dogs mean BFR was higher ($p<0.05$) in the femur than the jaws. Bisphosphonates decrease bone turnover at appendicular sites. Higher remodeling rates in the jaws of adults may predispose them to osteonecrosis and limit the ability of bone to respond to an injury or surgical insult. BFR decreases with age in the femur, but remains relatively elevated in the jaws.

The authors have no conflict of interest.

	Femur		Maxilla		Mandible	
Mean (SE)	MAR (μm/d)	BFR (%/yr)	MAR (μm/d)	BFR (%/yr)	MAR (μm/d)	BFR (%/yr)
Young	1.4 (0.1)	71.9 (6.5)	1.4 (0.1)	25.5 (3.1)	1.1(0.1)	51.0 (7.6)
Adult	1.3 (0.2)	6.4 (4.1)	1.5 (0.3)	19.1 (4.3)	1.5 (0.2)	36.9 (4.3)

Table 1.

P-7

Calcitriol And Phosphate Increase FGF23 Concentrations in XLH

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X-Linked hypophosphatemic rickets (XLH) causes rickets and osteomalacia due to renal phosphate wasting, with inappropriately low or normal 1,25-dihydroxyvitamin D concentrations. The *PHEX* mutation results in increased expression of fibroblast growth factor 23 (FGF23) and elevated circulating FGF23 concentrations. XLH is treated with high dose phosphate and calcitriol which could further increase FGF23 concentrations.

We measured FGF23 concentrations, before and during treatment with phosphate and calcitriol, in 8 subjects, aged 2 to 30 years, using an ELISA that detects only full-length FGF23. All pre-treatment FGF23 concentrations were elevated ranging from 127 to 1353 pg/ml (mean 349±408 pg/ml) (compared to a normal range <71 pg/ml). During therapy, post-treatment FGF23 concentrations ranged from 136 to 3270 pg/ml (mean 807±839 pg/ml). FGF23 concentrations increased after treatment in most subjects, but the elevation varied in extent and timing. FGF23 increased >20% in 5/8 and >100% in 3/8 subjects during treatment. FGF23 concentrations did not decrease in any subject. FGF23 concentrations correlated with both phosphate ($p=0.03$) and calcitriol doses ($p=0.004$). Controlling for combined treatment, only calcitriol dose remained a significant predictor of FGF23 concentrations ($p=0.01$).

Treatment of XLH with phosphate and calcitriol was associated with a concurrent increase in FGF23 concentrations. This effect is more strongly associated with the calcitriol dose. Increasing FGF23 concentrations may make therapy more difficult, or contribute to complications of therapy. Although more study is needed, it may be preferable to adjust therapy to minimize the effect on FGF23 concentrations.

Dr. Econs is a consultant for Kirin Pharmaceuticals, and holds the patent pending for FGF23. All other authors have no conflict of interest.

P-8

Comparison of Effects of Alendronate And Raloxifene on The Lumbar Bone Mineral Density, Bone Turnover, And Lipid Metabolism in Elderly Women With Osteoporosis

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The purpose of the present open-labeled prospective study was to compare the treatment effects of alendronate and raloxifene on the lumbar bone mineral density (BMD), bone turnover, and lipid metabolism in elderly women with osteoporosis. One hundred and twenty-two postmenopausal women with osteoporosis (mean age: 69.4 years) were randomly divided into two groups of 61 patients each: the alendronate group (5 mg daily) and the raloxifene group (60 mg daily). The BMD of the

lumbar spine (L1-L4), urinary level of cross-linked N-terminal telopeptides of type I collagen (NTX), and serum levels of alkaline phosphatase (ALP), total cholesterol (TC), high and low density lipoprotein cholesterols (LDL-C and HDL-C, respectively), and triglycerides (TG) were measured during the 12-month treatment period. There were no significant differences in the baseline characteristics between the two treatment groups. The trial could be completed in 50 patients in the alendronate group and 52 patients in the raloxifene group. Both alendronate and raloxifene increased the lumbar BMD (+8.0% and +2.4% at 12 months, respectively) following reductions of the urinary level of NTX (-44.6% and -34.5% at 3 months, respectively) and serum level of ALP (-17.7% and -9.6% at 12 months, respectively), with the effects of alendronate, however, being more pronounced than those of raloxifene. Only raloxifene reduced the serum levels of TC and LDL-C (-3.9% and -7.7% at 12 months, respectively) without any significant effect on the serum HDL-C and TG levels. No serious adverse events were observed in either group. The present study confirmed the greater efficacy of alendronate than raloxifene in increasing the lumbar BMD, through its effect of causing more marked reduction of the bone turnover than that by raloxifene, and some beneficial effects of raloxifeneon lipid metabolism in elderly women with osteoporosis.

