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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 MUSENCHELIMAL 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 Hypochondrodysplasia (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 (TDII). 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 vitro 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.

References

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...
The authors have no conflict of interest.

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/Pi-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 homoygous 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”, and 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, cryptogenic, 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 biochemical, clinical, and radiological criteria. The degree of cholestasis (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, CSMI's). 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=’ 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 (p=-0.558, 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) and (2) in correlation 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 cholestasis; (2) reduced also the muscular mass, independently of the bone turnover 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 jaws. 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 histoculture methods were used to evaluate dynamic histomorphometric (MAR-mineral apposition rate, BFR- bone formation rate) parameters.

**Table 1.**

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<thead>
<tr>
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<th>Femur</th>
<th>Maxilla</th>
<th>Mandible</th>
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<tbody>
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<td></td>
<td>MAR (μm/d)</td>
<td>BFR (%/yr)</td>
<td>MAR (μm/d)</td>
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<tr>
<td>Young</td>
<td>1.4 (0.1)</td>
<td>71.9 (6.5)</td>
<td>1.4 (0.1)</td>
</tr>
<tr>
<td>Adult</td>
<td>1.3 (0.2)</td>
<td>64.4 (4.1)</td>
<td>1.5 (0.3)</td>
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**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 histoculture 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 jaws. 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.**

**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
IN HEALING BONE

LIPUS has also been reported to be able to decrease the number of cementoblasts in enhancing bone growth and fracture healing. Not only in orthopedics, Low intensity pulsed ultrasound (LIPUS) has been reported to be effective in orthodontics, leading to serious medico-legal issues.

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CEMENTOBLASTS

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 test this, we subjected OCCM.30 cementoblasts (in comparison to MLO-Y4 osteocytes) to LIPUS (10mW/cm², 1.5 MHz frequency, 1 kHz repetition).

Cells were seeded on 35mm Petri dishes and grown till 90% confluent.
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 OCCR30 cells (Figure 1). Remarkably, OPG/RANKL ratio was slightly increased in OCCR30 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).

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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 pyrophosphatase (PPi) transporter gene *Ank*. *Ank* knockout (KO) mice show progressive radiopacity in cranial structures as well as metaphyseal widening and increased radiolucency in femurs. In addition, *Ank* KO mice develop decreased mobility of joints, similar to *Ank* KO 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* KO mice. However, MicroCT results show lower mineralization in cranial and long bones. Moreover, *Ank* KO 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 c1 (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 BMC 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 ARCHITECTURE OF THE LOWER LEG IN INDIVIDUALS WITH NEUROFIBROMATOSIS TYPE I 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 individuals without tibial dysplasia, we utilized peripheral quantitative computed tomography (pQCT).

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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-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 fixation of the limb. Reasonable good focus of the fluorescein 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 mechanotransduction, and provide new insights into drug delivery in bone and nutrient supply in tissue engineering scaffolds.

The authors have no conflict of interest.