The authors have no conflict of interest.

P-9

LRP5 IS VITAL FOR RESTORATION OF BIOMECHANICAL INTEGRITY IN HEALING BONE

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The importance of Wnt signaling in skeletal development and regulation is well documented, yet its role in fracture repair remains mostly unknown. At the cellular level, Wnt signaling is initiated by Wnt ligands binding to the Wnt co-receptor Lrp5. Homozygous null Lrp5 (Lrp5-/-) mice have low bone mass, concomitant with decreased mechanical properties. As Lrp5 is also known to be up-regulated during fracture repair, we hypothesized that fracture repair in Lrp5-/- mice would be compromised compared to wild-type littermates (Lrp5+/+). To test this hypothesis, closed fixed femoral fractures were generated unilaterally in Lrp5-/- (N=7) and Lrp5+/+ (N=6) mice.

Fracture repair was assessed *in vivo* by weekly radiography and *ex vivo* by pQCT, DXA and biomechanical (4-point bending) analyses (day 28 post-fracture). To correct for baseline phenotypic differences, results were normalized to contralateral intact femurs. No substantial differences in callus development were seen in the weekly radiographs or DXA and pQCT analyses. However, biomechanical testing revealed a gross hindrance in the mechanical properties of fractured femurs from Lrp5-/- mice, with reductions of 64-76% seen for ultimate load, stiffness, yield force, energy to yield and energy to ultimate force (all $p<0.05$). The striking mechanical impairment in Lrp5-/- calluses, combined with minimal radiographic differences, indicates that the newly formed bone is biomechanically unsound despite the fact that these calluses appear to undergo almost normal development and mineralization. We conclude that signaling through Lrp5 is critical for the establishment of biomechanical integrity in healing bone.

The authors have no conflict of interest.

P-10

LOW INTENSITY PULSED ULTRASOUND (LIPUS) INDUCES OPPOSITE METABOLIC RESPONSES OF OSTEOCYTES VERSUS CEMENTOBLASTS *IN VITRO*

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Serious root resorption (RR) occurs in approximately 20% of patients undergoing orthodontic treatment, leading to serious medico-legal issues. Low intensity pulsed ultrasound (LIPUS) has been reported to be effective in enhancing bone growth and fracture healing. Not only in orthopedics, LIPUS has also been reported to be able to decrease the number of resorption lacunae on the root surface during orthodontic tooth movement. However, the mechanism of LIPUS's effects on RR is unknown. We hypothesize that LIPUS prevents RR through inducing an anabolic response of cementoblasts. To testify this, we subjected OCCM.30 cementoblasts (in comparison to MLO-Y4 osteocytes) to LIPUS (30mW/cm^2, 1.5 MHz frequency, 1 kHz repetition).

Cells were seeded on 35mm Petri dishes and grown till 90% confluent.

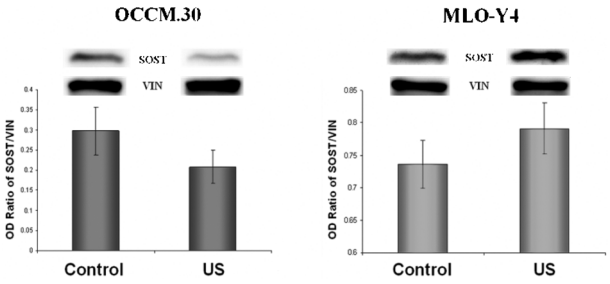

Figure 1. LIPUS oppositely regulates SOST protein production in MLO-Y4 osteocytes versus OCCM.30 cementoblasts. Both types of cells were subjected to LIPUS for 20 minutes followed by a post-incubation of 6 hours. The whole cell lysates were resolved through 10% SDS-PAGE and immunoblotted with antibodies against COX-2. It was found that LIPUS significantly increased the production of SOST in MLO-Y4 cells but decreased it in OCCM.30 cells. Representative WB results are shown. Values are expressed as mean ±SD (n=3, $p<0.05$ when compared to controls).

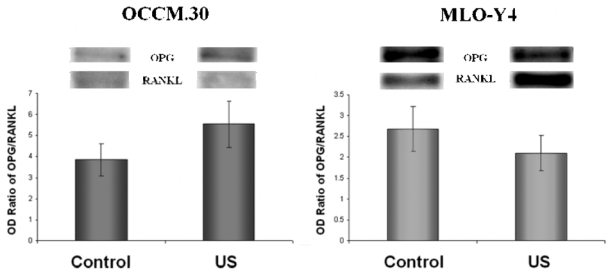

Figure 2. LIPUS oppositely regulates OPG/RANKL ratio in MLO-Y4 osteocytes versus OCCM.30 cementoblasts. Both types of cells were subjected to LIPUS for 20 minutes followed by a post-incubation of 6 hours. The whole cell lysates were resolved through 10% SDS-PAGE and immunoblotted with antibodies against OPG and RANKL. The densitometries of OPG and RANKL were determined and the ratio of OPG/RANKL calculated. As shown LIPUS significantly increased the ratio of OPG/RANKL in OCCM.30 cells, which was decreased in MLO-Y4 cells. Representative WB results are shown. Values are expressed as mean ±SD (n=3, $p<0.05$ when compared to controls).

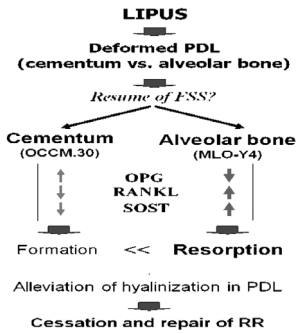

Figure 3. Hypothetical model of LIPUS's prevention and interception of the formation of root resorption in orthodontics.

Starved for 24 hours, the cells were subjected to LIPUS for 10 minutes to test signaling pathway or 20 minutes followed by 6 hours post-incubation to examine functional changes, using static cells as control. As found, in response to LIPUS, ERK1/2 activation, COX-2 production was increased in both cell types. SOST was increased in MLO-Y4 but decreased in OCCM.30 cells (Figure 1). Remarkably, OPG/RANKL ratio was slightly increased in OCCM.30 but dramatically decreased in MLO-Y4 cells (Figure 2). As a conclusion, LIPUS's prevention of RR is through not only promoting cementogenesis but more likely increasing osteoclastic alveolar bone resorption which indirectly alleviates the compression-induced hyalinization in periodontal ligament – a pathological basis for the formation of RR (Figure 3).

The authors have no conflict of interest.

P-11

Mutation in The *Ank* Gene Causes Craniometaphyseal Dysplasia (CMD)-Like Phenotype

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Craniometaphyseal dysplasia (CMD) is a rare craniotubular disorder characterized by progressive thickening of craniofacial bones concurrent with widened and radiolucent metaphyses in long bones. Here we study a knock-in (KI) mouse model expressing a deletion mutation (Phe377del) of the pyrophosphate (PPi) transporter gene *Ank*. $Ank^{KI/KI}$ bones show progressive radiopacity in cranial structures as well as metaphyseal widening and increased radiolucency in femurs. In addition, $Ank^{KI/KI}$ mice develop decreased mobility of joints, similar to $Ank^{ank/ank}$ and *Ank* null mice. Most $Ank^{+/KI}$ mice look and behave like wild type mice but over time develop an intermediate CMD-like phenotype. Consistent with clinical findings, DEXA shows increased bone mineral density and mineral content in skulls and jaws from $Ank^{KI/KI}$ mice. However, MicroCT results show lower mineralization in craniofacial and long bones. Moreover, $Ank^{KI/KI}$ mice display hyperostosis of calvariae and cranial base; narrowing of cranial neural foramina; and significant decrease in trabecular number and bone volume fraction (BVF) of club-shaped femurs. Multinucleated osteoclast formation *in vitro* is inhibited; however, the mechanism is still under investigation. Our observations suggest that the *Ank* mutation may cause the CMD-like phenotype in a dose and time-dependent manner.

Based on a comparable phenotype to the *Ank* null mice we propose that the CMD-causing *Ank* mutation is a loss of function mutation in PPi transporting activity. The unique CMD-like features in these mice suggest that a second molecular mechanism, rather than solely an abnormality in the extracellular pyrophosphate level, is involved in CMD pathogenesis.

The authors have no conflict of interest.

P-12

MLO-A5 Cells in a Porous Polyurethane Scaffold as a Model to Study The Mechanobiology of Bone Matrix Production in 3-D

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We have established a model to investigate the effects of mechanical loading on matrix production by bone cells in 3-D. Cells are seeded in porous polyurethane scaffolds and cultured in static conditions for at least 5 days. Cell-seeded scaffolds are subjected to bouts of mechanical loading at specified strains and frequencies in a sterile fluid filled chamber (Bose Electroforce 3200). We have used this model to investigate the effects of mechanical loading on matrix production by MLO-A5 late-stage osteoblast cells (donated by L. Bonewald). 2 bouts of 2 hours of compressive loading at 5% strain, 1 Hz caused final collagen content (sirius red staining) to be 50% higher in loaded samples compared with non-loaded controls ($p<0.05$). The effect was not changed by increasing the number of loading bouts, varying the days on which loading took place, or increasing the post-load culture time. However, reducing the loading period to 1 or 0.5 hours eliminated the difference in collagen content. To assess mineralization, loading was applied for 2 hours per day at days 5, 10 and 15 of culture on cells cultured with ascorbic acid and betaglycerophosphate. Calcium content (alizarin red staining) and scaffold stiffness were higher in loaded samples compared to non-loaded at all time-points, mineral content was five-fold higher by day 20 ($p<0.01$).

In conclusion, cyclic compressive loading of MLO-A5 cells in porous scaffolds in a Bose biodynamic chamber increases bone matrix production in a stimulus-dependent fashion and has the potential for use as a model to understand bone cell mechanobiology.

The authors have no conflict of interest.

P-13

Androgen Administration Has Therapeutic Advantages in The Hypogonadal, But Should be Approached With Caution in Healthy Adults

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Androgens are known to have pervasive effects on target tissues including muscle and fat, yet the effects on bone remain poorly characterized. To gain an insight into the cell types important for mediating androgen action, we constructed and compared two distinct transgenic lines of mice employing different α1 (I)-collagen promoter fragments to control skeletally-targeted androgen receptor (AR) overexpression. Histomorphometric and biomechanical analyses revealed compromised bone strength with AR overexpression in bone during development. The role of AR signaling in the adult was characterized *in vivo* using an experimental paradigm of hormone ablation followed by steroid replacement. Control and AR-tg mice were sham operated or gonadectomized at 3 months of age and the effect of nonaromatizable dihydrotestosterone (DHT) was determined after an 8-week delay, allowing for gonadectomy-induced changes to develop.

Following 6 weeks of treatment, the effects of androgen on bone and whole body composition was assessed by DXA. In control mice, systemic DHT administration significantly increased BMD and BMC in both sexes, reversing the loss sustained after a prolonged hypogonadal state. In contrast, in AR-tg mice DHT replacement did not improve either measure compared to placebo. DHT treatment was also beneficial to body composition, improving or fully restoring alterations in lean/fat mass after gonadectomy in control but not AR-tg mice. Further, DHT treatment in intact mice had a negative impact on body composition, reducing lean mass and increasing fat. These findings suggest androgen administration has therapeutic advantages in the hypogonadal, but should be approached with caution in healthy adults.

The authors have no conflict of interest.

P-14

Archtecture of The Lower Leg in Individuals With Neurofibromatosis Type 1 Using Peripheral Quantitative Computed Tomography

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Objective: The prototypical skeletal finding of neurofibromatosis type 1 (NF1) is unilateral tibial dysplasia. The unilaterality suggests a random second event. In support, we documented double activation of *NF1* in tibial pseudarthrosis tissue. However, we reported decreased BMD in *NF1* suggesting that NF1 haploinsufficiency also produces a generalized dysplasia. In order to investigate the bony architecture of the leg in NF1 individuals without tibial dysplasia, we utilized peripheral quantitative computed tomography (pQCT).

Methods: Measurements using pQCT (XCT-2000, Stratec) at the 66% and 4% site were obtained on both legs in NF1 individuals and the non-dominant leg in controls. Individuals with tibial dysplasia were excluded. The non-dominant leg of NF1 individuals were compared to controls adjusting for age, gender, tanner stage, height and weight. Dominant and non-dominant legs in NF1 were compared.

Results: NF1 individuals=93; Controls=448. Statistically significant decreases ($p<0.01$) were observed in all pQCT variables except for cortical BMD and thickness at the 66% site and tibial length. No statistically significant differences were observed between non-dominant and dominant lower legs in NF1 individuals except for a slight increase in strength strain index (66% site) of the non-dominant leg ($p=0.04$).

Conclusions: NF1 individuals without tibial dysplasia have a different bony architecture compared to individuals without NF1 based on pQCT measurements. The lack of significant differences between the non-dominant vs. dominant legs in NF1 individuals suggests that the differences between NF1 individuals and controls are generalized and not due to a unilateral subclinical tibial dysplasia in NF1.

The authors have no conflict of interest.

P-15

Treatment of Skeletally-Mature Beagle Dogs For 1-Year With 5-6X The Clinical Dose of BP Allows Significant Accumulation of Ages in Cortical Bone

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Bisphosphonates (BPs), such as risedronate (RIS) and alendronate (ALN), have been shown to be efficacious treatments for reducing the risk of osteoporotic fractures. However, the effects of long-term BPs' administration on the quality of bone's extra-cellular matrix (ECM) are relatively unknown. One important aspect of ECM quality is reflected in the accumulation of advanced glycation end-products (AGEs) by non-enzymatic glycation (NEG). NEG has been shown to occur in connective tissues, including bone and modify the properties relating to fracture resistance. Increased accumulation of AGEs has been demonstrated with increasing age in bone. Since bisphosphonates suppress turnover, leading to an overall increase in mean tissue age, we hypothesize that the reduction in tissue turnover may consequently increase the accumulation of AGEs. Furthermore, the accumulation of AGEs may adversely affect the fracture resistance of cortical bone.

This study documents the treatment of skeletally-mature beagle dogs for 1-year with 5-6x; the clinical dose of BP allows significant accumulation of AGEs in cortical bone. AGEs' accumulation is associated with a significant increase in tissue brittleness, as indicated by the significant decrement in post-yield work to fracture in high dose BP-treated animals ($p<0.001$) along with the significant inverse relationship between AGEs and work-to-fracture among all animals ($p<0.001$). In contrast to high doses, treatment with doses equivalent to those used for treatment of post-menopausal osteoporosis did not significantly alter AGEs' accumulation or mechanical properties of cortical bone. Dose-equivalents of RIS and ALN produced similar results for all properties measured in this study. These data implicate remodeling suppression with AGEs' accumulation, which in-turn influence fracture resistance at cortical bone sites. The levels of cortical bone turnover suppression achieved with clinical doses of BPs do not appear sufficient to allow AGEs' accumulation, thus sparing the bone from the associated decreases in fracture resistance. However, these data clearly show the negative consequences of AGEs accumulation in the skeleton, and implicate the level of remodeling suppression as the cause.

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P-16

Long Bone Dysplasia in neurofibromatosis Type 1

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Objective: Neurofibromatosis type 1 (NF1) is diagnosed clinically based on the presence of 2 of 7 criteria as developed by a panel of experts in 1987. The sixth criterion focuses on skeletal findings and is stated as follows: "A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex, with or without pseudoarthrosis". The wording for this criterion is misleading. A better description of what constitutes a distinctive osseous lesion is needed to effectively use this sixth criterion.

Methods: We reviewed all radiographs of the lower leg of patients with the following diagnostic codes: (pseudarthrosis of bone, pseudarthrosis of tibia, neurofibromatosis generalized, neurofibromatosis of tibia, and non-union of fracture) at the Shriners Hospital for Children Intermountain over 52 years between 1950 and 2002. Radiographs with instrumentation or fracture of the tibia were excluded in order to assess the initial radiographic findings of tibial dysplasia. A total of 26 cases fitting the above criteria were identified.

Results: Anterolateral bowing was observed in 25/26 (one individual had posterolateral bowing). Cortical thickening near the apex of the bowing was observed in 27/27 individuals. The fibula was dysplastic in 21/26 individuals. The most characteristic radiographic findings in a dysplastic tibia prior to fracture are anterolateral bowing with medullary canal narrowing and cortical thickening that is greatest at the maximum point of bowing, usually located near the distal third of the tibia.

Conclusions: We propose to eliminate the example of "thinning of the cortex, with or without pseudarthrosis" used in the diagnostic criterion. We suggest that anterolateral bowing of the distal third of the leg, with or without fracture or pseudarthrosis, is a more appropriate description of the primary finding a clinician will use to fulfill the sixth diagnostic criterion for NF1. Clarification of this diagnostic criterion is important for the clinician and for research protocols utilizing these criteria for inclusion in therapeutic trials. Appropriate interpretation of these distinctive osseous lesions will improve understanding of the natural history and pathophysiology of NF1.

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P-17

Solute Transport Among Osteocytes in Live Animals

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Introduction: Osteocytes, the most numerous cells in bone, are essential for bone to sense and adapt to mechanical stimuli and to remodel damaged tissue. Since osteocytes are completely encased in mineralized bone matrix, their survival and function are entirely dependent on the transport of solutes (metabolites, growth factors, cytokines, and other signaling molecules) through the lacunar-canalicular system (LCS). However, little is known about the mechanisms involved in moving biological molecules to and from osteocytes *in vivo*. This reflects a lack of methods available to study these questions under real-time conditions in living animals.

Methods and results: We developed a new imaging method based on Fluorescence Recovery After Photobleaching (FRAP) that allowed measurement of solute movement in LCS in live animals. This method has been used by us to quantify solute diffusion in newly sacrificed bone (Wang et al., 2005, PNAS 102:11911). The current study aimed to quantify solute convection due to mechanical loading and/or hydrodynamic blood pressure. Our working hypothesis is that convection due to mechanical loading is the primary mechanism for moving large molecules in the LCS. To begin to test this hypothesis, the following studies were performed.

Live imaging without loading: Adult B6 mice were anesthetized and received a dose of sodium fluorescein via tail vein injection. After the medial anterior surface being exposed, the hind limb was fixed in a custom fluid chamber and time series of confocal images were obtained from the tibial mid-shaft. Motions caused by breathing, heart beating, and muscle contraction were greatly reduced in this preparation, possibly due to the usage of anesthesia and rigid fixing of the limb. Reasonable good focus of the fluorescent lacunar-

canalicular system was obtained during 2-5 minute imaging session. *In vivo* FRAP was performed on 6 lacuna and the effective diffusion coefficient for sodium fluorescein was found to be greater than that found *in situ*. We are investigating the possible reasons including the difference in blood pressure and LCS permeability between the *in vivo* and *in situ* preparations.

Live imaging with loading: We designed and fabricated a loading device that allowed us to image AND apply well-controlled mechanical strains to bones in live mice. Using this device, we applied cyclic intermittent compression (2 sec loading -4 sec imaging -2 sec loading) on the knee joint while the ankle was fixed. The stroke displacements were set to 50, 100, 150 and 200 microns with 1 second ramping up and 1 second ramping down, producing cyclic (peak-to-peak) forces of 0.8, 1.5, 3.0, and 4.8 N. Good focus was obtained for displacements no larger than 150 micron, which were used for all the following studies.

FRAP in mechanically loaded live bone: We are currently performing FRAP using the intermittent loading regimen. Mechanical loading seems to speed up the recovery in these experiments. To analyze the data, a two-leveled mathematical model has been developed to predict pore fluid pressure at the whole bone level and tracer recovery at the LCS level. The model predicts 8- to 500-fold increase of recovery rates for various sized molecules in loaded vs. unloaded bones. The overall trend of recovery is found to follow a damping process, validating the usage of intermittent loading regimen. We will fit our experimental data in the model to estimate the LCS permeability, the most sensitive parameter in the model, and then to obtain the fluid velocity and transport characteristics such as Peclet numbers in the LCS.

Conclusions: We have tested the feasibility and worked out a protocol to measure solution convection in mechanically loaded live bone. A two-leveled mathematical model has been developed to analyze the experimental data. These studies will help delineate the transport mechanisms that are essential for osteocyte viability and bone mechano-transduction, and provide new insights into drug delivery in bone and nutrient supply in tissue engineering scaffolds.

The authors have no conflict of interest.